L08a

Microbial behaviour: Quorum sensing, biofilm, symbioses…. the power of many… towards multicellularity

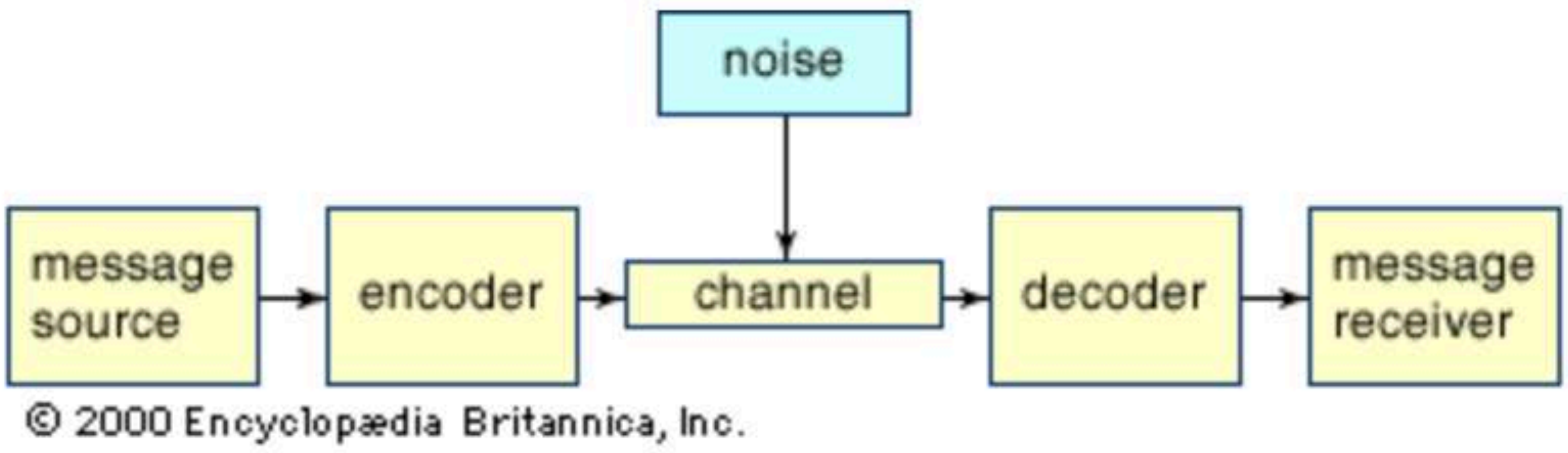
7-Ecologia microbica: cenni su concetto di specie ed evoluzione, ambienti microbici e cicli biogeochimici degli elementi

8-Comportamento: quorum sensing, biofilm, simbiosi microbiche con macrorganismi fino all'essere umano

9-Tecniche di biorisanamento, biomining, biotecnologie e produzione biocombustibili

Microbial communication

Communications



Encyclopædia Britannica

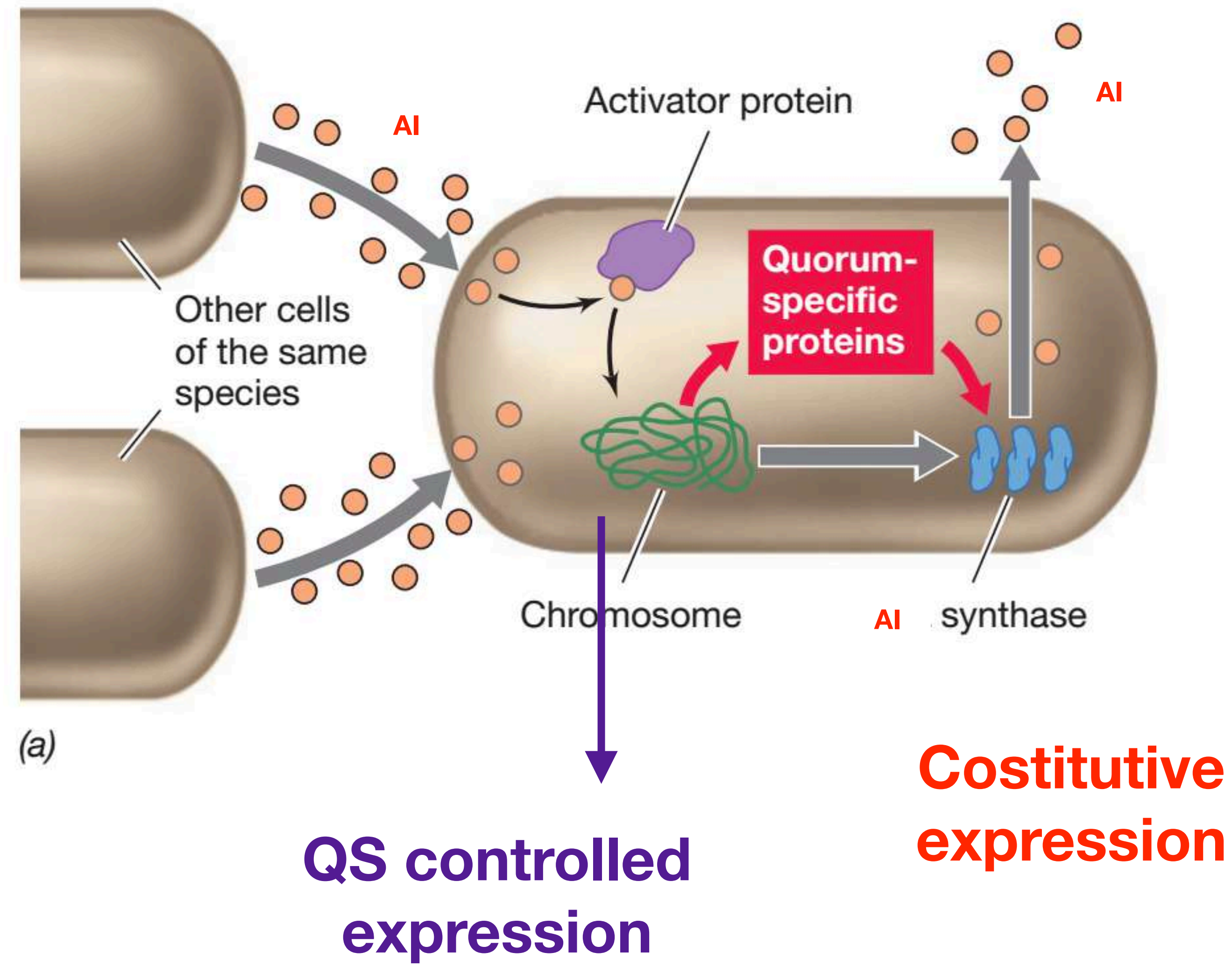
Signal over noise

Shannon's communication model
Consider a simple telephone conversation:

1. A person (message source) speaks into a telephone receiver (encoder), which converts the sound of the spoken word into an electrical signal.
2. This electrical signal is then transmitted over telephone lines (channel) subject to interference (noise).
3. When the signal reaches the telephone receiver (decoder) at the other end of the line it is converted back into vocal sounds.
4. Finally, the recipient (message receiver) hears the original message.

Quorum Sensing, I

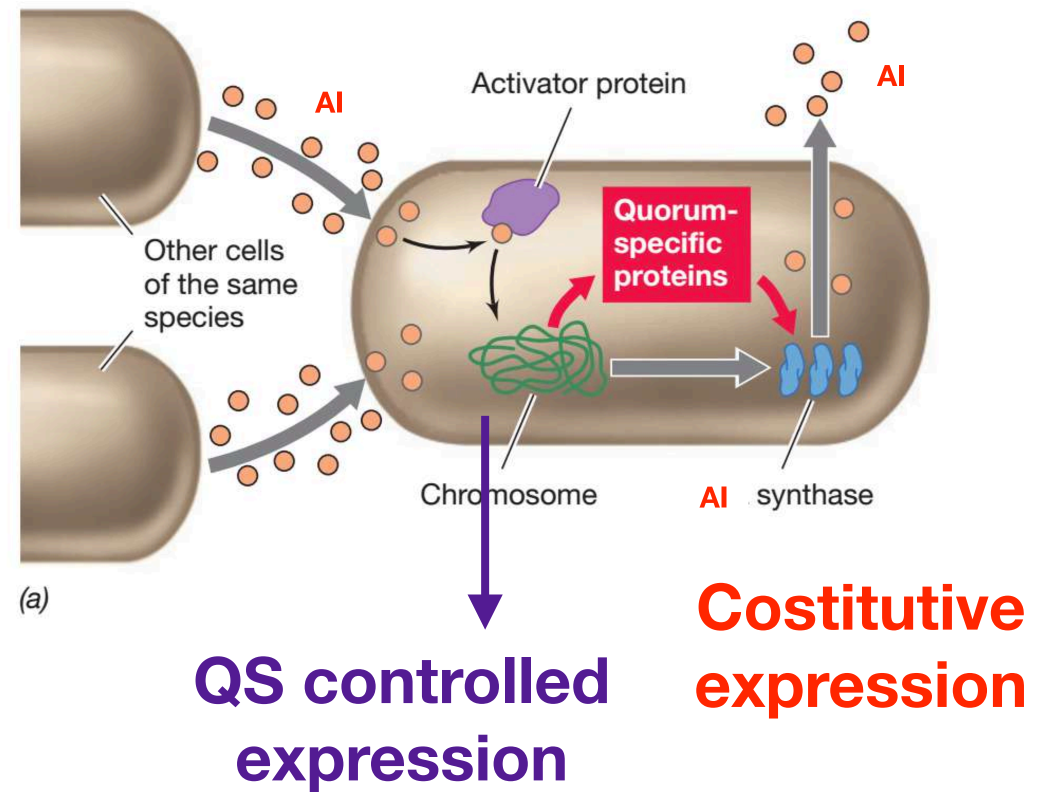
- Quorum sensing (QS) is a process of bacterial **cell-to-cell chemical communication**
- Production, detection, response to extracellular signalling molecules: **autoinducers (AIs)**
- Quorum sensing allows **groups of bacteria** to **synchronously alter behaviour** in response to changes in the population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



