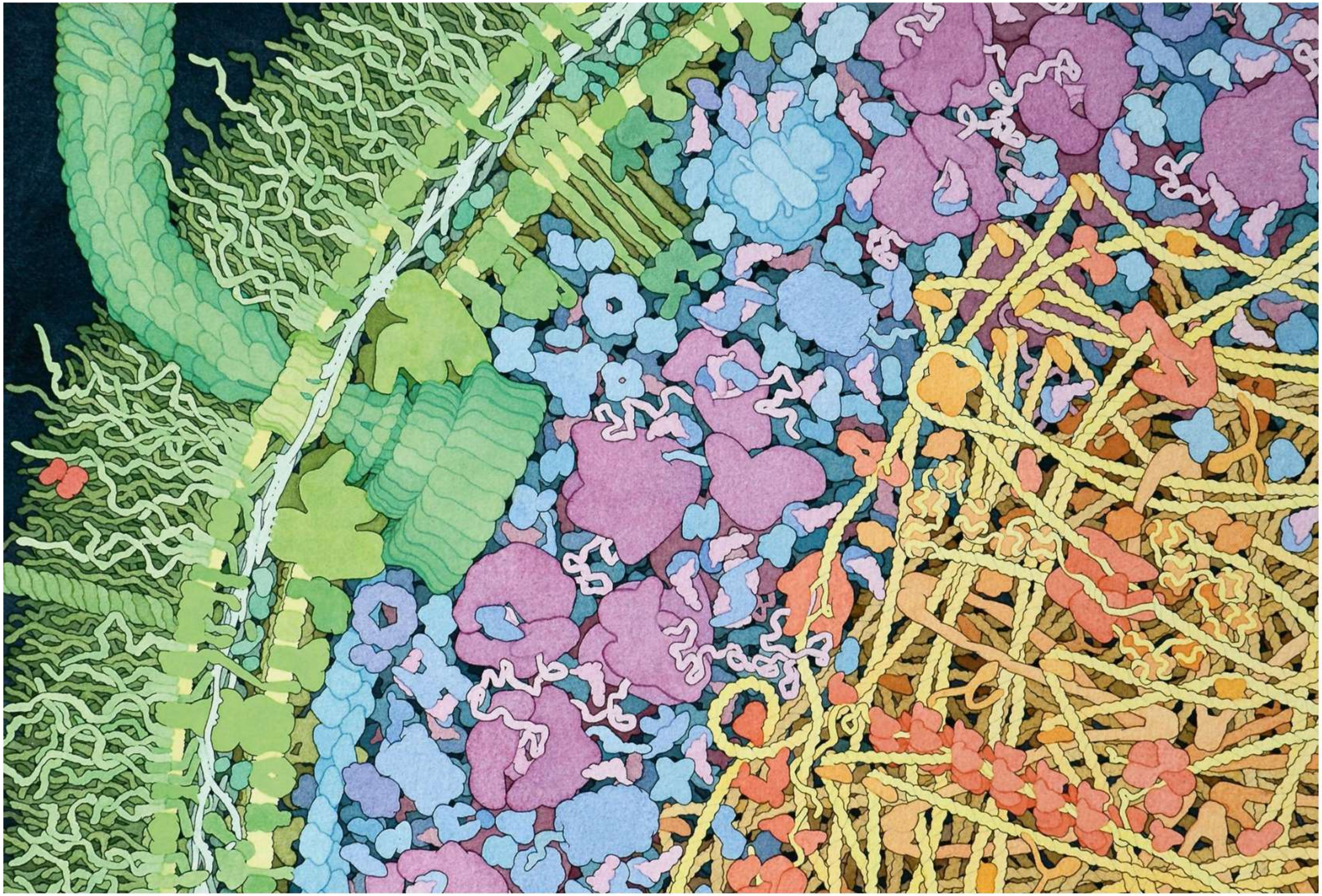
L02b

Recap L02a

Understanding structure and function, II

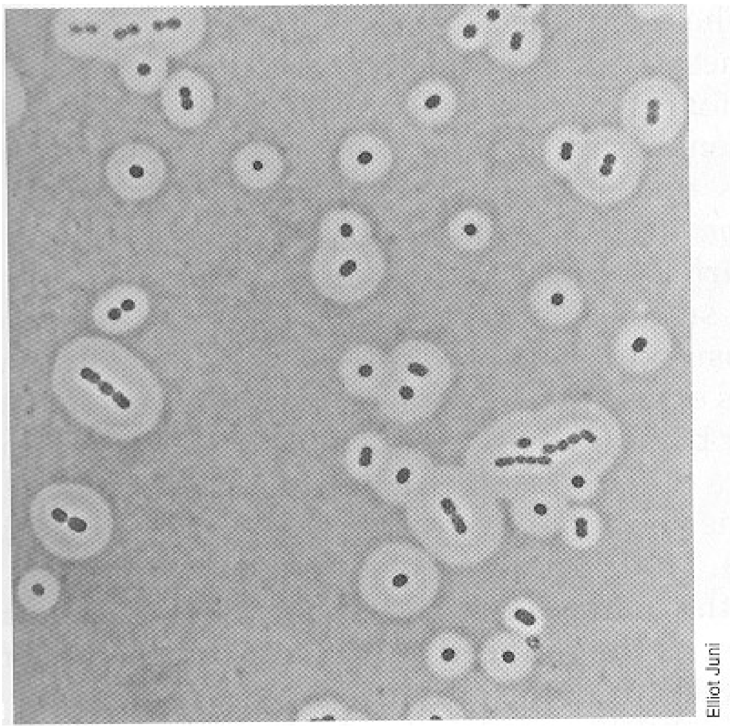


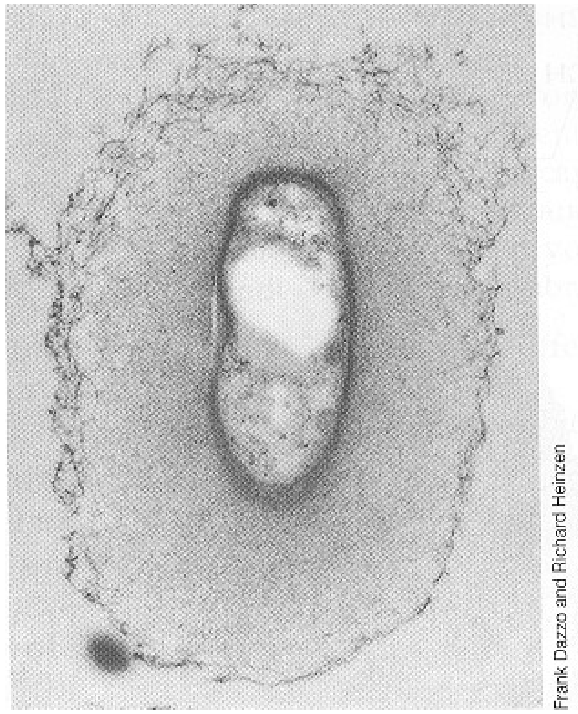
David S. Goodsell

