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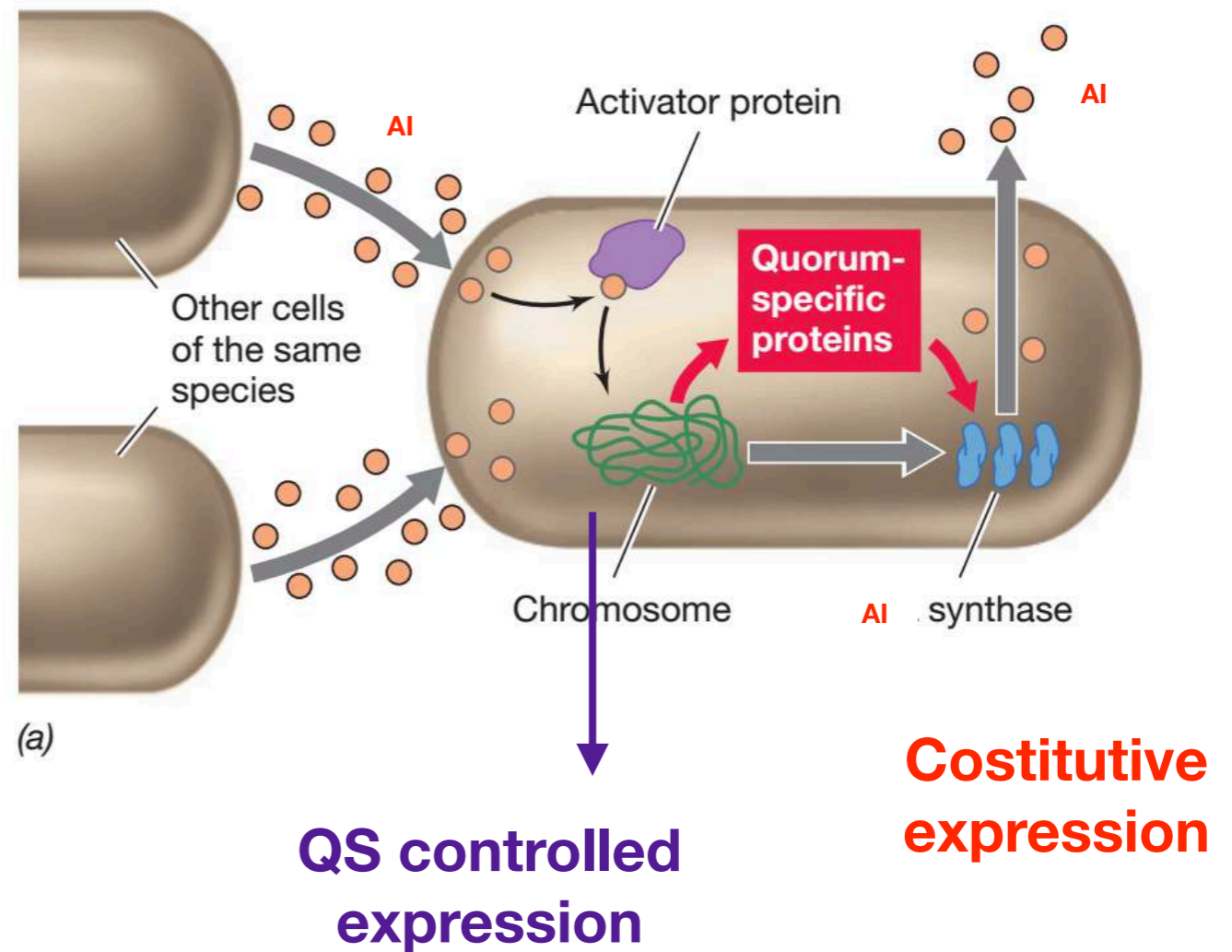
Recap

Microbial interactions: Quorum sensing, biofilm, symbioses

**[https://www.ted.com/talks/
bonnie_bassler_how_bacteria_talk?
utm_campaign=tedsread&utm_medium=referral&utm_sourc
e=tedcomshare](https://www.ted.com/talks/bonnie_bassler_how_bacteria_talk?utm_campaign=tedsread&utm_medium=referral&utm_source=tedcomshare)**

Quorum Sensing, I

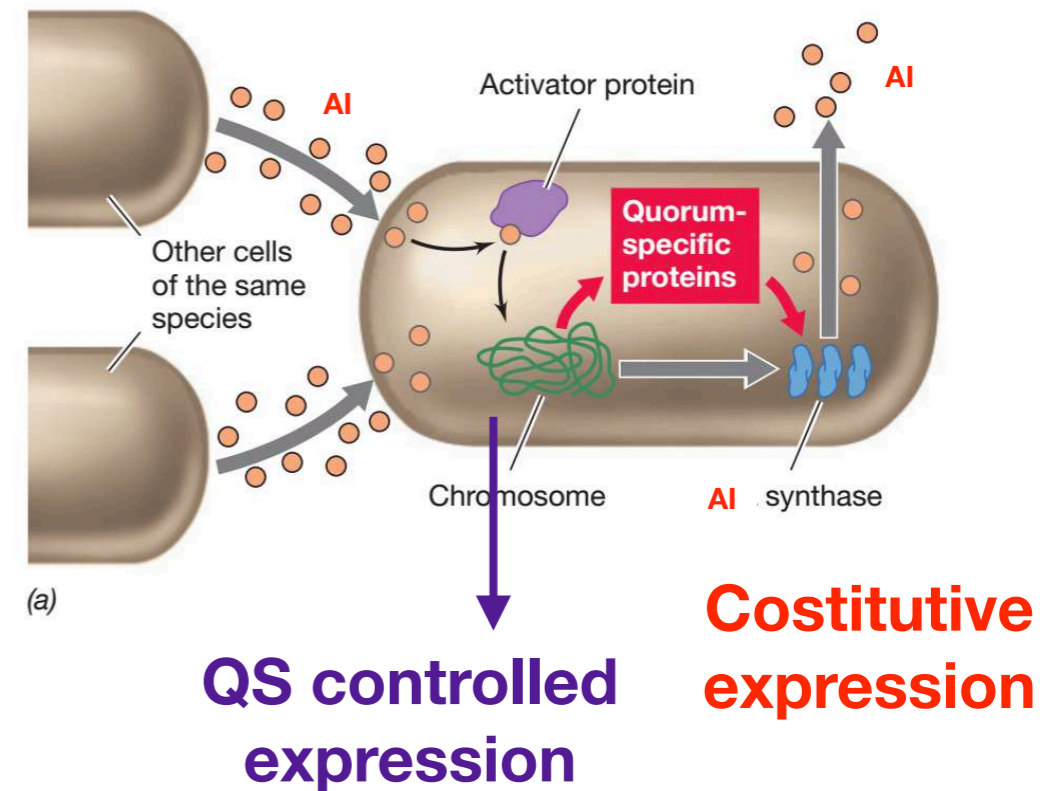
- Quorum sensing (QS) is a process of bacterial **cell-to-cell chemical communication**
- Production, detection, response to extracellular signalling molecules: **autoinducers (AIs)**
- Quorum sensing allows **groups of bacteria to synchronously alter behaviour** in response to changes in the population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



Madigan et al. 2020

Quorum Sensing, II

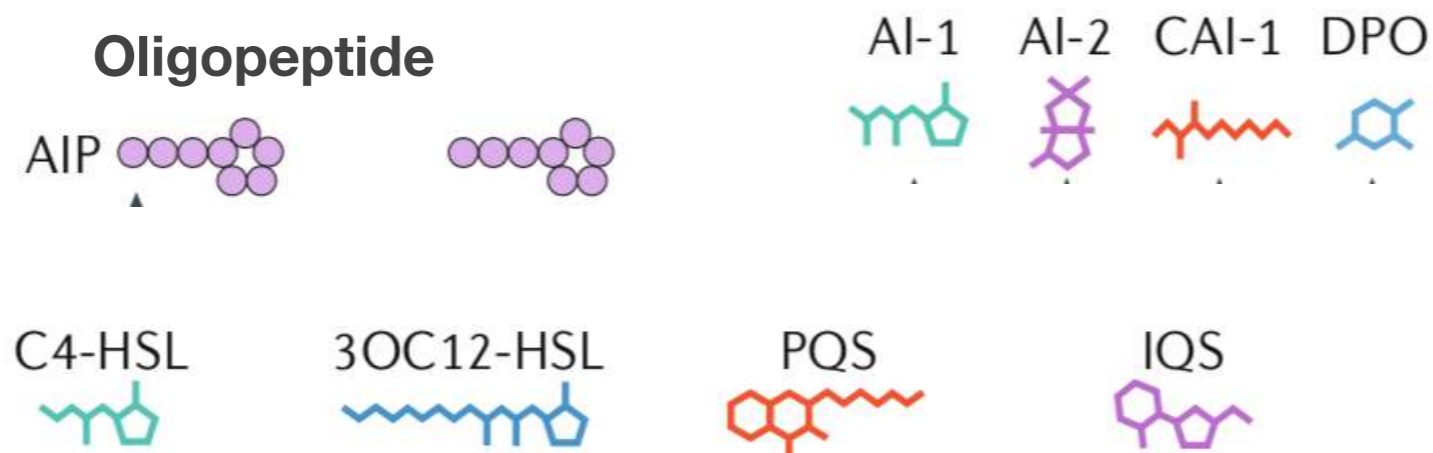
- QS is **global regulatory control**
- QS present in Gram -, Gram + and Archaea
- Many Bacteria respond to the presence in their surroundings of other cells of their own species, and in some species, regulatory pathways are controlled by the cell abundance of their own kind
- QS is regulatory mechanism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



Madigan et al. 2020

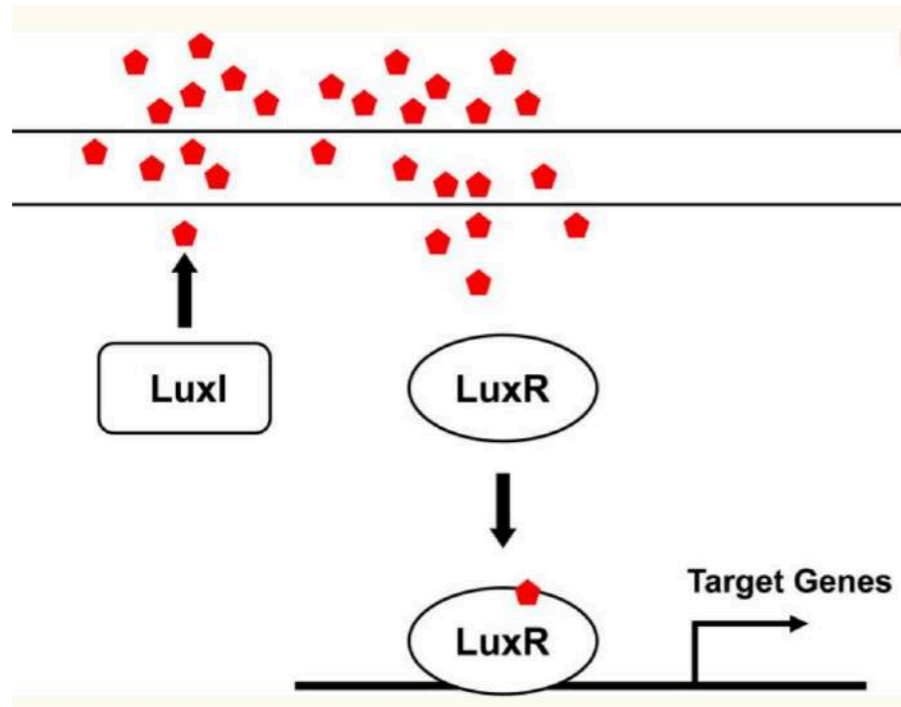
Quorum Sensing, III

- Examples are: **bioluminescence, virulence factor production, secondary metabolite production, competence for DNA uptake, biofilm formation, species composition**
- Autoinducer (AI) is species specific and **freely diffuse in & out**
- **Diverse** chemical structure
- **Constitutively expressed**
- **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

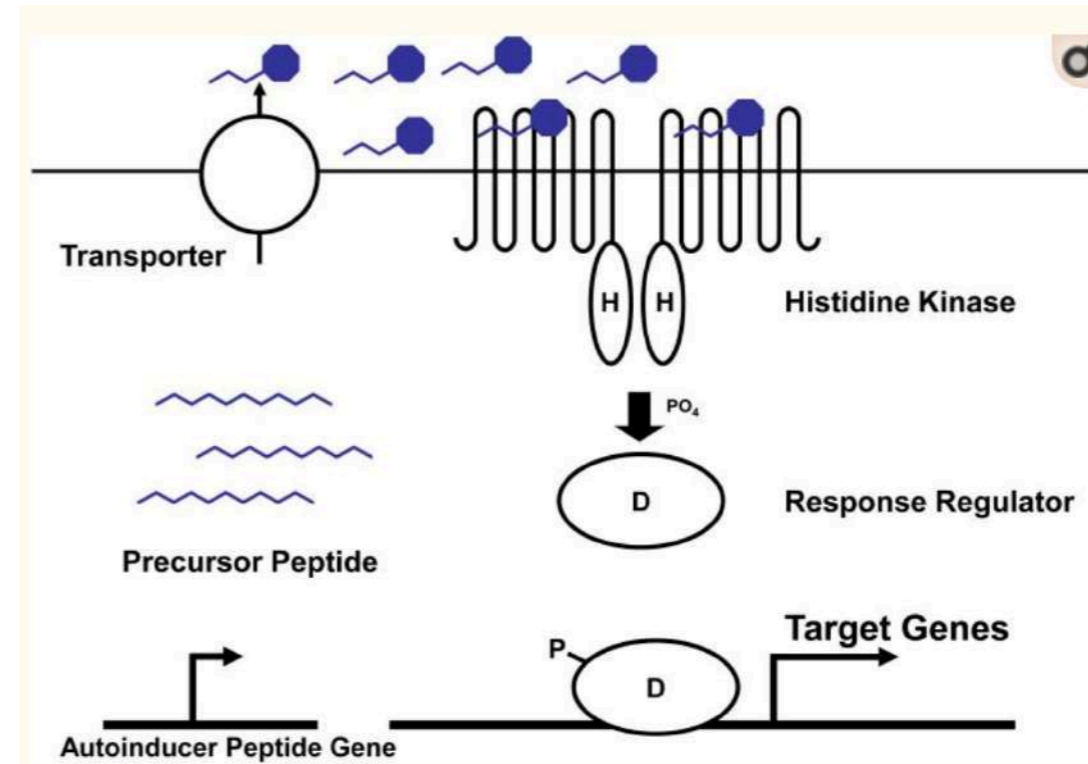


Mukherjee & Bassler, 2019

Gram - & Gram +

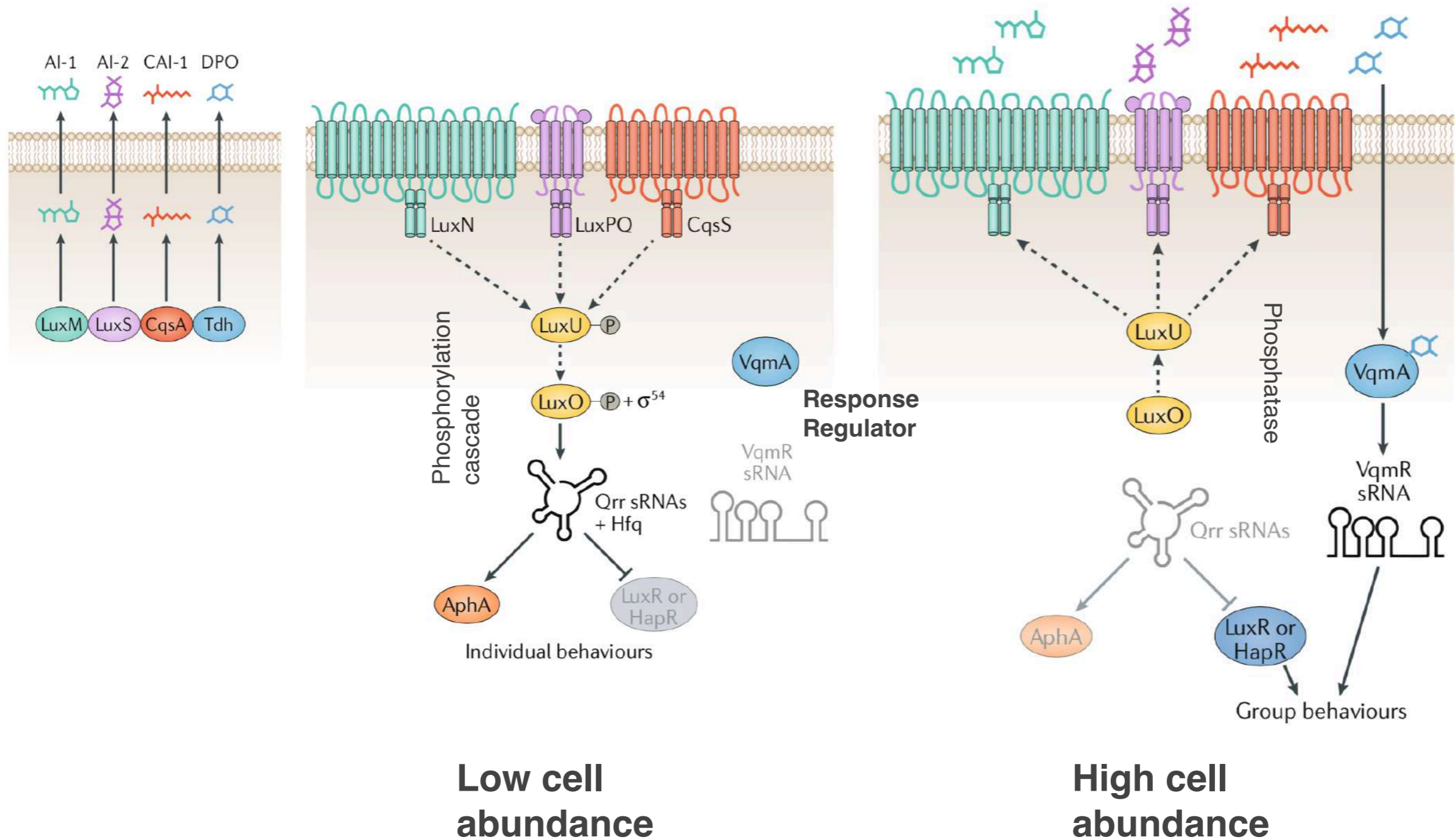


- LuxI is AI synthase
- LuxR is AI cytoplasmic receptor & **transcriptional activator**
- *luxICDABE* operon
- Gene transcription
- Induction of more AI production



