# L02c: Metabolic Diversity and Ecophysiology of Marine Microbes

0. Ethics in science, plagiarism and AI

1. Microbes in the Marine Environment

**2. Metabolic Diversity and Ecophysiology of Marine Microbes**

3. Diversity of Marine Bacteria, Archaea, Viruses and unicellular Eukaryotes

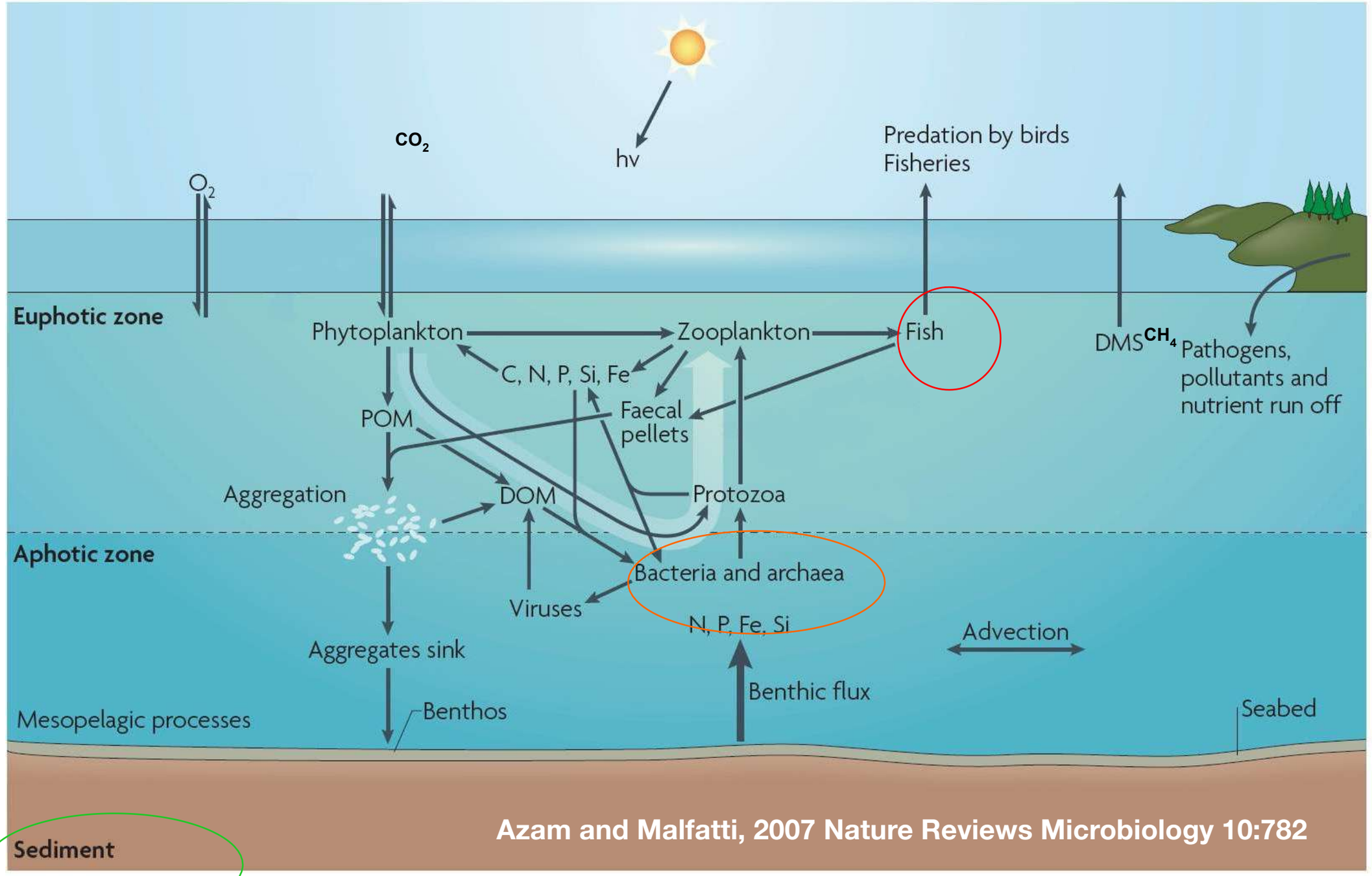
4. Microbes in Ocean Processes: Carbon and other nutrients

5. Marine Microbial Symbioses and Diseases

6. Marine Microbes, Climate and Society

# Healthy Ocean <—> Functioning Ocean

# Functioning Ocean <—> Functioning C biogeochemical cycle




Azam and Malfatti, 2007 Nature Reviews Microbiology 10:782

**Table 3.2** Nutritional categories of microorganisms

| Energy source | Carbon source | Hydrogen or electron source | Representative examples |
|---|---|---|---|
| **Photolithoautotrophy** | | | |
| Light | $CO_2$ | Inorganic | Cyanobacteria<br>Purple sulfur bacteria<br>Phototrophic protists |
| **Photoorganoheterotrophy** | | | |
| Light | Organic compounds | Organic compounds or $H_2$ | Purple non-sulfur bacteria<br>Aerobic anoxygenic bacteria<br>Proteorhodopsin-containing bacteria and archaea[a] |
| **Chemolithoautotrophy** | | | |
| Inorganic | $CO_2$ | Inorganic | Sulfur-oxidizing bacteria<br>Hydrogen bacteria<br>Methanogens<br>Nitrifying bacteria and archaea |
| **Chemoorganoheterotrophy** | | | |
| Organic compounds | Organic compounds | Organic compounds | Wide range of bacteria and archaea<br>Fungi<br>Phagotrophic protists |
| **Mixotrophy** (combination of lithoautotrophy and organoheterotrophy) | | | |
| Inorganic | Organic compounds | Inorganic | Some sulfur-oxidizing bacteria, e.g. *Beggiatoa* |
| **Mixotrophy** (combination of photoautotrophy and organoheterotrophy) | | | |
| Light + organic compounds | $CO_2$ + organic compounds | Inorganic or organic | Phagotrophic photosynthetic protists (some flagellates and dinoflagellates) |



Munn, 2020

# Microbial energy generating metabolic pathways shaping Earth ecosystem

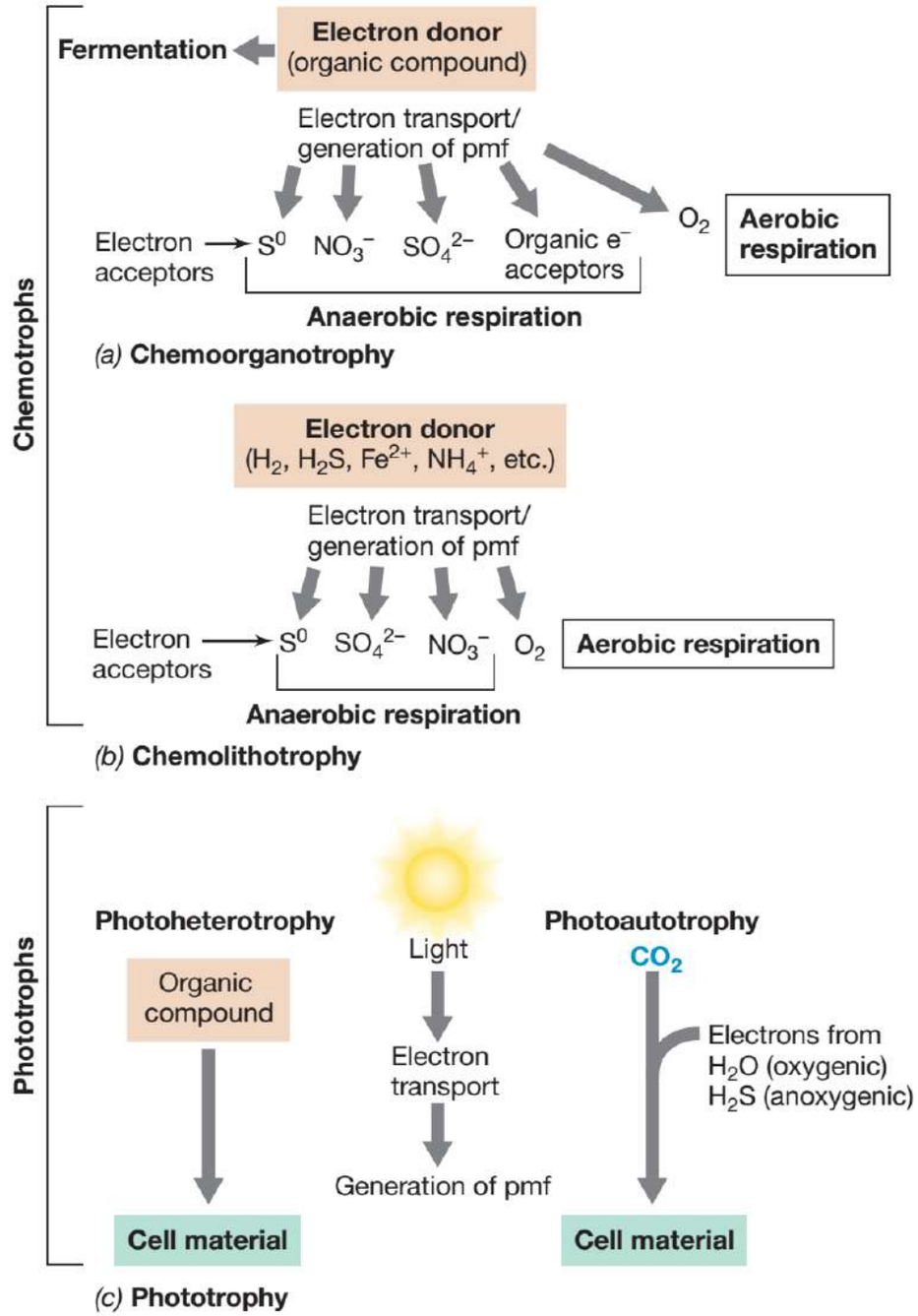
**•Oxygenic Photosynthesis**
ATP and NADPH are made in large amounts
Produces oxygen as a bi-product during splitting of water for reducing power

**•Anoxygenic Photosynthesis**
ATP made in large amounts
Reduction of NADP does not involve water; hence no oxygen produced

**•Aerobic Respiration**
ATP and NADH are made in abundance
Requires oxygen

**•Anaerobic Respiration**
Lower ATP yield than aerobic respiration; NAD easily reduced
Requires electron acceptor other than oxygen

**Fermentation**
Little ATP, no net NAD reduction, MOST SIMPLE SYSTEM




Madigan et al. 2018

# Microbial diversity and metabolic pathways to survive in the environment

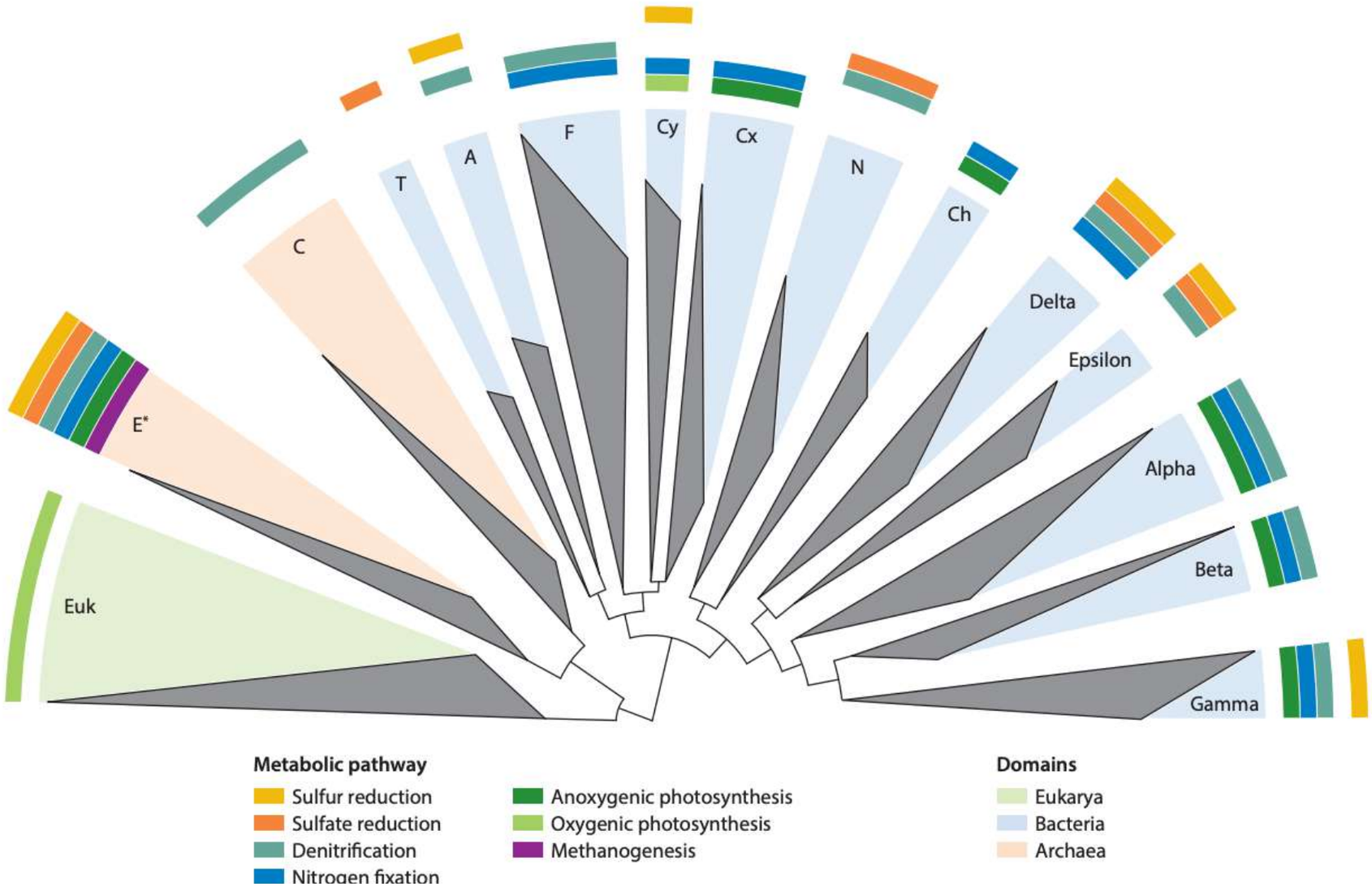



**Figure 1**

Distribution of selected metabolic pathways on the 16S rRNA tree of life. The tree (constructed with ARB; 104) was edited for clarity and shows selected bacterial and archaeal taxa. The area of each branch is proportional to the total number of 16S rRNA sequences present in the database. Metabolic pathways were assigned based on physiological data (**Supplemental Table 2**). Sulfate reduction includes sulfite and thiosulfate reduction pathways. **Euryarcheata* are capable of bacteriorhodpsin-based photosynthesis only. Abbreviations: A, *Aquificae*; Alpha, *Alphaproteobacteria*; Beta, *Betaproteobacteria*; C, *Crenarchaeota*; Ch, *Chlorobi*; Cx, *Chloroflexi*; Cy, *Cyanobacteria*; Delta, *Deltaproteobacteria*; E, *Euryarchaeota*; Epsilon, *Epsilonproteobacteria*; Euk, *Eukarya*; F, *Firmicutes*; Gamma, *Gammaproteobacteria*; N, *Nitrospirae*; T, *Thermodesulfobacteria*.

Jelen et al. 2016

# Microbial energy generating metabolic pathways shaping Earth ecosystem

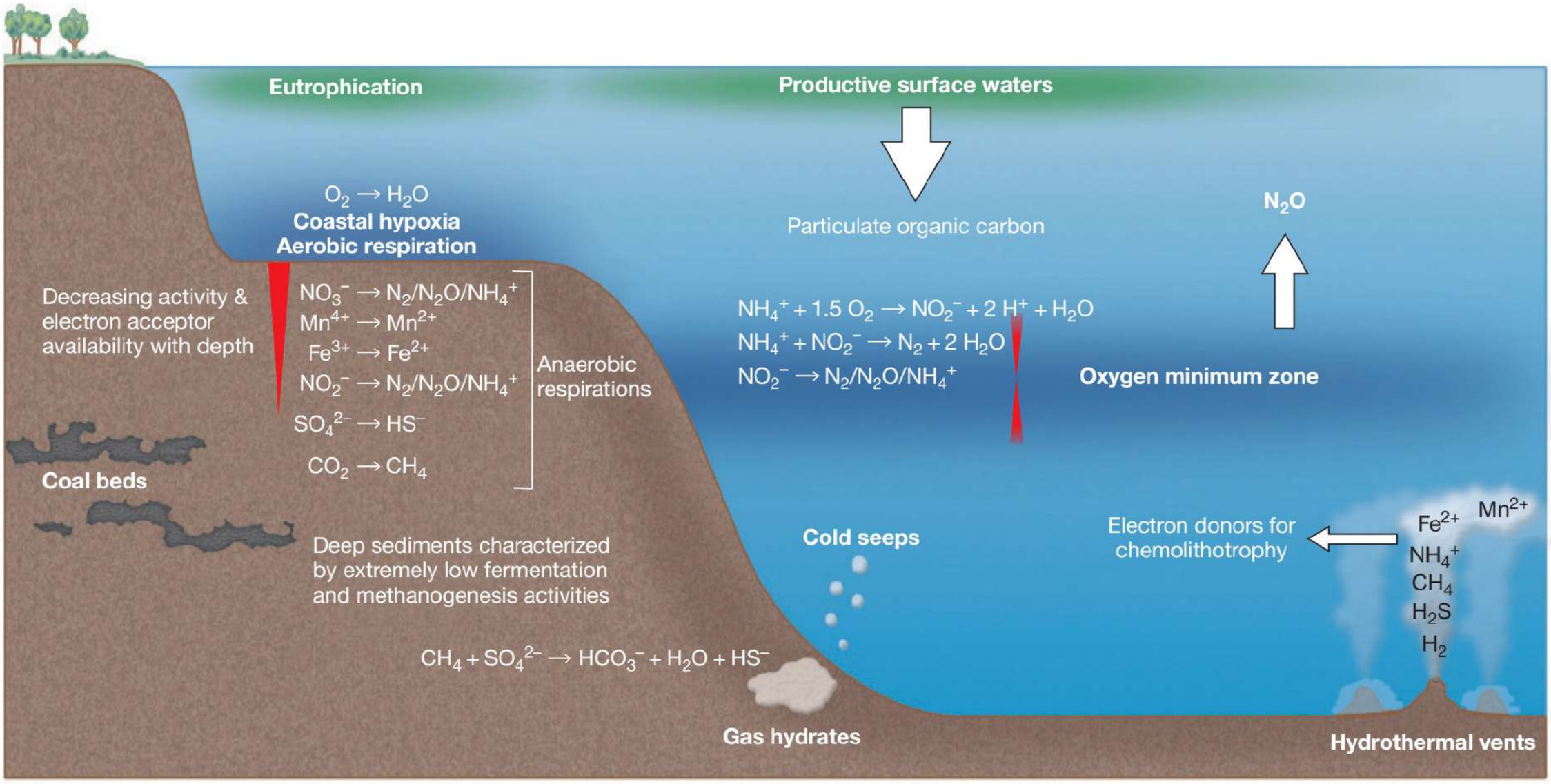
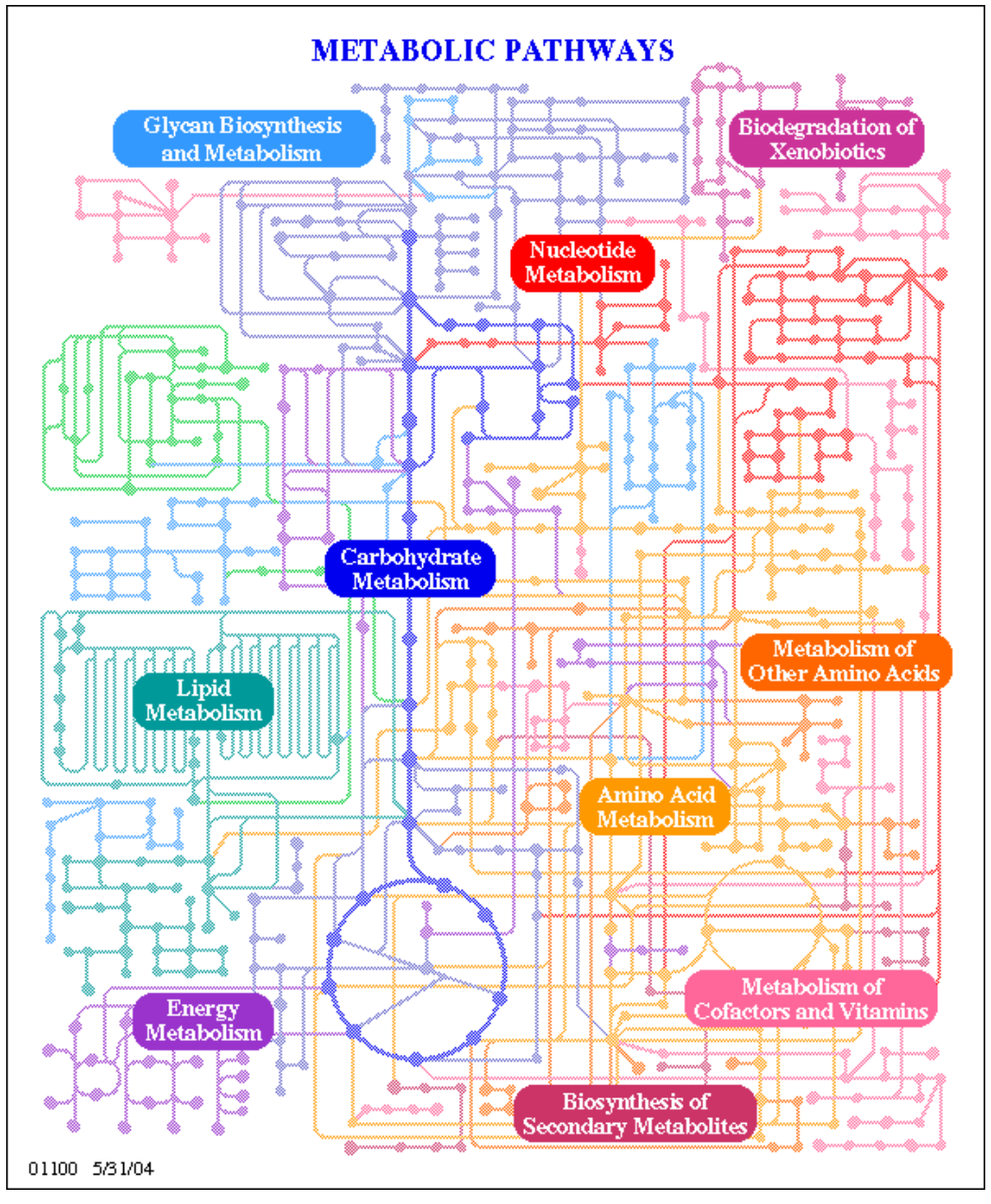



**Figure 20.20 Diversity of marine systems and associated microbial metabolic processes.** Decreasing electron acceptor availability with depth into the sediment or with increasing distance into an oxygen minimum zone is indicated by red wedges. Sulfate becomes limiting only at greater depths in marine sediments. The indicated metabolic diversity is covered in Chapter 14.