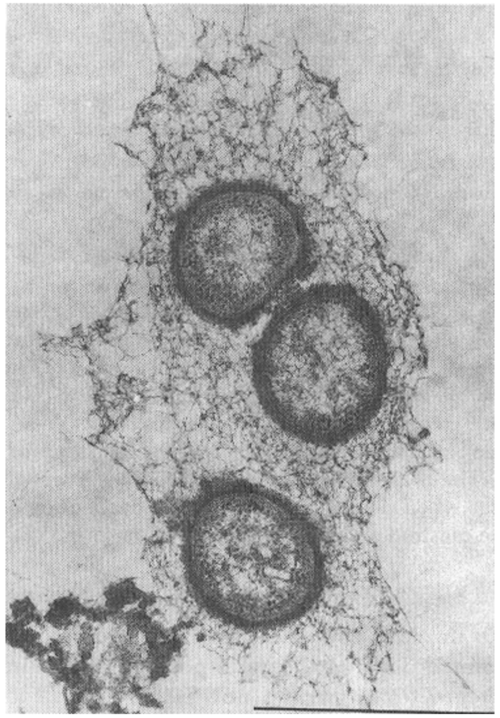
Interaction with the environment at the microscale

- Carbon sources
- Energy sources
- Defence and Offence
- Navigating in the environment
- Propelling in the environment

