Playing by the Rules:
Structural and Spatial Organization of Biofilm Communities

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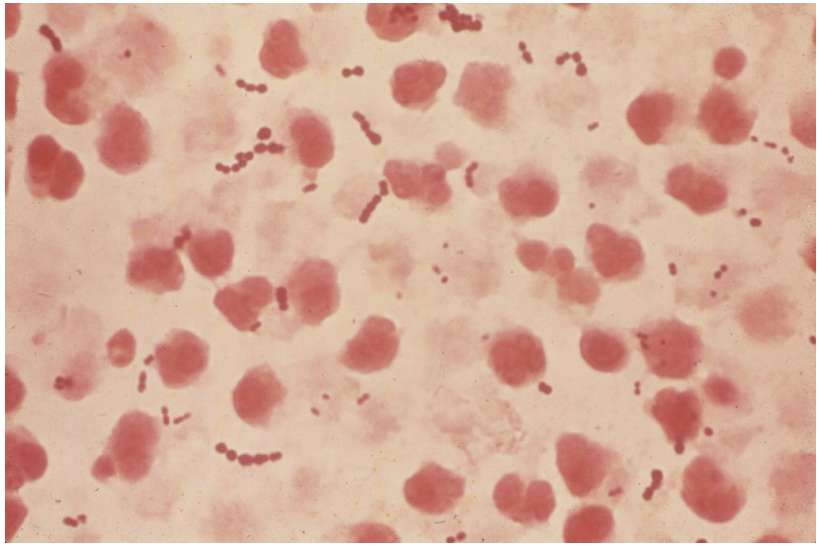
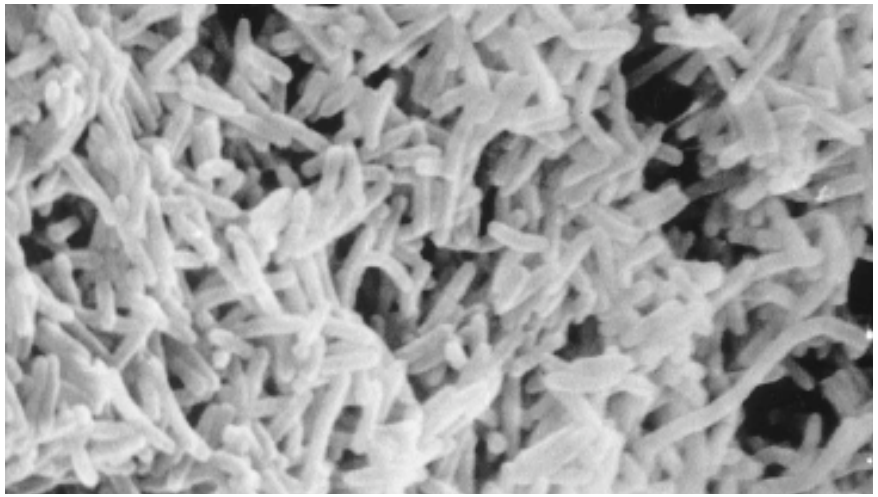
How do bacteria grow?

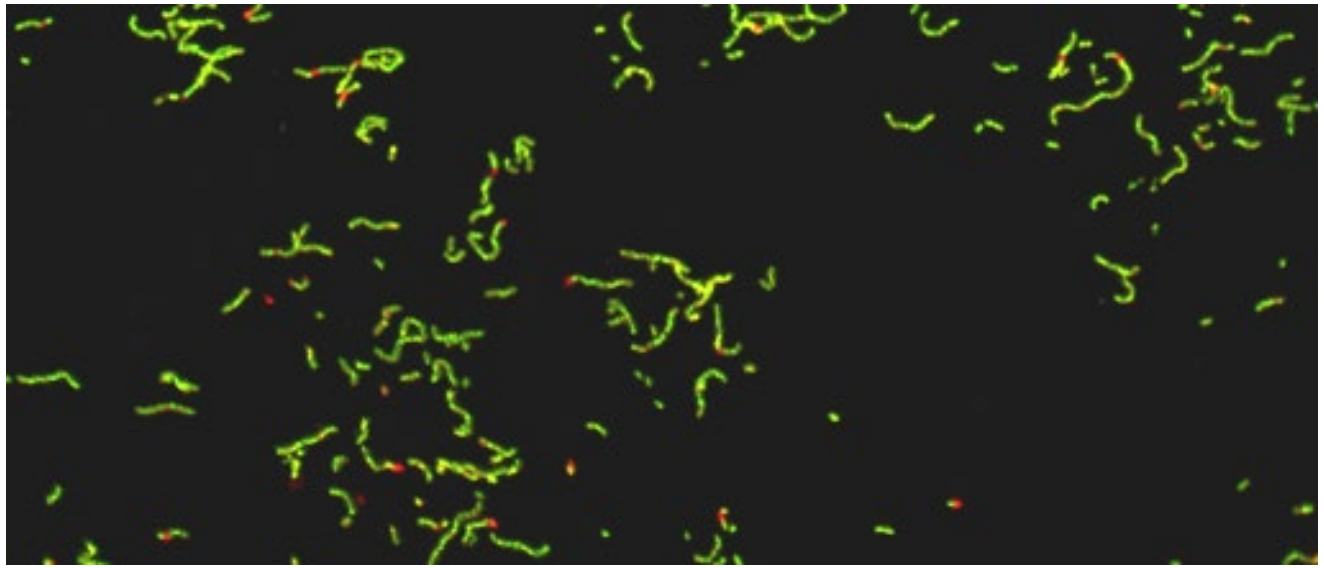


Planktonic Cells
(e.g. in blood during septicemia)

Biofilms



Adherent Cells



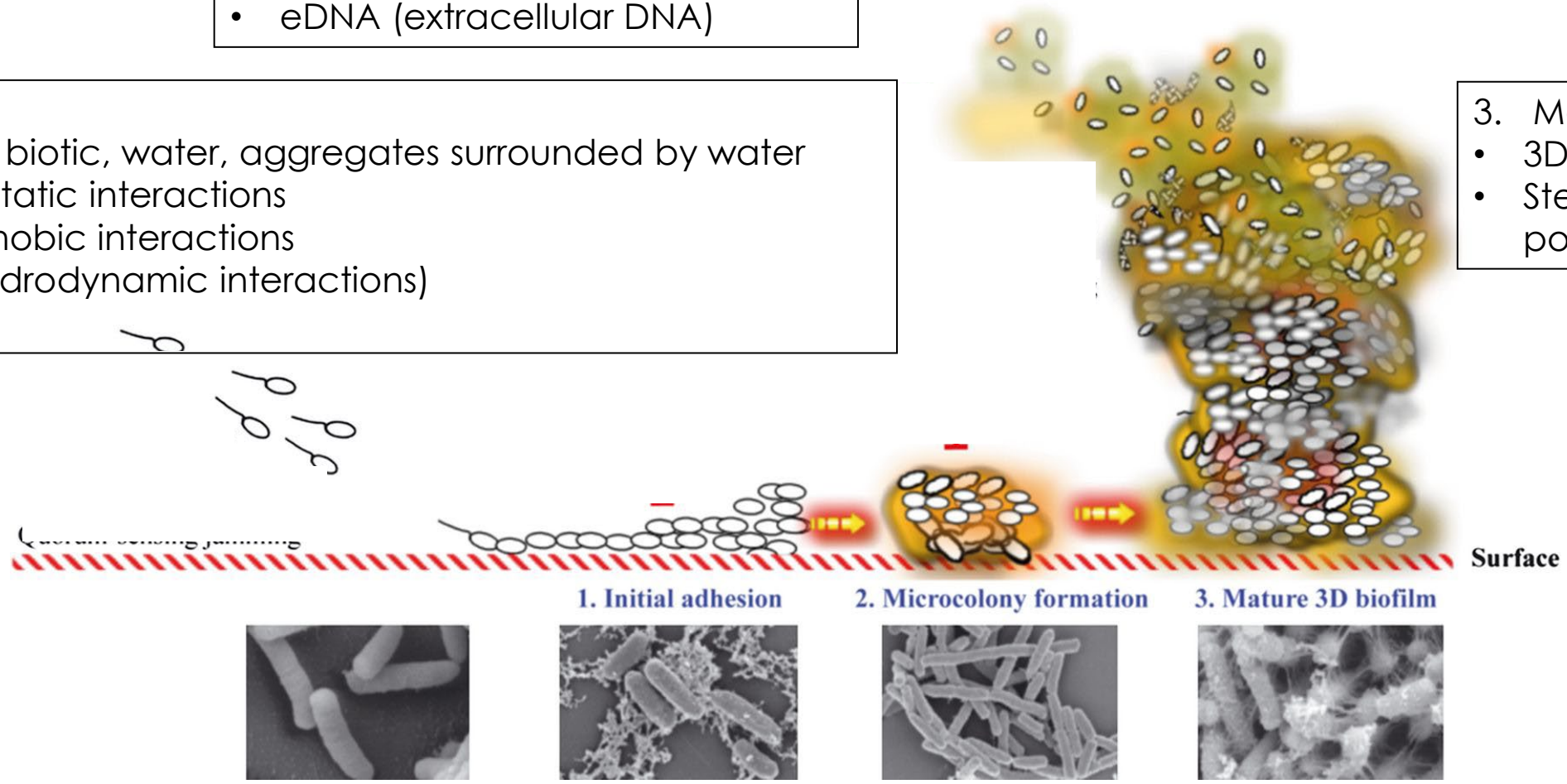
What is a biofilm?

2. Matrix can be composed of
- polysaccharides
- Protein (including amyloids)
- eDNA (extracellular DNA)

4. Cellular detachment
- in some species

1. surface
- abiotic, biotic, water, aggregates surrounded by water
- electrostatic interactions
- hydrophobic interactions
- flow (hydrodynamic interactions)

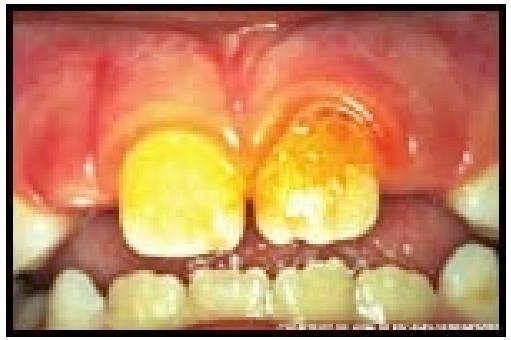
3. Mature biofilm
- 3D structure
- Steady state population



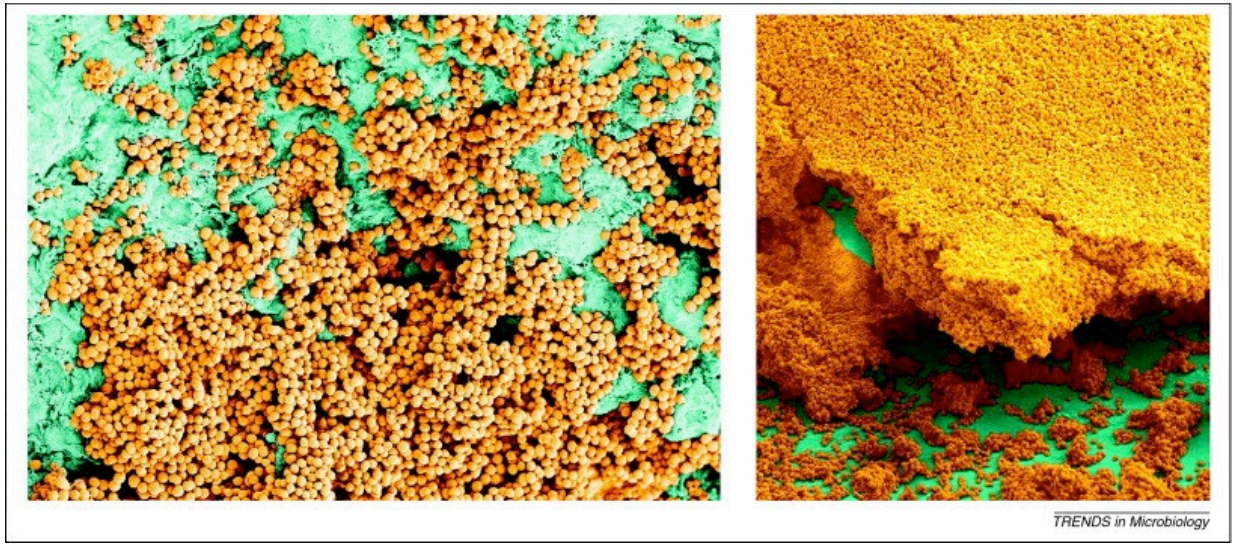
Rendueles and Ghigo, FEMS Microbiol. Rev., 2012

Where are biofilms?

Kitchen drainpipe

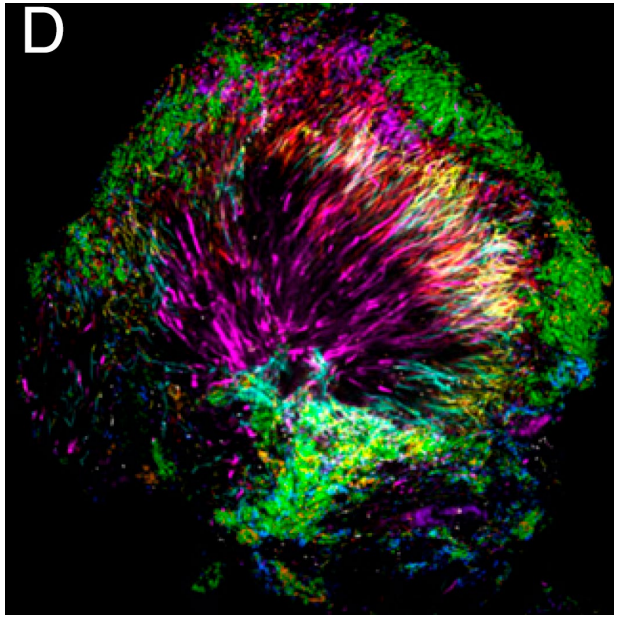


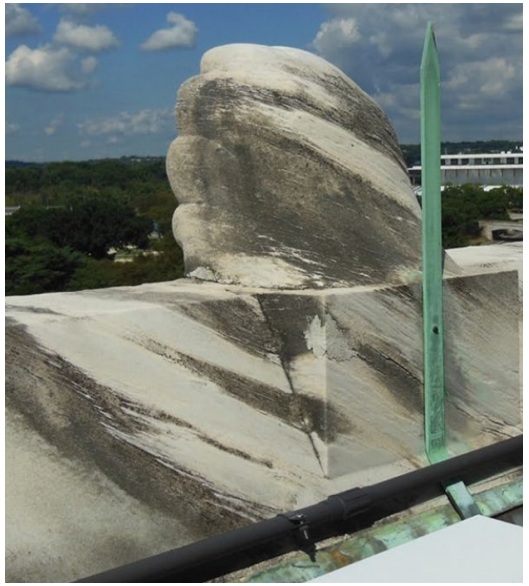
Dental plaque
(microscale)

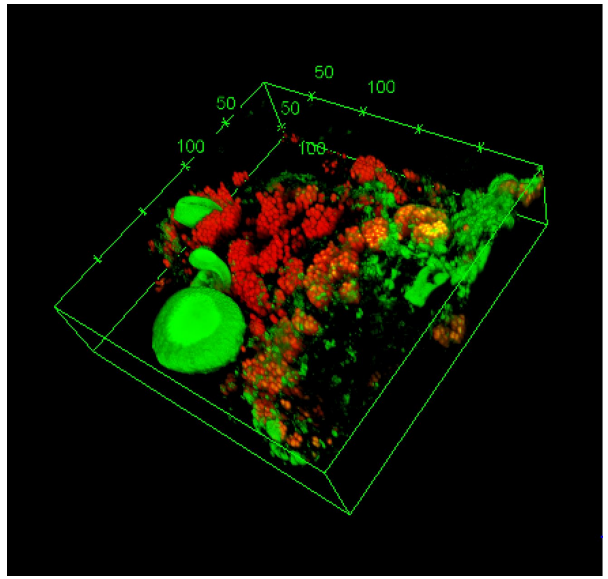


Staphylococcus aureus
on heart valve and endotracheal tube

Streams

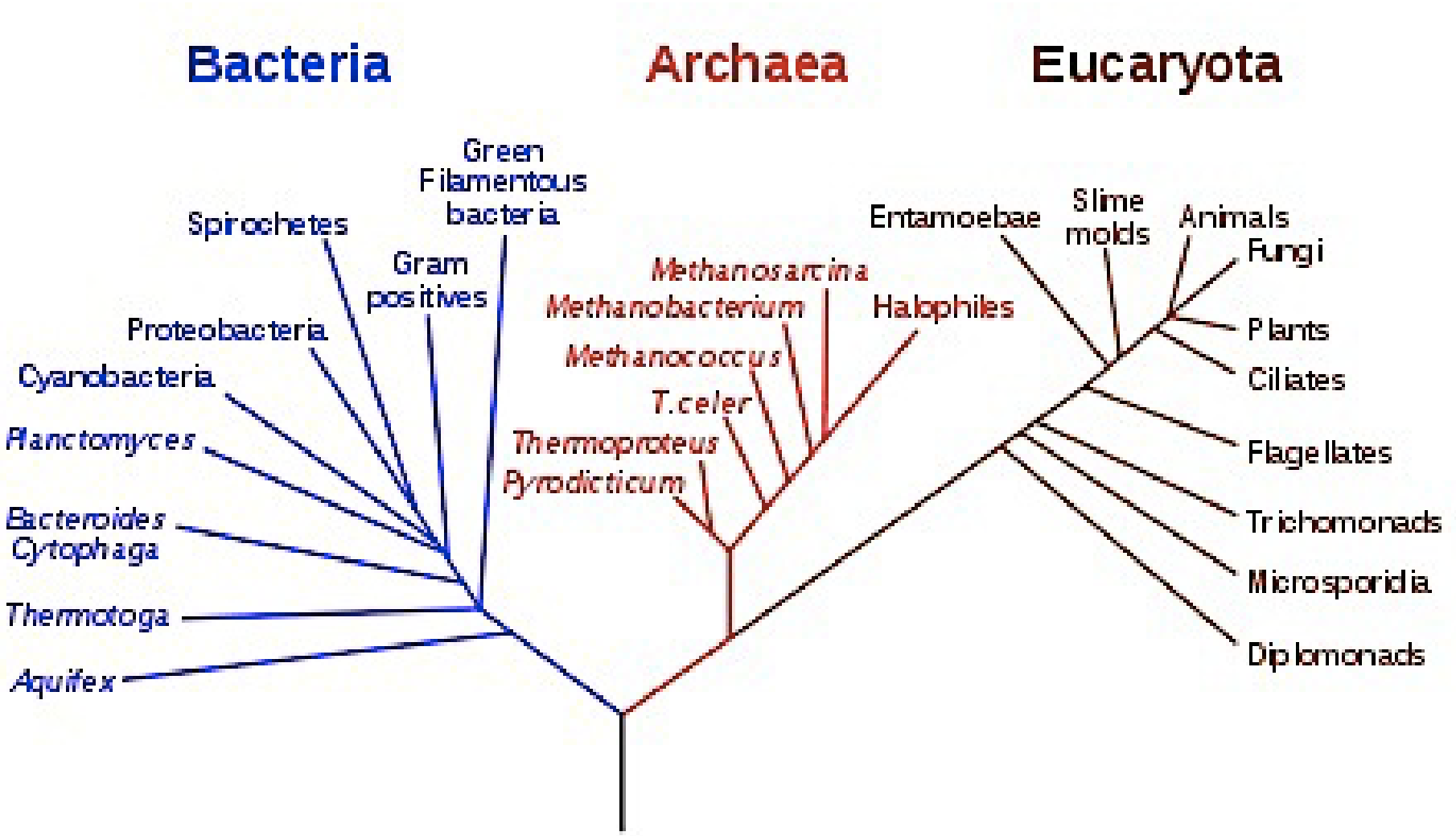






Marble Monument

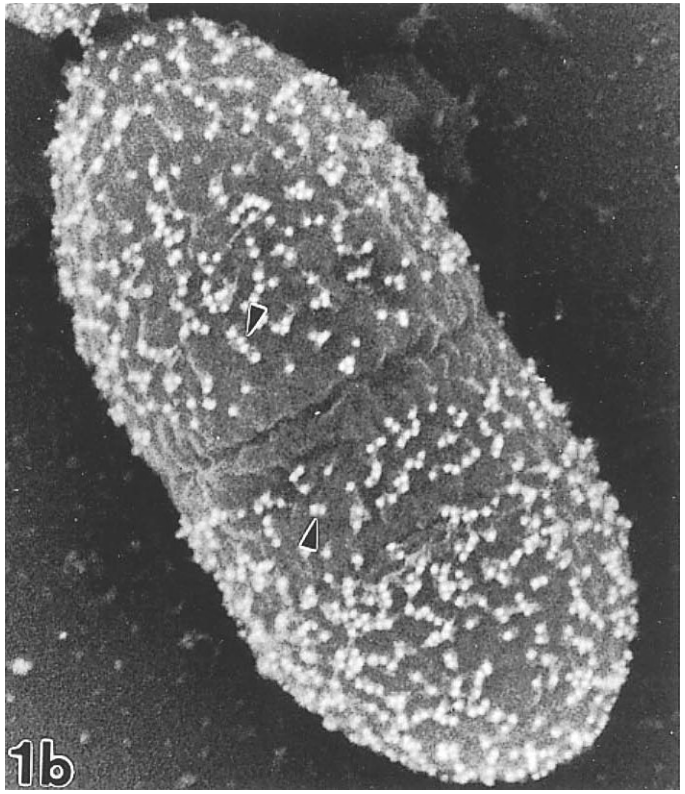
Bacterial Naming Phylogeny
(Evolutionary Relatedness 16S rRNA gene)