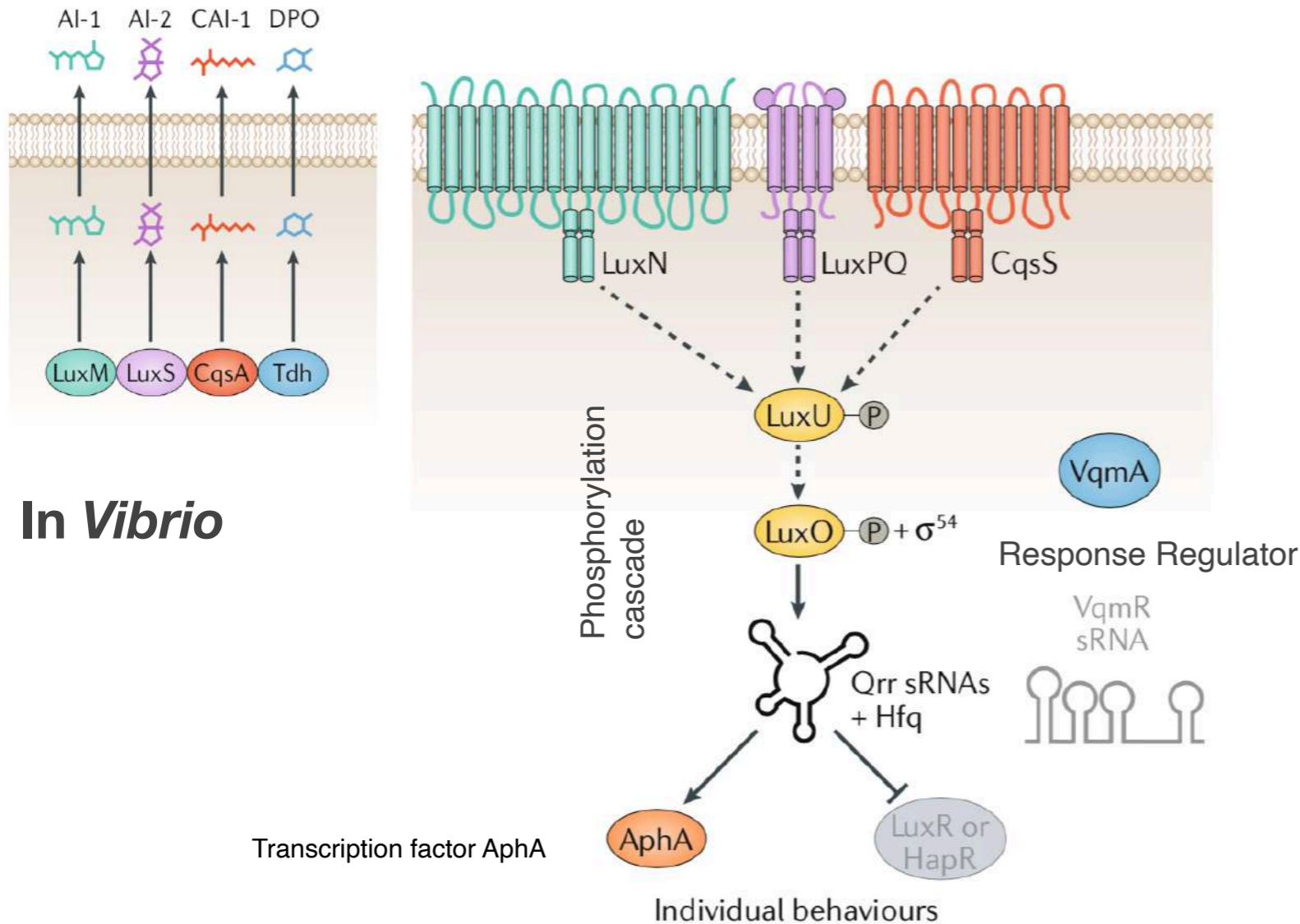
- Peptide binding to membrane-bound receptor
- Autophosphorylation activity
- P to cognate response regulator (RR)
- RR → DNA-binding factors
- Gene transcription
- Induction of more AI production

Individual vs Group behaviour



Individual vs Group behaviour

Low cell density

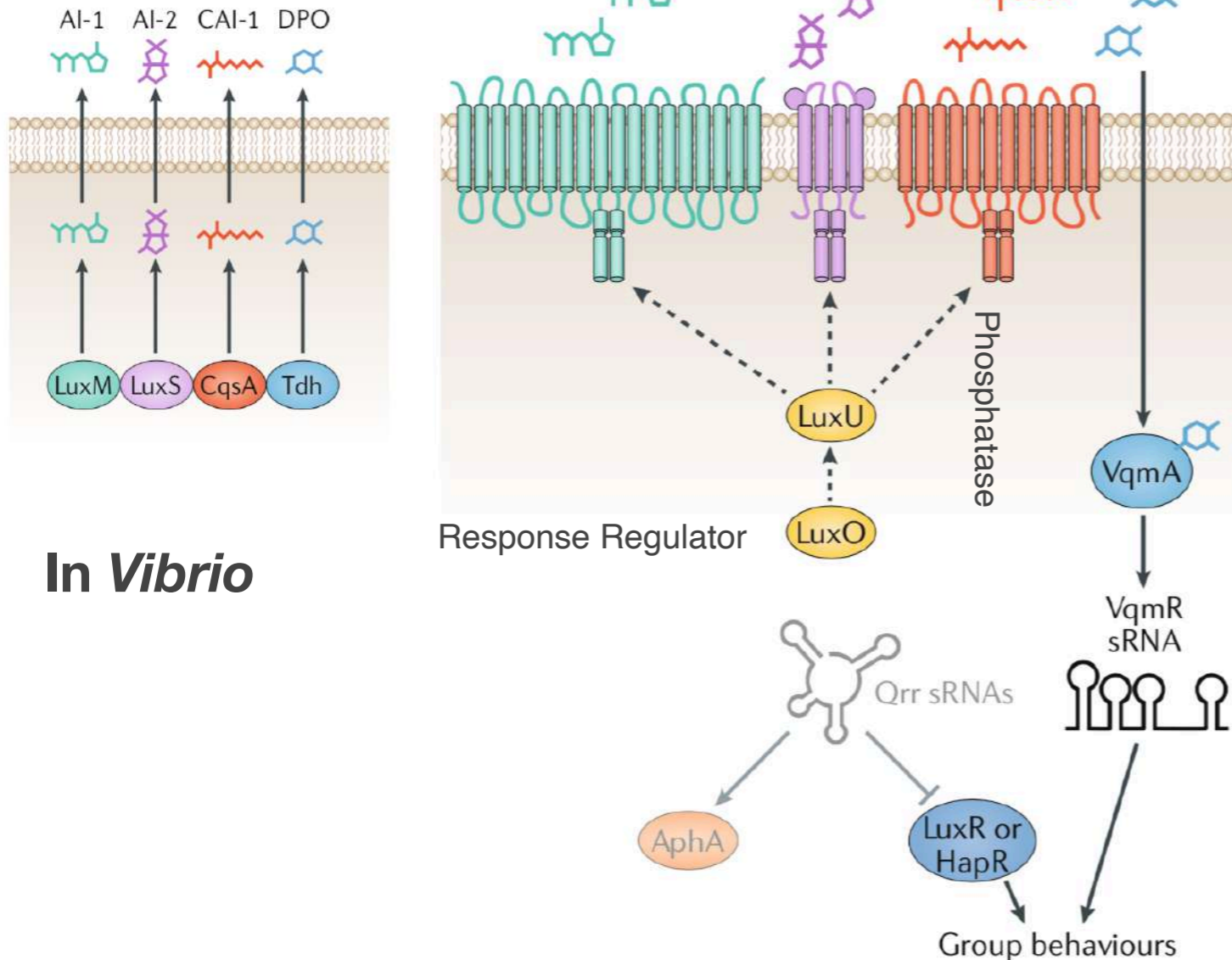


In Vibrio

- At low cell density, the receptors are kinases that transfer phosphate to LuxO via LuxU
- Phospho-LuxO, with σ^{54} , 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 low and high cell density master regulators, AphA and LuxR, respectively

Individual vs Group behaviour

High cell density

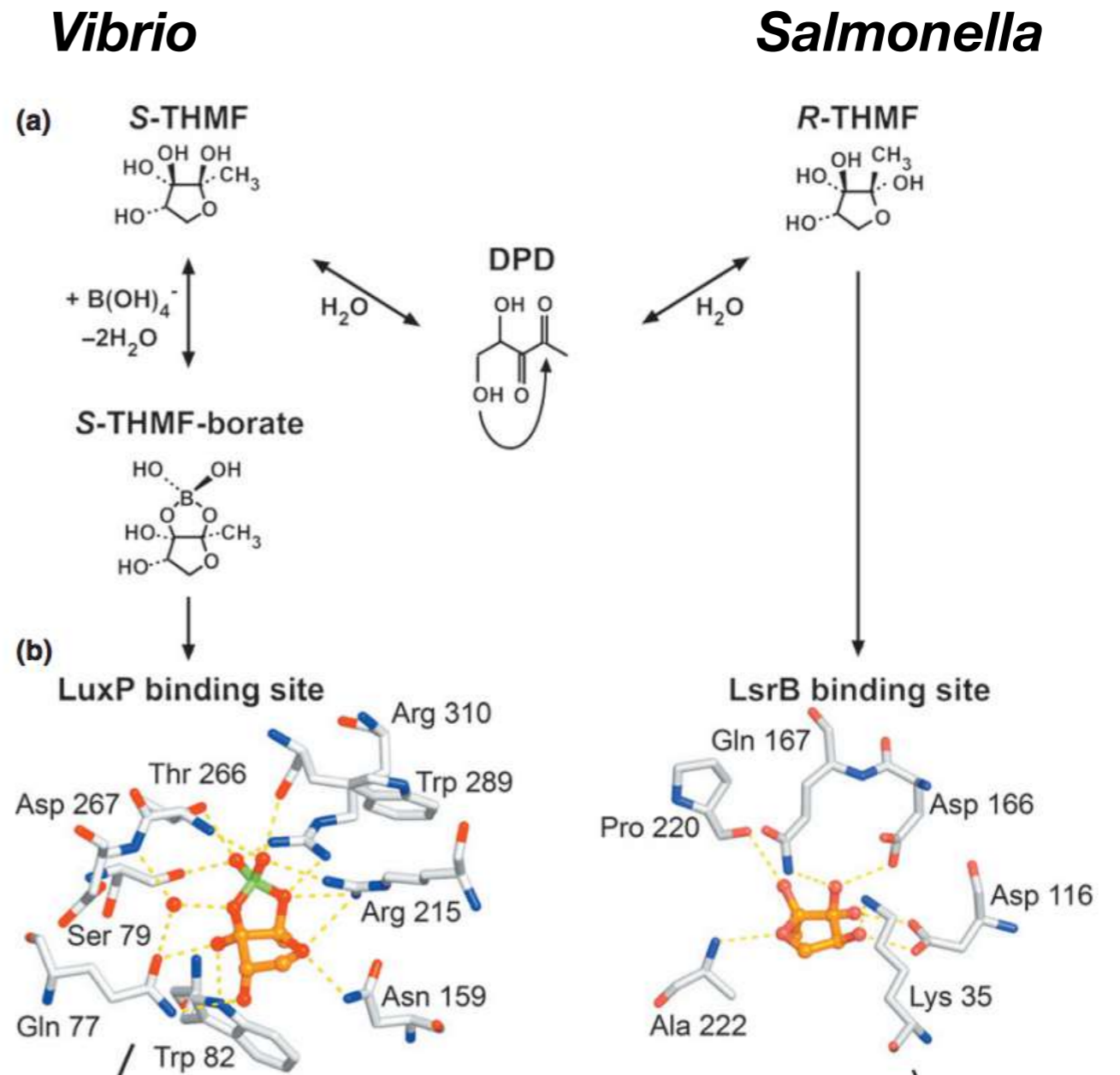


In *Vibrio*

- 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

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

INTRA-INTER SPECIES COMMUNICATION

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

INTRA-INTER SPECIES COMMUNICATION

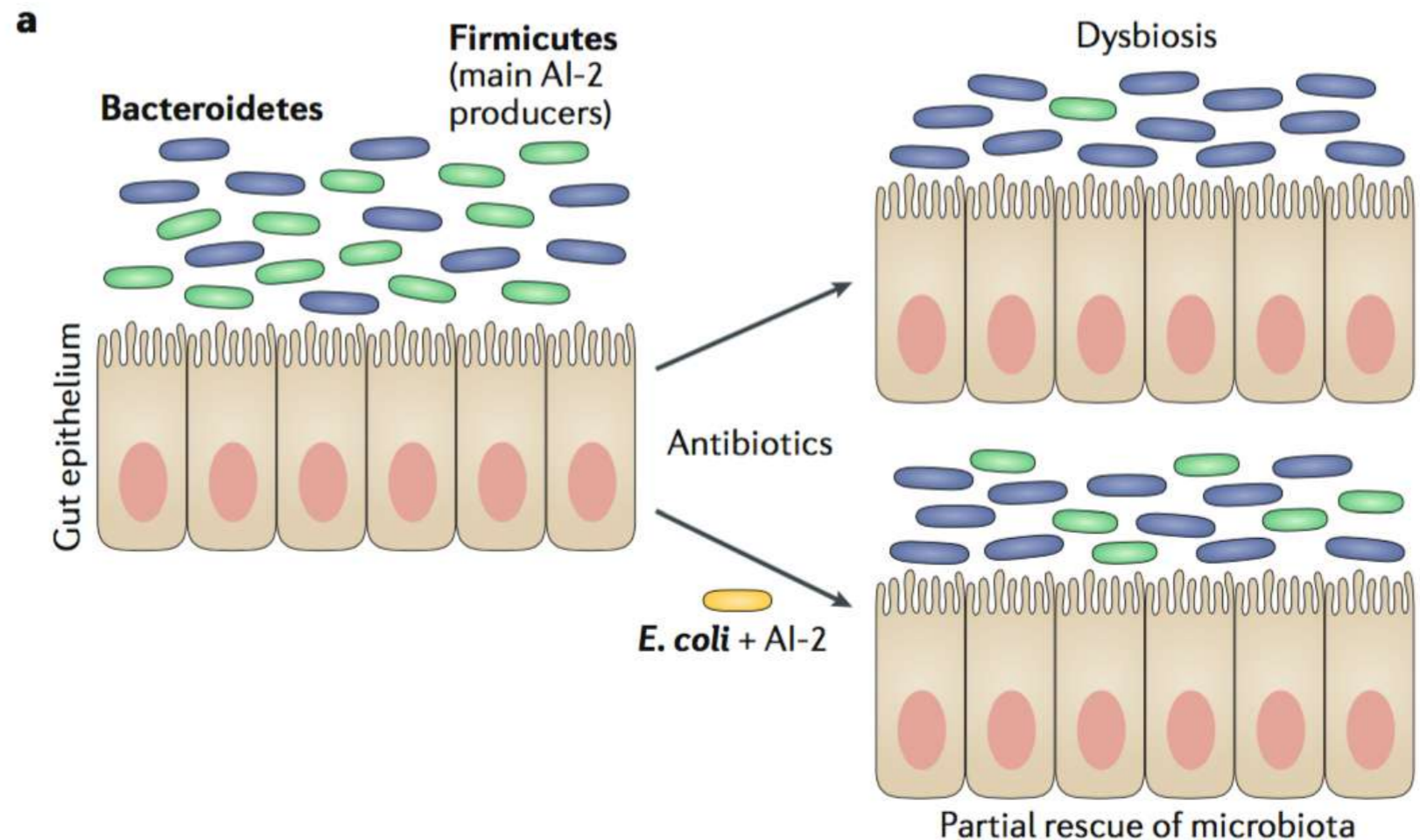
Table 1. Continued

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

Pereira et al., 2012

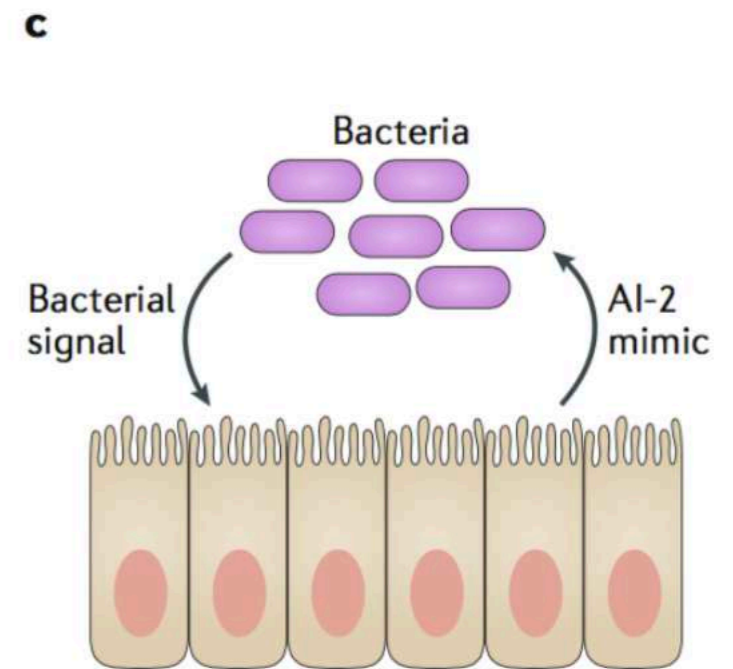
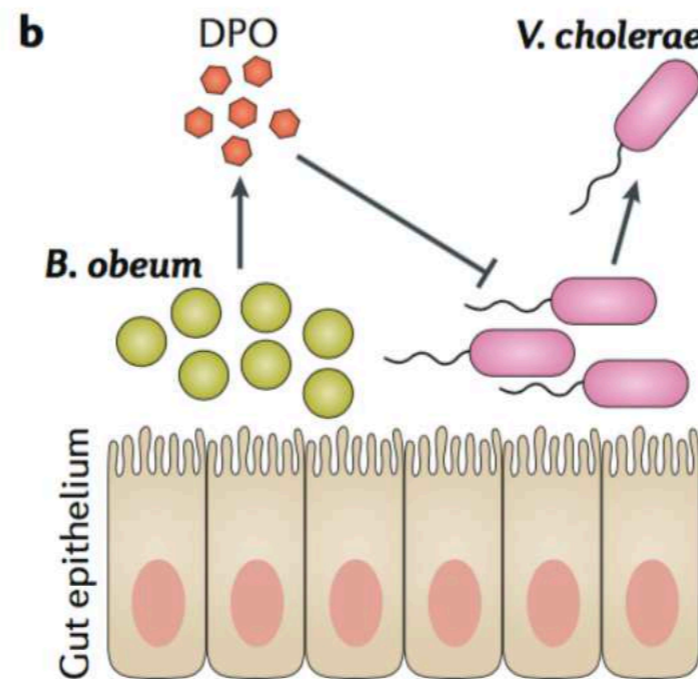
QS and the host microbiota, I

