# A1 RB-1 : « Biofilms microbiens : structure, diversité et fonctions émergentes »

# Architecture of microbial societies

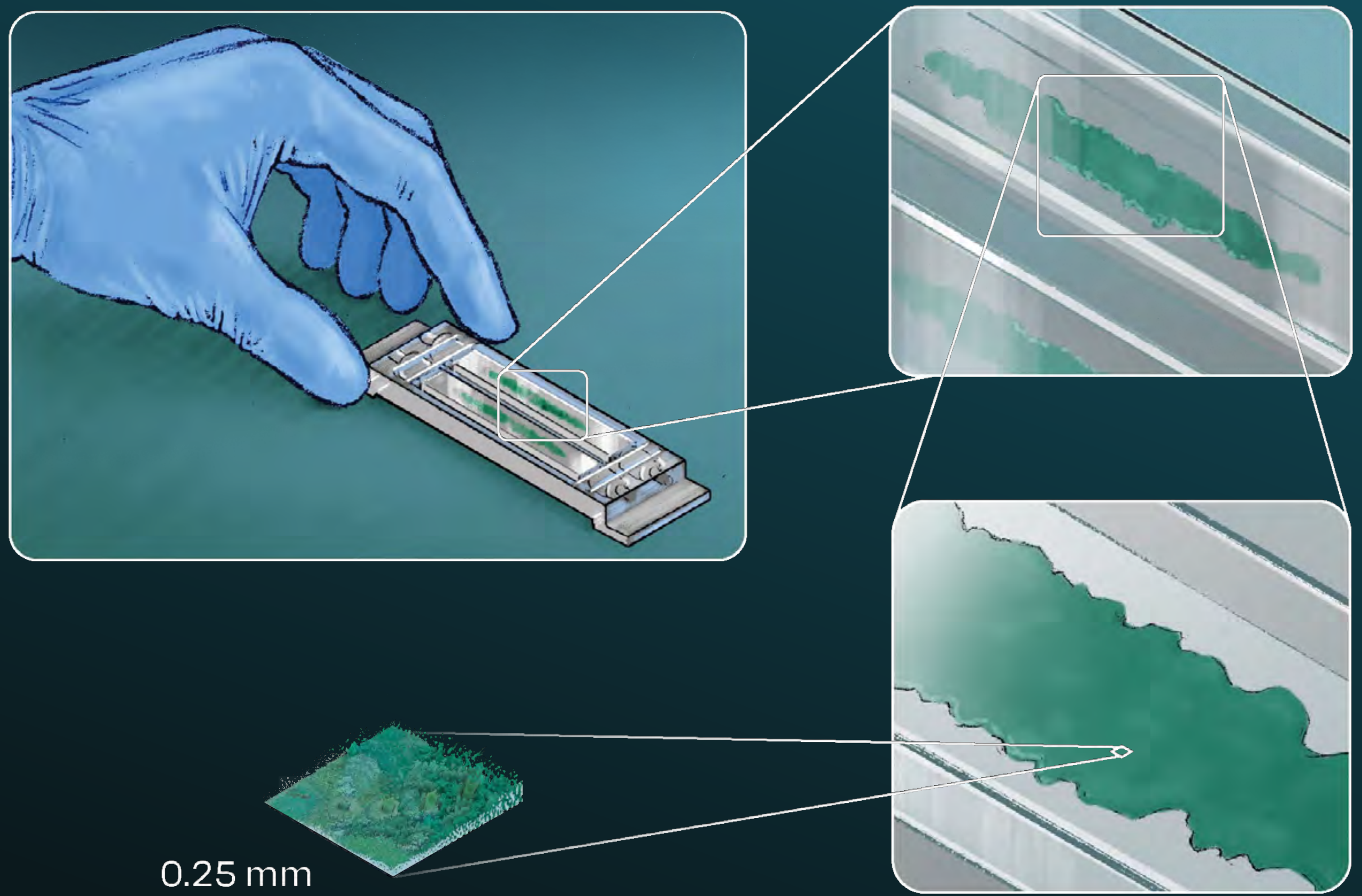
spatiality, cooperation and the emergence of biofilms

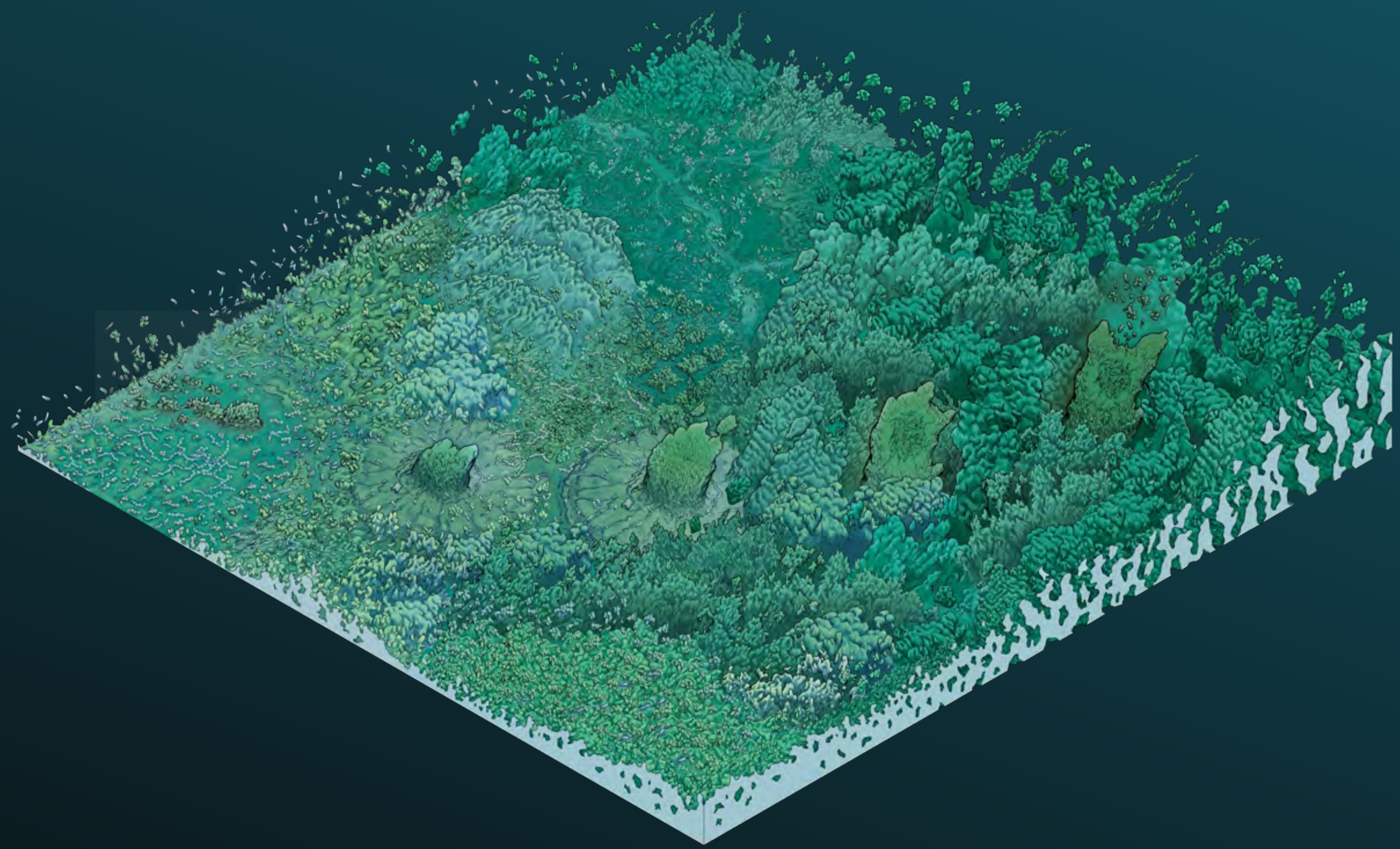
# The biofilm as a microbial city

Architecture, dynamics and life cycle

# Experimental context of biofilm cycle

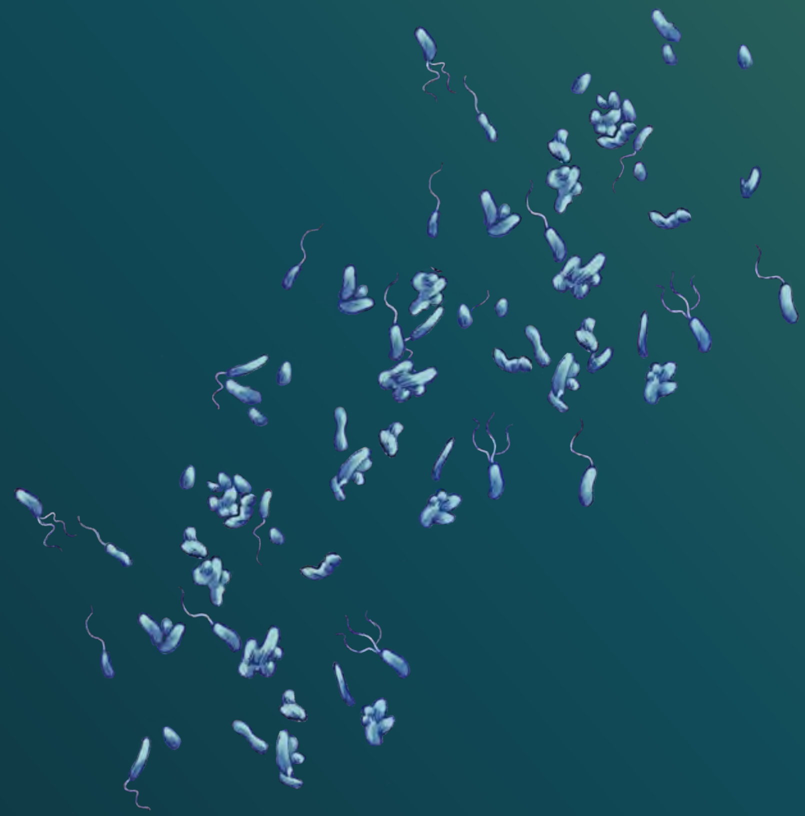
The processes illustrated take place within a region measuring just one-sixteenth of a square millimetre in a biofilm grown in a laboratory flow cell channel





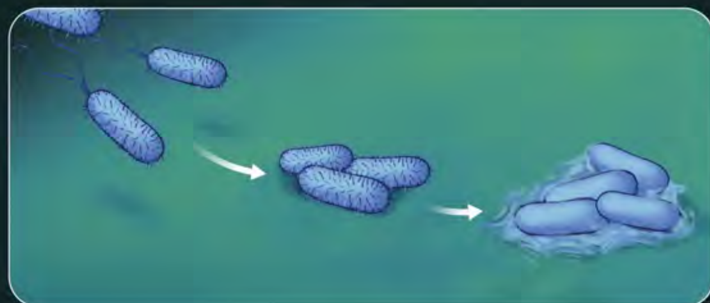
## Transport to surface

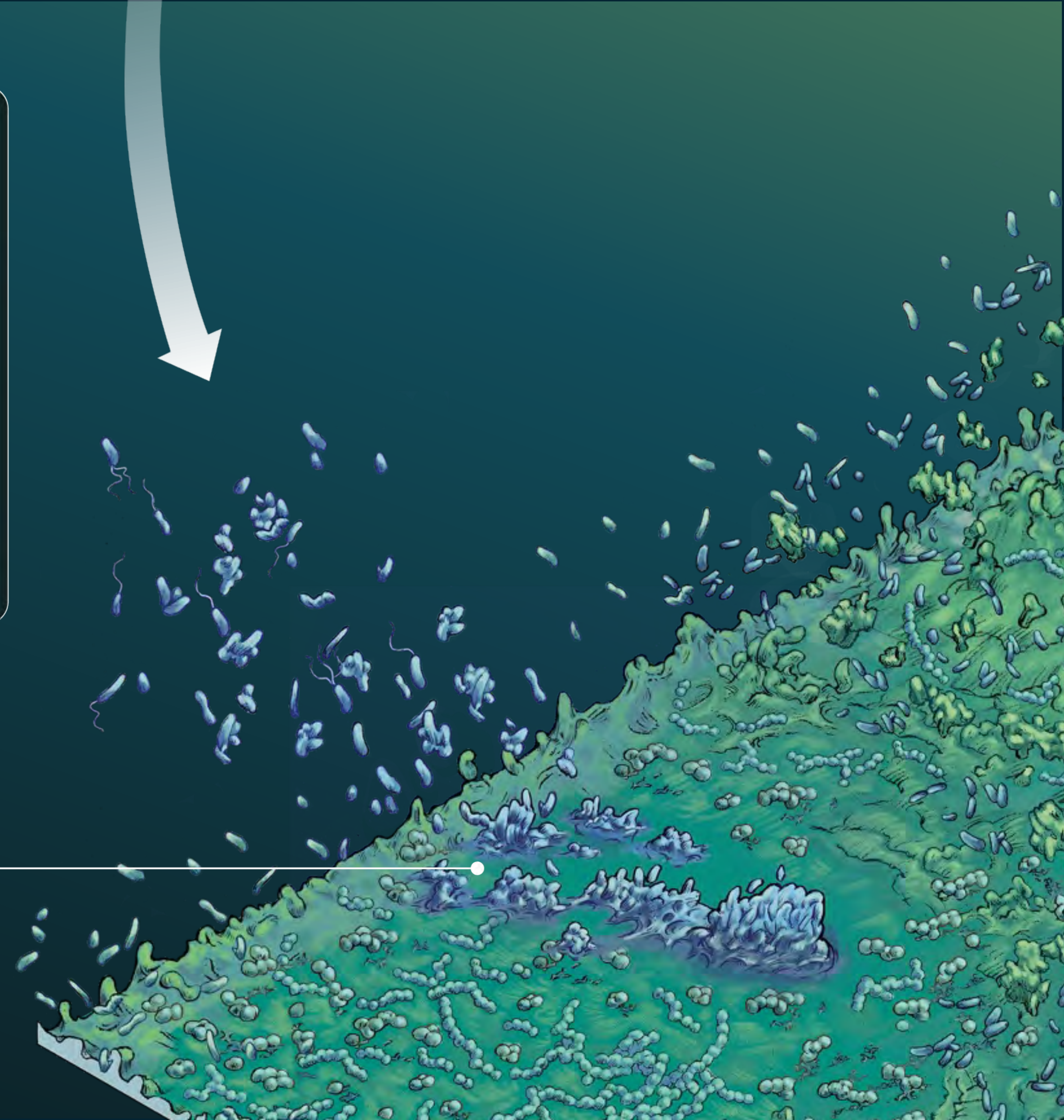
Cells reach surfaces via passive flow, motility, or as pre-formed aggregates



## Initial adhesion and surface engagement

Individual cells and aggregates approach the surface, establish in itial contact, and begin forming early clusters through adhesion and matrix production.

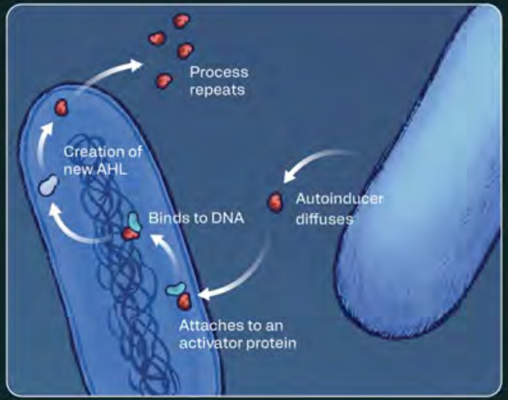


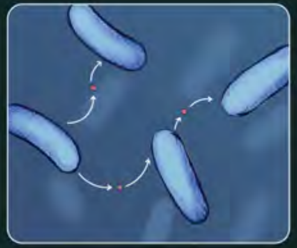


# Early biofilm developpement

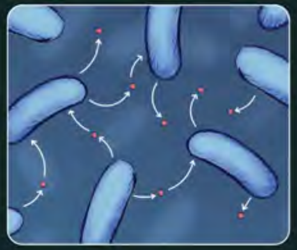
Clonal expansion and matrix secretion create structured multicellular clusters.