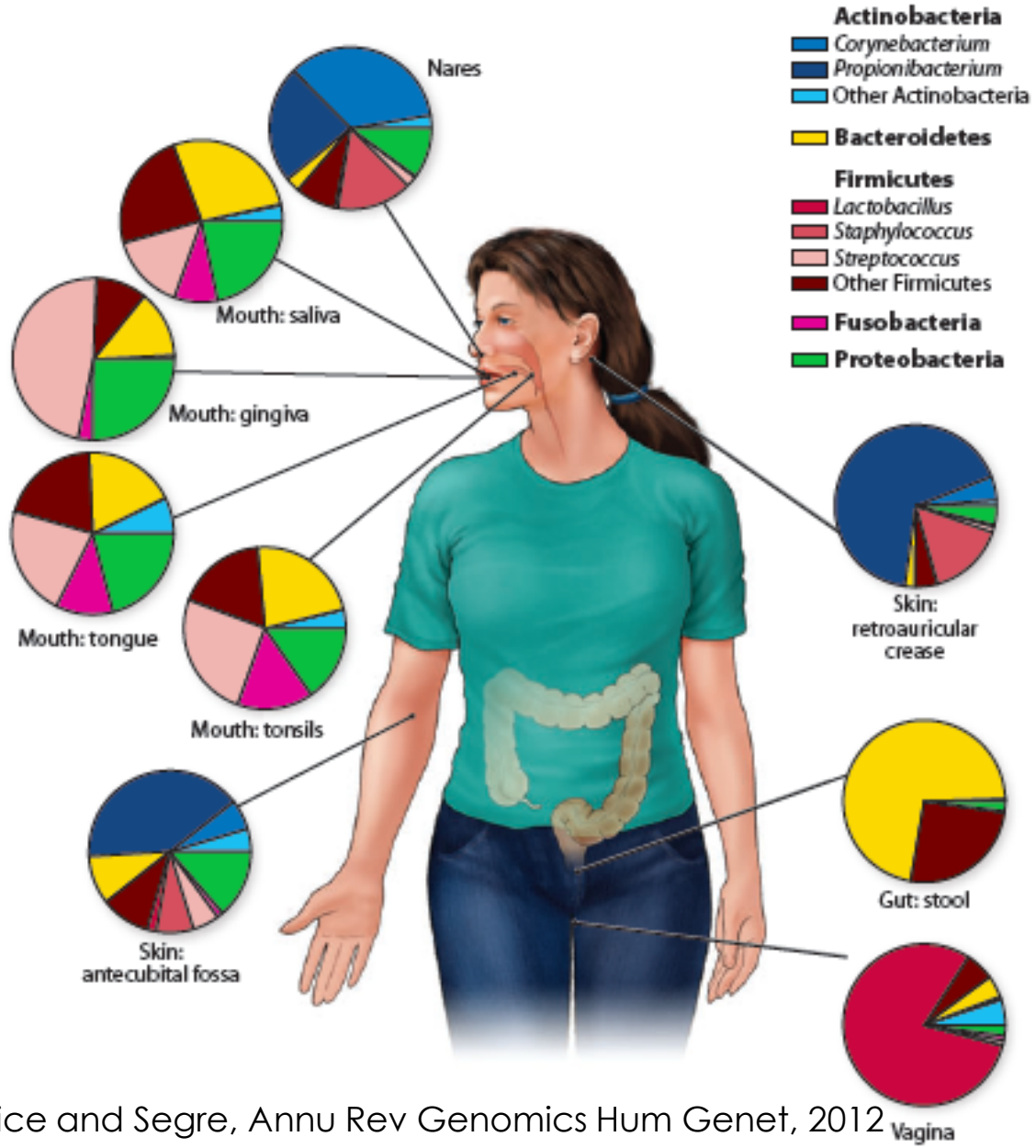
dendrogram or tree

Bacterial taxonomy

Kingdom	Bacteria
Phylum	Firmicutes
Class	Bacilli
Order	Lactobacillales
Family	Enterococcaceae
Genus	*Enterococcus*
Species	*faecalis*



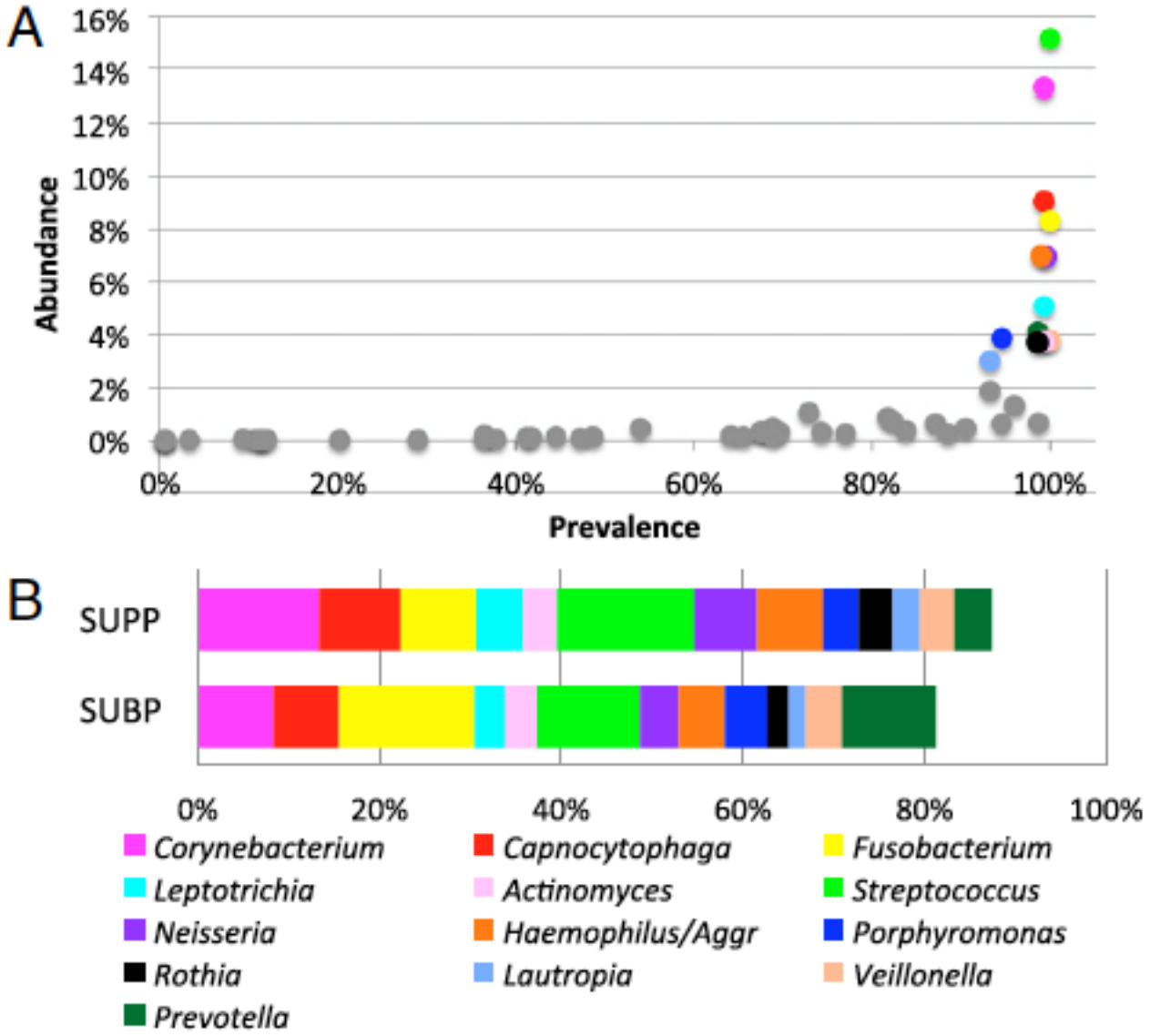
Biofilms in promoting human health



Grice and Segre, Annu Rev Genomics Hum Genet, 2012

- Can be composed of hundreds of different species
- Many of the bacterial species have never been cultured in the laboratory
- Highly variable from person to person
- Studies focus on general properties shared by bacteria in the same genus, family or phylum
- Major example is that many bacteria can break down non-digestible fiber in the diet -> use it to produce energy for growth -> produce a byproduct of short chain fatty acids (scfa) that promote human health

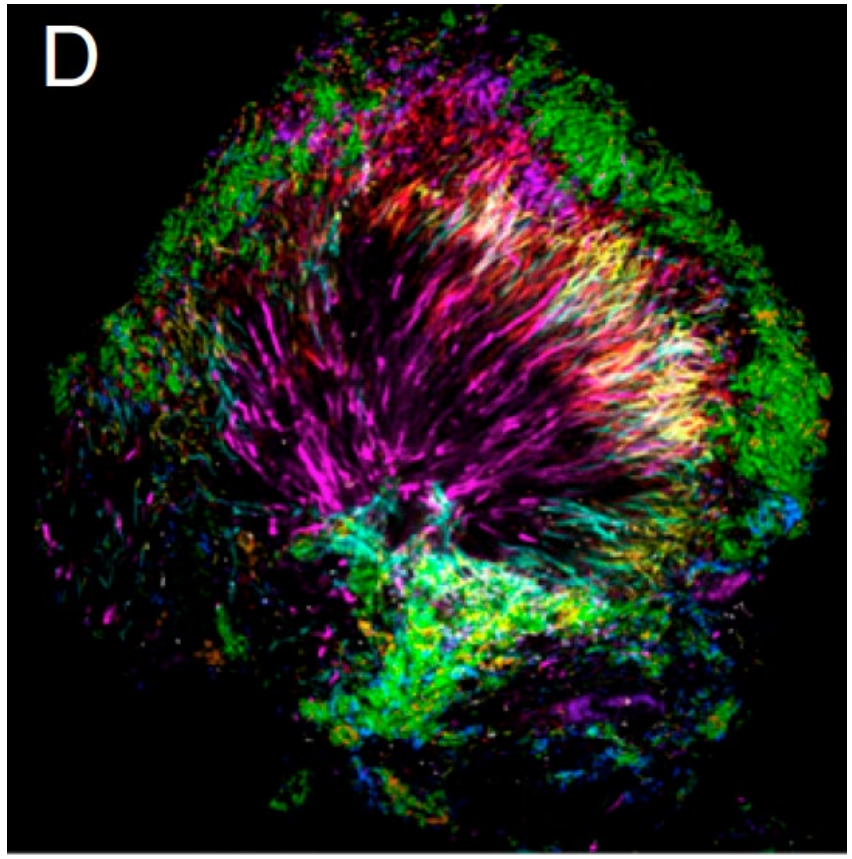
Microbiota are highly evolved complex ecologies
Example oral microbiome



- There more than 700 species of bacteria in the oral microbiome
- In 148 patients a few genera (composed of multiple species) were present in the highest numbers (abundance) and in almost all patients (prevalence)
- The organization of major members of the microbiota can be studied using fluorescently labeled to probes to visualize the bacteria present in the biofilm using CLASI-FISH

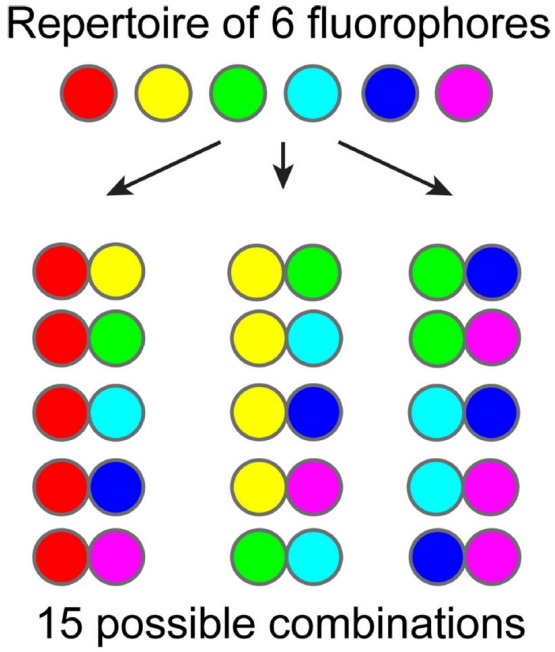
CLASI-FISH microscopy

Borisy Lab: Sys. Appl. Microbiol. 2012, PNAS 2016, Cell Host Microbe, 2020

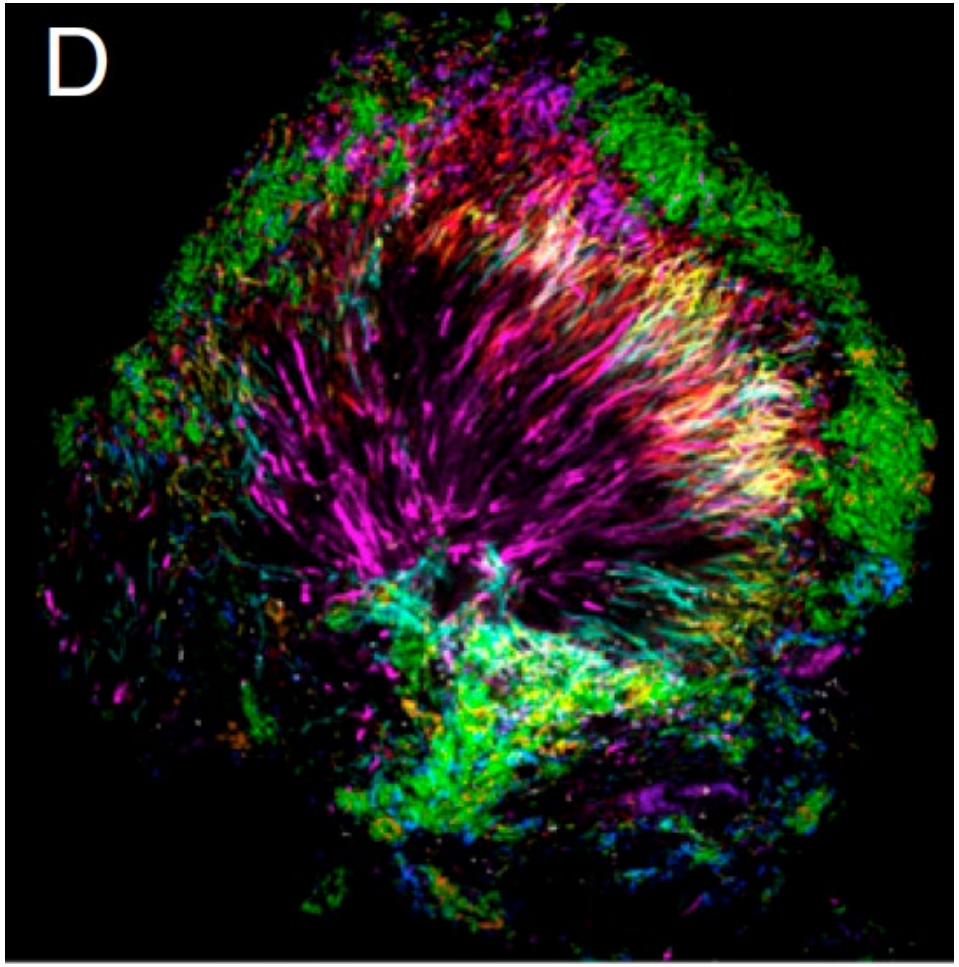


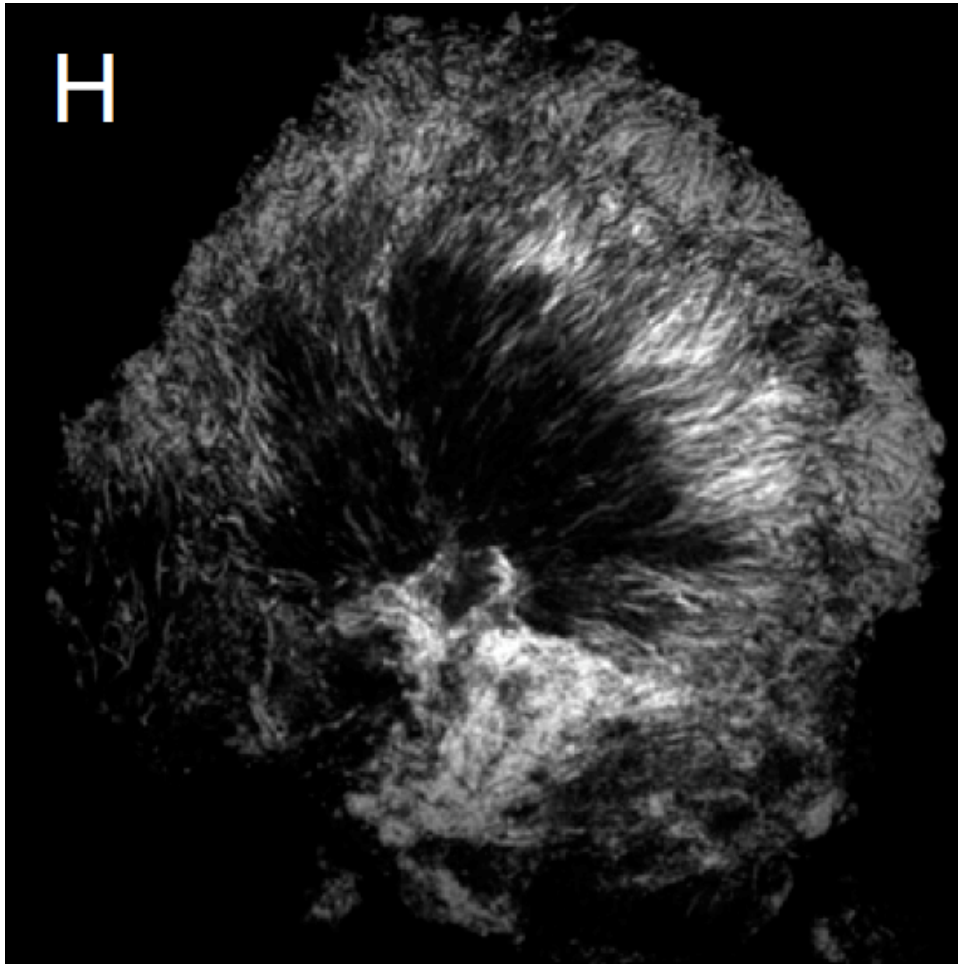
Corynebacterium
Streptococcus
Capnocytophaga
Haemophilus/Aggr.
Porphyromonas
Neisseriaceae
Fusobacterium
Leptotrichia
Bacteria (Eub338)

- 2D-microscopy with fluorescent probes to label the different genera of bacteria
- Each genera is labeled by 2 colors
- Each combination is assigned a unique color
- The organization of the community can be visualized

