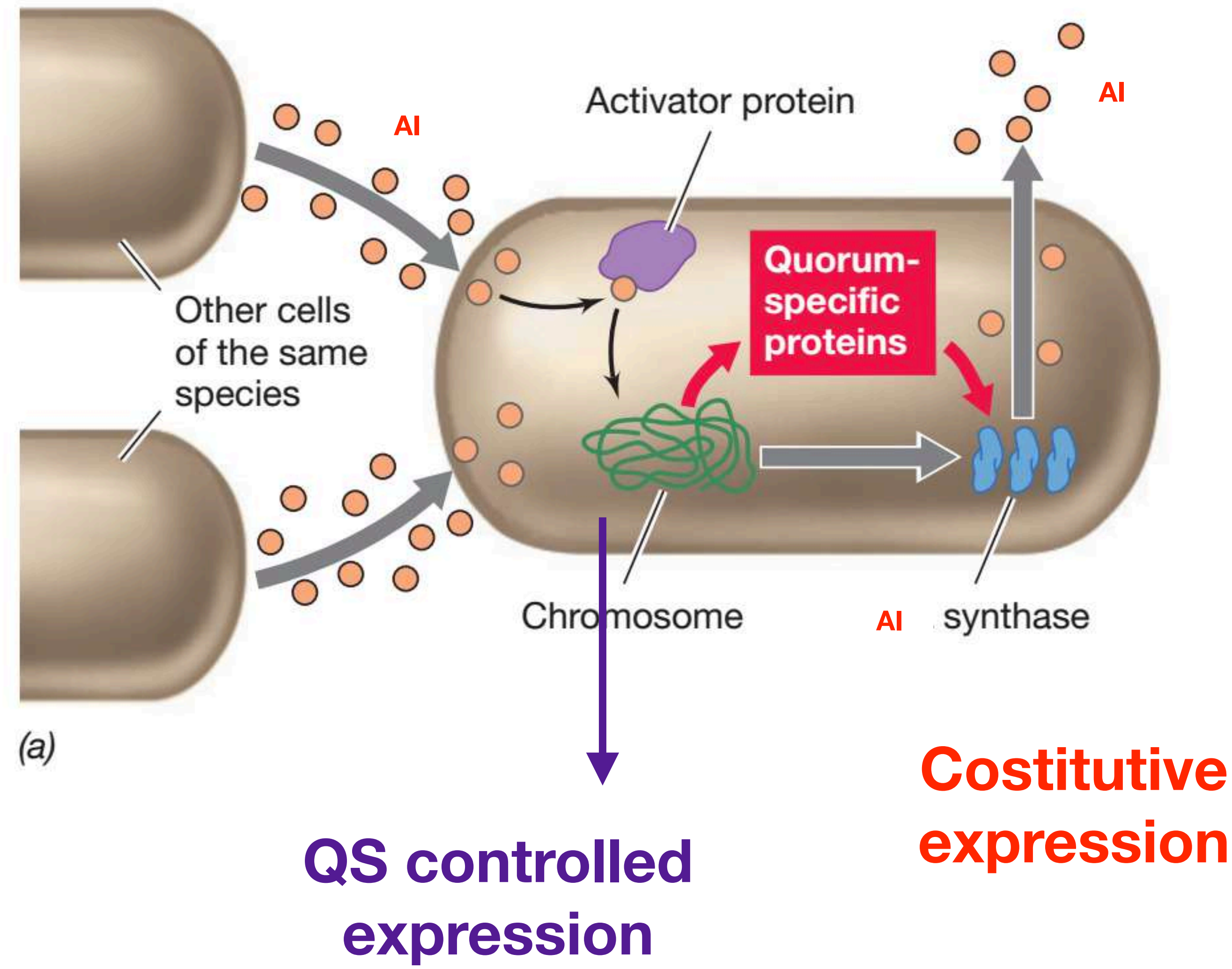
L07a

Recap

Microbial interactions: Quorum sensing, biofilm, symbioses

Quorum Sensing, I

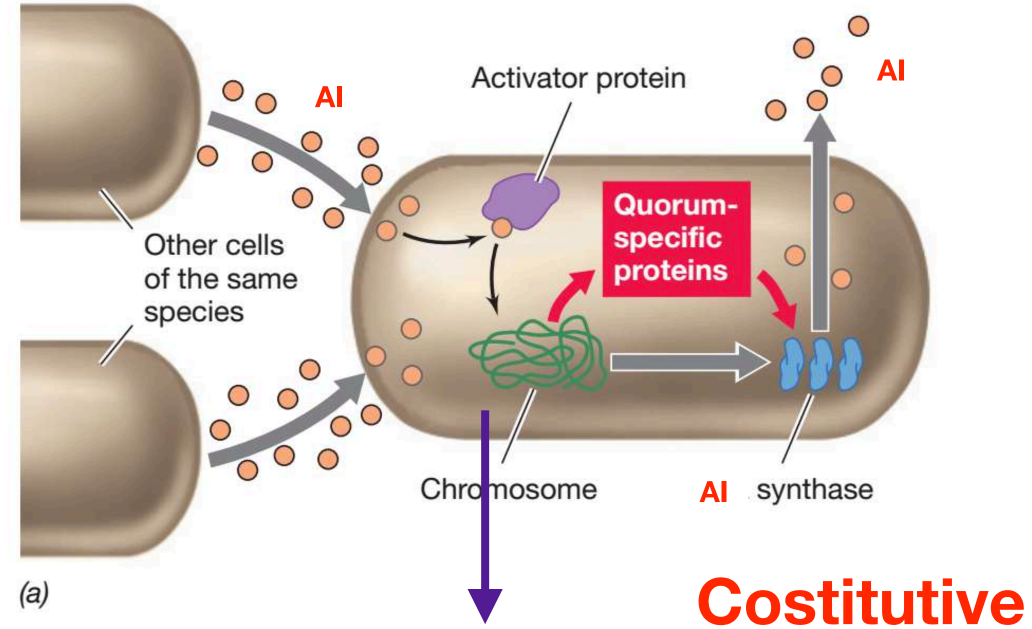
- Quorum sensing (QS) is a process of bacterial **cell-to-cell chemical communication**
- Production, detection, response to extracellular signalling molecules: **autoinducers (AIs)**
- Quorum sensing allows **groups of bacteria** to **synchronously alter behaviour** in response to changes in the population abundance and species composition of the vicinal community
- “Quorum” means “sufficient numbers”



Madigan et al. 2020

Quorum Sensing, II

- QS is **global regulatory control**
- QS present in Gram -, Gram + and Archaea
- Many Bacteria respond to the presence in their surroundings of other cells of their own species, and in some species, regulatory pathways are controlled by the cell abundance of their own kind
- QS is regulatory mechanism that assesses population abundance—> successful coordinate expression at population level **(not necessarily entire population)**



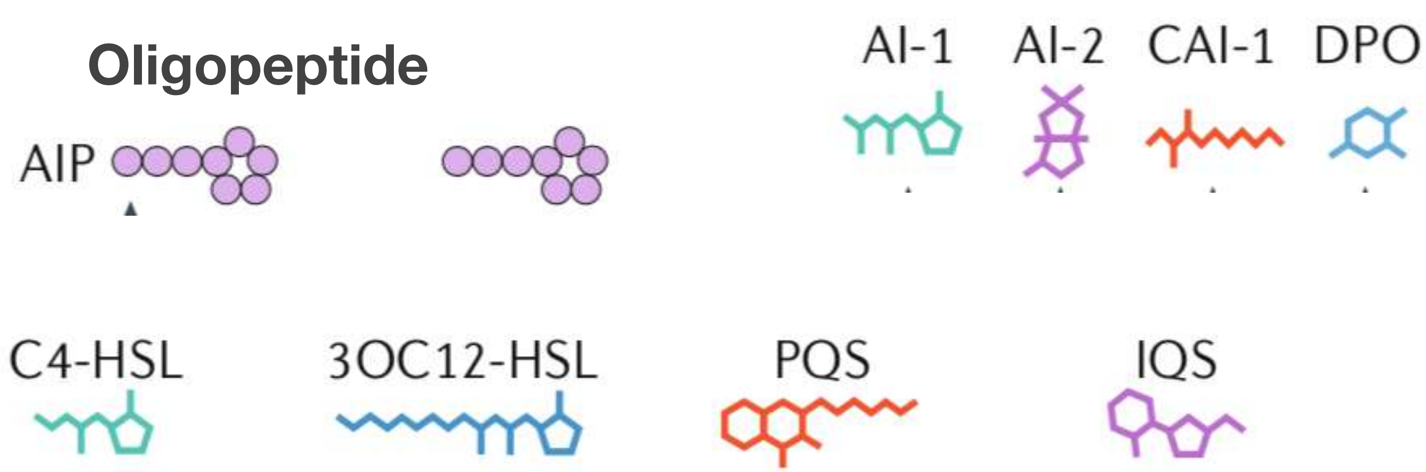
QS controlled expression

Costitutive expression

Madigan et al. 2020

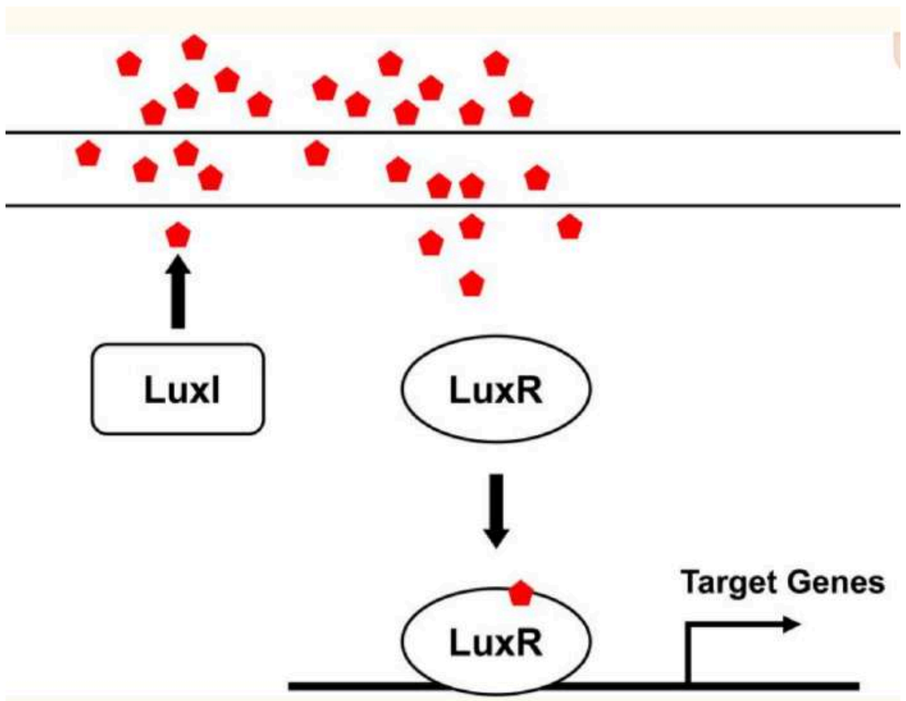
Quorum Sensing, III

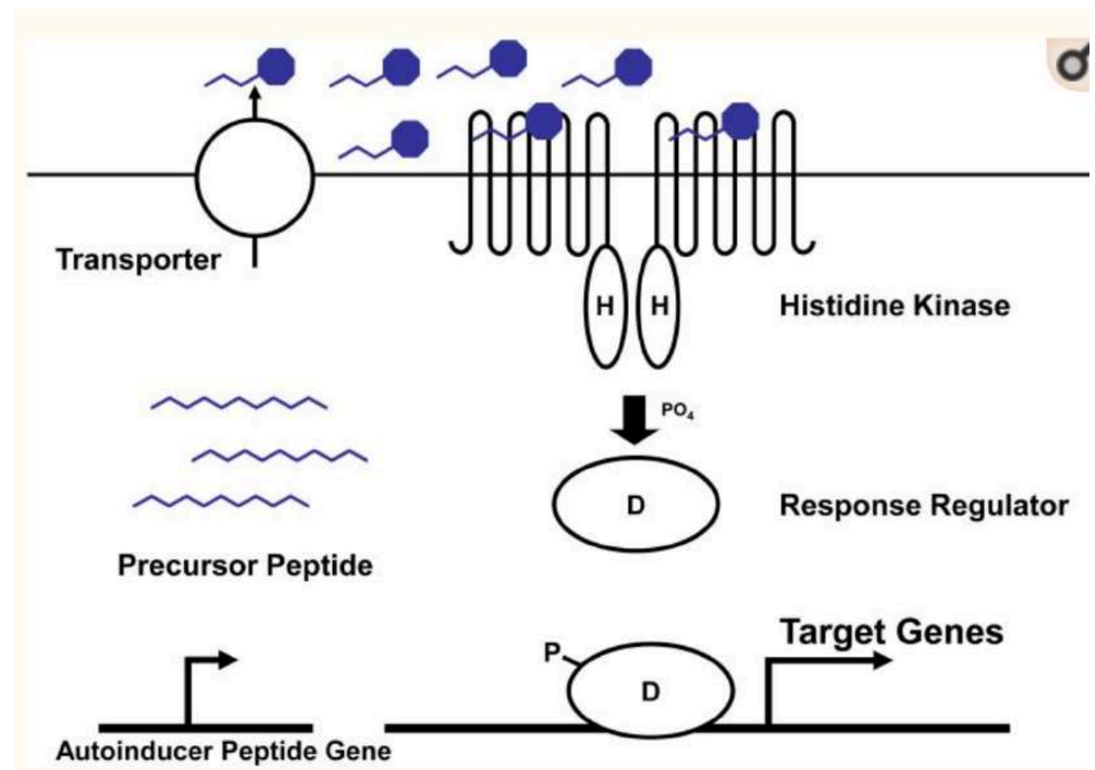
- Examples are: bioluminescence, virulence factor production, secondary metabolite production[7], competence for DNA uptake, biofilm formation, species composition
- Autoinducer (AI) is species specific and **freely diffuse in & out**
- **Diverse** chemical structure
- **Same bacterium can have diverse AIs**
- AI reaches high concentrations inside the cell only if many cells are nearby, each making same AI
- In cytoplasm, AI binds to a specific **transcriptional activator protein or a sensor kinase of a two-component system** —> triggering transcription of specific genes



Mukherjee & Bassler, 2019

Gram - & Gram +



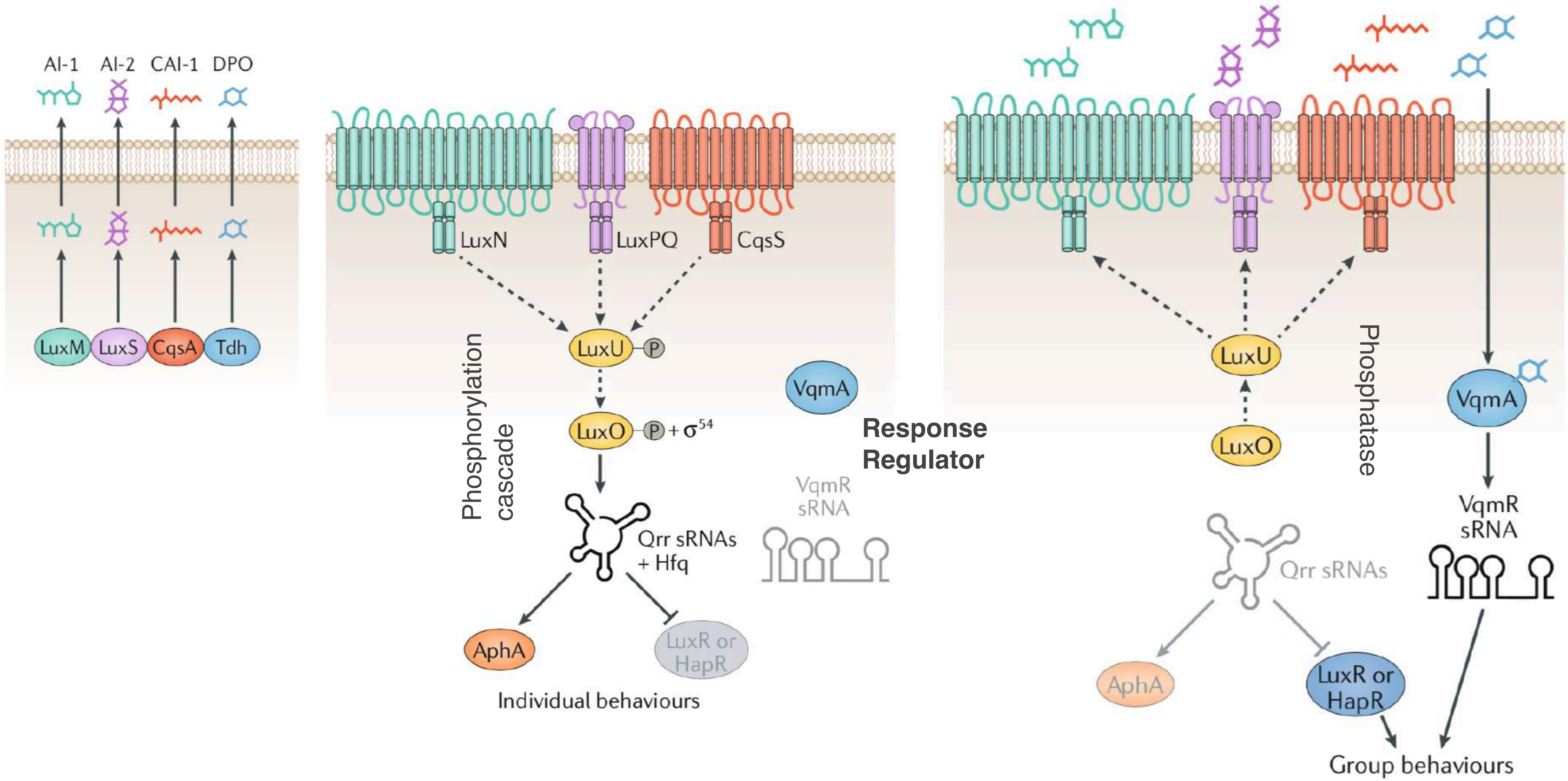


- LuxI is AI synthase
- LuxR is AI cytoplasmic receptor & **transcriptional activator** *luxICDABE* operon
- Gene transcription
- Induction of more AI production