Capsules and Slime Layers


Elliot Juni


Frank Dazzo and Richard Heinzen

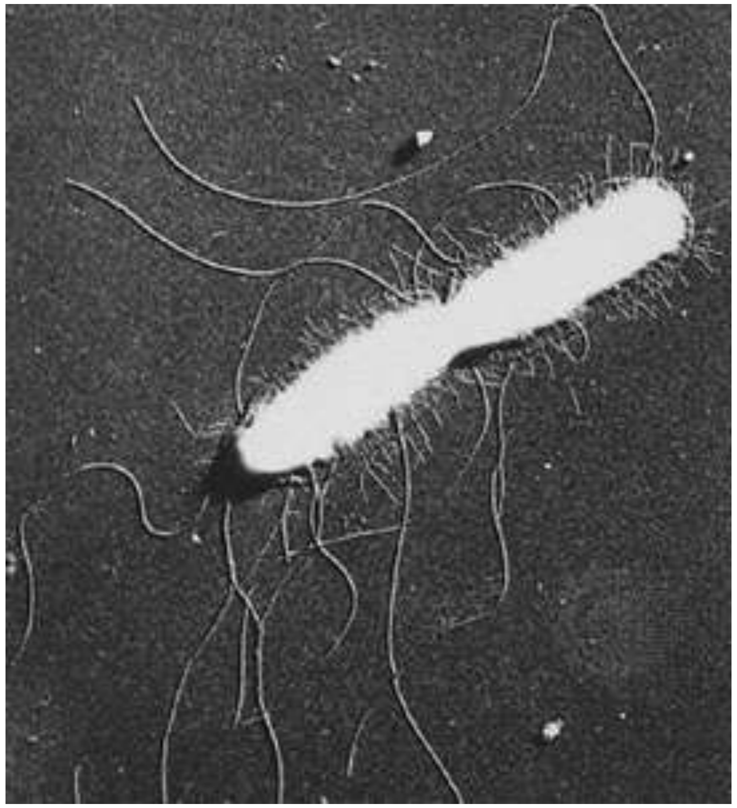


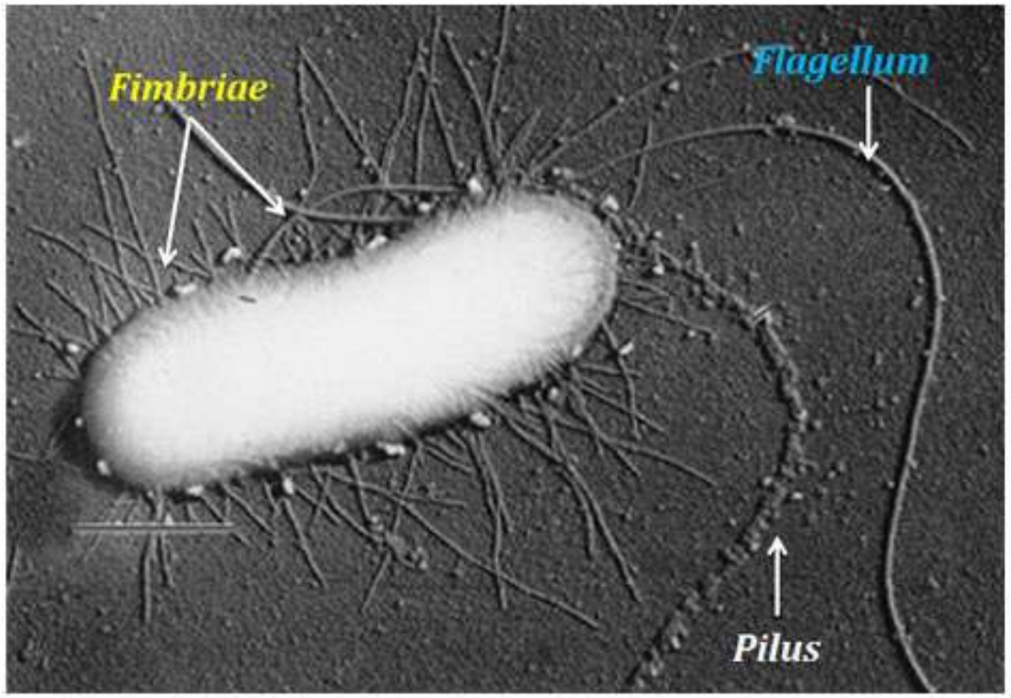
Madigan et al. 2018

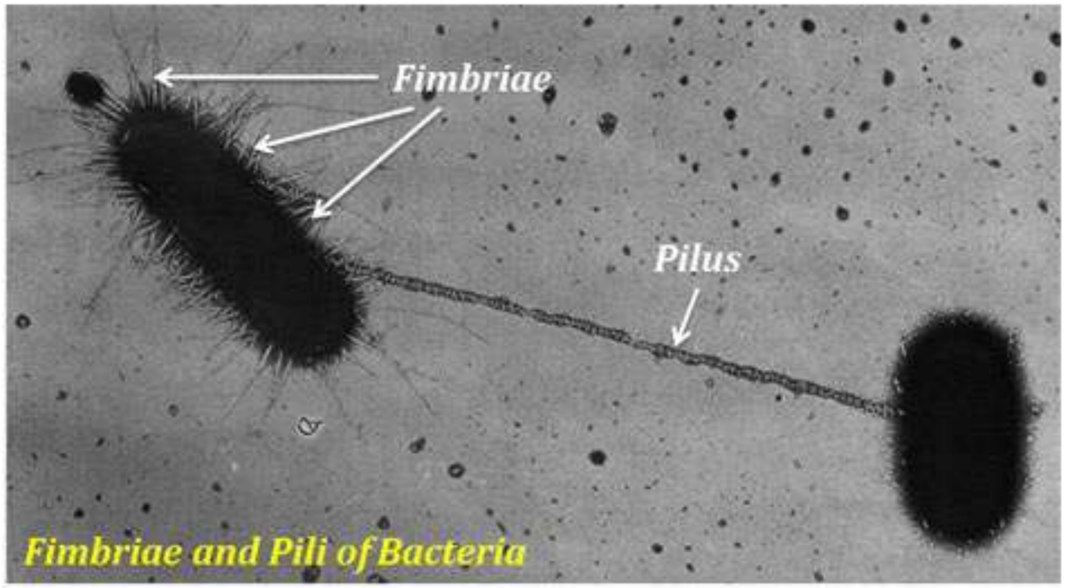
- Polysaccharide and glycoproteins outside the wall and outer membrane (different charge)
- Capsule poly-CHO extremely diverse in composition and structure (*E. coli* strains ~80)
- Can greatly increase effective cell volume
- May hold hydrolytic enzymes and scavenge metals and radionuclides
- May help attachment to surfaces; biofouling
- Defense against protozoa and viruses

Pili & Fimbriae

Pili & Fimbriae are thin (2–10 nm in diameter) filamentous structures made of proteins, pilin, that extend from cell surface