Madigan et al. 2020

Quorum Sensing, II

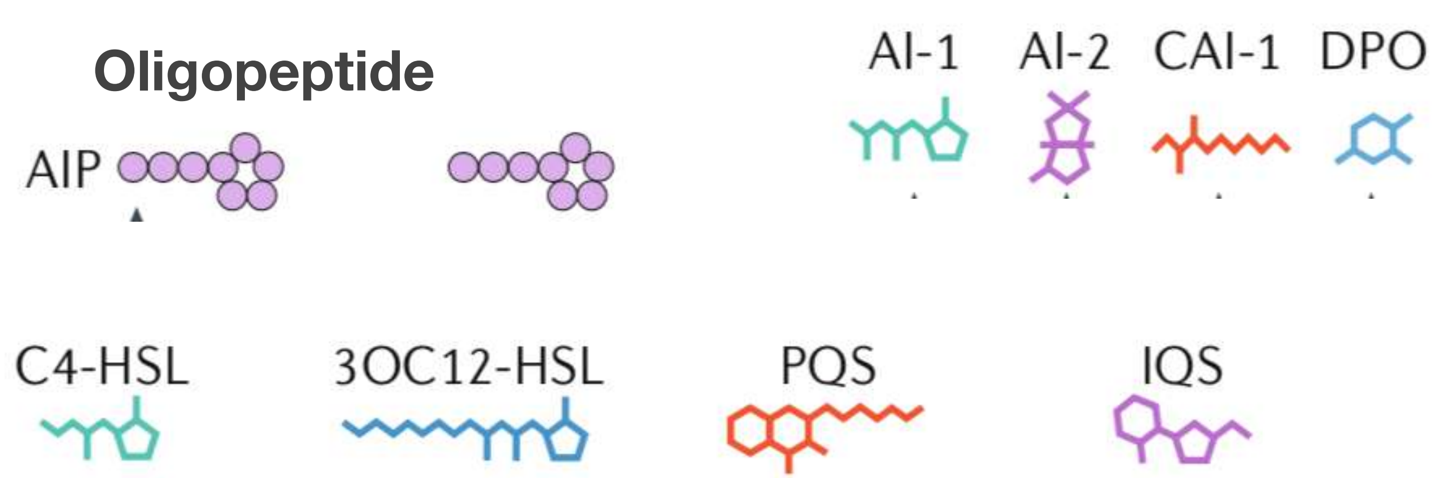
- QS is **global regulatory control**
- QS present in Gram -, Gram + and Archaea
- Many Bacteria **respond to the presence in their surroundings of other cells of their own species**, and in some species, regulatory pathways are controlled by the cell abundance of their own kind
- QS is regulatory mechanism that assesses population abundance—> successful coordinate expression at population level **(not necessarily entire population)**
- Examples are: bioluminescence, virulence factor production, secondary metabolite production, competence for DNA uptake, biofilm formation, species composition



Madigan et al. 2020

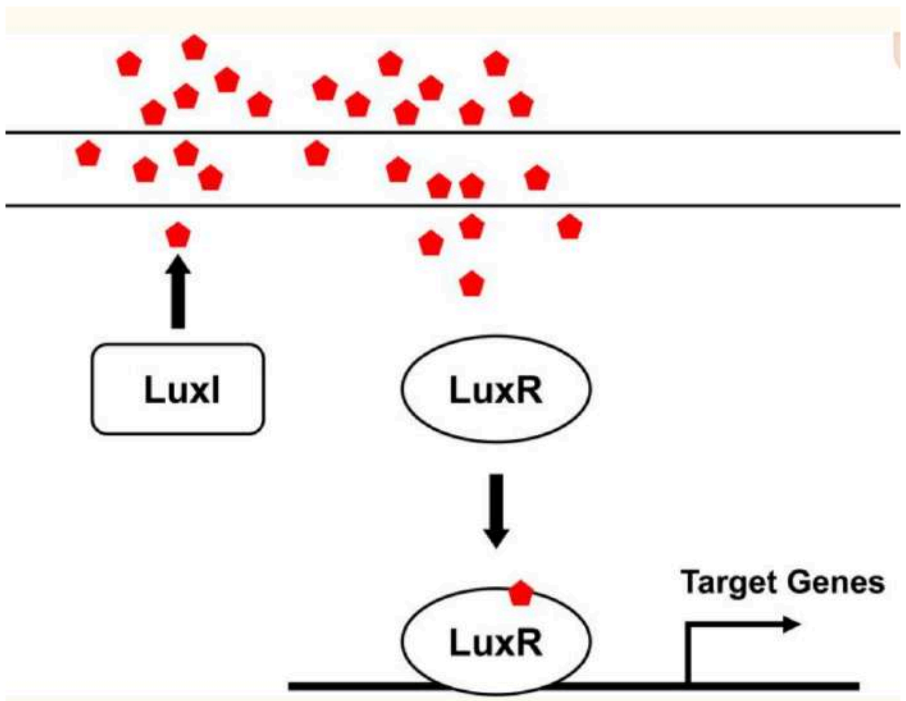
Quorum Sensing, III

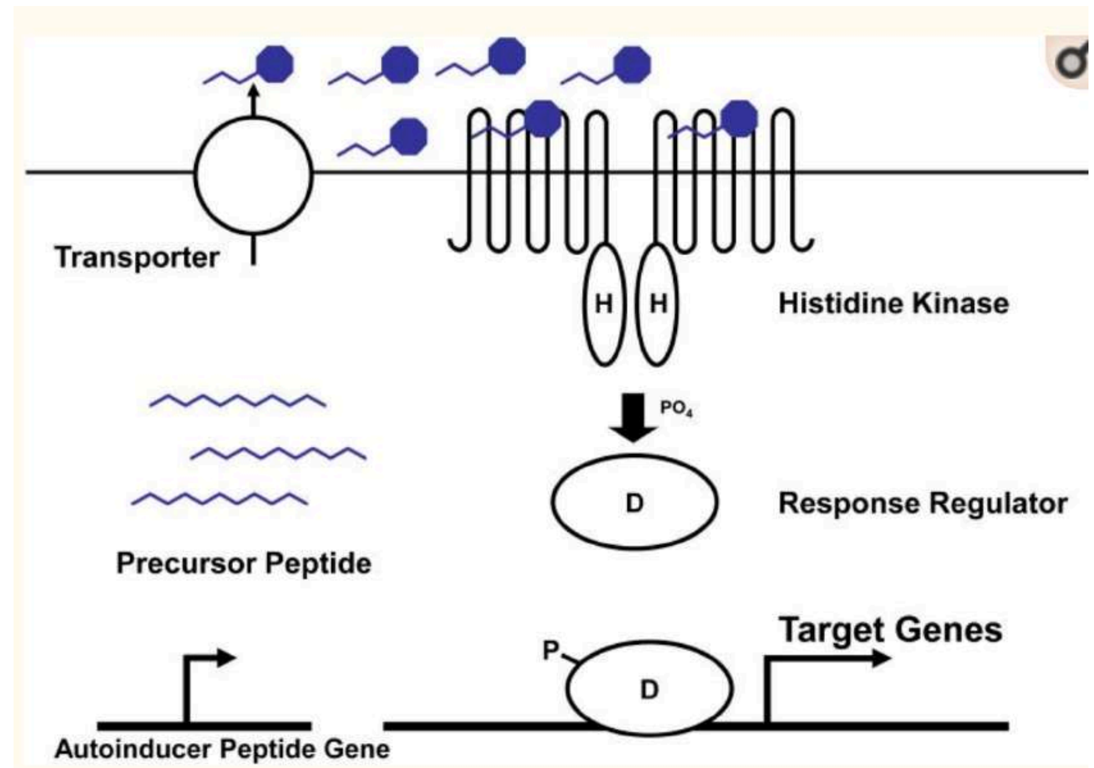
- Autoinducer (AI) is species specific and **freely diffuse in & out**
- **Diverse** chemical structure
- **Same bacterium can have diverse AIs**
- AI reaches high concentrations inside the cell only if many cells are nearby, each making same AI
- In cytoplasm, AI binds to a specific **transcriptional activator protein or a sensor kinase of a two-component system** —> triggering transcription of specific genes
- Intra-species QS by AI-2, different species can coordinate gene expression