- Peptide binding to membrane-bound receptor
- Autophosphorylation activity
- P to cognate response regulator (RR)
- RR —> DNA-binging factors
- Gene transcription
- Induction of more AI production

Ng & Bassler, 2009

Individual vs Group behavior



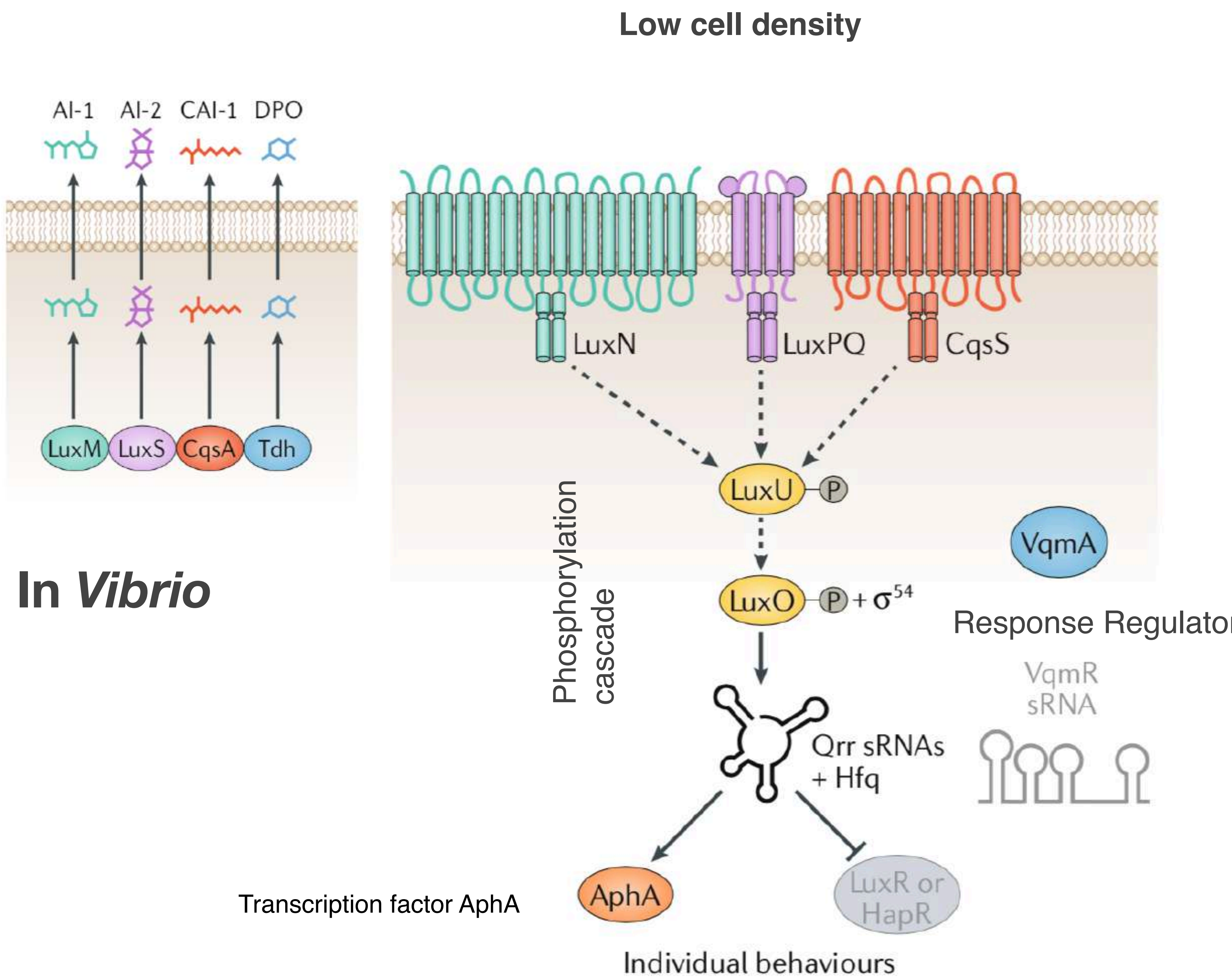
Low cell abundance

High cell abundance

Mukherjee & Bassler, 2019

Individual vs Group behavior

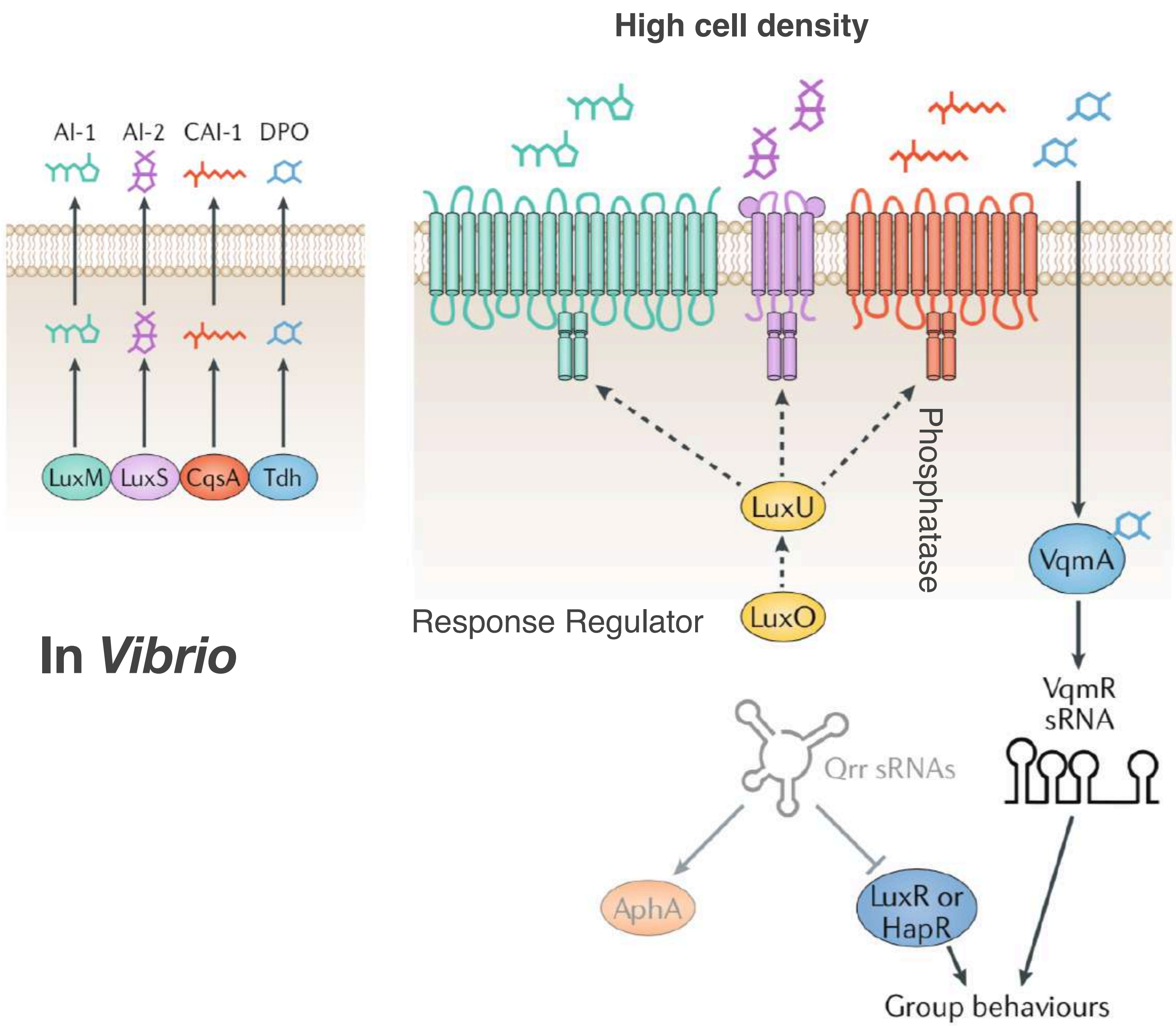
In *Vibrio*



- **At low cell density (LCD), the receptors are kinases that transfer phosphate to LuxO via LuxU**
- **Phospho-LuxO, with σ54, activates transcription of genes encoding regulatory small RNAs (sRNAs)**
- **The sRNAs, called Qrr1-5 (Quorum Regulatory RNA), with the Hfq chaperone, activate or repress translation of target mRNAs**
- **They activate and repress translation of the LCD and high cell density (HCD) master regulators, AphA and LuxR, respectively**

Mukherjee & Bassler, 2019

Individual vs Group behavior



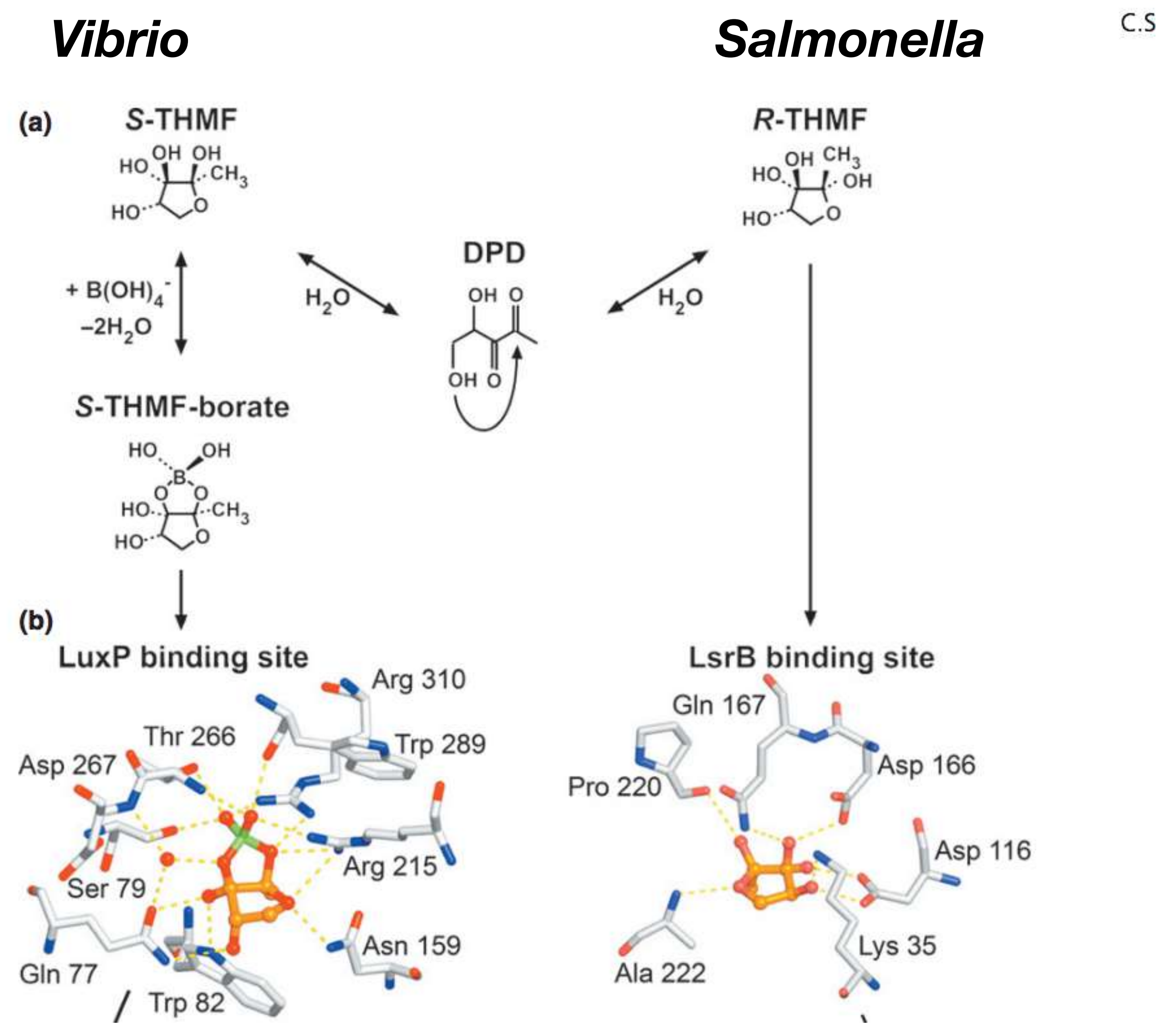
In *Vibrio*

- **At high cell density, AIs bind their receptors and phospho-flow through the circuit reverses**
- **AphA is no longer activated, and LuxR is no longer repressed**
- **AI-1, AI-2, CAI-1 and DPO, corresponding receptors function as phosphatase**
- **Instead of AphA, LuxR or HapR is produced, which mediates group behaviors**

Mukherjee & Bassler, 2019

INTRA-SPECIES COMMUNICATION

- Intra-species QS by AI-2
- LuxS product DPD spontaneously undergoes cyclization and hydration reactions in solution to form R-THMF (detected by *Salmonella Typhimurium*) and S-THMF
- S-THMF in presence of boron, reacts to form S-THMF-borate (detected by *Vibrio harveyi*)
- Regulates: motility, biofilm, virulence, siderophores, enzyme, bioluminescence



INTRA-INTER SPECIES COMMUNICATION

Table 1. Functions regulated by AI-2 signal*

Species	Functions regulated by AI-2	AI-2 receptor	References
Actinobacillus pleuropneumoniae	Biofilm formation†, adherence to host cells and growth in iron-limited medium	Unknown	Li *et al.* (2011)
Actinomyces naeslundii and *Streptococcus oralis*	Mutualistic biofilm formation	Unknown	Rickard *et al.* (2006)
Aggregatibacter actinomycetemcomitans	Biofilm formation	LsrB and RbsB	Shao *et al.* (2007a,b)
Bacillus cereus	Biofilm formation†	LsrB‡	Auger *et al.* (2006)
Borrelia burgdorferi	Increased expression of the outer surface lipoprotein VlsE†	Unknown	Babb *et al.* (2005)
Escherichia coli EHEC	Chemotaxis towards AI-2, motility and HeLa cell attachment	LsrB‡	Bansal *et al.* (2008)
Escherichia coli K12	Biofilm formation and motility† AI-2 incorporation and chemotaxis towards AI-2	LsrB‡ LsrB	Xavier & Bassler (2005a), Gonzalez Barrios *et al.* (2006), Hegde *et al.* (2011)
Haemophilus influenzae strain 86-028NP	AI-2 incorporation and biofilm formation	RbsB	Armbruster *et al.* (2011)
Helicobacter pylori	Motility	Unknown	Rader *et al.* (2007), Shen *et al.* (2010), Rader *et al.* (2011)
Moraxella catarrhalis	Biofilm formation and antibiotic resistance†	Unknown	Armbruster *et al.* (2010)
Mycobacterium avium	Biofilm formation†	Unknown	Geier *et al.* (2008)
Pseudomonas aeruginosa	Virulence factor production	Unknown	Duan *et al.* (2003)