## Quorum sensing: density-dependent coordination of gene expression



Low density



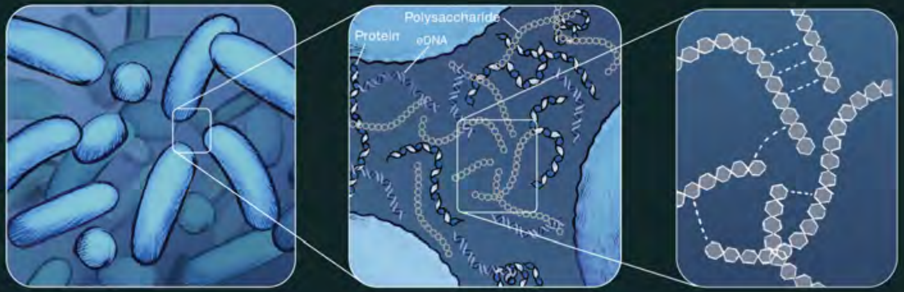
High density

At low cell density, signaling molecules remain below threshold. As density increases, accumulated signals trigger intracellular responses that alter gene expression and coordinate collective behaviors.

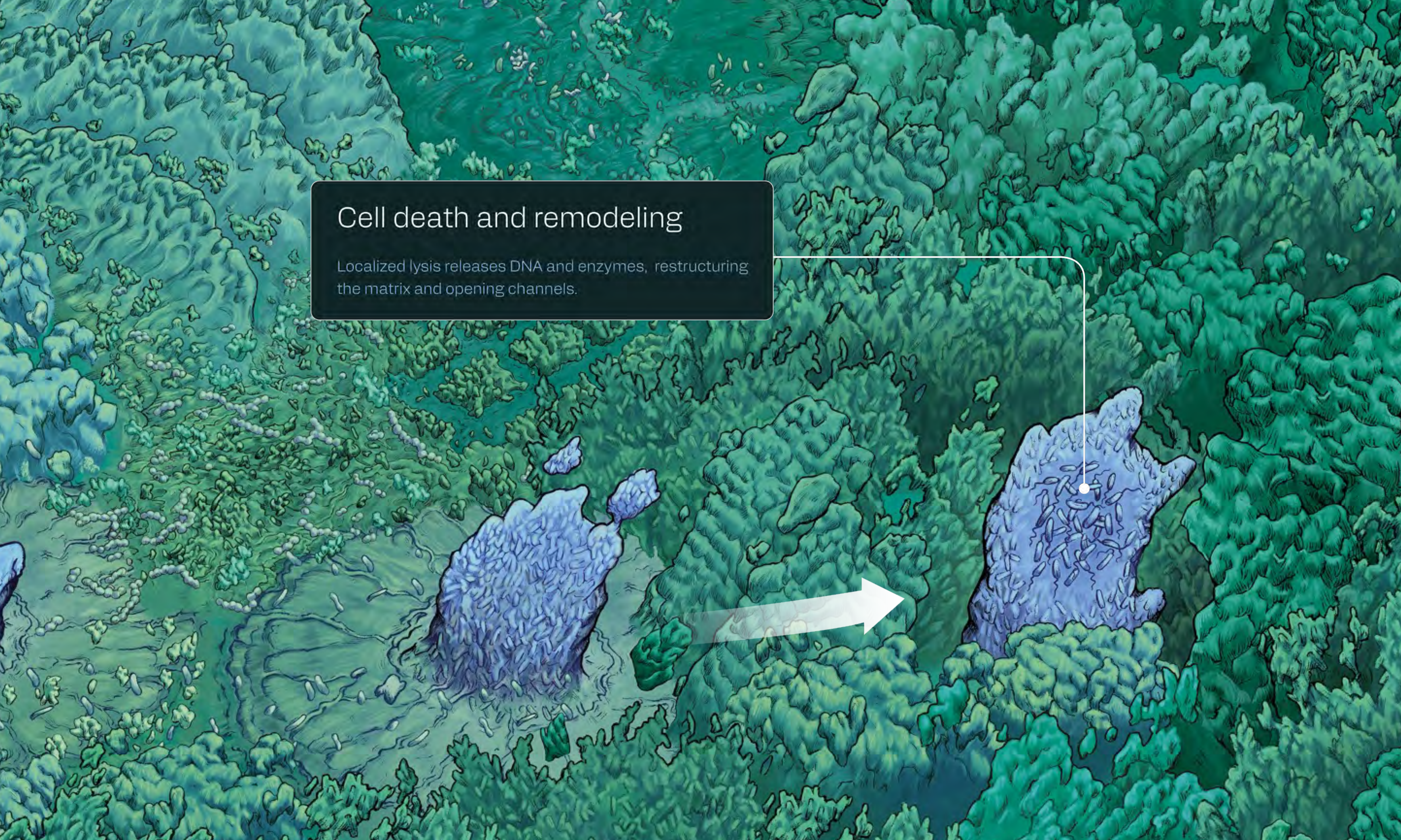
# Biofilm maturation

Complex 3D architecture develops, characterized by increased extracellular matrix production and spatial organization.

## Biofilm matrix: an interconnected network of biopolymers

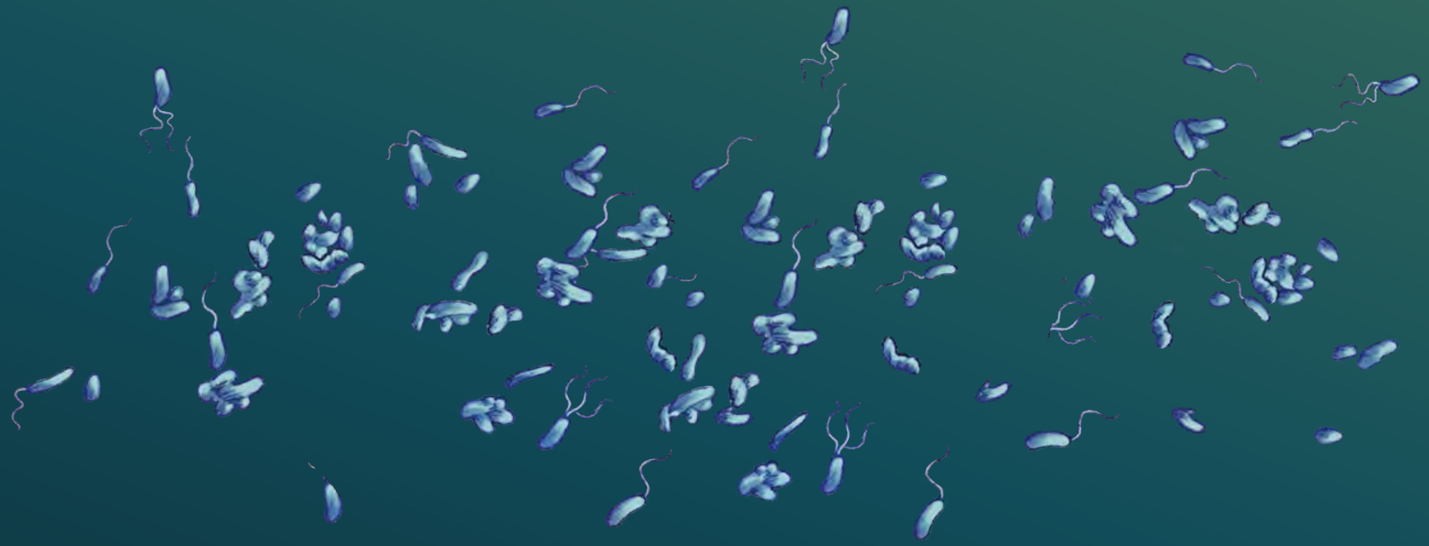



Progressive zoom reveals the extracellular matrix as a hydrated network of diverse biopolymers — polysaccharides, proteins, and extracellular DNA — structured into a viscoelastic gel.

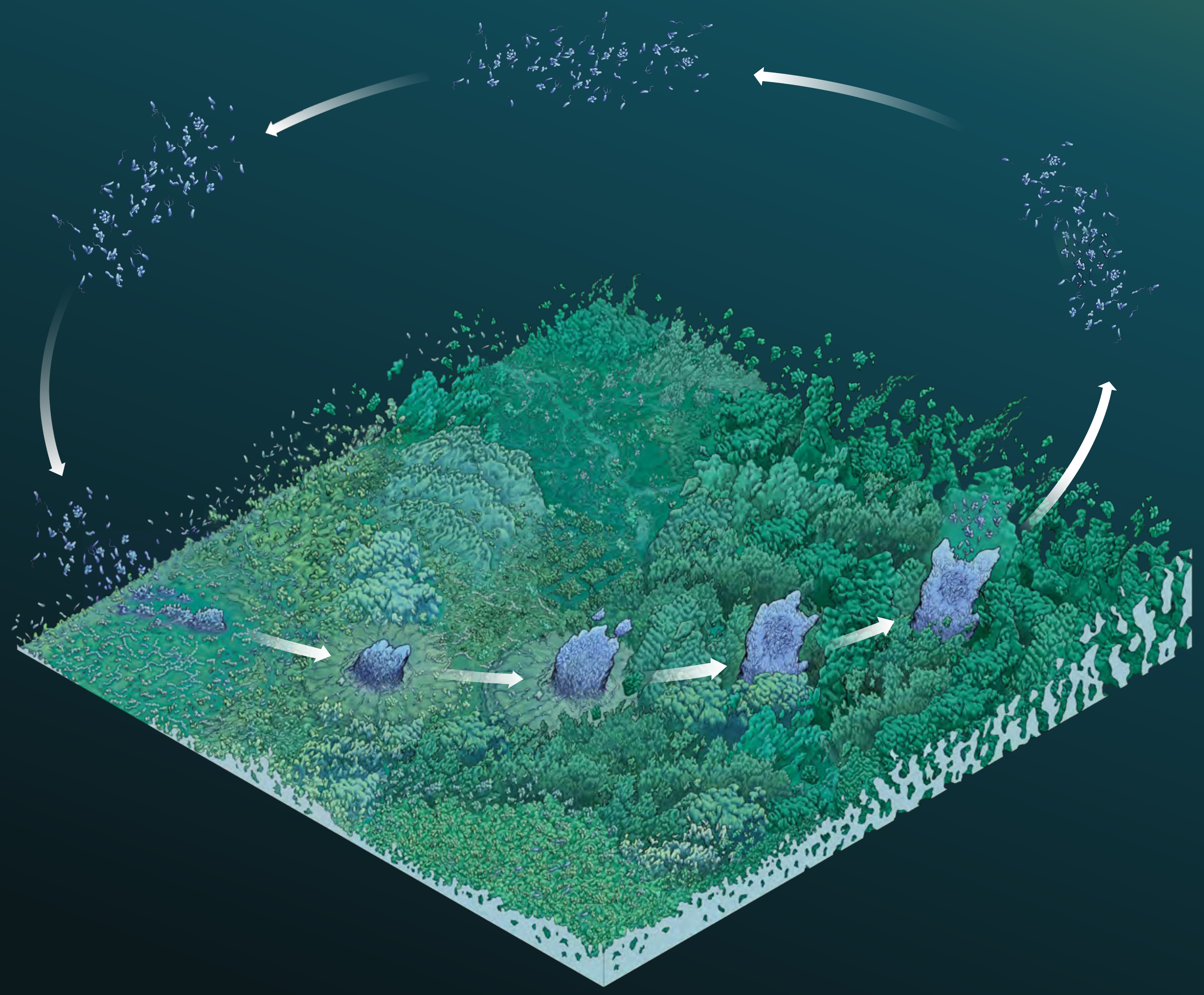

Cell death and remodeling
Localized lysis releases DNA and enzymes, restructuring the matrix and opening channels.

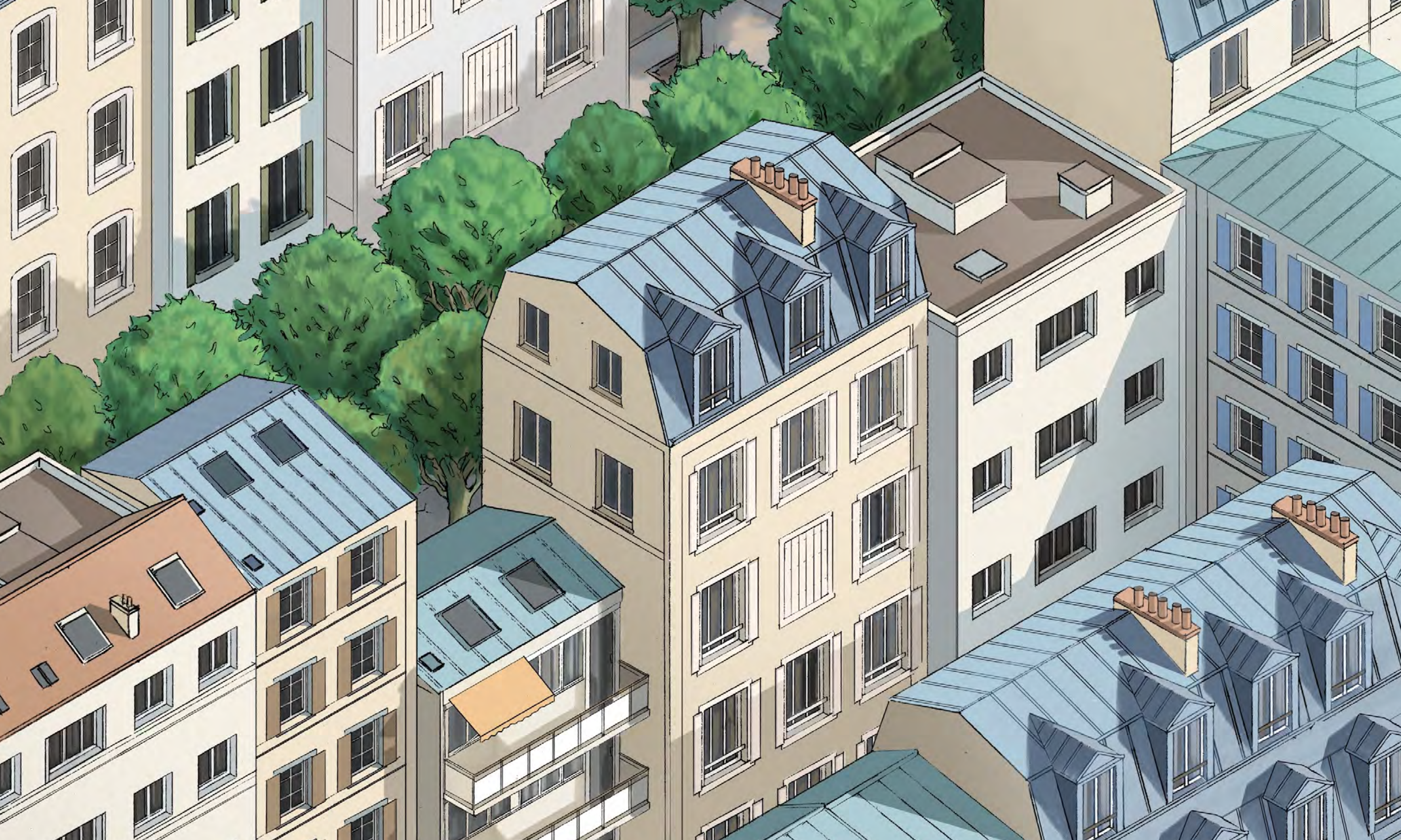

Dispersal
Cells detach actively or passively in response to environmental or internal signals.