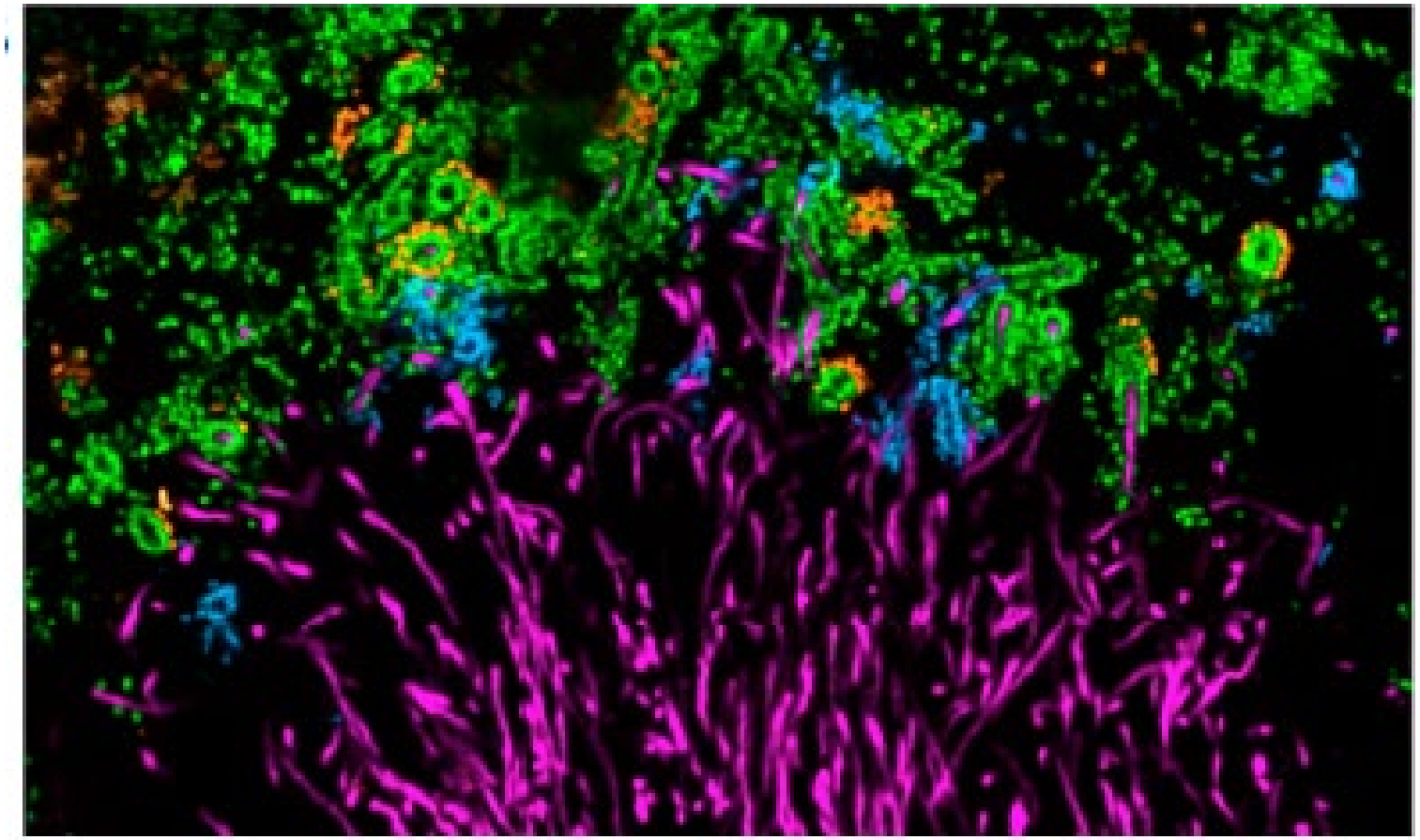


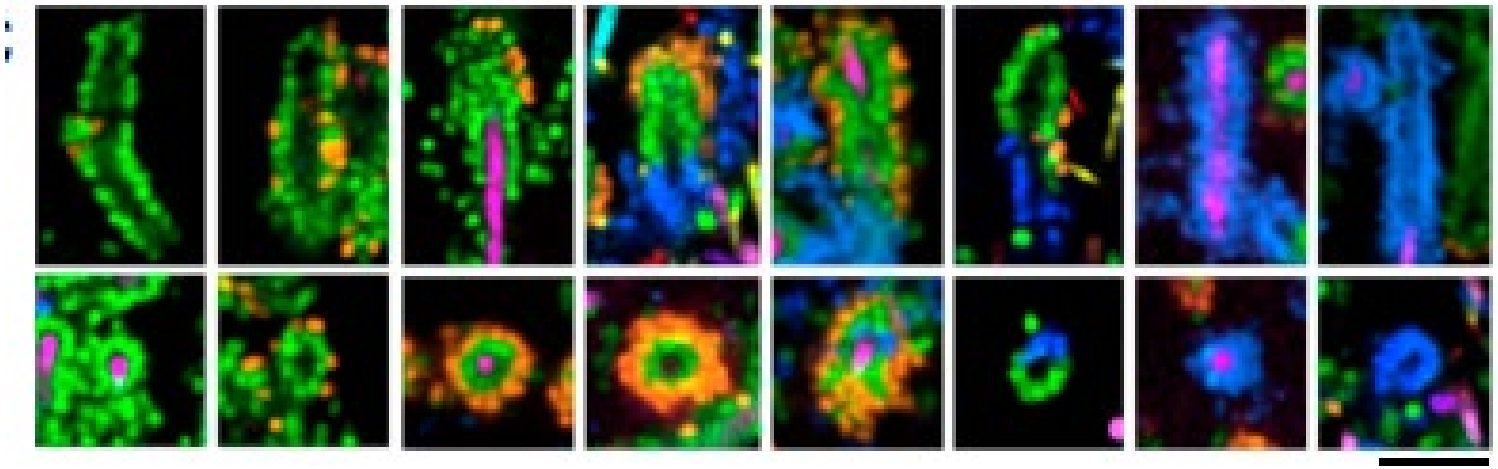
- All colored probes vs universal probe confirm that most bacteria in the microbiota are the highly abundant genera (can represent multiple species)
- Uncolored bacteria represent the highly variable diverse bacteria present in lower numbers



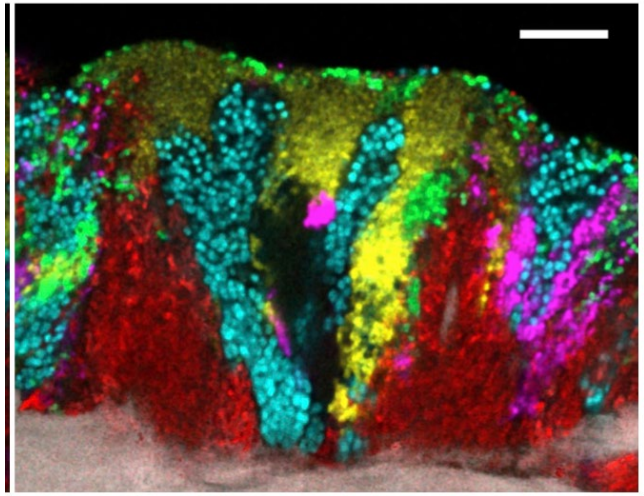


Corynebacterium *Capnocytophaga* *Porphyromonas* *Fusobacterium* *Bacteria (Eub338)*
Streptococcus *Haemophilus/Aggr.* *Neisseriaceae* *Leptotrichia*





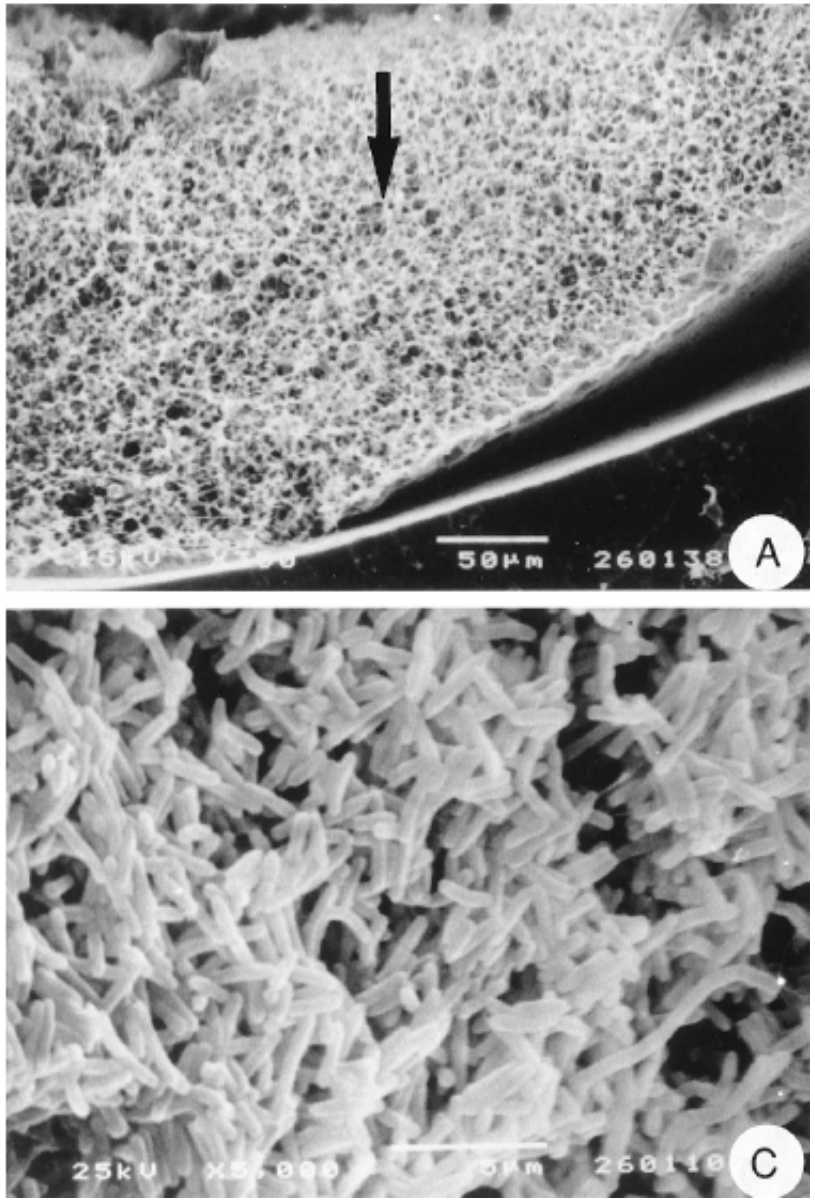
- Distinct subcommunities can be visualized
- Invitro and metabolic studies suggest the communities can depend on each other
- They often are completely dependent on each other dividing the labor of producing compounds necessary to survive
- This also occurs in the gastrointestinal tract



Detrimental biofilms

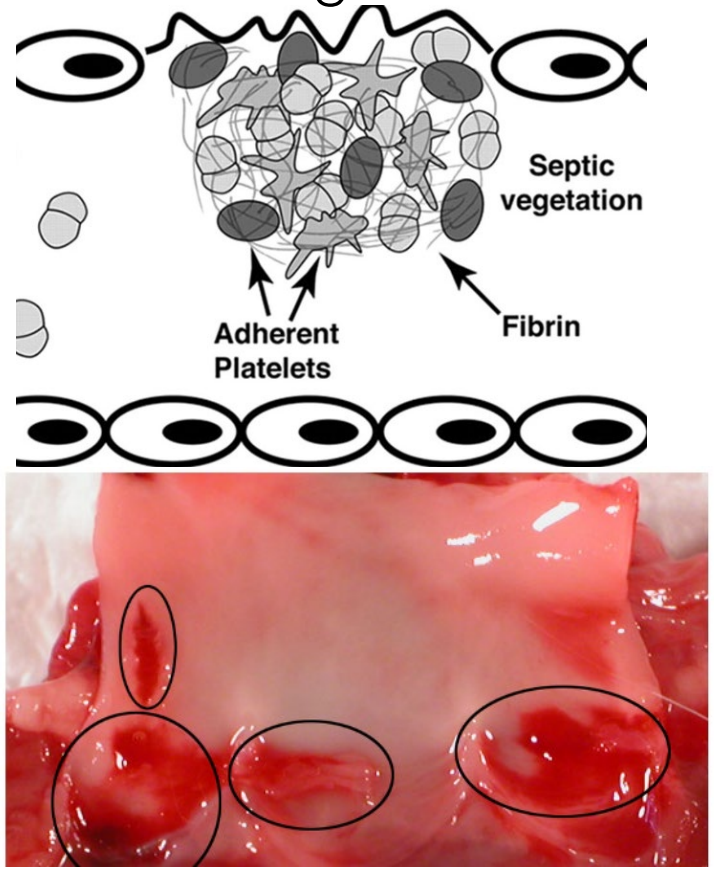
- These communities are often monospecies biofilms composed of a single bacterial species that are pathogens (disease-causing)

Pseudomonas aeruginosa
Urinary catheters



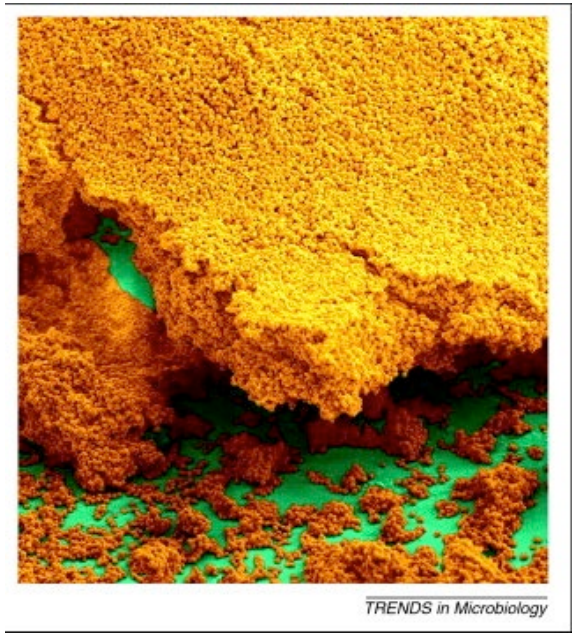
Stickler *et al.*, Appl. Enviro. Microbiol. 64:3486

Enterococcus faecalis
Heart vegetations

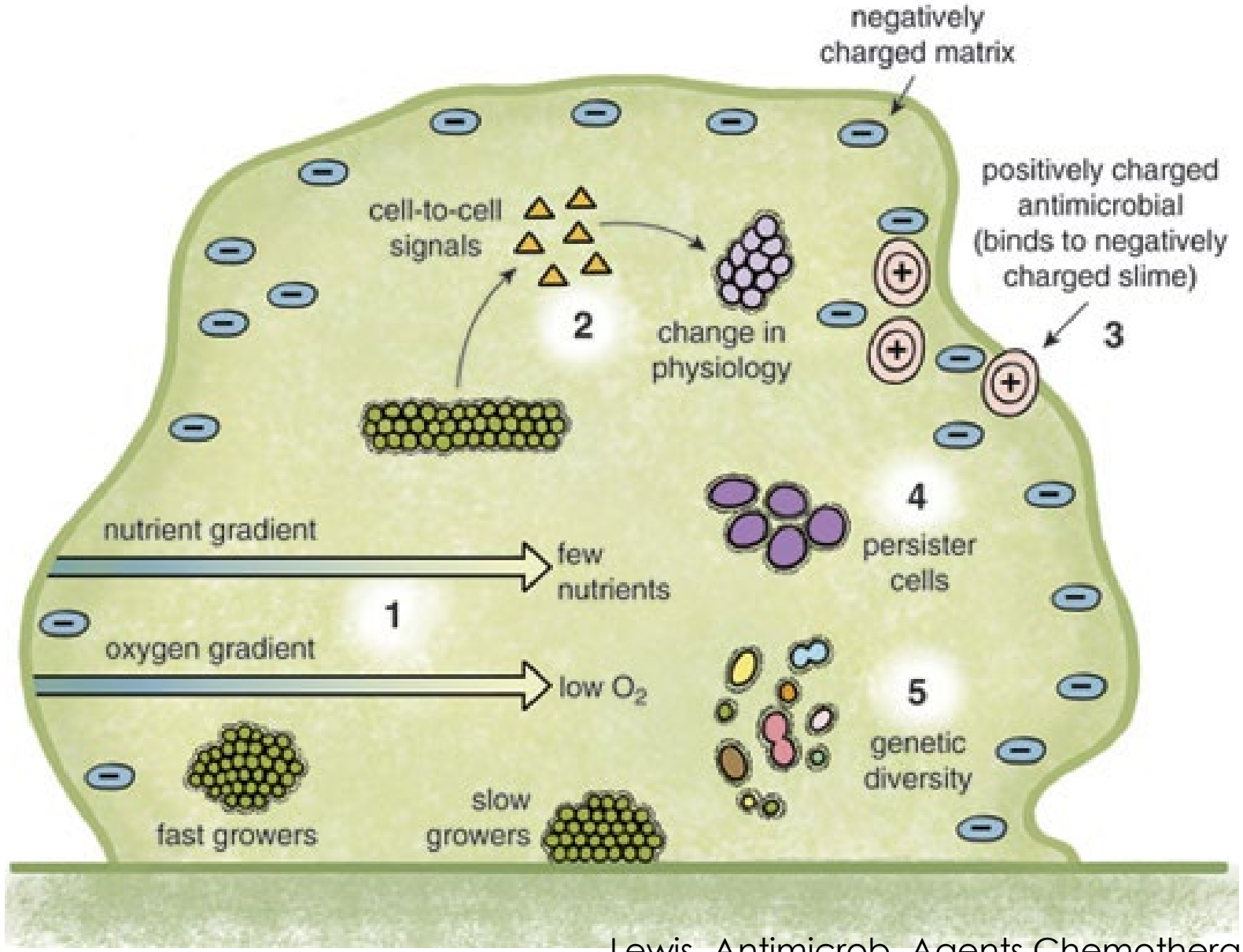


Schlievert PlosOne 2010

Staphylococcus aureus
Endotracheal tube

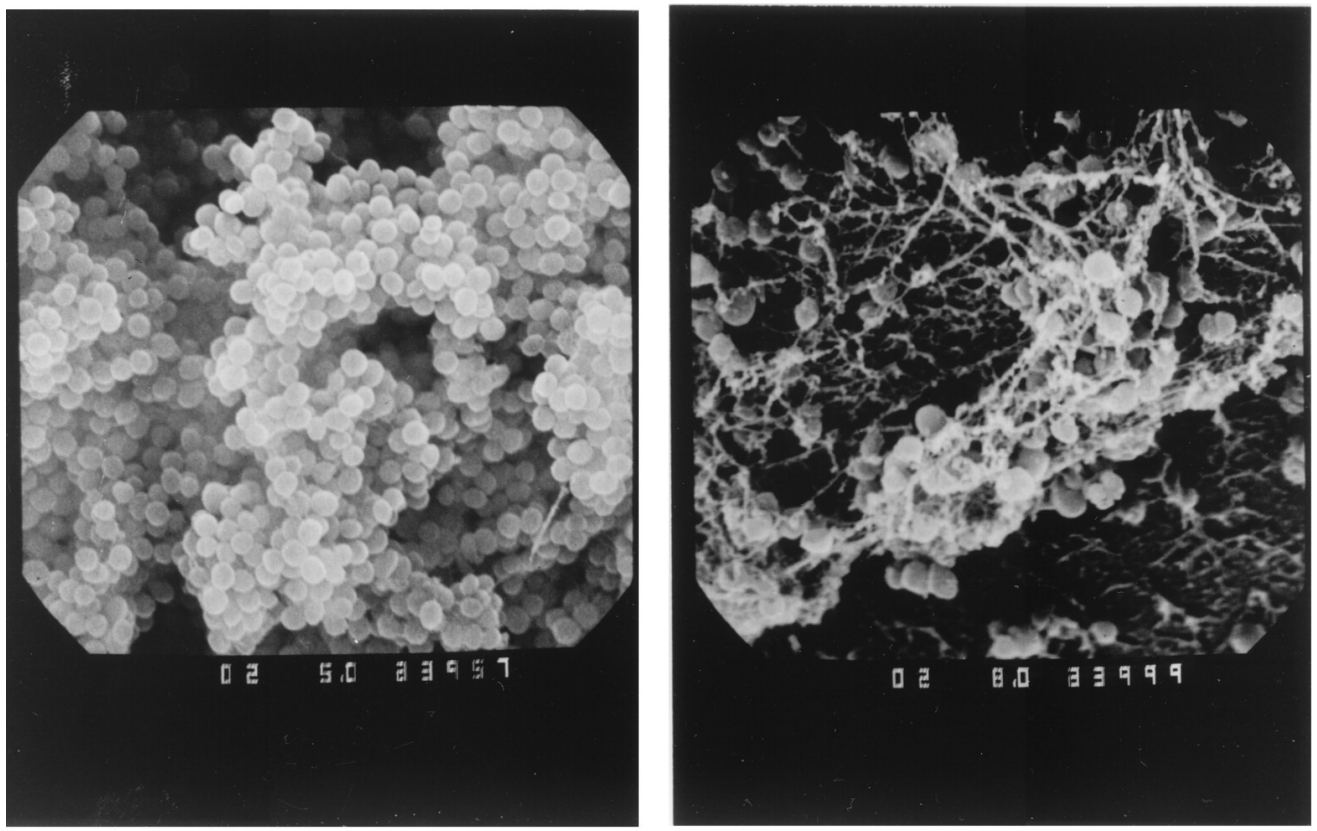


Treatment of biofilms is a major medical problem due to decreased sensitivity to antibiotics