Pereira et al., 2012

INTRA-INTER SPECIES COMMUNICATION

Salmonella enterica ssp. *enterica* serovar Typhimurium	Pathogenicity island 1 gene expression and invasion into eukaryotic cells	LsrB‡	Taga *et al.* (2001, 2003), Miller *et al.* (2004), Choi *et al.* (2007, 2012)
	AI-2 incorporation	LsrB	
Sinorhizobium meliloti	AI-2 incorporation	LsrB	Pereira *et al.* (2008)
Staphyloccocus aureus	Capsular polysaccharide gene expression and survival rate in human blood and macrophages	Unknown	Zhao *et al.* (2010)
Staphylococcus epidermidis	Expression of phenol-soluble modulin peptides, acetoin dehydrogenase, gluconokinase, bacterial apoptosis protein LrgB, nitrite extrusion protein and fructose PTS system subunit	Unknown	Li *et al.* (2008)
Streptococcus anginosus	Susceptibility to antibiotics	Unknown	Ahmed *et al.* (2007)
Streptococcus intermedius	Haemolytic activity, biofilm formation and susceptibility to antibiotics	Unknown	Ahmed *et al.* (2008, 2009)
Streptococcus gordonii	Biofilm formation	Unknown	Saenz *et al.* (2012)
Streptococcus gordonii and *Streptococcus oralis*	Mutualistic biofilm formation	Unknown	Saenz *et al.* (2012)
Streptococcus pneumoniae	Biofilm formation	Unknown	Vidal *et al.* (2011)

Pereira et al., 2012

INTRA-INTER SPECIES COMMUNICATION

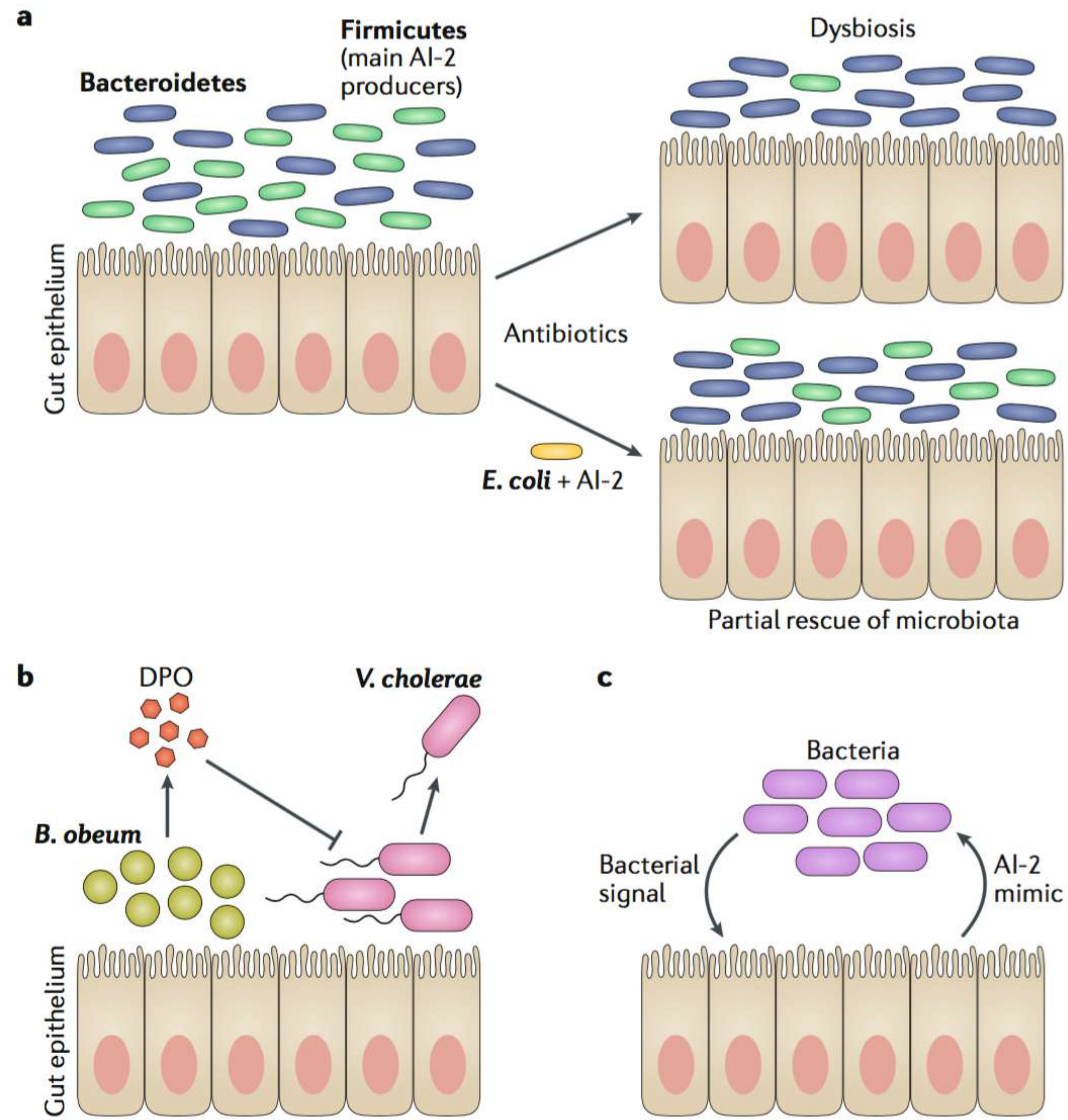
Table 1. Continued

Species	Functions regulated by AI-2	AI-2 receptor	References
Vibrio cholerae	Biofilms, protease and virulence factor production, and competence	LuxP	Jobling & Holmes (1997), Miller *et al.* (2002), Zhu *et al.* (2002), Hammer & Bassler (2003), Antonova & Hammer (2011)
Vibrio harveyi	Bioluminescence, colony morphology, siderophore production, biofilm formation, type III secretion and metalloprotease production	LuxP	Bassler *et al.* (1993, 1994), Lilley & Bassler (2000), Chen *et al.* (2002), Mok *et al.* (2003), Henke & Bassler (2004a, b), Waters & Bassler (2006)

Pereira et al., 2012

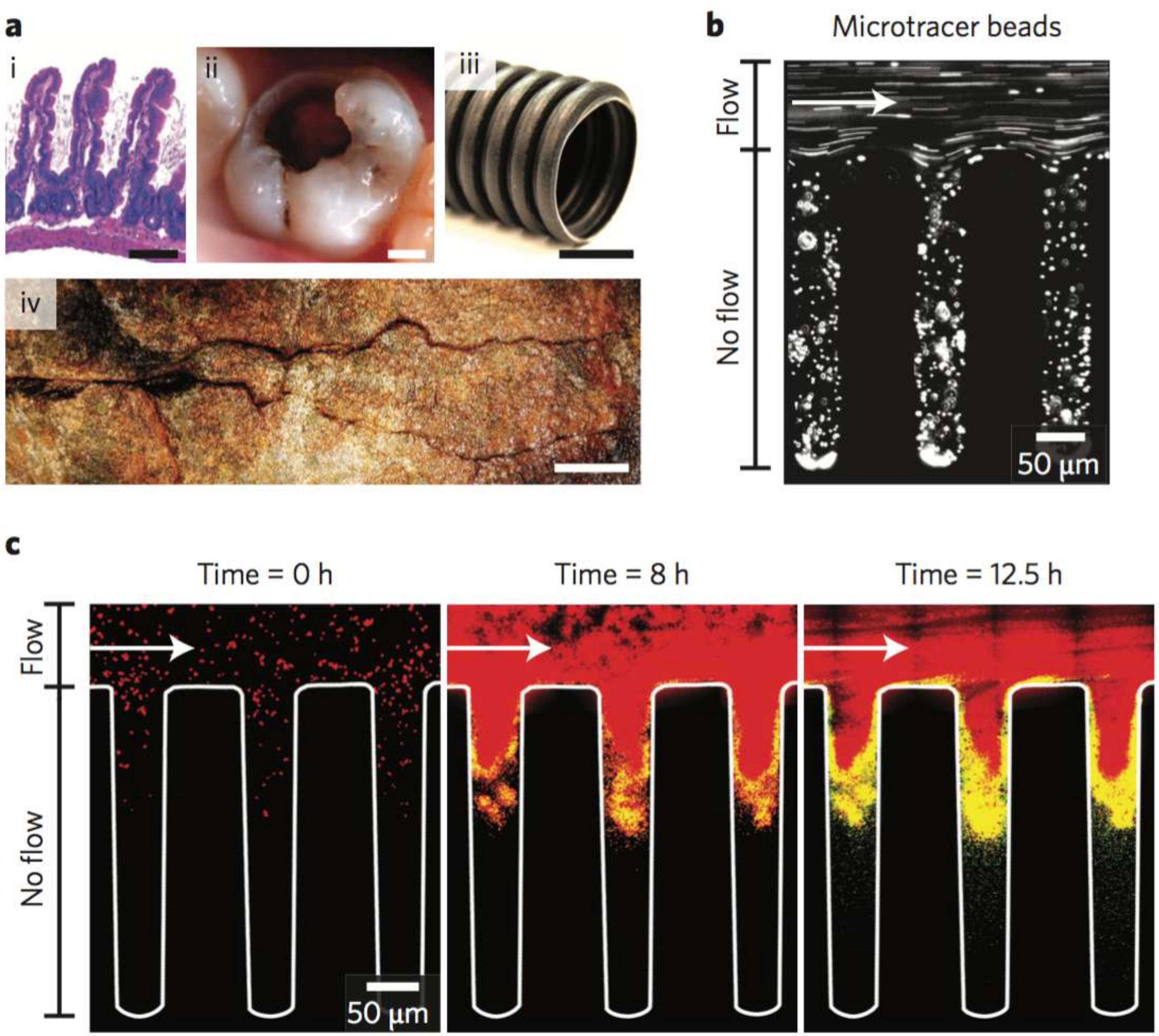
QS and the host microbiota

- Quorum sensing can control the species composition of the gut microbiota
- Disruption of the normal microbiota composition by antibiotic treatment leads to a reduction in AI-2-producing bacteria (and AI-2 levels), resulting in dysbiosis.
- Gut commmensal bacterium *Blautia obeum* can produce the DPO autoinducer, and DPO is speculated to inhibit colonization by *Vibrio cholerae*, possibly providing protection against this pathogen
- Communication between mammalian epithelial cells and bacteria: epithelial cells release an AI-2 mimic in response to bacteria, and this AI-2 mimic is detected by bacterial colonizers —> modulation bacterial quorum sensing



Mukherjee & Bassler, 2019

QS in the microenvironment



Kim et al., 2016

Flow networks with crevices or pores: the small intestine of mice (image courtesy of A. Ismail) (i), tooth cavities (image courtesy of W. Lee) (ii), corrugated industrial pipes (iii) and cracks in rocks (iv)

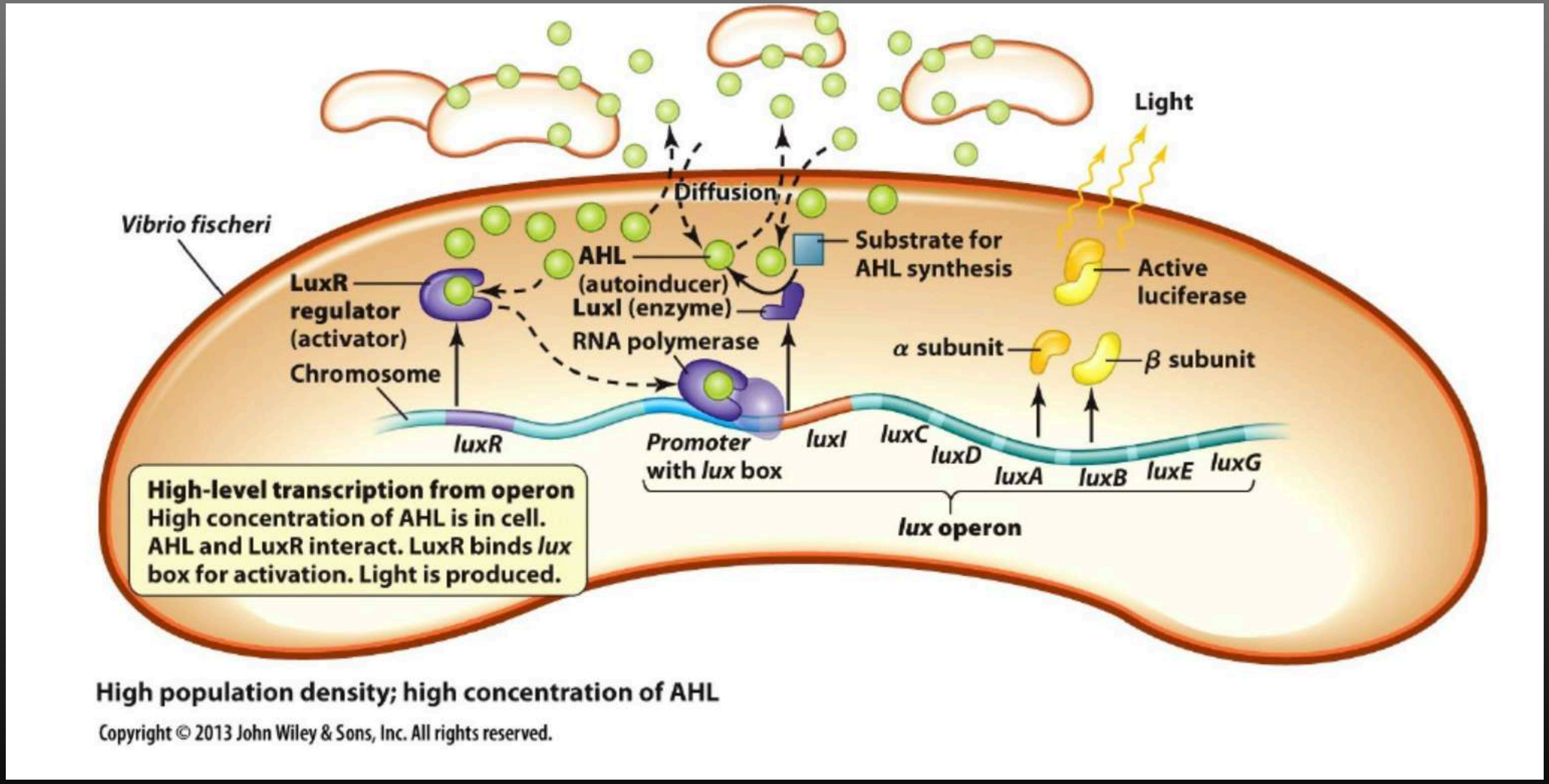
Scale bars, 120 µm, 10 mm, 2 cm and 5 cm

- **Residence time of AIs is key for QS**
- **Flow conditions interferes with QS —> washing AI**
- **Biofilm vs free-living microbes**
- **Other microbes can respond/ produce INTRA-SPECIES AIs**
- **Host can produce AIs**