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

INTRA-INTER SPECIES COMMUNICATION, I

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, II

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, III

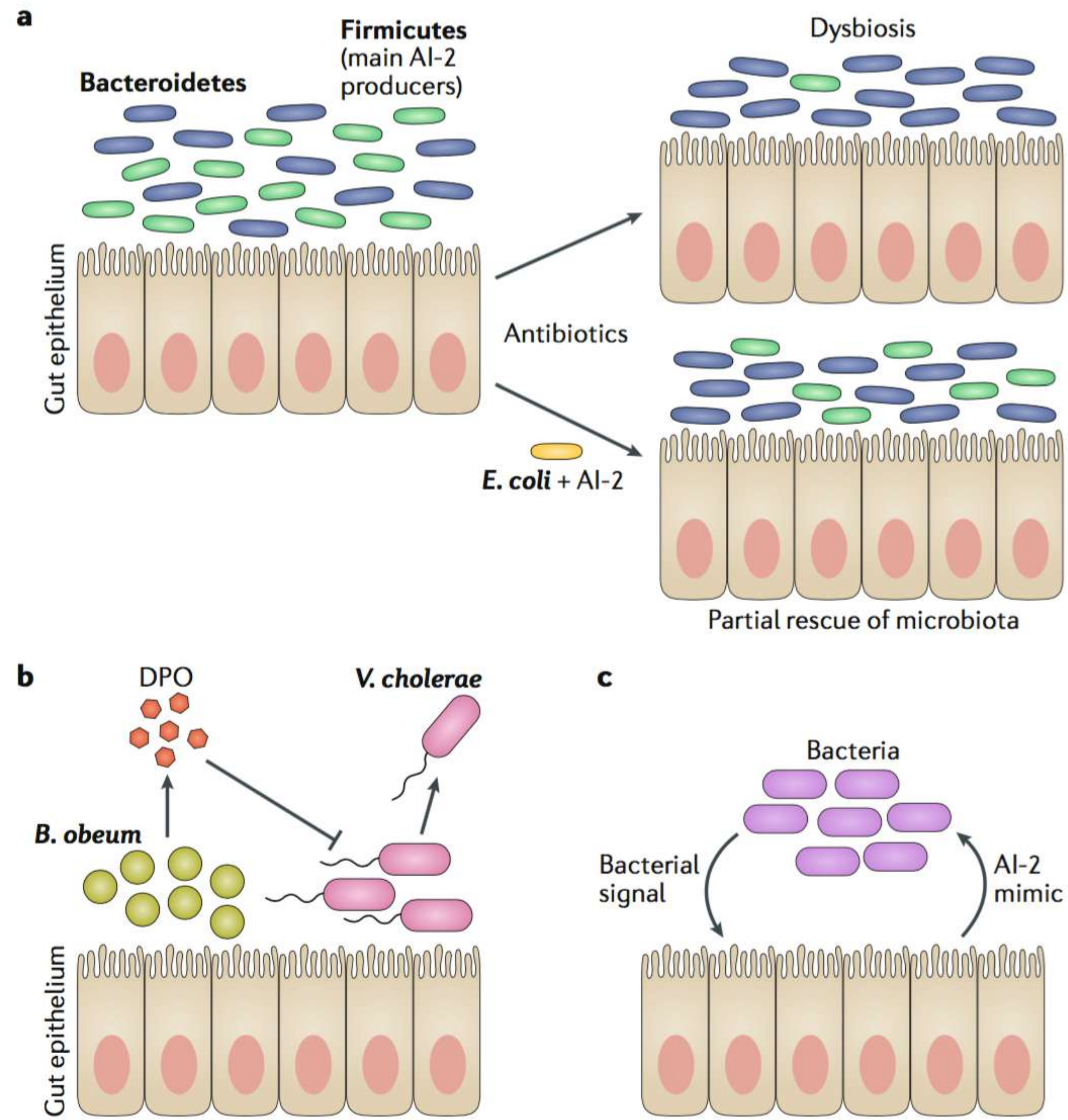
Table 1. Continued

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

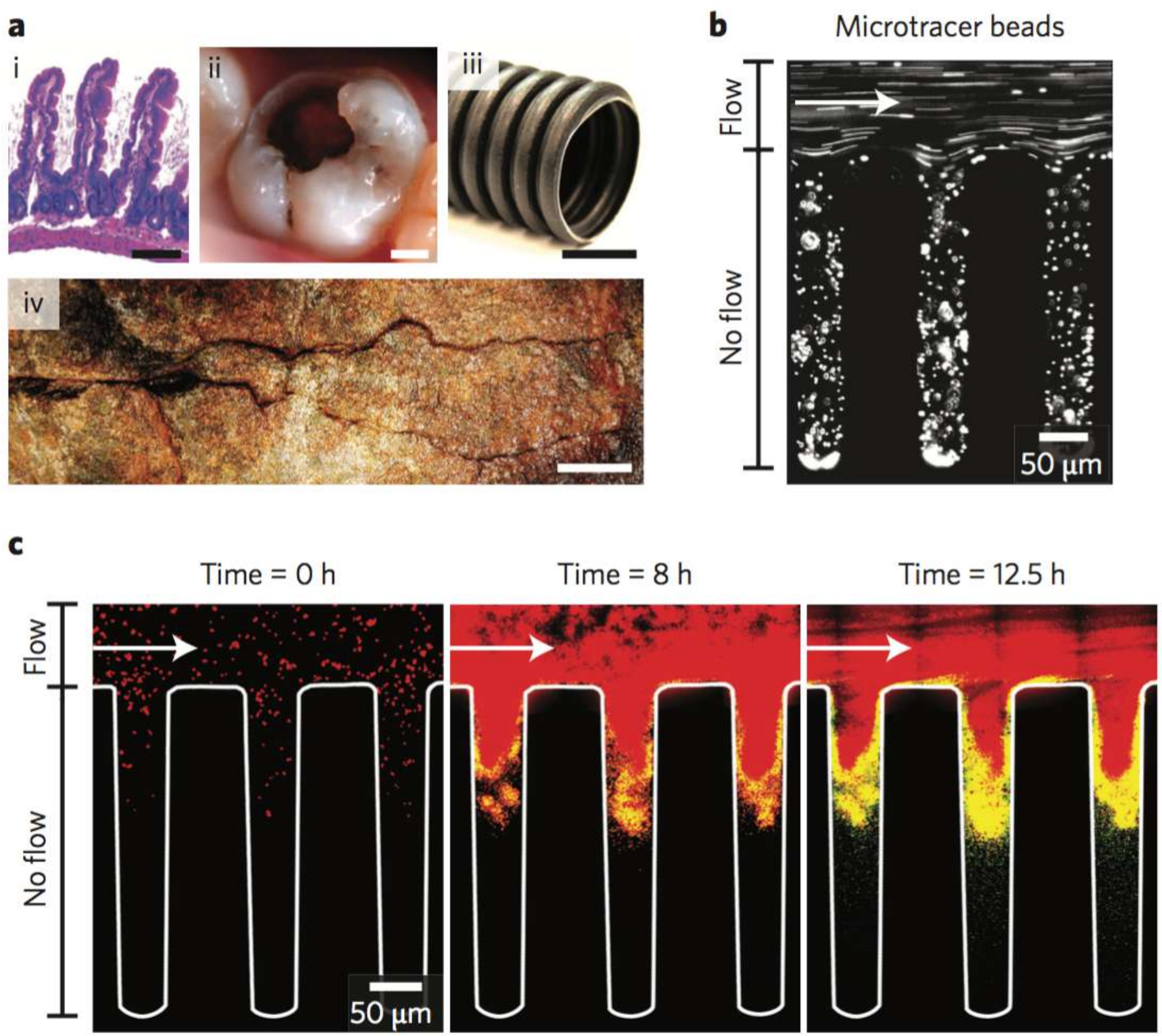
QS and the host microbiota

- 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

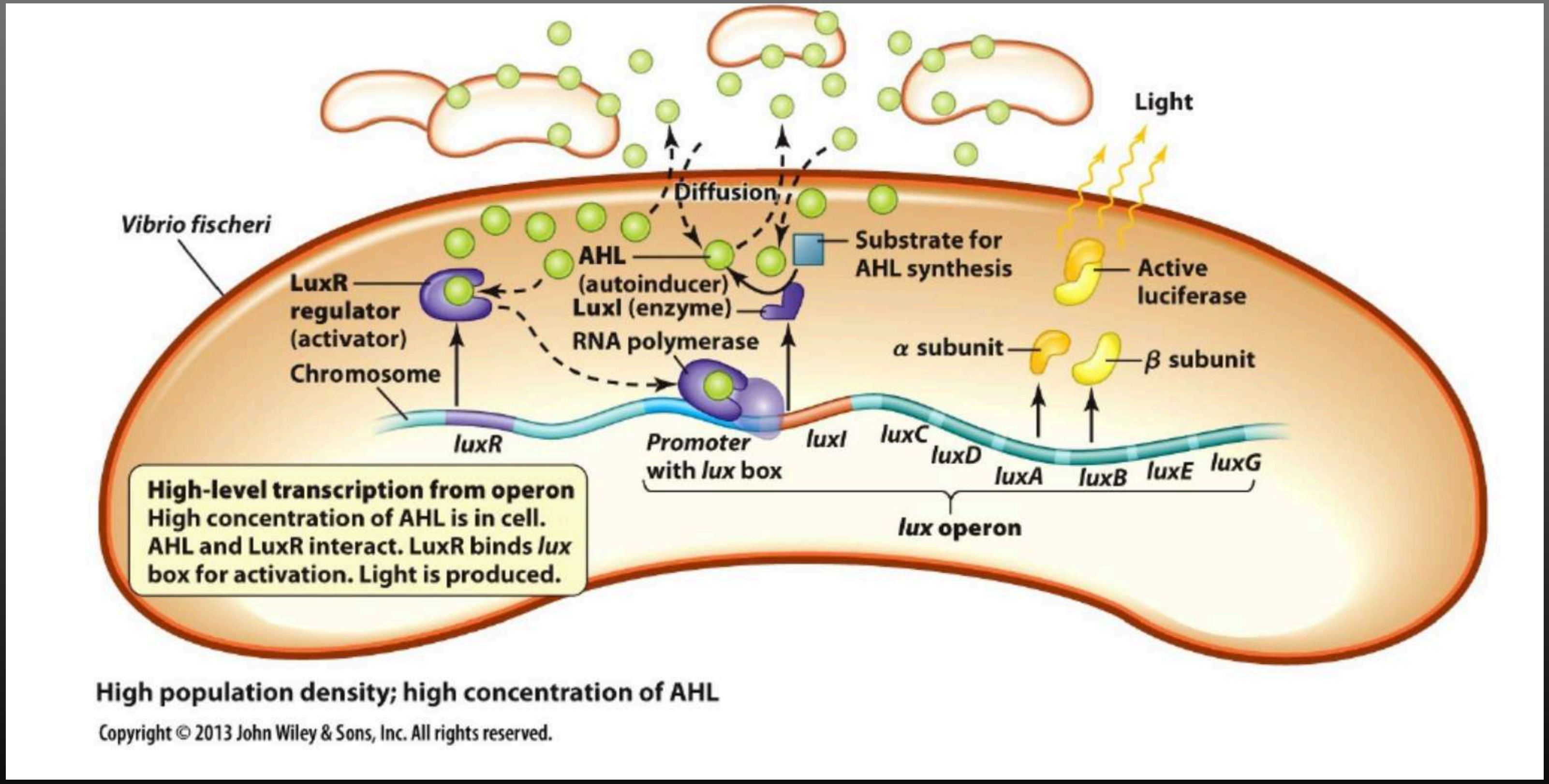
- **Residence time of AIs is key for QS**
- **Flow conditions interferes with QS —> washing AI**
- **Biofilm vs free-living microbes**
- **Other microbes can respond/ produce INTRA-SPECIES AIs**
- **Host can produce AIs**