**Madigan et al. 2018**

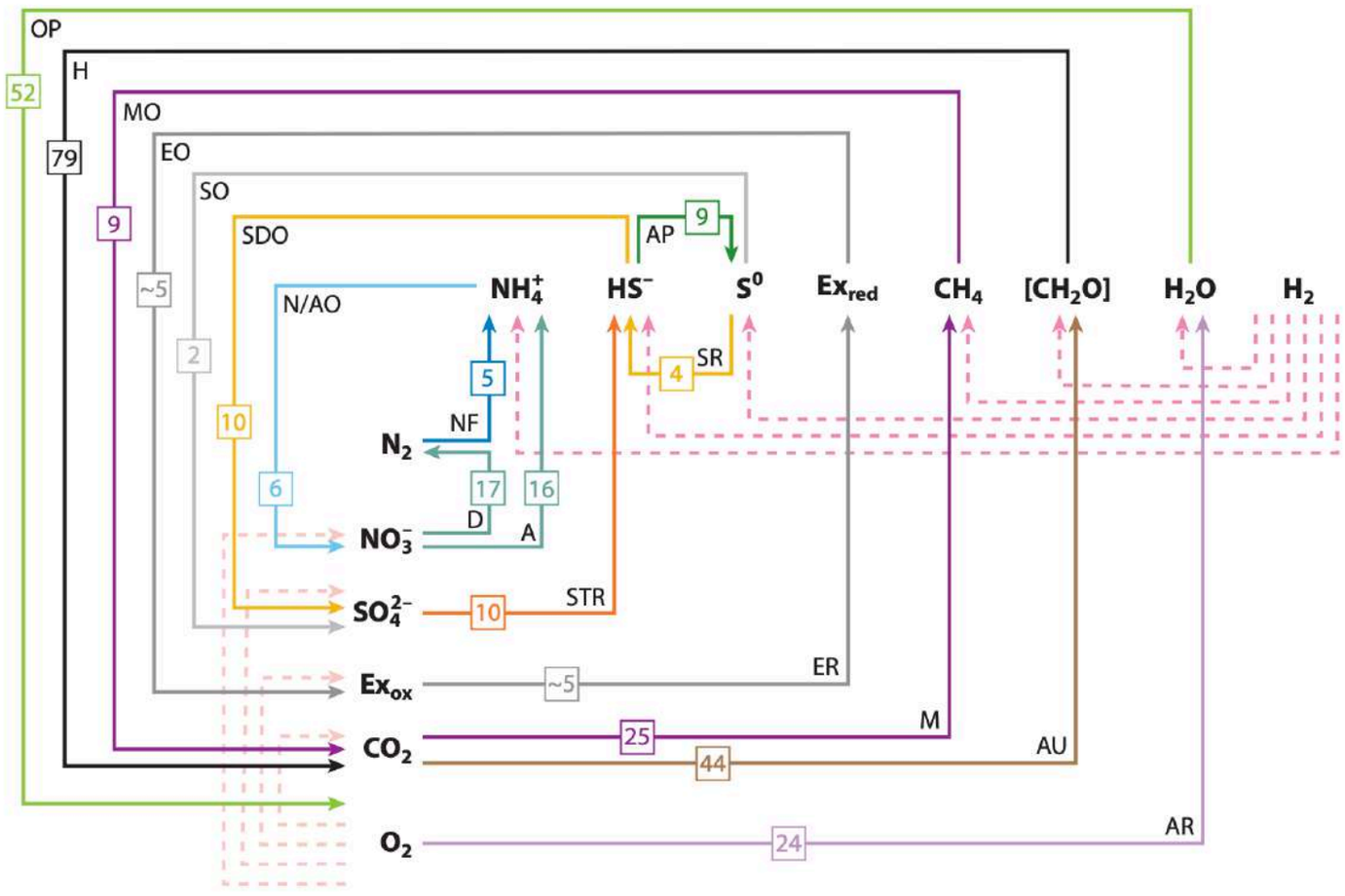
# Integrative approach

**Metabolic pathways evolved to utilize available substrates produced as end products of other types of microbial metabolism, either by modification of existing metabolic pathways or by using established ones in reverse**




https://www.genome.jp/

## Oxidative reaction




## Reductive reaction

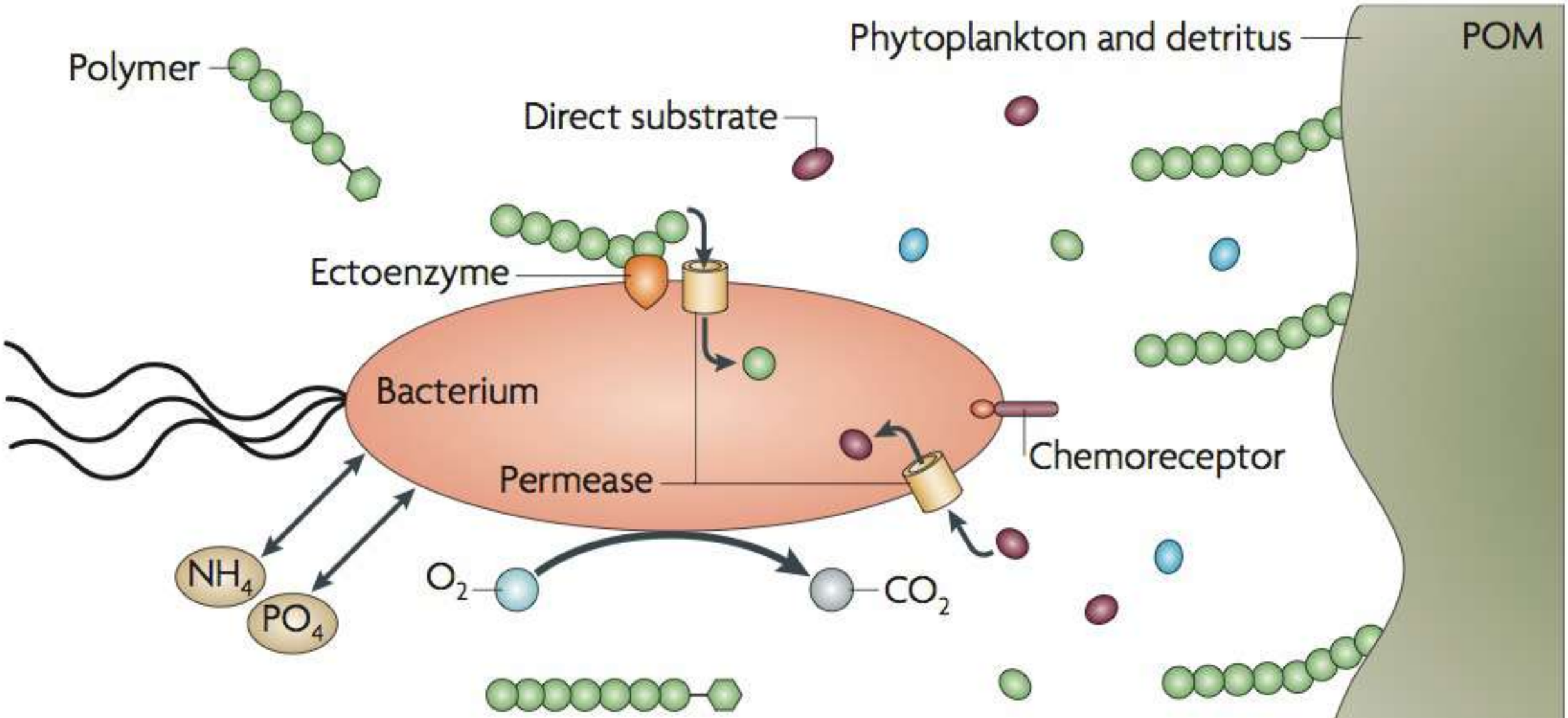
Jelen et al. 2016

*A, ammonification; AP, anoxygenic photosynthesis; AR, aerobic respiration; AU, autotrophy; D, denitrification; Exox, other elements oxidation; Exred, other elements reduction; H, heterotrophy; M, methanogenesis; MO, methane oxidation/methanotrophy; N/AO, nitrification/ammonia oxidation; NF, nitrogen fixation; OP, oxygenic photosynthesis; SDO, sulfide oxidation; SO, sulfur oxidation; SR, sulfur reduction; STR, sulfate reduction*

# Adaptive strategies of microbes in the ocean

- Hydrolysis-uptake coupling: permease and ectoenzyme
- Two-component system: chemoreceptors
- Motility
- Quorum sensing
- Biofilm
- Genome architecture

# Adaptive strategies of heterotrophic bacteria in the ocean




by Farooq Azam

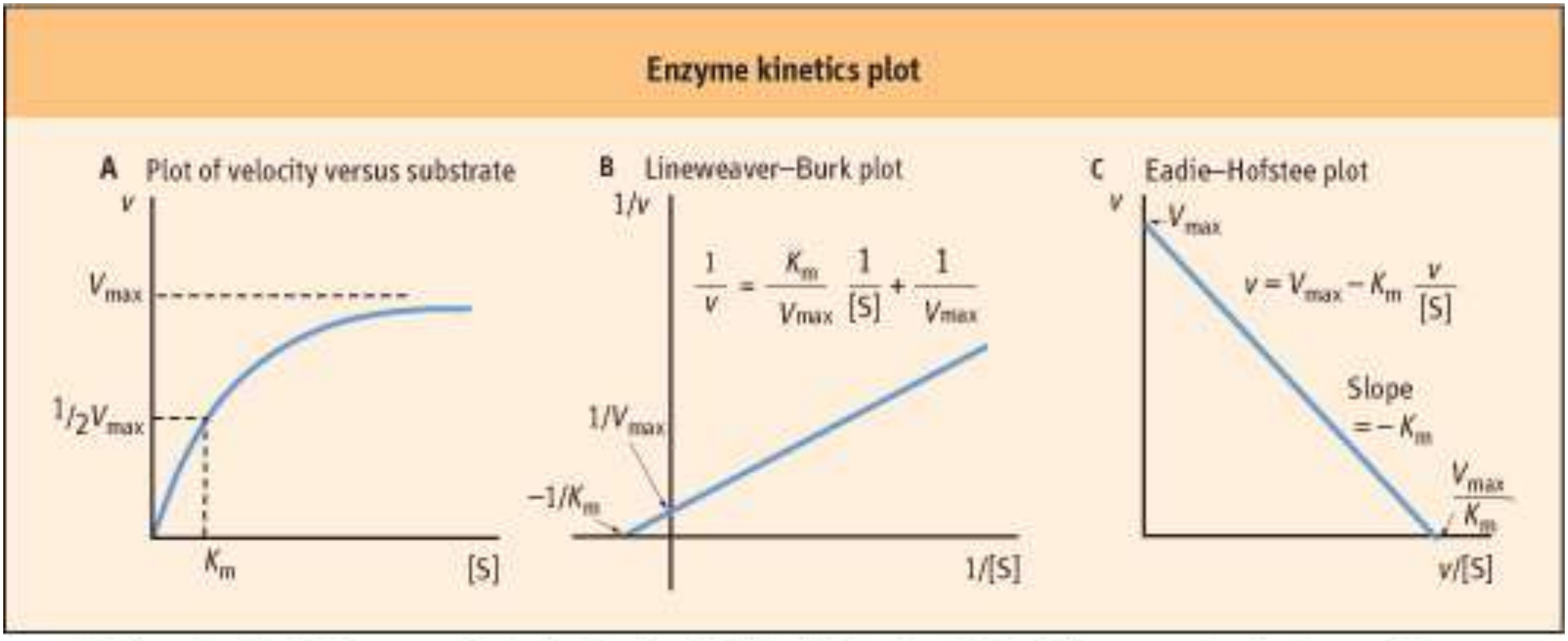
Azam and Malfatti, 2007 Nature Reviews Microbiology 10:782

- Motility, environmental sensing, permeases and cell-surface hydrolases
- Adapted fine biochemical strategies to interact with organic matter natural and human-created

## Hydrolysis-uptake coupling: permease and ectoenzyme

## Spatial coupling to optimize growth

## Compared to steady environments of equal average concentration, fluctuating environments reduce growth rate by up to 50%



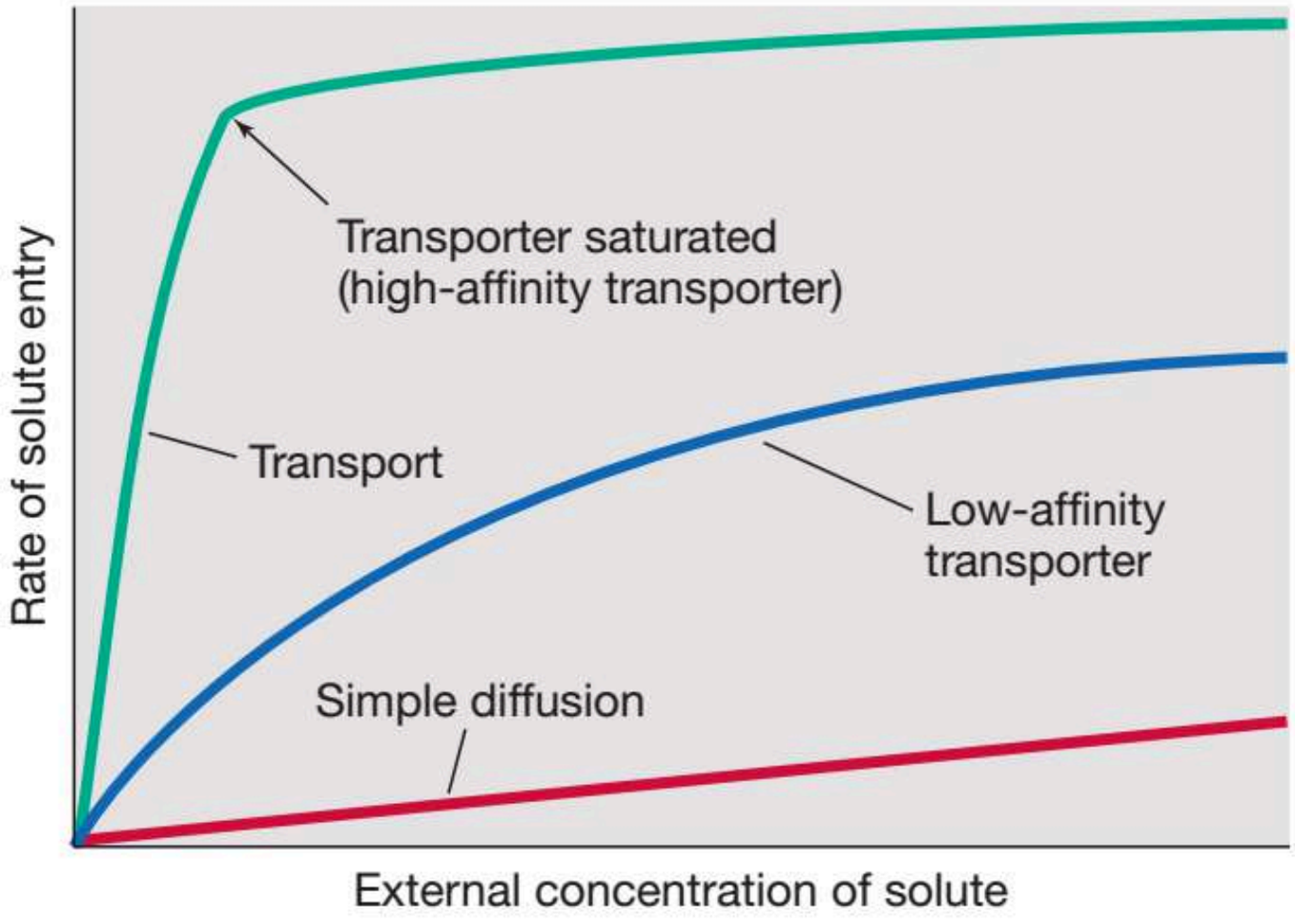

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$K_m$ (substrate concentration required for half-maximal activity)

The rate of the reaction approaches the maximum velocity ($V_{max}$)

# Microbial adaptations to increase uptake of molecules

## Microbial interfaces, the membranes as hotspots of activities




**Low-concentration**

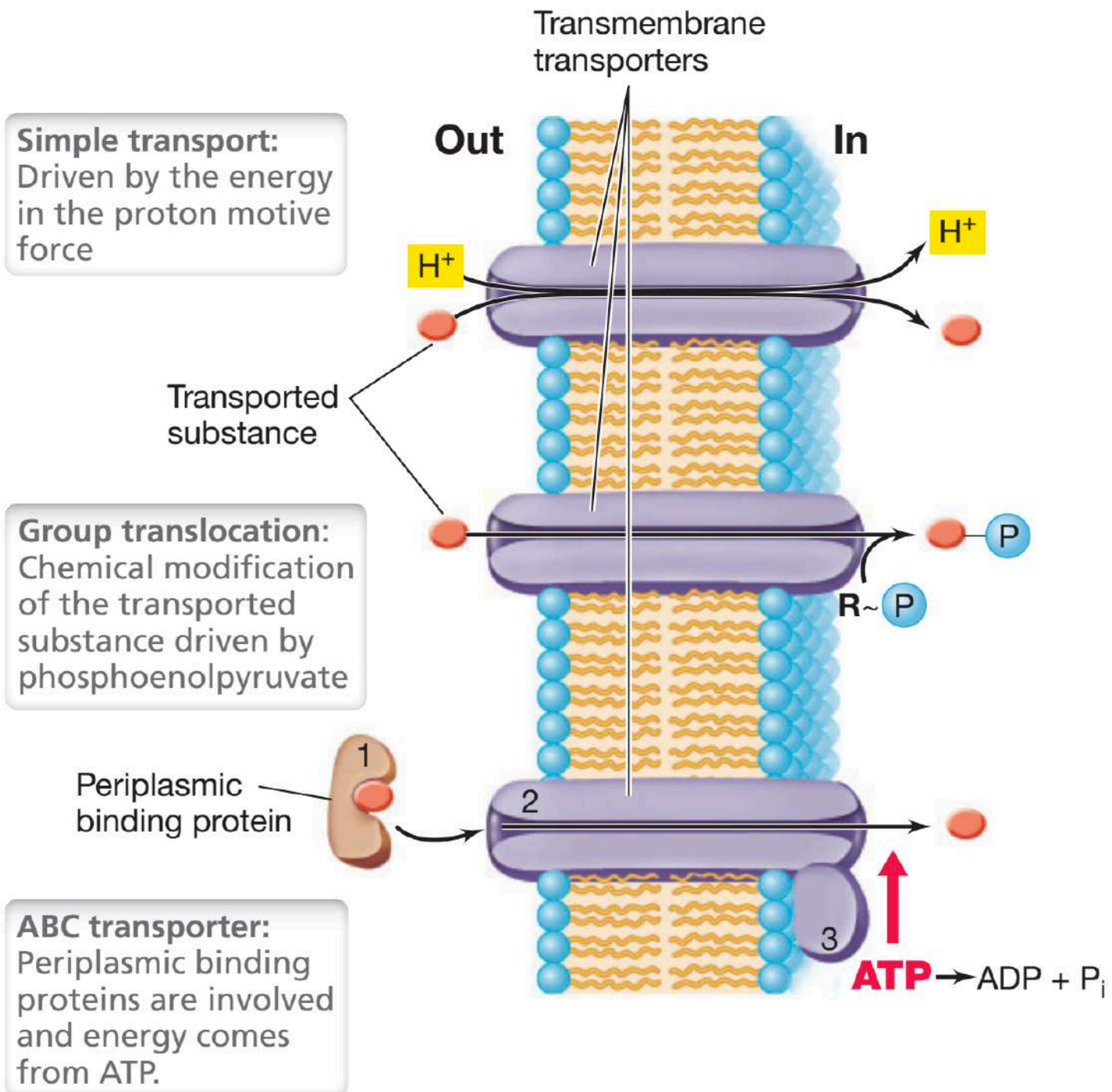
**High-concentration**

**Figure 2.8 The importance of transport in membrane function.** In transport, the uptake rate shows saturation at relatively low external concentrations. Both high-affinity and low-affinity transport systems are depicted.