Staphylococcus aureus: Red, QS-off cells (costitutive plasmid), Yellow, QS-on cells (QS control plasmid)

lux operon in *Aliivibrio fischeri*
(old name *Vibrio fischeri)*

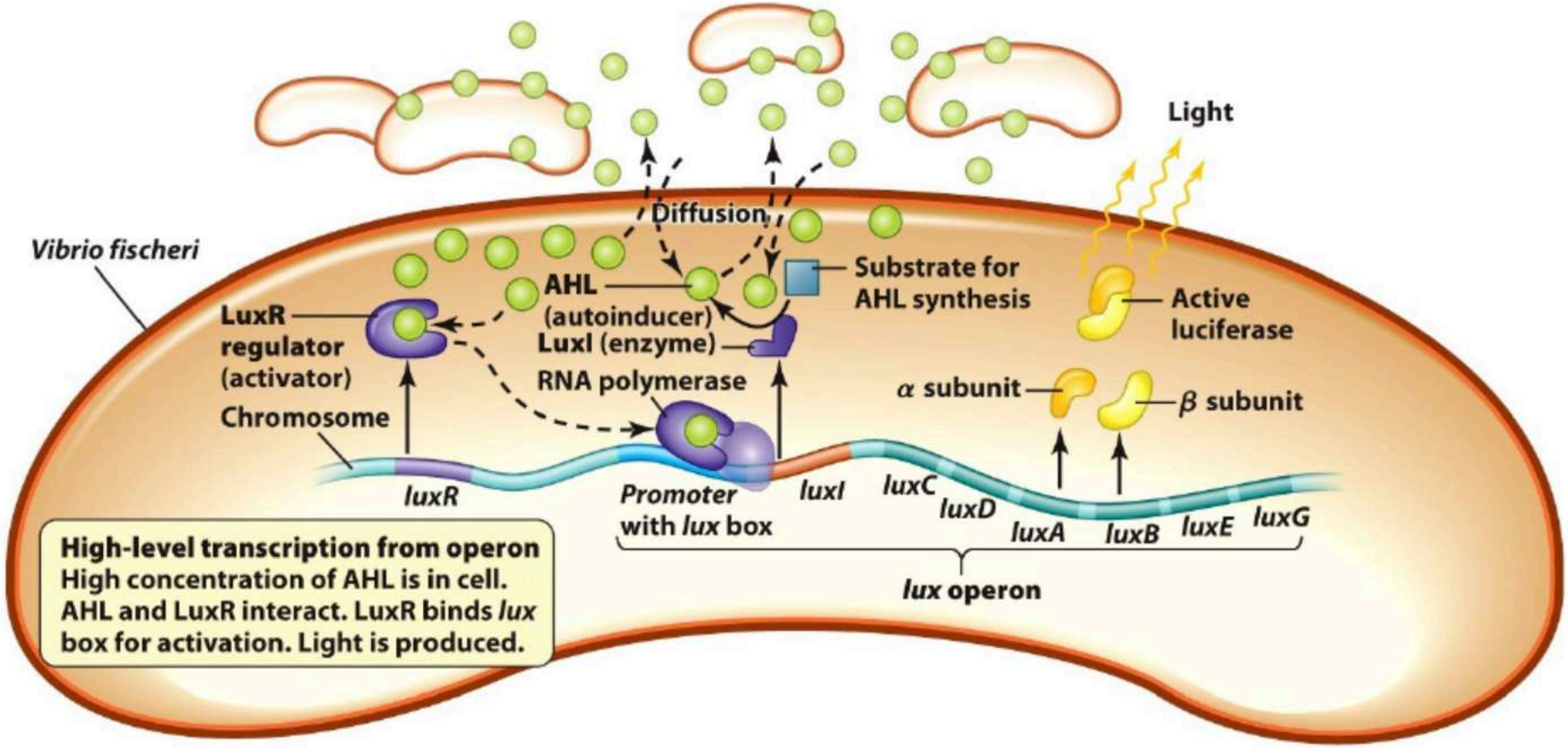
- Acyl homoserine lactones (AHLs)—> light emission in the bobtail squid by *Aliivibrio fischeri* (old name *Vibrio fischeri)*
- In the light organ of its symbiotic host squid *Euprymna scolopes, Aliivibrio fischeri* may attain 10^9–10^{10} cells/cm^3 and a single cell may emit ~10^3 photons/s
- Light production by luciferase that is encoded by lux operon



TODD BRETL UNDERWATER PHOTOGRAPHY

lux operon in *Aliivibrio fischeri*

(old name *Vibrio fischeri*)

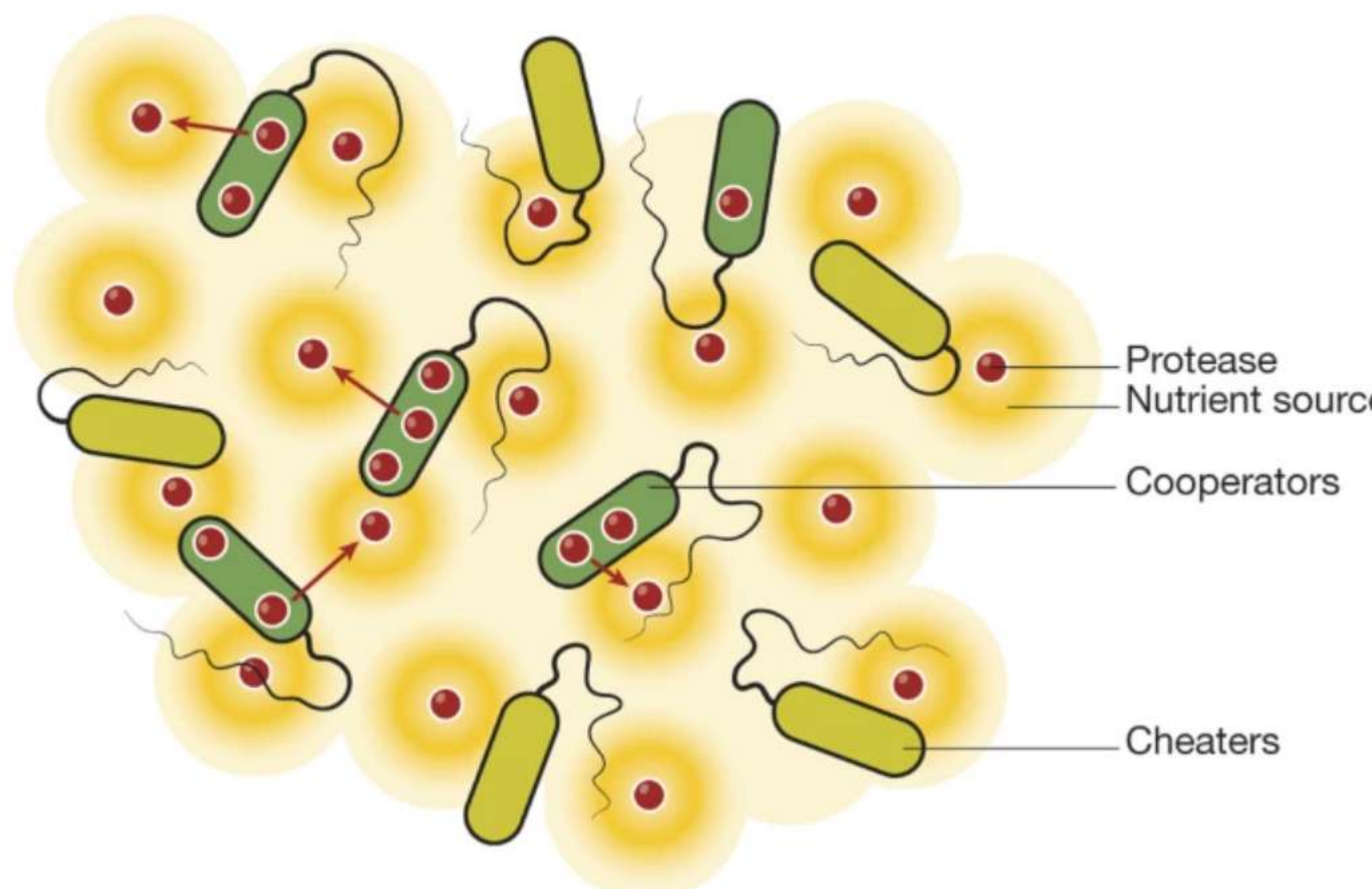


High population density; high concentration of AHL

Social cheating & Social policy, I

- **Mutants** evolve naturally within communities —> social **cheating not sharing energetic costs of producing molecules**
- **Cooperative behaviors provide a collective benefit**, but are considered **costly** for the **individual**
- Bacteria frequently secrete **extracellular biomolecules** to capture nutrients from the environment, hydrolyze solid nutrient sources, construct biofilm communities
- **Some secreted substances** can be **used by non producing** cells and are thus considered to be **public goods**

Figure 2: Social cheating in QS populations.

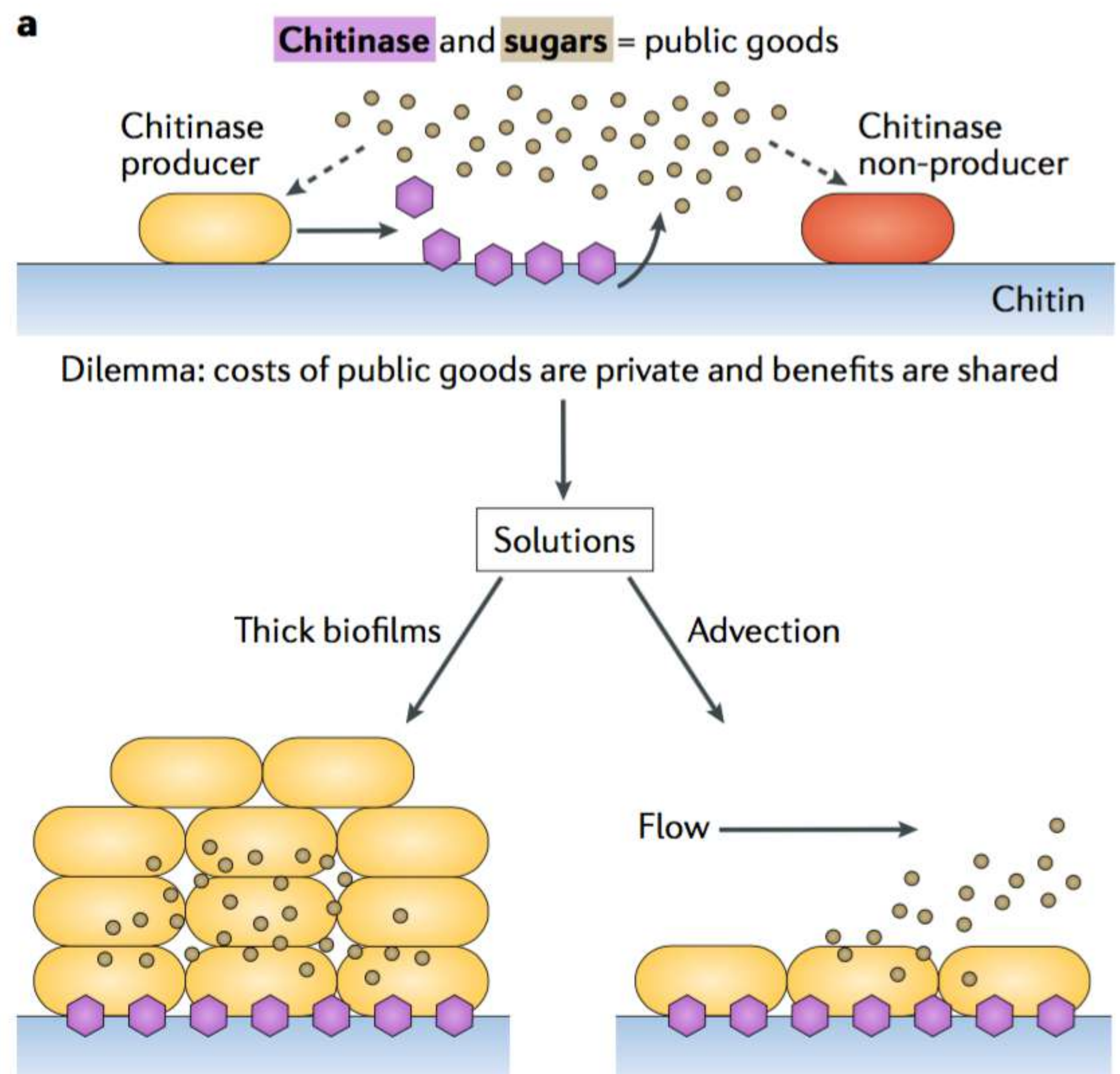


Whiteley et al., 2017

Social cheating & Social policy, II

- **Quorum-sensing-driven co-regulation of two metabolic enzymes**, one that serves as a **public good** and one that serves as a **private good**, can provide an **incentive that reduces social cheating** and prevents the collapse of the wild-type population (not favorable as earlier)
- Social policing: A strategy in which quorum-sensing bacteria **link production** of **costly private goods** to production of public goods **to punish** non-producers and thereby prevent emergence of social cheater (part of the metabolic pathway is under QS)

Vibrio cholerae



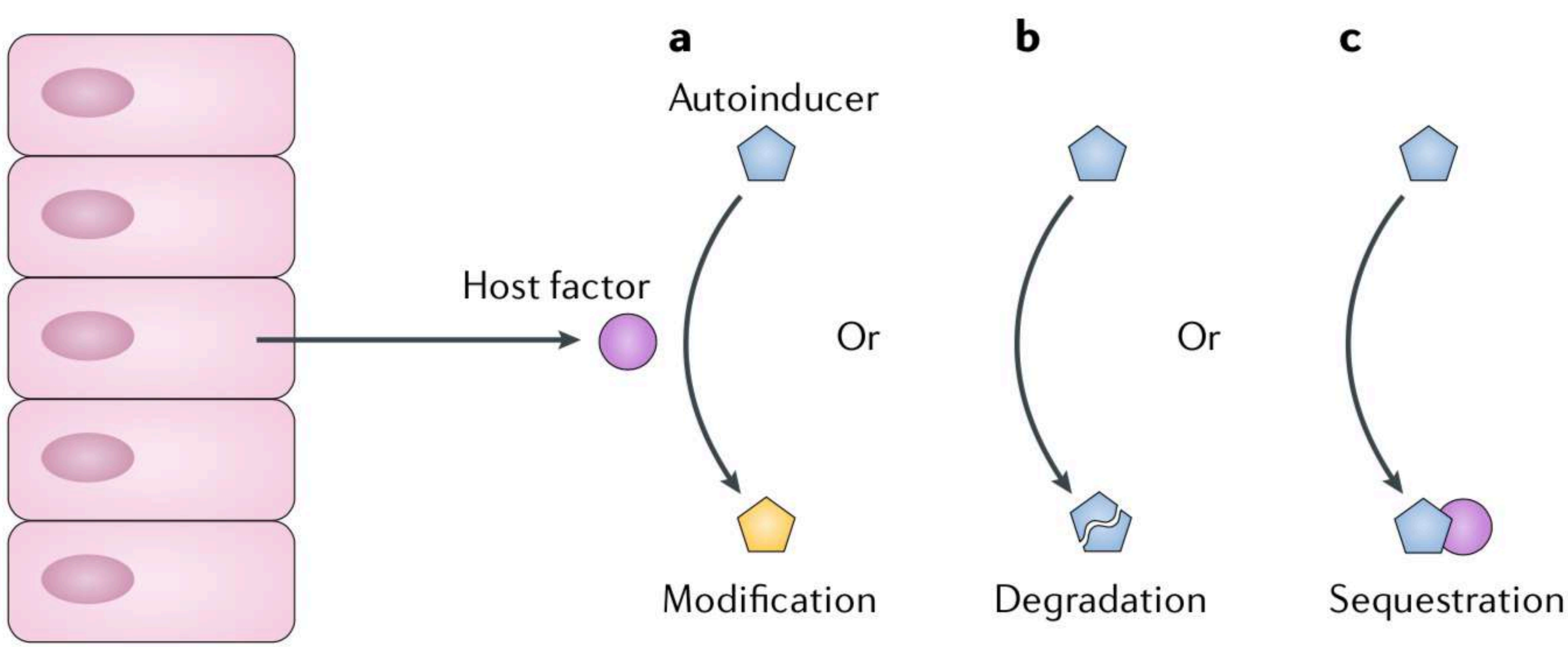
Mukherjee & Bassler, 2019

Quorum Quenching

- Quenching: host strategy to avoid bacterial infection
- **Silencing the communication by chemical interference**
- Eukaryotic quorum-quenching mechanisms include:

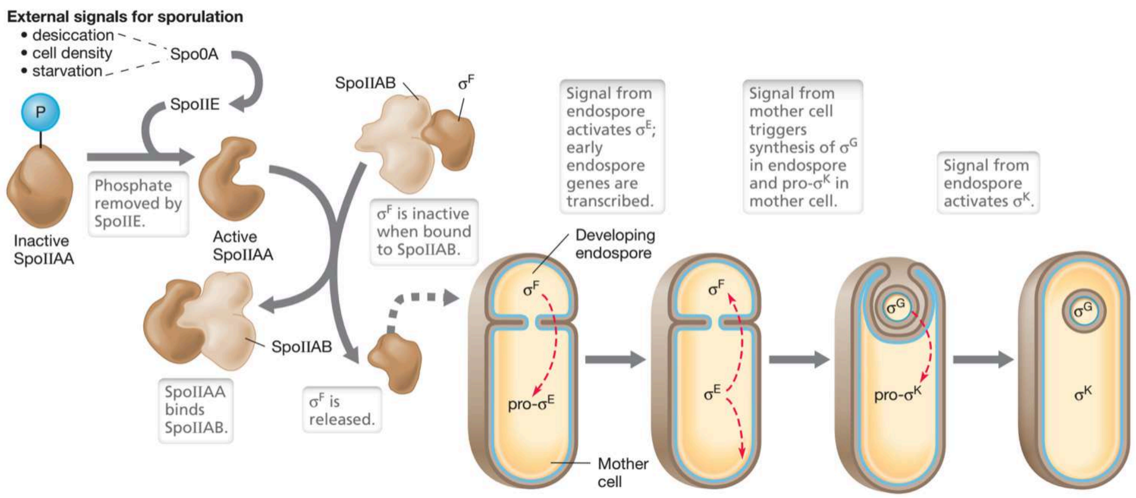
A. Production of halogenated furanones by the red algae *Delisea pulchra* that function as QS-receptor antagonists