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

lux operon in *Aliivibrio fischeri*

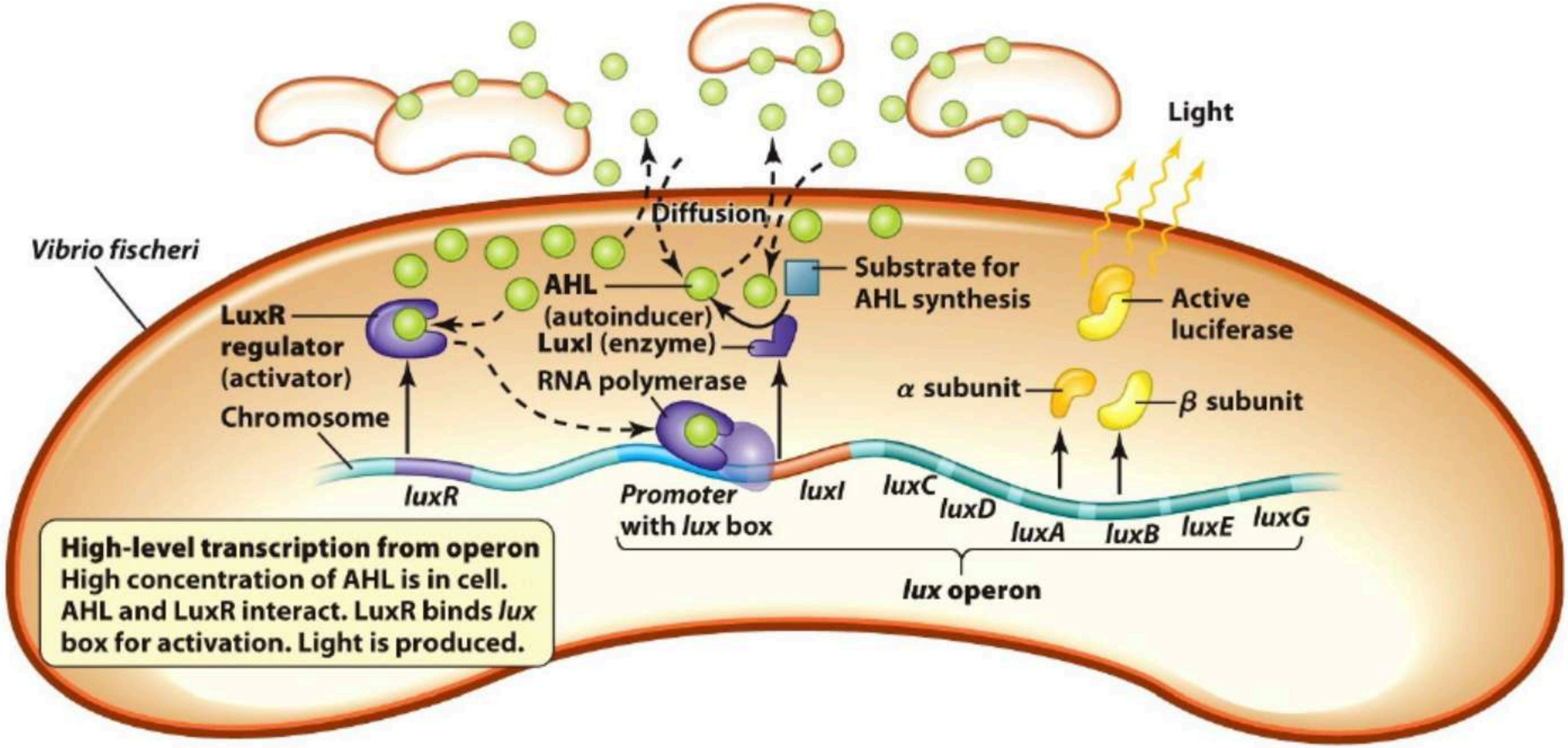
(old name *Vibrio fischeri*)

- 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*)



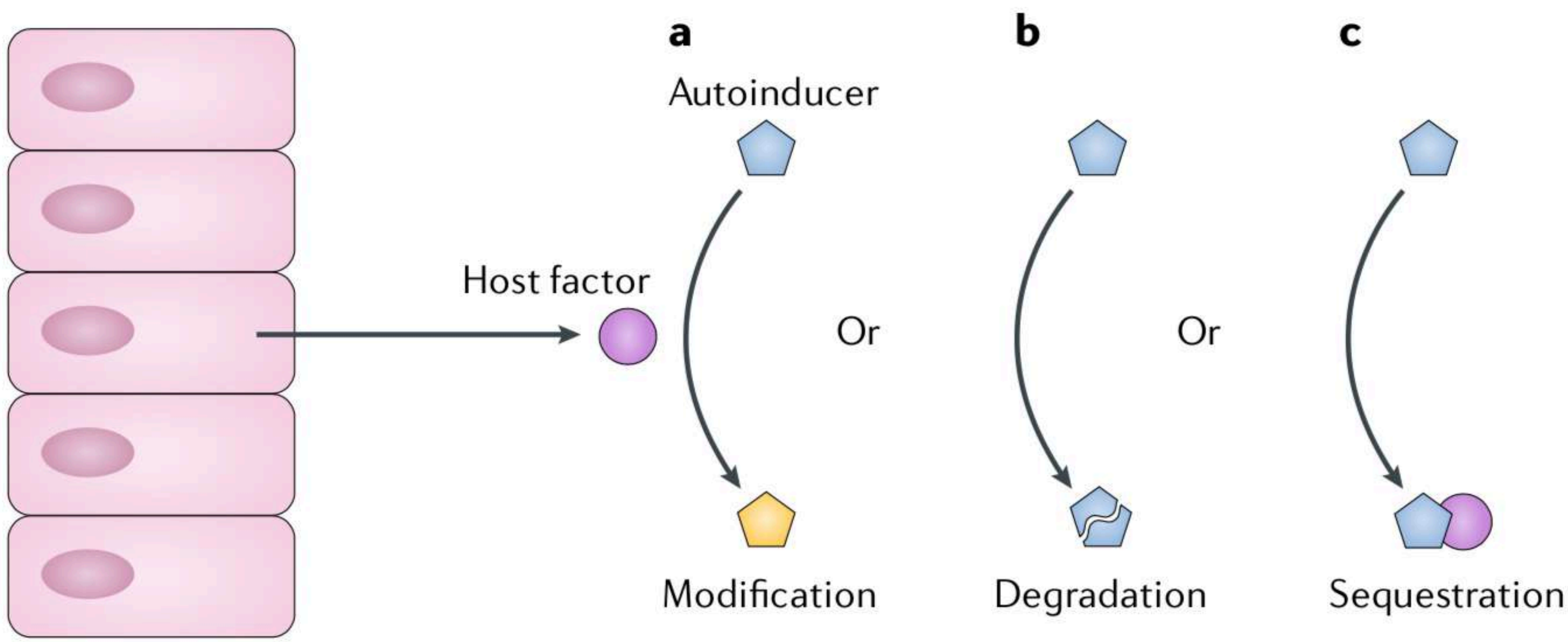
High population density; high concentration of AHL

Quorum Quenching

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

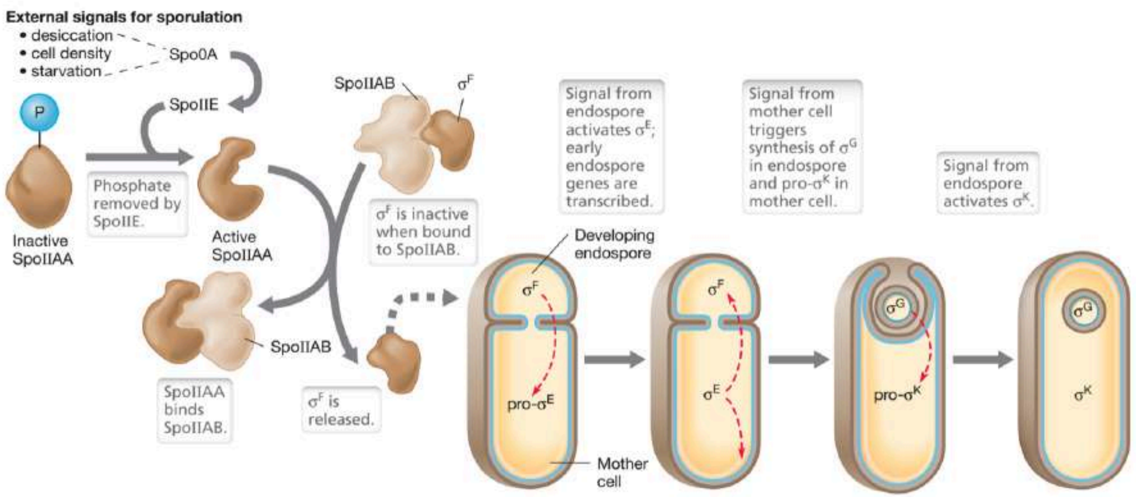
A. Production of halogenated furanones by the red algae *Delisea pulchra* that function as QS-receptor antagonists

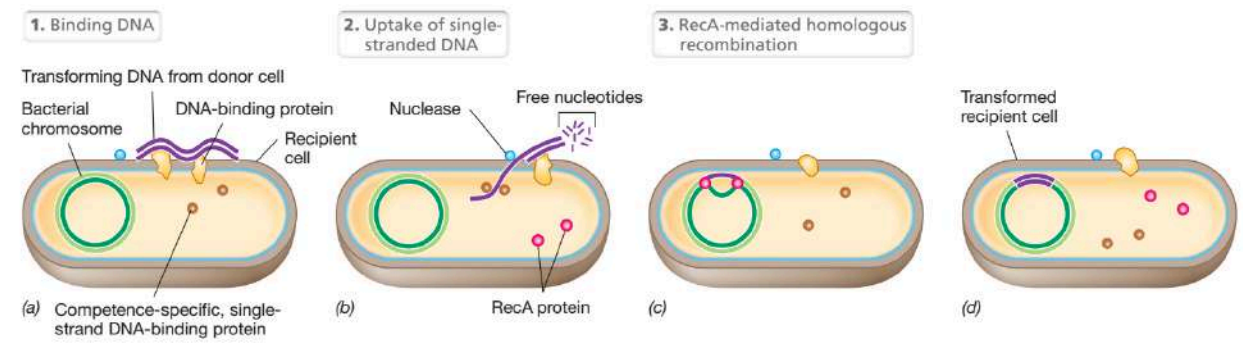
B. Mammalian-produced paraoxonases that function as lactonases that hydrolyse AI