- 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

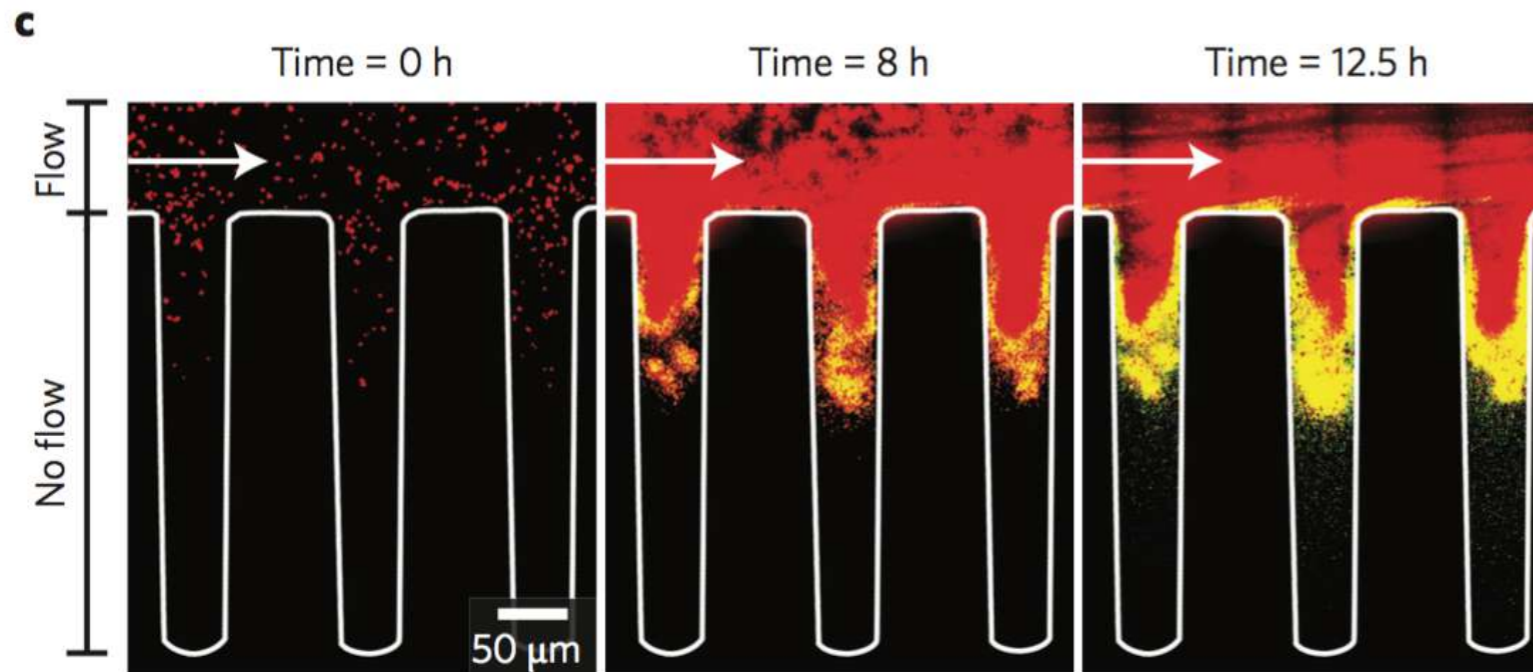
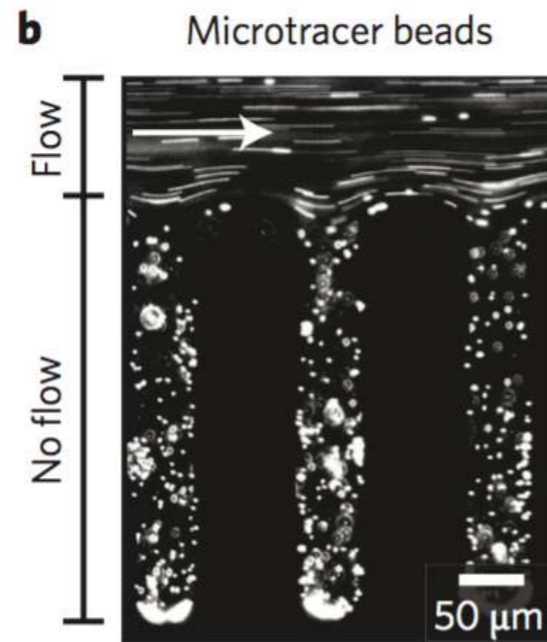
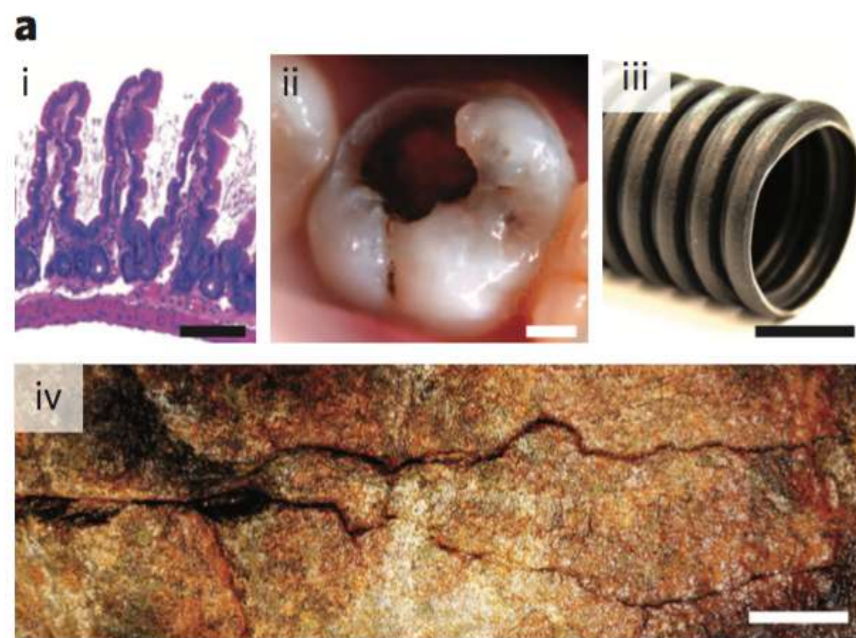


QS and the host microbiota, II

- Gut commensal 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



QS in the microenvironment



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

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

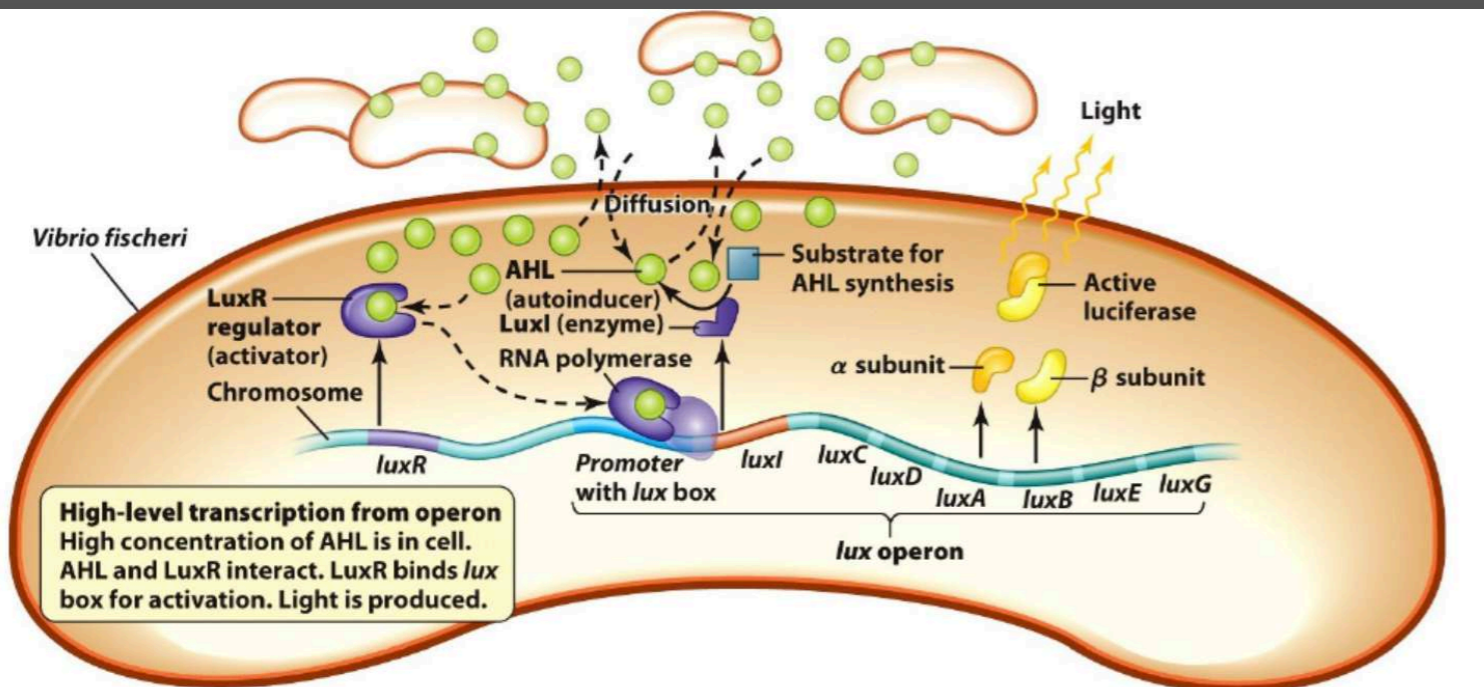
Flow networks with crevices or pores: the small intestine of mice (image courtesy of A. Ismail) (i), tooth cavities (image courtesy of W. Lee) (ii), corrugated industrial pipes (iii) and cracks in rocks (iv)

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

Kim et al., 2016

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/cm³ and a single cell may emit $\sim 10^3$ photons/s
- Light production by luciferase that is encoded by lux operon



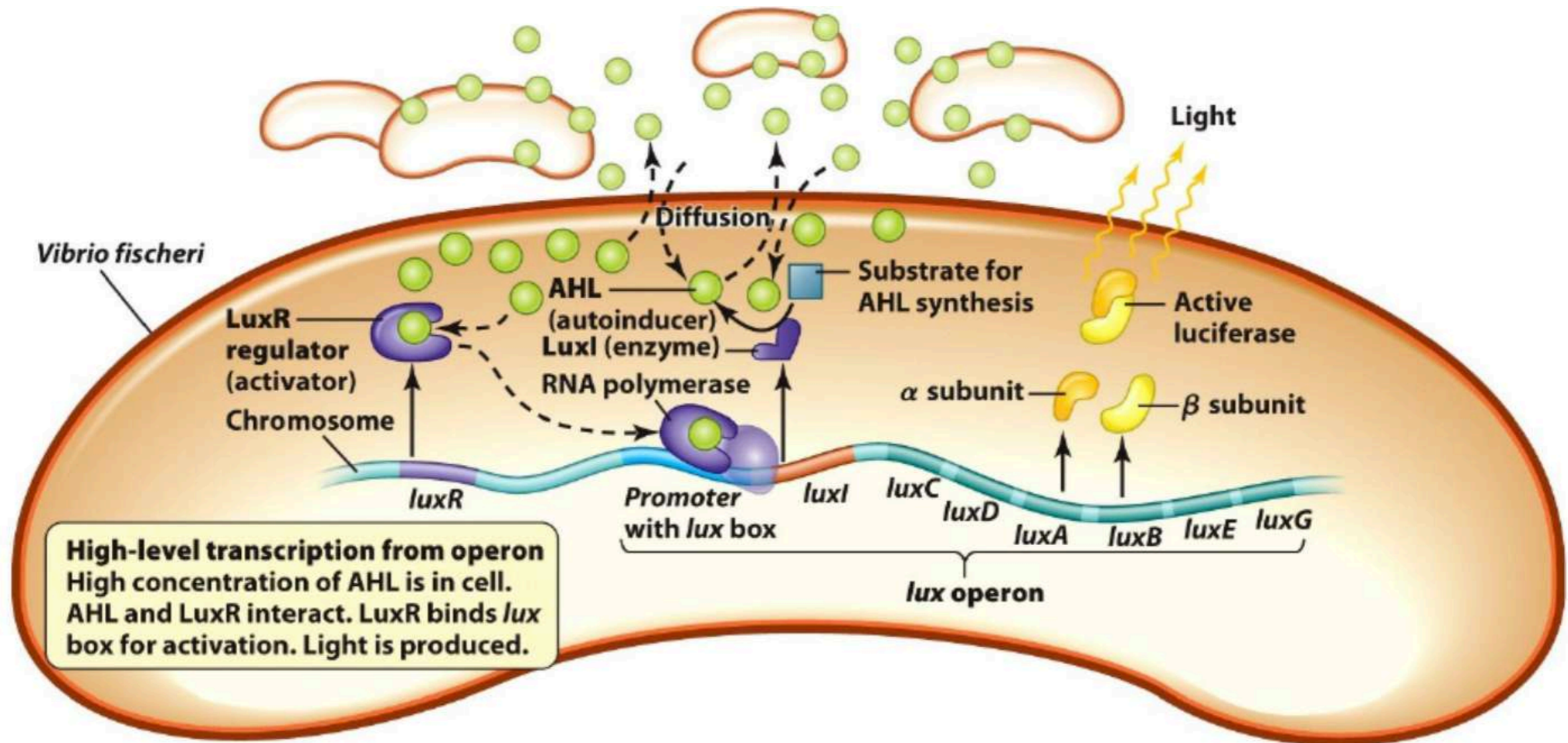
High population density; high concentration of AHL

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TODD BRETL UNDERWATER PHOTOGRAPHY

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



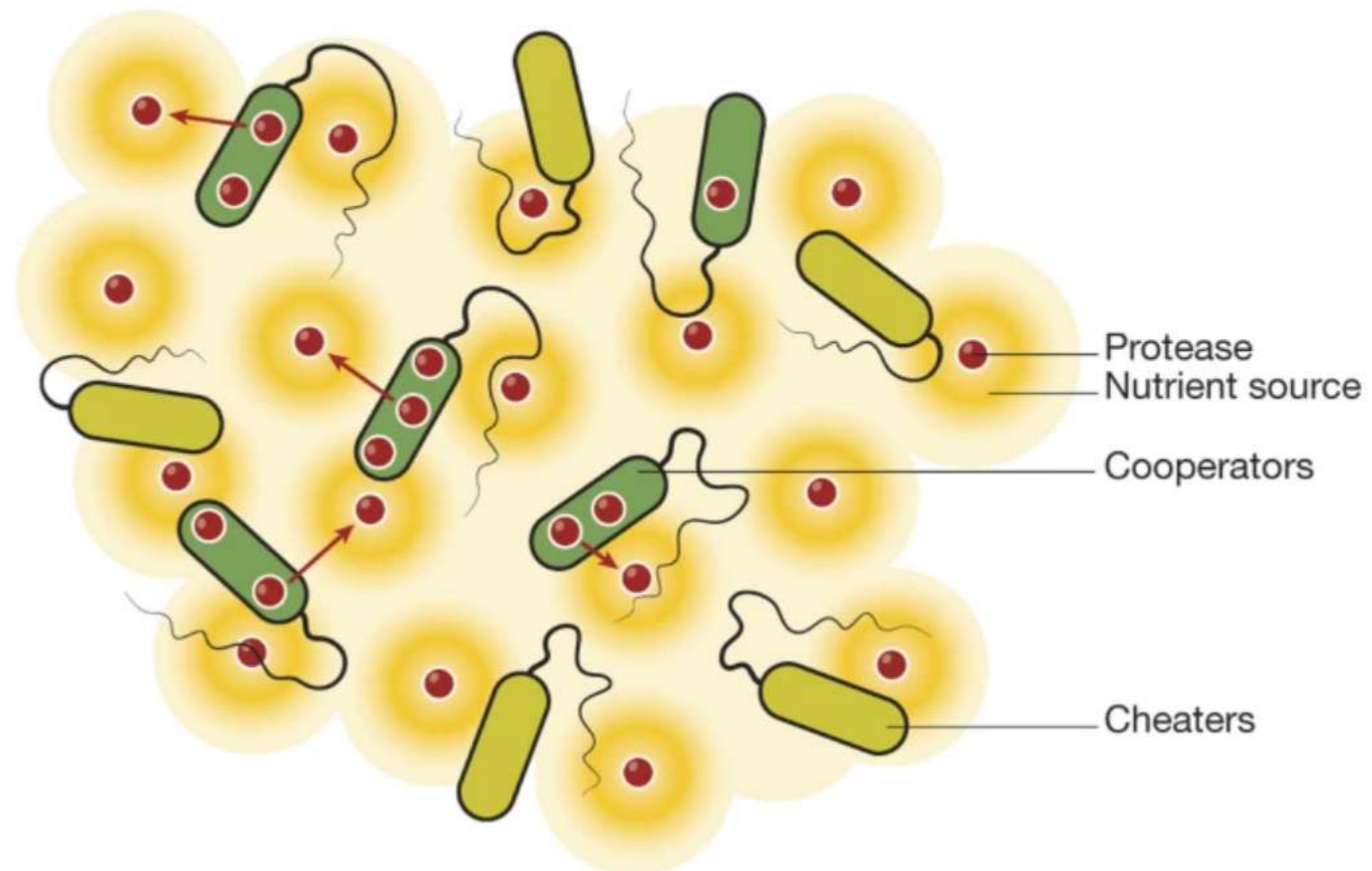
High population density; high concentration of AHL

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Social cheating & Social policy, I

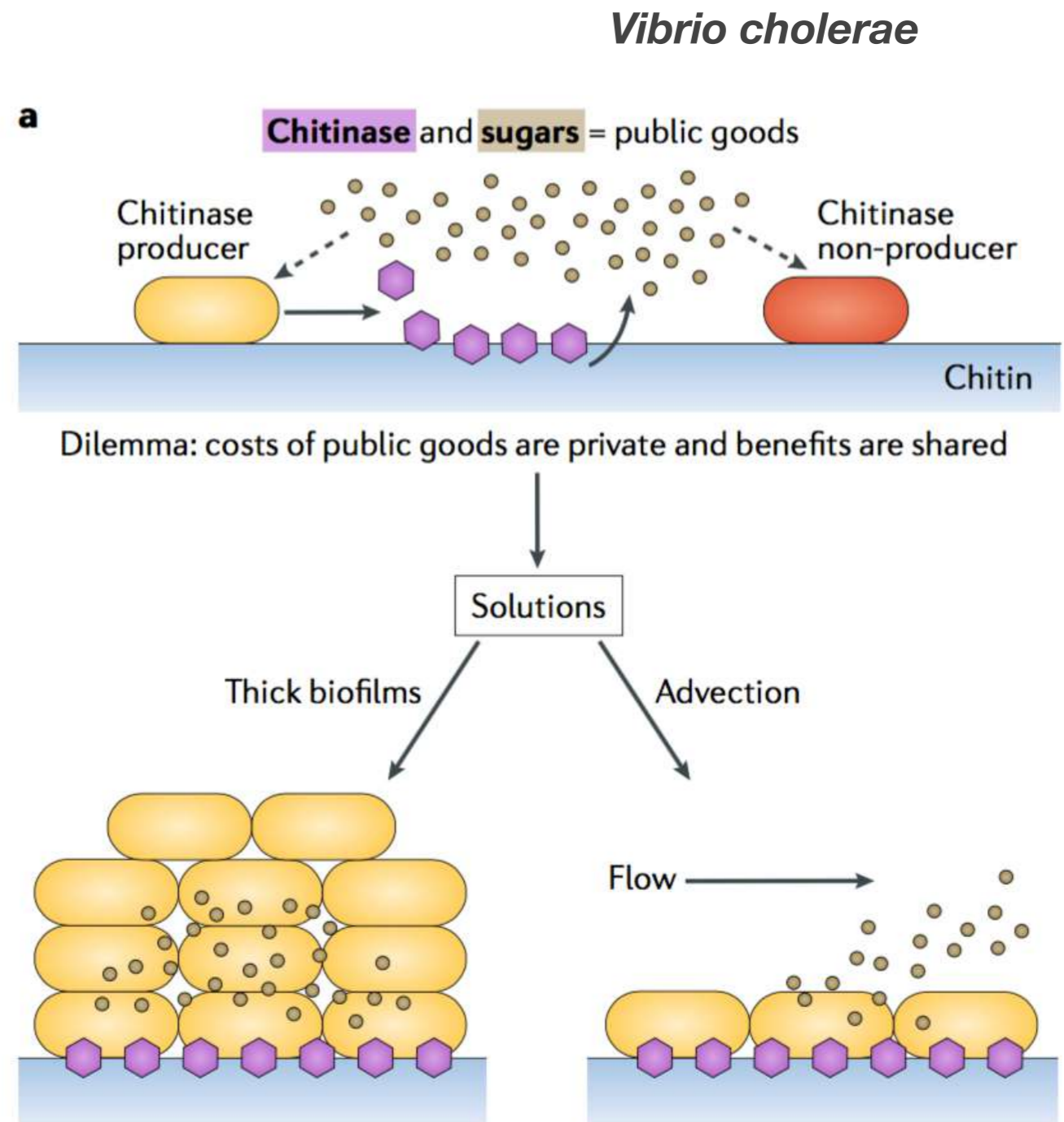
- **Mutants** evolve naturally within communities → **social cheating not sharing energetic costs of producing molecules**
- **Cooperative behaviours provide a collective benefit**, but are considered **costly** for the **individual**
- Bacteria frequently secrete **extracellular biomolecules** to capture 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.