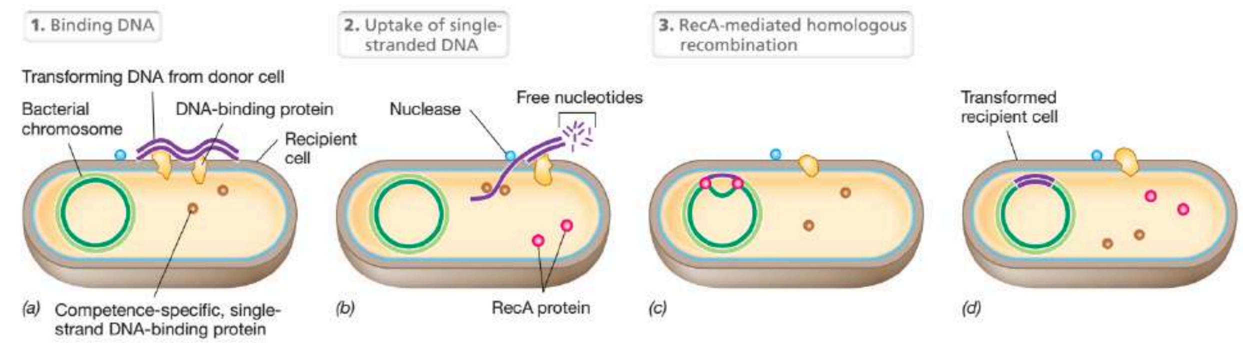
B. Mammalian-produced paraoxonases that function as lactonases that hydrolyse AI



Mukherjee & Bassler, 2019

Quorum sensing *in Gram +*



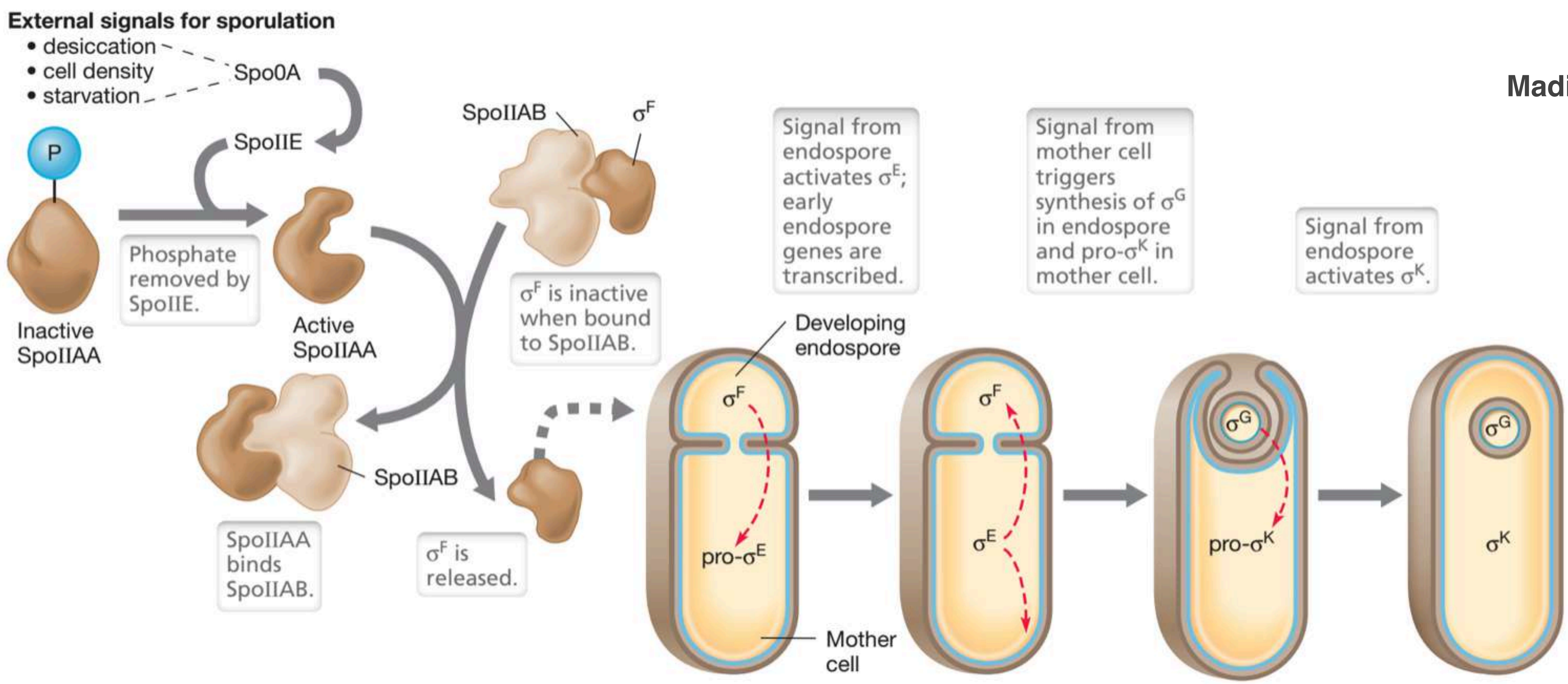


Madigan et al. 2020

- QS two-component system
- Sporulation —> Endospore formation as **response to adverse conditions** (starvation, desiccation, growth-inhibitory temperatures)
- DNA competence
- Regulation of pathogenicity
- Pheromones ComX, competence
- Pheromones CSF, sporulation

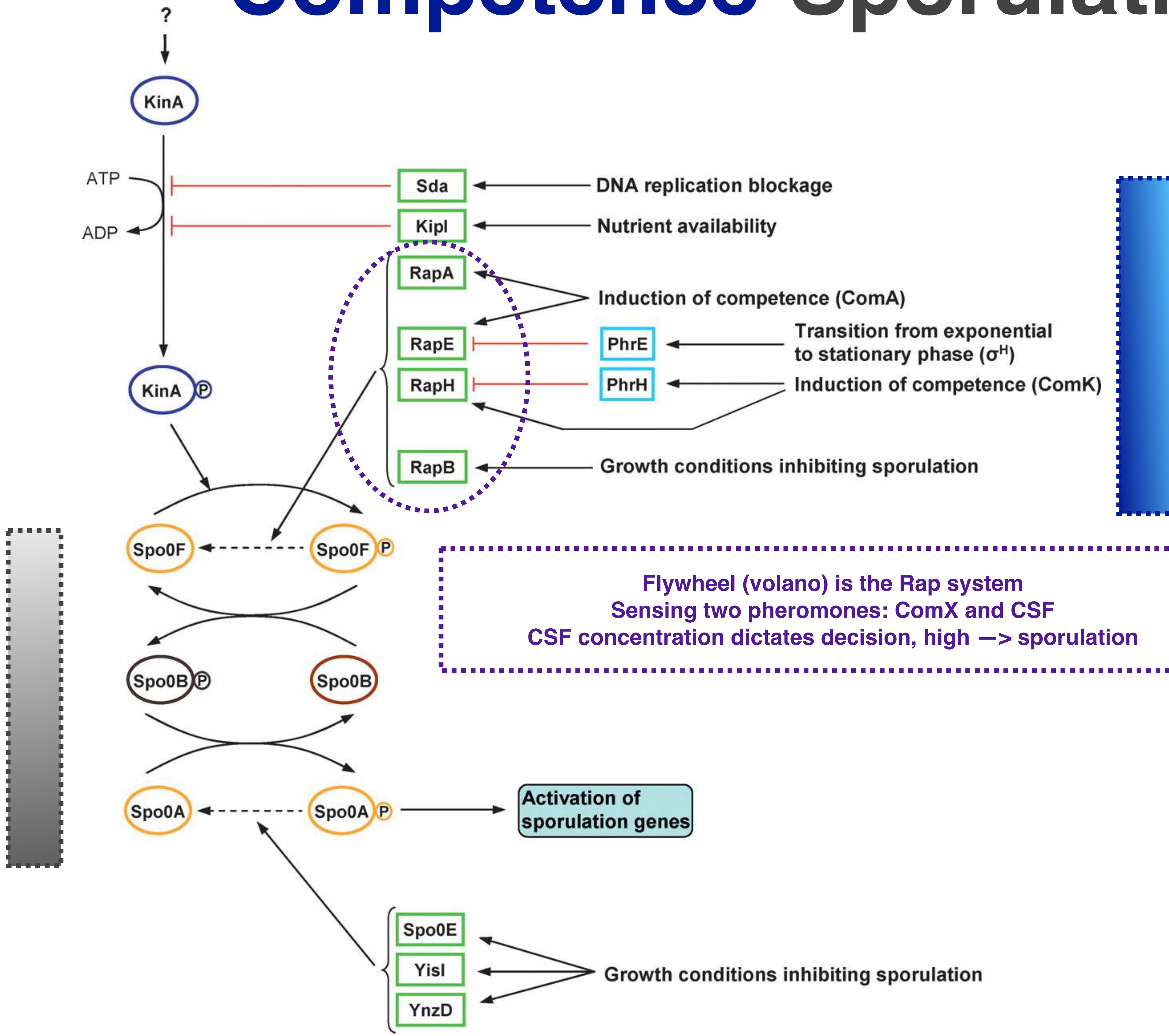
Bacillus subtilis: Sporulation

Madigan et al. 2020



- Endospore formation as **response to adverse conditions** (starvation, desiccation, growth-inhibitory temperatures)
- Spore germinates when favorable conditions return
- Prior to endospore formation, **cell divides asymmetrically**—> smaller cell develops into the endospore
- Mother cell surrounds spore, bursts in the end
- Sporulation entails the activity of **>500 genes over the course of ≈10 h**
- When **Spo0A is highly phosphorylated —> sporulation proceeds**
- Spo0A controls expression of several **sporulation-specific genes and sigma factors**
- Sigma factors in the mother and in developing spores
- Sigma factors have different timing and interactions a formation of a mature spore

Competence-Sporulation

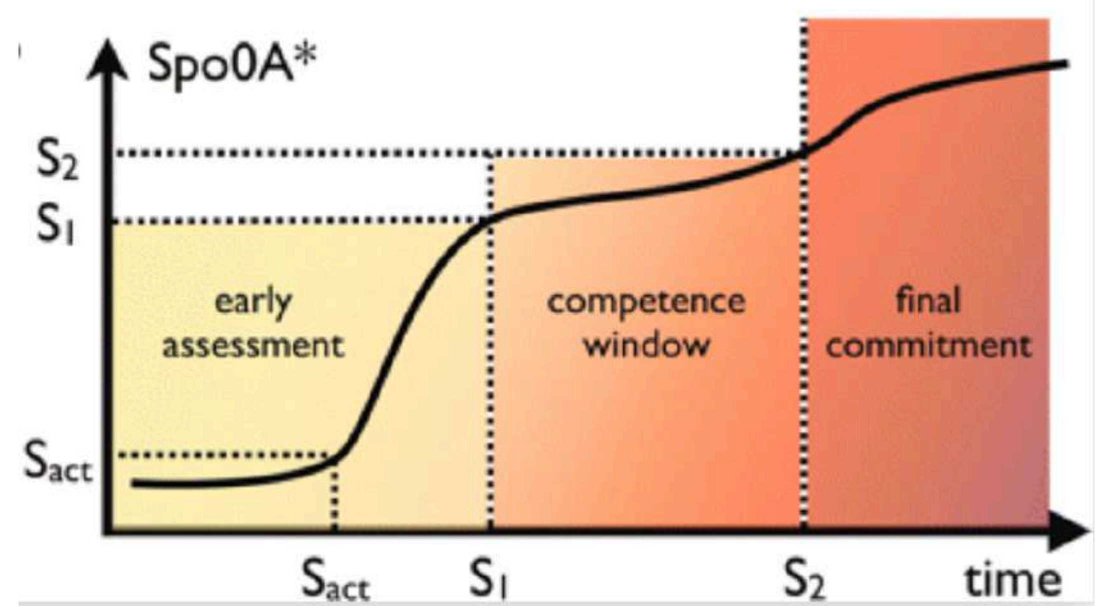


Flywheel (volano) is the Rap system
Sensing two pheromones: ComX and CSF
CSF concentration dictates decision, high —> sporulation

Mitrophanov & Groisman, 2008

Decision making: competence vs sporulation

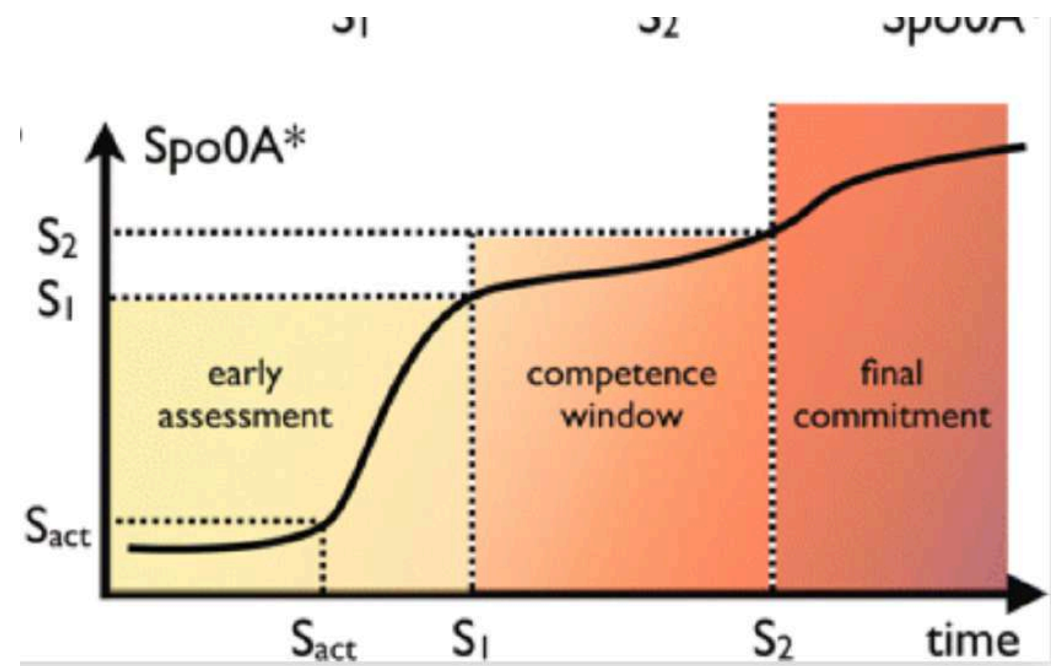
- *B. subtilis* **monitors** its environment via **5 sensor kinases**
- Adverse conditions—> **phosphorylation** of several proteins sporulation factors, culminating with **sporulation factor Spo0A**
- *B. subtilis* with **Spo0A-P secrete a toxic protein —> lyses nearby cells**
- Cells in the process of sporulation make an **antitoxin protein to protect** themselves against the effects of their own toxic protein
- Strategy in which survival of a few (as opposed to all) cells of the species in a population is a priority and is facilitated by the **sacrifice of other cells of the same species**



Schultz et al., 2019

Decision making: competence vs sporulation

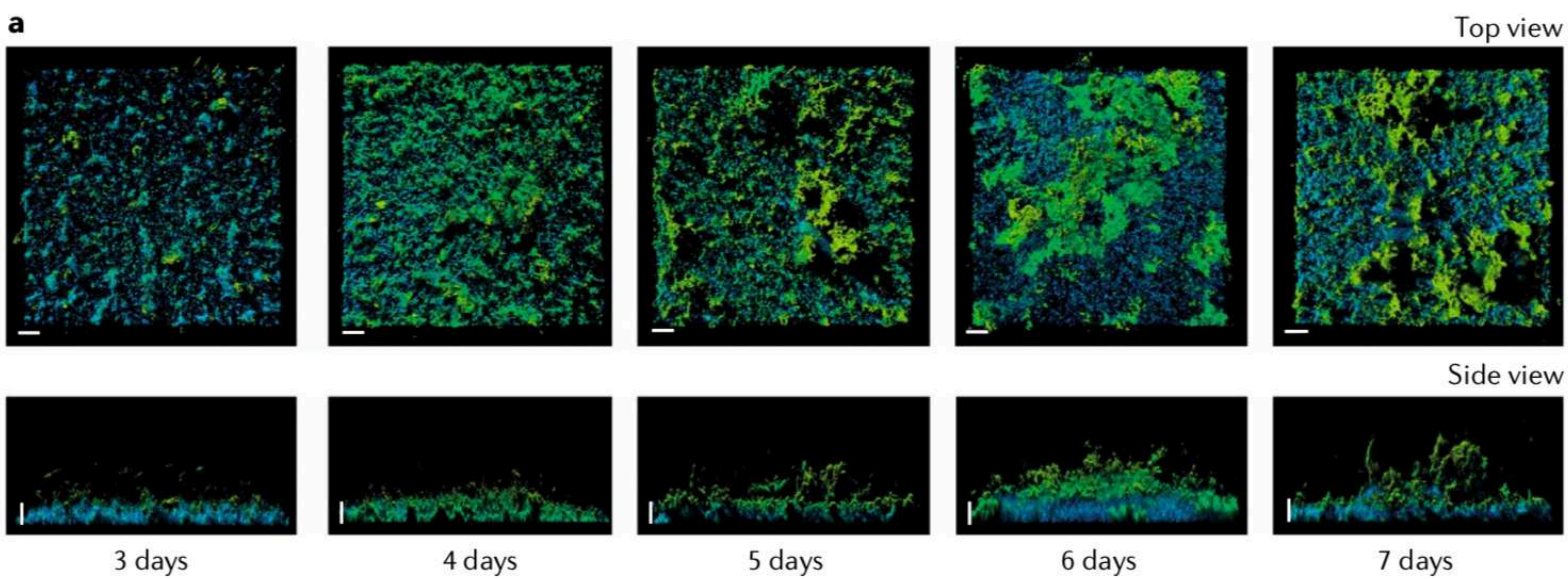
- **Spo0A dynamic** (sporulation, response to stimuli) **is linked to ComK** dynamic (competence, QS)
- On their path toward sporulation, the individual cells can opt for the differentiated state of **competence, triggered by ComK** (the competence master regulator) exceeding a certain threshold level
- In this state cell can take up **exogenous DNA from lysed cells**—> DNA repair and occasionally even as **new genetic information** to enable resisting the encountered stress
- **Competence is not a permanent genetic state**, after several hours the **cell switches back to vegetative growth on its path toward sporulation**