Madigan et al. 2018

# Uniporters, symporters and antiporters

## Periplasmic space



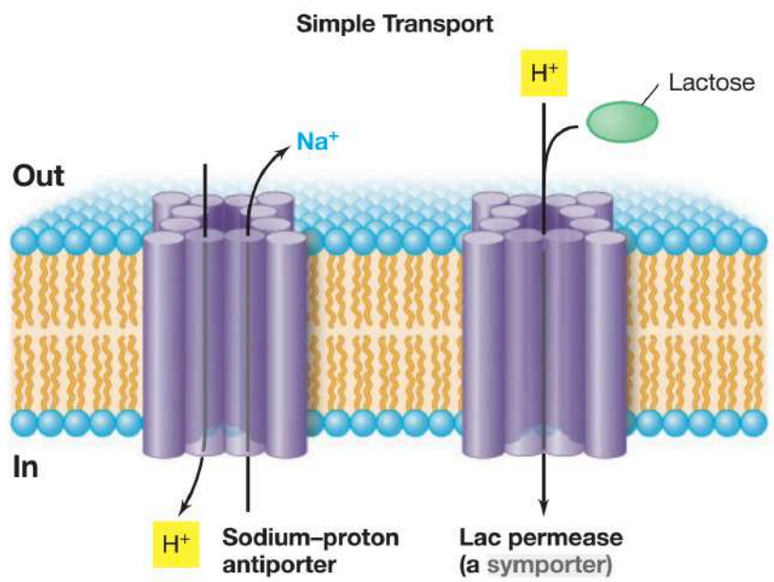

## Cytoplasm

**Uniporter: Cause unidirectional transport (through membrane spanning protein)**

**Symporter: Transport substrate along with $H^+$ (or $Na^+$)**

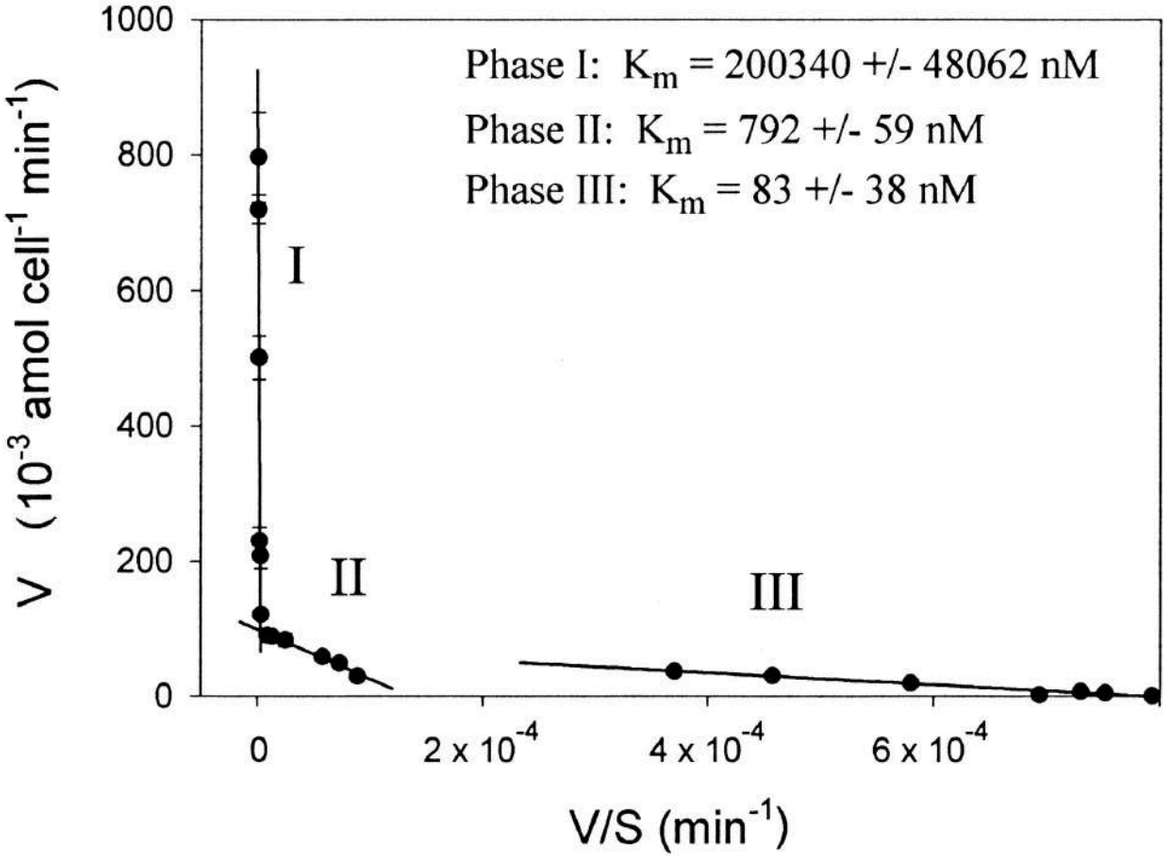
**Antiporter: Substrate and $H^+$ (or $Na^+$) transported in opposite directions**

**[Require PMF, Proton Motive Force]**




Madigan et al. 2018

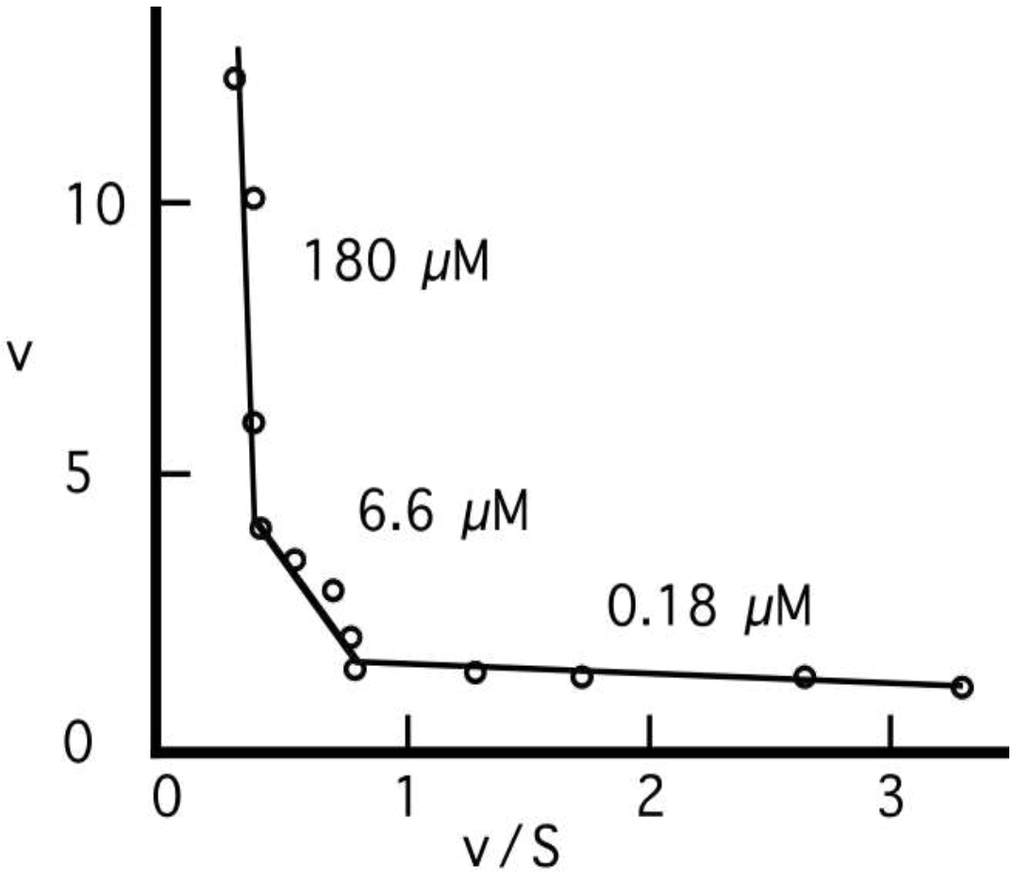
# Microbes can modulate uptake kinetics

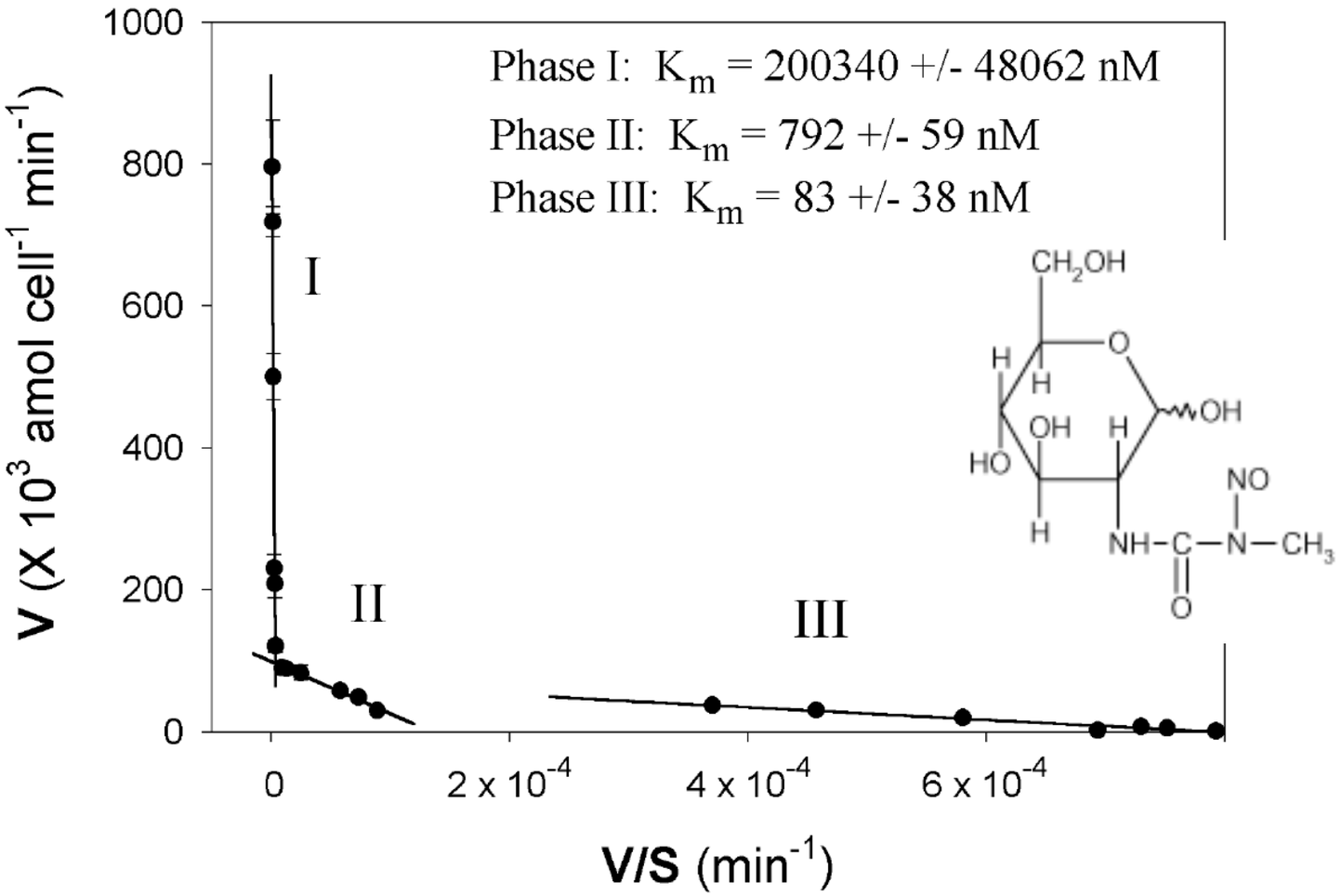



- Low-Flux and High-Flux systems to use patchy DOM
- Eadie-Hoftsee plot of NAG uptake data for isolate JSL 12-2 (γ-*Proteobacteria*) —> N-acetyl-D-glucosamine (NAG)
- Microbes perceive substrate gradients in the environment
- Glucose and C-AMP have multiphasic uptake systems

**Riemann and Azam, 2002**

# Multiphasic kinetics: Do bacteria experience substrate gradients/ patches?

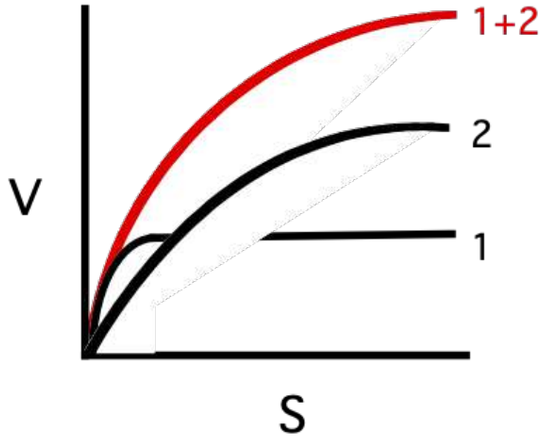



**D-GLUCOSE permease**
**(Nissen et al, 1984 MEPS 16:155-160)**

**N-Acetylglucosamine PTS common in marine bacteria**
**(Riemann and Azam, AEM, 2002)**
**-In ~50% marine isolates; natural assemblage, too!**
**-Facultative aerobes common in waters off SIO?**
**-STZ (streptozotocin; alkylating agent; breaks down DNA) as assay**

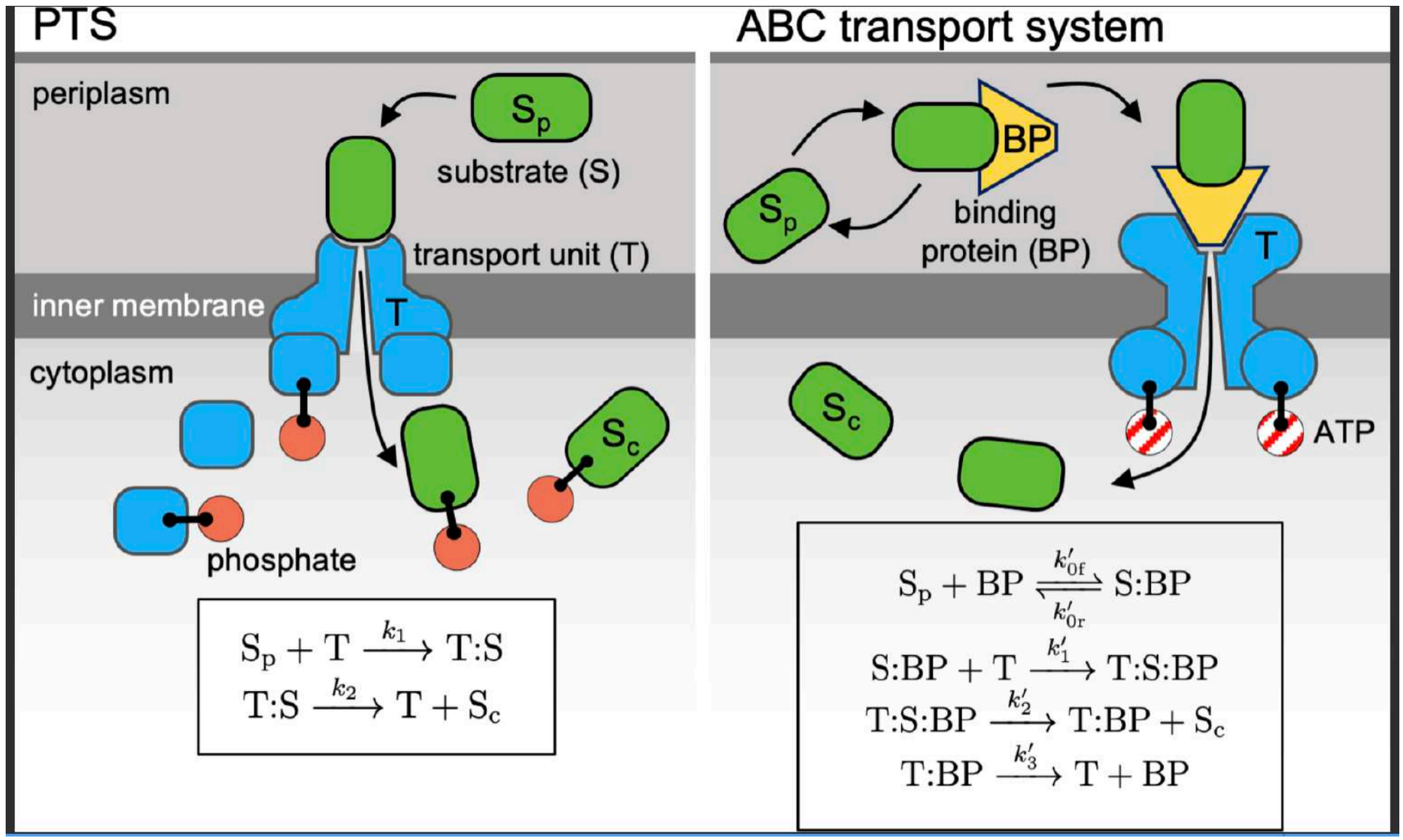
**Low-flux and High-flux systems:**
- **Bacteria perceive environmental substrate gradients?**
- **Tight hydrolysis-uptake coupling?**




# From Farooq Azam

# Copiotrophs *vs.* Oligotrophs

While prototypical copiotrophs, like *Vibrios*, possess numerous phosphotransferase systems (PTS), prototypical oligotrophs, such as SAR11, lack PTS and rely on ATP-binding cassette (ABC) transporters, which use binding proteins




Norris et al., 2021

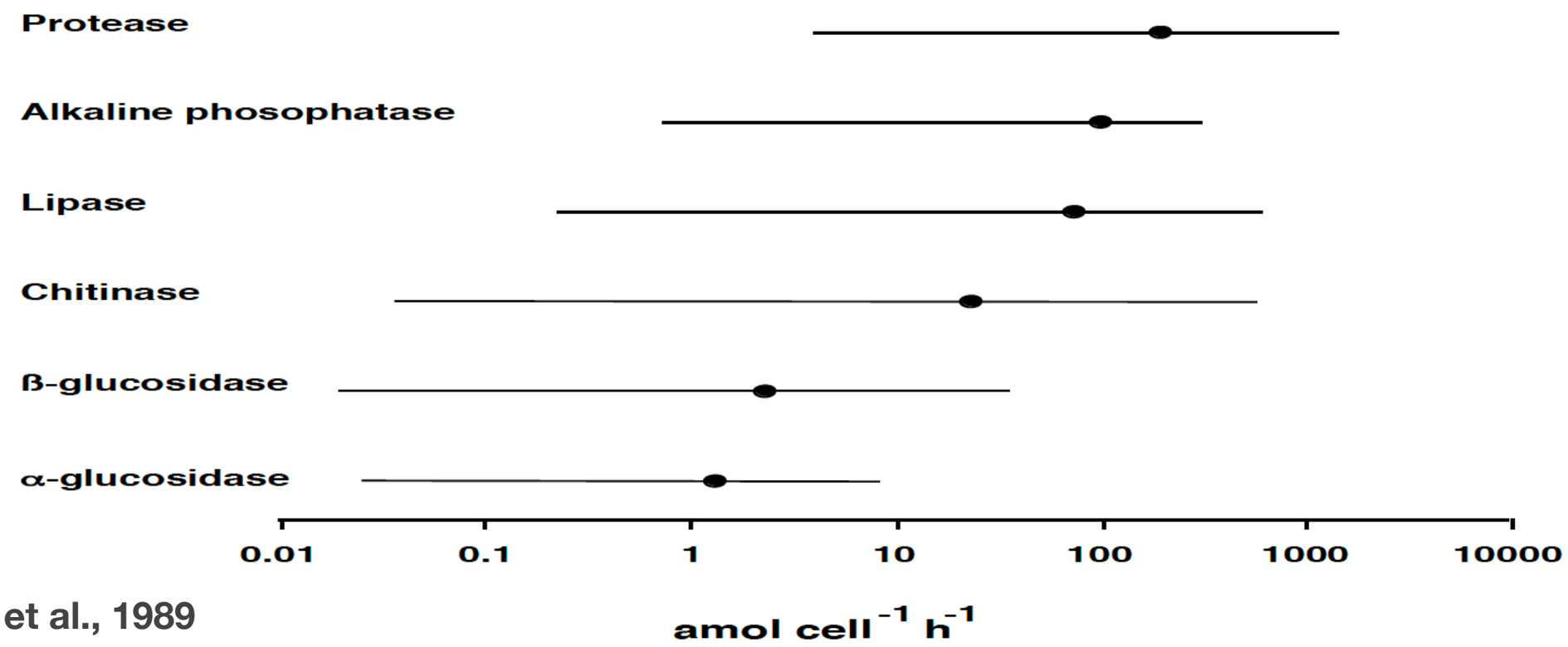
# Oligotrophic-copiotrophic life style

**Table 2 Characteristics of oligotrophic and copiotrophic bacteria in the oceans**

| Property | Oligotrophs | Copiotrophs | Comment |
|---|---|---|---|
| Maximum growth rate | $<0.2\ d^{-1}$ | $>1\ d^{-1}$ | See main text |
| Variation in growth | Small | Large | See main text |
| Abundance | High | Low | |
| Genome size | Small | Large | Divide at 2 Mb? |
| Versatility | Low | High | E.g., organic carbon use |
| Hydrolyases | − | + | E.g., chitinase |
| Periplasmic proteins | ++ | + | E.g., transporters |
| Siderophores | − | + | |
| Motility | − | + | |
| Chemotaxis | − | + | |
| Quorum sensing | − | + | |
| CRISPR/Cas | − | + | Phage defenses |

The −, +, and ++ indicate the absence, presence, and enhanced presence of the indicated property, respectively. Table based on studies by Lauro et al. (2009) and Yooseph et al. (2010) and arguments presented in the main text.