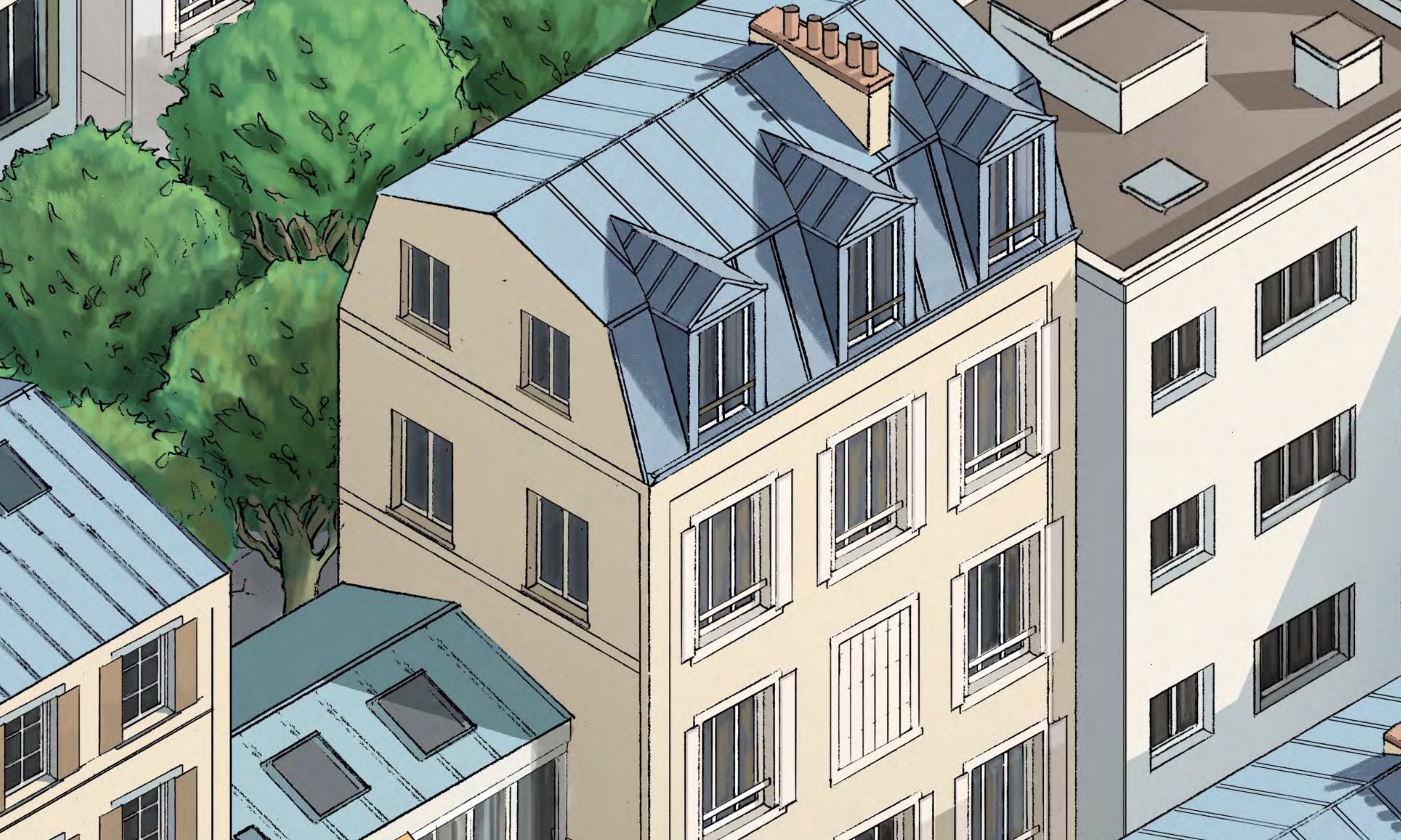

Released cells
Ready for new environments

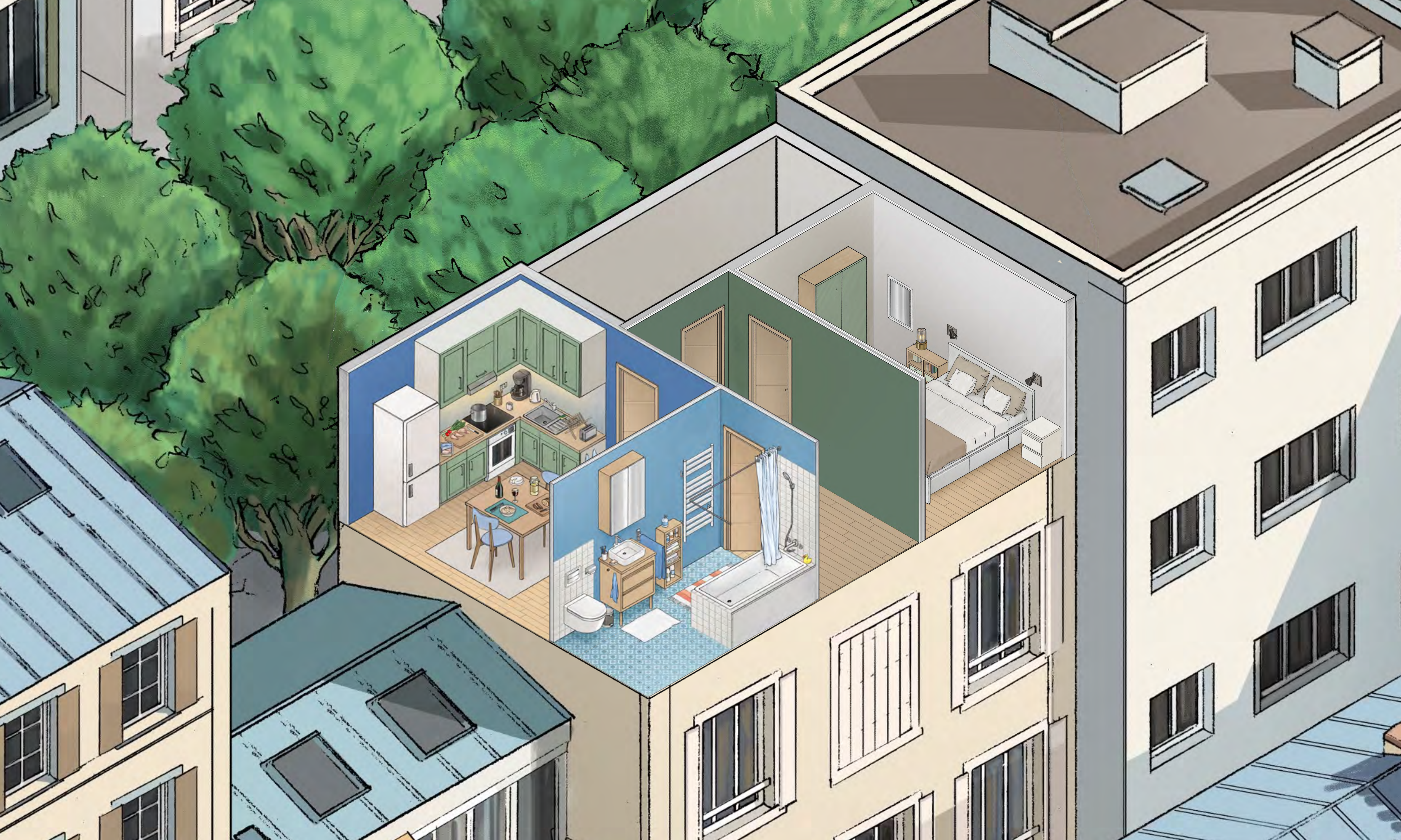


Planktonic free-living state

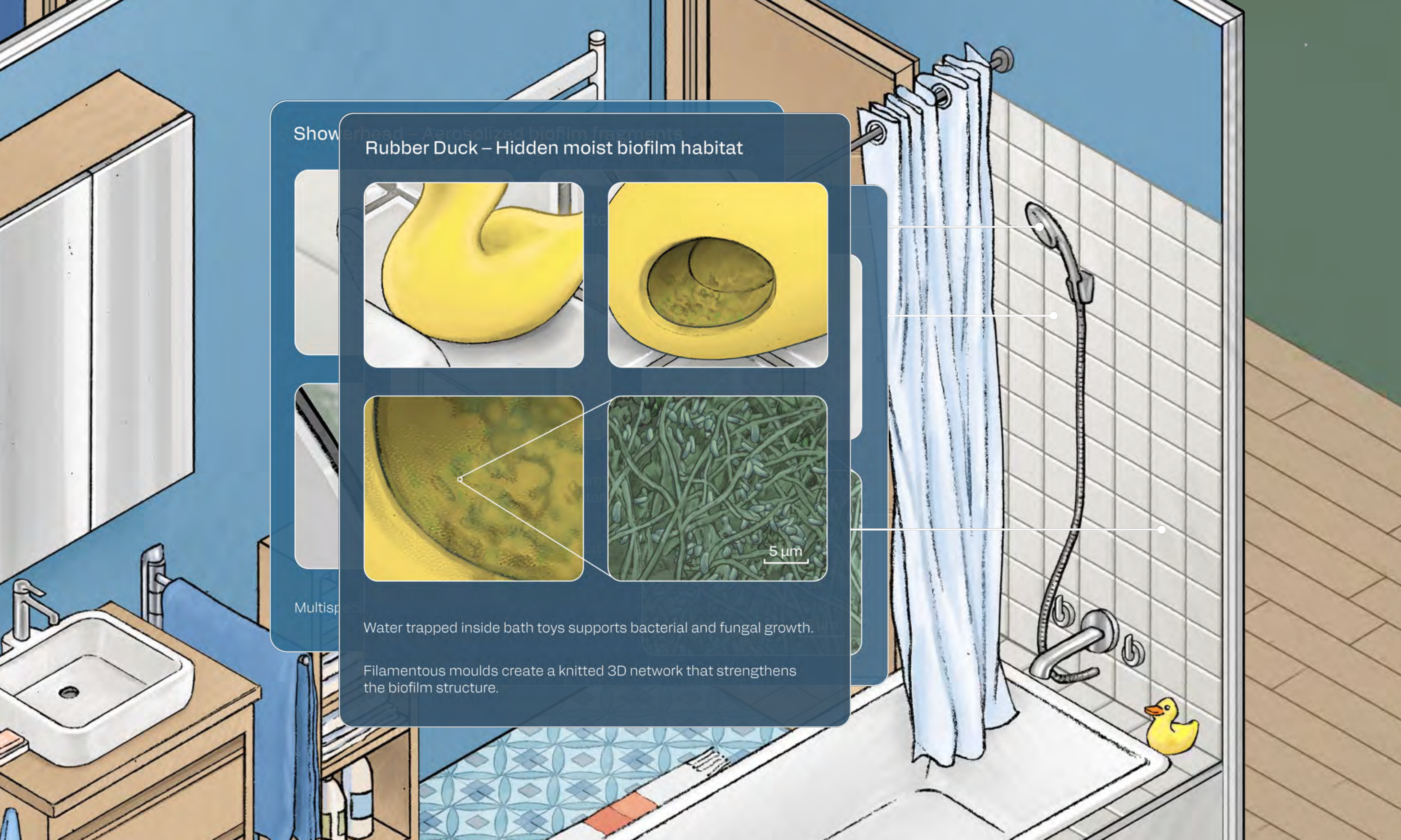






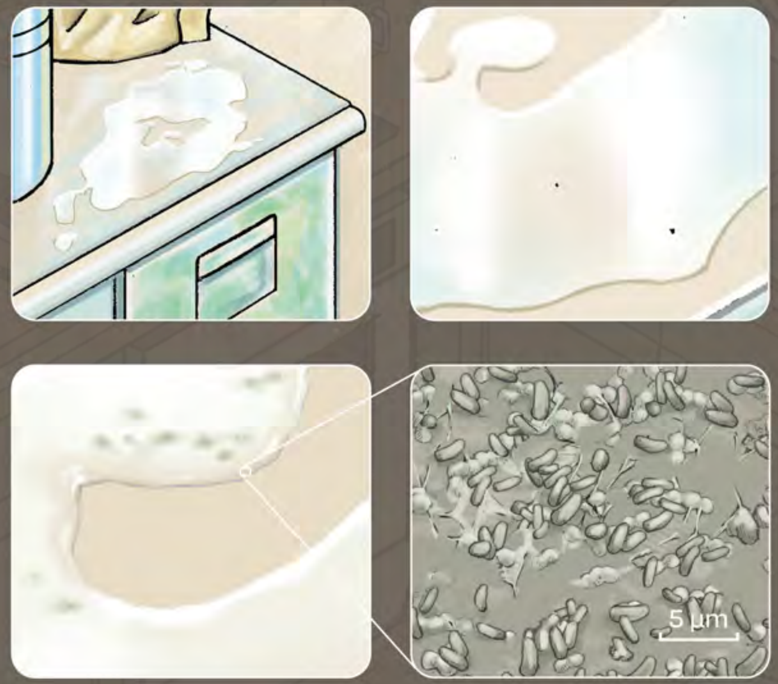



Faucet Base – Mineral-embedded bacterial biofilm
5 µm
Bacteria are embedded in a dense EPS matrix that also traps limescale, forming a cement-like structure.


Rubber Duck – Hidden moist biofilm habitat
5 µm
Water trapped inside bath toys supports bacterial and fungal growth.
Filamentous moulds create a knitted 3D network that strengthens the biofilm structure.


Kefir – Probiotic biofilm grains
Natto – Sticky Bacillus biofilm
5 µm
A sticky threads forms the matrix of a ***Bacillus subtilis*** biofilm
Rich in vitamin $K_2$, enzymes, and microbial compounds with benefits for digestion, immunity, and heart health.

## Fridge – Cold-resistant listeria biofilms

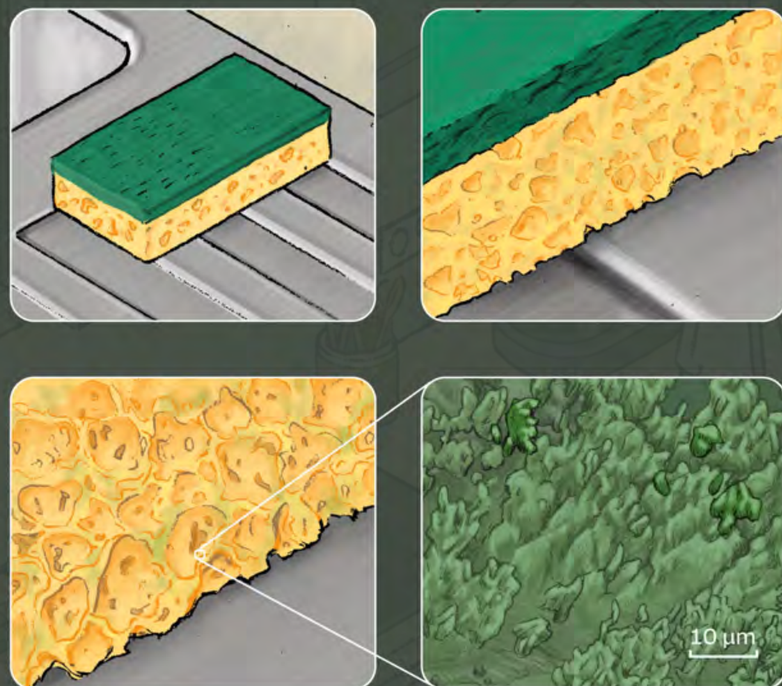



In cold, damp areas of the fridge, ***Listeria monocytogenes*** can form persistent biofilms.

It can survive cleaning and cause severe infections, especially in vulnerable individuals.