Schultz et al., 2019

Biofilm, I

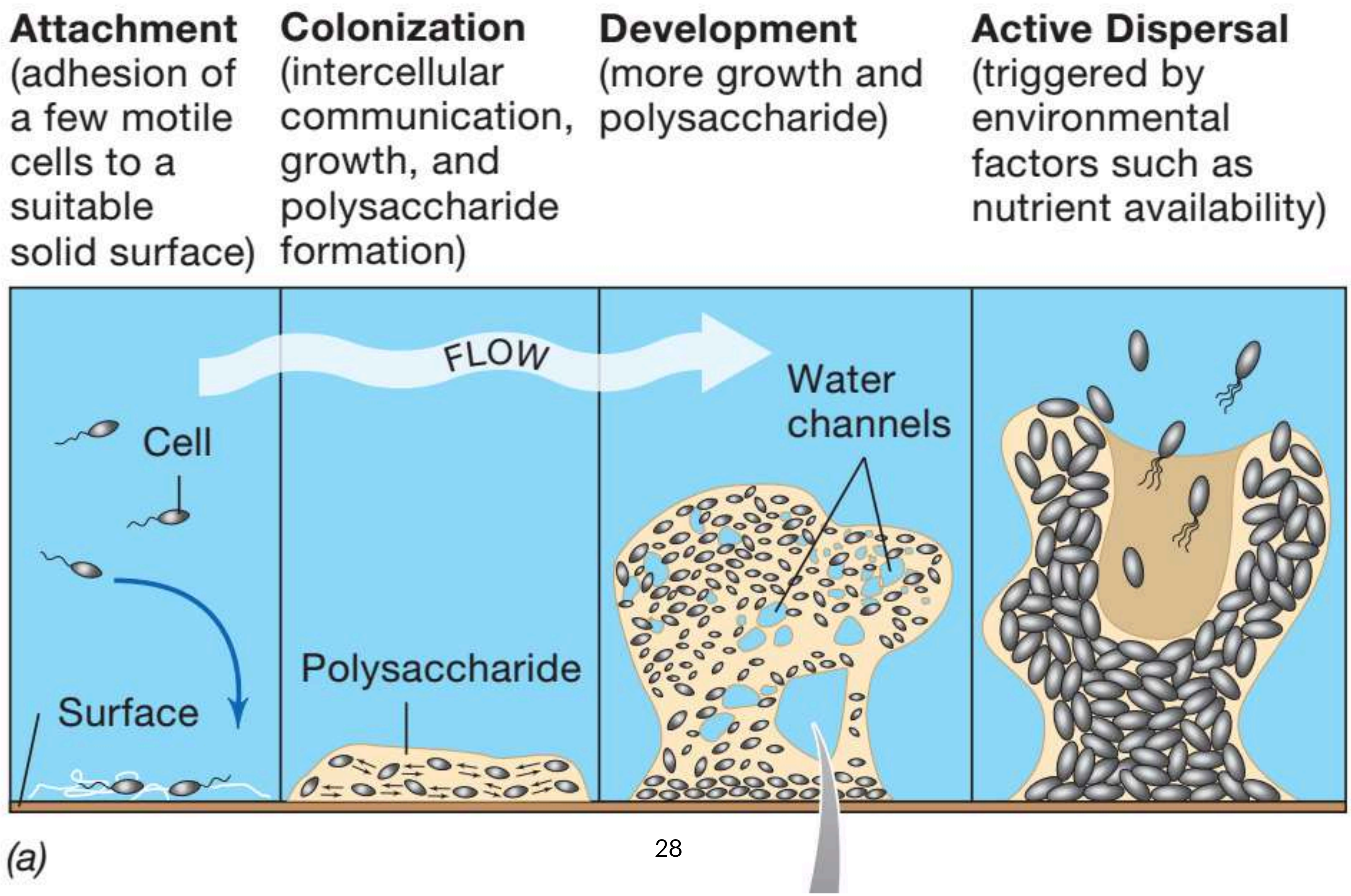
- Cells with suspended lifestyle, called **planktonic growth** vs **sessile cells** —> attaching on surfaces and forming biofilm
- A biofilm is an attached **polysaccharide matrix** containing embedded bacterial cells
- Some biofilms form multilayered sheets with different organisms present in the individual layers: microbial mat (phototrophic and chemotrophic bacteria in hot spring outflows, in marine intertidal regions)



Wolferen et al., 2019

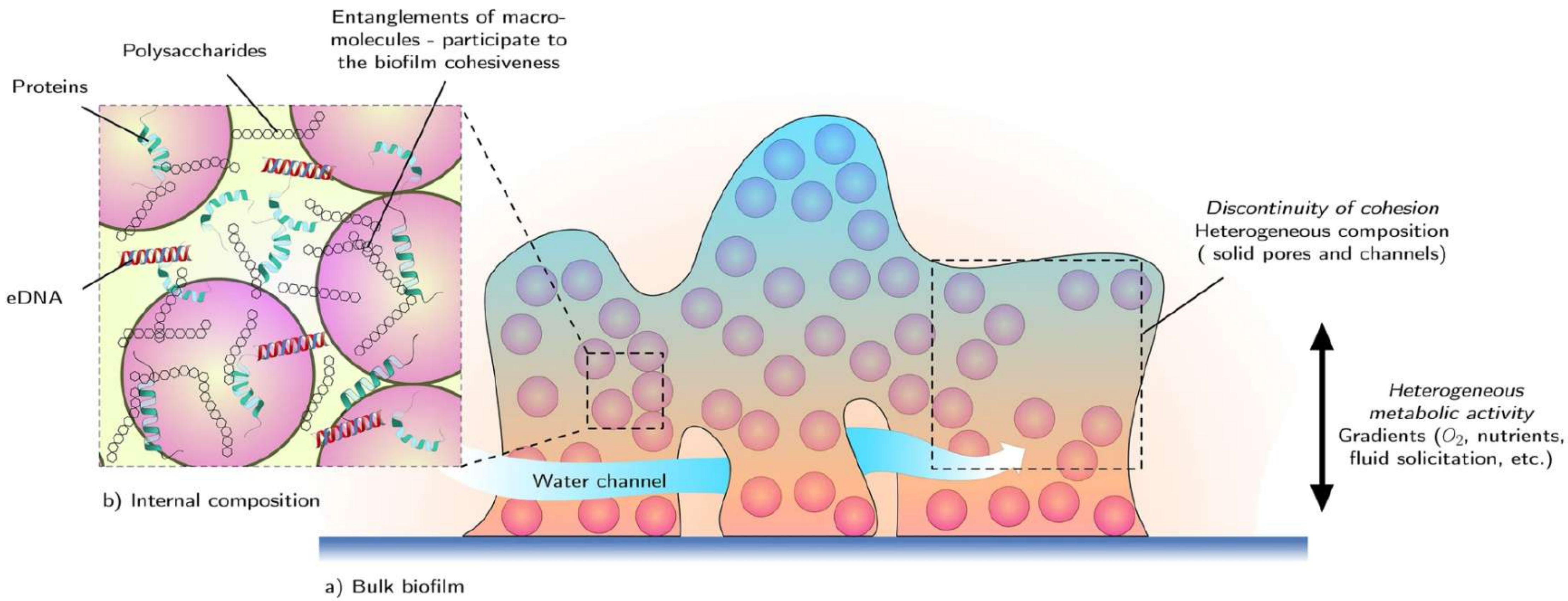
Biofilm, II

- Biofilms form in stages: (1) attachment, (2) colonization, (3) development, (4) dispersal
- **Very dynamic, very diverse**



Madigan et al. 2018

Biofilm as a heterogeneous microenvironment

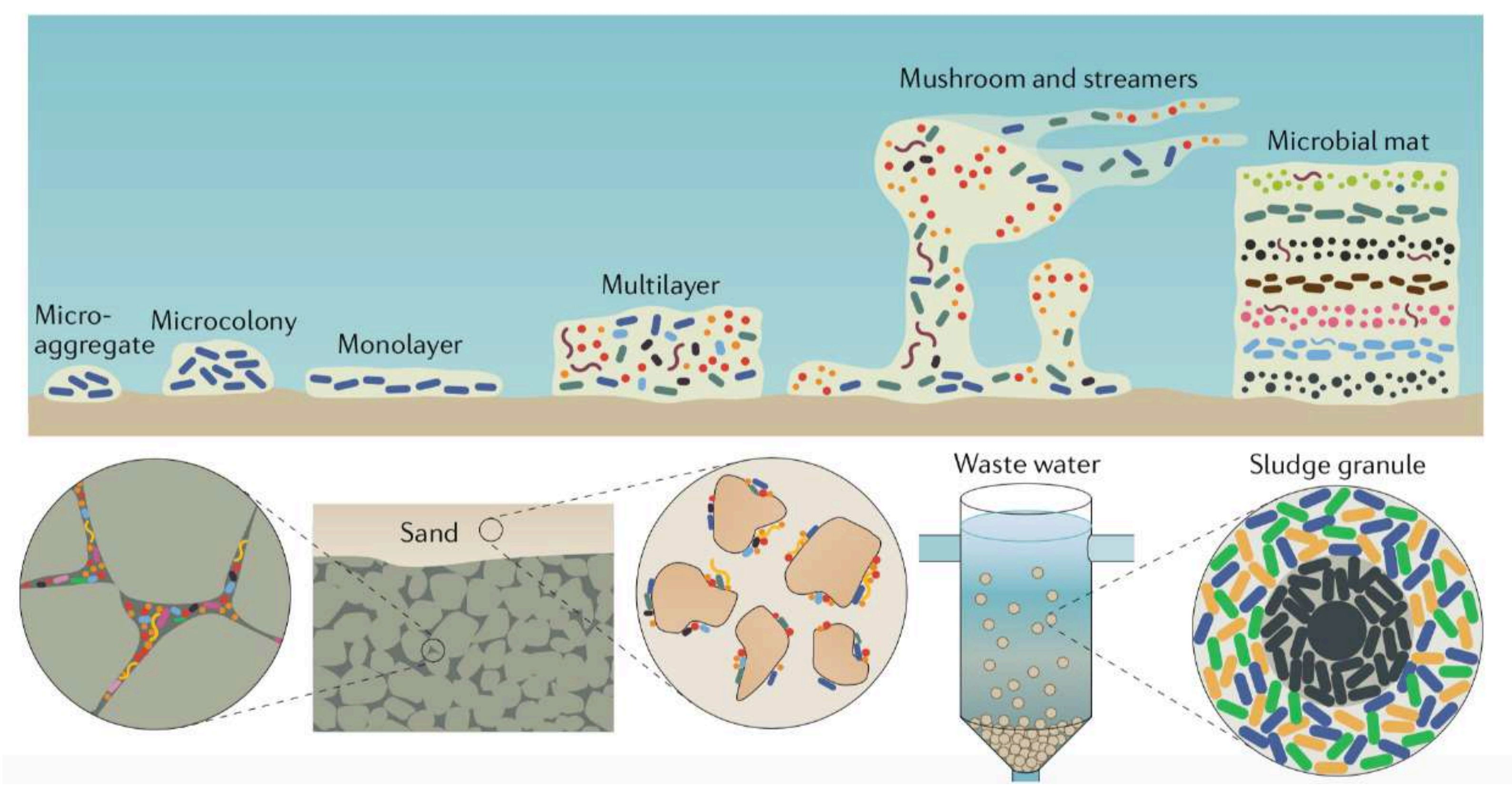


Boudarel et al., 2018

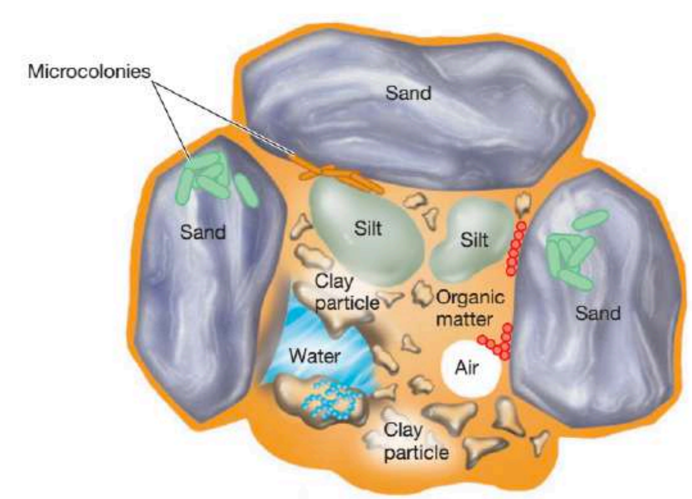
- **Extracellular polymeric substances (EPS)** secreted by the cells dwelling inside (Hall-Stoodley et al., 2004)
- EPS is usually a mixture of **polysaccharides, proteins, extracellular DNA (eDNA)**, and other minor components
- Matrix proteins (in V.c.: RbmA, Bap1, and RbmC) structure networks among polymeric substances and cells
- **Rheology is the study of viscoelastic materials**: materials that have both solid and liquid properties (Billings et al., 2015): 1) Elastic modulus, which is the stiffness of the biofilm at small deformation; 2) Yield strain, which is how much deformation a biofilm can sustain before it fails (Kovach et al., 2017): flow or attack by grazers; 3) The product of the elastic modulus and the yield strain defines the yield stress, which is the minimum force needed to cause a biofilm to fail
- Absence of matrix proteins can cause structure to swell resulting in an increased yield strain but at the expense of a highly reduced elastic modulus

Biofilm, III

- **Viscous** structure **3D** environment is formed in **synergy with the flow**
- Viscous structured 3D environment **prevents harmful** chemicals (e.g. antibiotics or other toxic substances) from penetrating —> **pathogens** (e.g. artificial heart valves and joints, and indwelling devices, such as catheters; cystic fibrosis are caused by a tenacious bacterial biofilm that fills the lungs and prevents gas exchange)
- Viscous structured 3D environment is a **favorable environment,** prevent cells from being washed away into a potentially less favorable habitat
- Biofilms as **barrier to bacterial grazing by protists (size-predation) and virus (low diffusion)**
- Biofilms cause fouling and plugging of water distribution systems and can form in fuel storage tanks, where they contaminate the fuel by producing souring agents such as H_2S