# Microbes display wide range of enzymatic activities

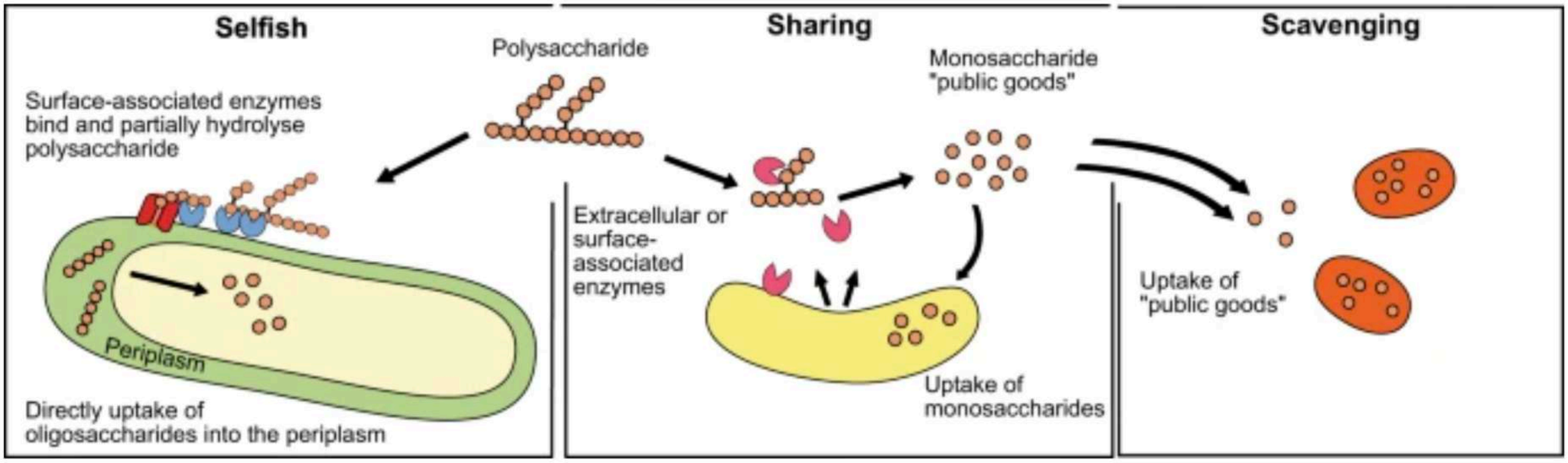



**Martinez et al., 1989**

- Digestion: Ectoenzymes (Protease, Lipase, Nuclease, Phophatase, Glucoside)
- Ectoenzymes: cut at the periphery of the molecules not `endo' cut
- Substrate specificity
- Adaptation for bacteria-POM interaction —> microbial biochemical pressure on POM and DOM
- Inhibited by monomers and building blocks
- Diverse microbes diverse speed of processing organic matter
- Cell surface hydrolases: $10^{2}$-$10^{4}$ x variability in cell-specific activity

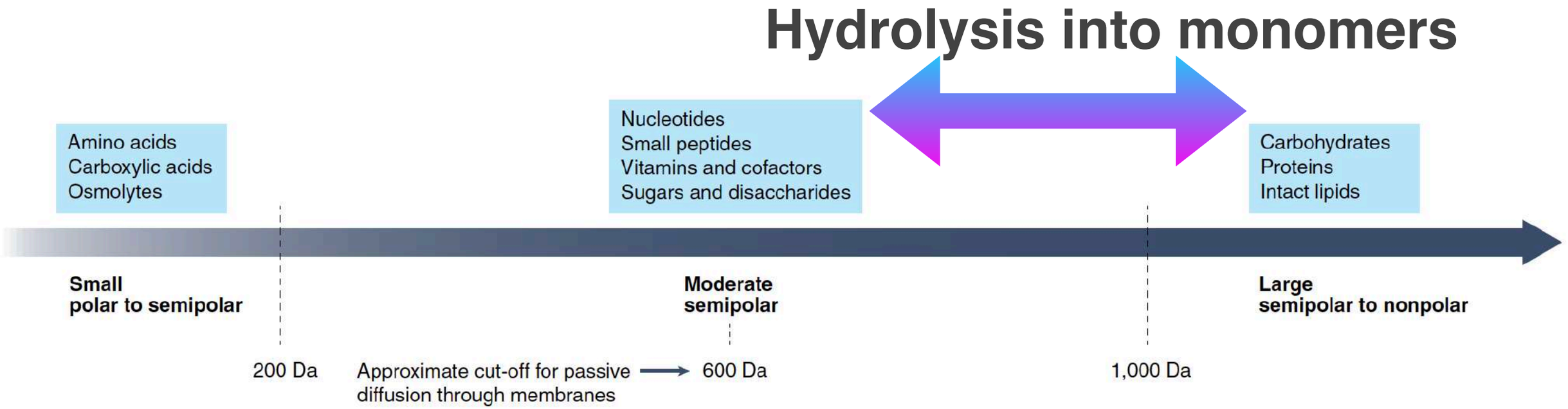
# Microbes use structurally-selective extracellular enzymatic strategies for high molecular weight DOM fraction

Reintjes et al., 2018



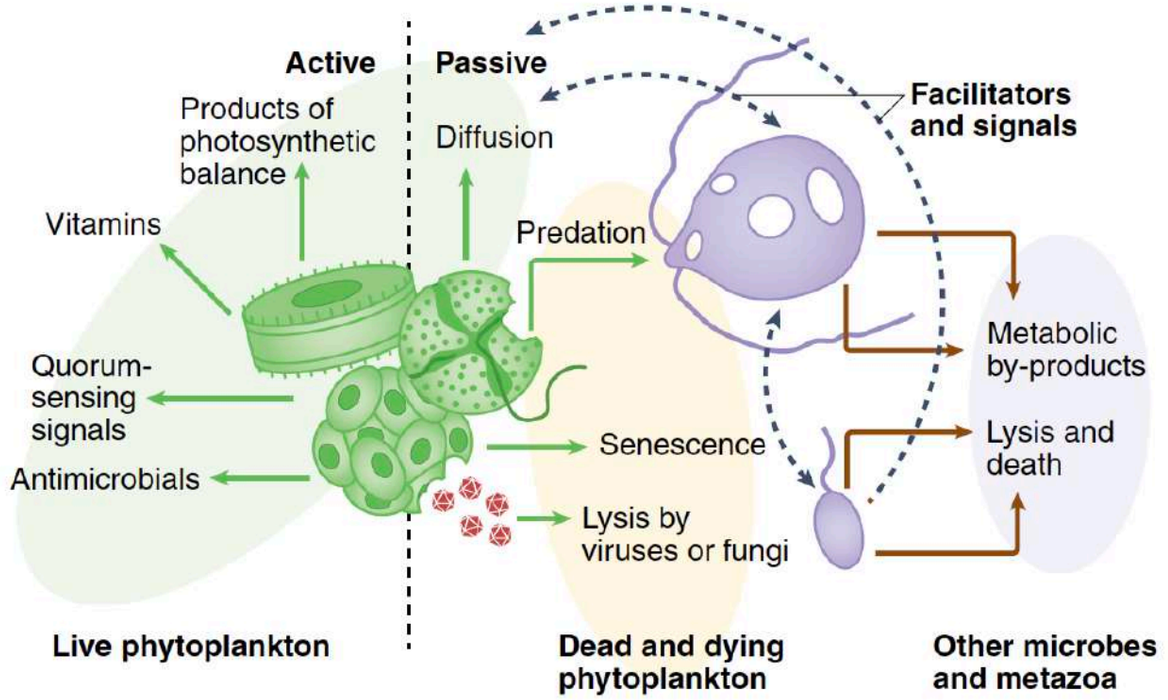

Schematic diagram of three main mechanisms of HMW substrate utilisation. Selfish: cells use surface associated enzymes to bind and partially degrade polysaccharides, which are directly taken up into the periplasm for further degradation with little to no production of extracellular hydrolysis products. Sharing: cells use surface-associated or ‘free’ extracellular enzymes to degrade polysaccharide to sizes suitable for uptake. Causes production of extracellular hydrolysis products (public goods). Scavengers: cells do not or cannot produce enzymes for the hydrolysis of polysaccharides, but take up the hydrolysis products produced by other organisms

# DOM size and reactivity




Actively photosynthesizing marine phytoplankton generate exometabolites (metabolites released into surrounding seawater) that form a pool of carbon named ‘extracellular release’ or ‘dissolved primary production

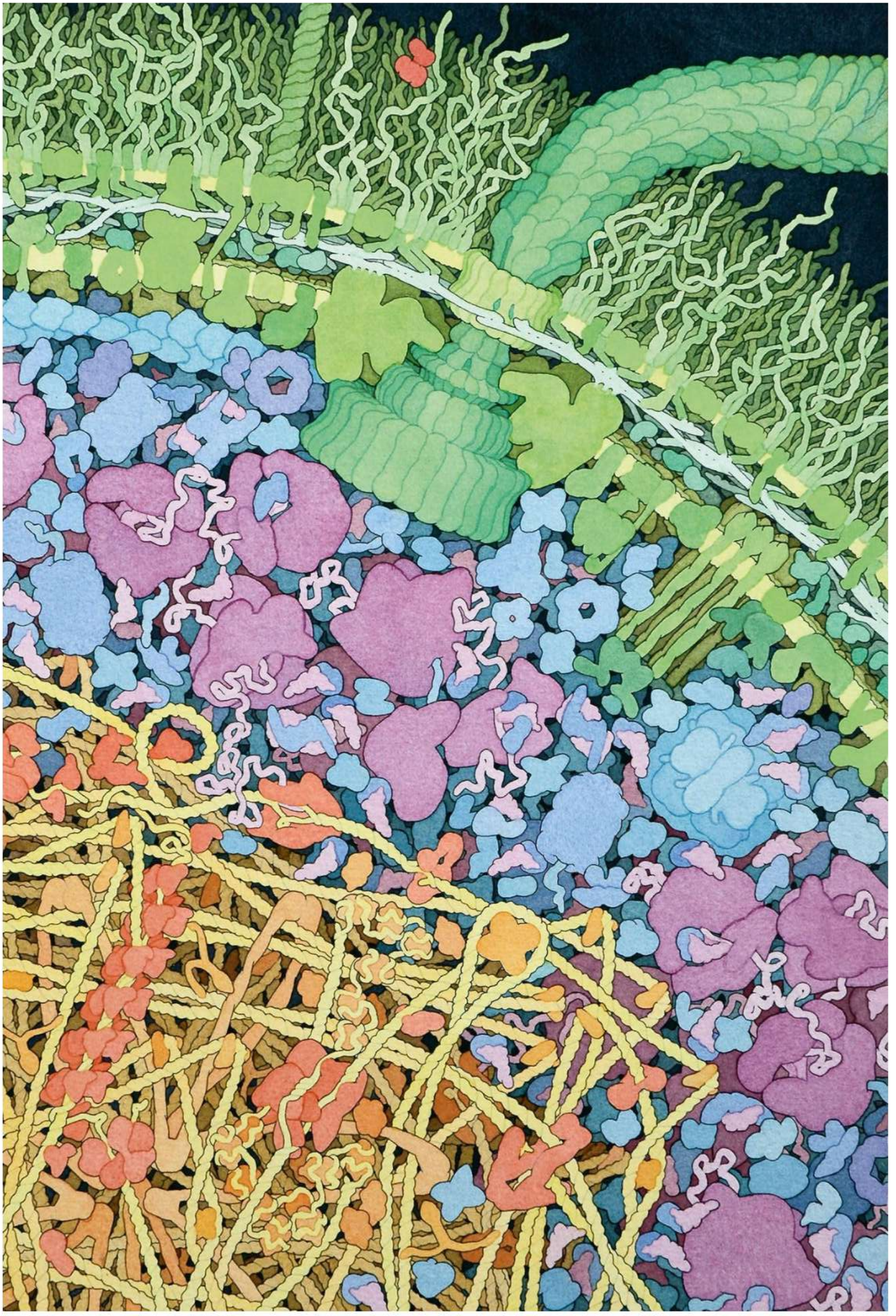
Green arrows indicate substrate metabolites derived from primary production, whereas brown arrows indicate those from secondary production




Moran et al., 2022

# Two-component system: chemoreceptors

# David S. Goodsell



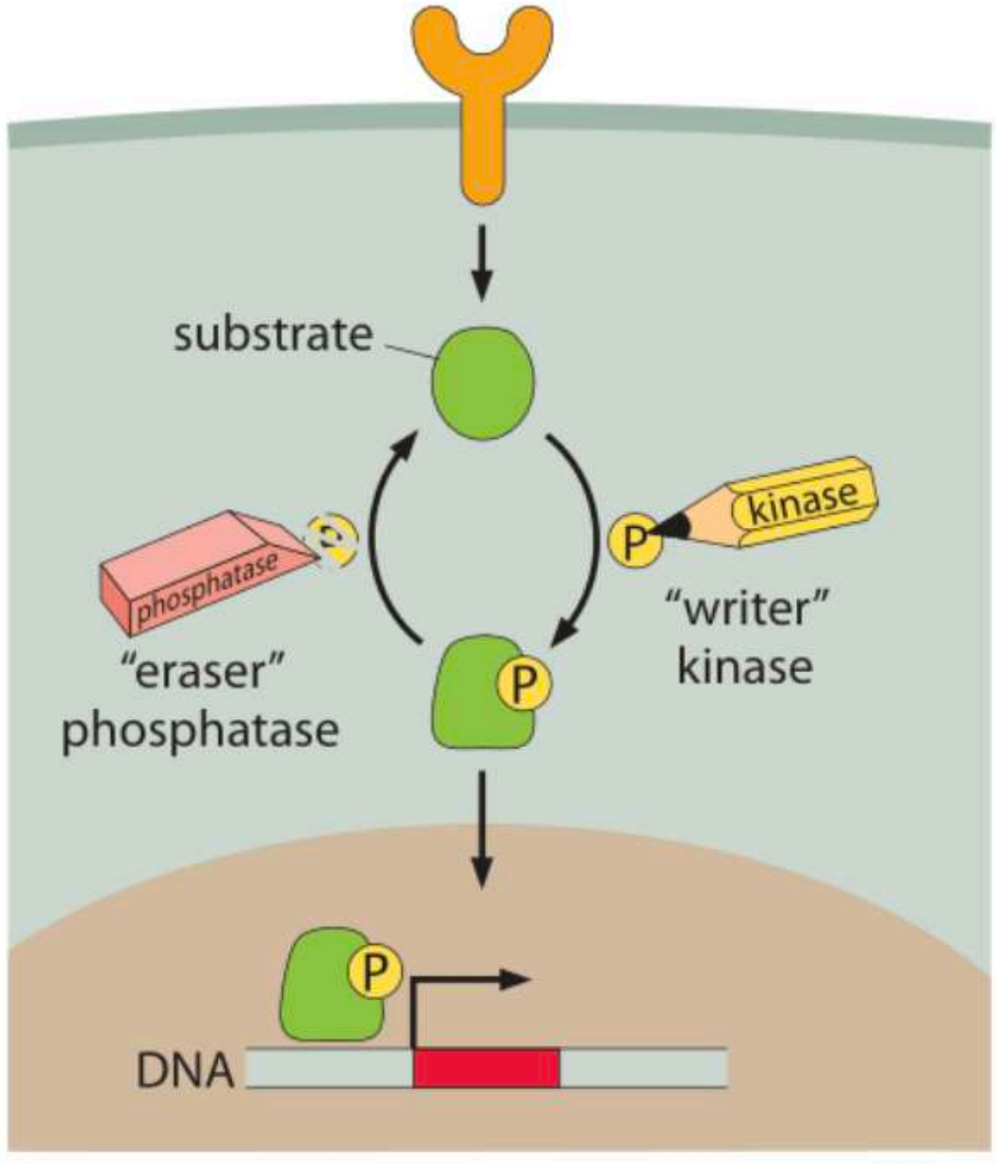
# Microbial signaling network

Two-component signal transduction systems are a predominant means by which microbes respond to their environments

• Receptor histidine kinase that senses a specific signal and translates that input into a desired output through the phosphorylation of its cognate response regulator

• Strategy for coupling changes in the environment to changes in cellular physiology