Mukherjee & Bassler, 2019

Quorum sensing *in Gram +*



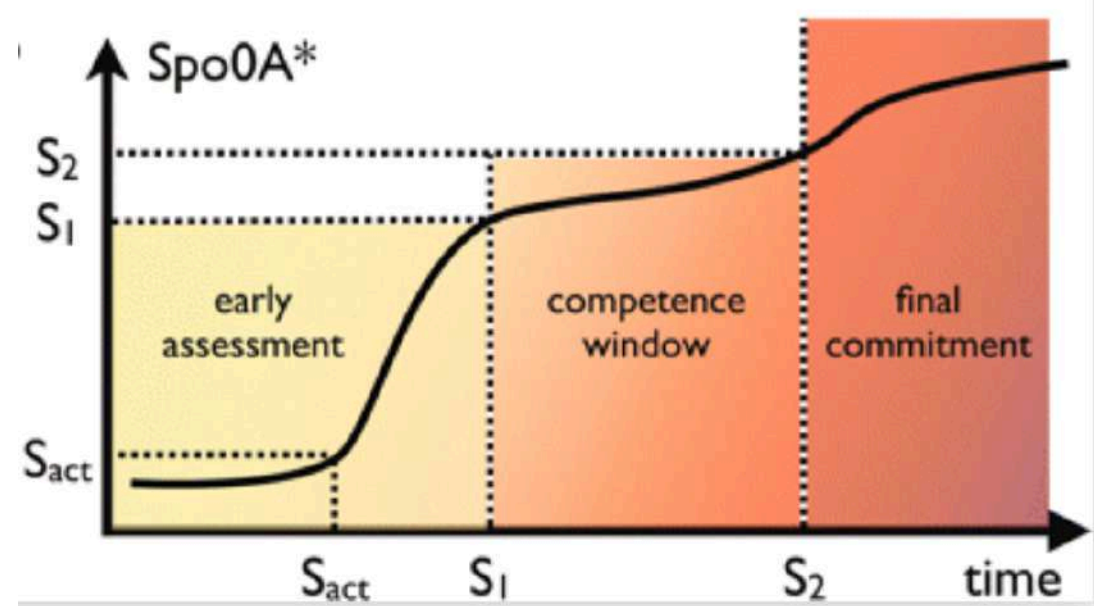


Madigan et al. 2020

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

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**
- 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

Microbial volatile organic compounds in intra-kingdom and inter-kingdom interactions

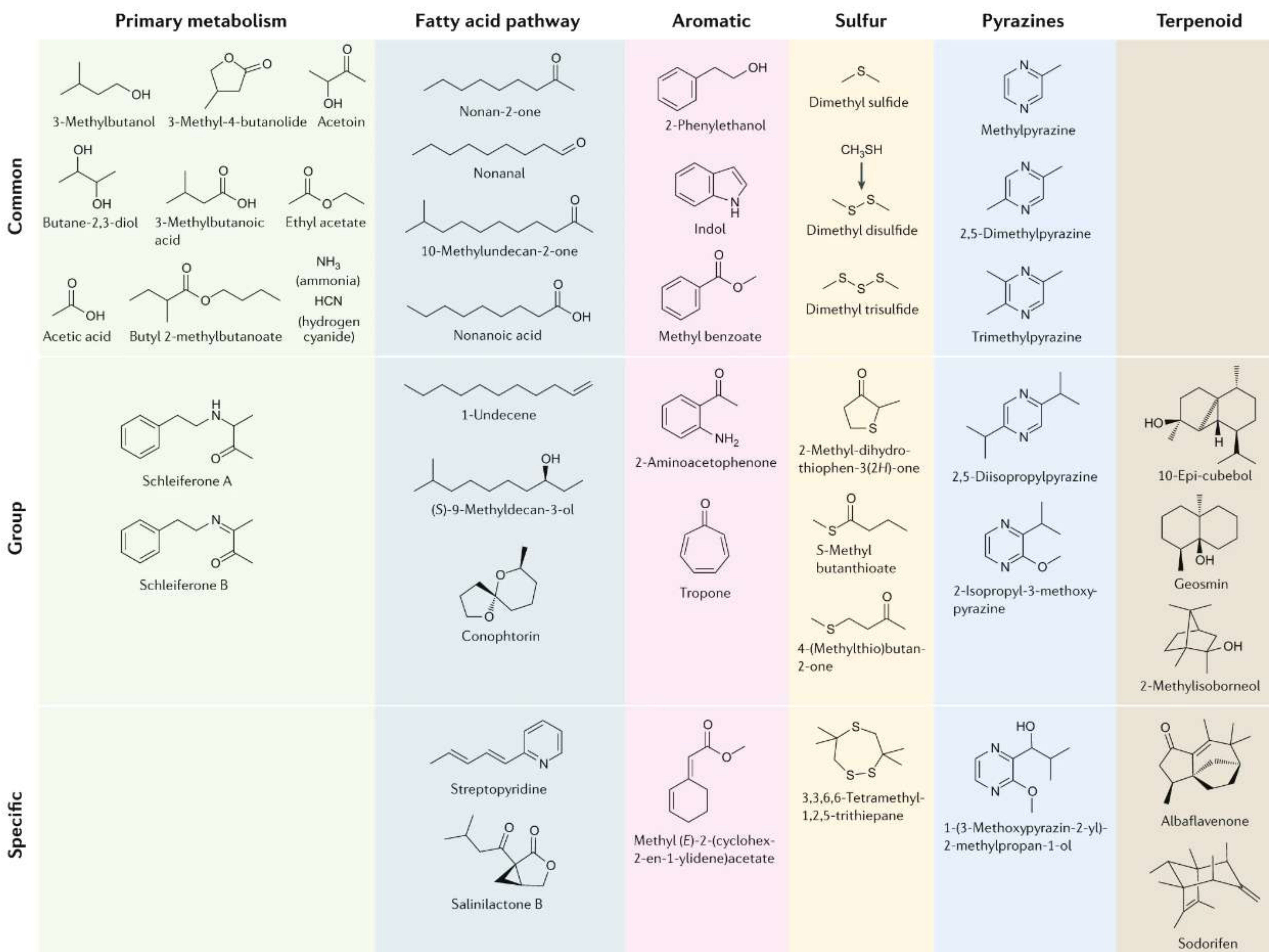
Microorganisms (bacteria, archaea, fungi and protists) use **chemical** signals as a primary source of information —> quorum sensing

Signals in **intra-kingdom** and **inter-kingdom** interactions at **low concentration** and over **long distances (>20 cm)**

Microbial **volatiles** are compounds that can be detected in the gas phase of a microbial culture

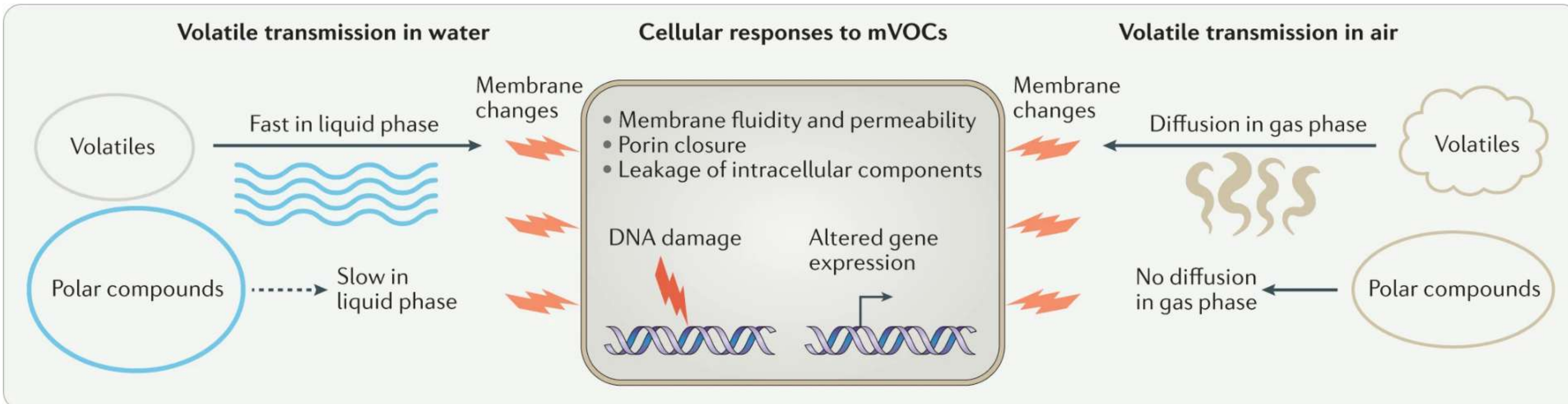
Unique physico-chemical properties: they are **small molecules (<300 Da)**, with up to **two functional groups** and the ability to **easily diffuse in air and water**

Major biosynthetic pathways of microbial volatile organic compounds



Weisskopf et al., 2021

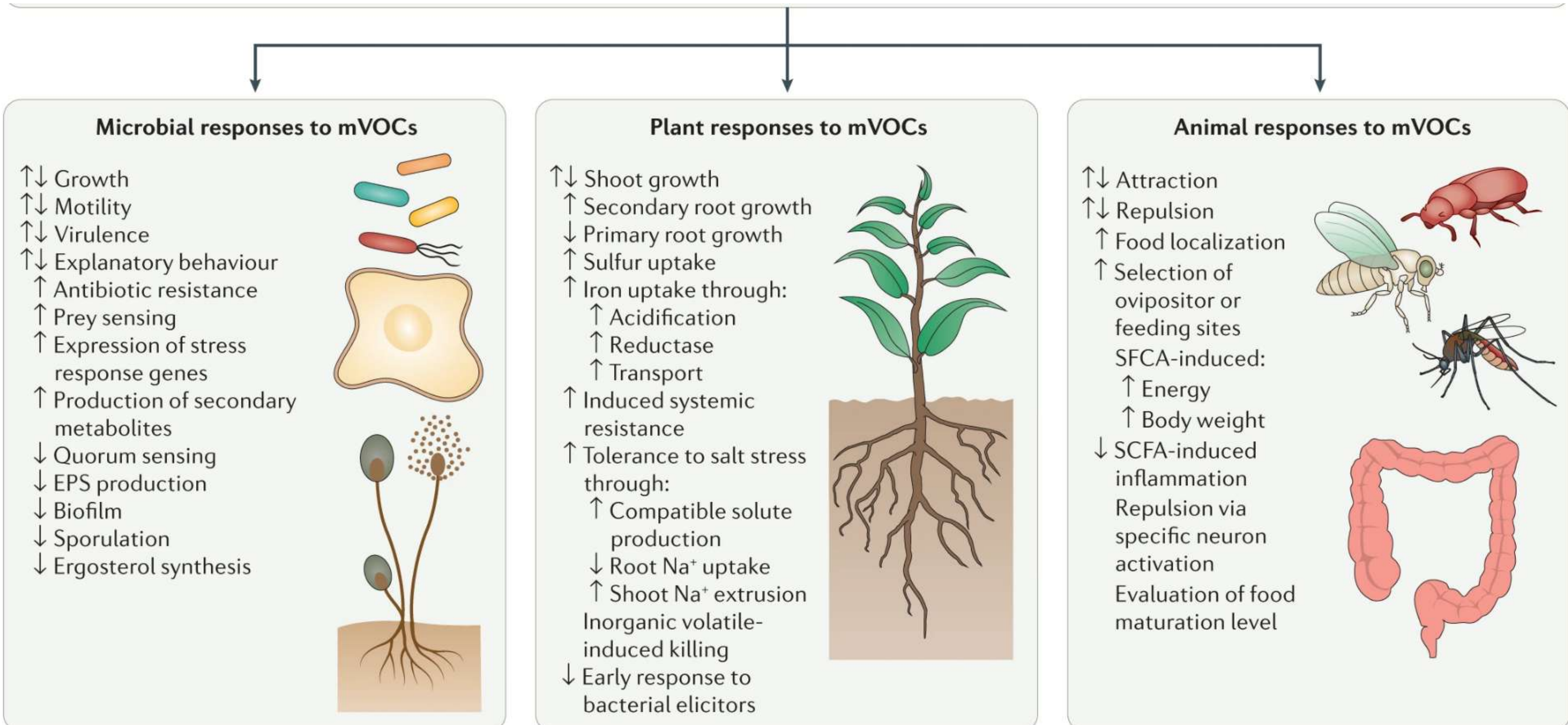
Modes of diffusion of microbial volatiles