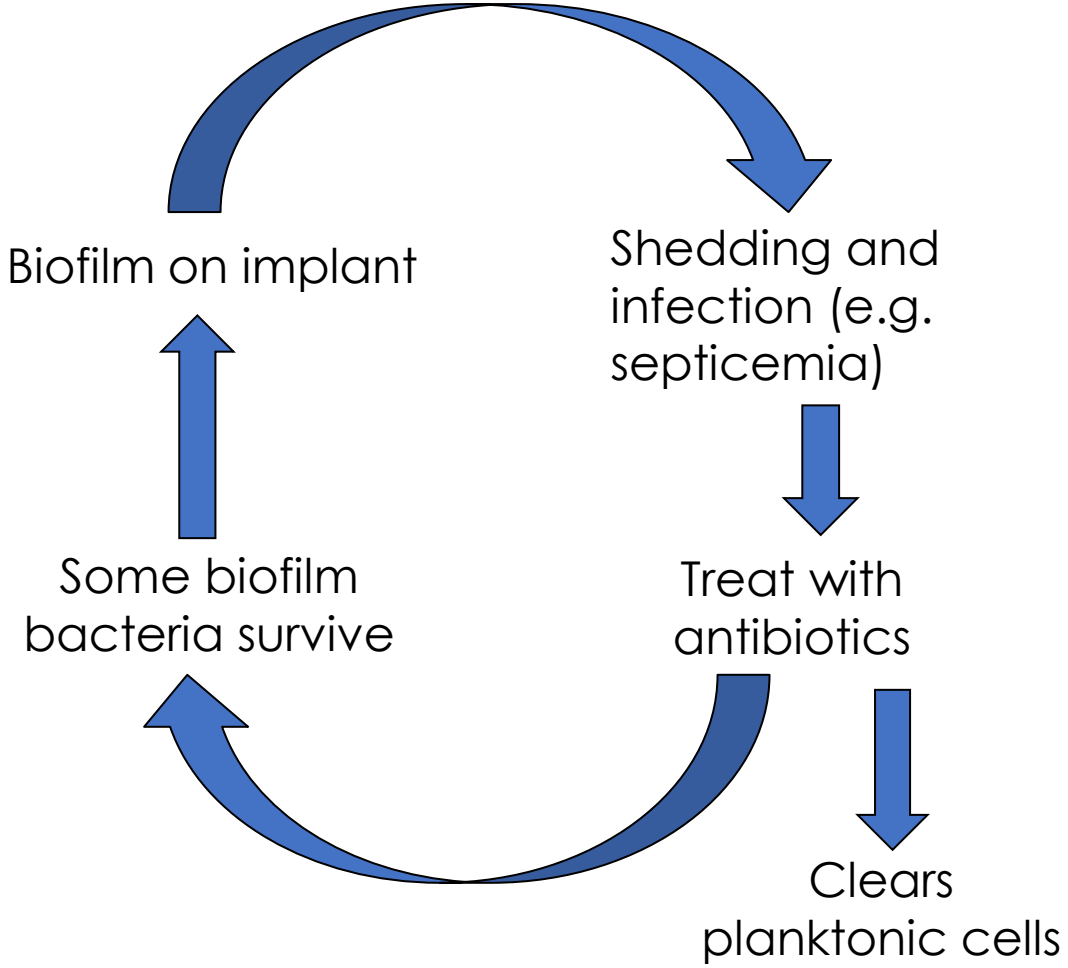


Lewis, Antimicrob. Agents Chemotherapy, 2001

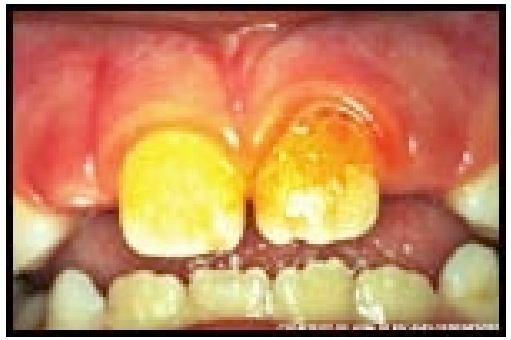
Biofilm resistance to antibiotics can lead to infection cycles



Dunne, 2002, Clin Microbiol Rev, 15(2):155



Kitchen drainpipe



Dental plaque
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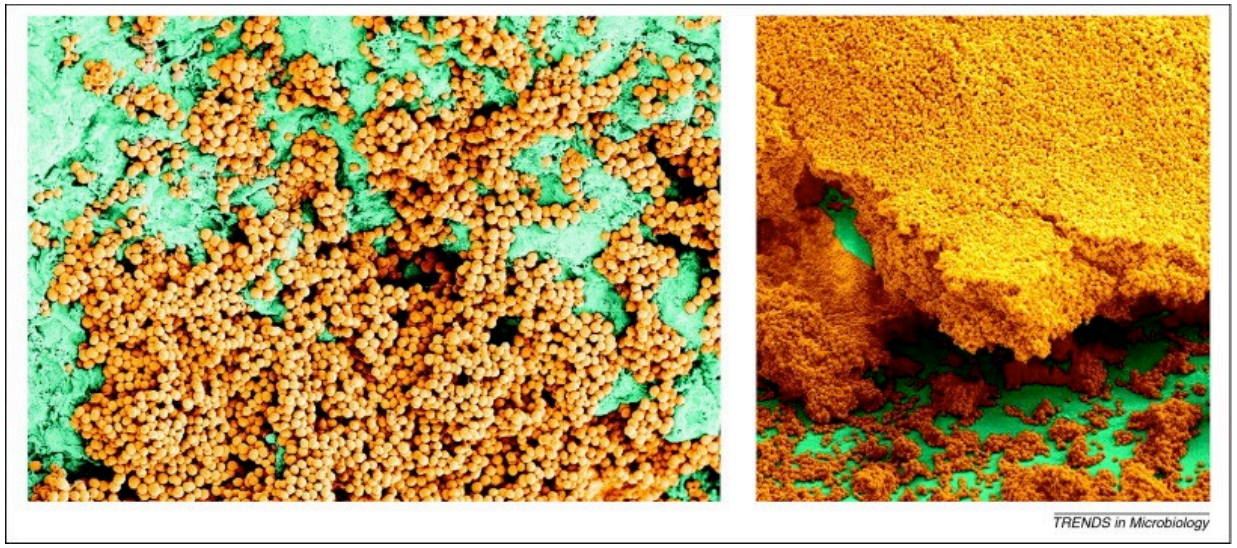

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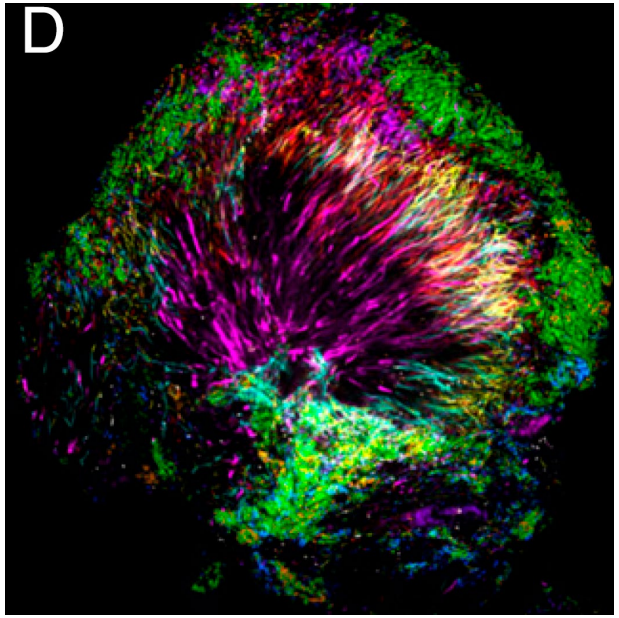
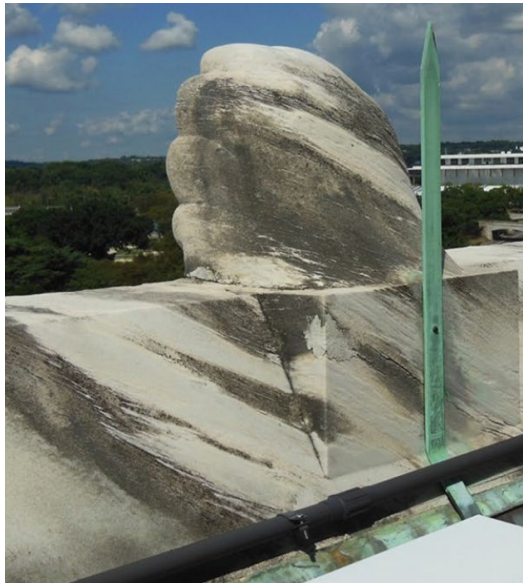
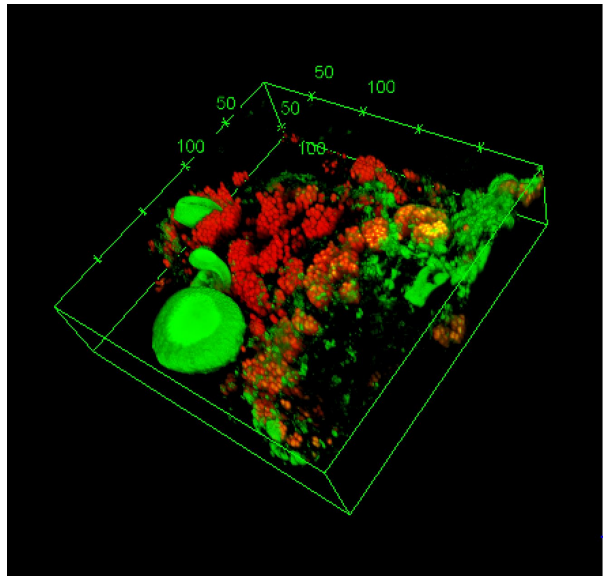
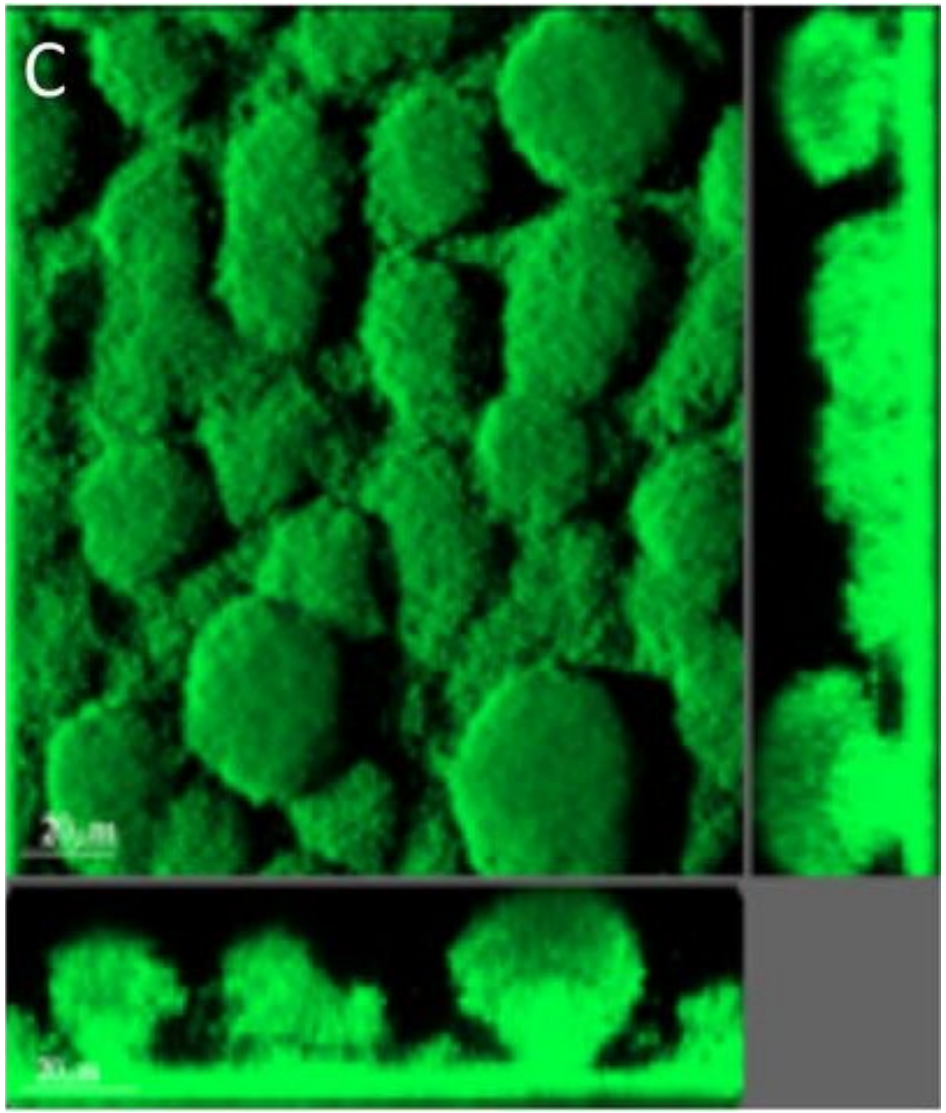






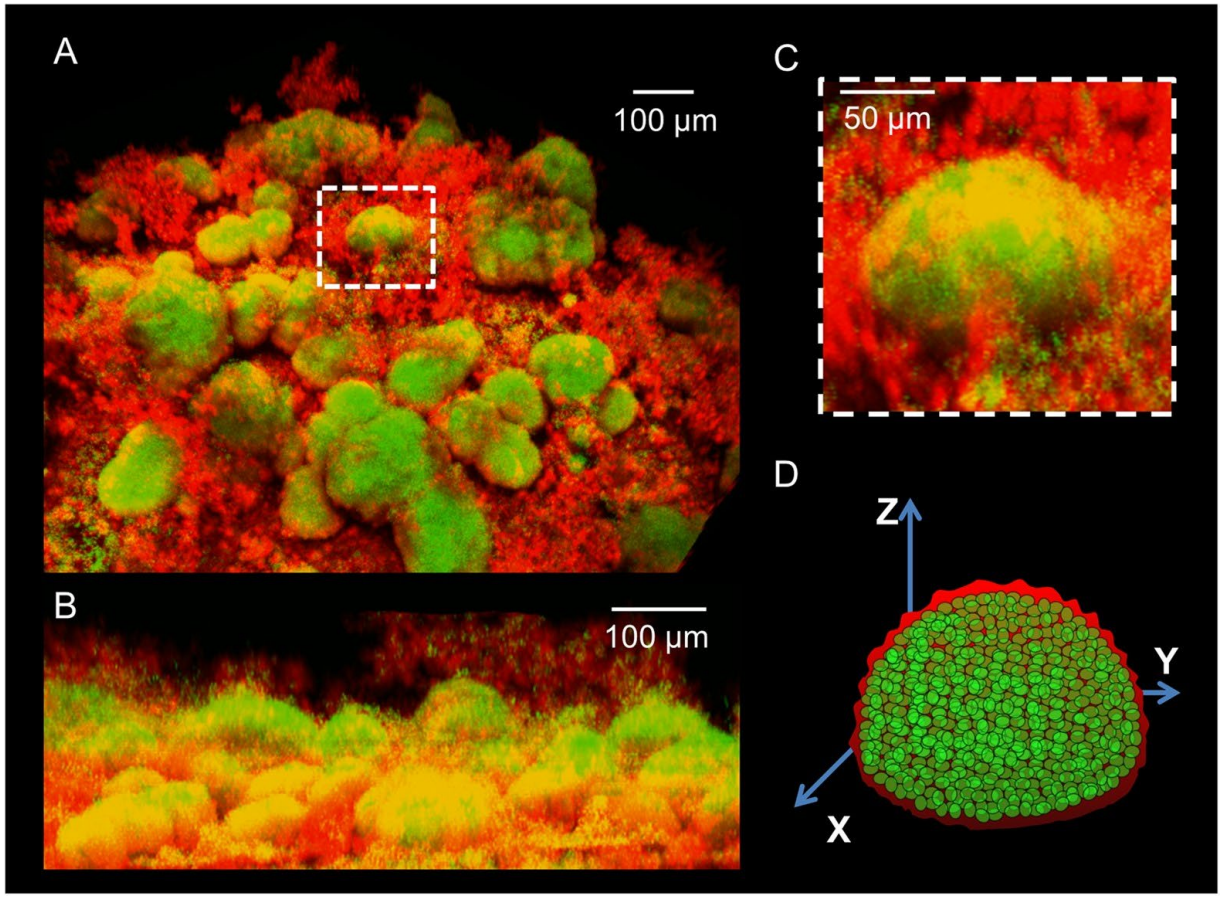
Marble Monument

Biofilm Shapes and Spatial Arrangements

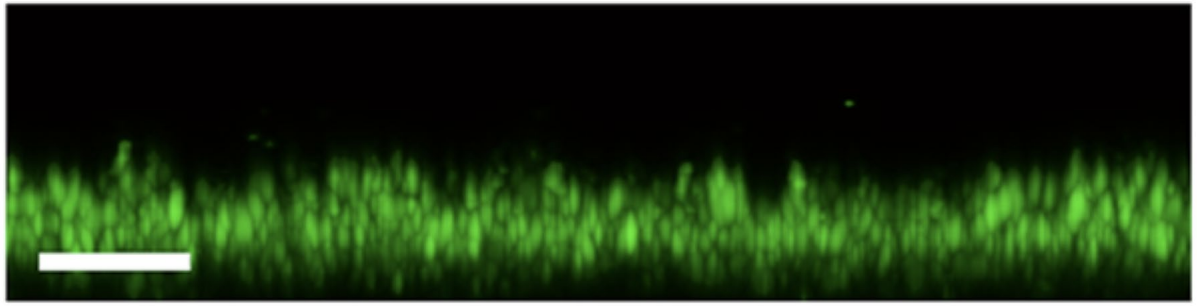


Pseudomonas aeruginosa
under flow
Diggle Lab 2015 J Mol Biol

Streptococcus mutans
Koo Lab 2016 Scientific Reports



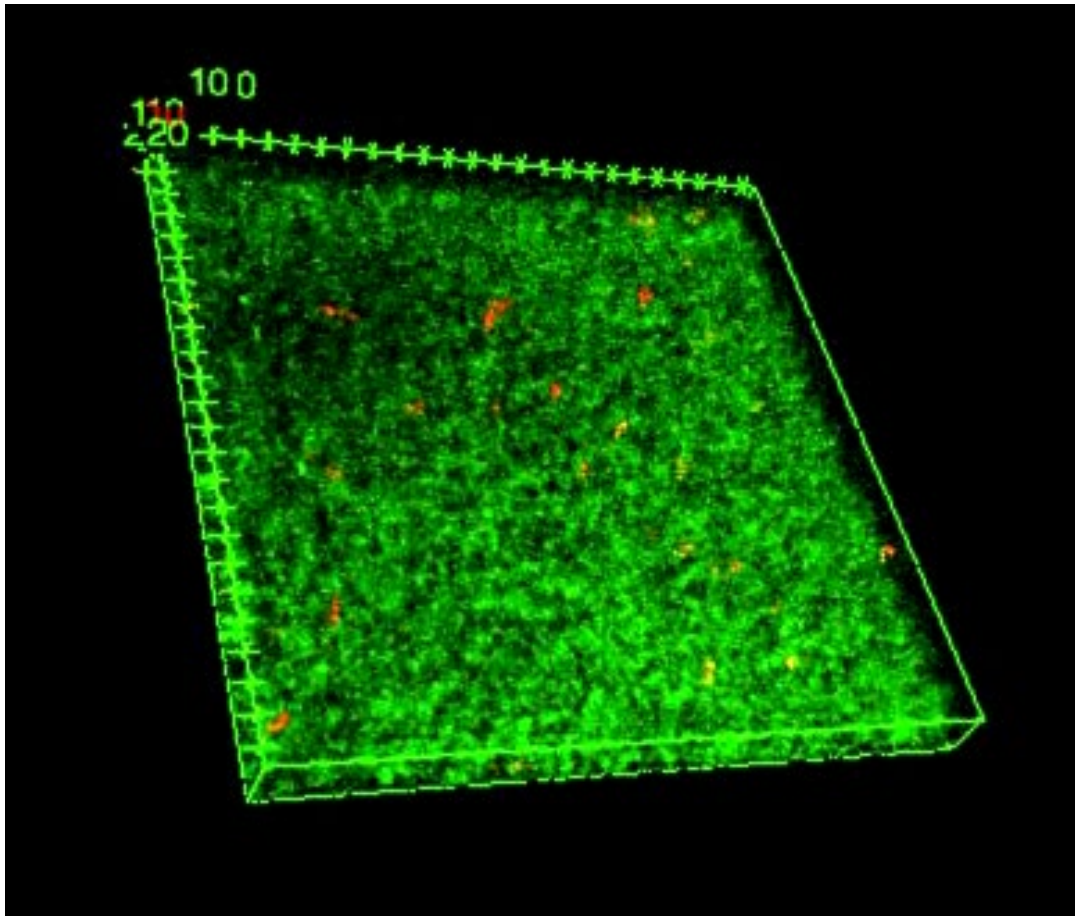
Pseudomonas aeruginosa
Collins Lab 2013 PLOS ONE