- Biofilm in between the sediment grains
- Biofilm in waste water treatment

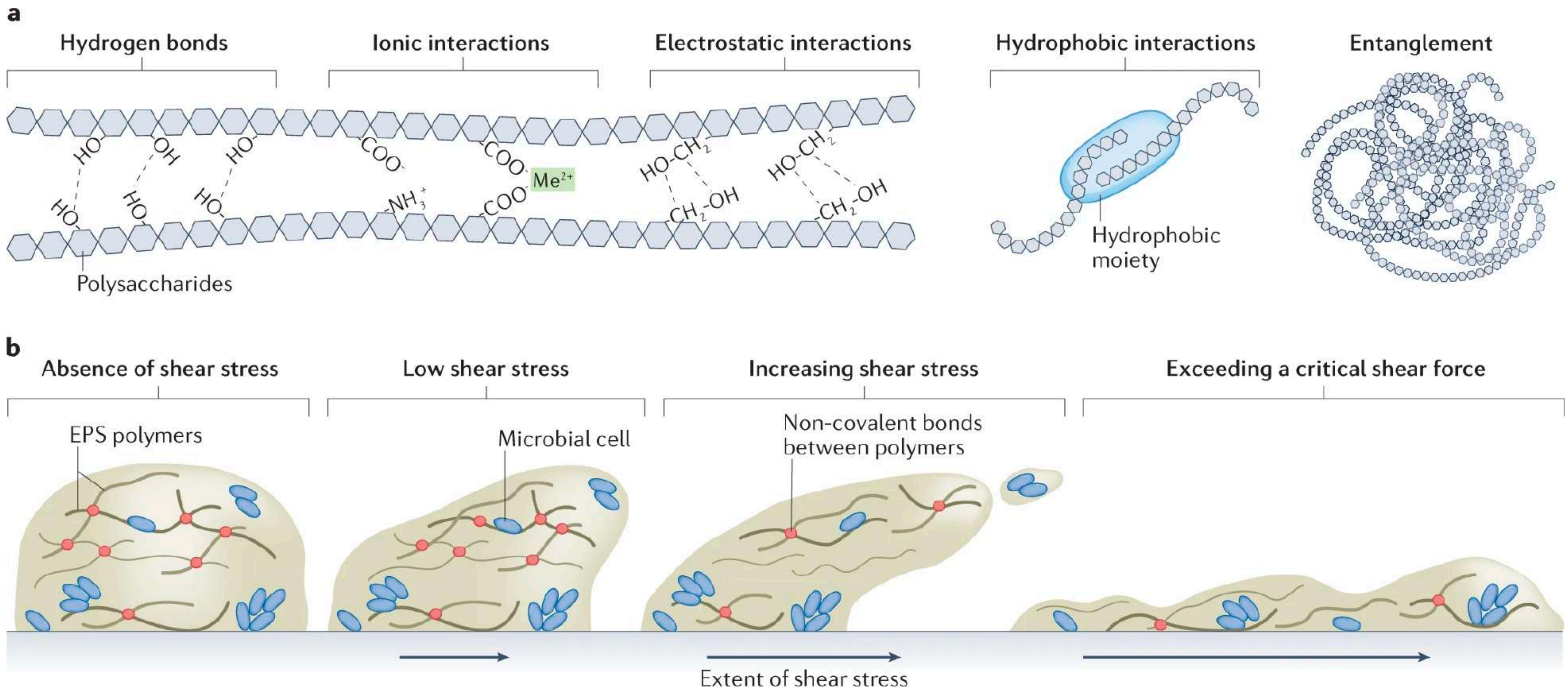


Flemming & Wuertz, 2019

Madigan et al. 2018

Mechanical properties of the biofilm matrix

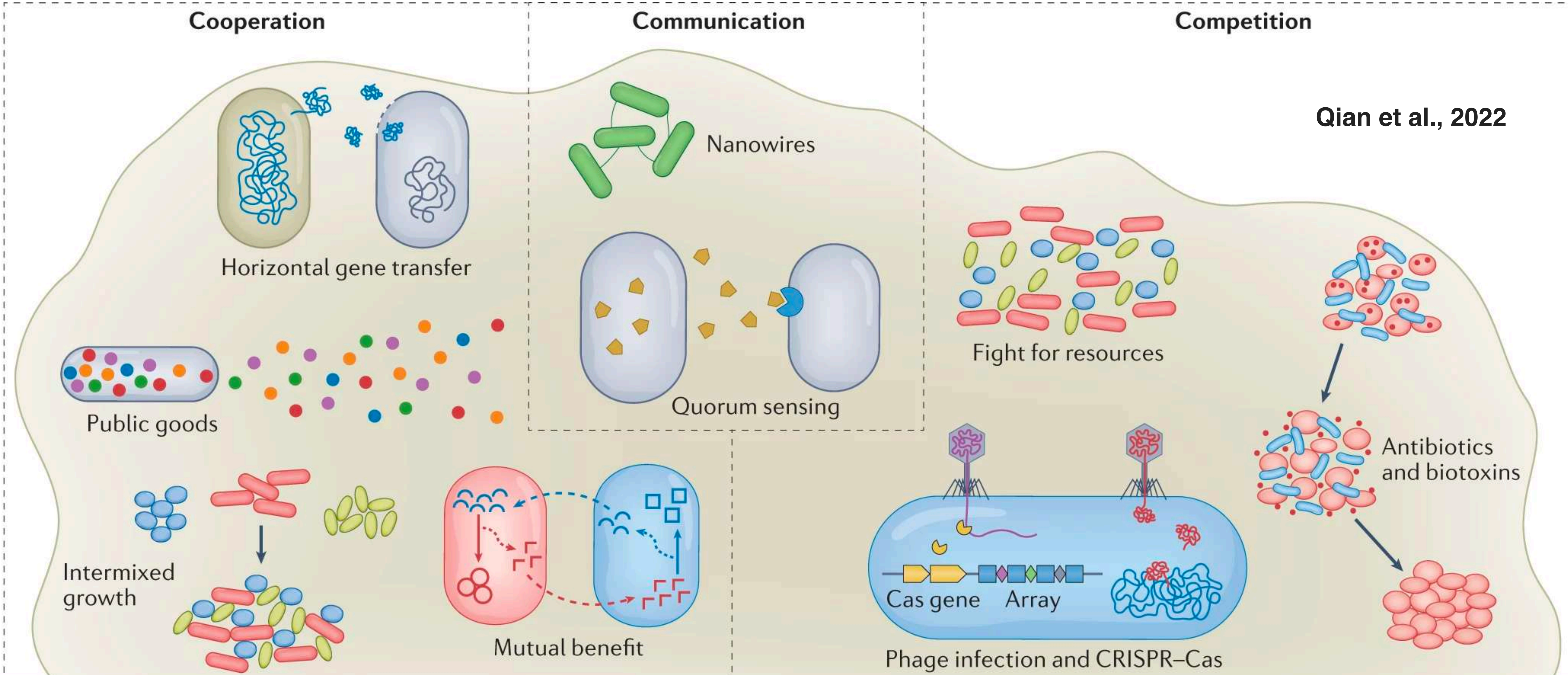
Extracellular Polymeric Substances (EPS)



Flemming et al., 2023

- In the absence of stress, the biofilm structures are undisturbed
- At low shear stress, the biofilm can immediately stretch out and spring back when the stress is removed, which is mediated by intermolecular forces among matrix components —> viscoelastic solid behaviour
- As shear increases, the biofilm flows more as bonds begin to break and polymers move past each other, and when the shear stress is removed the remaining bonds slowly pull the biofilm back but it never regains its original form; patches of the biofilm can be torn off —> highly viscous liquid behaviour
- After exceeding a critical shear force, many intermolecular forces are broken, the polymers are gliding past each other and the biofilm flows similar to a low-viscosity liquid, sometimes in ripples. When the shear stress is removed, the biofilm does not regain any of its form but new intermolecular bonds form providing stability to the new form

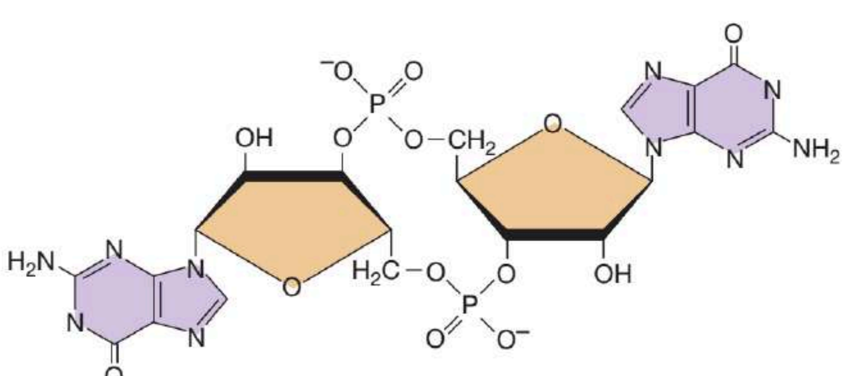
Microbial interactions in biofilms



- Cooperation can help microorganisms gain advantages, for example, through compounds that promote collaboration, the uptake of nutrients and horizontal gene transfer.
- Competition is pervasive in multispecies biofilms owing to limited space and resources; it drives evolution and has an essential role in shaping the biofilm structure and physiological activities.
- Chemical communication (such as quorum sensing) and electrical communication (such as nanowires) regulate social behaviours in microbial communities

Biofilm formation

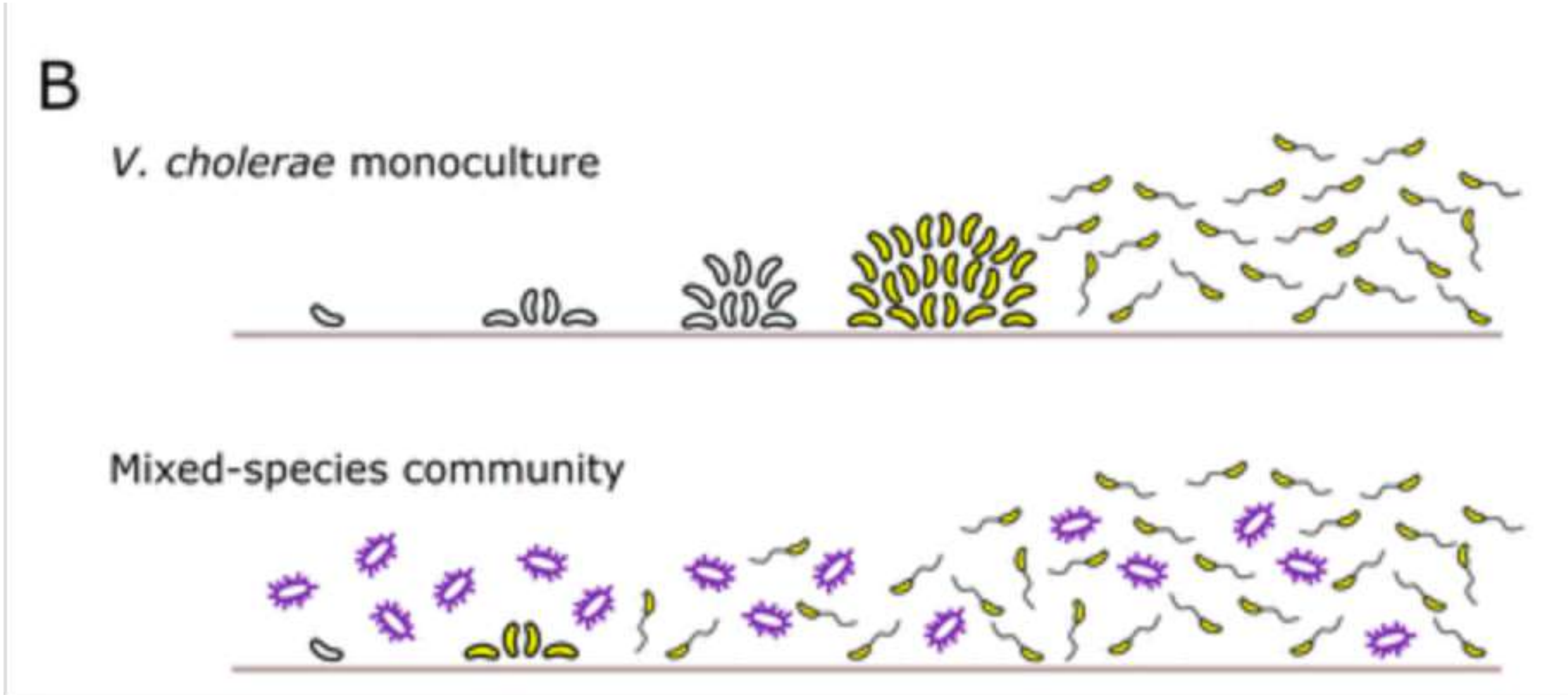
- **Molecular coating** on surfaces
- Cell attachment
- Attachment of a cell to a surface is a signal for the expression of biofilm-specific genes —> intercellular signaling molecules and extracellular polysaccharides
- Once committed to biofilm formation, a previously suspended (planktonic) cell typically **loses its flagella and becomes nonmotile**
- **Switch** from planktonic to biofilm growth in many bacteria is **triggered by the cellular accumulation of the regulatory nucleotide cyclic di-guanosine monophosphate (c-di-GMP)**
- c-di-GMP binds to proteins **reducing** activity of the **flagellar motor**, **regulates** cell **surface proteins required for attachment**, mediates the **biosynthesis of extracellular matrix polysaccharides** of the biofilm



- **Mushroom-shaped microcolonies up to 0.1 mm high** and contain **millions of cells**
- **QS, exchange DNA, antibiotic resistance, heavy metal resistance**

Madigan et al. 2018

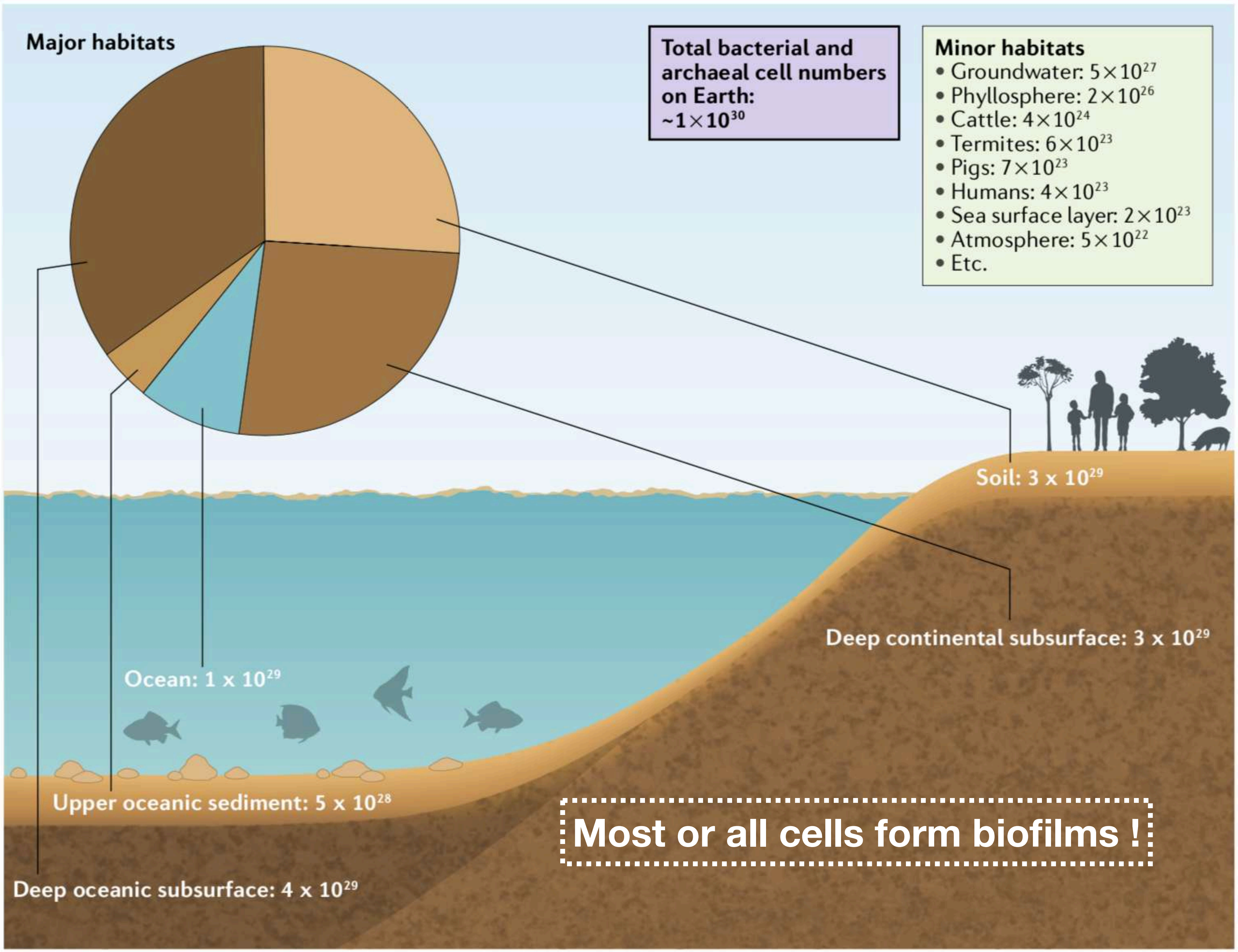
Biofilm formation and dispersal: *V. cholerae*