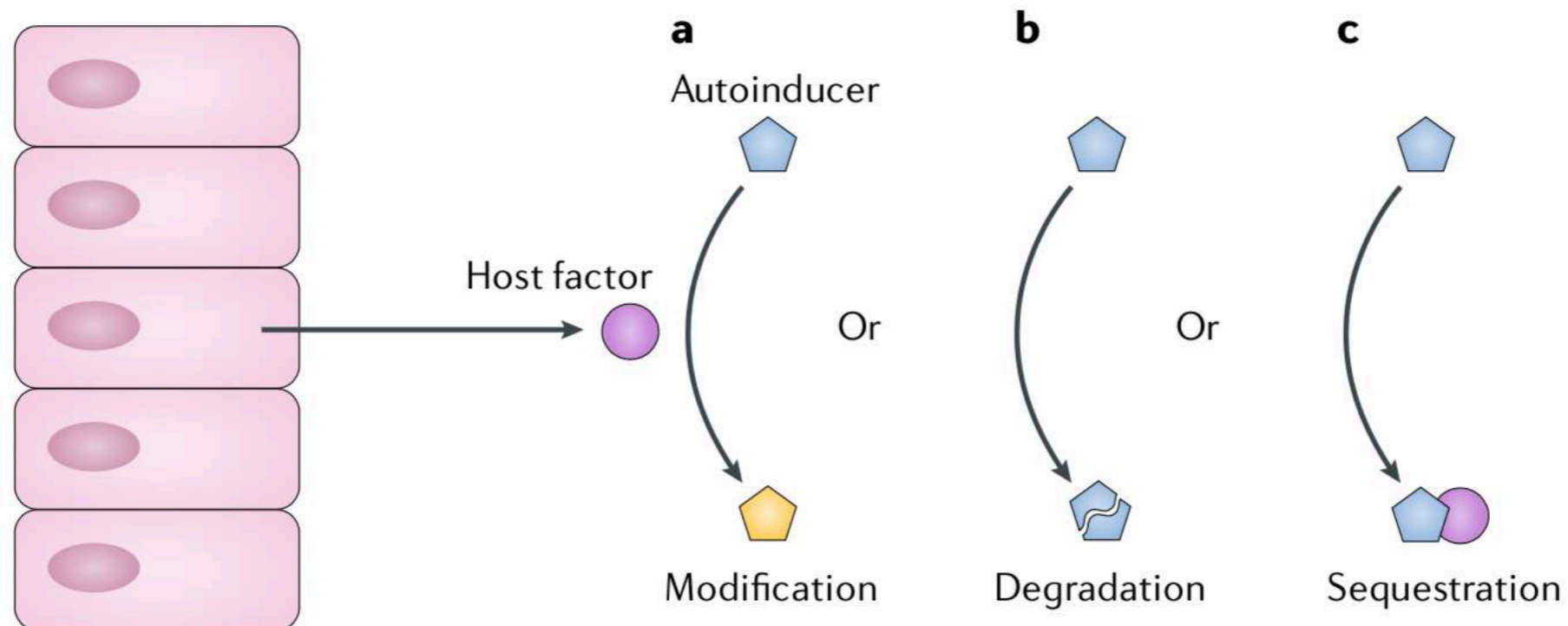
Social cheating & Social policy, II

- **Quorum-sensing-driven co-regulation of two metabolic enzymes.** One serves as a **public good** and the other 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)

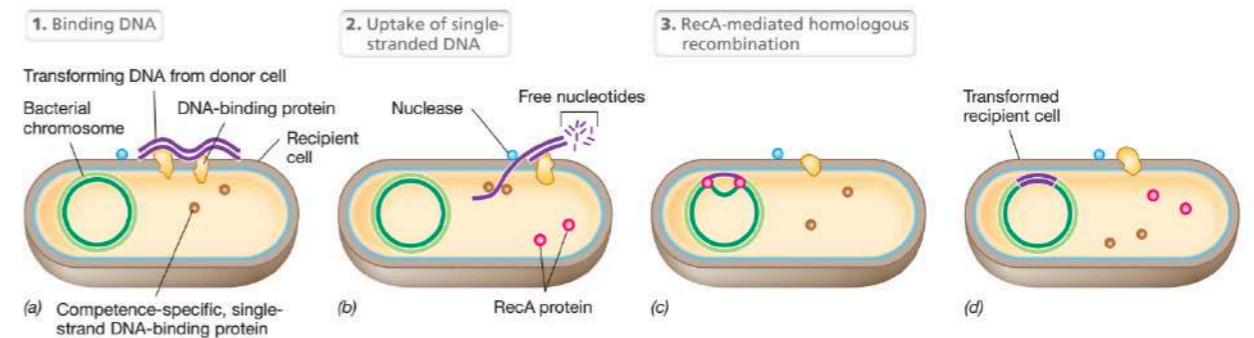
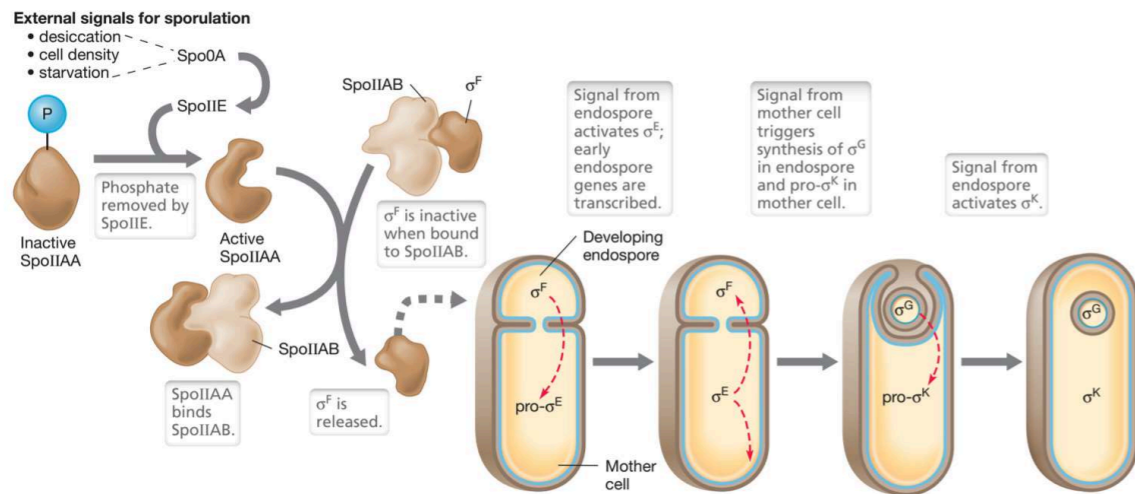


Quorum Quenching

- 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



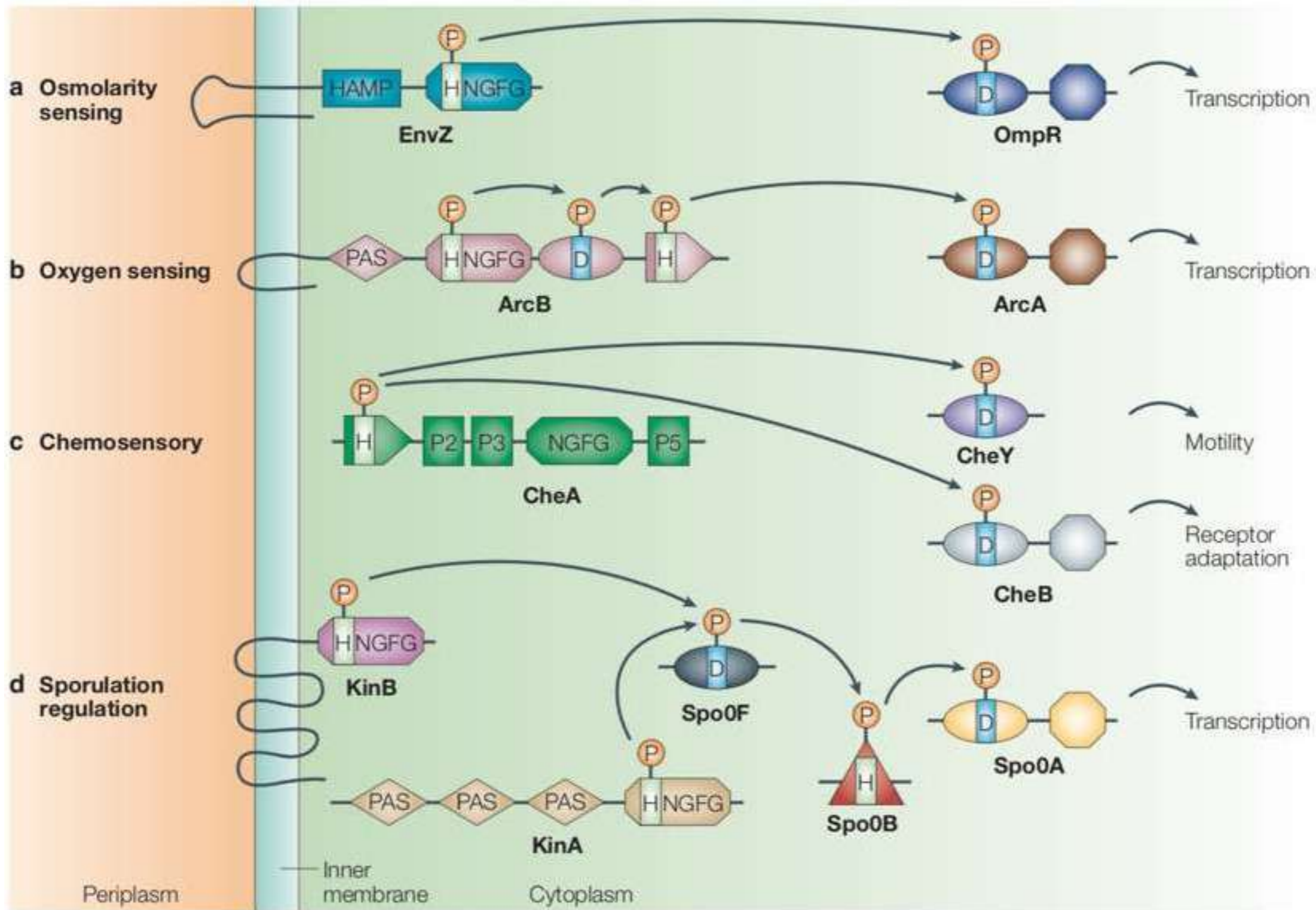
Quorum sensing *in Gram +*



Madigan et al. 2020

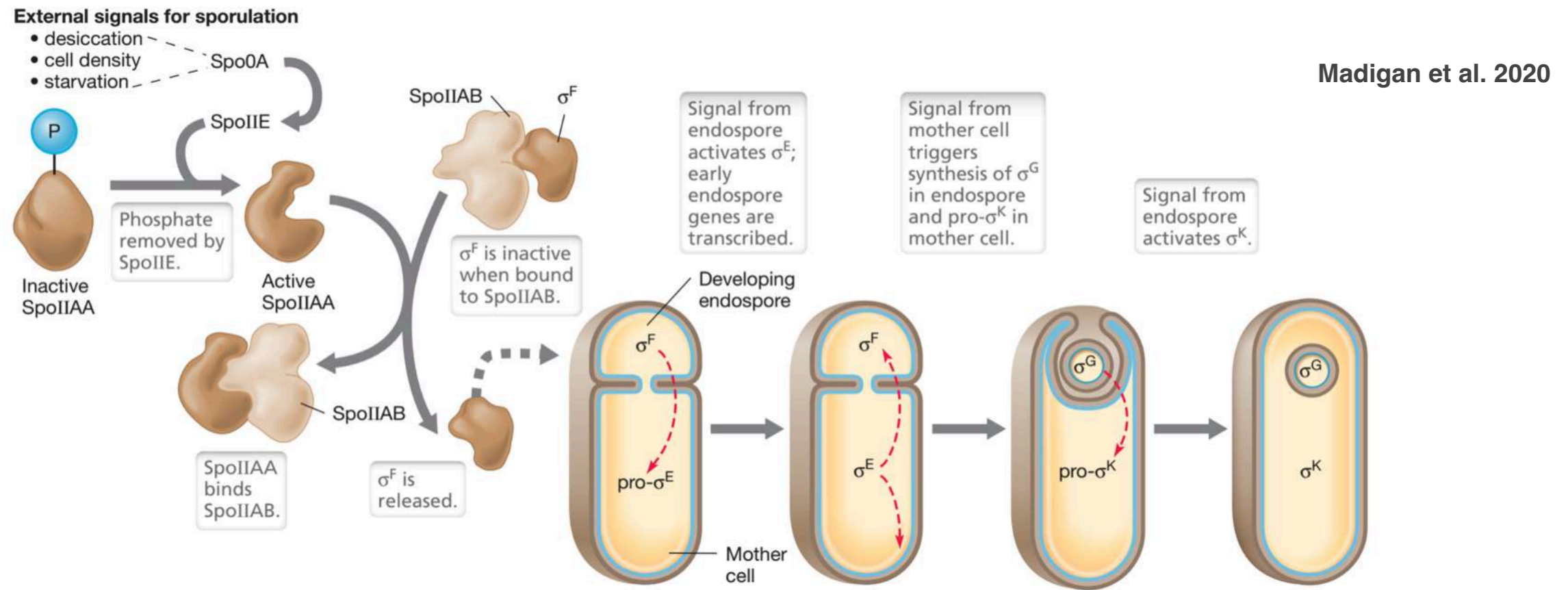
- QS two-component system
- Sporulation —> Endospore formation as **response to adverse conditions** (starvation, desiccation, growth-inhibitory temperatures)
- DNA competence
- Regulation of pathogenicity
- **Pheromones ComX, competence**
- **Pheromones CSF, sporulation**

Two-component systems



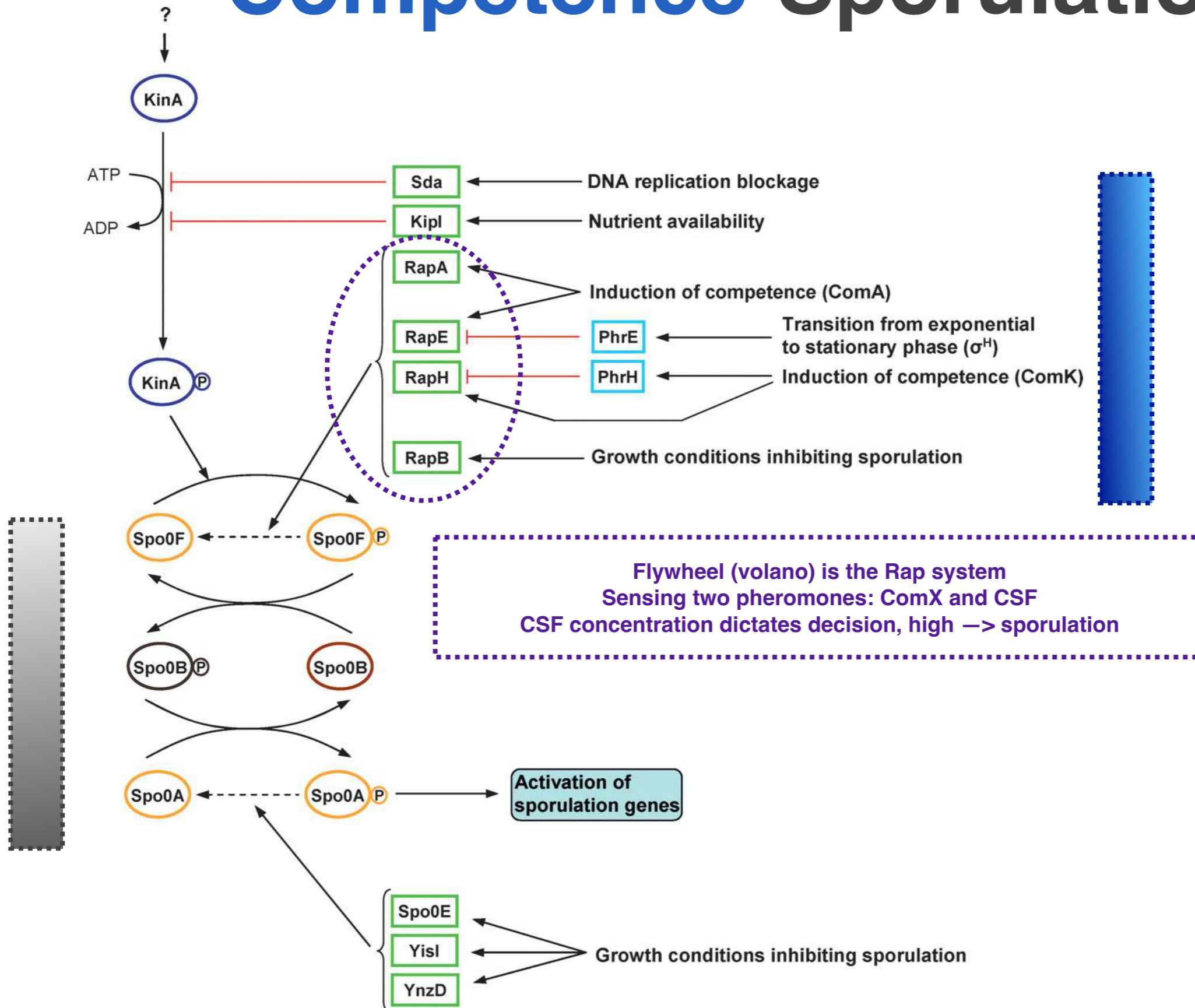
- Phototaxis

Bacillus subtilis: Sporulation



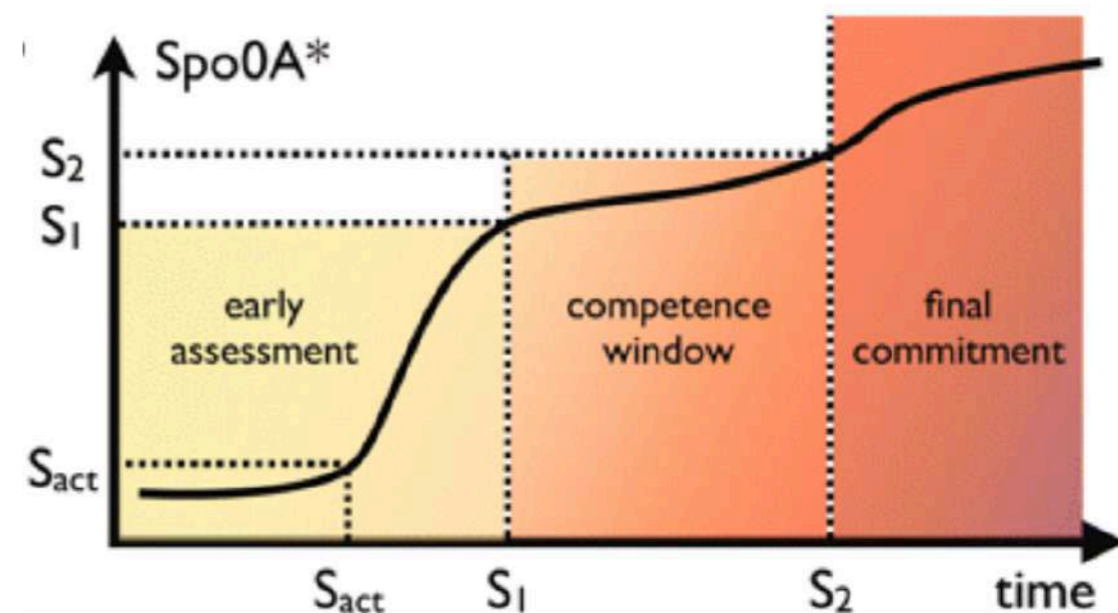
- Endospore formation as **response to adverse conditions** (starvation, desiccation, growth-inhibitory temperatures)
- Spore germinates when favorable conditions return
- Prior to endospore formation, **cell divides asymmetrically** —> smaller cell develops into the endospore
- Mother cell surrounds 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