Viscoelasticity

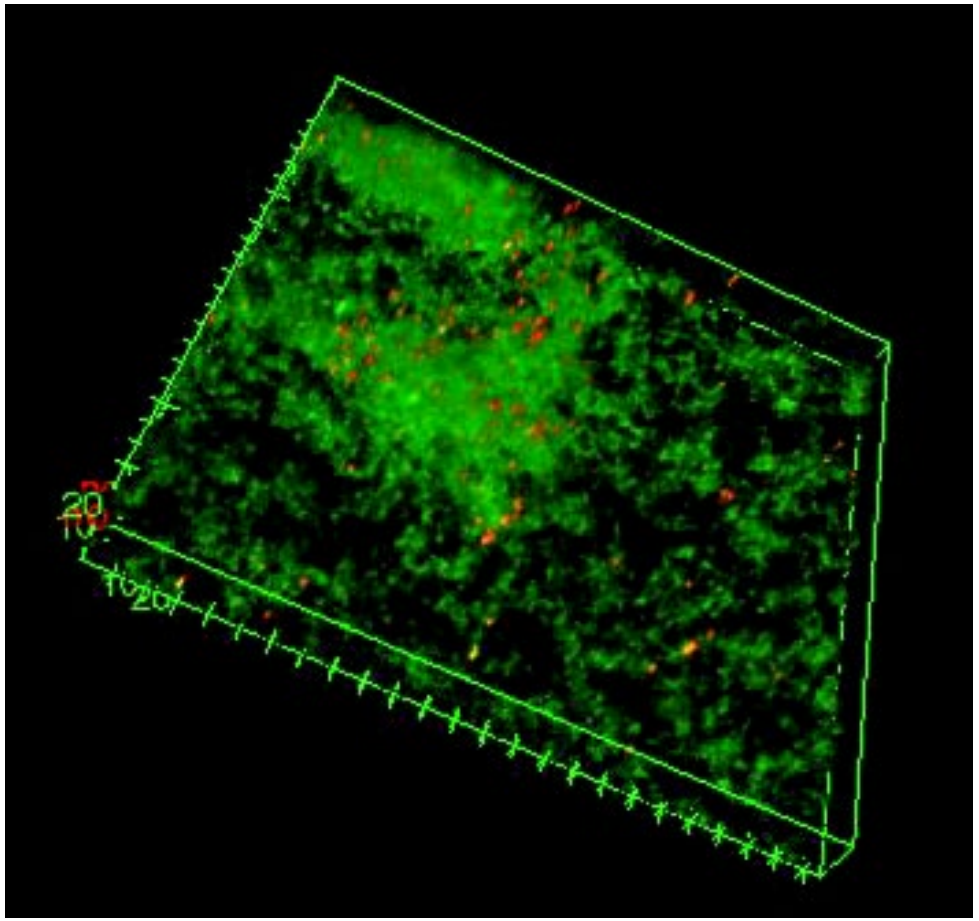
Viscous

Enterococcus faecalis

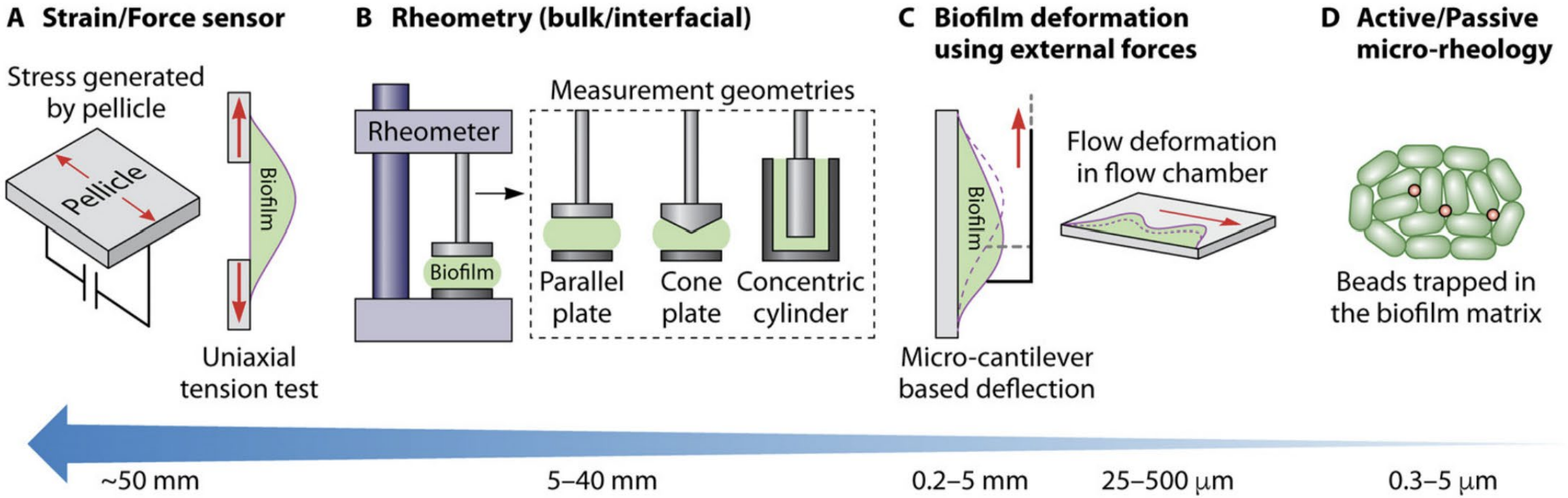


Rigid

Escherichia coli



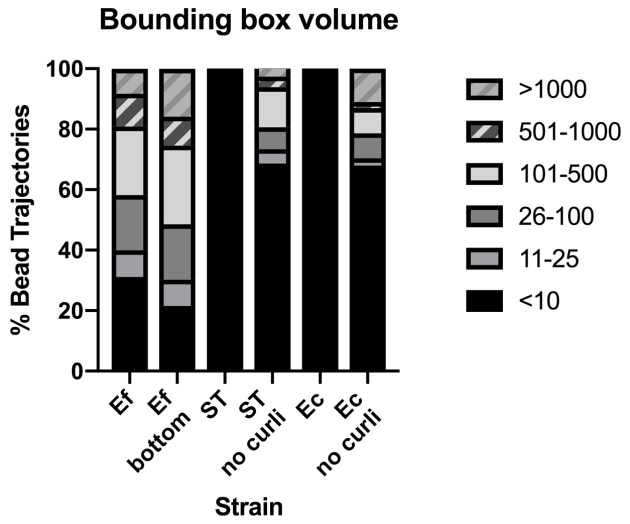
Measurement of viscoelastic properties at different length scales

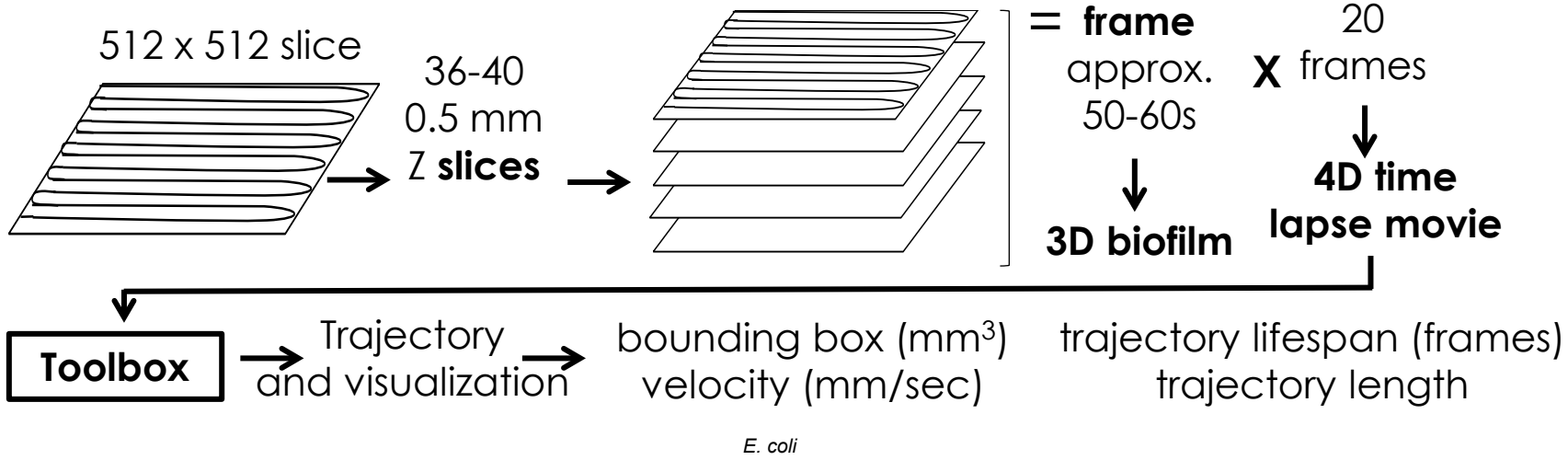


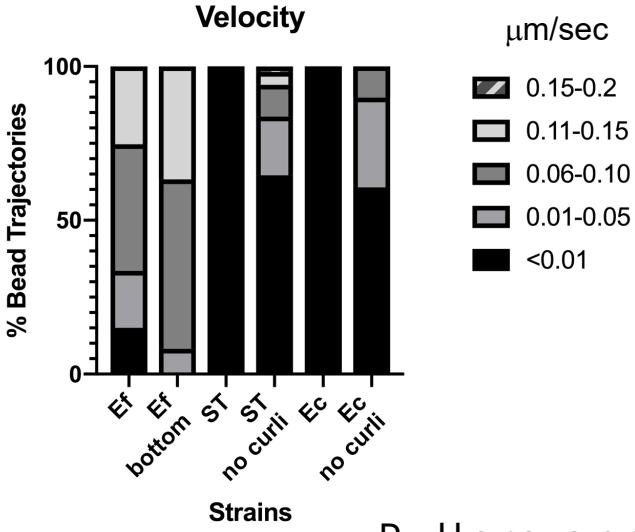
2D method

Carlton, et. al, Curtis Lab, J. Bact. 2019

3D microscale material properties measurements

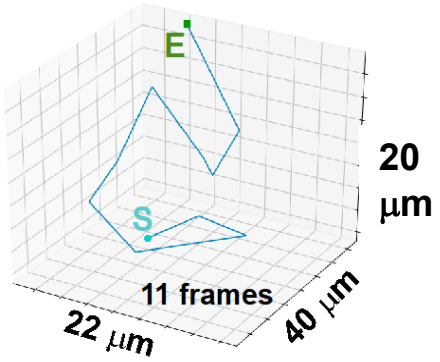


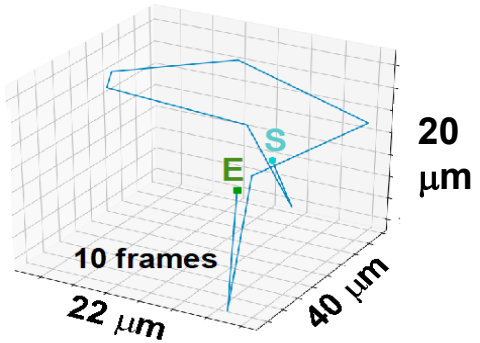




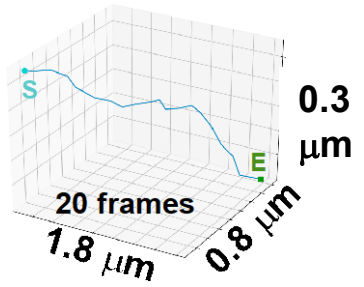
E. coli

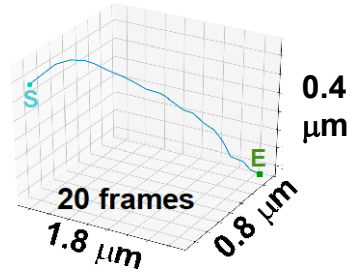
E. faecalis



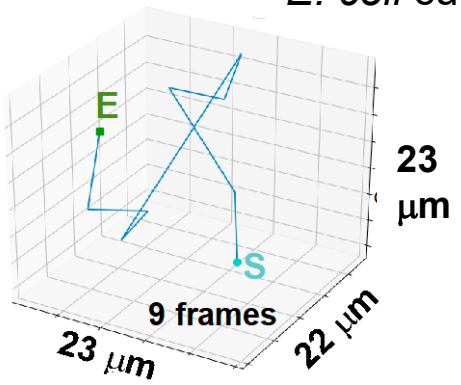


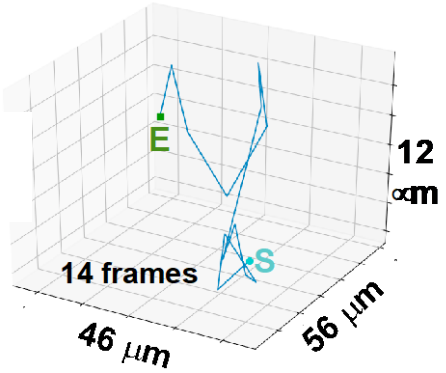
E. coli





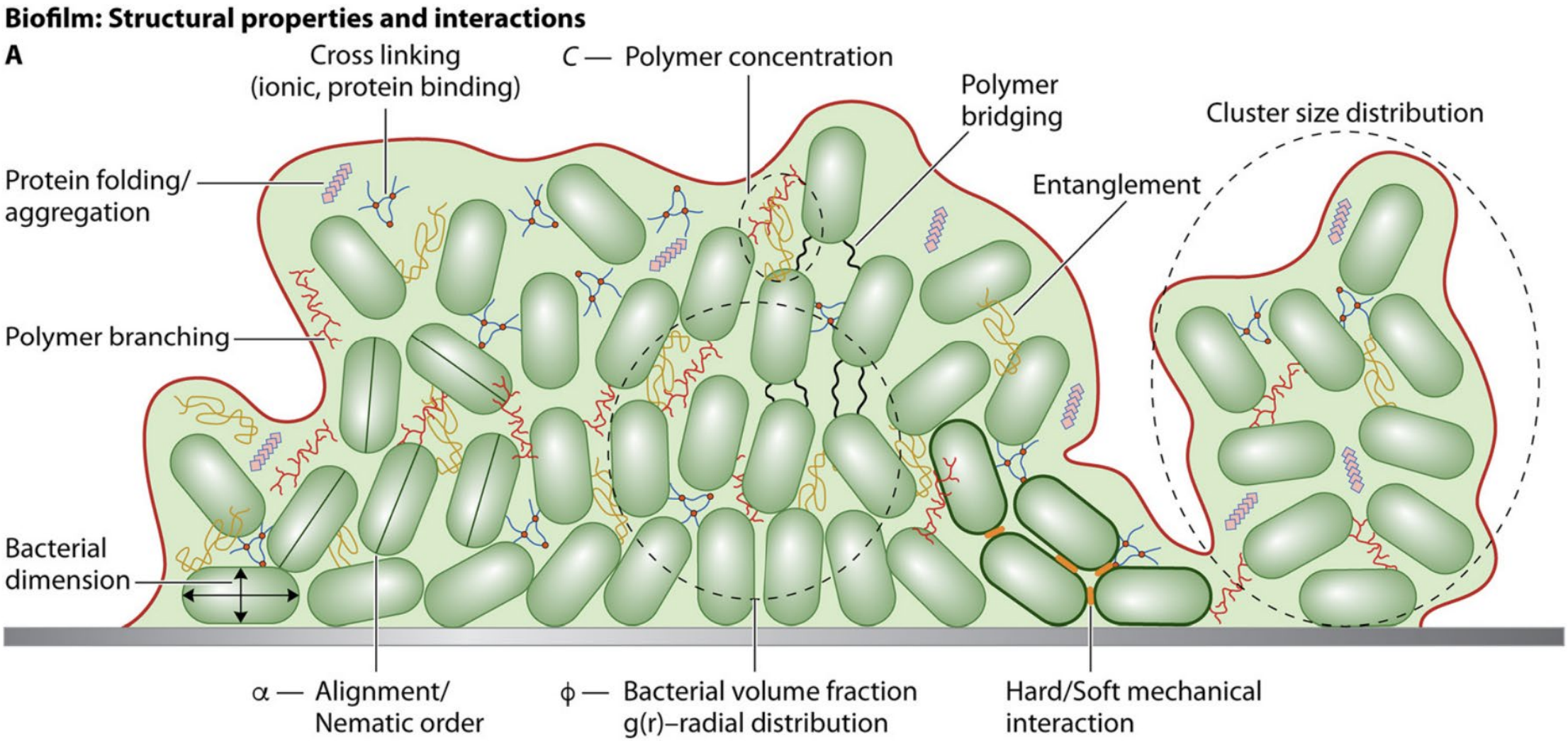
E. coli curli mutant





Buttaro and Queisser Labs

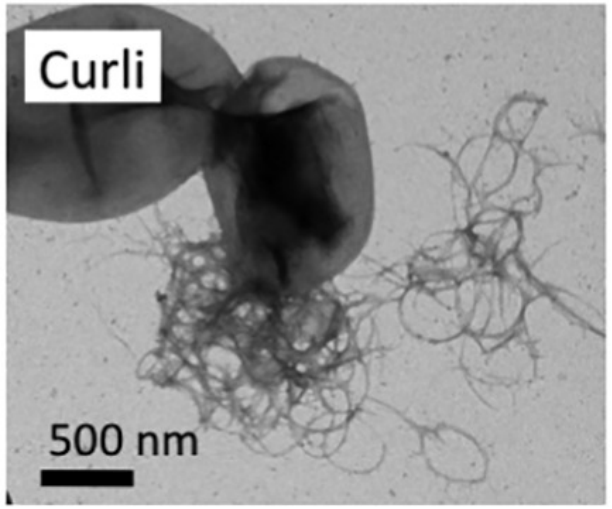
What influences architecture and viscoelasticity?



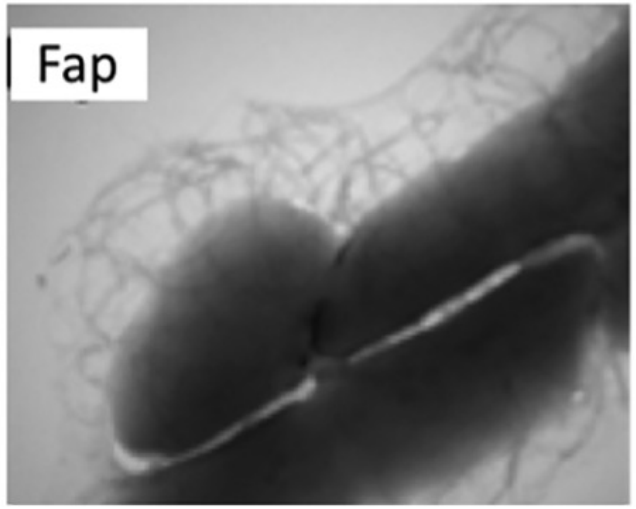
Carlton, et. al, Curtis Lab, J. Bact. 2019