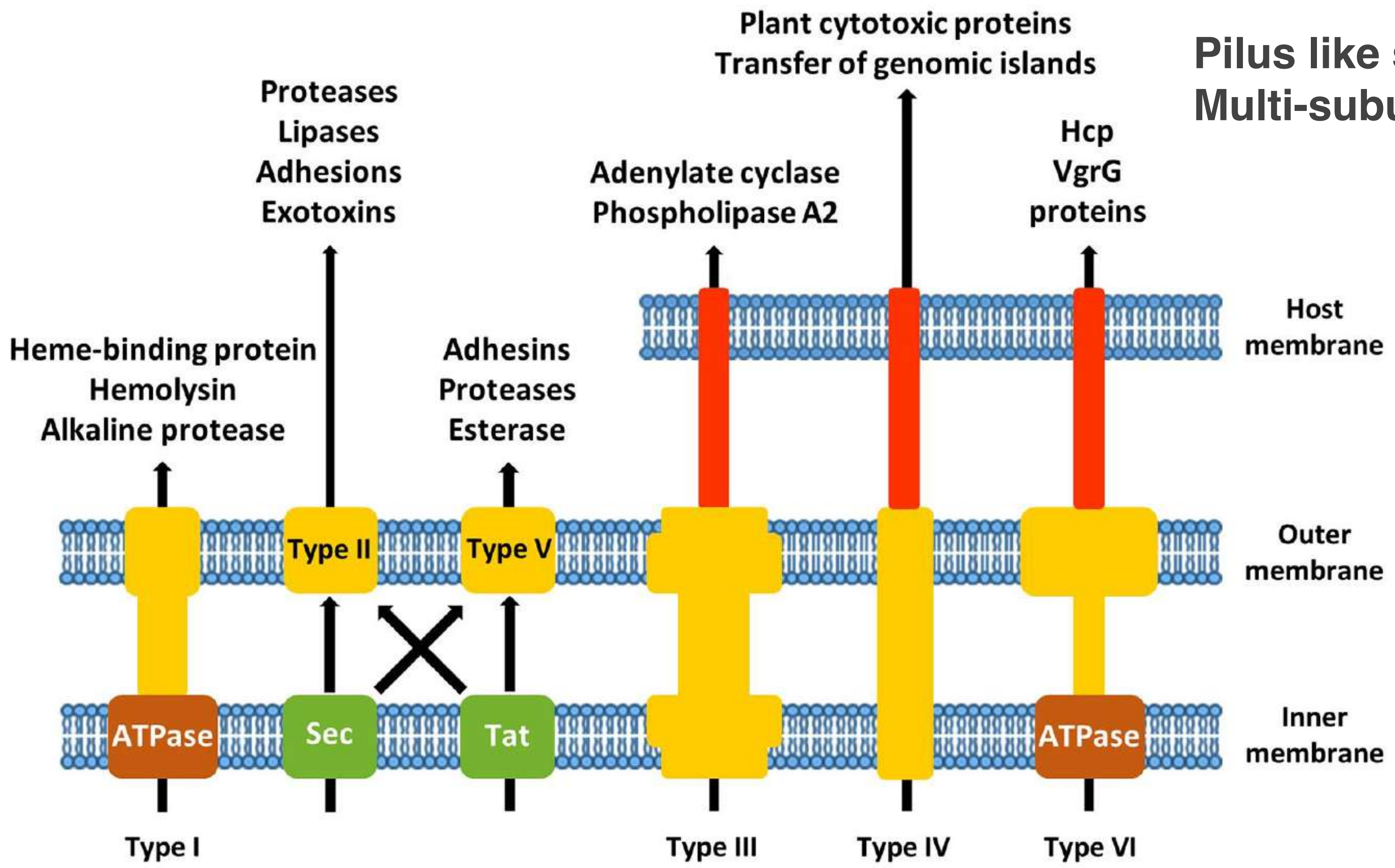






https://www.easybiologyclass.com/bacterial-cell-surface-structures-and-appendages-flagella-fimbriae-and-pili/