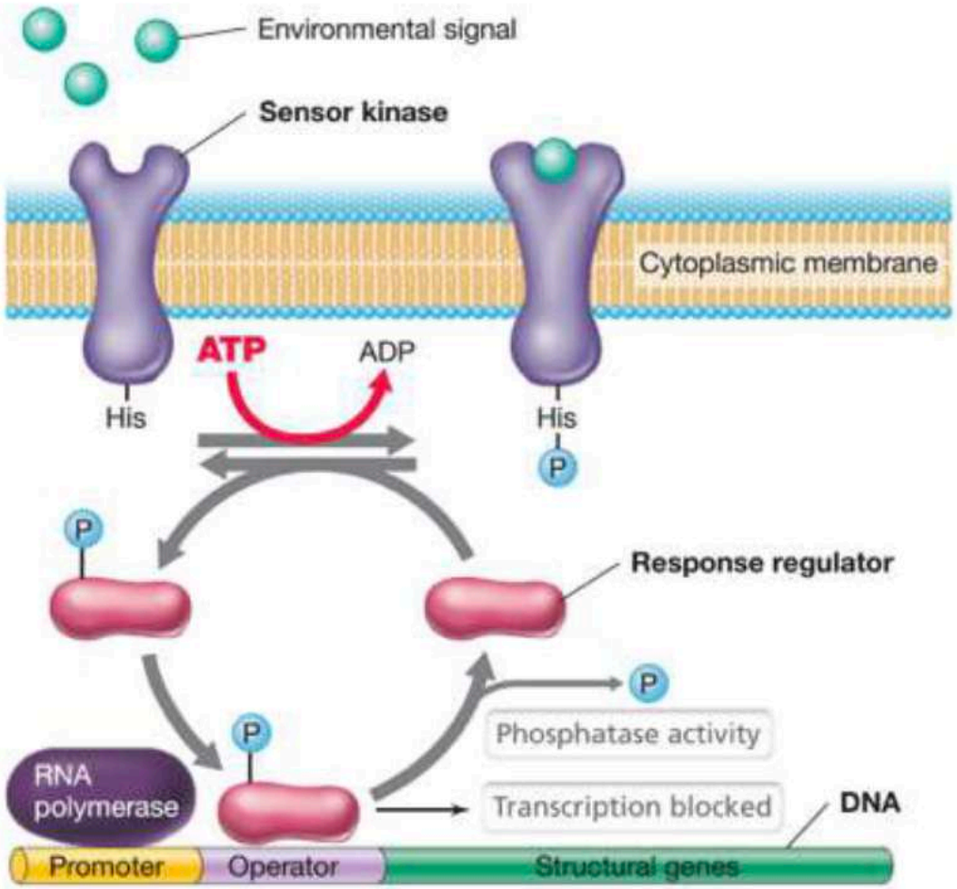
• Cognate histidine kinases and response regulators coevolve to maintain their interaction and to avoid cross-talk with other pathways




http://book.bionumbers.org/what-are-the-absolute-numbers-of-signaling-proteins/

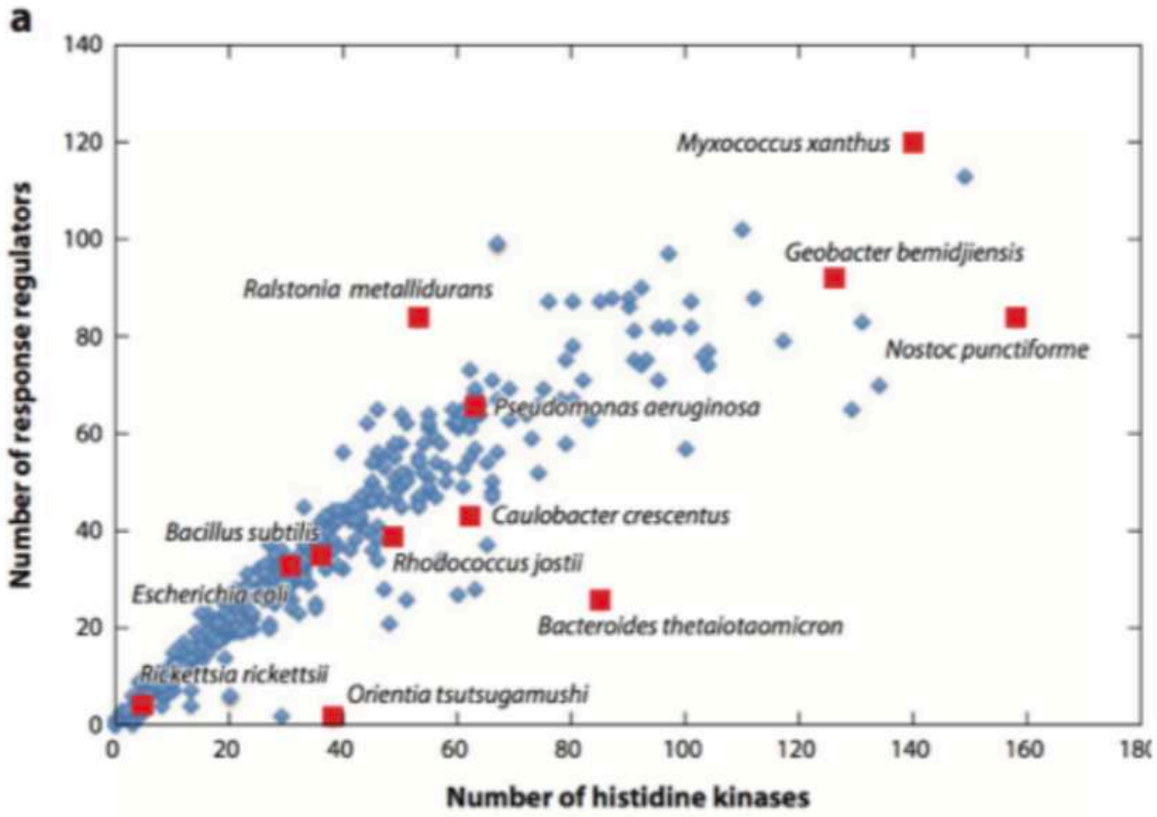
# Sensing and Signal Transduction

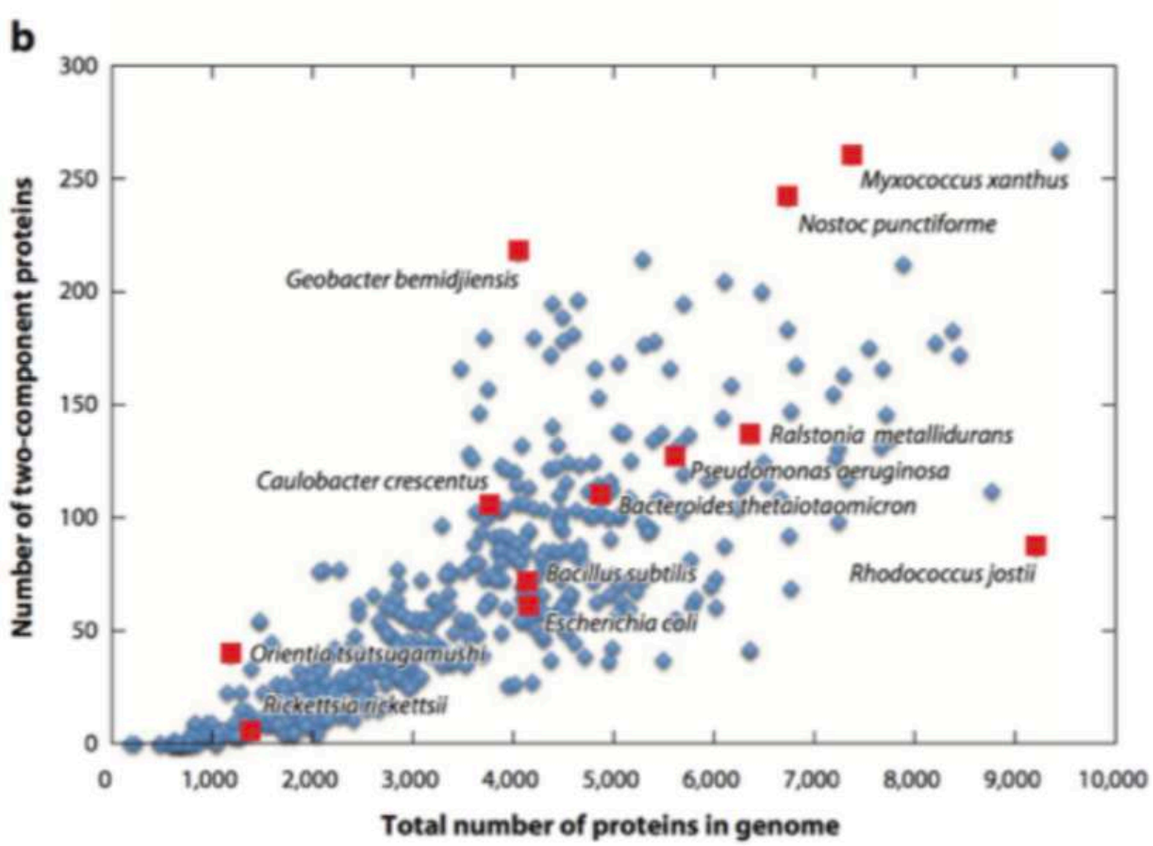
- Signal transduction systems contain two parts, they are called **two-component regulatory systems**
- Specific **sensor kinase** protein usually located in the cytoplasmic membrane, and a **response regulator** protein, present in the cytoplasm
- A kinase is an enzyme that **phosphorylates** compounds, typically using phosphate (P) from ATP, **autophosphorylation** at a specific histidine residue on the protein (histidine kinases)




Madigan et al. 2020

# Diversity of two-component signaling gene content in bacterial genomes

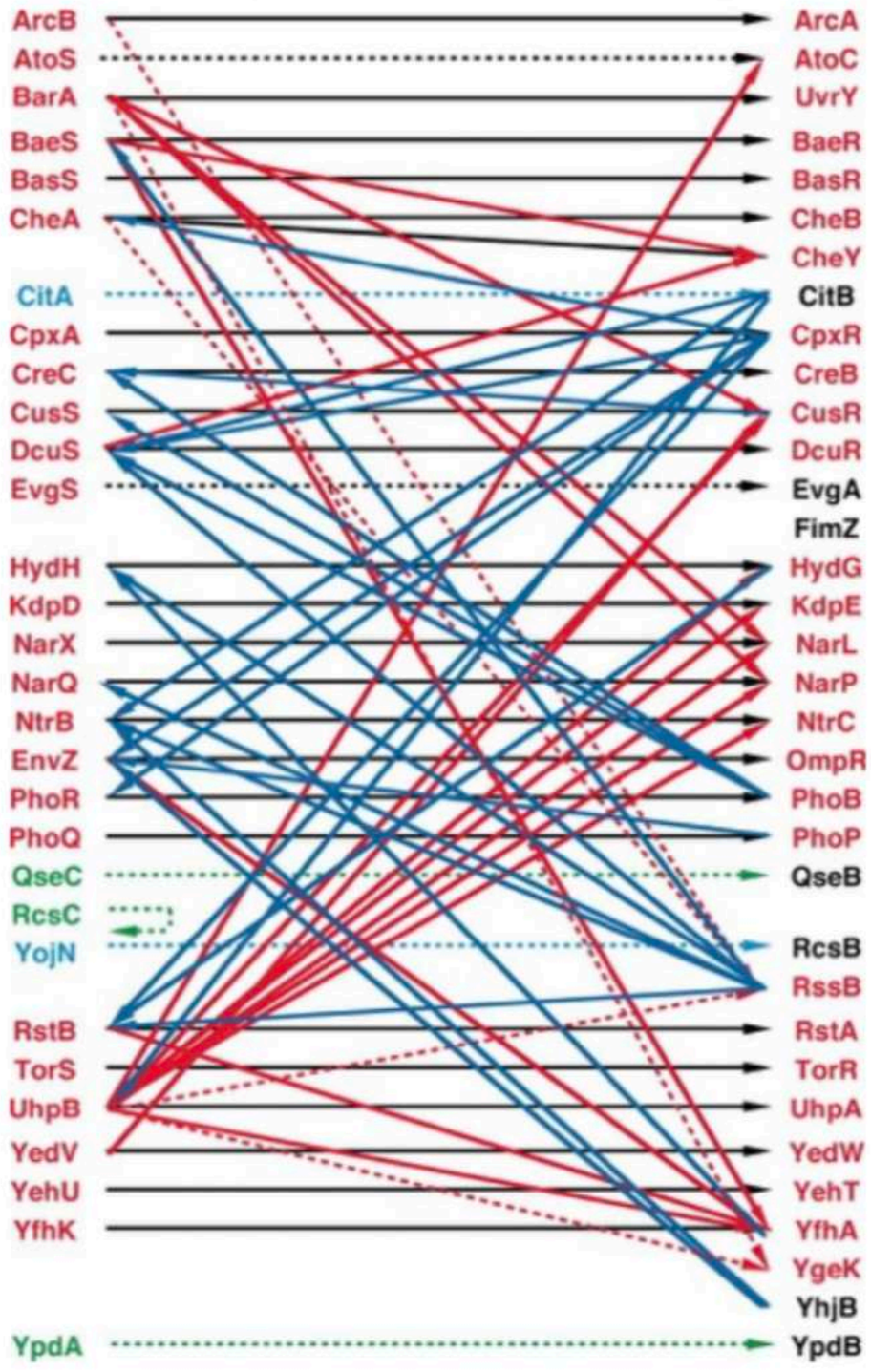



Capra & Laub, 2012

- **Most genomes** contain **equal numbers of kinases and regulators**, as pathways typically comprise one kinase and one cognate regulator
- When the ratio is not **1:1**, there are usually **more kinases than regulators**, suggesting that **response regulators may sometimes integrate signals from multiple kinases**
- Each plot is based on 504 bacterial genomes

# Cross-talk in trans-phosphorylation between non-cognate HK-RR pairs




**BLACK: cognate**

**RED: non cognate**

**BLUE: interference by non cognate RR —> the presence of non-cognate RR enhanced dephosphorylation of HK**

# Kinetic pattern of HK self-phosphorylation in vitro

**Quick signal transduction**

| | | | | |
|---|---|---|---|---|
| ArcB | ⇄ | *p*ArcB → | *p*ArcA | Anaerobibiotic respiration |
| NarQ | ⇄ | *p*NarQ → | *p*NarP | Nitrite & Nitrate utilization |
| NtrB | ⇄ | *p*NtrB → | *p*NtrC | Nitrogen assimilation |
| HydH | ⇄ | *p*HydH → | *p*HydG | Zinc/lead efflux system |

**Medium signal transduction**

| | | | | |
|---|---|---|---|---|
| CpxA | ⇄ | *p*CpxA → | *p*CpxR | Protein degradation |
| CusS | ⇄ | *p*CusS → | *p*CusR | Copper/silver efflux system |
| DcuS | ⇄ | *p*DcuS → | *p*DcuR | Fumarate respiration |
| KdpD | ⇄ | *p*KdpD → | *p*KdpE | Pottasium transport |
| NarX | ⇄ | *p*NarX → | *p*NarL | Nitrite & Nitrate utilization |
| TorS | ⇄ | *p*TorS → | *p*TorR | TMAO respiration |
| UhpB | ⇄ | *p*UhpB → | *p*UhpA | G6P transport |
| YehU | ⇄ | *p*YehU → | *p*YehT | Unidentified |
| YfhK | ⇄ | *p*YfhK → | *p*YfhA | Unidentified |
| BarA | ⇄ | *p*BarA → | *p*UvrY | Carbon storage regulator |
| BasS | ⇄ | *p*BasS → | *p*BasR | Unidentified |
| EnvZ | ⇄ | *p*EnvZ → | *p*OmpR | Outer membrane proteins |
| PhoR | ⇄ | *p*PhoR → | *p*PhoB | Phosphate assimilation |
| PhoQ | ⇄ | *p*PhoQ → | *p*PhoP | Magnesium transport |
| RstB | ⇄ | *p*RstB → | *p*RstA | Unidentified |
| YedV | ⇄ | *p*YedV → | *p*YedW | Unidentified |

**Slow signal transduction**

| | | | | |
|---|---|---|---|---|
| BaeS | ⇄ | *p*BaeS → | *p*BaeR | Envelop stress response |
| CheA | ⇄ | *p*CheA → | *p*CheB | Chemotaxis modulation |
| | | | *p*CheY | Flagellar motor switch |
| CreC | ⇄ | *p*CreC → | *p*CreB | Unidentified |



Kaneyoshi Yamamoto et al. J. Biol. Chem. 2005;280:1448-1456



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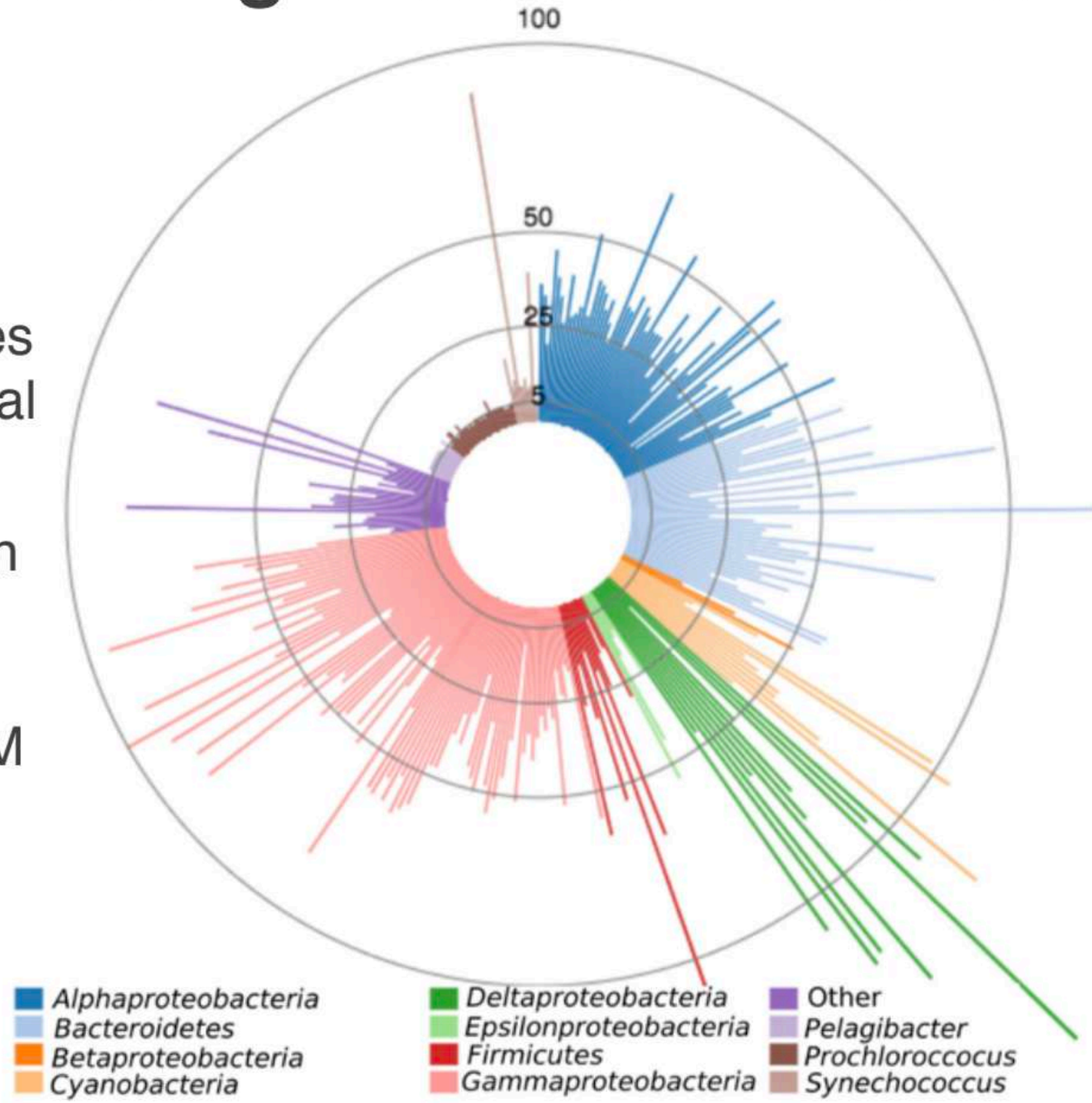
# Affinity of sensor kinases for signal molecules

| System | Function | Signal molecule |
|---|---|---|
| ArcB/ArcA | Sensing of oxygen and redox states | Quinones reflecting the redox state |
| NarX/NarL | Nitrate and nitrite respiration | Nitrate, nitrite |
| CitA/CitB | Transport and anaerobic metabolism of citrate | Citrate |
| CheA/CheY | Chemotaxis | Chemoattractants, e.g., serine and aspartate |
| FixL/FixJ | Nitrogen fixation | $O_2$, CO, NO |
| LovK/LovR | Bacterial cell attachment | Blue light |
| TodS/TodT | Degradation of benzene derivatives | Monoaromatic compounds |
| NtrB/NtrC | Nitrogen utilization | 2-ketoglutarate, glutamine |
| KdpD/KdpE | $K^+$ supply | $K^+$ |
| VanS/VanR | Antibiotics | Vancomycin |
| EnvZ/OmpR | Osmolarity | ? |
| KinB/Spo0F | Sporulation | ?, ATP as cosignal? |
| BvgS/BvgA | Virulence | Temperature, sulfate ions, nicotinic acid |
| LuxQ/LuxO | Quorum sensing | AI-2 |
| DesR/DesK | Lipid modification | Temperature |