In bacteria, mVOCs have also been shown to **modulate antibiotic resistance** (for example, 1-methylthio-3-pentanone, 2-aminoacetophenone and trimethylamine), **quorum sensing** (for example, dimethyl disulfide and dimethyl trisulfide) and **biofilm formation** (for example, indole and 1-butanol)

Weisskopf et al., 2021

Responses in microorganisms, plants and animals to mVOCs



Weisskopf et al., 2021

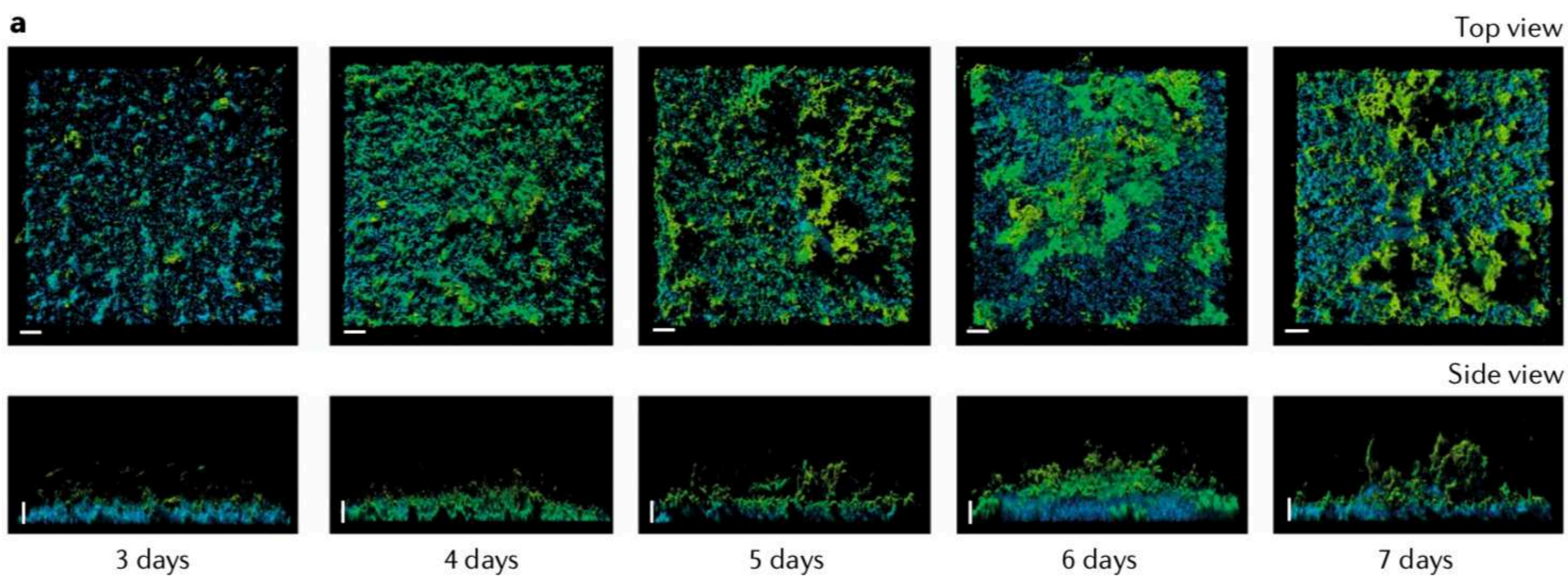
Biofilm

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

Biofilm, I

- Cells with suspended lifestyle, called **planktonic growth** vs **sessile cells** —> attaching on surfaces and forming biofilm
- A biofilm is a very **heterogeneous** attached **polysaccharide matrix, with proteins and extracellular DNA** 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)
- Specific gene activation, cell reprogramming
- Adaptive behaviour

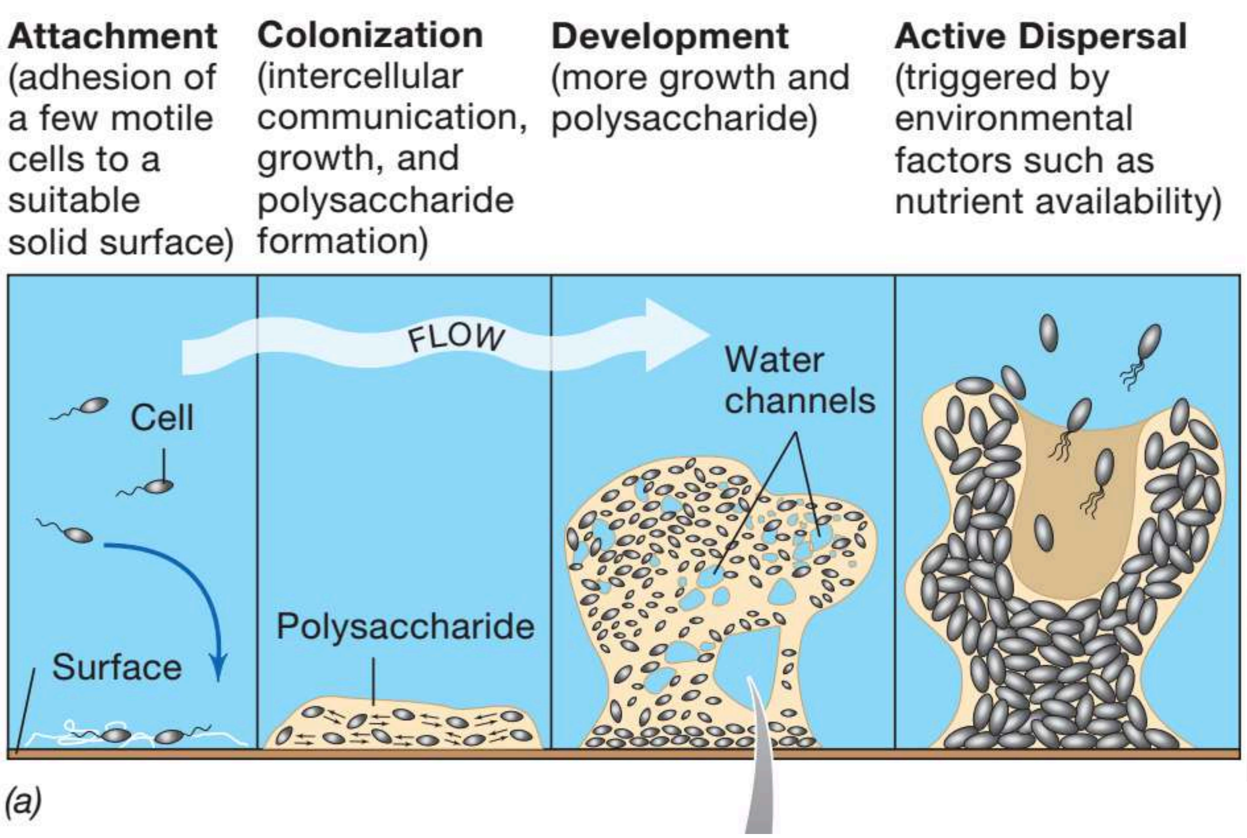


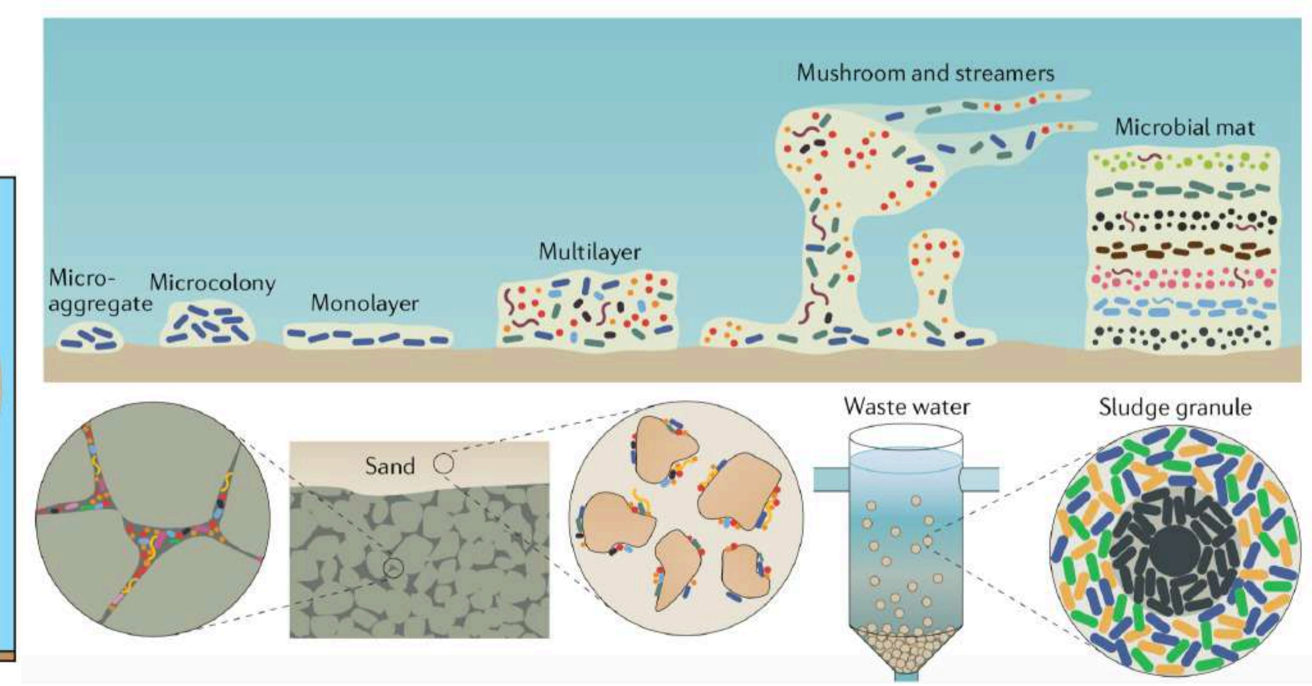
Wolferen et al., 2019

Biofilm, II

- Biofilms form in stages: (1) attachment, (2) colonization, (3) development, (4) dispersal
- Molecular coating of the surface
- **Very dynamic, very diverse**
- **Steep gradient of nutrients and oxygen**
- **Protection against grazers, viruses, antibiotics, drugs and metals**