Secretion systems, I

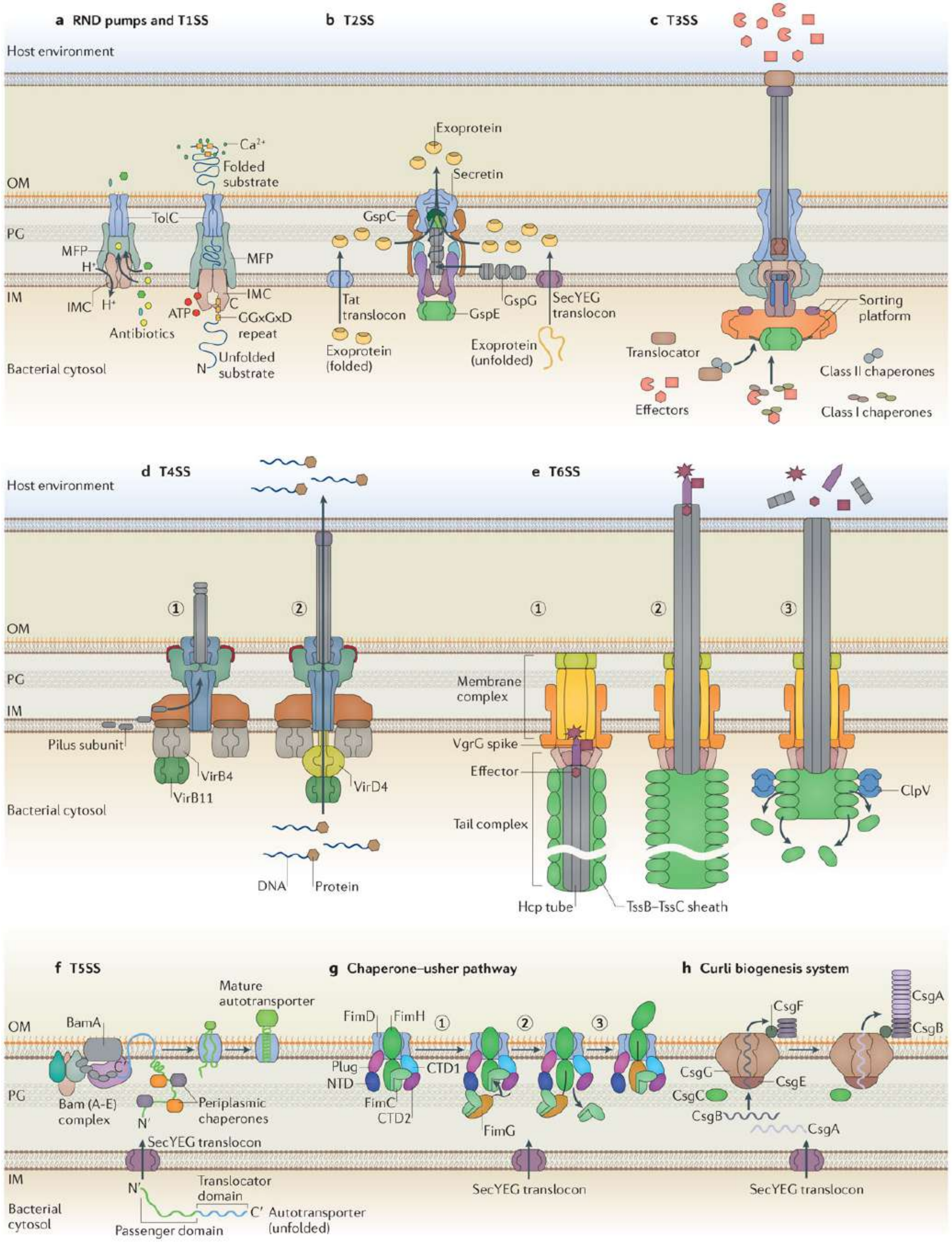


Pilus like system
Multi-subunit-secretion system

Depluverez et al., 2016

- Bacterial secretion is the process by which bacteria **release substances** to their **surroundings**, including **other cells**
- Bacteria achieve this using dedicated secretion systems that **transport molecules**
- Such as factors involved in **bacterial pathogenesis**, so called **effectors**
- Specialized macromolecular nanomachines that secrete a wide range of substrates, i**ncluding small molecules, proteins and DNA , important in host cell adherence as an initial step in colonization and pathogenesis**

Secretion systems, II