Amyloids

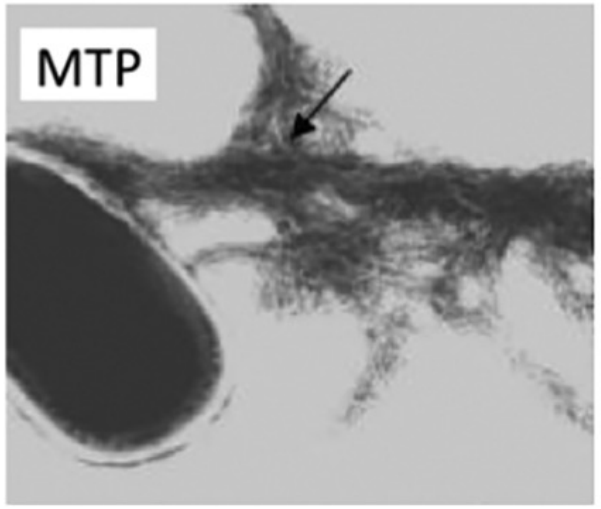
E. coli



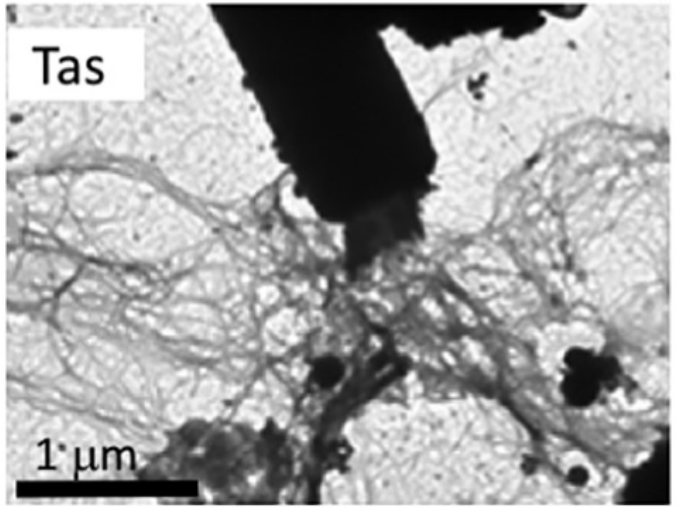
Pseudomonas aeruginosa

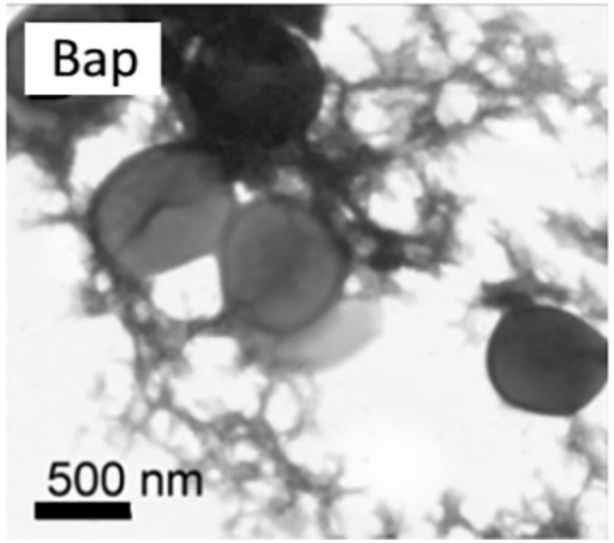


Mycobacterium tuberculosis

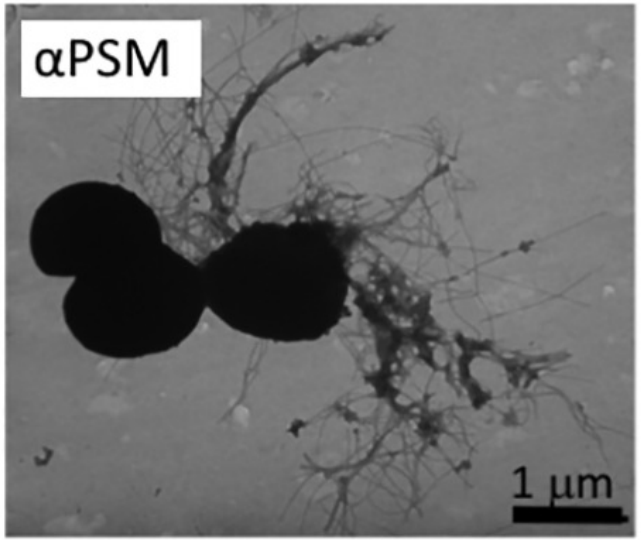


Bacillus cereus

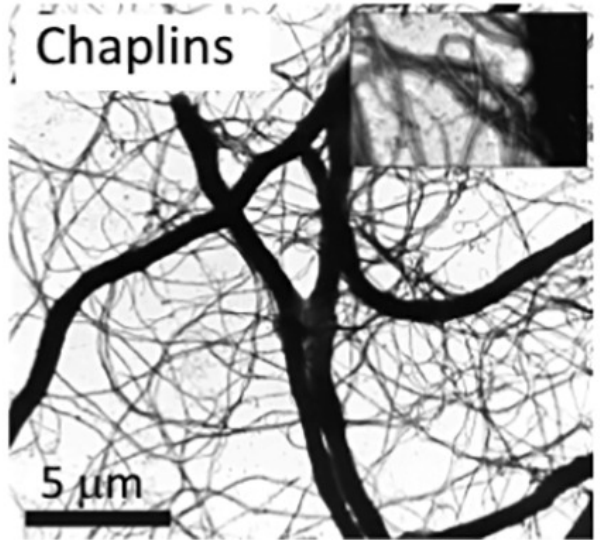




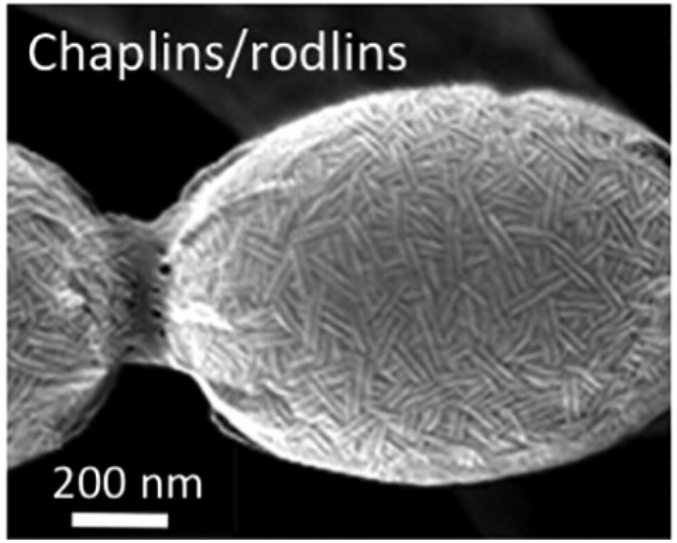
Staphylococcus aureus



Staphylococcus aureus fibrillar form

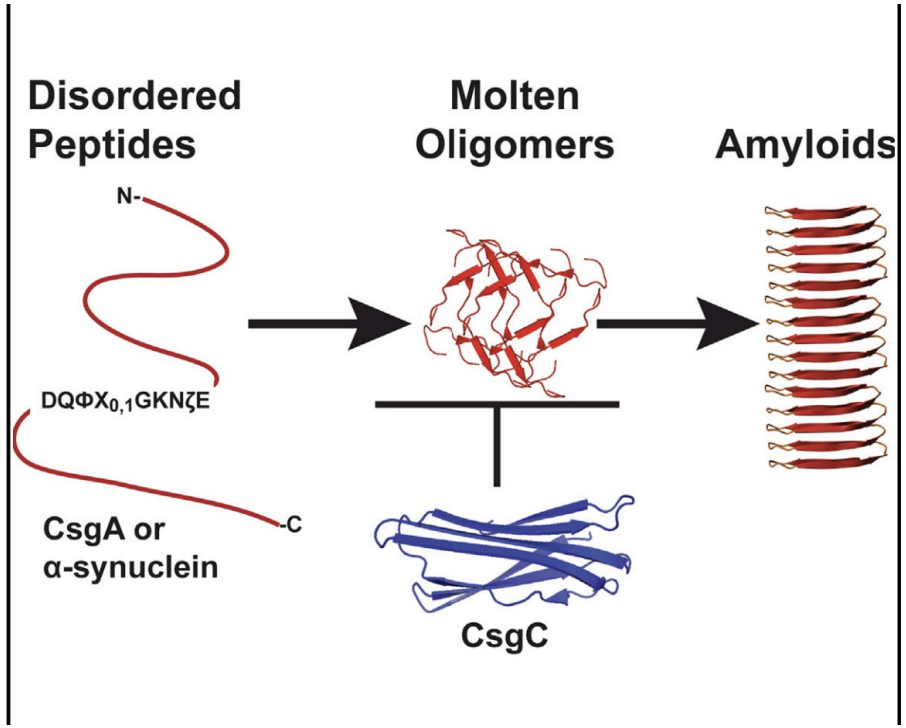


Streptomyces coelicolor fibrillar form



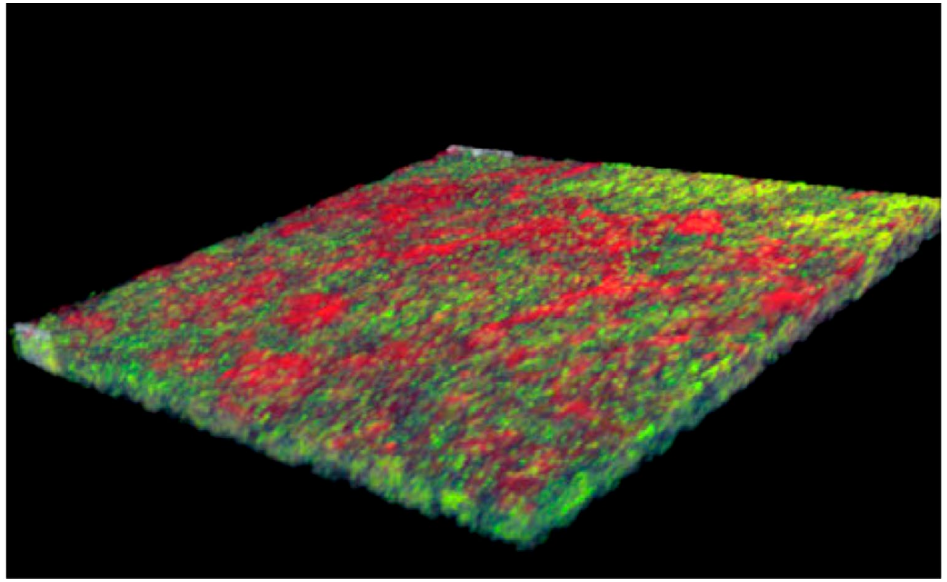
Streptomyces coelicolor spore coat

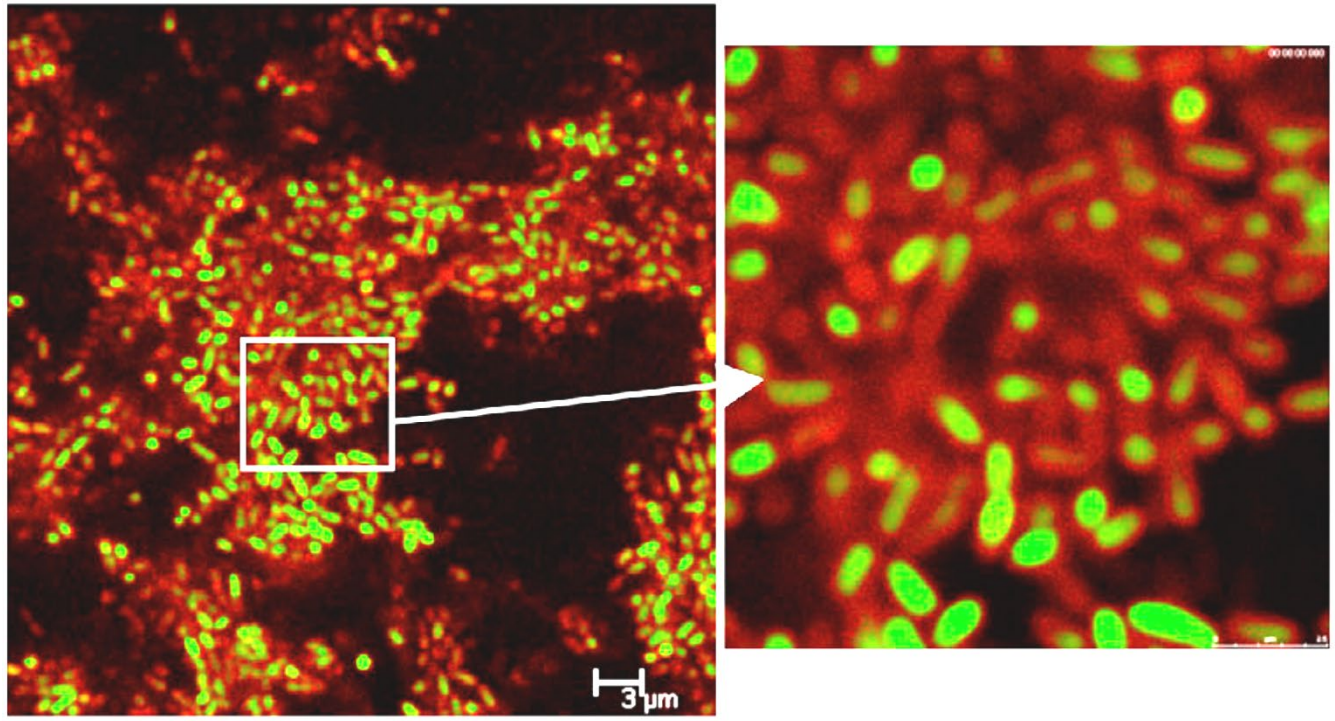
Van Gervan et.al. J Mol Biol 2018



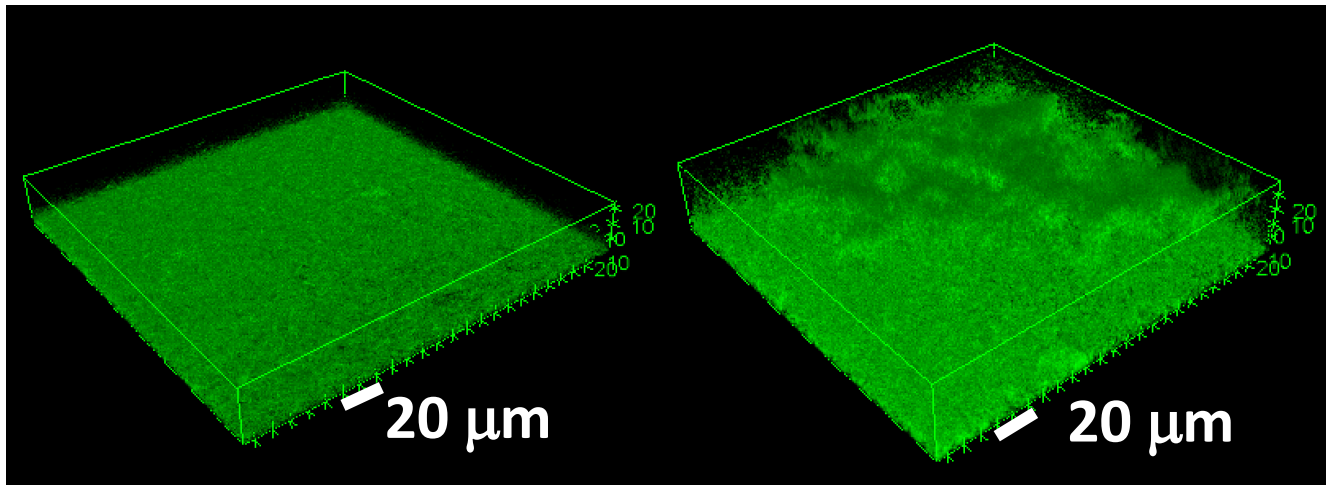
Amyloids and Rigid Structures

fold into beta-sheets
ordered rigid structures
conferring rigidity to the biofilm





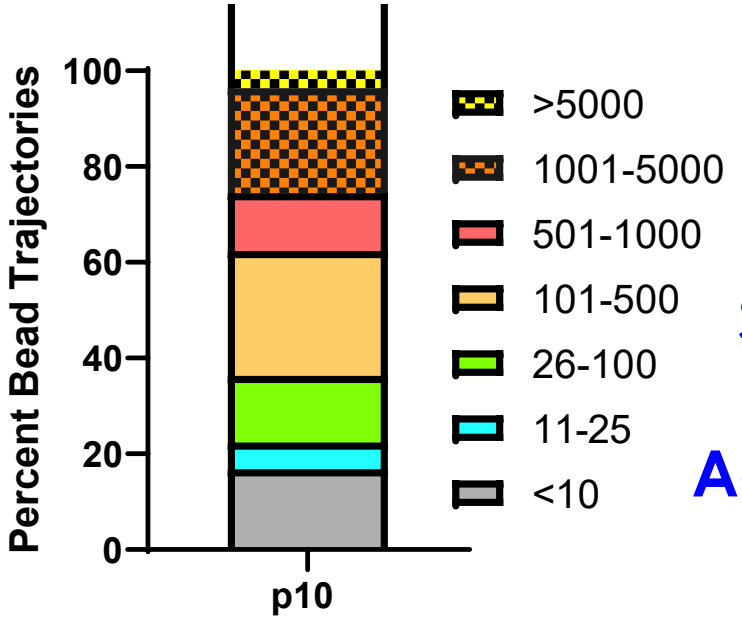
Salmonella Typhimurium
Buttaro and Tukel Labs



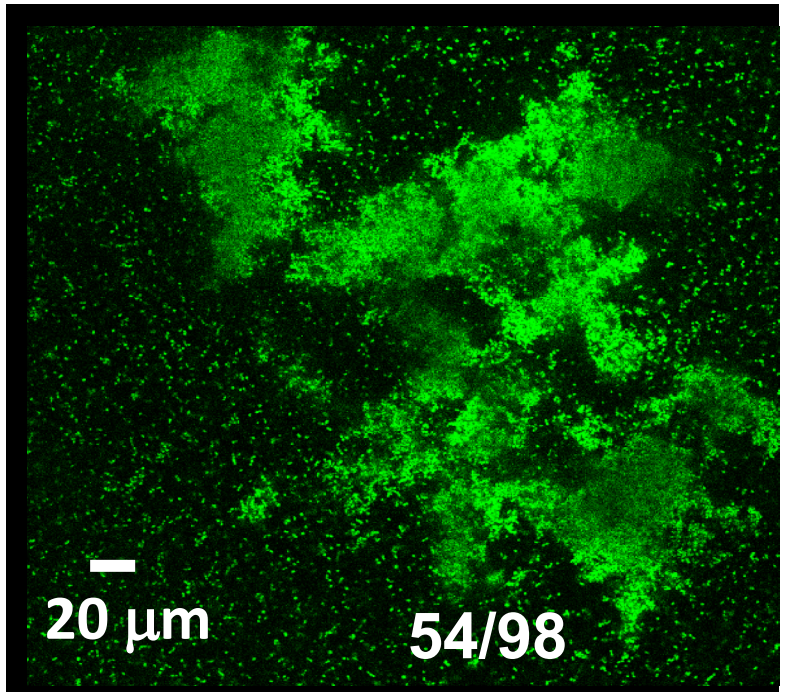
Enterococcus faecalis

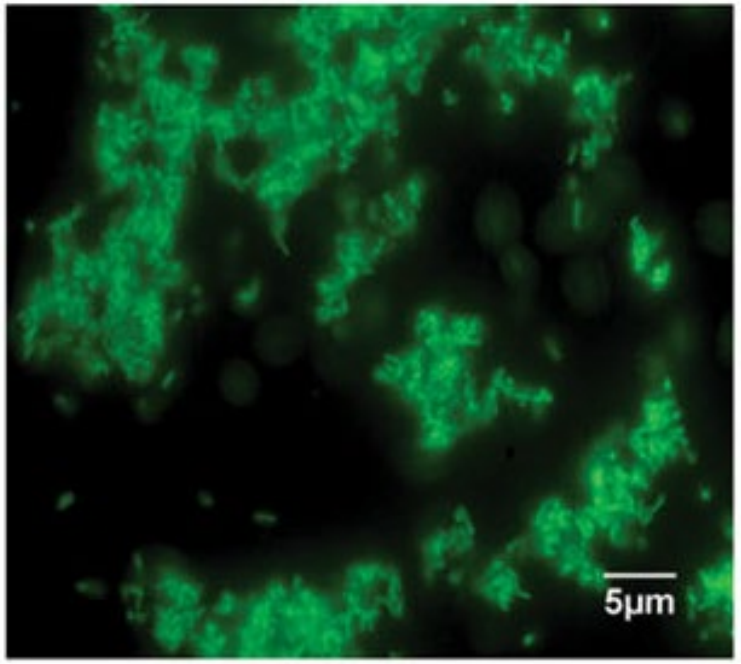
no plasmid

plasmid pCF10



Rigid Structures by Aggregation





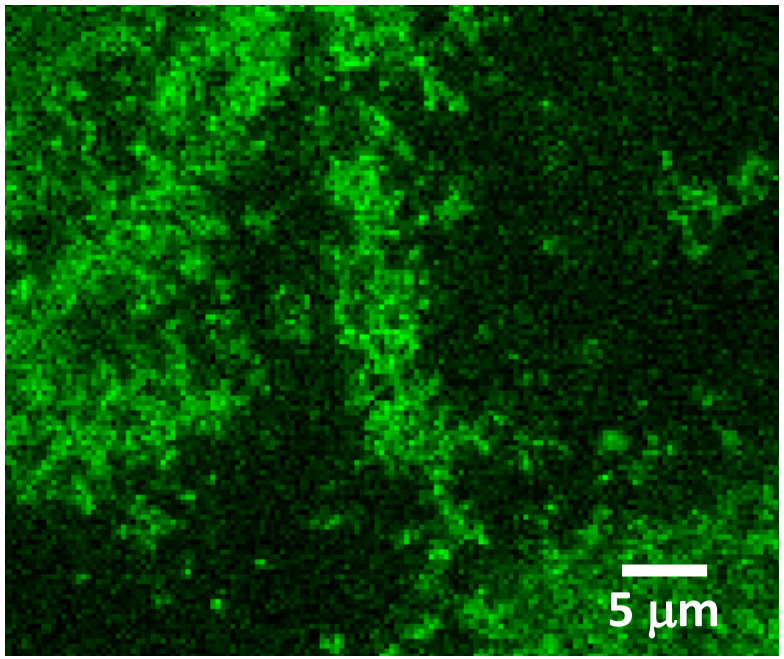


Fig. 1B *E. coli* Ag43^{EDL993} from Vo, JL *et al.*, Biofilms and Microbiomes, 2022