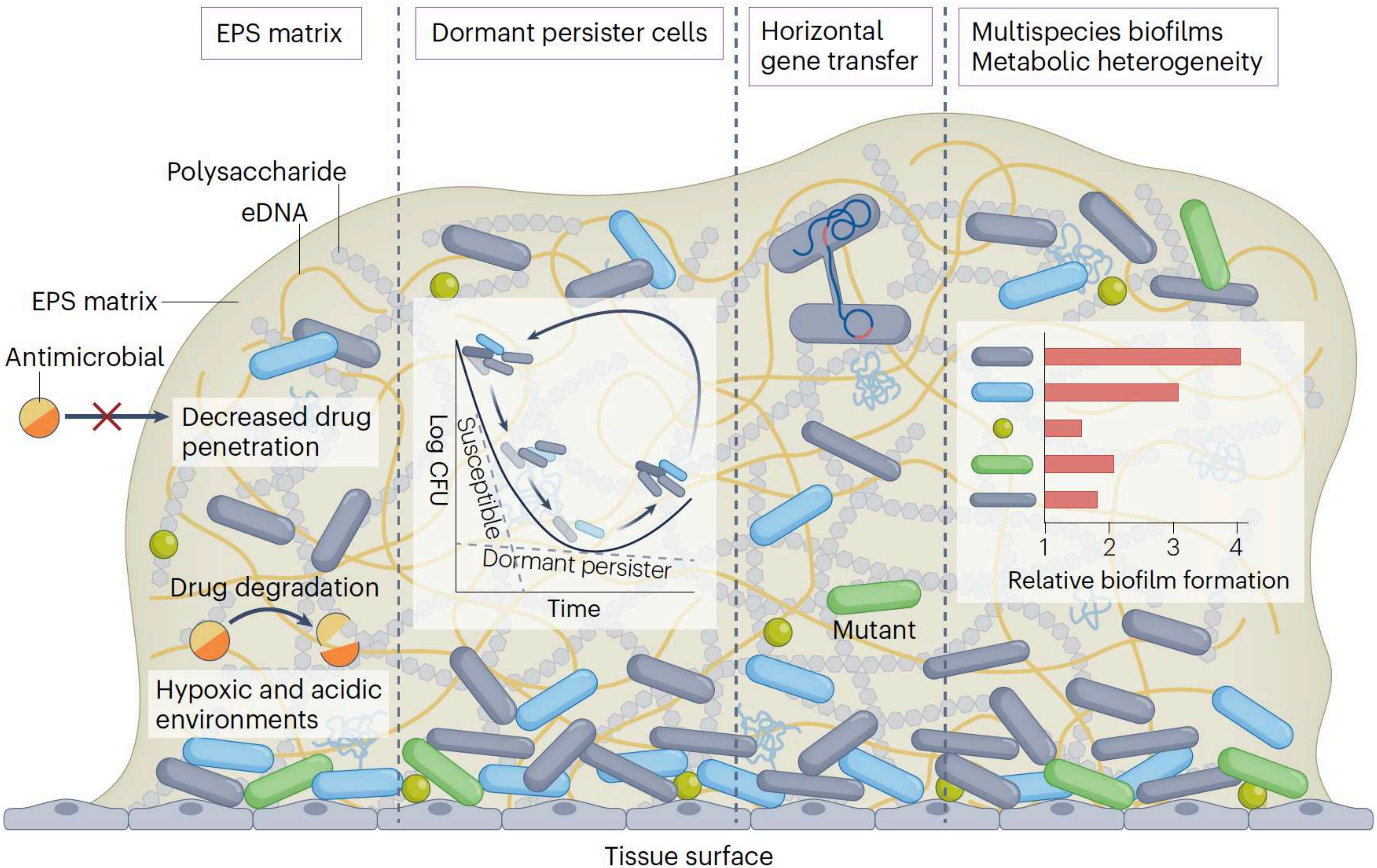
Madigan et al. 2018





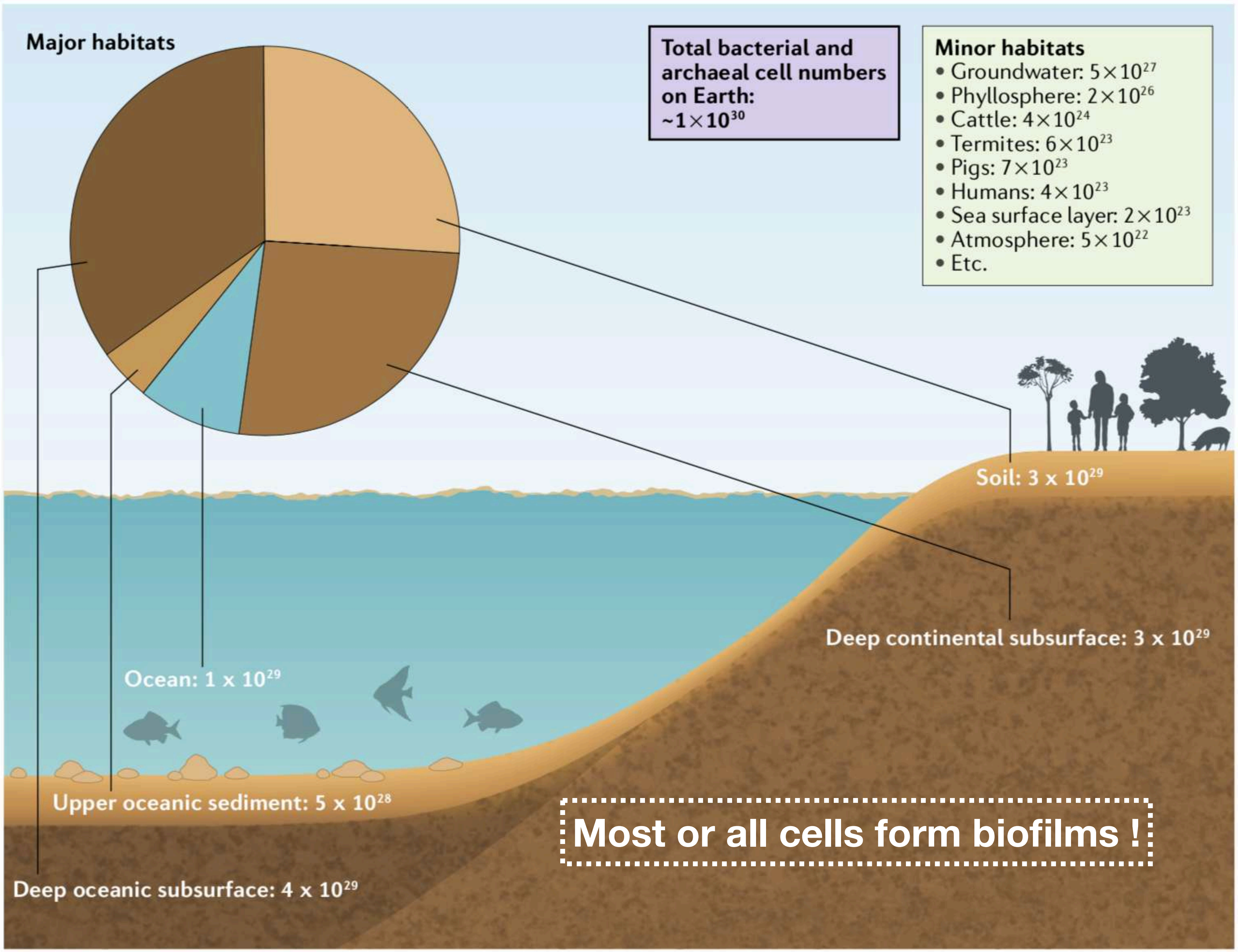
Flemming & Wuertz, 2019

Biofilm features



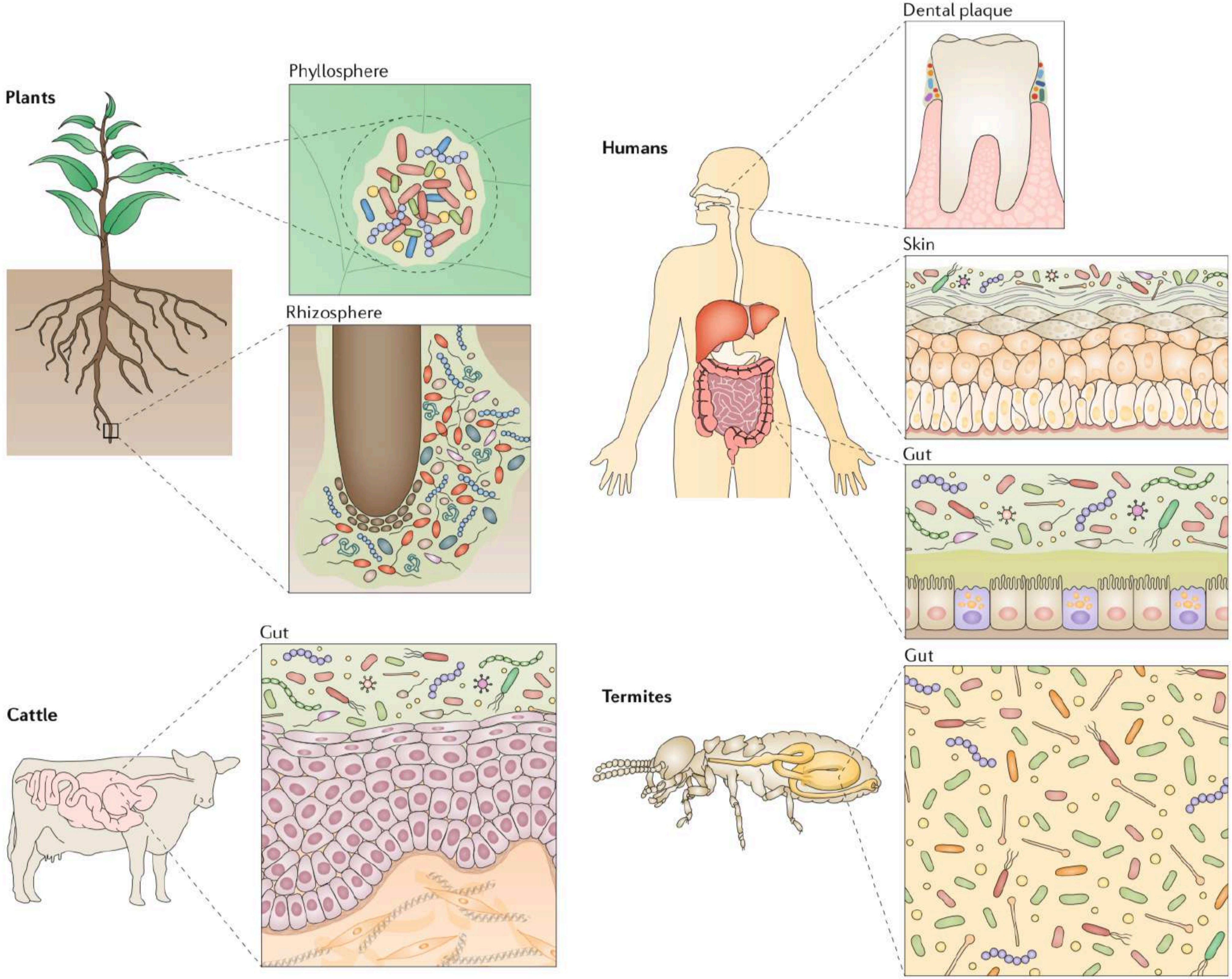
Choi et al., 2023

Biofilm as a microbial habitat



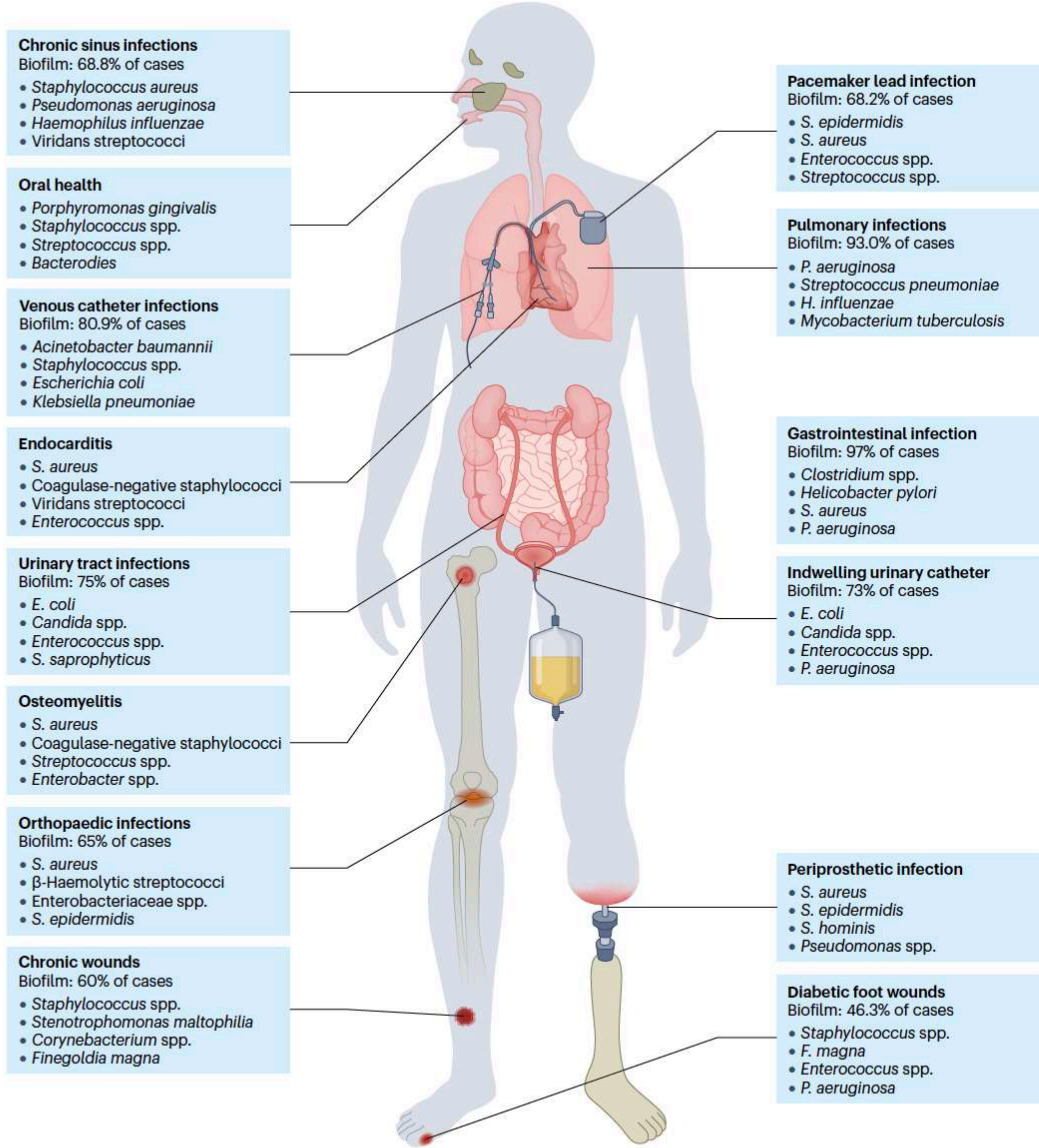
Flemming & Wuertz, 2019