Nature Reviews | Microbiology

Specialized macromolecular nanomachines that **secrete** a wide range of substrates, including small molecules, proteins and DNA , important in host cell adherence as an initial step in colonization and pathogenesis

All double-membrane-spanning secretion systems (T1SS, T3SS, T4SS and T6SS) use a one-step mechanism, such that substrates are transported directly from the bacterial cytoplasm into the extracellular space or into a target cell

T2SS, T5SS, Chaperon-usher and curli two-step translocases because they depend on either the Sec or Tat system; no ATP

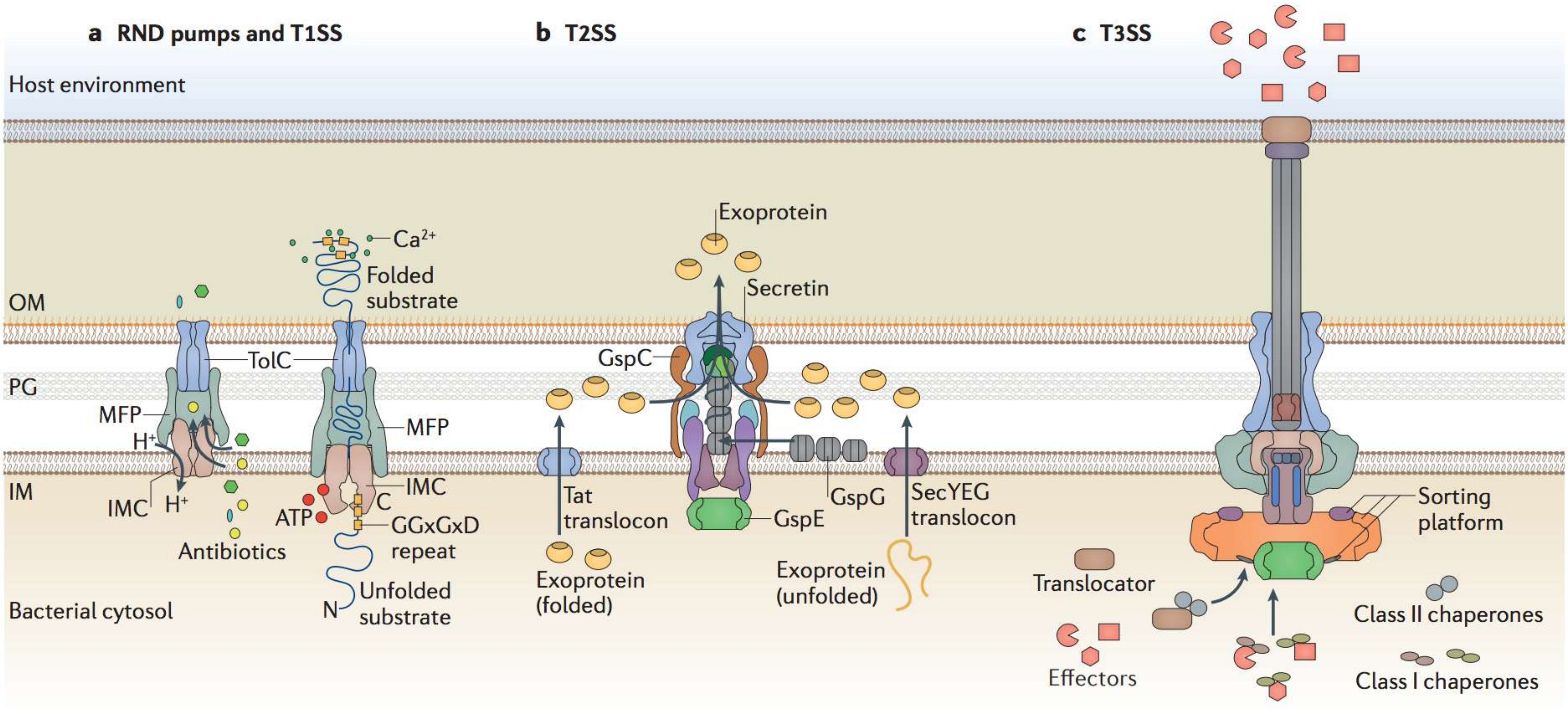
T7SS, *Mycobacterium* (Gram positive)