Bridges & Bassler, 2020

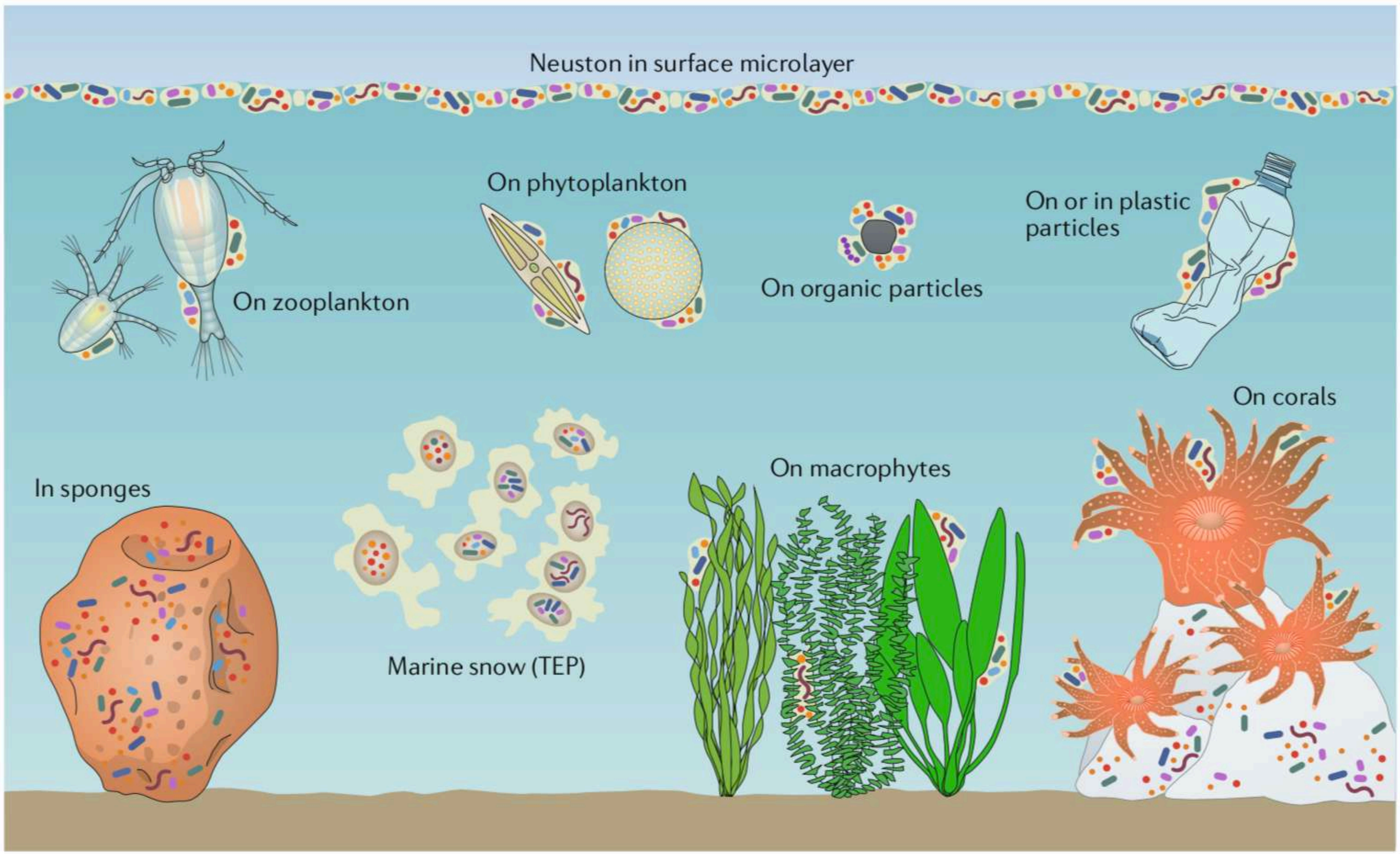
- At low cell densities, when QS autoinducers are absent, *V. cholerae* forms biofilms
- At high cell densities, when autoinducers have accumulated, biofilm formation is repressed and dispersal occurs
- CAI-1, is used to measure *Vibrio* abundance
- AI-2, is a broadly-made universal autoinducer —> *V. cholerae* to assess the total bacterial cell density of the vicinal community
- Both autoinducers must be present simultaneously for repression of biofilm formation to occur
- CAI-1 produced by *V. cholerae* engages its cognate CqsS receptor at very low cell densities vs AI-2 at high cell density
- *V. cholerae* uses CAI-1 to verify that some of its kin are present before committing to the high-cell-density quorum-sensing mode, but it is, in fact, the universal autoinducer AI-2, that sets the pace of the *V. cholerae* quorum-sensing program

Biofilm habitat



Flemming & Wuertz, 2019

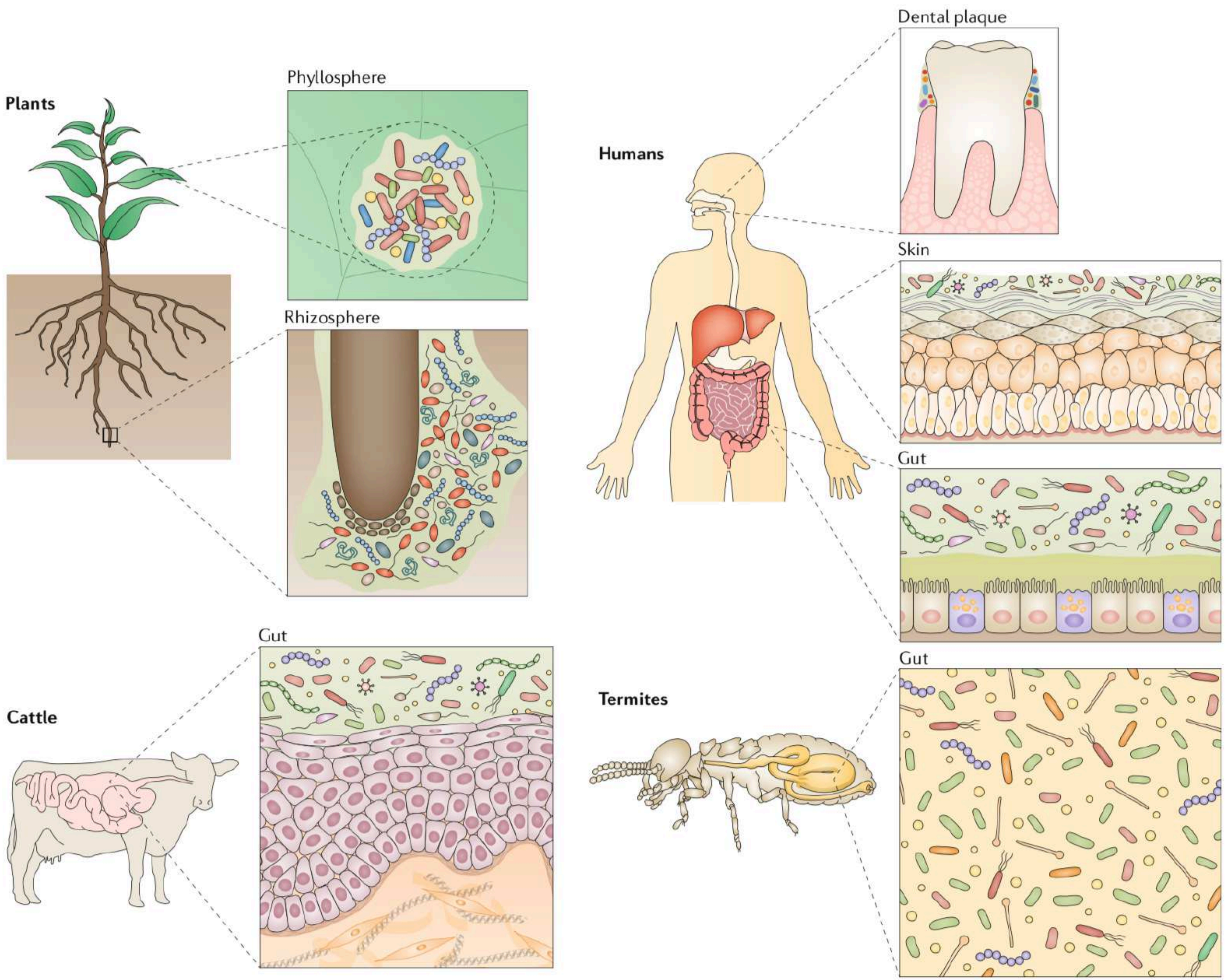
Marine biofilms



Molecular coating and living or not surfaces provide 3D structure for biofilm formation

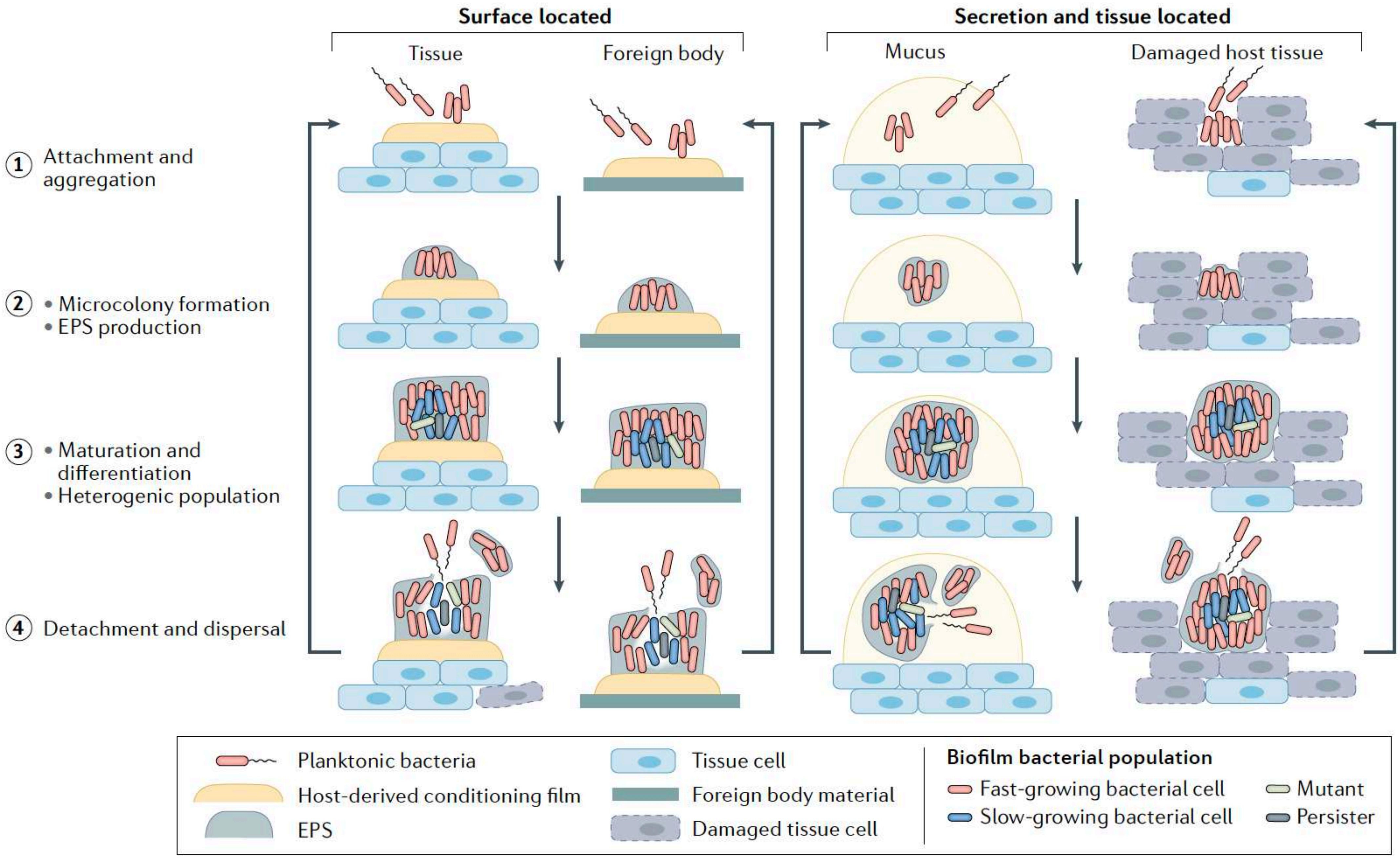
Flemming & Wuertz, 2019

Eukarya as microbial biofilm



Flemming & Wuertz, 2019

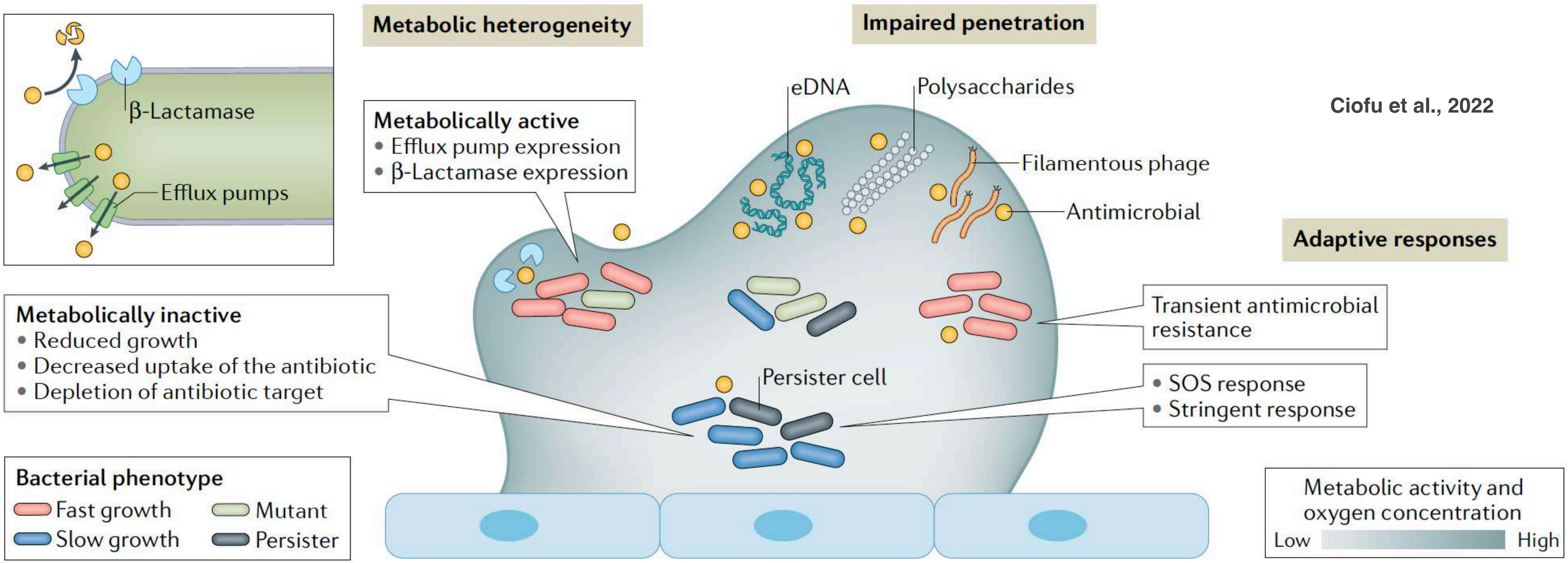
Biofilm types in Eukarya



EPS, extracellular polymeric substance.

Ciofu et al., 2022

The mechanisms of antimicrobial tolerance of a biofilm



Ciofu et al., 2022

Tolerance
A tolerant population is characterized by a slow rate of killing due to slow growth and low metabolic activity of the bacterial cells, requiring a longer time to kill 99% of the bacterial population than a susceptible population despite the similar values of the minimum inhibitory concentration of antibiotics against both populations.

Resistance
A resistant population is characterized by the lack of killing by antibiotic concentrations above the minimum inhibitory concentration of antibiotics against the susceptible bacterial population.

Biofilm in sum

Box 2 | **Key features of biofilms**

- Microbial aggregates at interfaces: solid–liquid, solid–gas, liquid–liquid and liquid–gas
- Genetic response to surface adhesion
- Extracellular polymeric substances matrix, mainly consisting of polysaccharides, proteins and extracellular DNA (eDNA), which forms a ‘house for biofilm cells’ and provides mechanical stability
- Gradients resulting in heterogeneous microenvironments in biofilms
- Wide variety of habitats supporting biodiversity
- Retention of extracellular enzymes in a matrix, for example, providing an external digestion system
- Matrix-stabilized microconsortia that enable synergistic use of nutrients
- Water retention and protection against dehydration
- Nutrient acquisition by sorption and retention
- Recycling of nutrients
- Enhanced tolerance to disinfectants, biocides and other stressors
- Enhanced intercellular communication (signalling), regulation of matrix synthesis, detachment and virulence factors, among others
- Access to extracellular genetic information (eDNA)
- Facilitated horizontal gene transfer by conjugation, transduction and transformation
- Collective, coordinated behaviour (regulated by signalling molecules)

NB: our expanded biofilm definition implies cellular organization at a higher level with associated emergent properties, even if not all key features are present.

Flemming & Wuertz, 2019