Krell et al. 2010

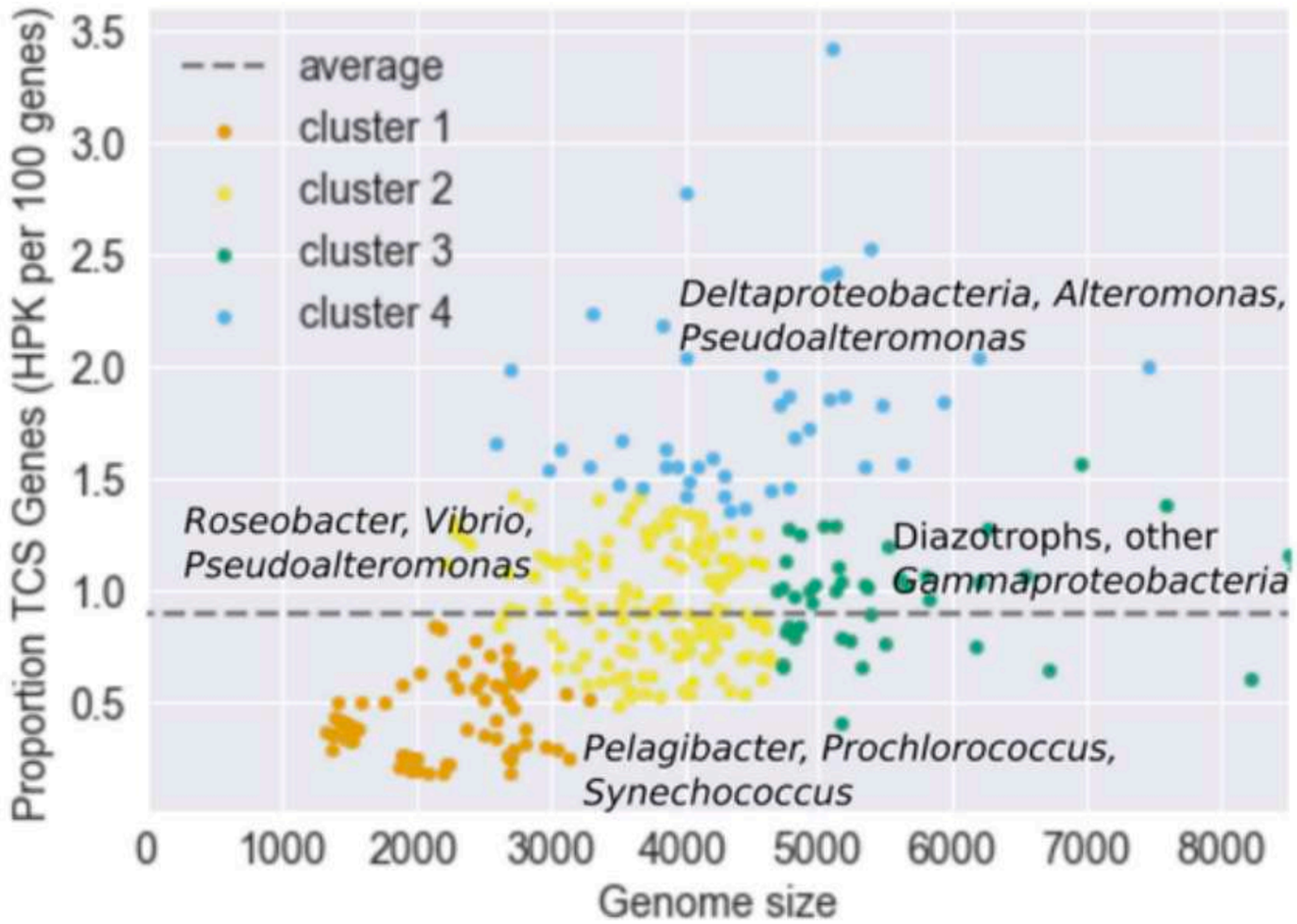
# Two-Component Sensing in Marine Bacteria

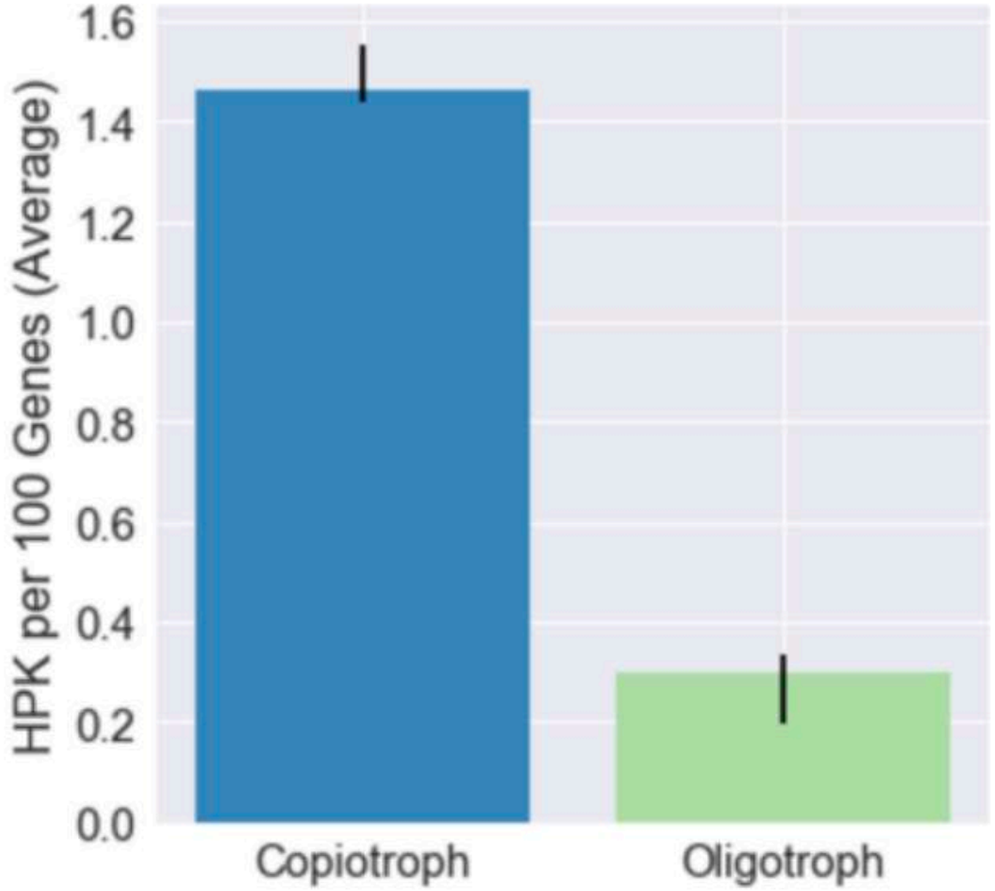
- Number of histidine kinase sensory genes in the genomes of 328 diverse marine bacterial species
- Number of histidine kinases in data set ranges from 1 (*Pelagibacter*) to 174 (*Desulfovibrio inopinatus* DSM 10711)




**Held et al., 2019**

# Two-Component Sensing in Marine Bacteria

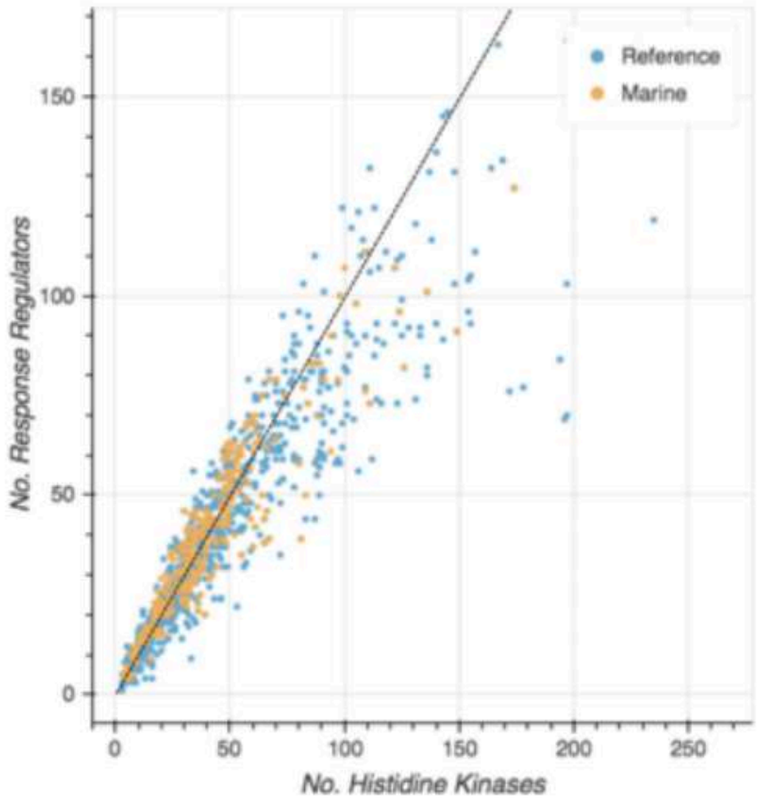


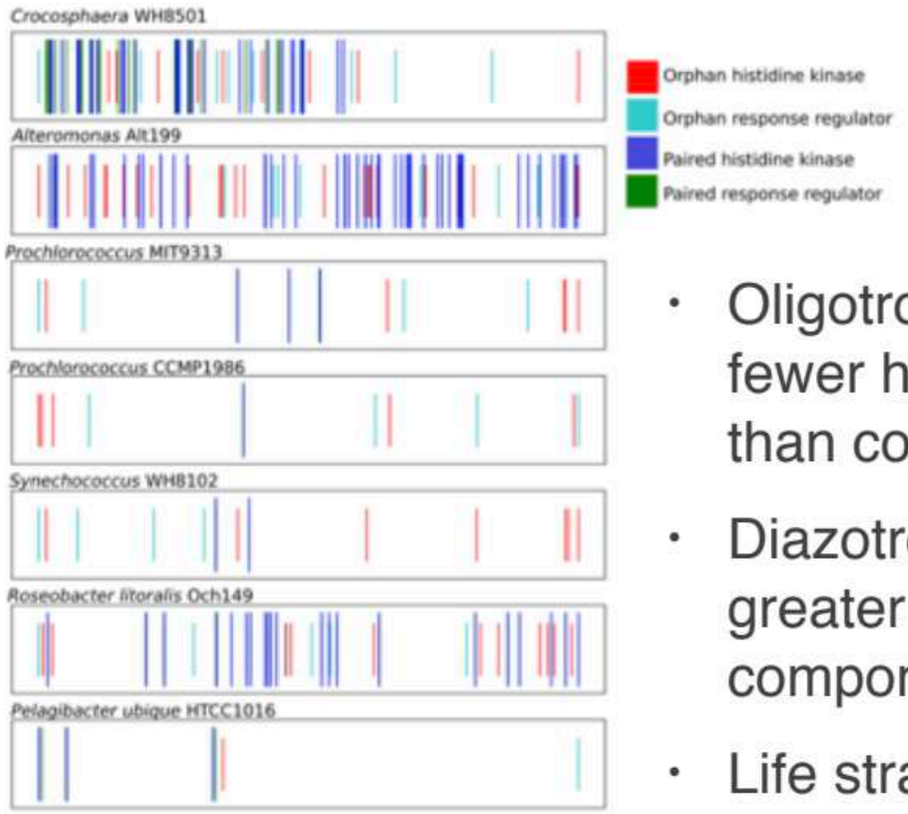




- Diverse groups along nutrient range
- TCS indicate insight of lifestyle

**Held et al., 2019**

# Adaptability continuum







- Oligotrophs have significantly fewer histidine kinases per gene than copiotrophs
- Diazotrophy is associated with greater numbers of two-component system genes
- Life strategy related to two-component systems

**TABLE 1** Characteristics of copiotrophs versus oligotrophs and their TCS system genes[a]

| Genus | Example genome size (bp) | Example % growth rate (per day) | Lifestyle(s) | HPK/100 genes | RR/HPK ratio | % hybrid HPKs | Reference |
|---|---|---|---|---|---|---|---|
| *Pelagibacter* | 1,200–1,400 | 0.4–0.58 | Oligotroph | 0.387 | 0.83 | Typically 0 | 59 |
| *Prochlorococcus* | 1,200–2,000 | 0.51–0.83 | Oligotroph | 0.76 | 1.22 | Typically 0 | 60 |
| *Synechococcus* | 1,500–3,000 | 1 | Oligotroph | 1.005 | 1.23 | 0–40 | 60 |
| *Trichodesmium* | ~5,000 | 0.29 | Oligotroph | 0.694 | 0.753 | 15–35 | 61 |
| *Crocosphaera* | ~6,000 | 0.5 | Oligotroph | 0.723 | 0.992 | ~35 | 62 |
| *Roseobacter* | ~5,000 | 1.45 | Varies/copiotroph | 0.755 | 0.991 | 10–40 | 63 |
| *Vibrio* | ~5,000 | Up to 14.3 | Copiotroph | 1.25 | 1.07 | 25–50 | 64 |
| *Alteromonas* | 4,000–4,500 | 6 | Copiotroph | 1.43 | 1.06 | ~40 | 65 |
| *Pseudoalteromonas* | 3,000–5,000 | ~30 | Copiotroph | 1.5 | 1.1 | 40–50 | 66 |

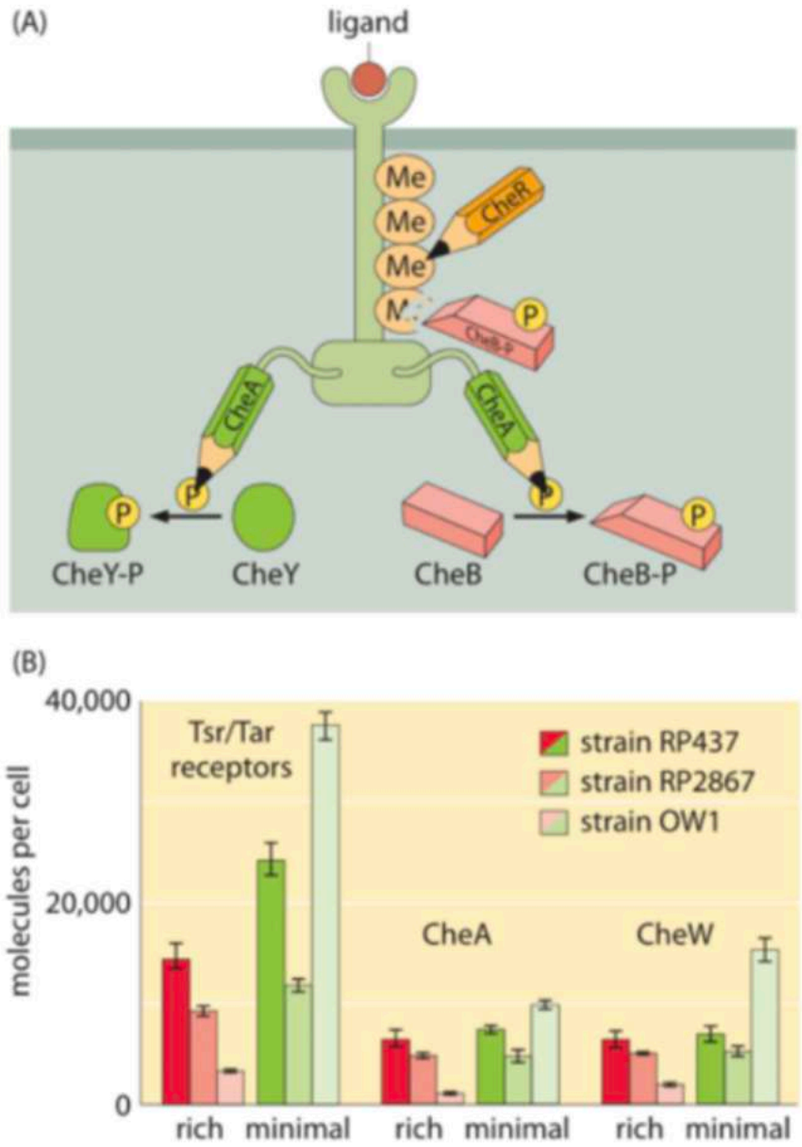
[a]HPK, histidine kinase; RR, response regulator.

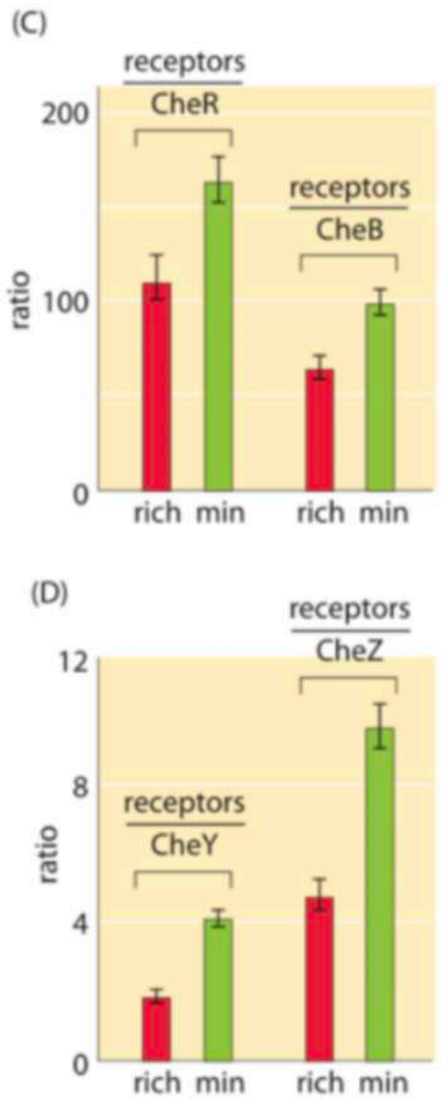
Held et al., 2019

# Chemotaxis, I

- **Chemokinesis: random movements, in absence of a concentration gradient of chemoattractant**
- **Chemotaxis: directional movement along a + gradient of chemoattractant**

# Census of the molecules of the bacterial chemotaxis signaling pathway




(A) Schematic of molecular participants involved in bacterial chemotaxis

(B) Number of chemotaxis receptor molecules and number of CheA and CheW (which connects Tsr/Tar receptors to CheA) molecules

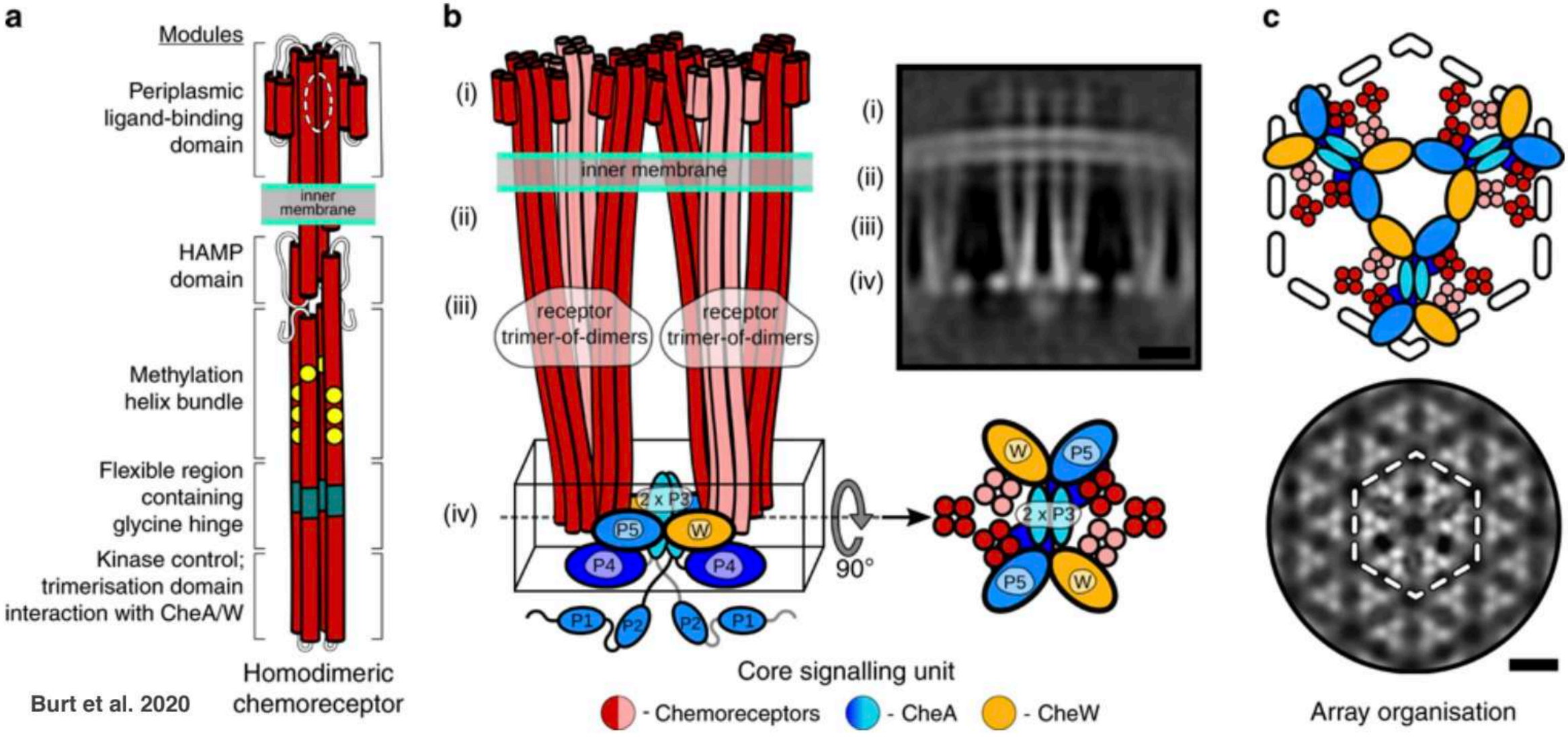
(C) Ratio of number of receptors to CheR and CheB for both rich and minimal media

(D) Ratio of number of receptors to CheY and CheZ (the phosphatase of CheY) for both rich and minimal media

- Results are shown for different strains and for different growth media
- By immunblotting

**(http://book.bionumbers.org/what-are-the-absolute-numbers-of-signaling-proteins/; adapted from M. Li et al., J. Bact. 186:3687, 2004.)**