Stress Induction of Amyloids

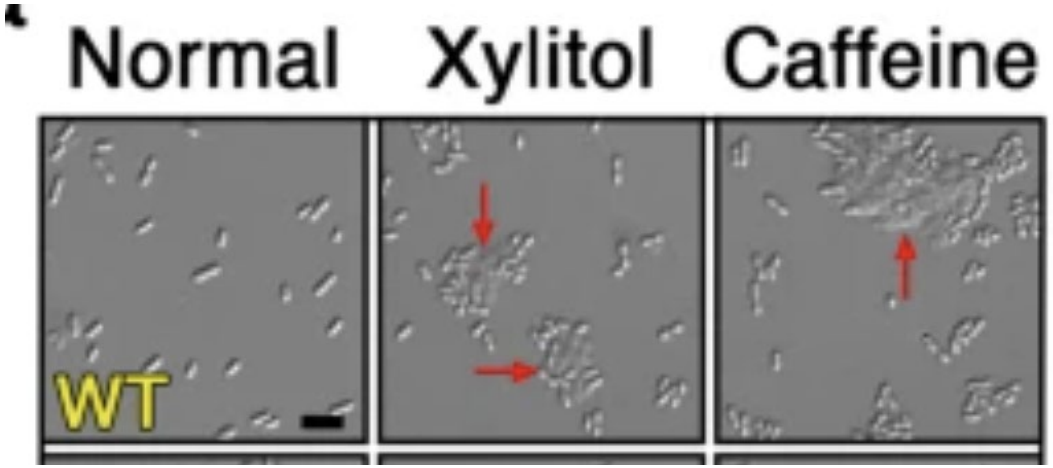
E. coli

- low temperature
- envelope stress
  - low osmotic conditions- not enough salt
  - high osmotic conditions – too much salt
- metals such as nickel
- stationary phase (collection of conditions unfavorable for rapid bacterial growth)

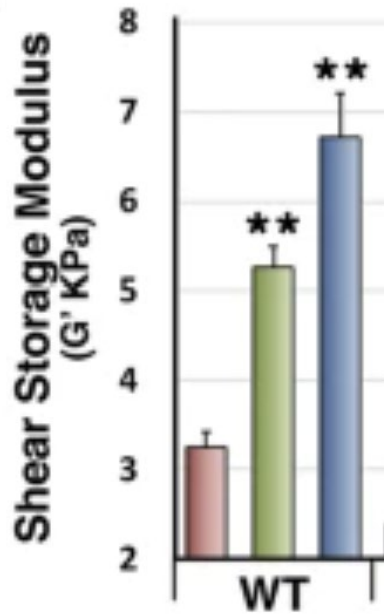
Bacillus subtilis

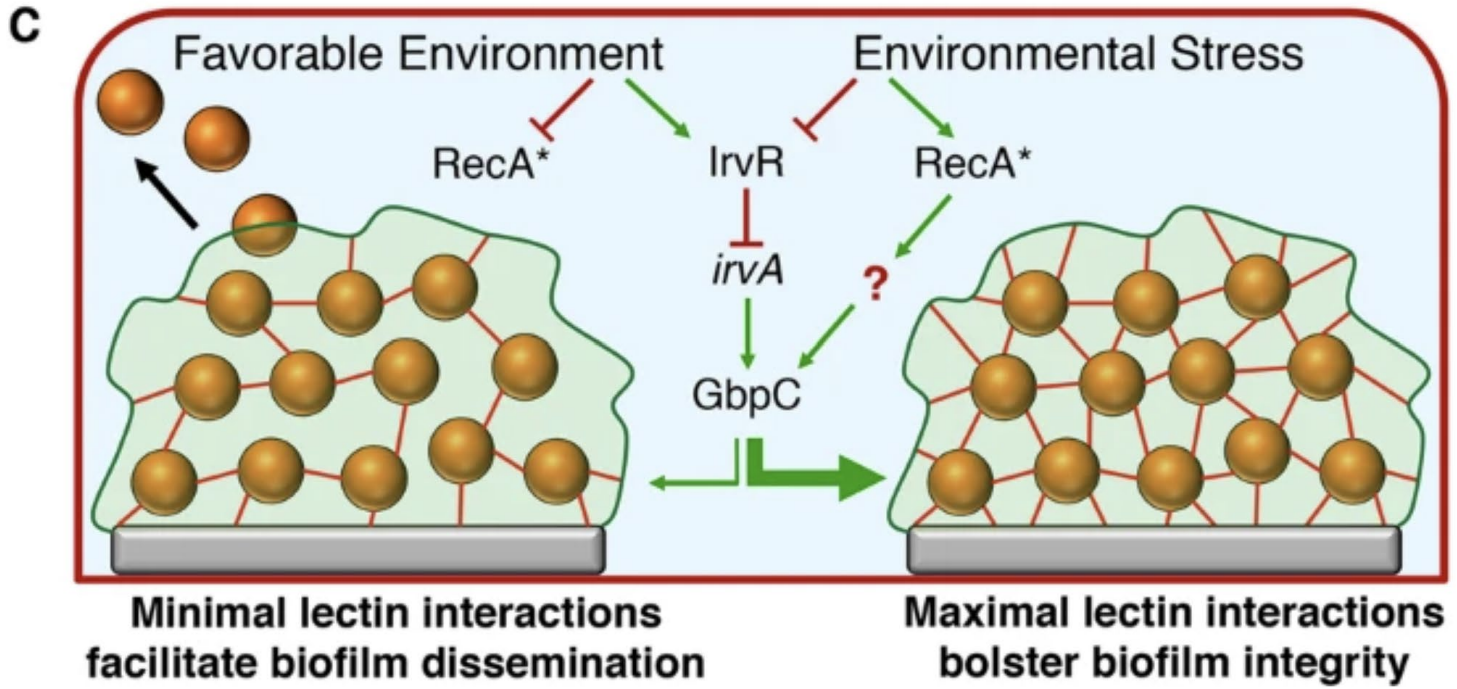
- starvation
- high cell density (lots of competition)
- potassium leakage
- membrane damage (antibiotics)
- during spore formation – a dormant and resistant form of bacteria - amyloids can become part of the spore

Stress Induction of Rigid Structures by Aggregation



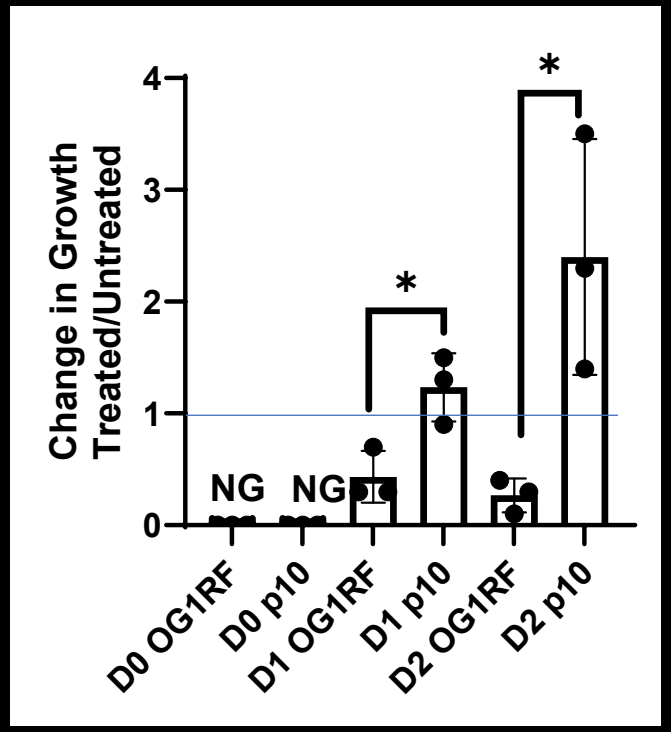
- Xylitol and Caffeine remodel *Streptococcus mutans* biofilms
- aggregation cannot be disrupted by sonication
- increased shear stress
- makes aggregation between the cells tighter





Antibiotic Resistance

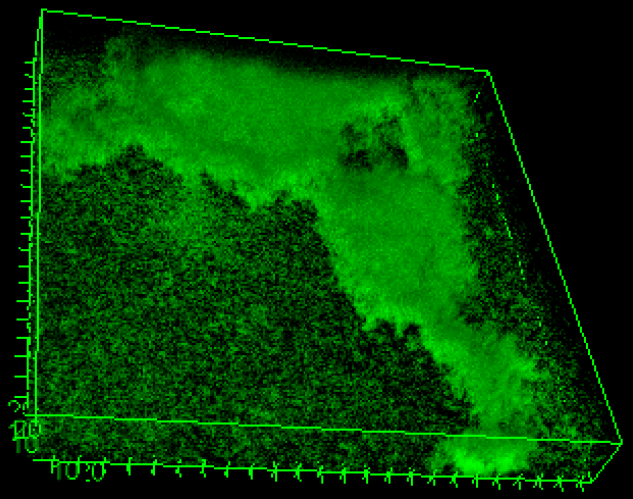
Continued growth in the presence of 100X MIC erythromycin increased size of rigid structures and protected microenvironments