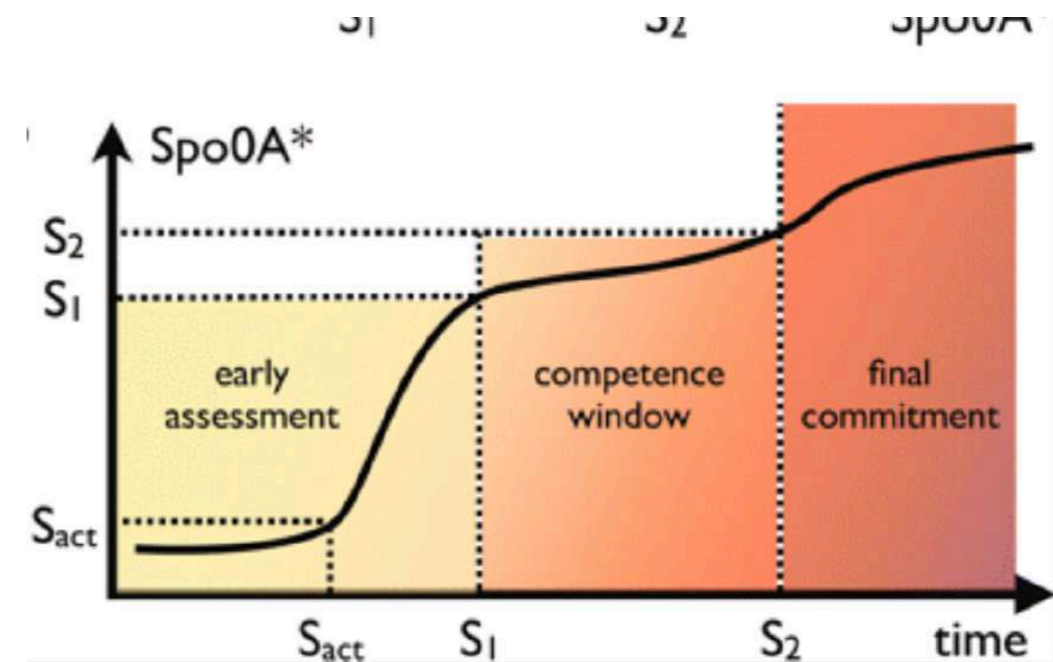
Decision making: competence vs sporulation, I

- *B. subtilis* monitors its environment via **5 two-component systems**
- 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**



Decision making: competence vs sporulation, II

- **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**



Biofilm definition

Biofilms can broadly be defined as dynamic **self-constructed accumulations of microorganisms that produce a matrix of extracellular**

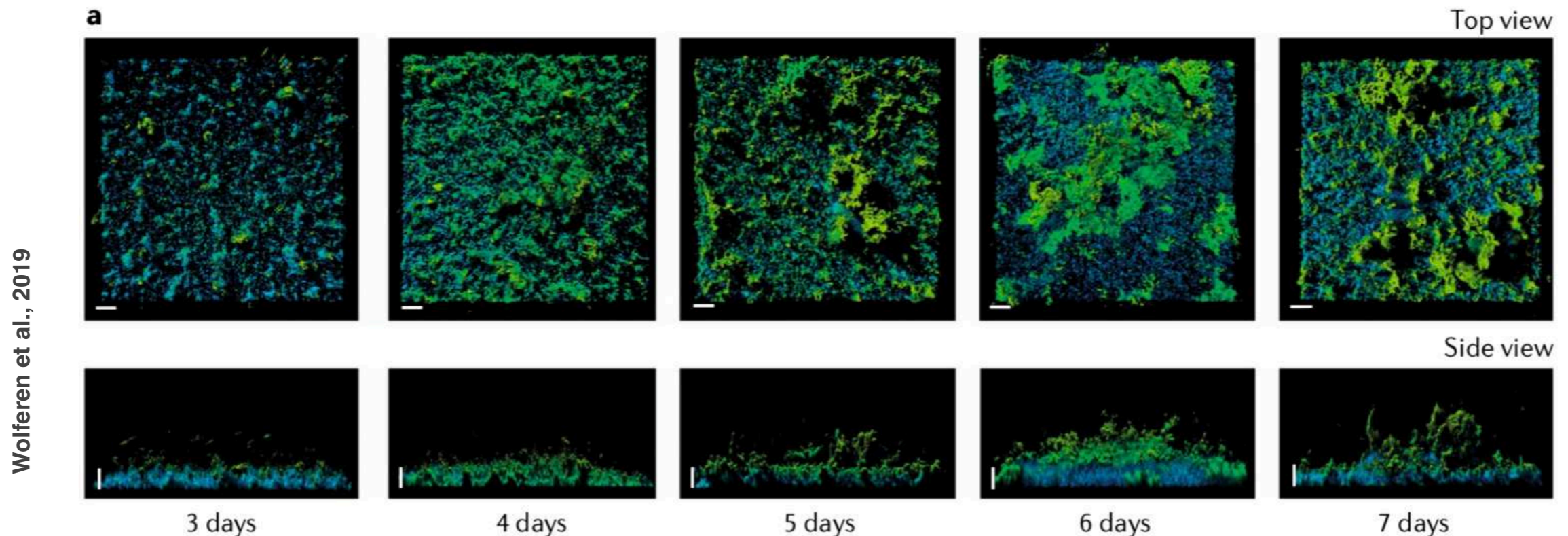
biopolymers that is, extracellular polysaccharides (EPSs)

The **collective behaviour** of bacteria within biofilms promotes **communication and interaction to ensure propagation and survival**

Microbial abundance ranging from 10^8 to 10^{11} cells g^{-1} wet weight

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 microbial 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)



Biofilm, II

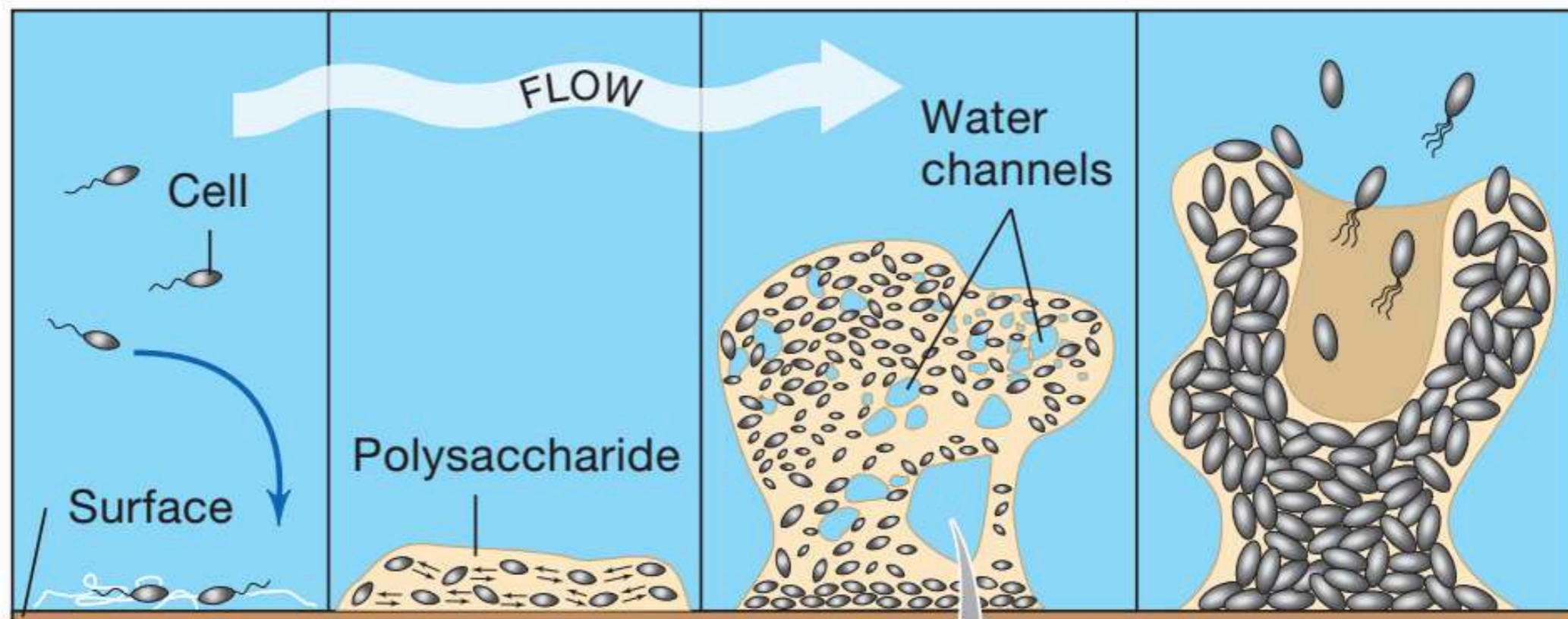
- Biofilms form in stages: (1) attachment, (2) colonization, (3) development, (4) dispersal
- **Very dynamic, very diverse**

Attachment
(adhesion of a few motile cells to a suitable solid surface)

Colonization
(intercellular communication, growth, and polysaccharide formation)

Development
(more growth and polysaccharide)

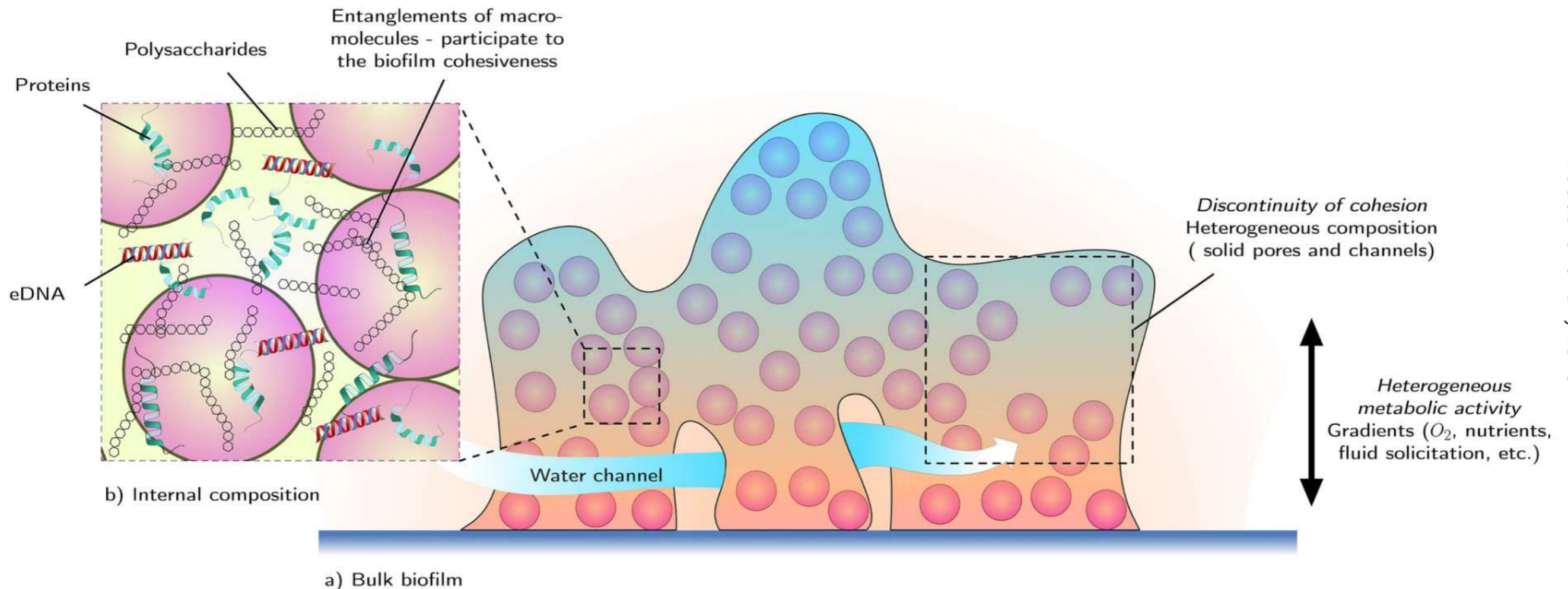
Active Dispersal
(triggered by environmental factors such as nutrient availability)



(a)

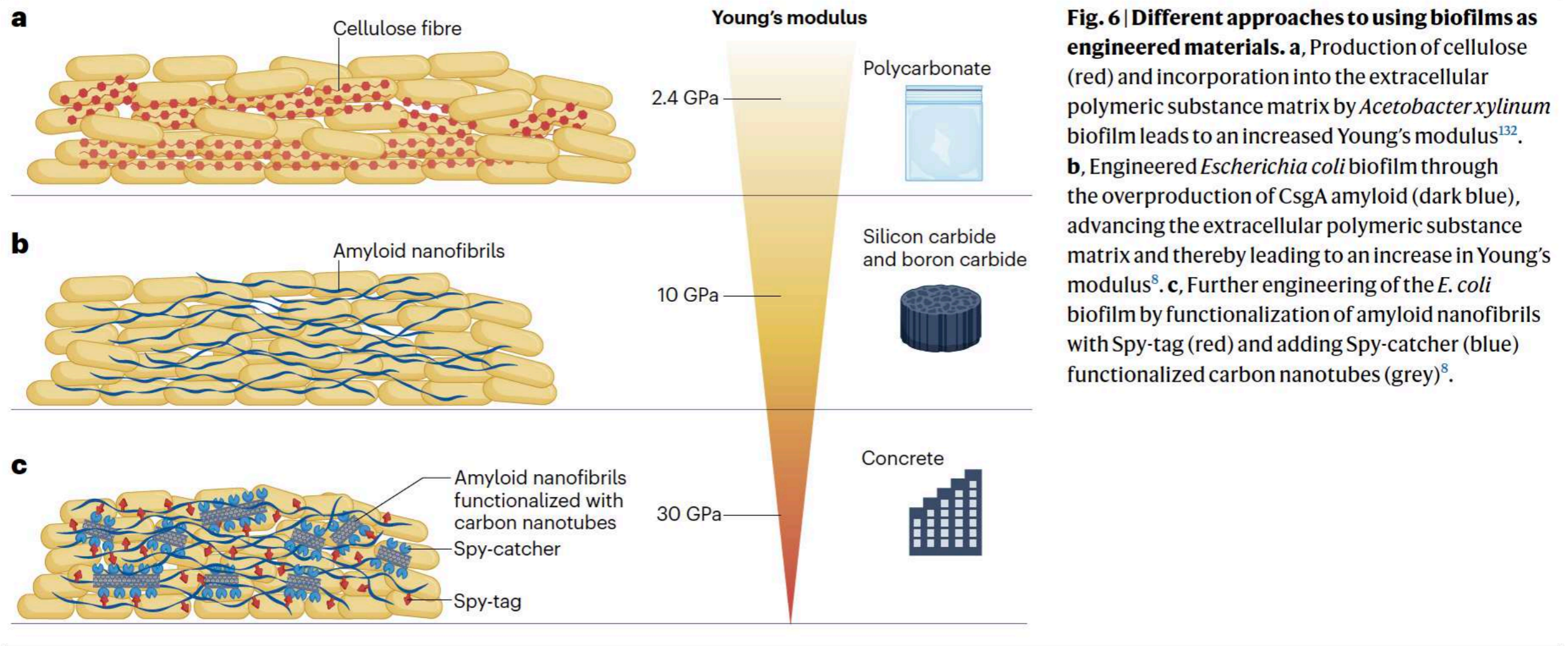
Biofilm as a heterogeneous microenvironment

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.cholerae*.: RbmA, Bap1, and RbmC) structure networks among polymeric substances and cells

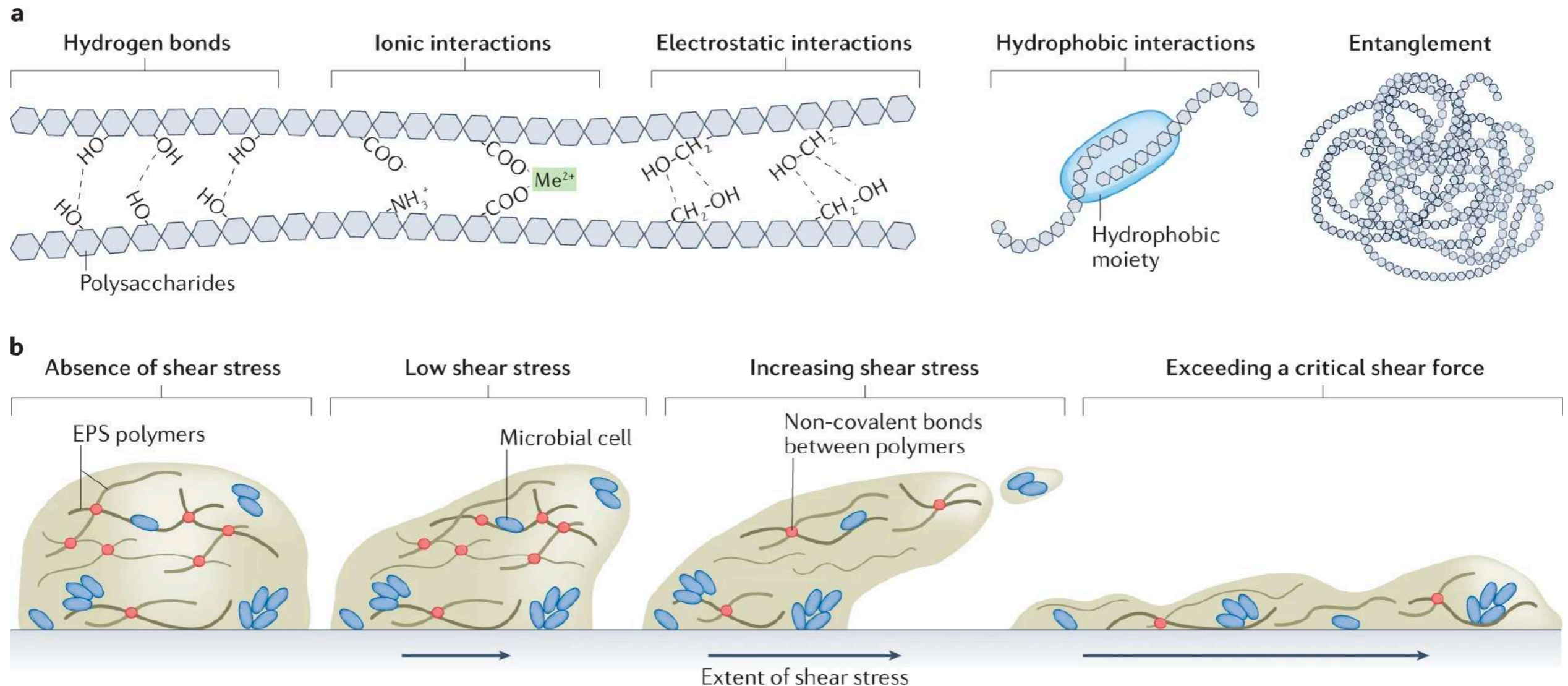
Biofilm as a viscoelastic material



- **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

Mechanical properties of the biofilm matrix

Extracellular Polymeric Substances (EPS)