# Overview of chemoreceptor array architectures

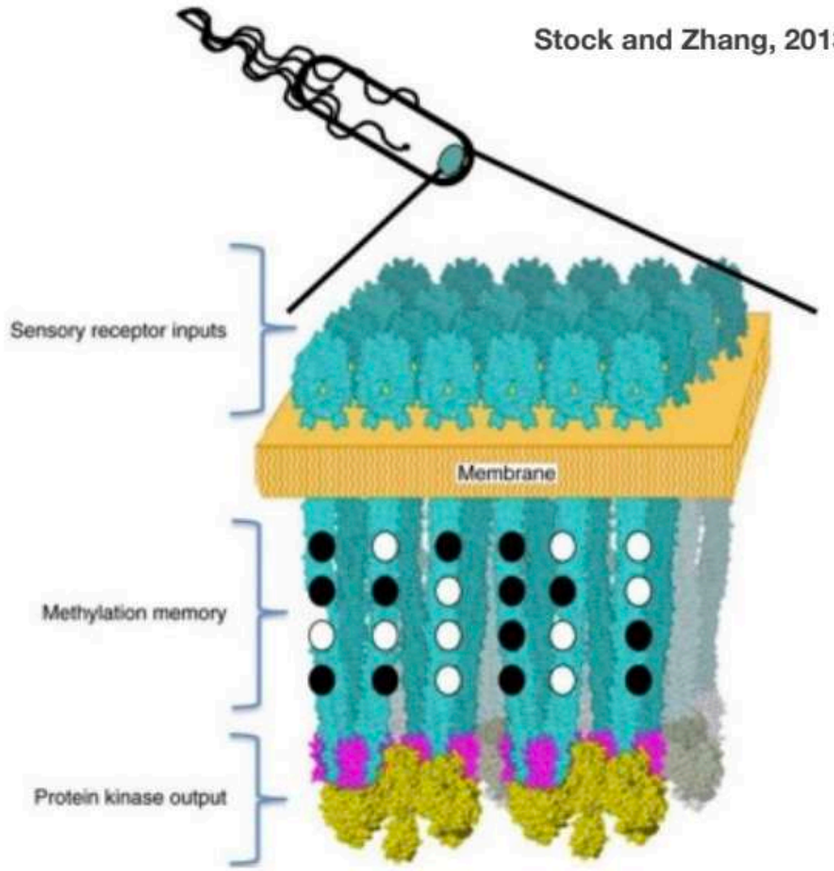


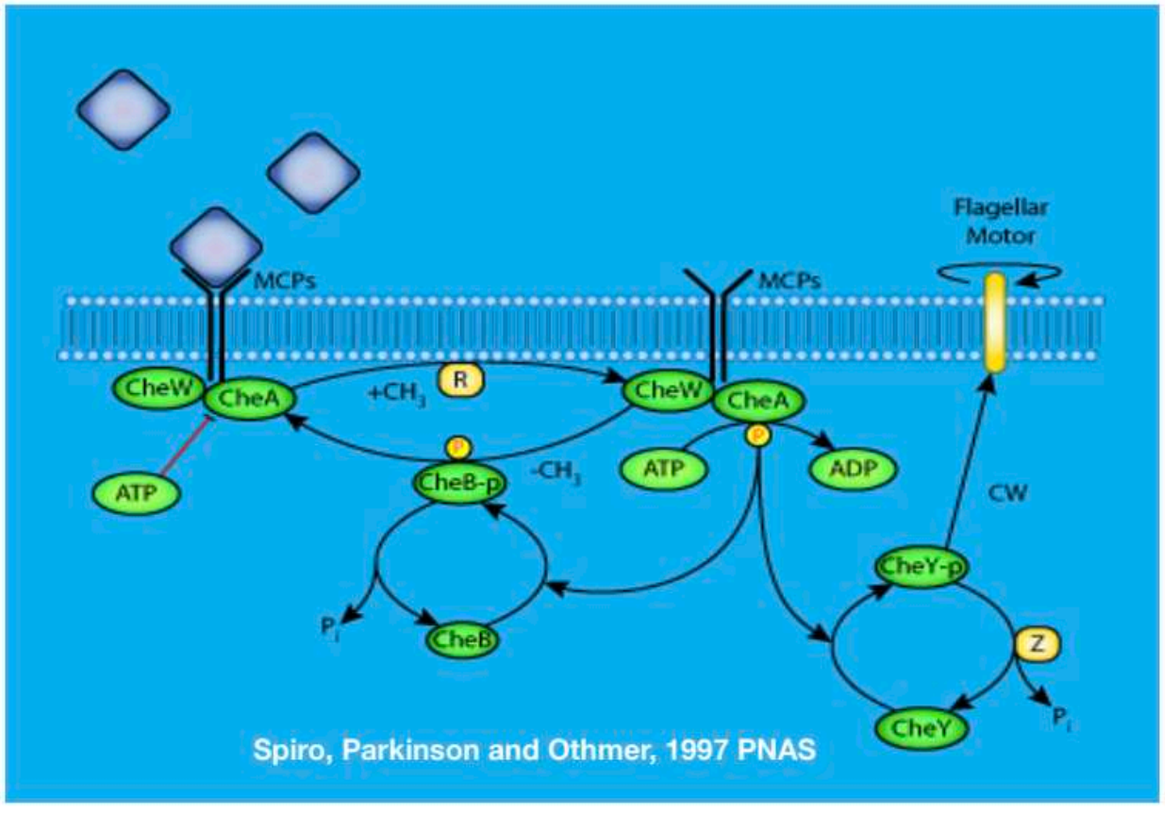

**scale bar 10 nm**

- *Escherichia coli* has four canonical MCPs that share a common functional architecture
- Two numerically predominant ones are Tar (aspartate and maltose sensor) and Tsr (serine and autoinducer 2 sensor)
- Cryo-electron tomography and subtomogram averaging

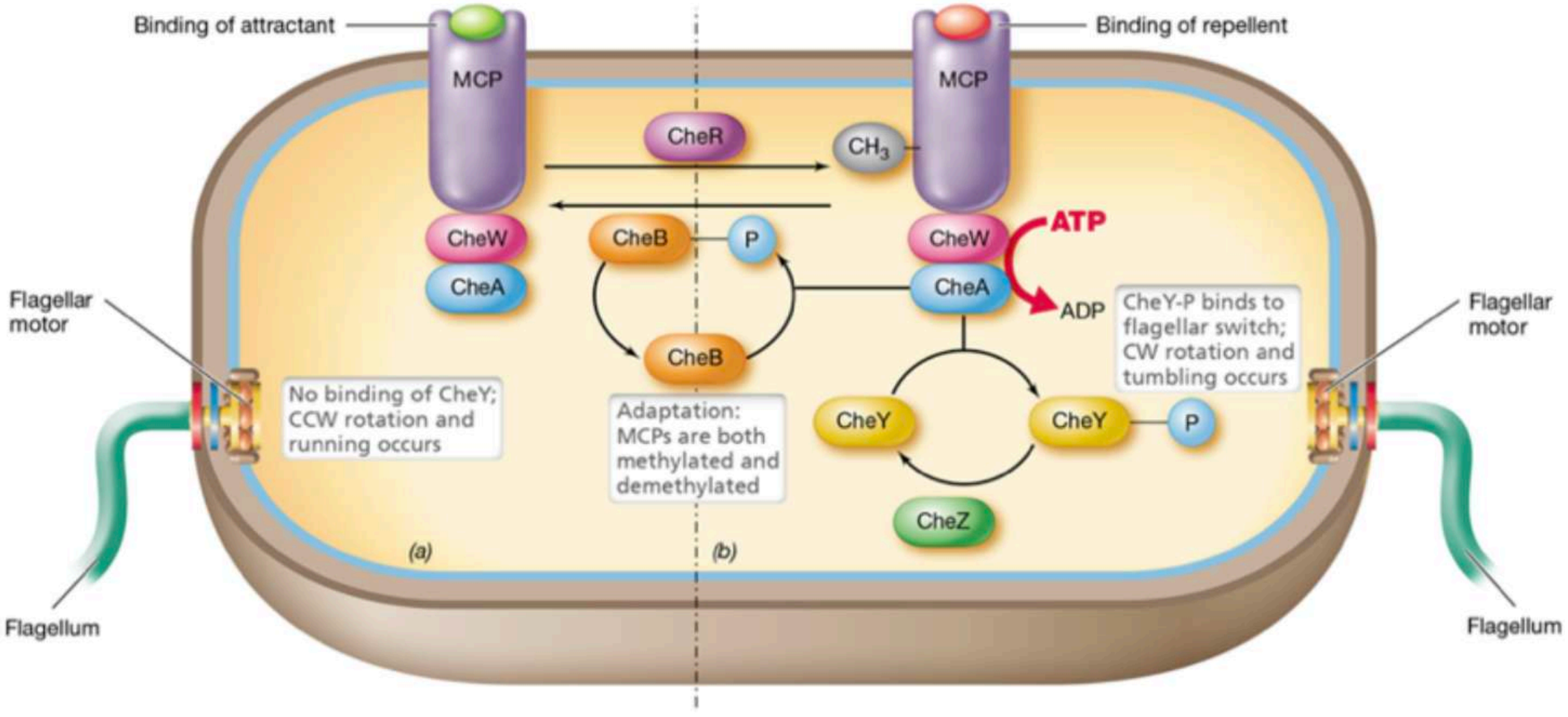
# Chemotaxis, II

- **Cells** are **too small** to sense **spatial** gradients of a chemical
- Cells can **respond** to **temporal gradients**
- **Sensing the change in concentration** of a chemical (attractant or repellent) **over time** rather than the absolute concentration of the chemical stimulus —> **signal-to-noise ratio**
- Two-component system modulates activities in pre-existing proteins: flagellum machinery
- **MCP (methyl-accepting chemotaxis proteins): several sensory** proteins reside in cytoplasmic membrane and sense attractants or repellents
- **Nanobrain**, chemoreceptor clusters



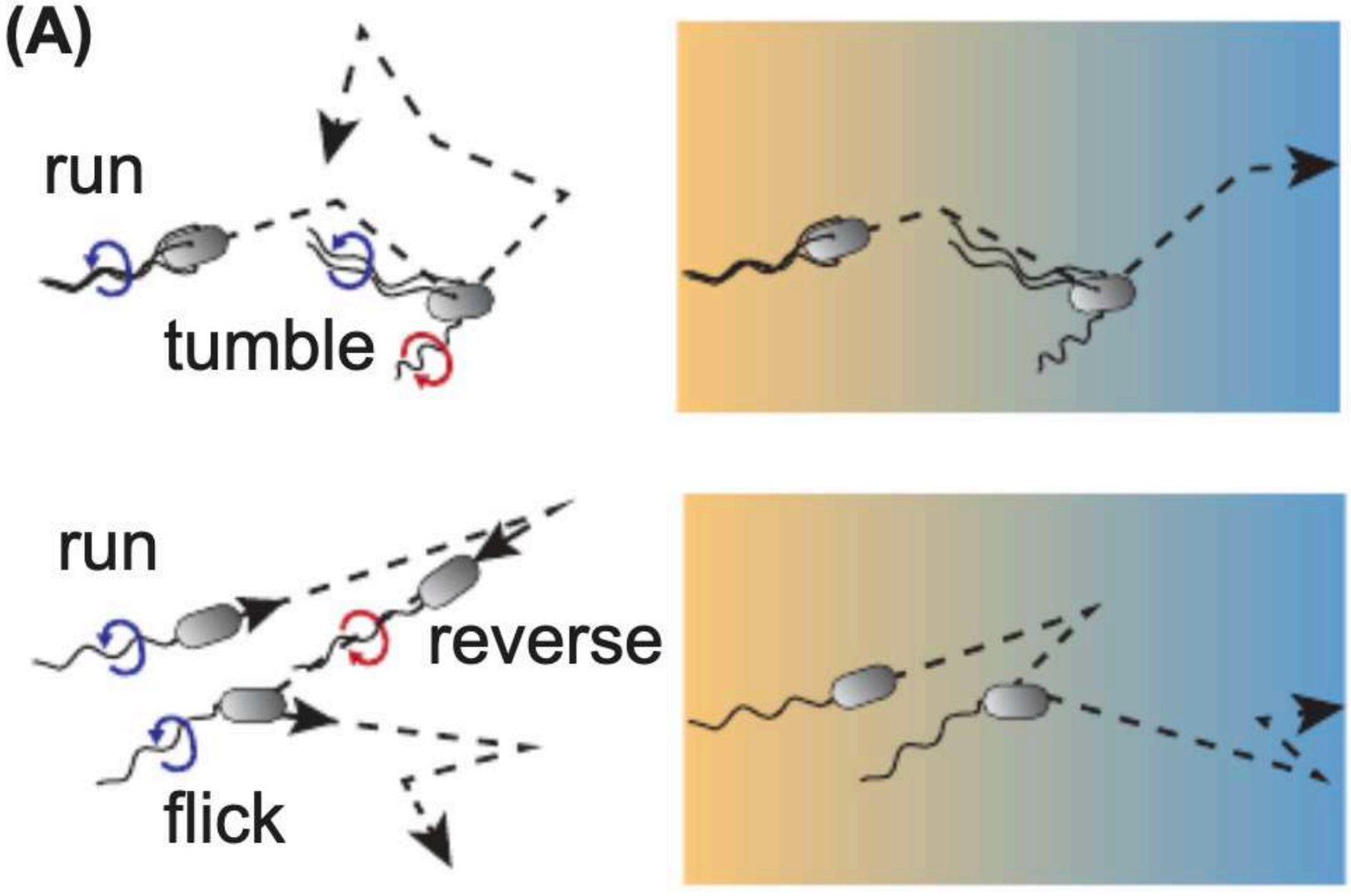



# Chemotaxis architecture




Madigan et al. 2020

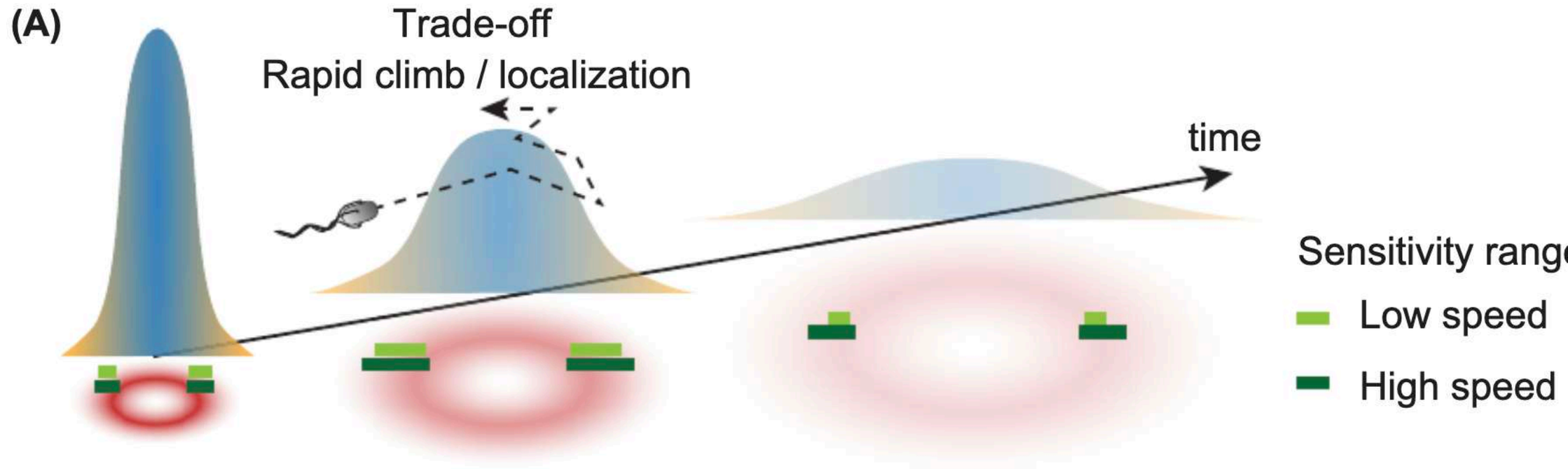
# Chemotactic behavior




Colin et al. 2021

- Two prominent types of bacterial fagellar motility patterns, run-tumble and run-reverse-flick swimming
- Both types of swimming lead to effective diffusion in homogeneous environments and get biased by the chemotaxis pathway to climb up physicochemical gradients

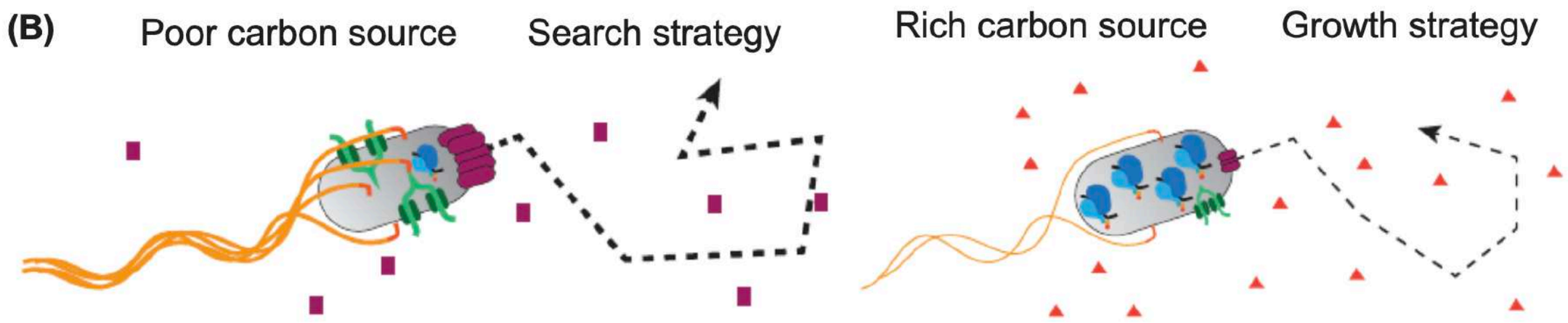
# Trade-offs in chemotactic behaviour




Colin et al. 2021

- Chemotactic response to time varying concentration profiles that could result from diffusive spreading of attractant patch needs to balance rapid gradient climbing and localization at the peak
- Higher swimming velocity expands sensitivity range of bacterial chemotaxis, particularly in shallow gradients (right), but incurs additional energetic costs

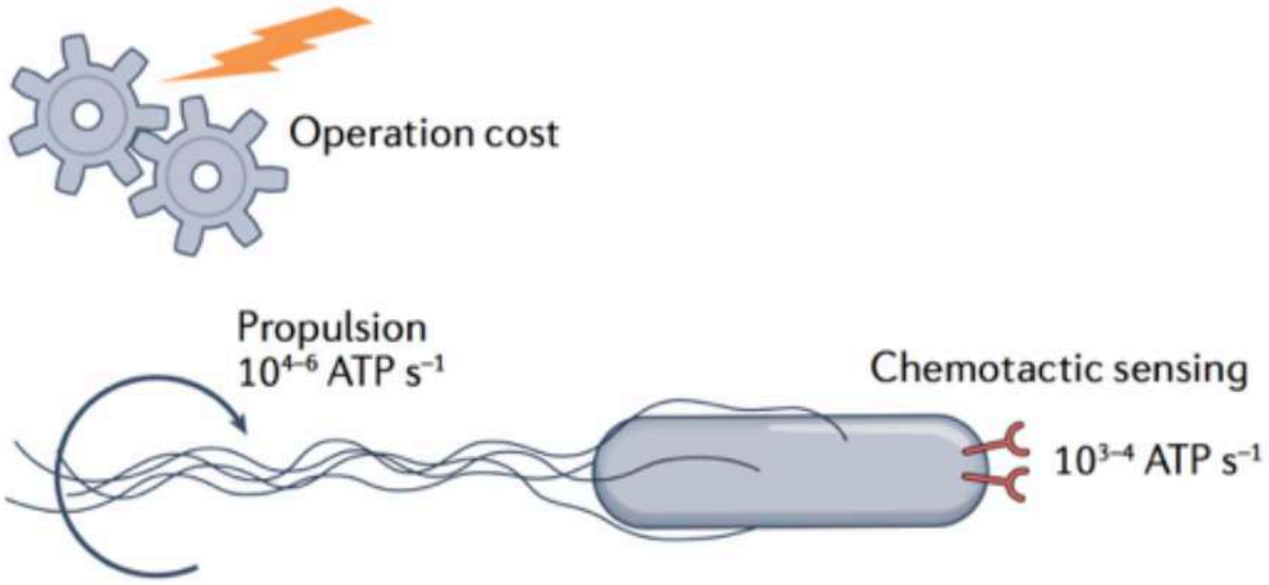
# Regulation of chemotaxis

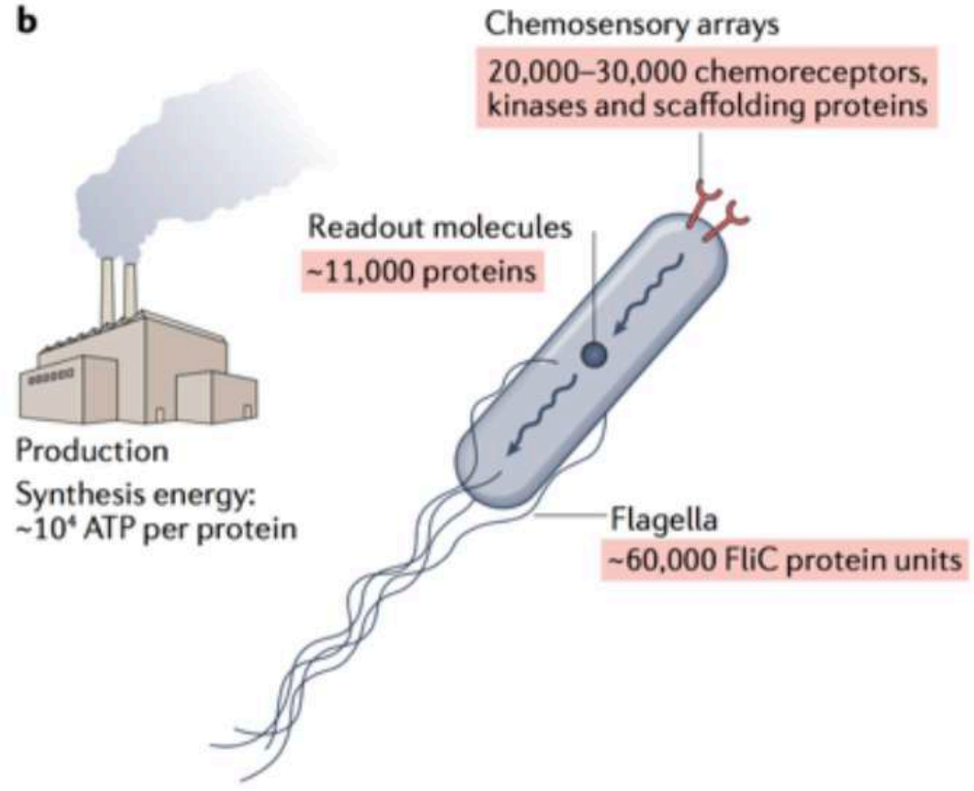



Colin et al. 2021

- Motility and nutrient uptake are regulated antagonistically with biosynthetic machinery dependent on the nutritional quality of the carbon source
- During growth in poor carbon sources (left), motility is upregulated in proportion to potentially higher advantage provided by chemotaxis towards sources of additional nutrients (search strategy)
- In rich carbon sources(right), motility is downregulated to enable higher investment into biosynthetic machinery (growth strategy)