Cutting Board – Cross-contamination risk
Raw chicken can carry *Salmonella*, which may contaminate the board.
Using the same board for raw vegetables can transfer pathogens to ready-to-eat foods
10 µm

## Sponge – A microbial hotspot

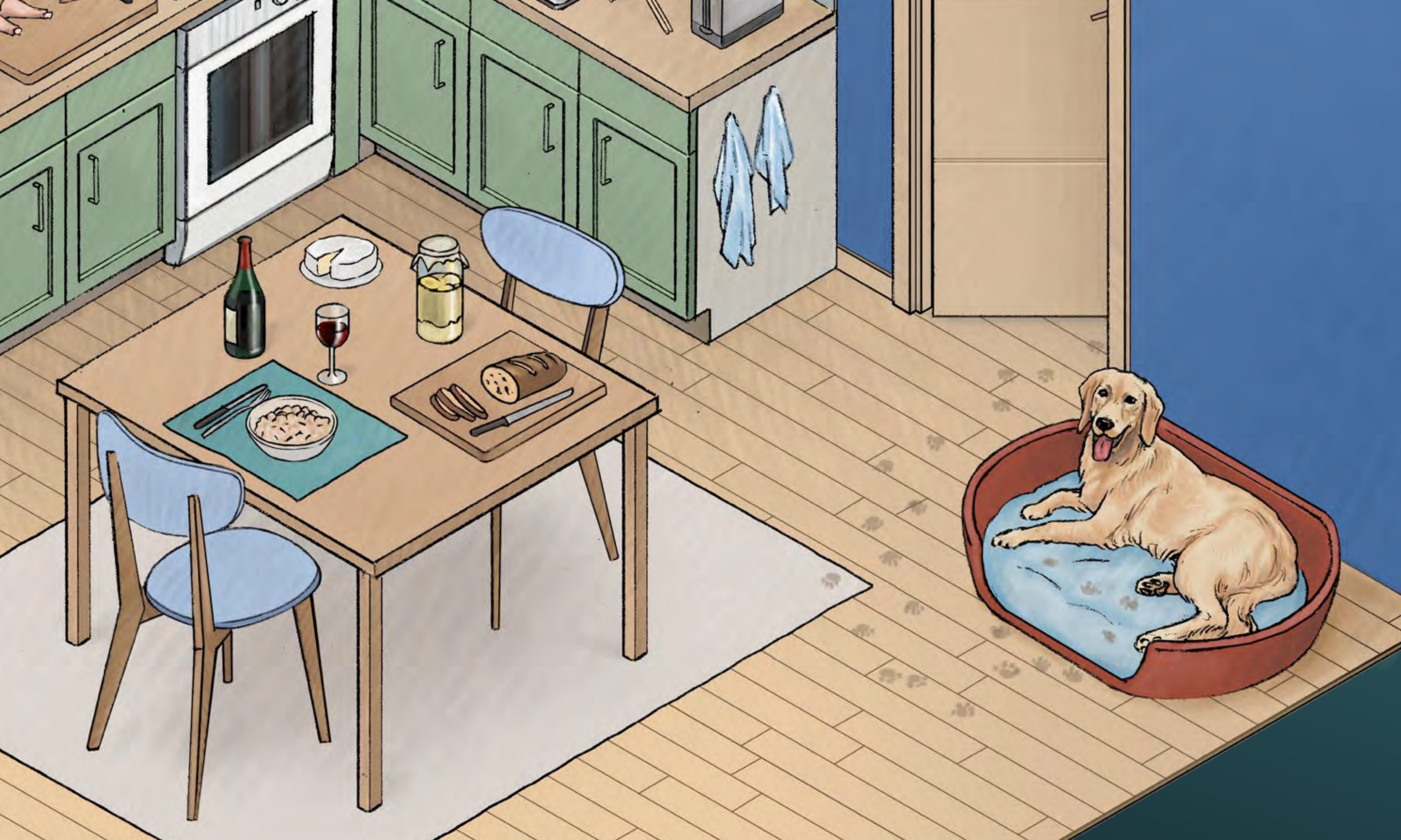


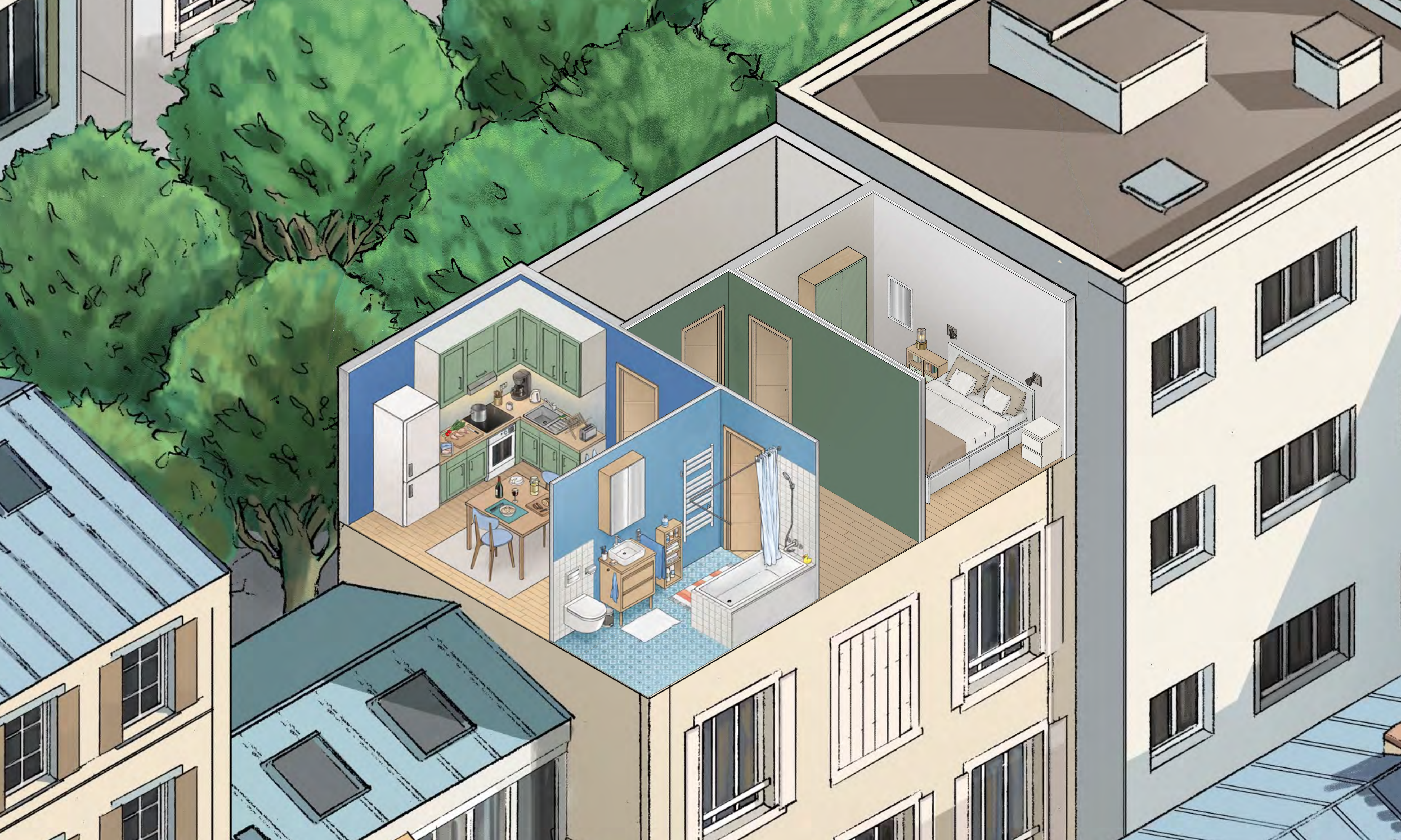

Moisture, food residues, and porous structure allow dense biofilms to develop, making the sponge a major source of cross-contamination.

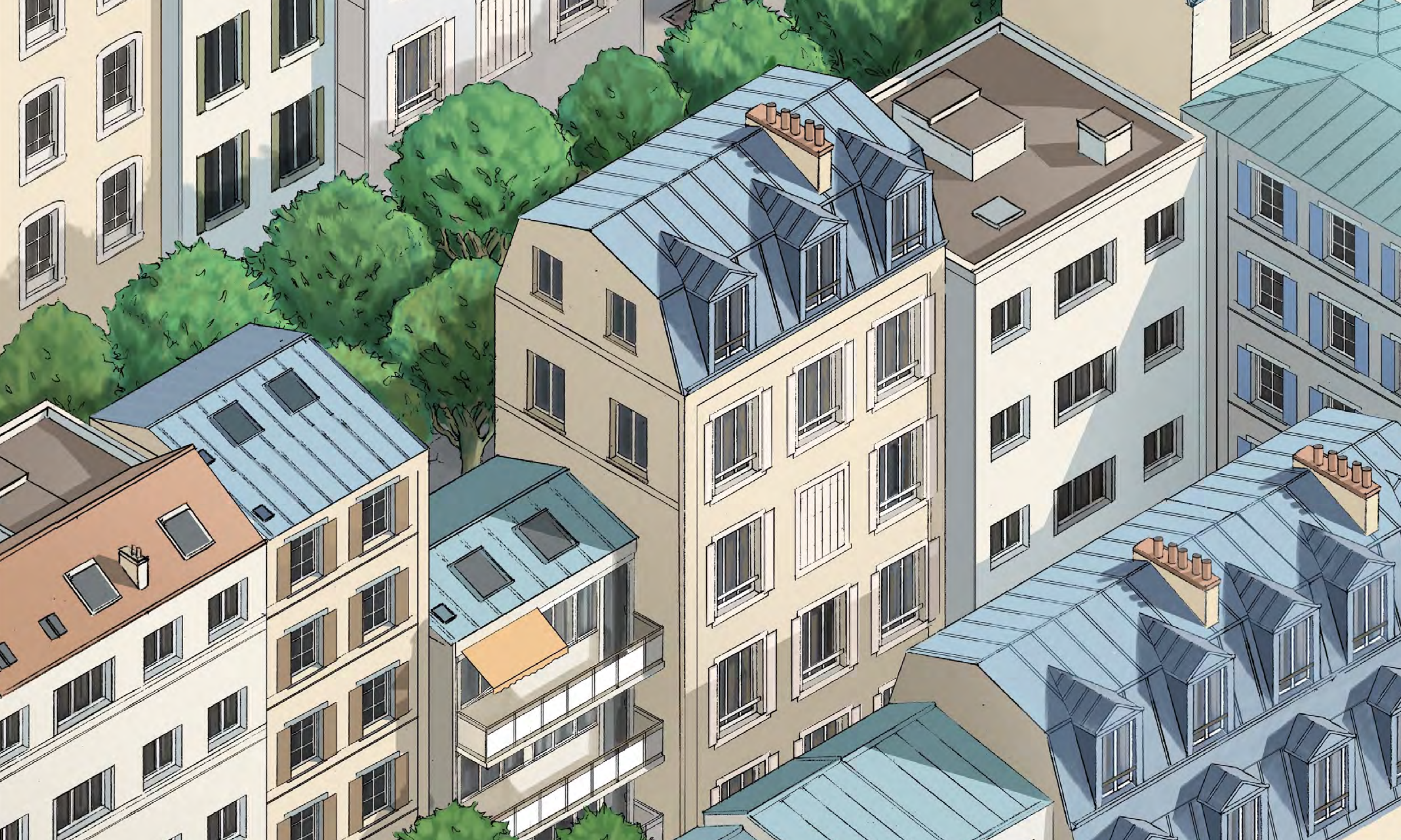
## Coffee Maker – Biofilms and off-Flavors


Moisture and coffee residues allow biofilms to grow at the base of the filter holder.

It can alter the taste and smell of your coffee if not regularly cleaned.



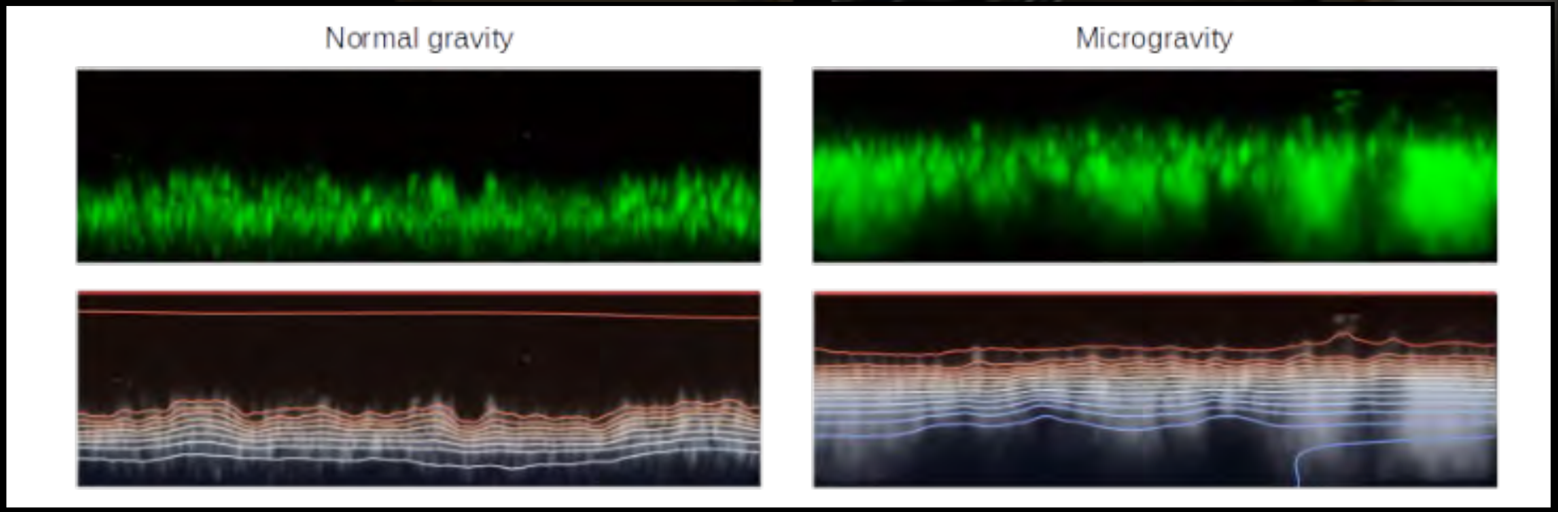





INRAE MIMA2 Imaging Center

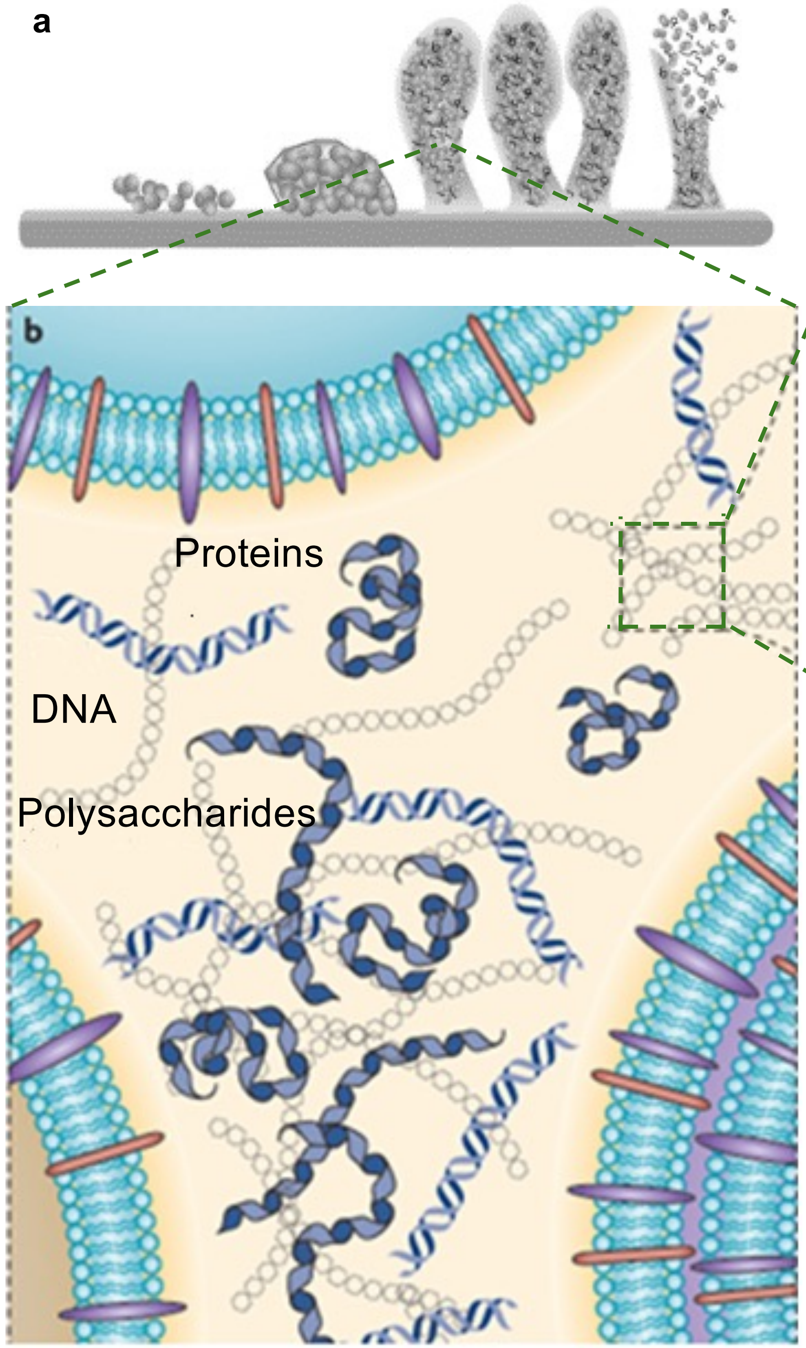
# Biofilms everywhere on Earth…

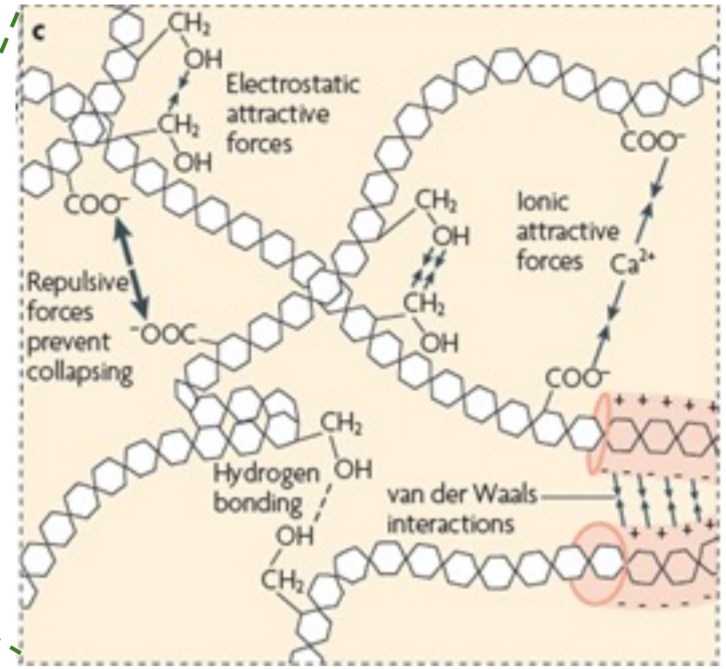
# …and beyond!