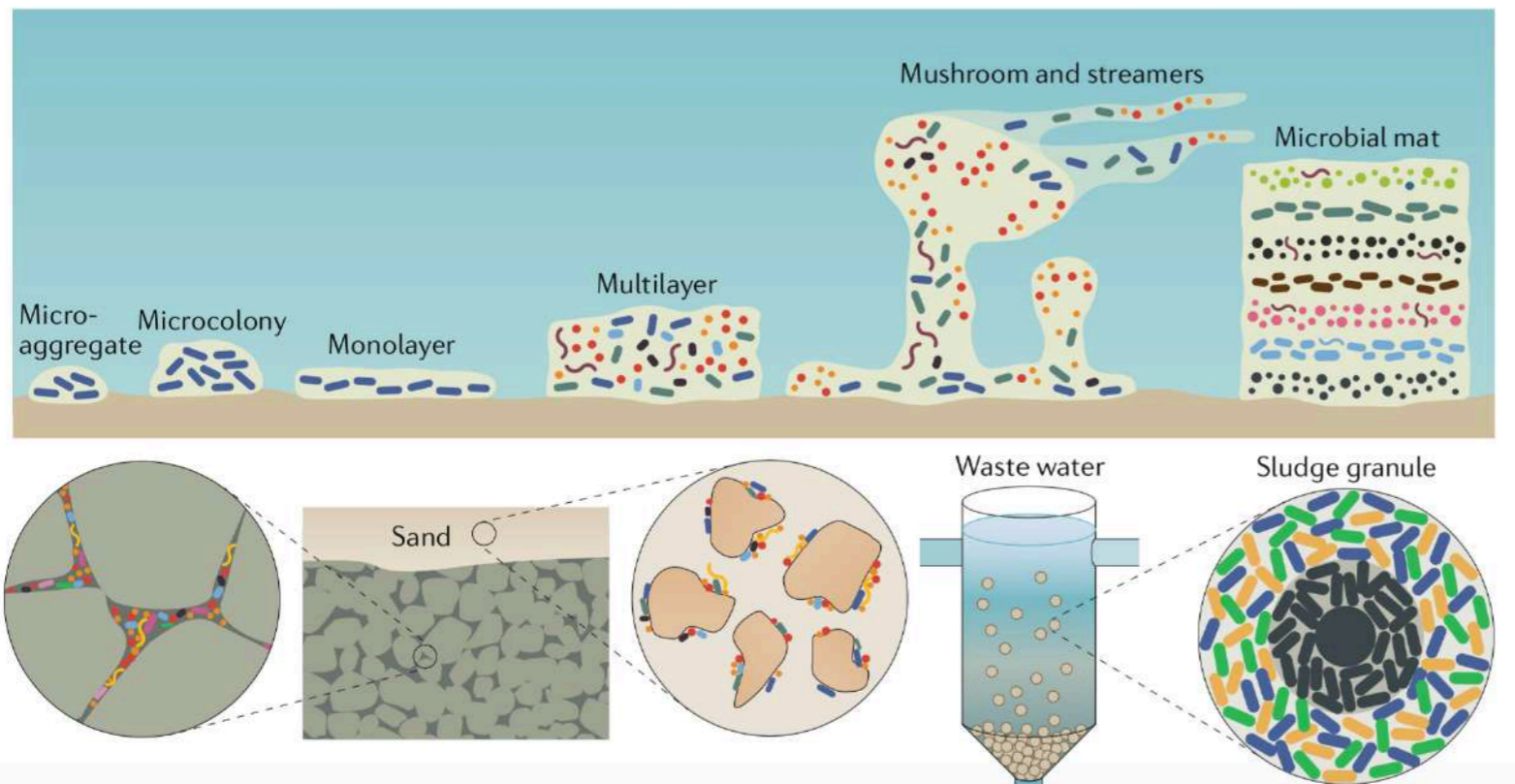


Flemming et al., 2023

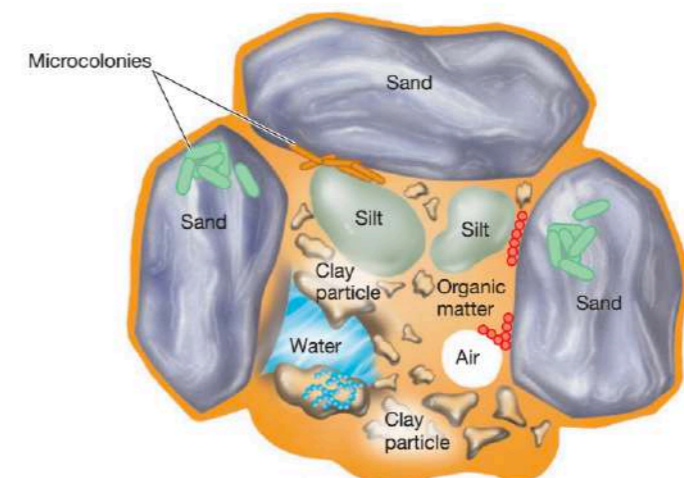
- In the absence of stress, the biofilm structures are undisturbed
- At low shear stress, the biofilm can immediately stretch out and spring back when the stress is removed, which is mediated by intermolecular forces among matrix components → viscoelastic solid behaviour
- As shear increases, the biofilm 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

Biofilm, III

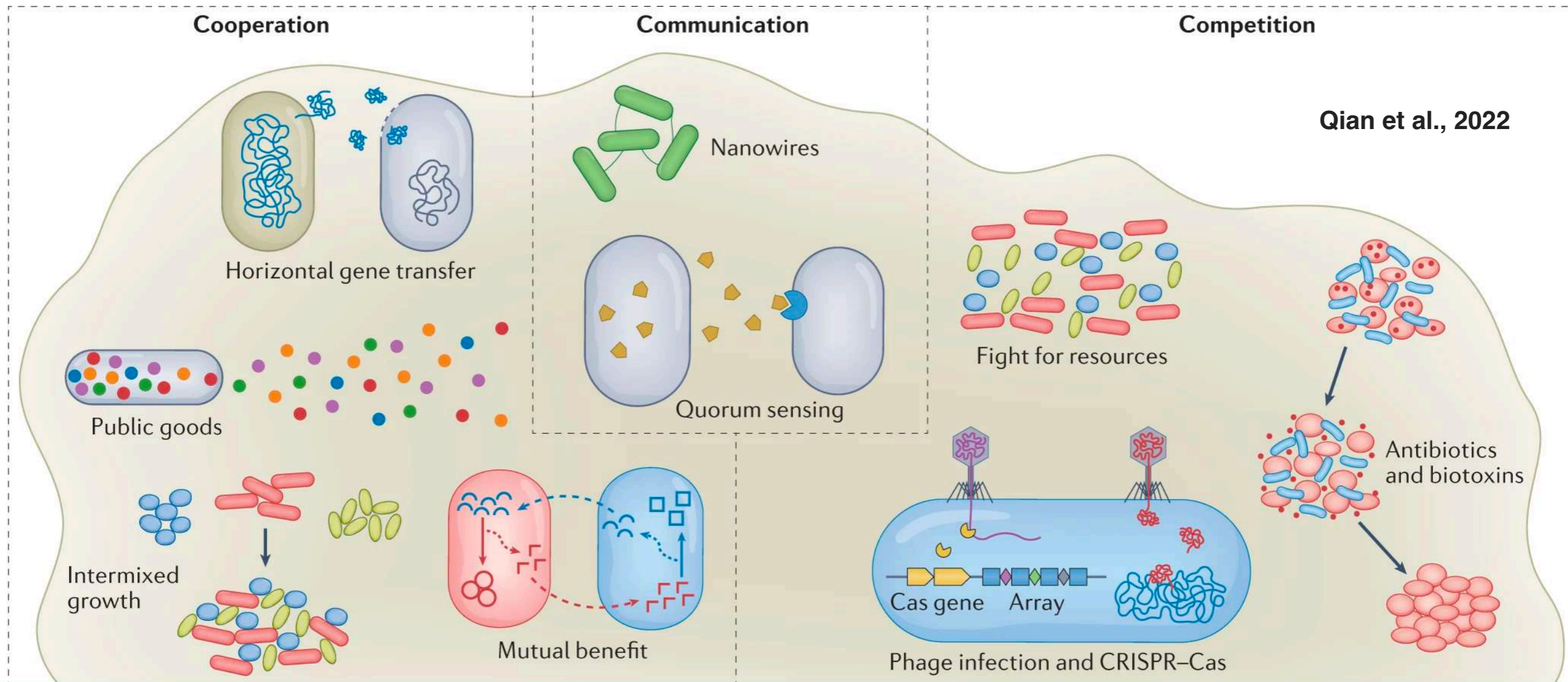
- **Viscous** structure **3D** environment is formed in **synergy with the flow**
- Viscous structured 3D environment **prevents harmful** chemicals (e.g. antibiotics or other toxic substances) from penetrating → **pathogens** (e.g. artificial heart valves and joints, and indwelling devices, such as 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 H₂S



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

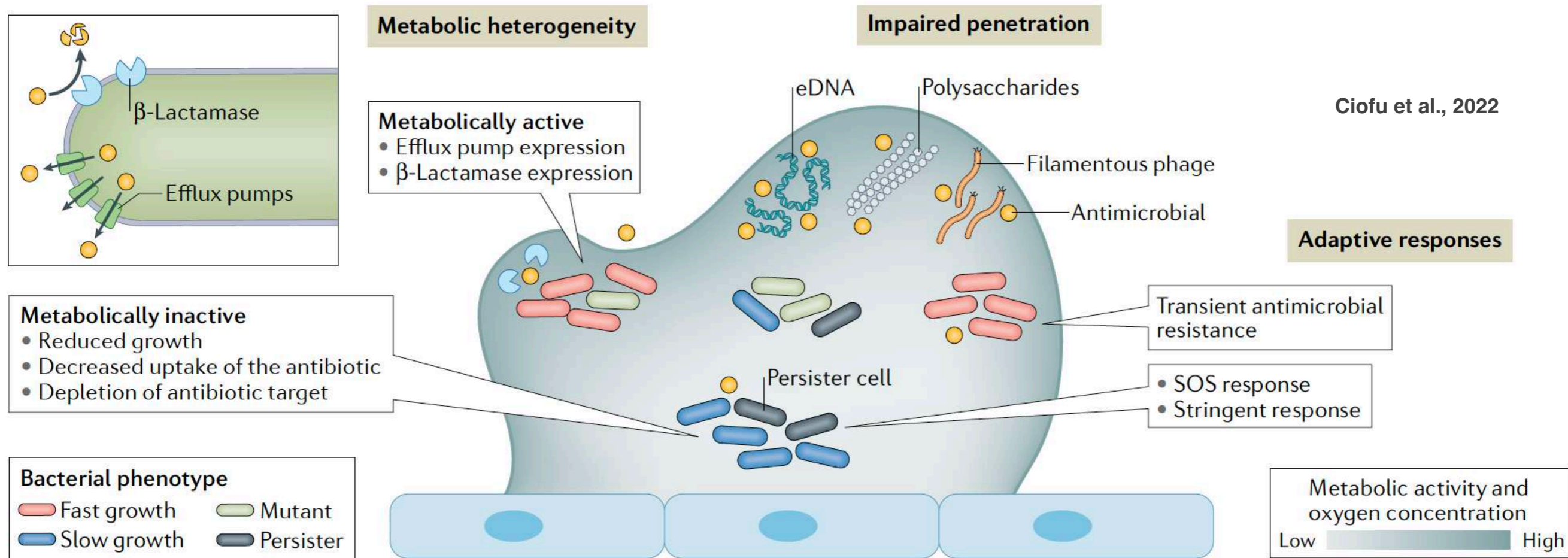


Microbial interactions in biofilms



- 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

The mechanisms of antimicrobial tolerance of a biofilm



Tolerance/Persistence

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

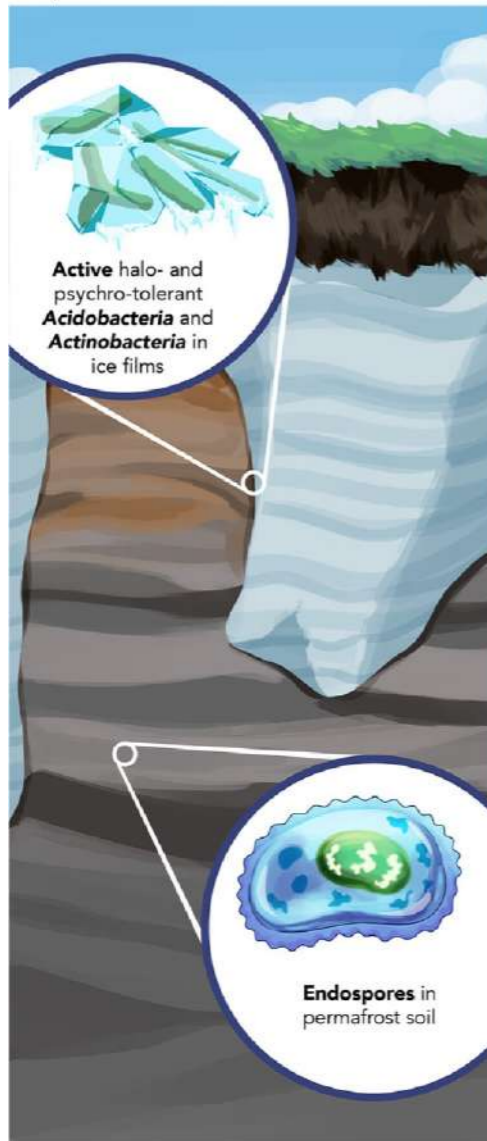
Resistance

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

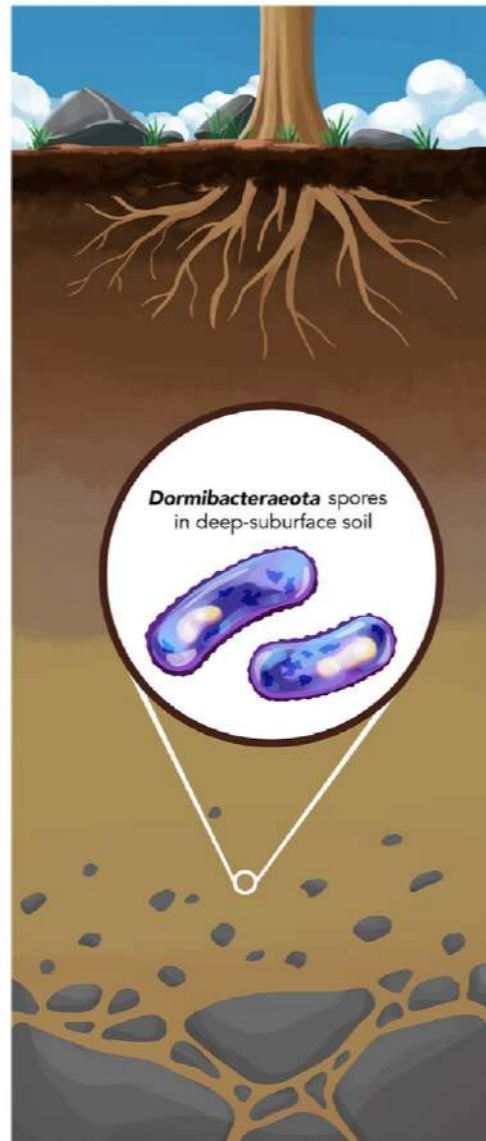
Dormancy

a temporary, adaptive, state of reduced metabolic activity within an extended period of arrested growth that enables a microbe to maintain viability under unfavorable environmental conditions