# Relative cost of bacterial chemotaxis



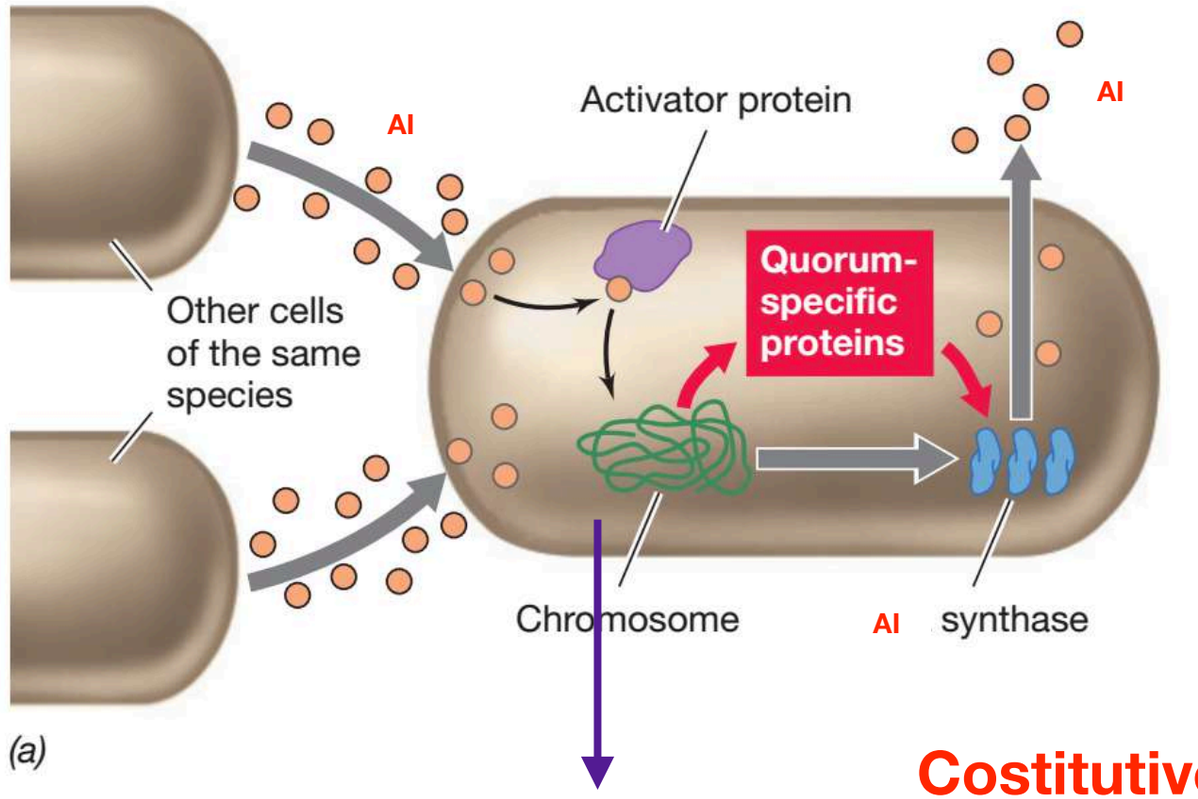




- **Metabolism** fuels chemotaxis
- **Informed foraging** and **cue-based navigation**
- **Increase growth rate in a better environment**

**Keegstra et al., 2022**

# Quorum Sensing, I

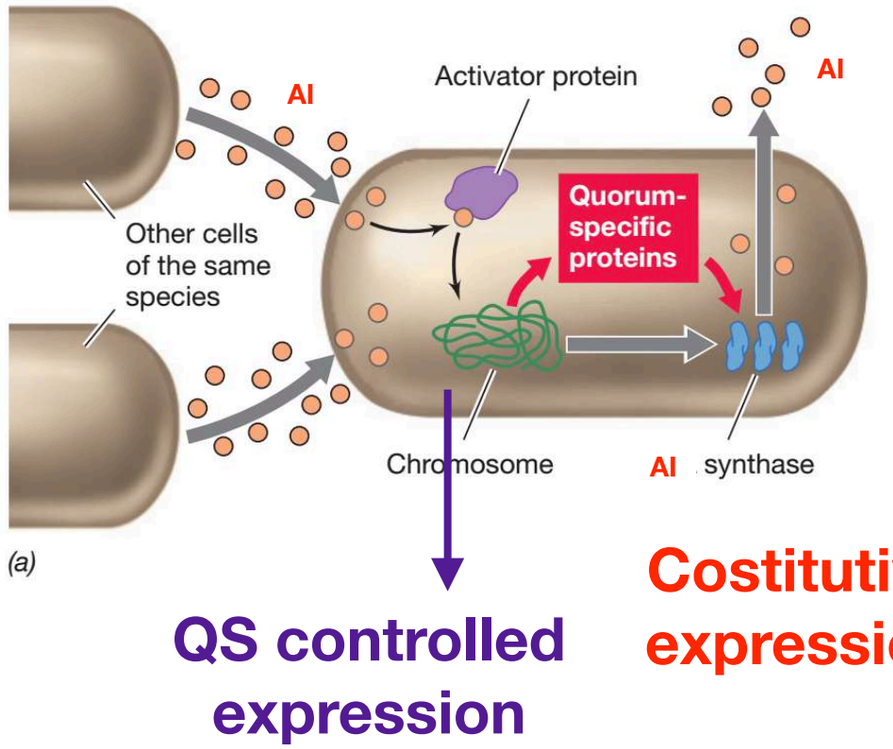
- Quorum sensing (QS) is a process of bacterial **cell-to-cell chemical communication**
- Production, detection, response to extracellular signalling molecules: **autoinducers (AIs)**
- Quorum sensing allows **groups of bacteria** to **synchronously alter behaviour** in response to changes in the population 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)**
- **Individual <—> coordinated group behaviour**




Madigan et al. 2020

# 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†<br>AI-2 incorporation and chemotaxis towards AI-2 | LsrB‡<br>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

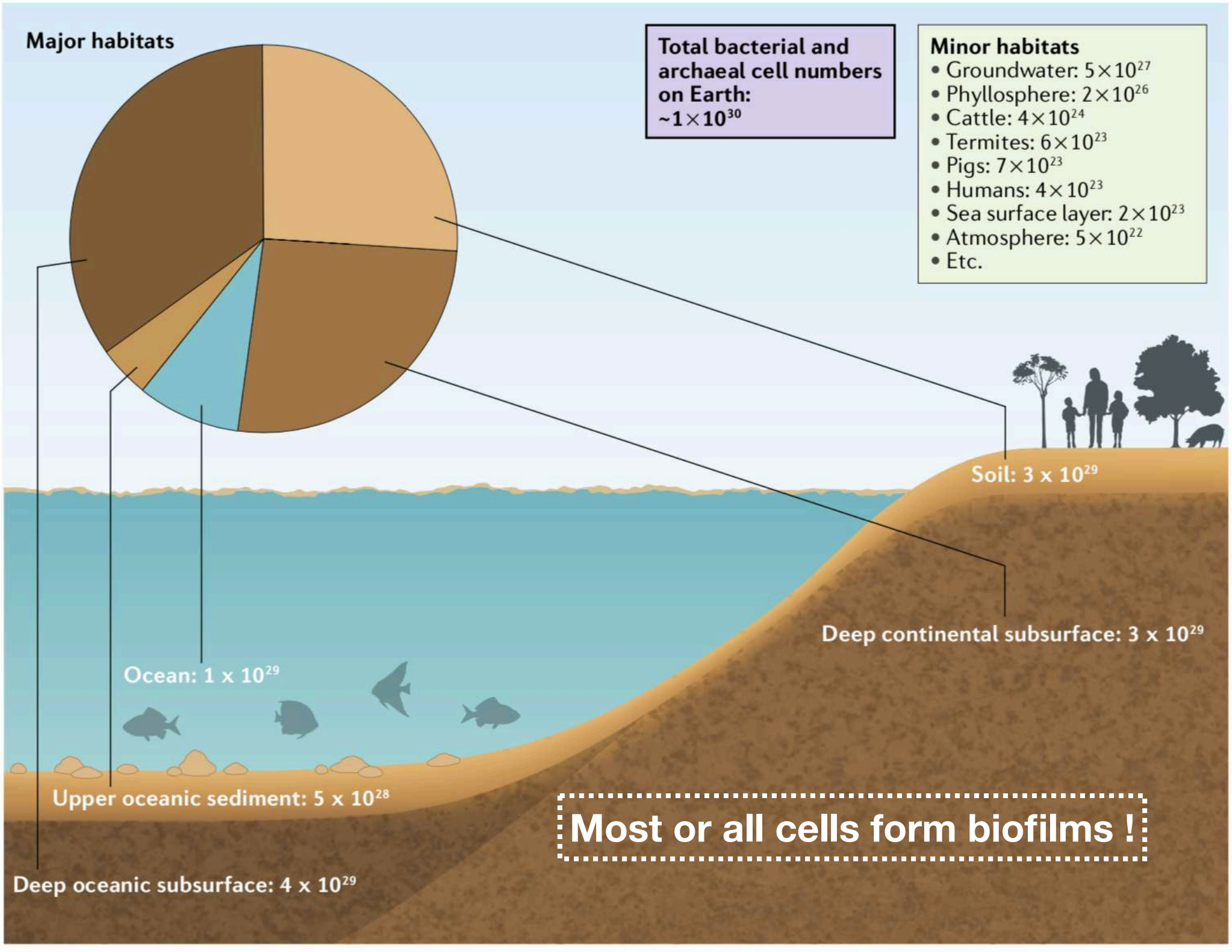
**Table 1.** Continued

| 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**

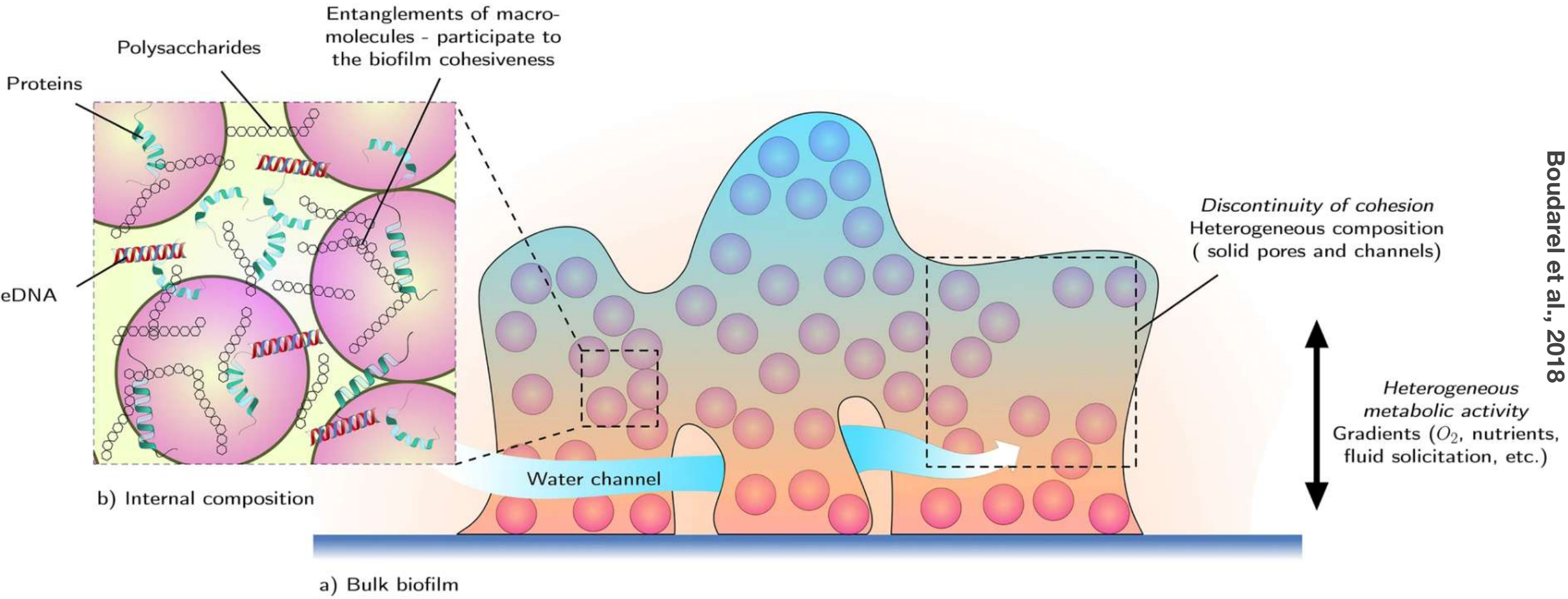
Sporulation
DNA uptake

# Biofilm habitat




Flemming & Wuertz, 2019

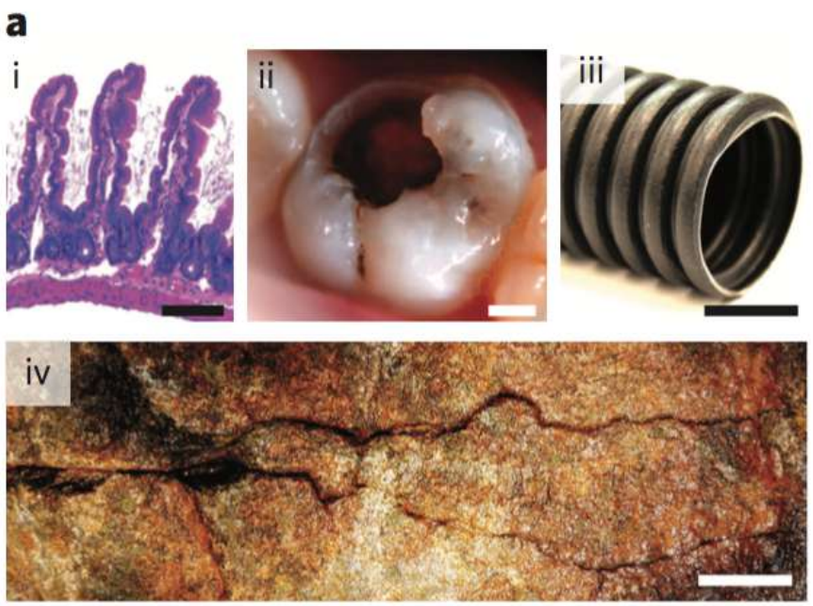
# Biofilm

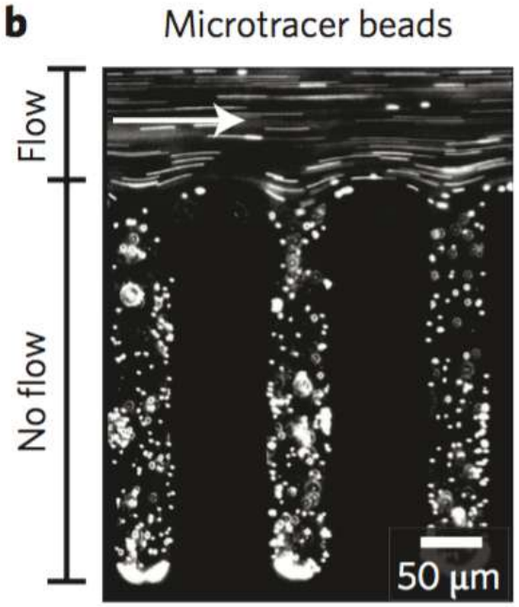


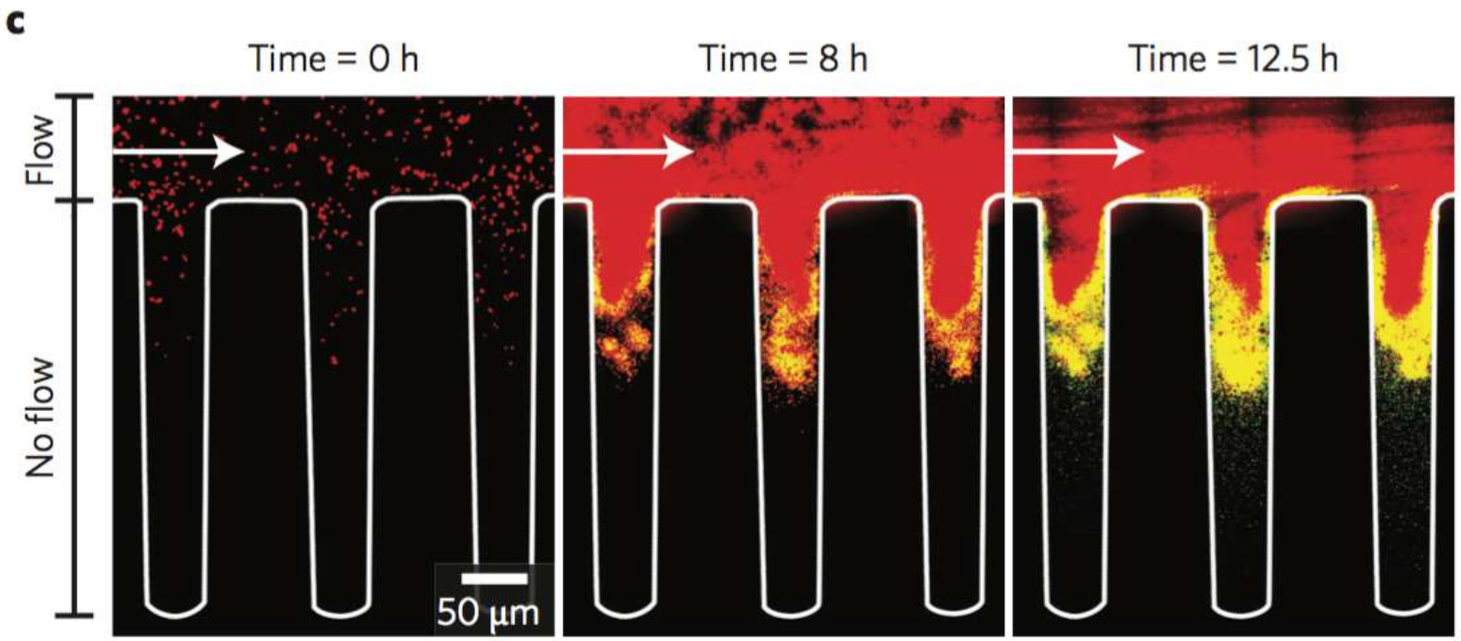

Boudarel et al., 2018

- 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)
- DNA, protein and sugar: very viscous ad dynamic

# 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)

# 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**