Marra et al. (2023)

# The slime matrix




(Flemming et Wingender, Nat. Rev. Microbial., 2010)

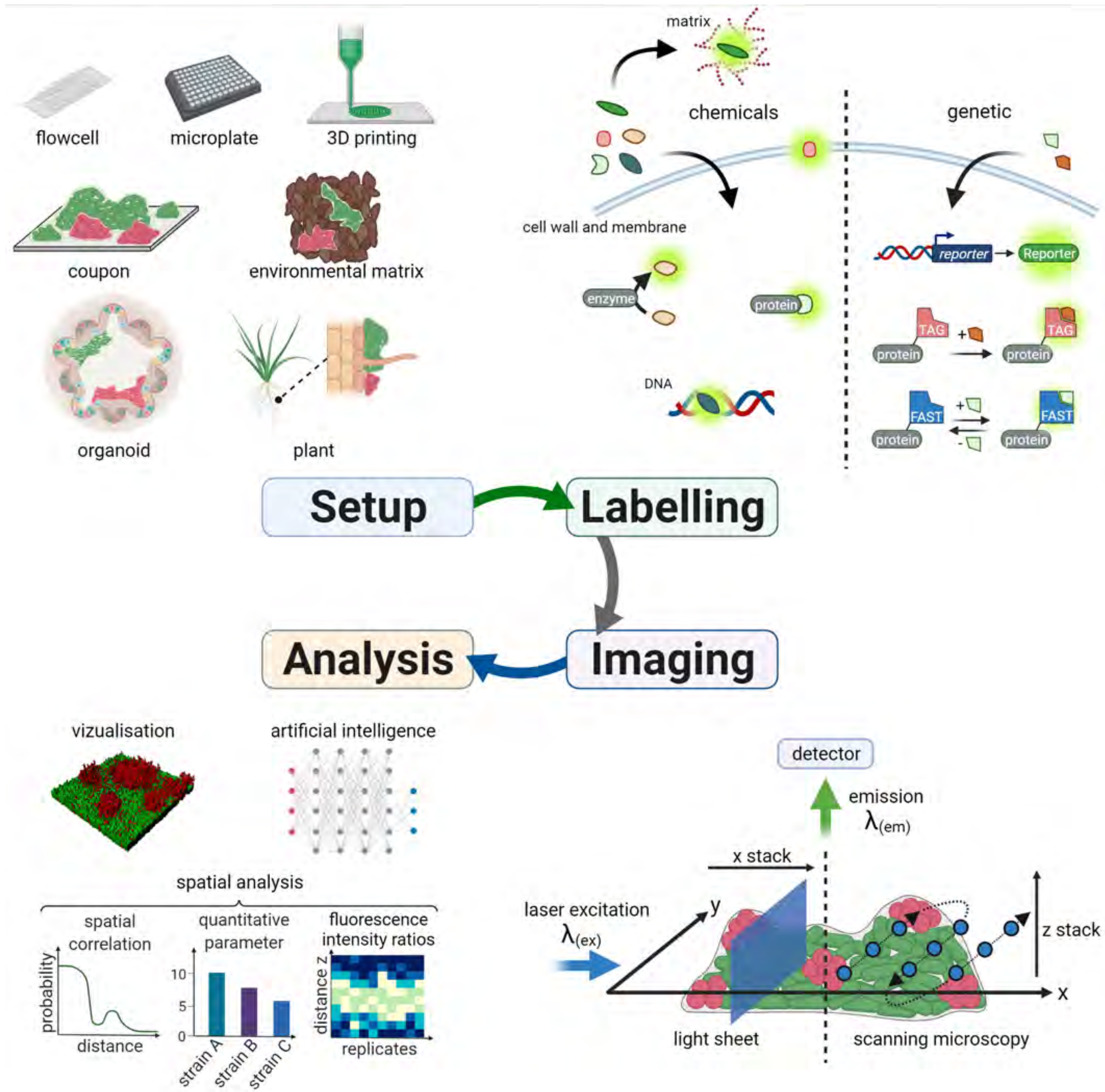
**-EPS= Extra Polymeric Substances**

-Water, polysaccharides, proteins, eDNA, lipids…

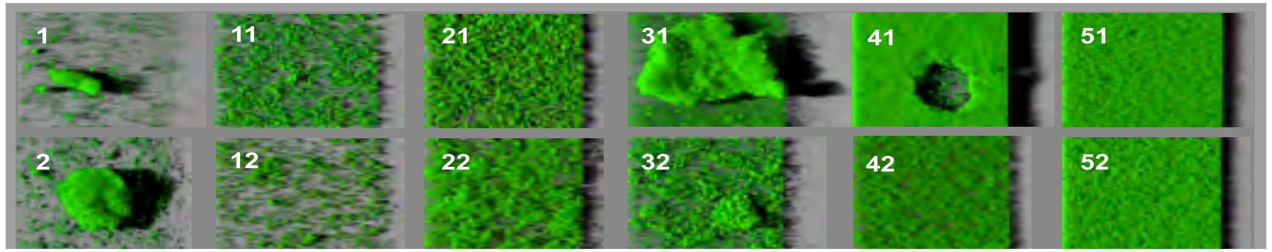
-EPS cohesion by a network of week bonds

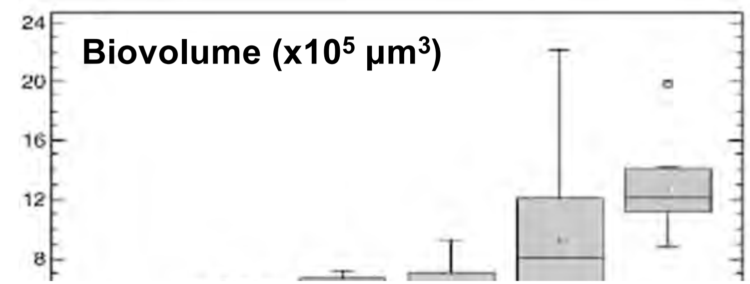
-EPS= the immediate environment of biofilm cells, determine their local living conditions

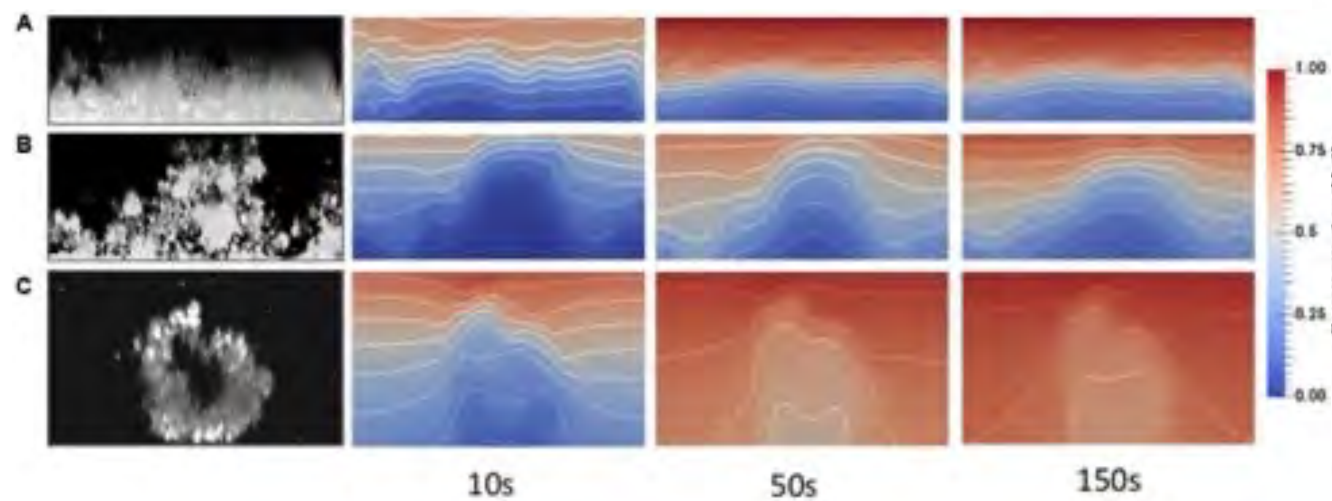
# Worflow to analyse biofilm by microscopy

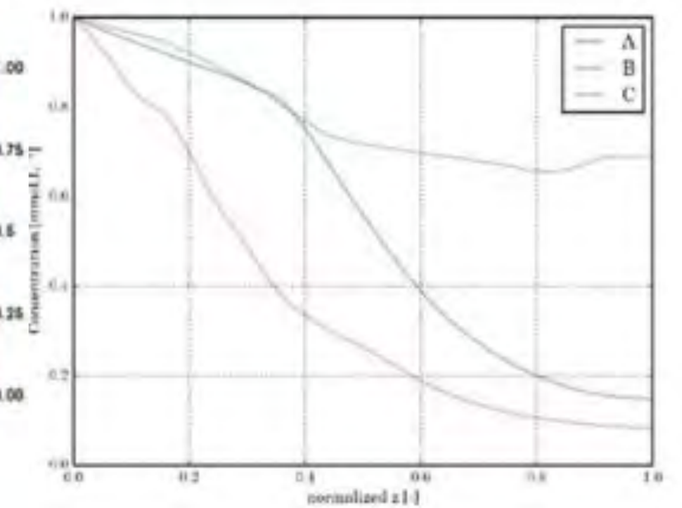


# Diversity in biofilm spatial organisation



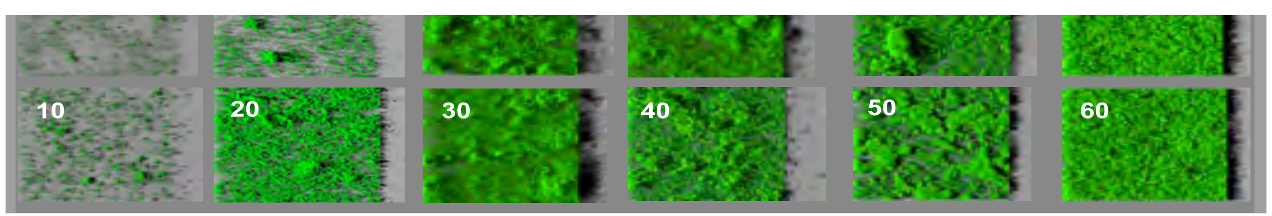



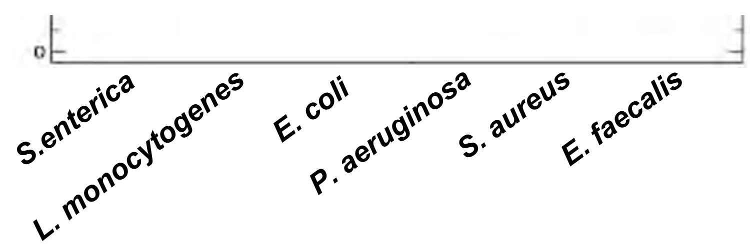




**Modeling of diffusion in biofilm of various architecture**

*(A) Escherichia coli, (B) Pseudomonas putida, (C) Salmonella enterica*.

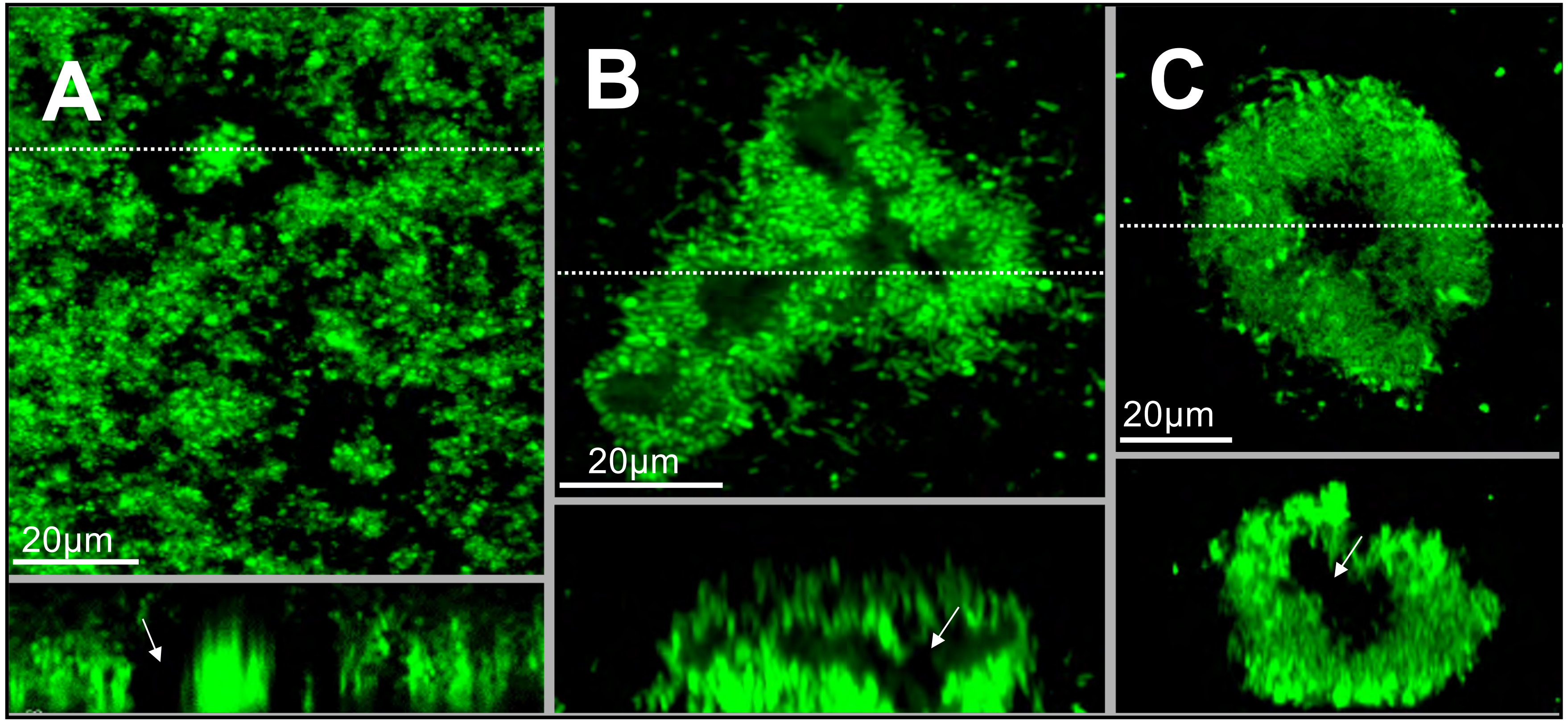


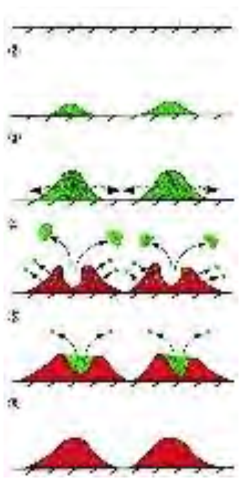



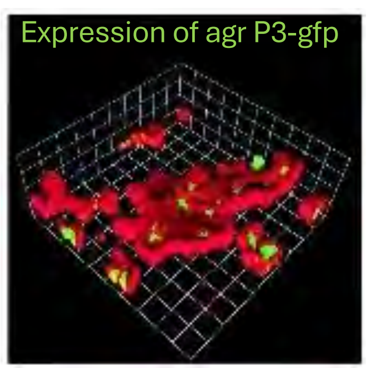

Bridier et al. *J. Microbio. Methods*, 2010

# Hollow voids & localized cell death

*S.aureus* (strain 47) *P.aeruginosa* (strain 36) *S.enterica* (strain 2)