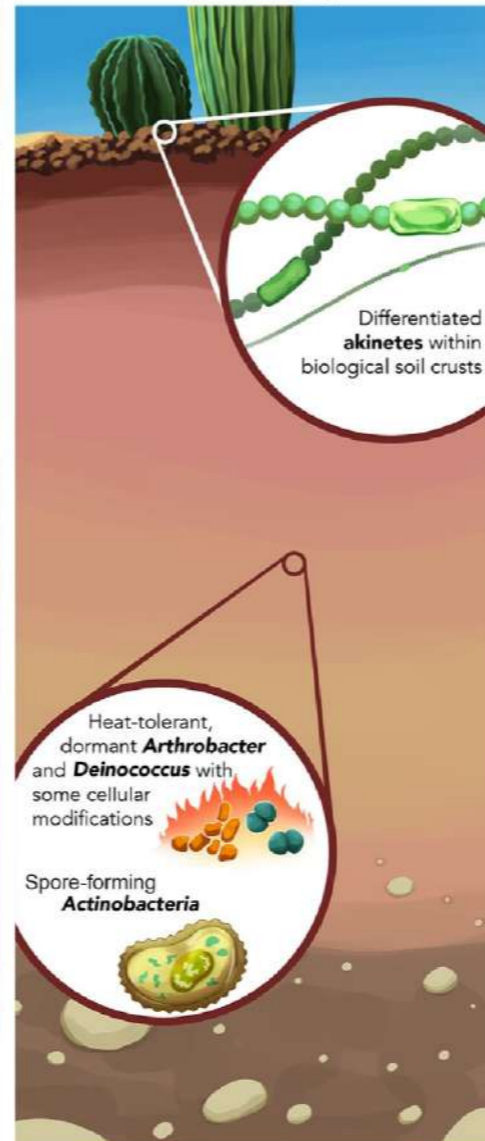
(A) permafrost



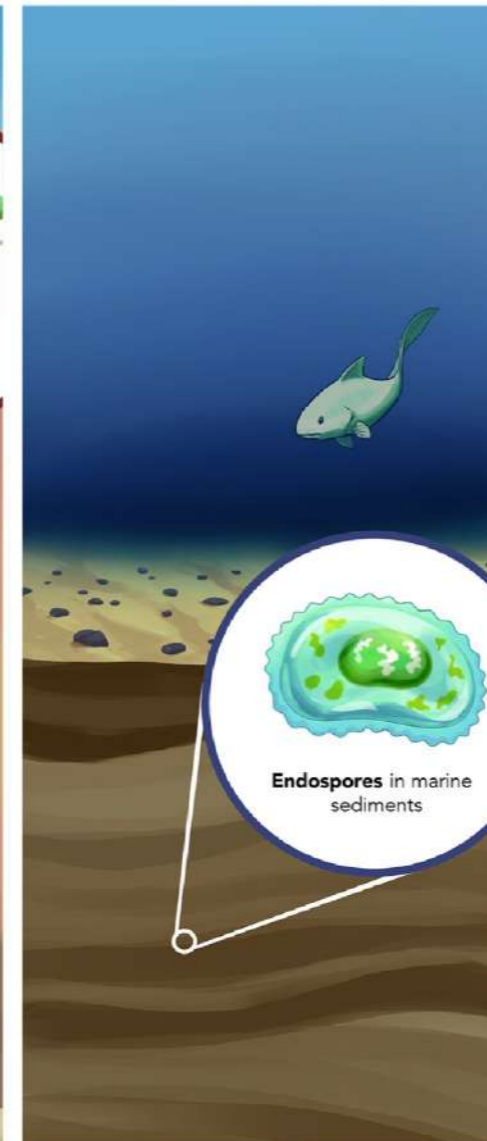
(B) deep subsurface soil



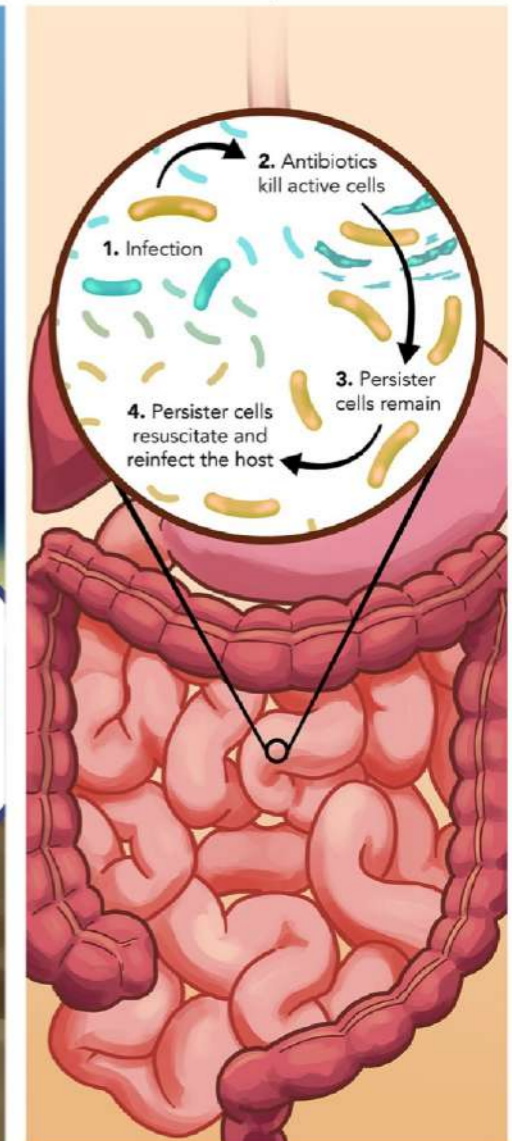
(C) arid and desert ecosystems



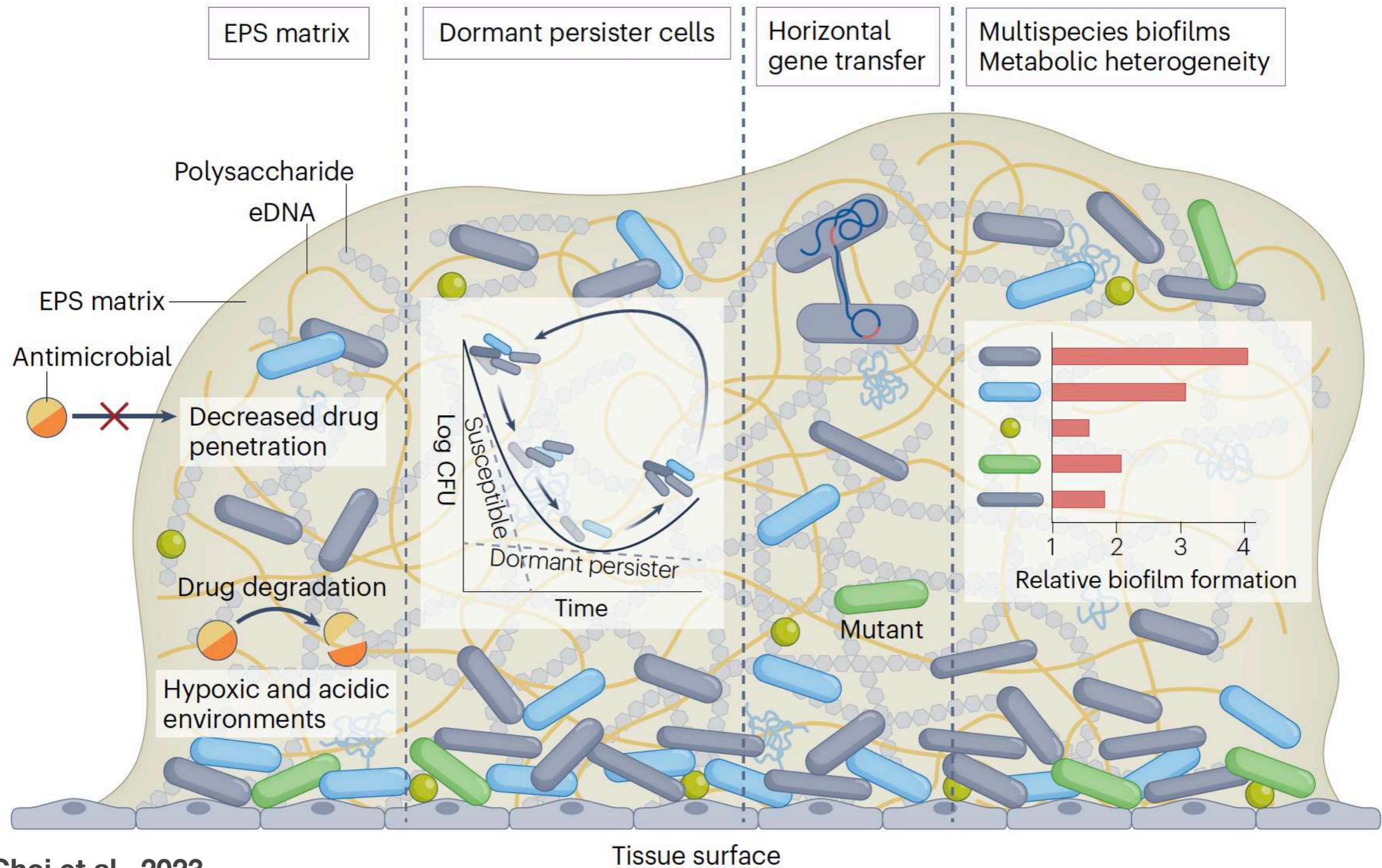
(D) marine sediments



(E) the human body

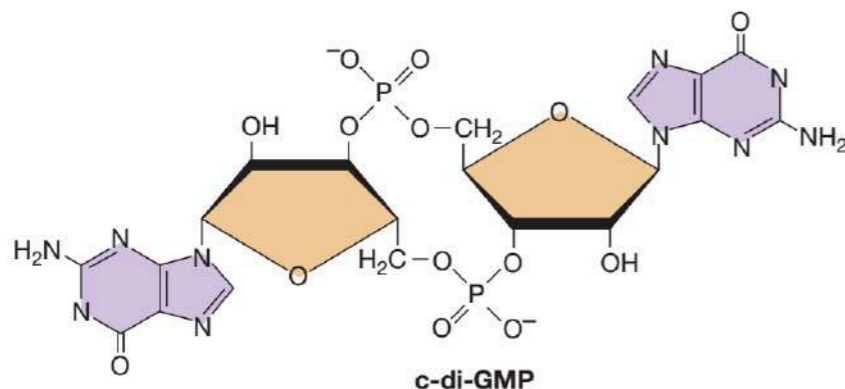


Diverse adaptive strategies in the biofilm



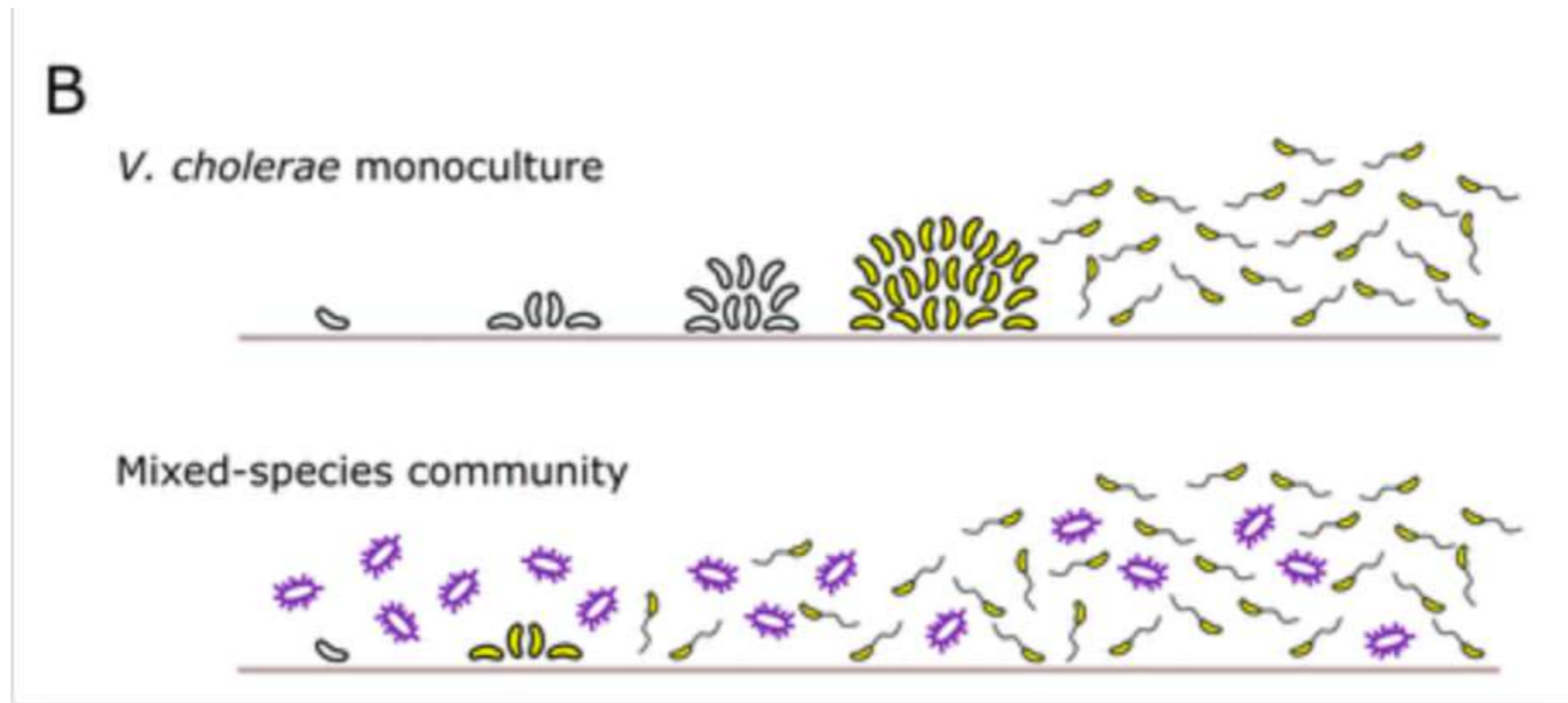
Biofilm formation

- **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**

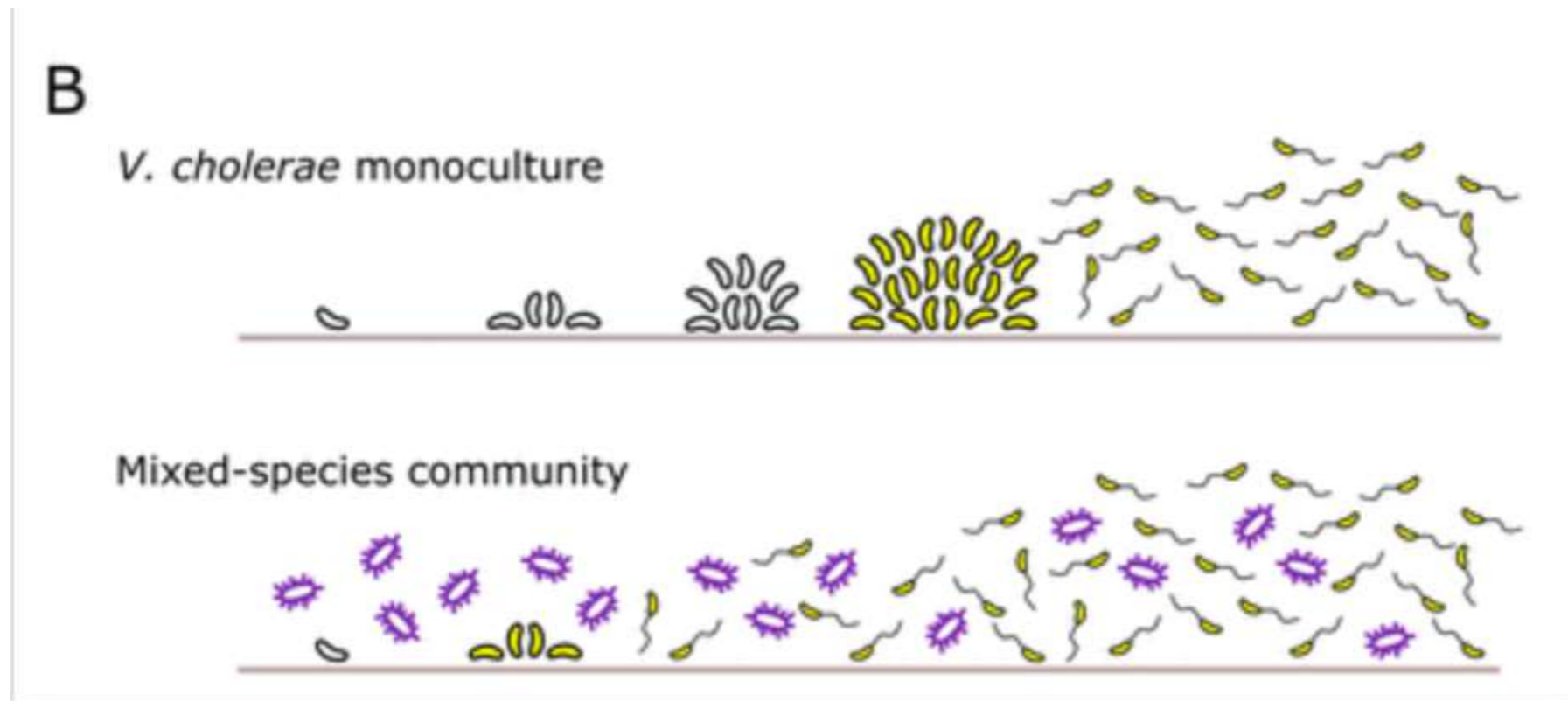
Biofilm formation and dispersal: *V. cholerae*



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

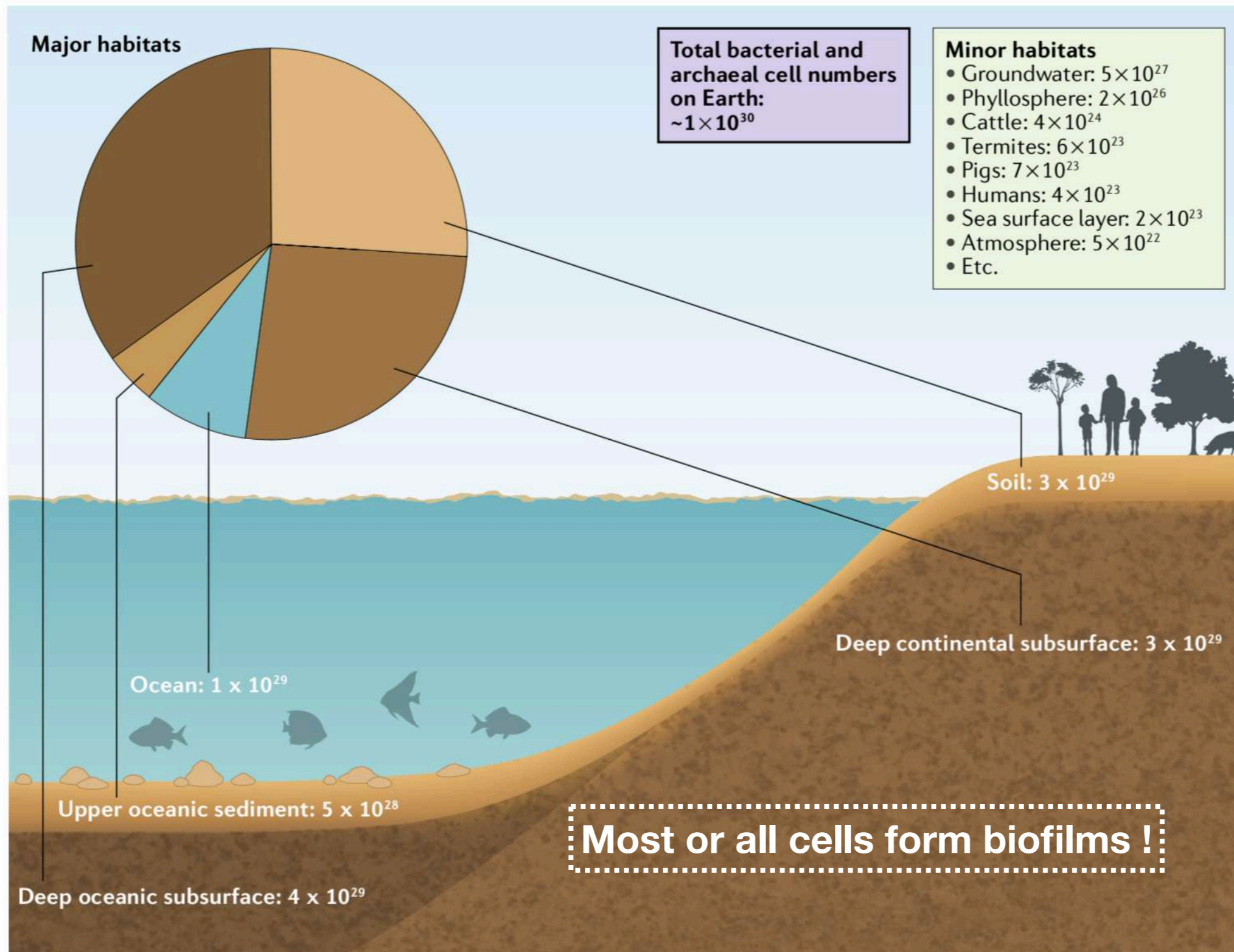
Biofilm formation and dispersal: *V. cholerae*