None secretion systems is constitutively active

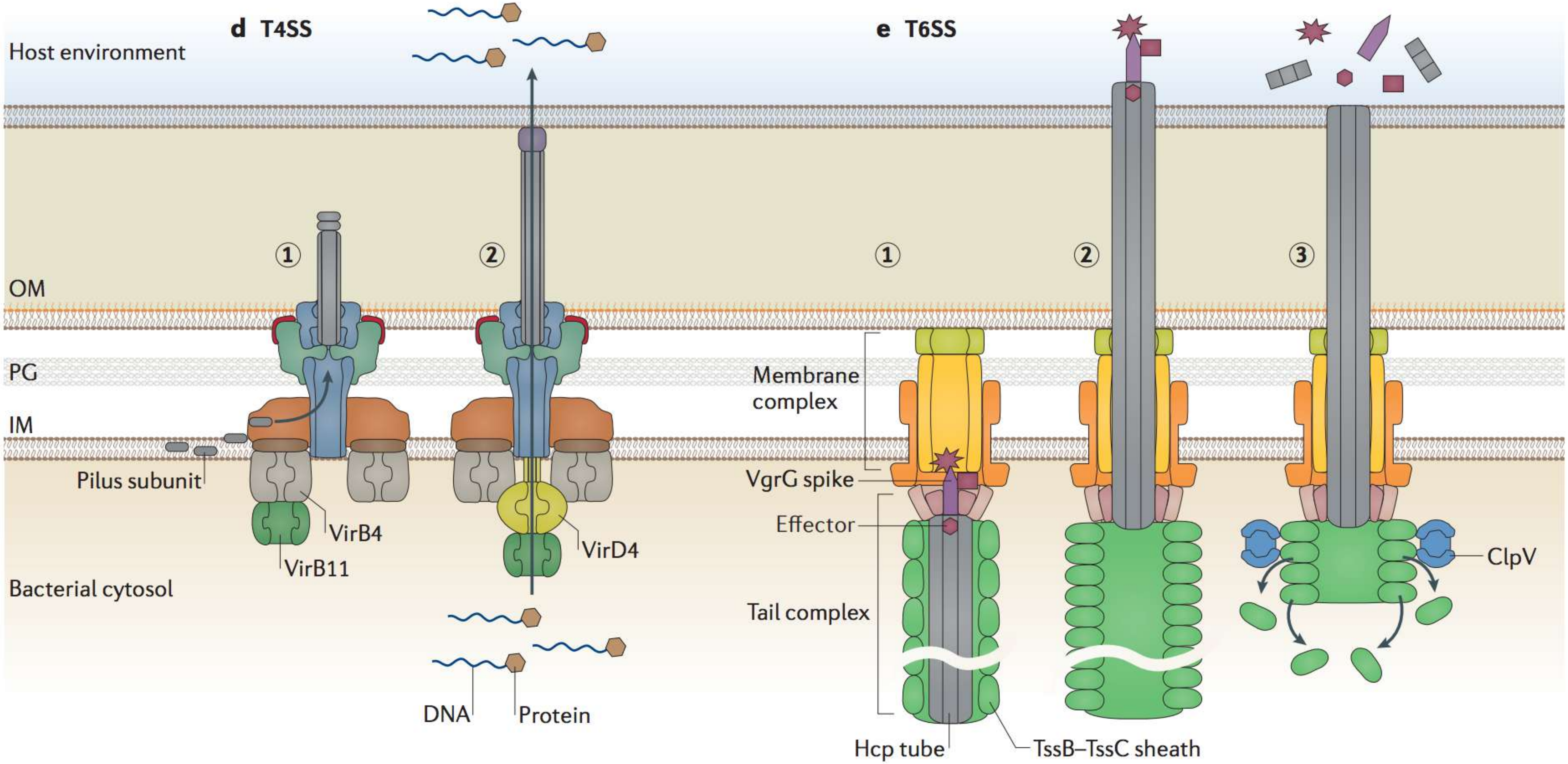
Hyp.: secretion may be triggered by the recognition of host receptors by specialized adhesion molecules called adhesins