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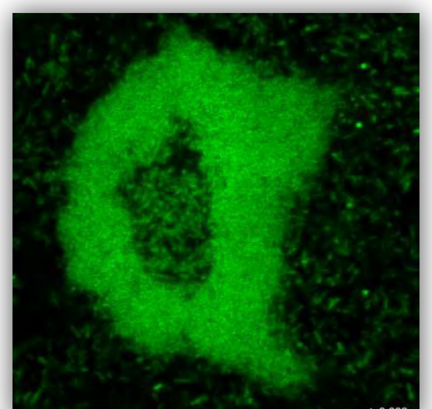
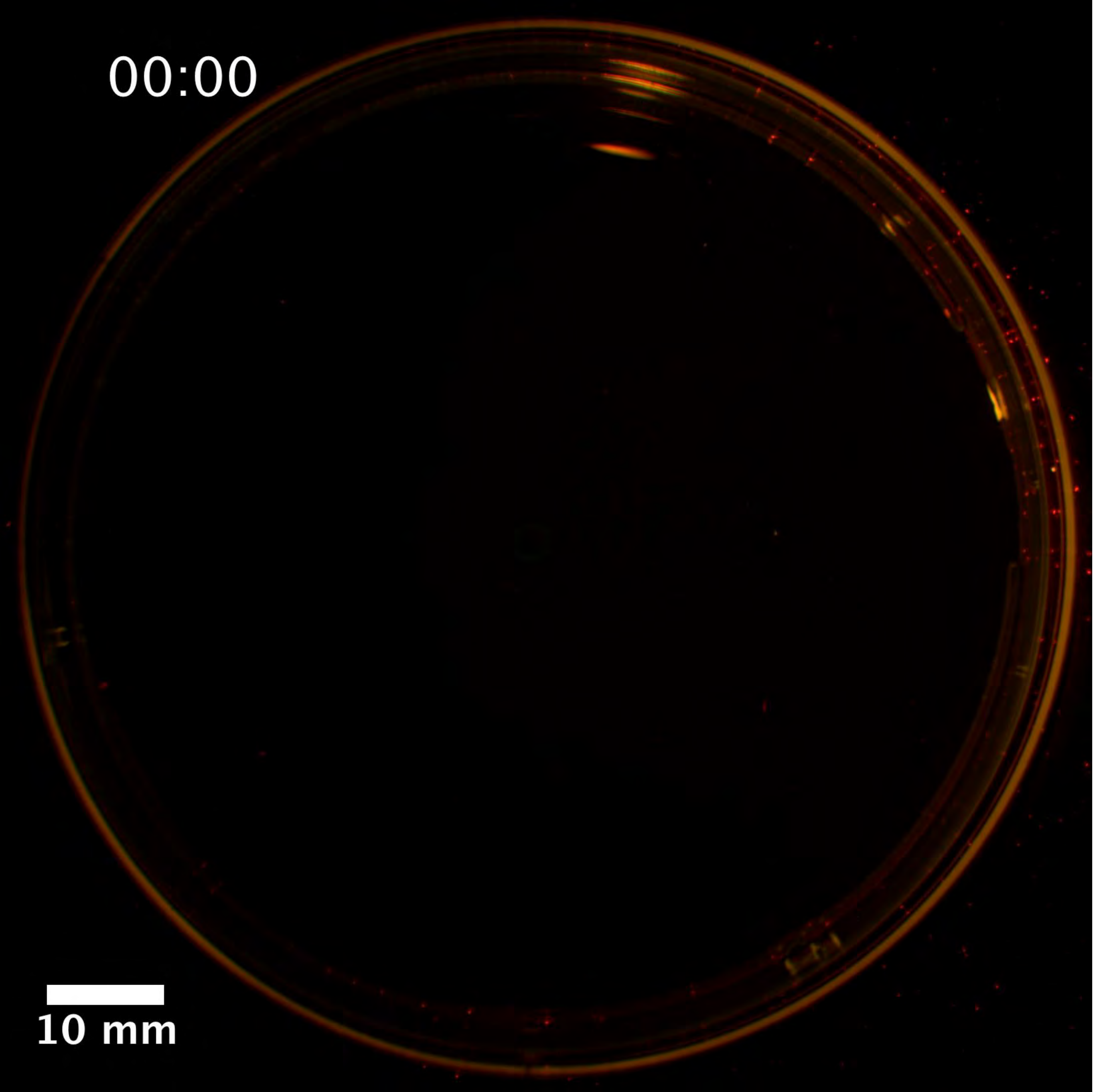
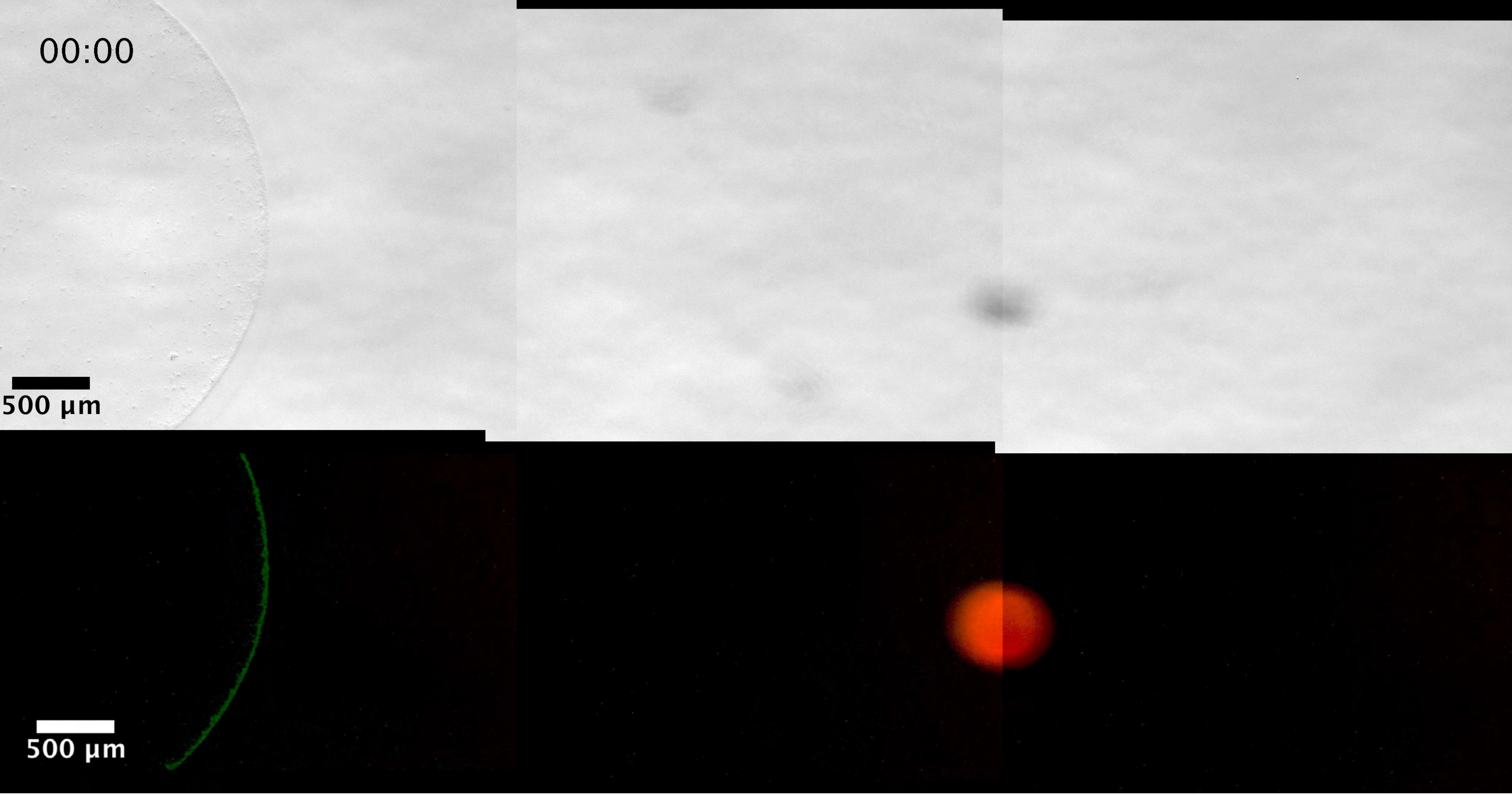