Eukarya as microbial biofilm



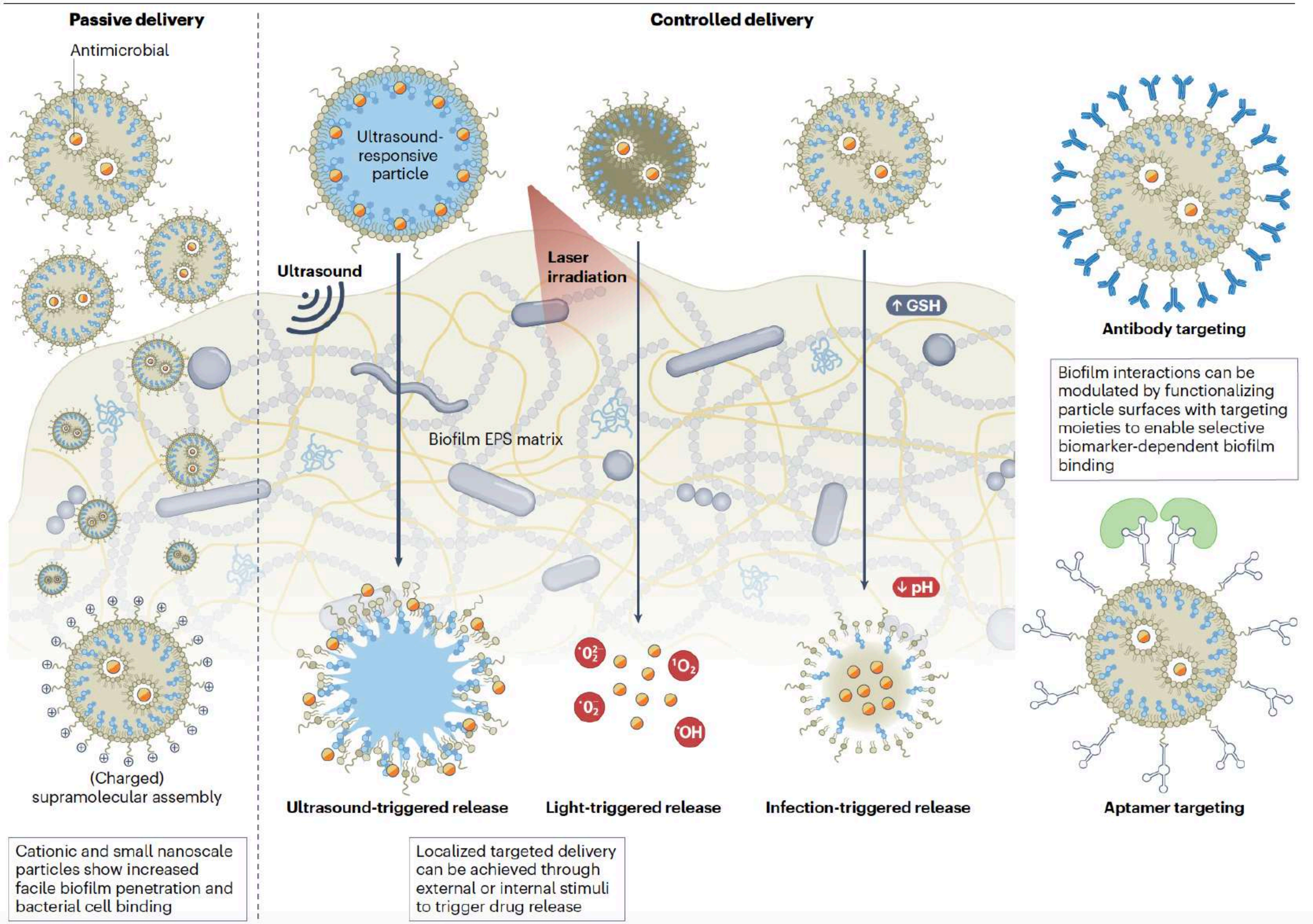
Flemming & Wuertz, 2019

Sites of common clinical biofilm-associated infection



Choi et al., 2023

Supramolecular assembly delivery strategies in biofilm



Choi et al., 2023

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