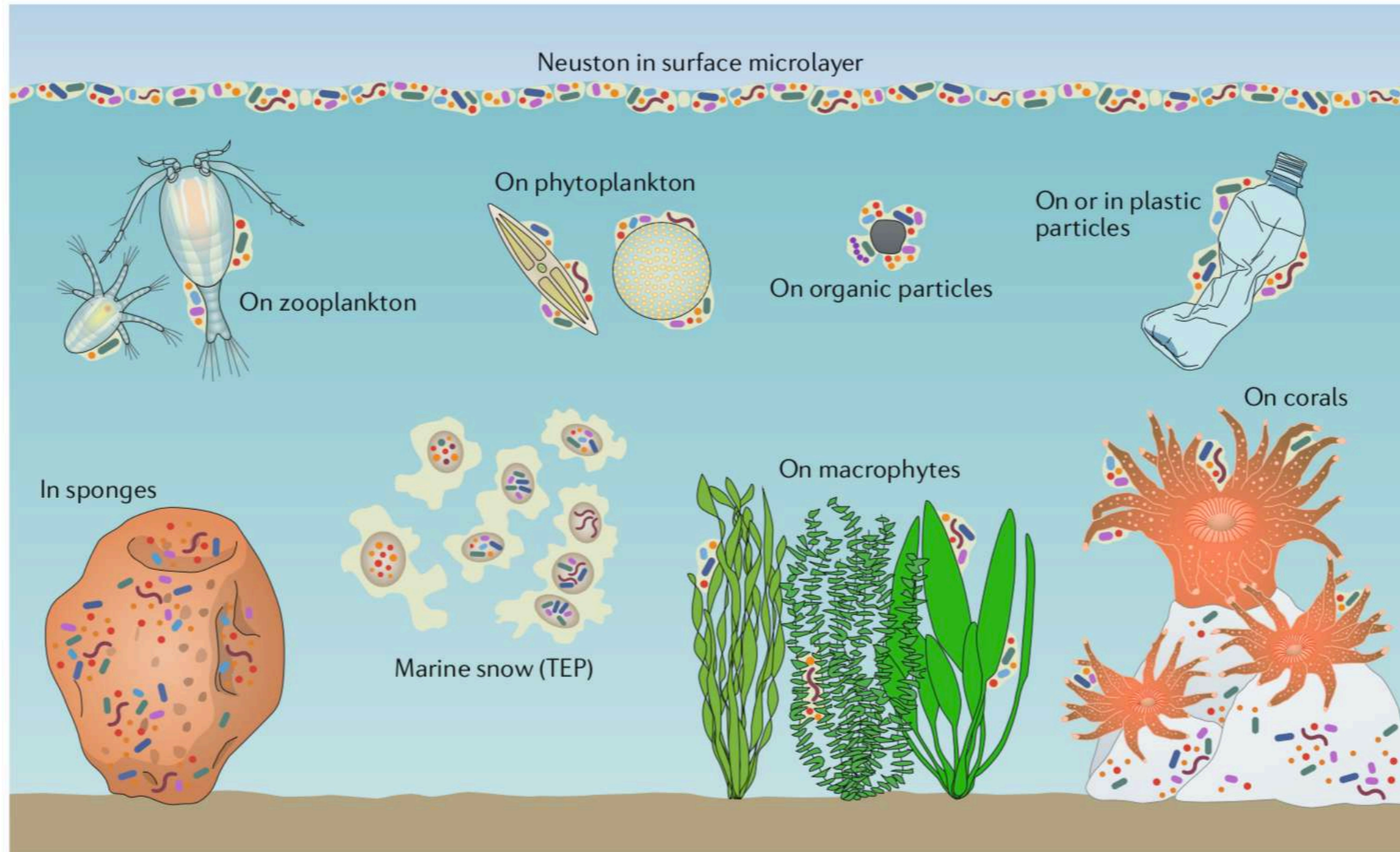
Bridges & Bassler, 2020

- **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

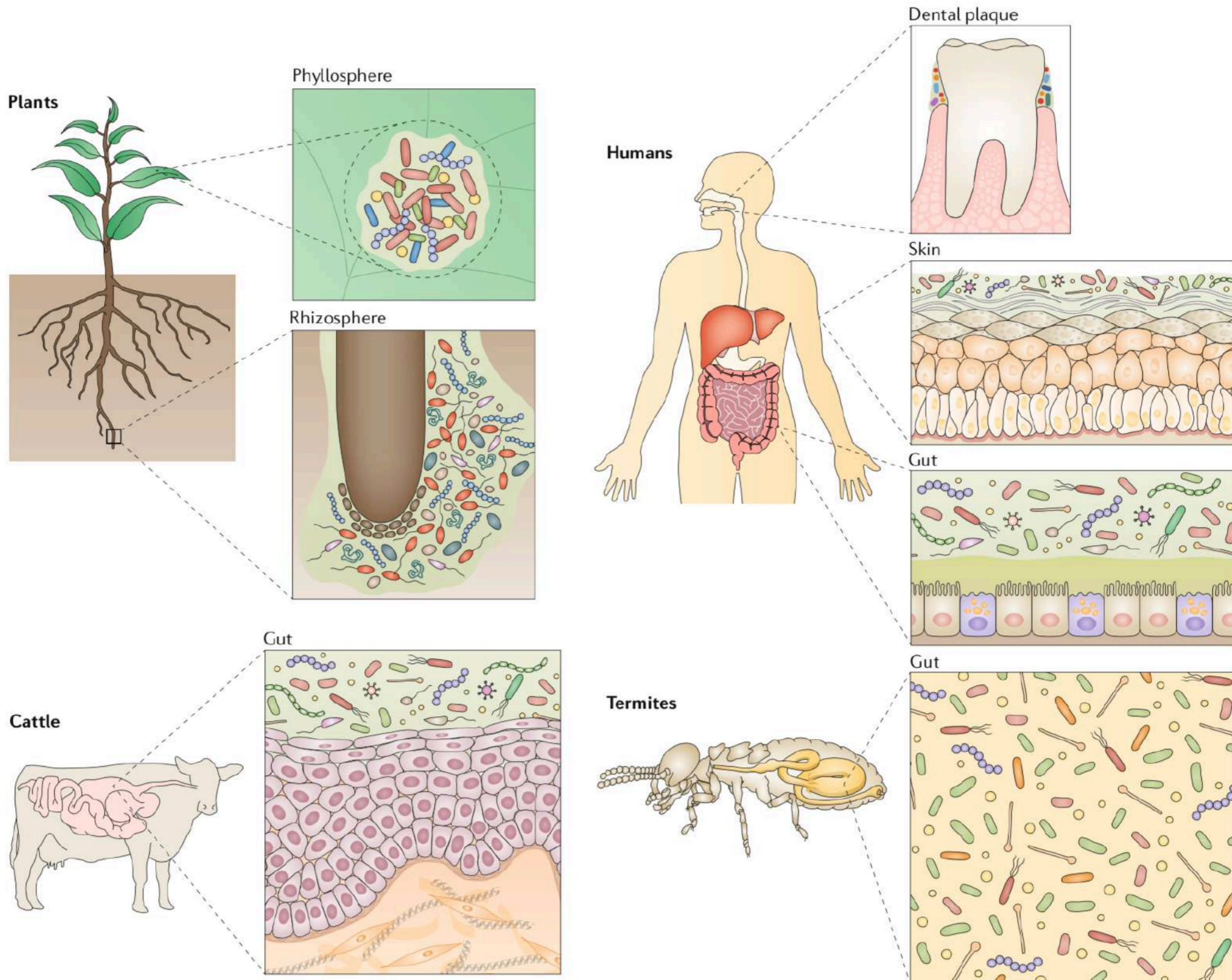


Marine biofilms

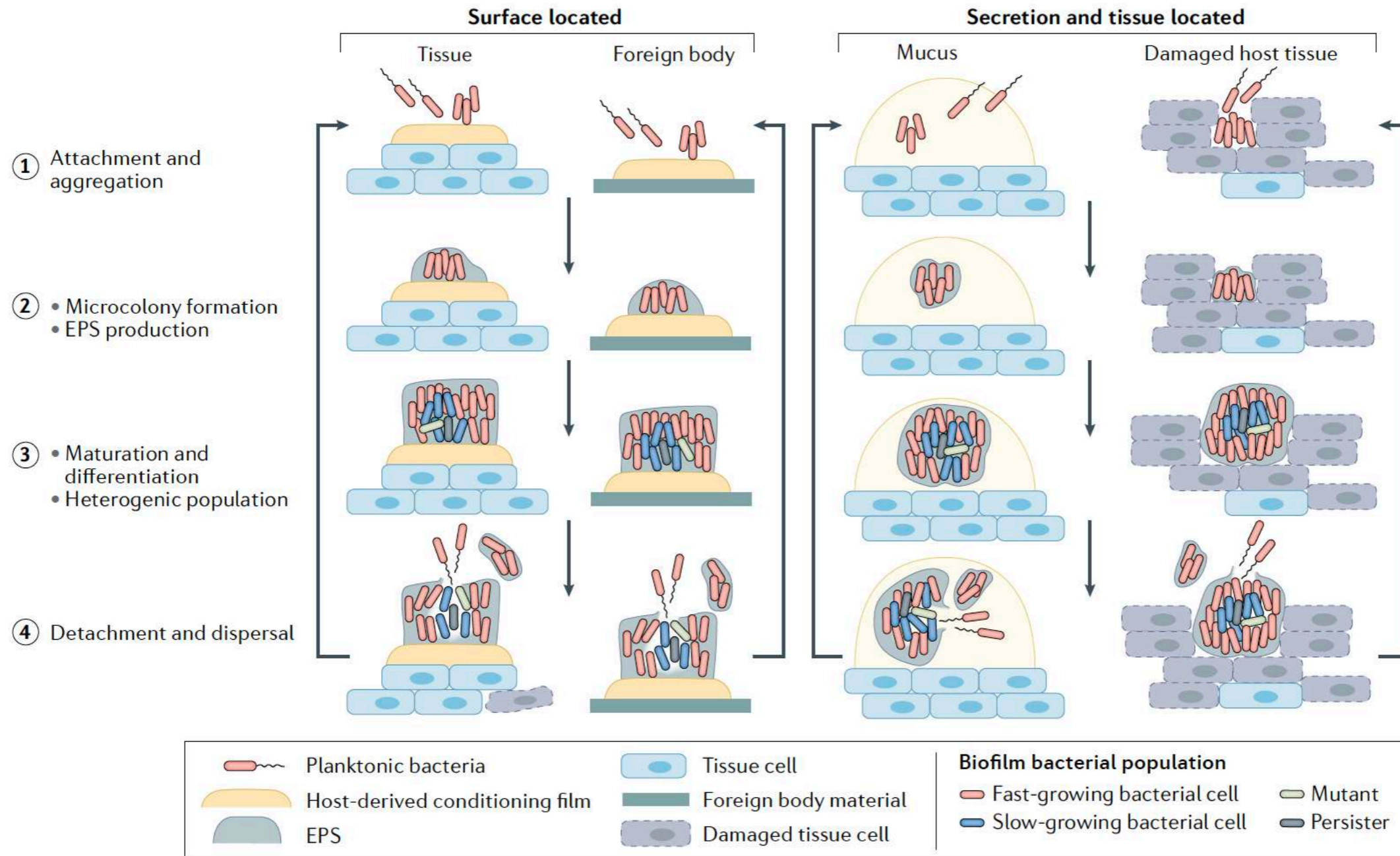


Molecular coating and living or not surfaces provide 3D structure for biofilm formation

Eukarya as microbial biofilm

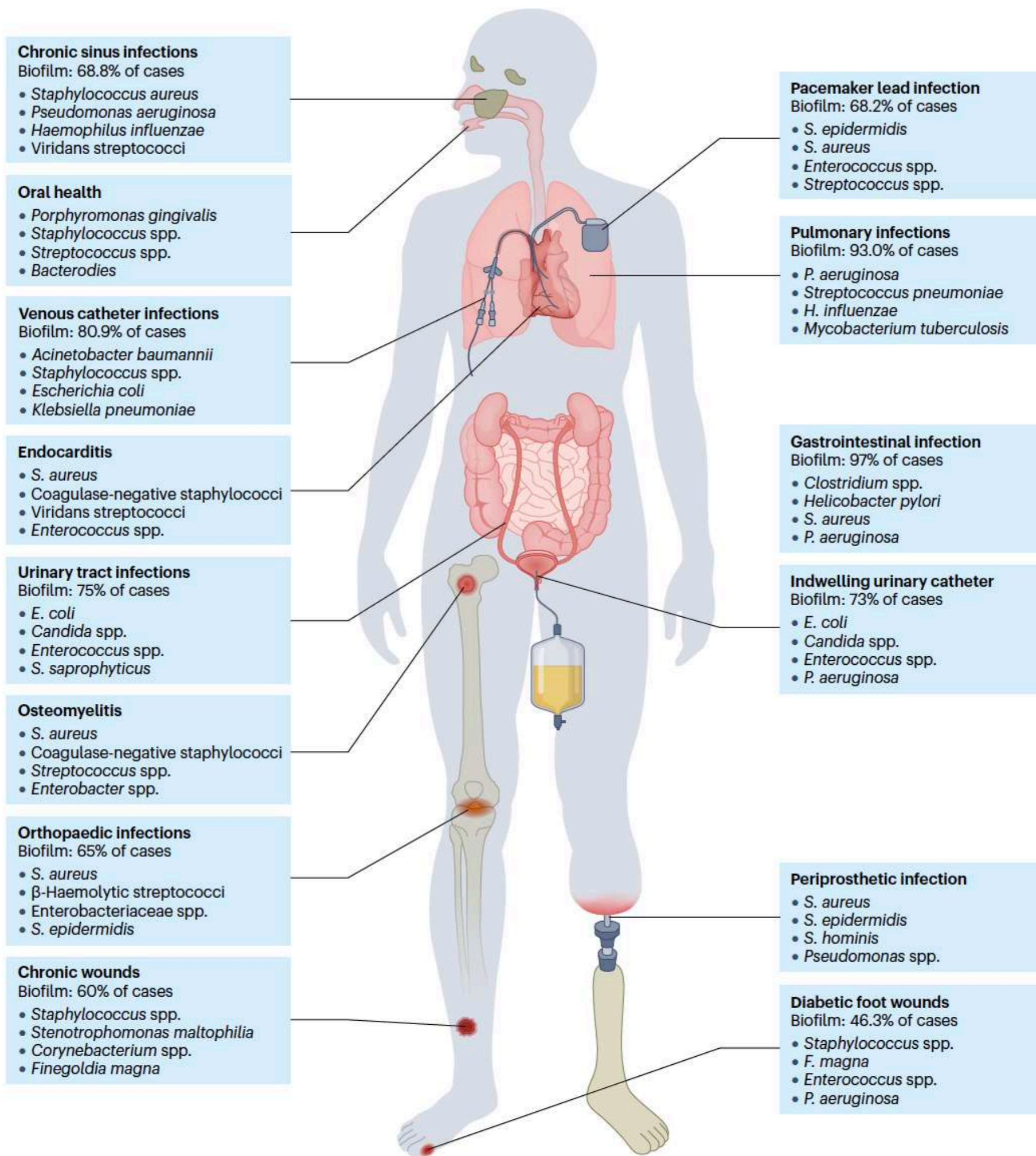


Biofilm types in Eukarya

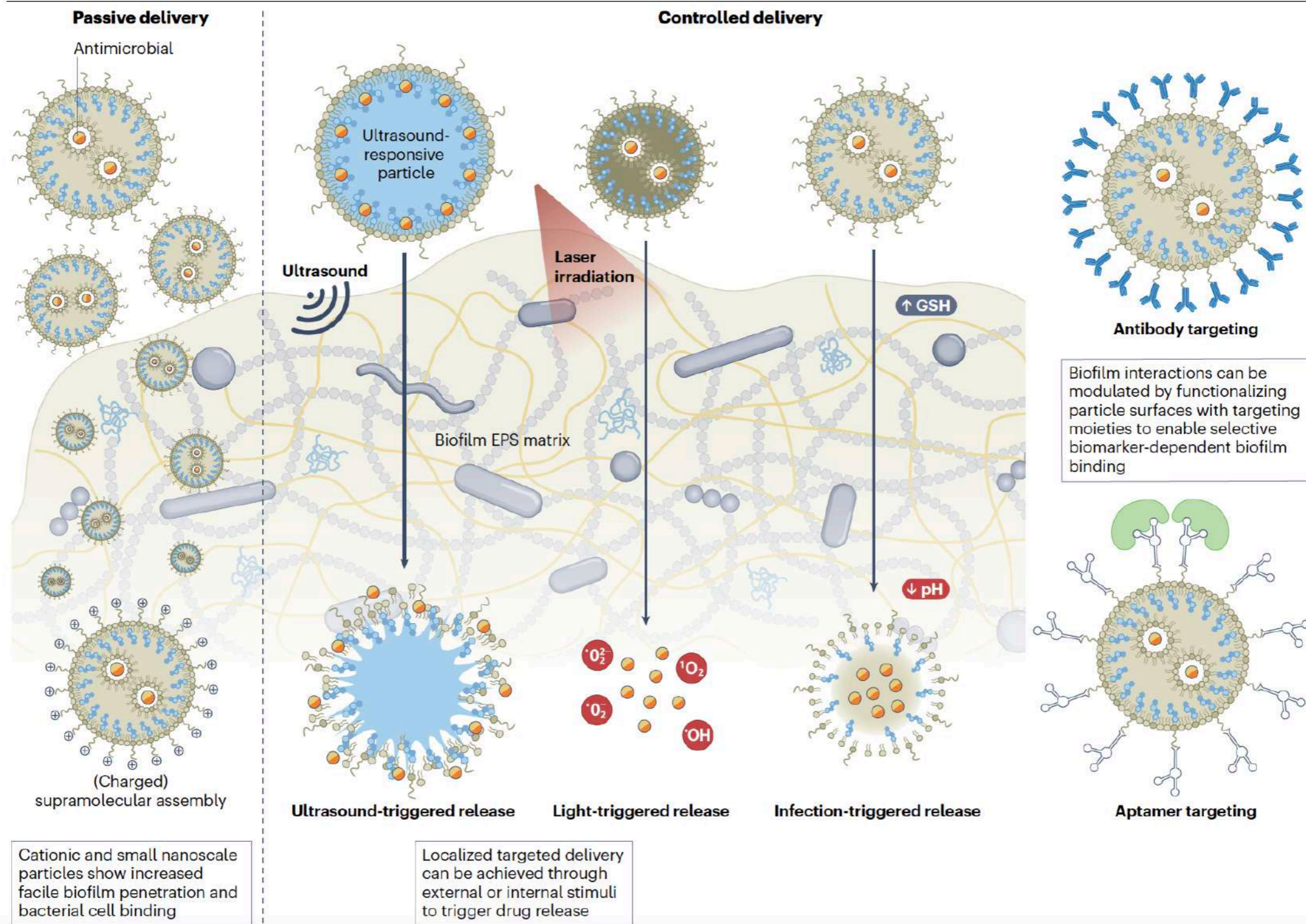


EPS, extracellular polymeric substance.

Sites of common clinical biofilm-associated infection



Supramolecular assembly delivery strategies



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.