# Diversification of cell types

Swarming model of *Bacillus subtilis*

*B.subtilis Phag-**GFP** PtapA-**mKate2***




*B.subtilis Phag-**GFP** PtapA-**mKate2***

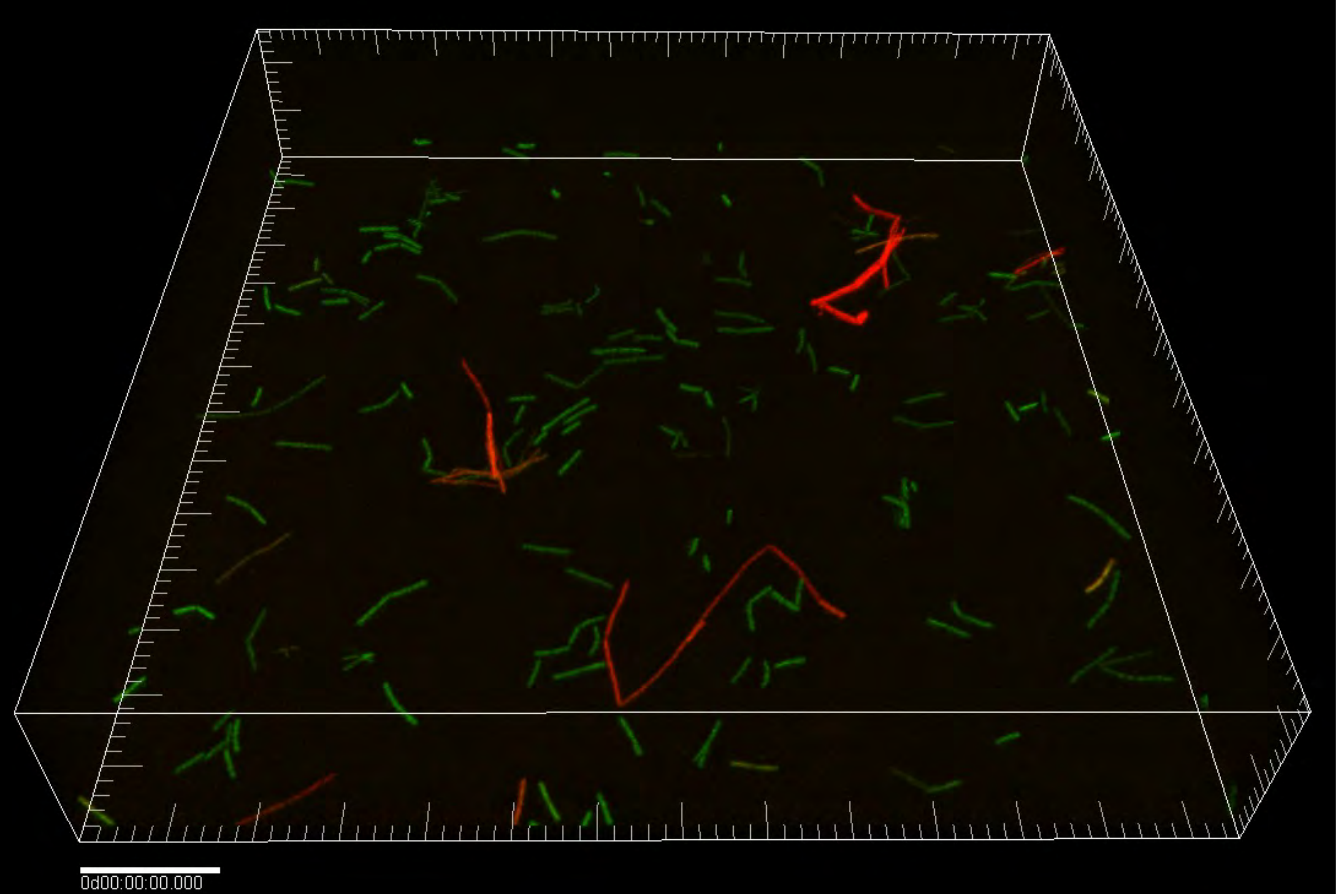
# Diversification of cell types

Macrocolony model of *Bacillus subtilis*

*B.subtilis Phag-**GFP** PtapA-**mKate2***

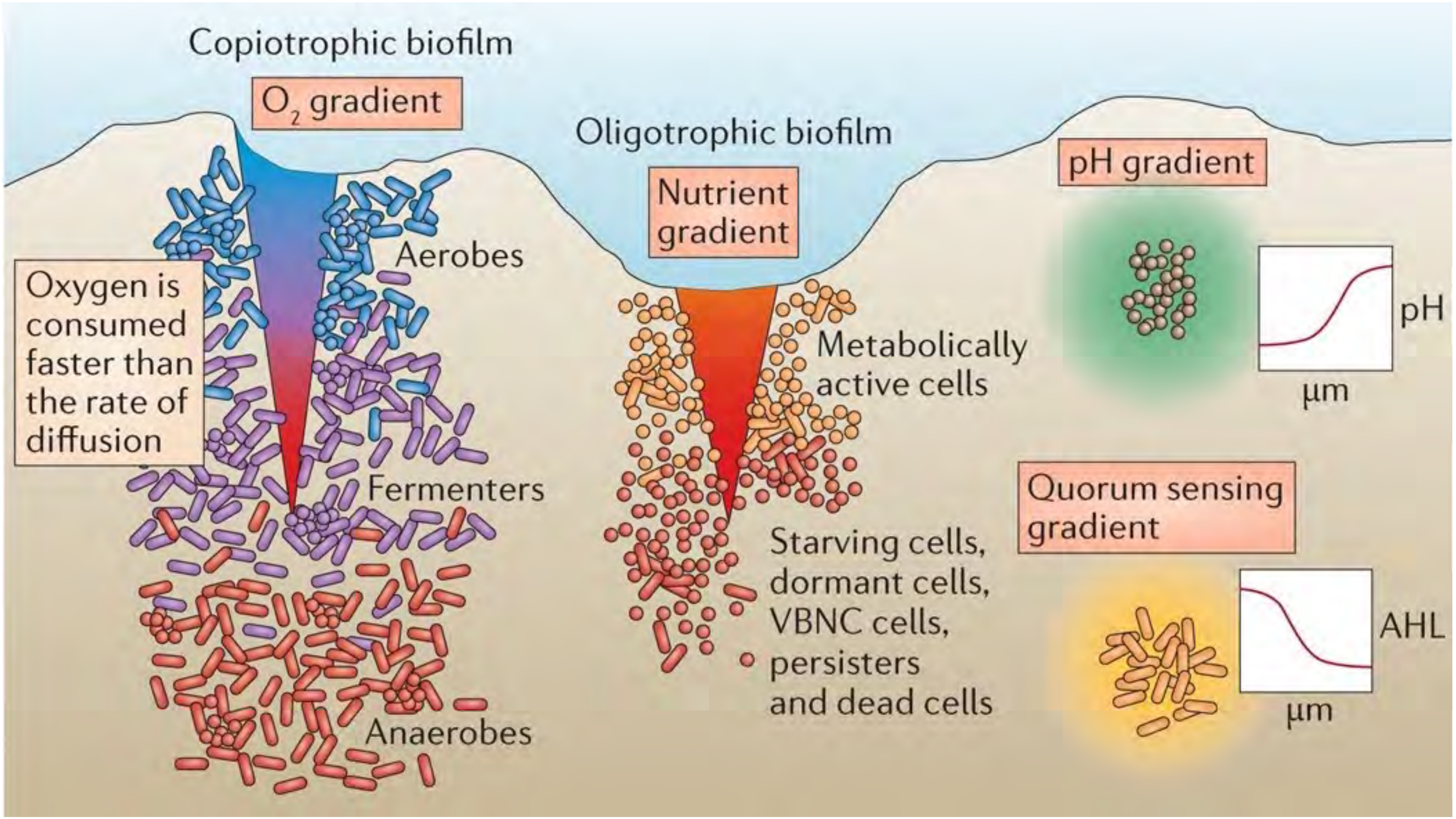
# Diversification of cell types

Submerged biofilm model of *Bacillus subtilis*

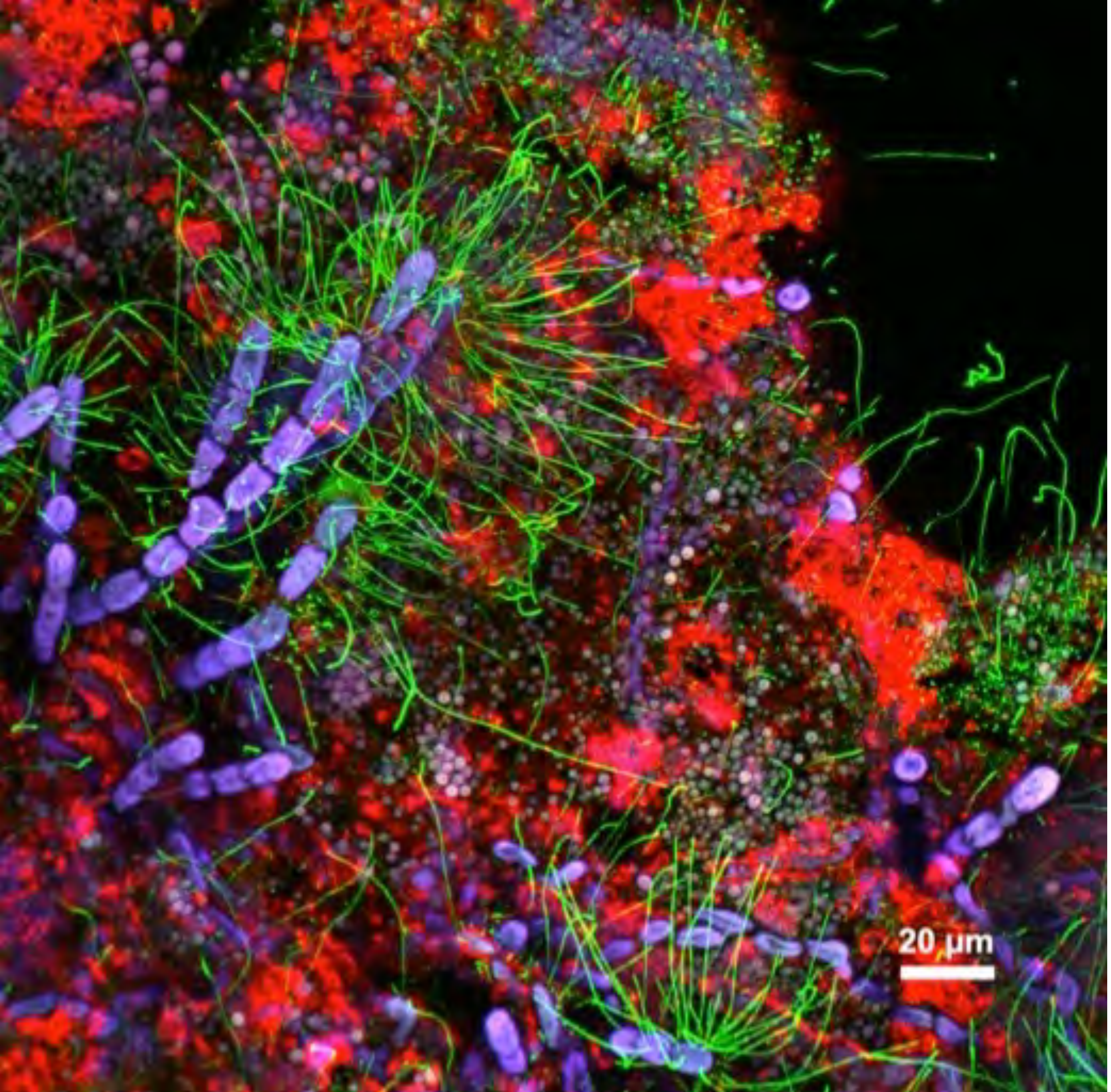



*B.subtilis Phag-GFP PtapA-mKate2*

## Matrix gradients provide different localized habitats and physiological heterogeneity




*Flemming et al., Nature Reviews Microbiology, 2016*



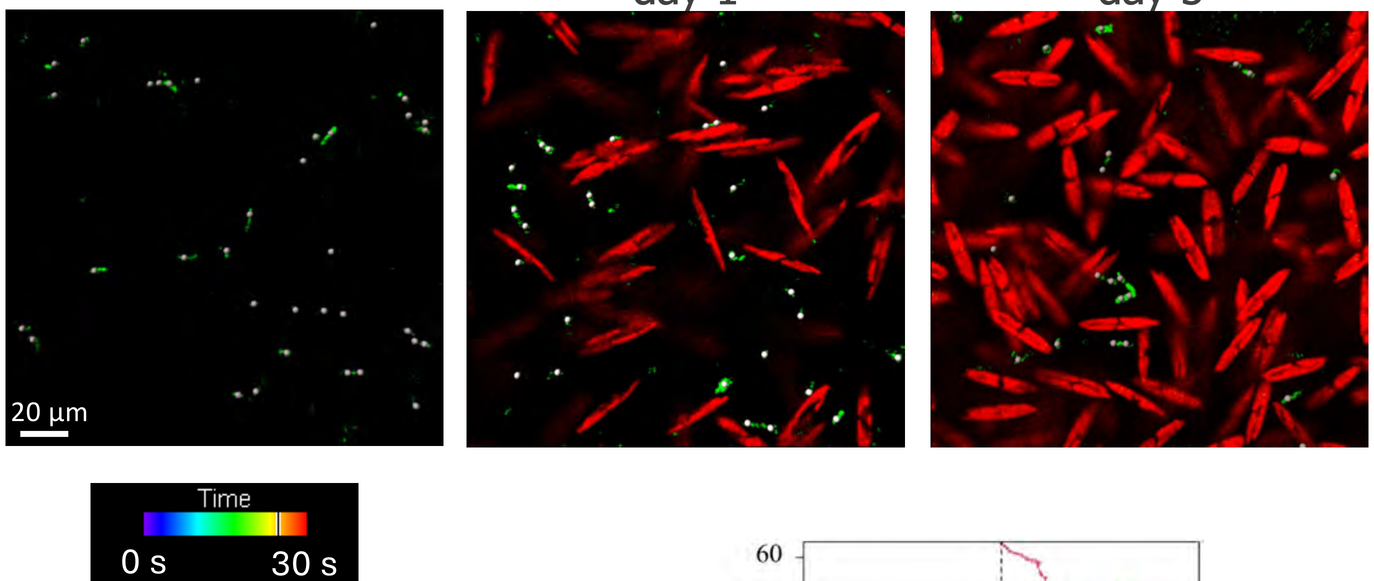

A natural biofilm is a microscopic jungle !

# *Bacillus thuringiensis* swimming in *Cylindroteca closterium* biofilm

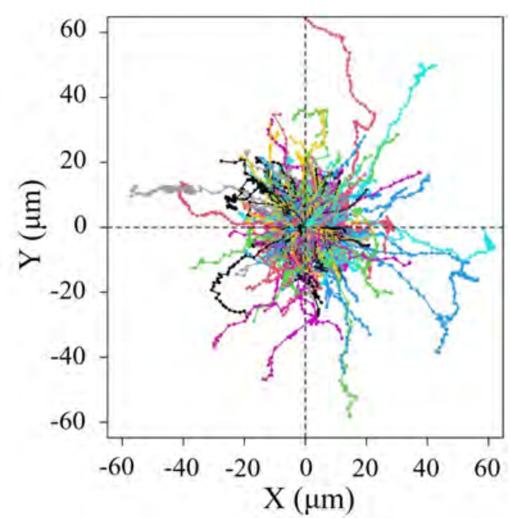
Control

Bt407-GFP
day 1

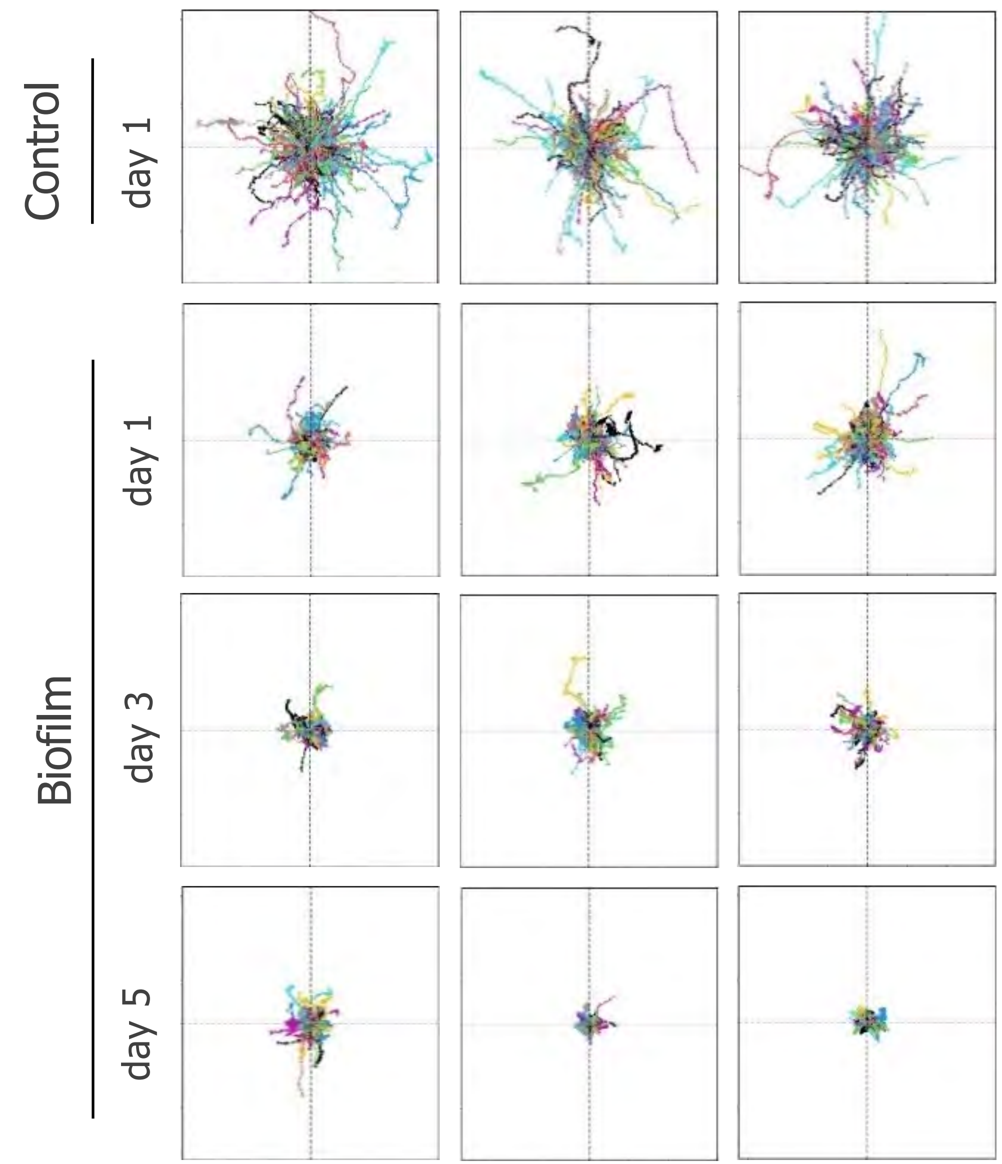
Bt407-GFP
day 5




Analysis of bacterial motility by trajectory spreading:

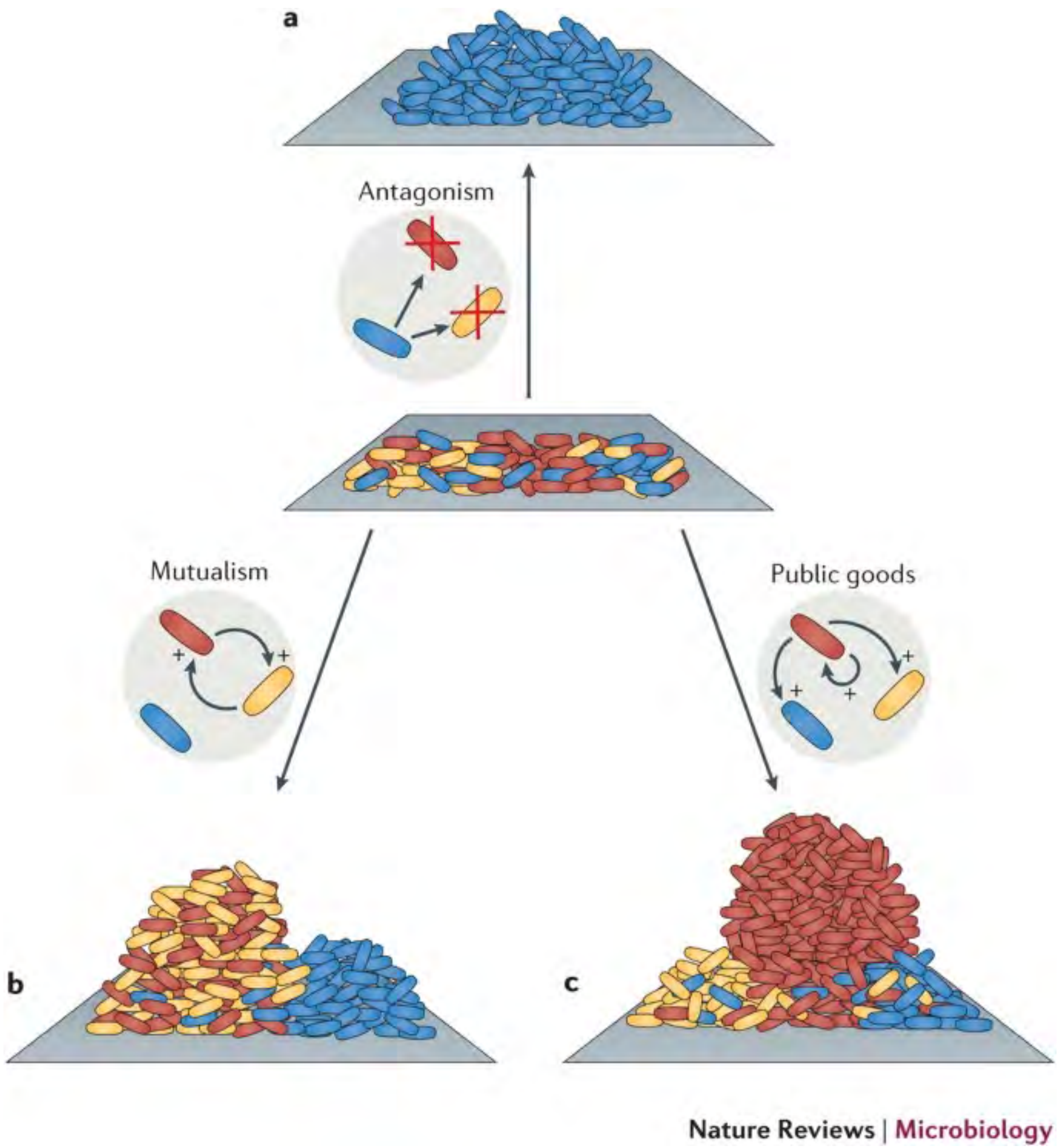


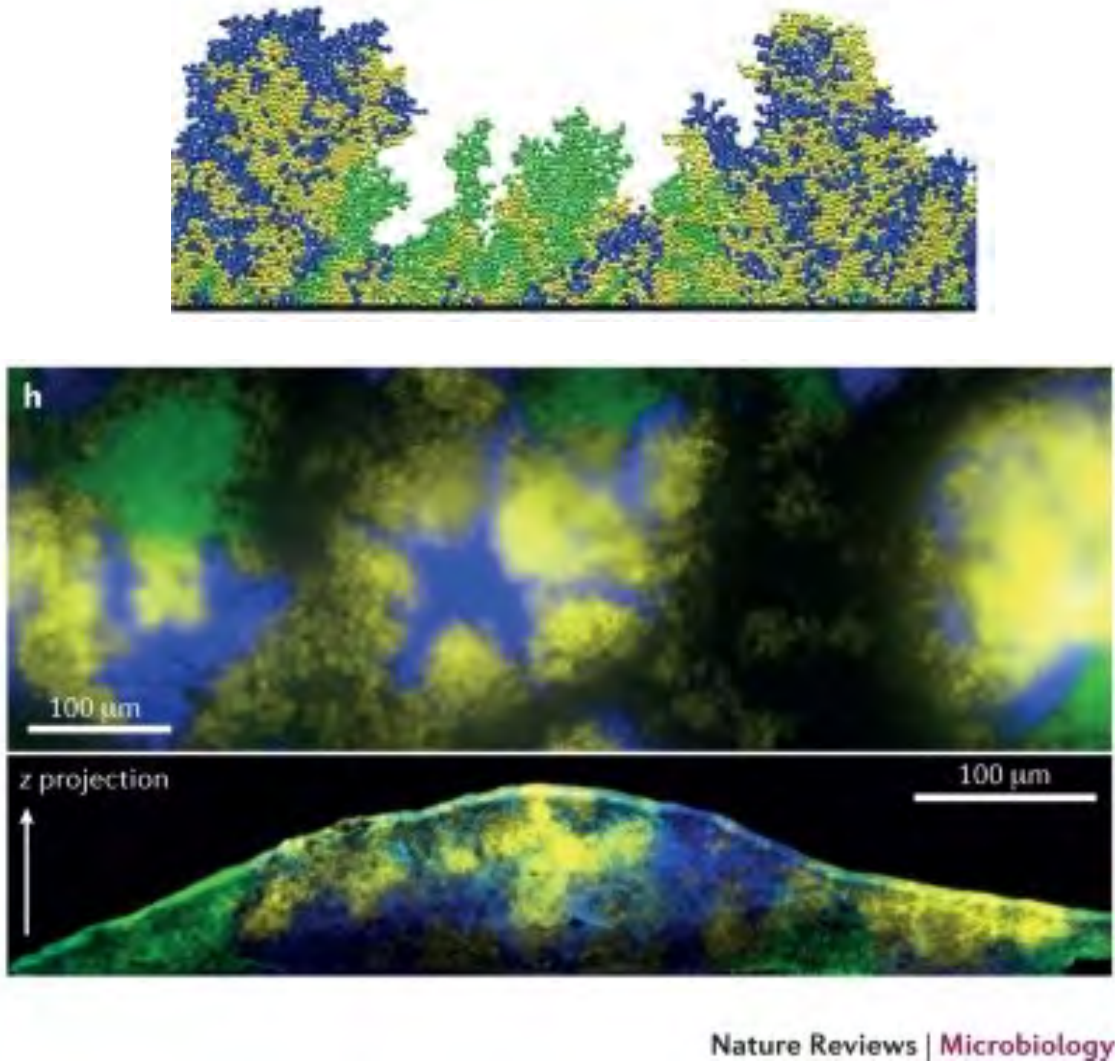

Trajectories at different time points :




*Manceau et al. In preparatio*

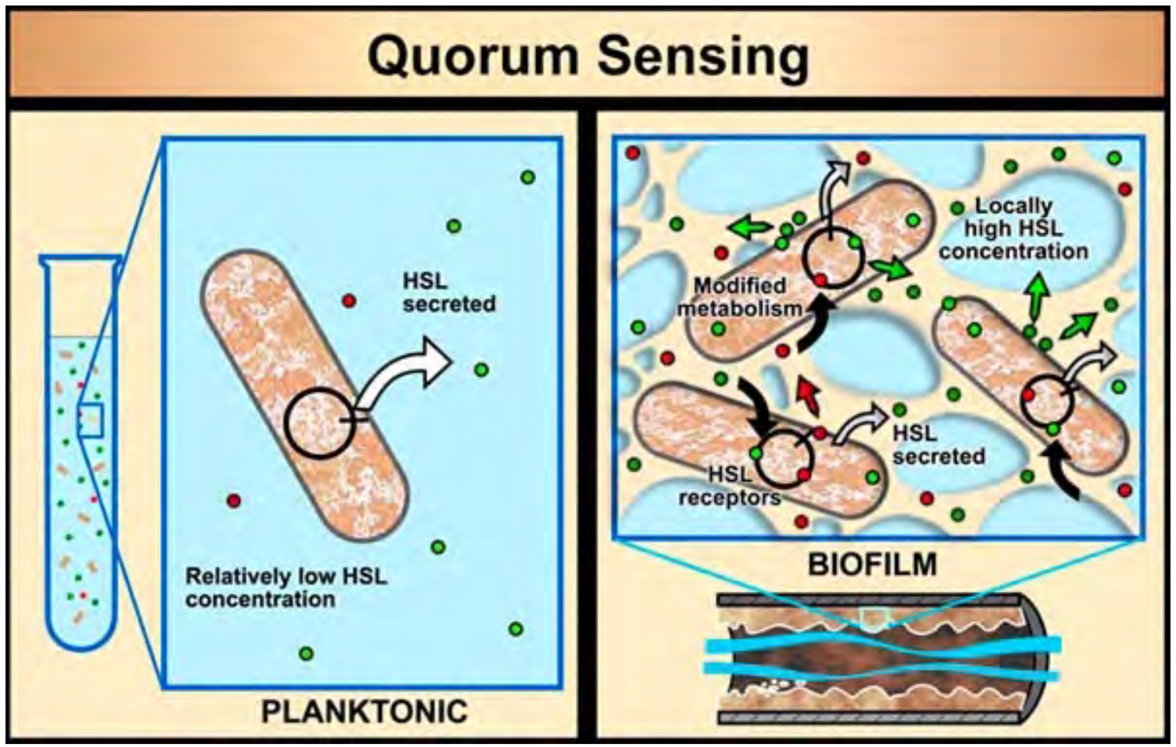
# Are biofilms cooperative communities or microbial battlefields?

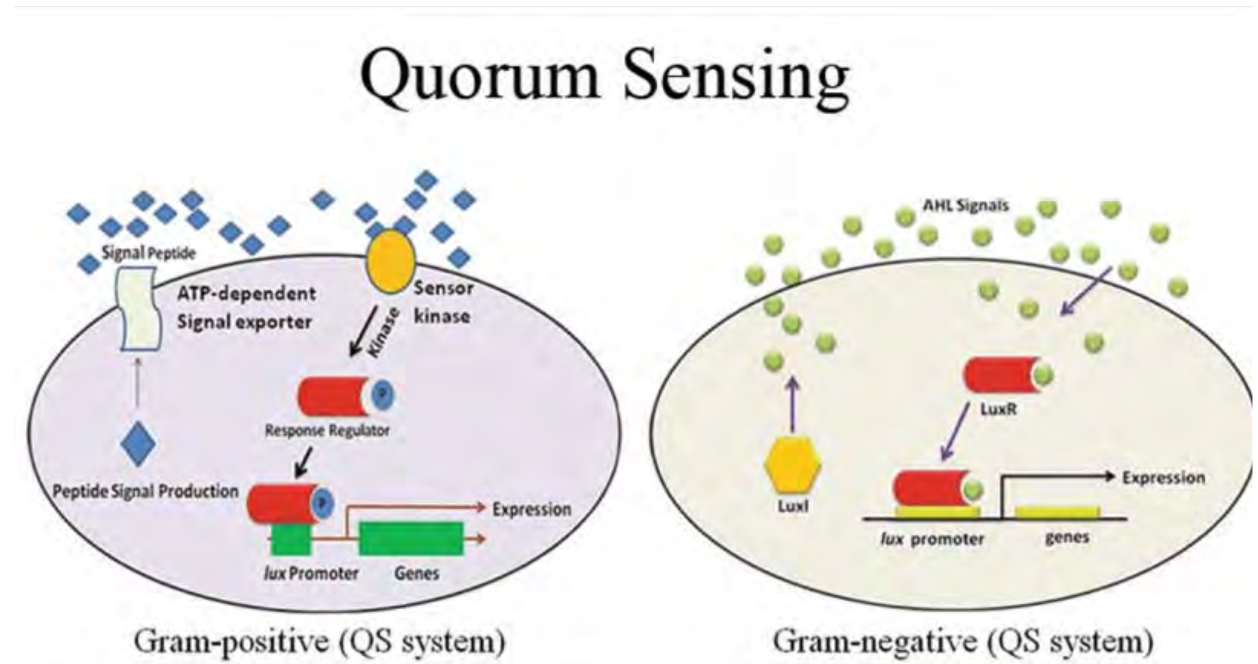


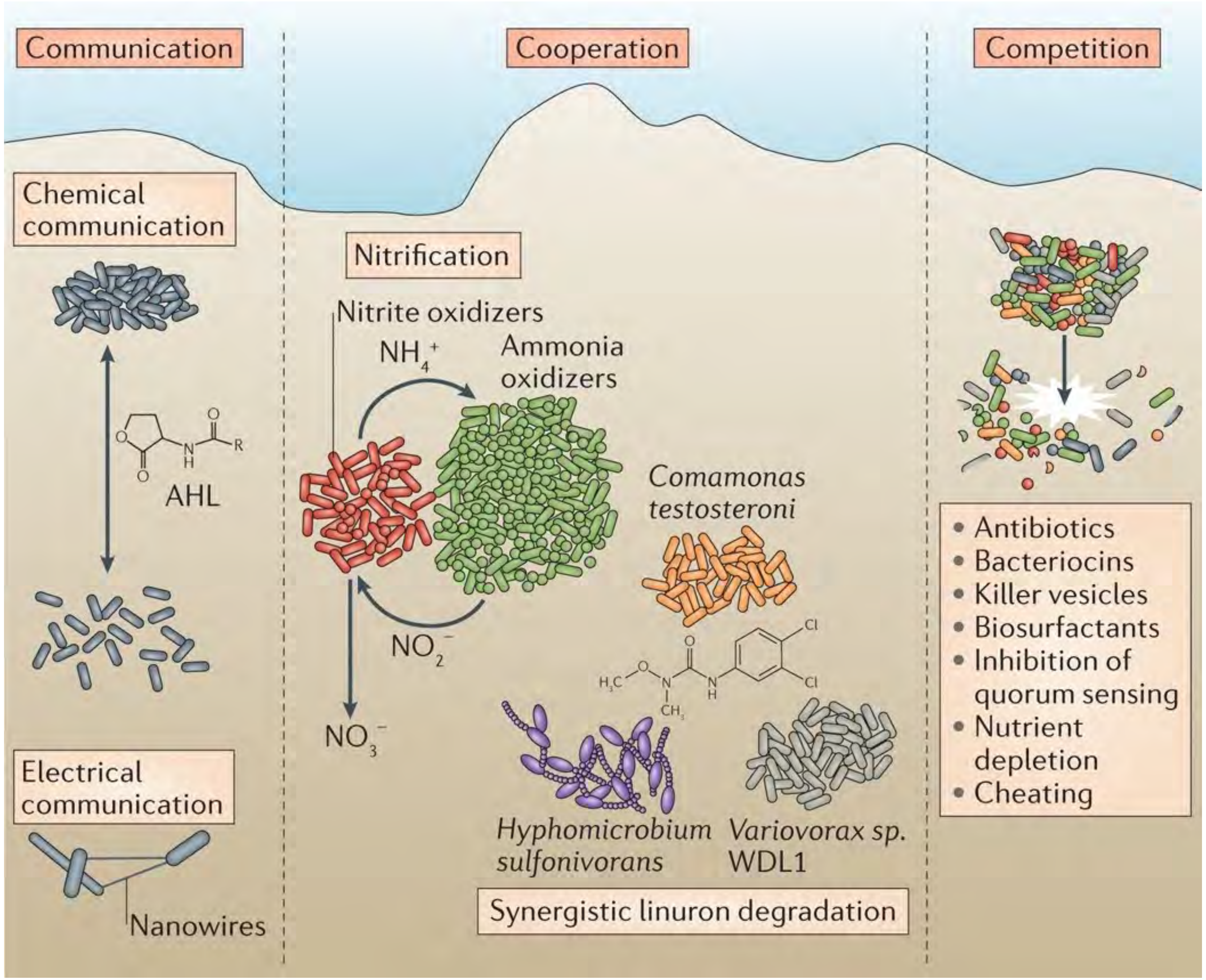

*Nadell et al., Nature Reviews Microbiology, 2016*

# Quorum sensing and regulation of biofilm formation



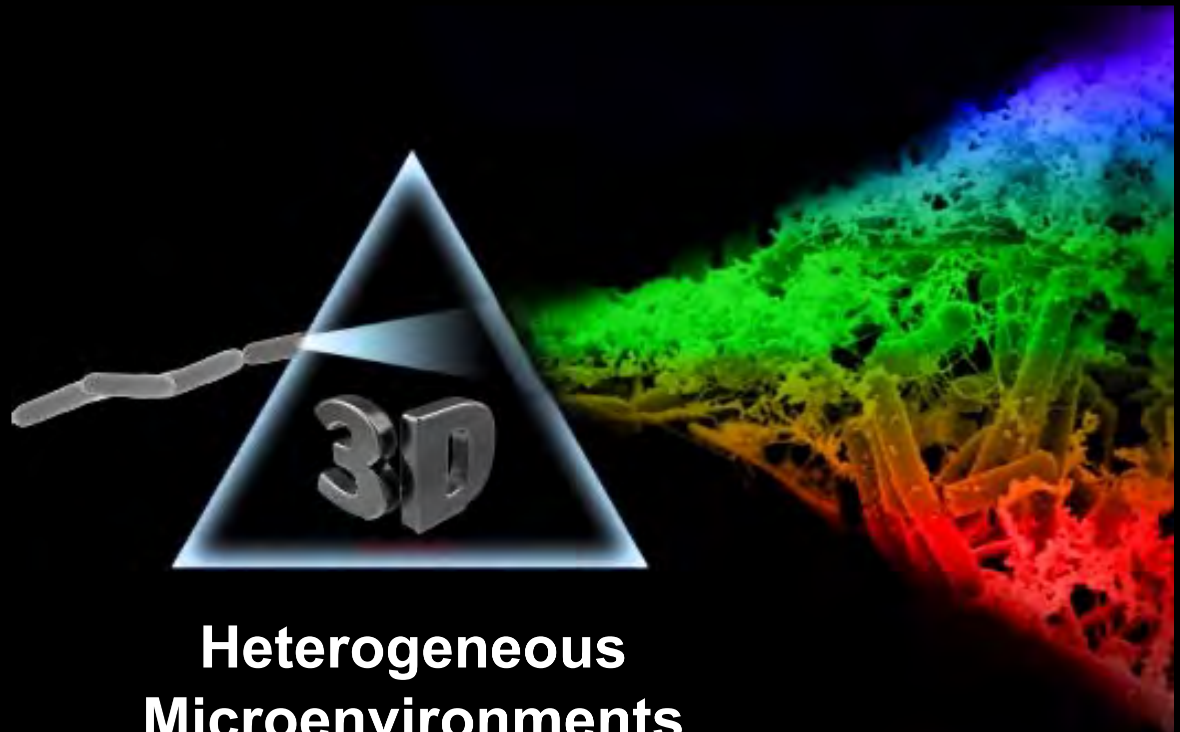



# Social interaction and dynamics remodling of biofilm comunity




*Flemming et al., Nature Reviews Microbiology, 2016*

## Biofilms & Spatially Organized Communities `




**Heterogeneous Microenvironments**

-gradients (gas, nutrients, inhibitors, QS)
-substratum microdomains
-background microbiota & predators

**Diversification of cell types**

-Patterns of gene expression, single-cell physiology
-Heterogeneity of cell behaviors (growth, survival, virulence, social phenotypes)

**>>Emerging community functions**