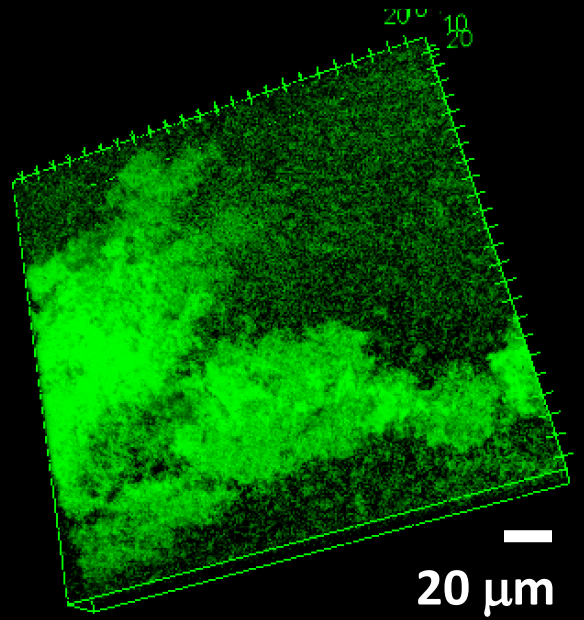


Raiyu Ayanto

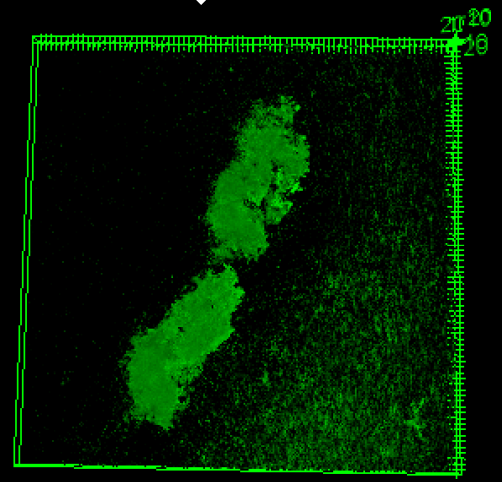
Optic Bottom
24-well

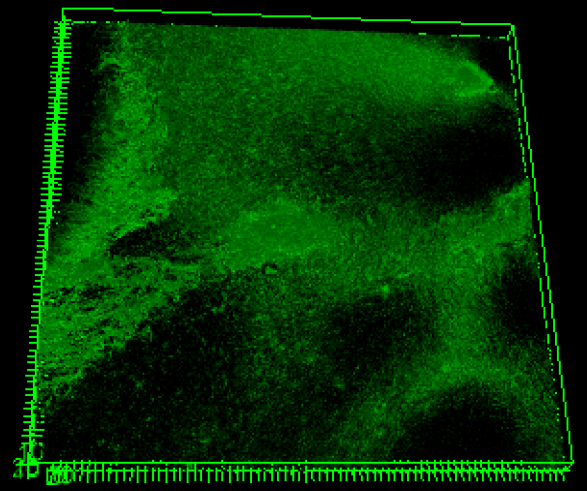
Flow Cell



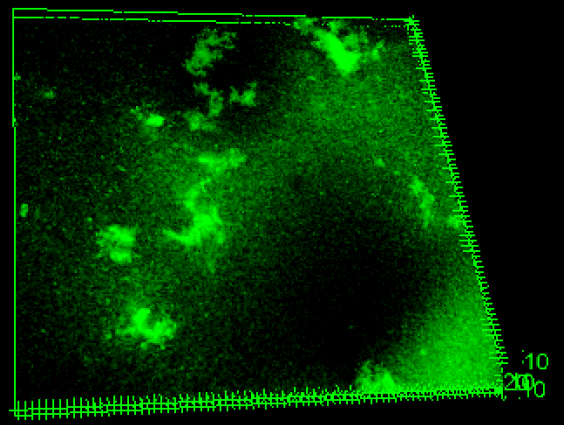


20 µm

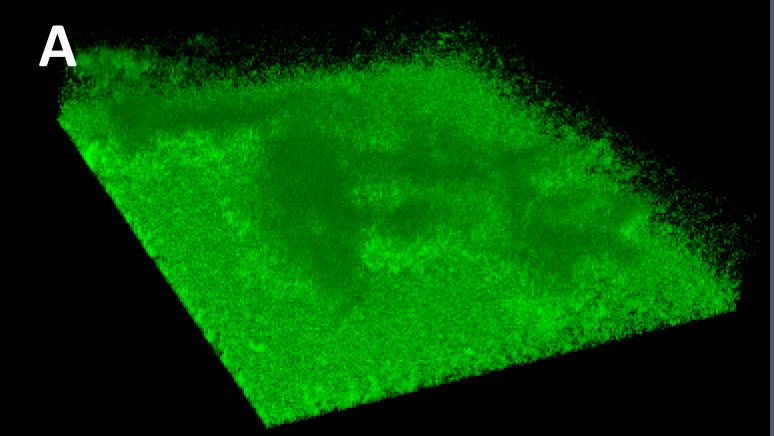


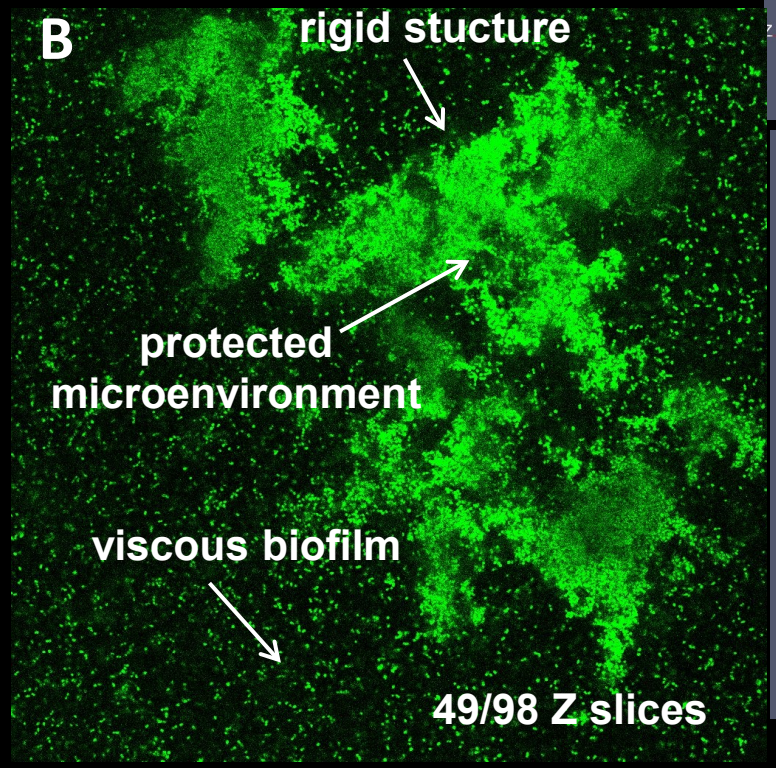


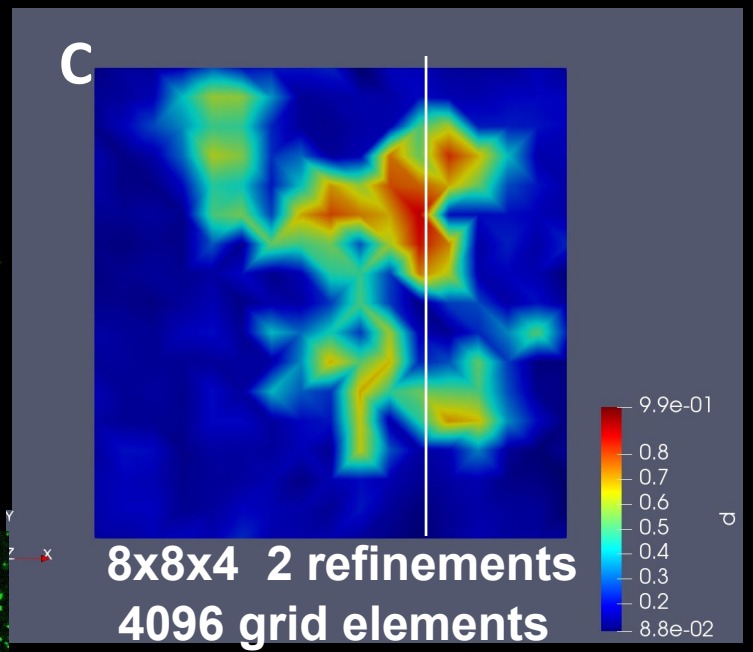
Bella Bentivogli

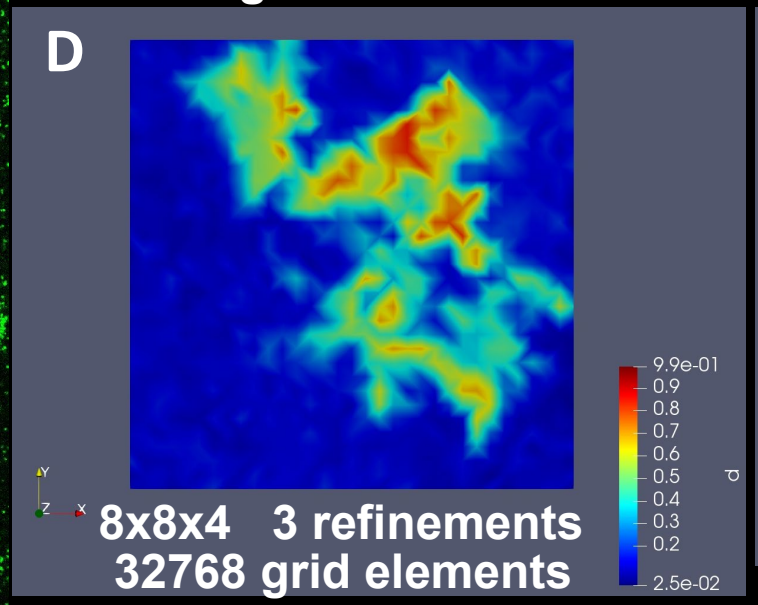


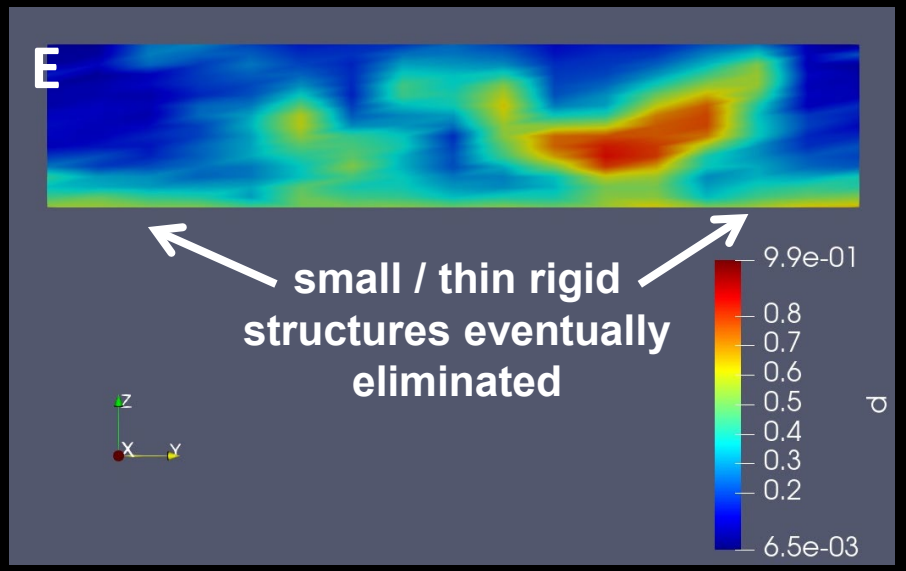
100 µm



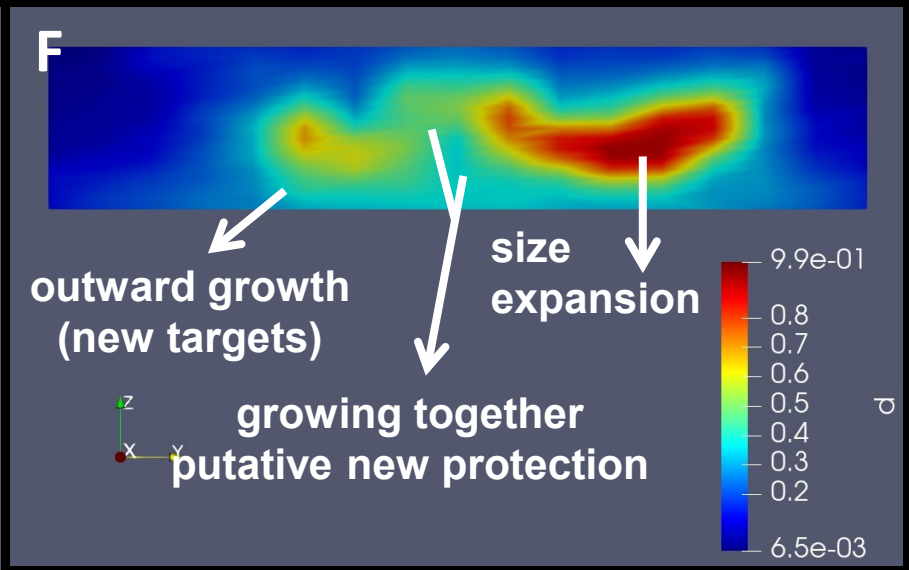








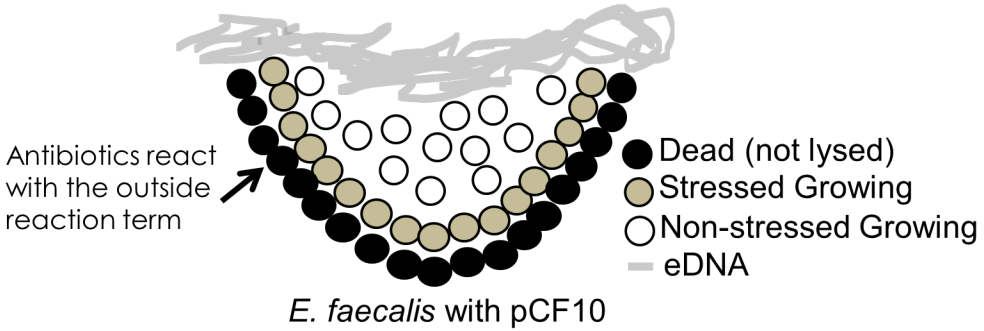
Time = 0 hours



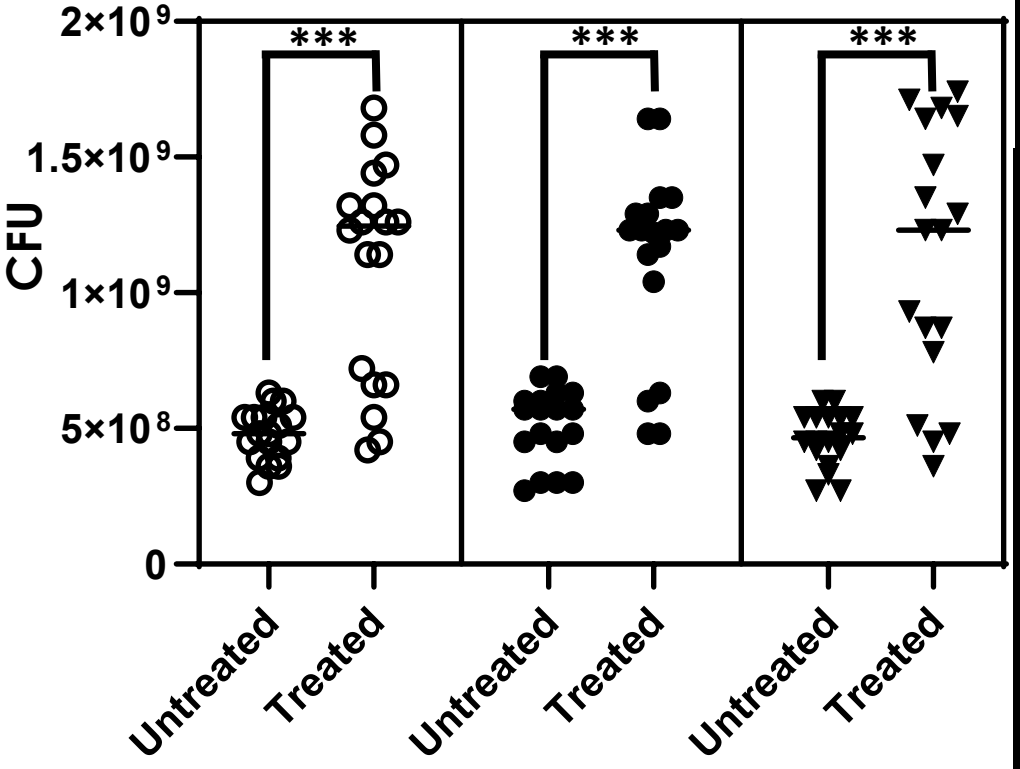
Time = 12 hours

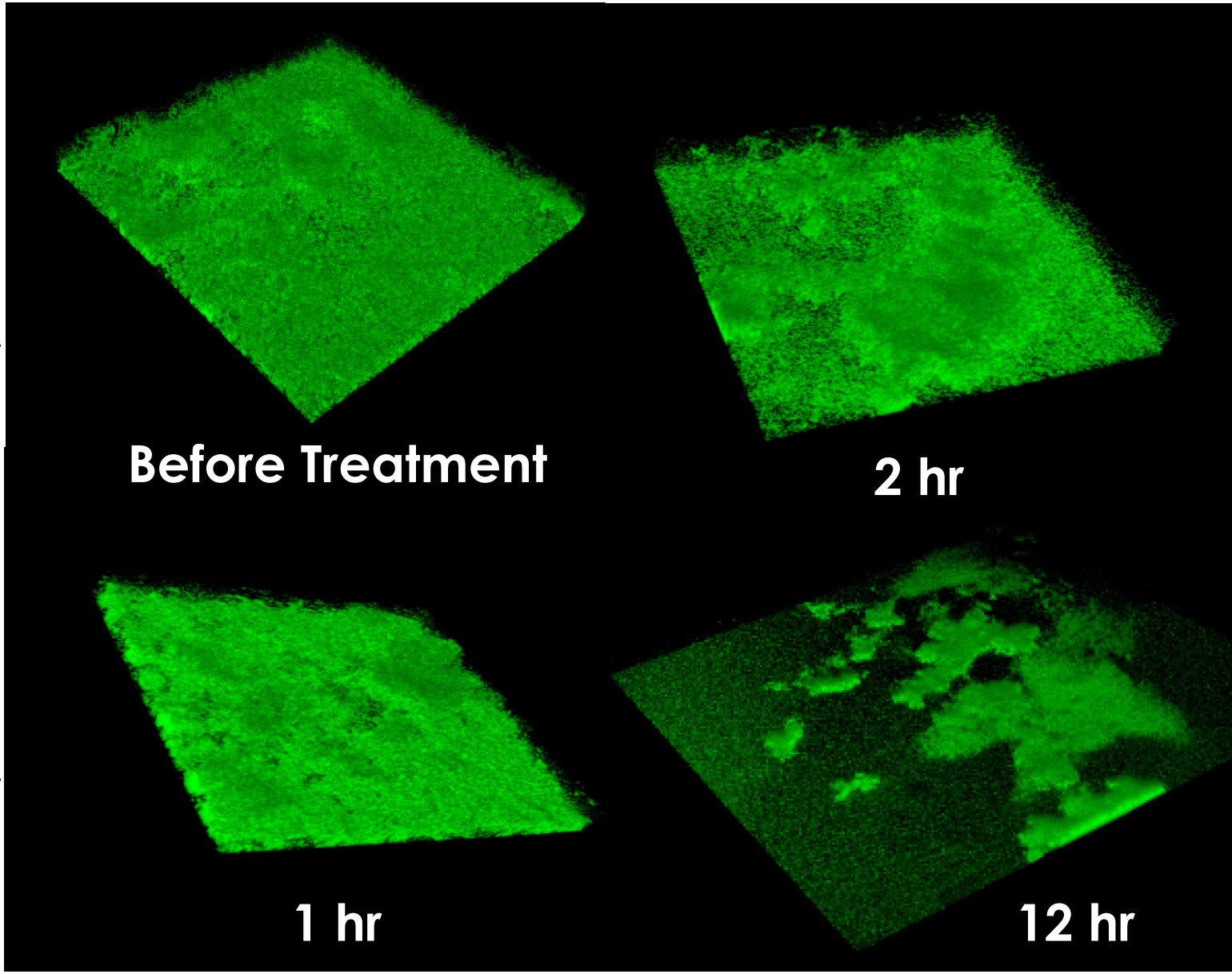
Gilliian Queisser (Department of Mathematics) Brandi Henry

Is multicellularity a means to quickly adapt and respond?

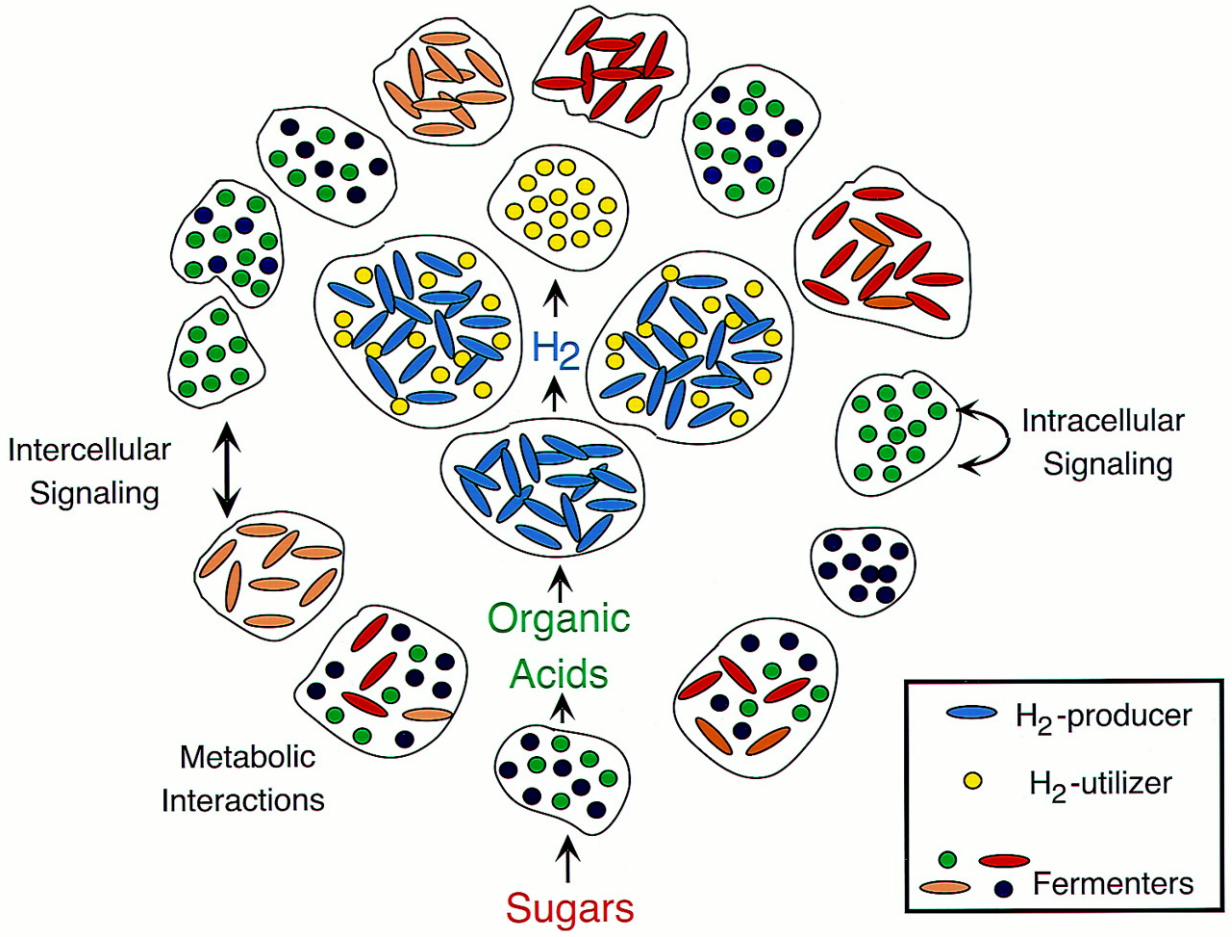


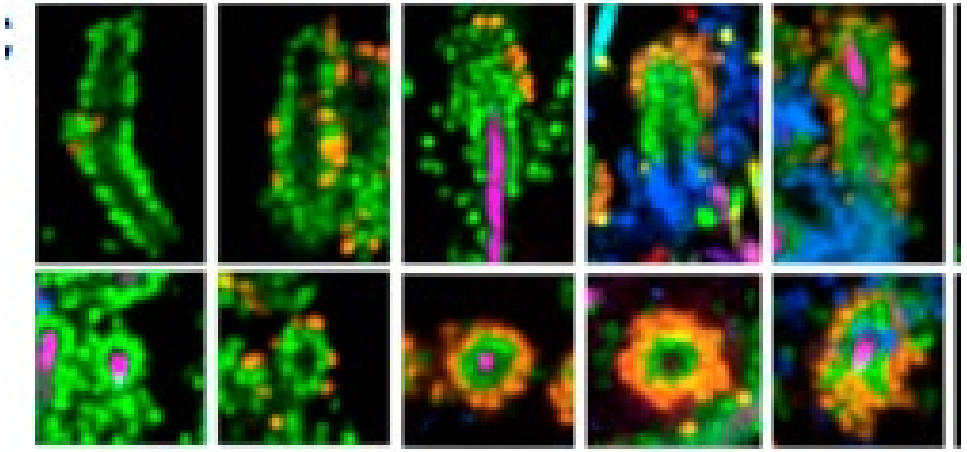
Increase in steady state bacterial numbers in first hour of antibiotic treatment





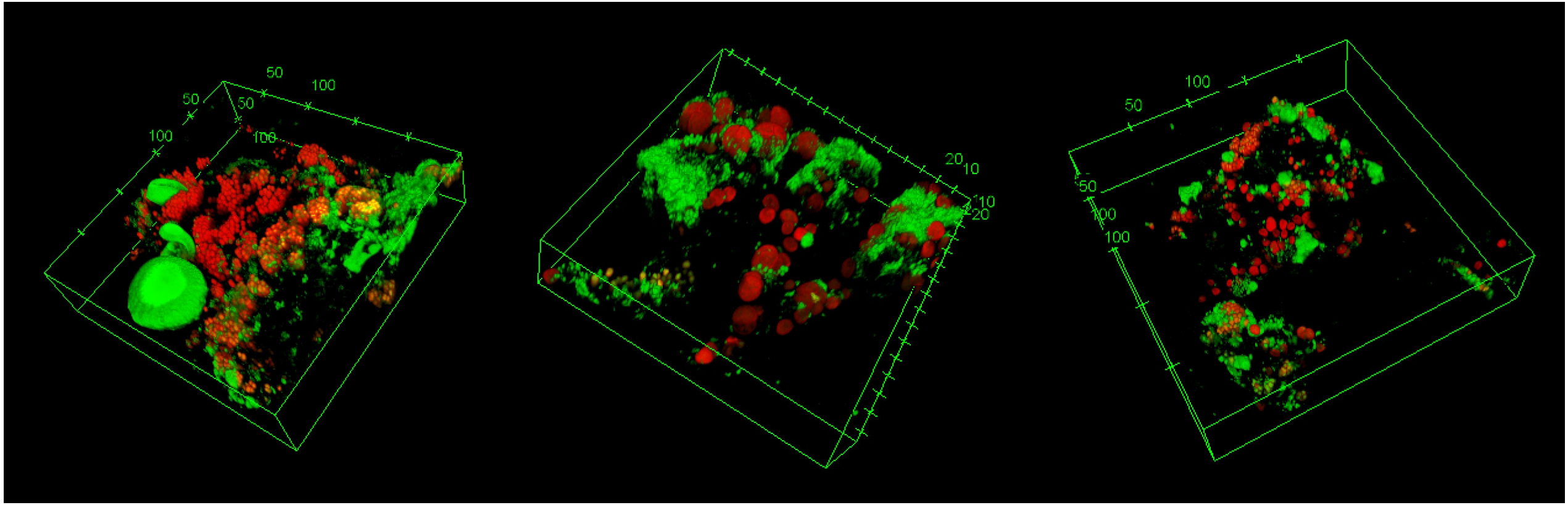
Building interdependent communities: models in extreme environments





- Small interdependent communities are present in the GI microbiota as well
- What governs the organization of these communities?
- Are they organized on the basis of diffusion length interaction?

Building interdependent communities: models in extreme environments



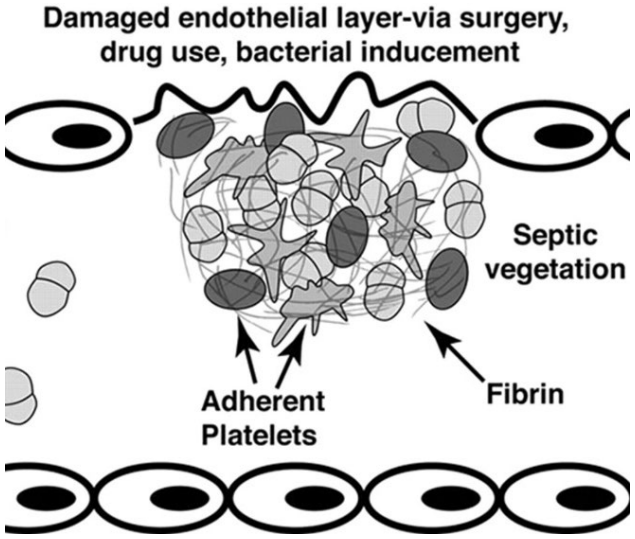
Merchant Exchange Building
Philadelphia

Jefferson Memorial
Washington DC

Federal Hall
New York City

Red – Cyanobacteria – use sunlight for energy (photosynthesis) and fix carbon for growth
Green – Heterotrophs – use carbon from Cyanobacteria for energy and growth
Cyanobacteria can be found alone. Heterotrophs cannot.
What do they provide? And what are the physical rules for their association?

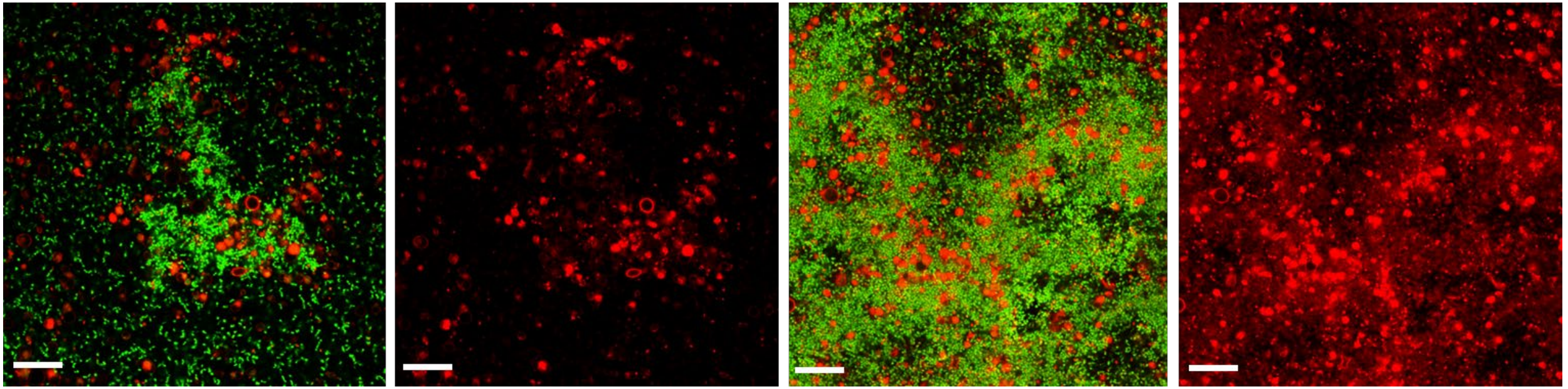
Rigid structures may increase virulence



E. faecalis endocarditis

- bacteria in biofilms, fibrin, and platelets form a vegetation on heart valves
- pCF10 causes rigid structures to form
- pCF10 increases the size of vegetations on the heart valves

- Human platelets were added to *E. faecalis* biofilms
- platelets are labelled red
- bacteria are green
- unactivated platelets are the same size as bacteria
- activated platelets are bigger



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