Gunasinghe et al., 2017
Costa et al., 2015

Costa et al. 2015

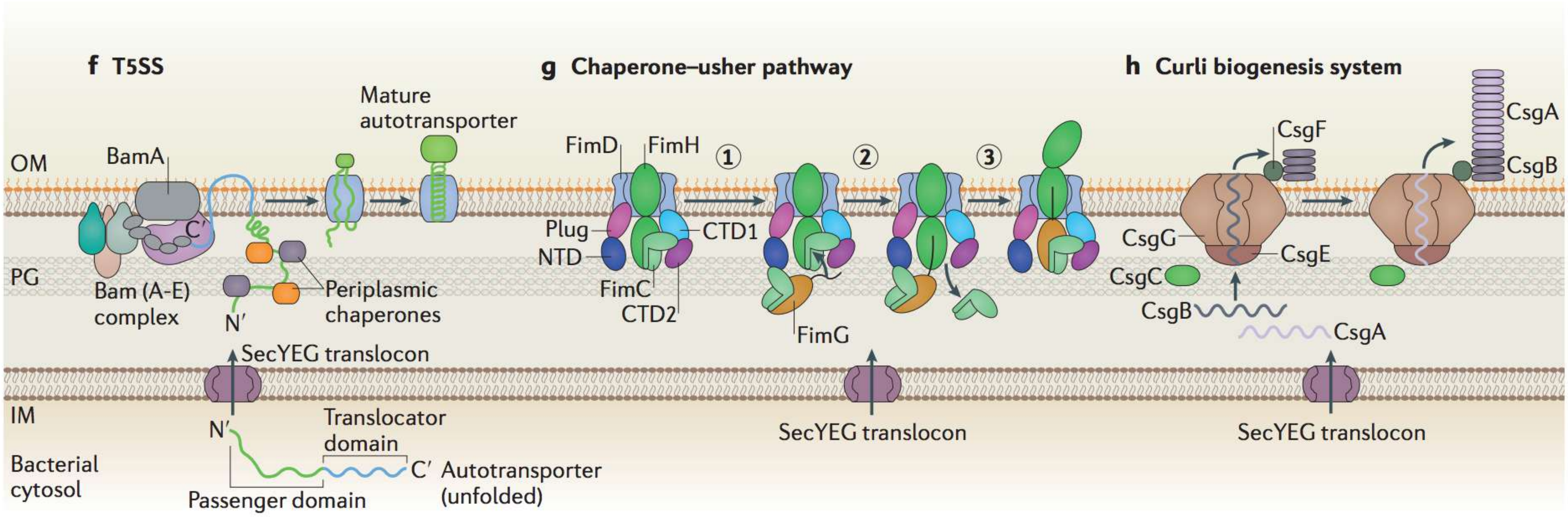


- **Resistance–nodulation–division (RND) pumps, antibiotics and small exogenous compounds**
- **Type I secretion system (T1SS), ATPase (nutrient acquisition and virulence)**
- **Type 2 secretion system (T2SS), Tat and Sec (folded and unfolded), ATPase (enzyme, toxins)**
- **Type 3 secretion system (T3SS), effectors, form a pore in host, proteins to help the process**
- **effectors modulate or subvert specific host cell functions, thereby promoting bacterial invasion and colonization**



Costa et al. 2015

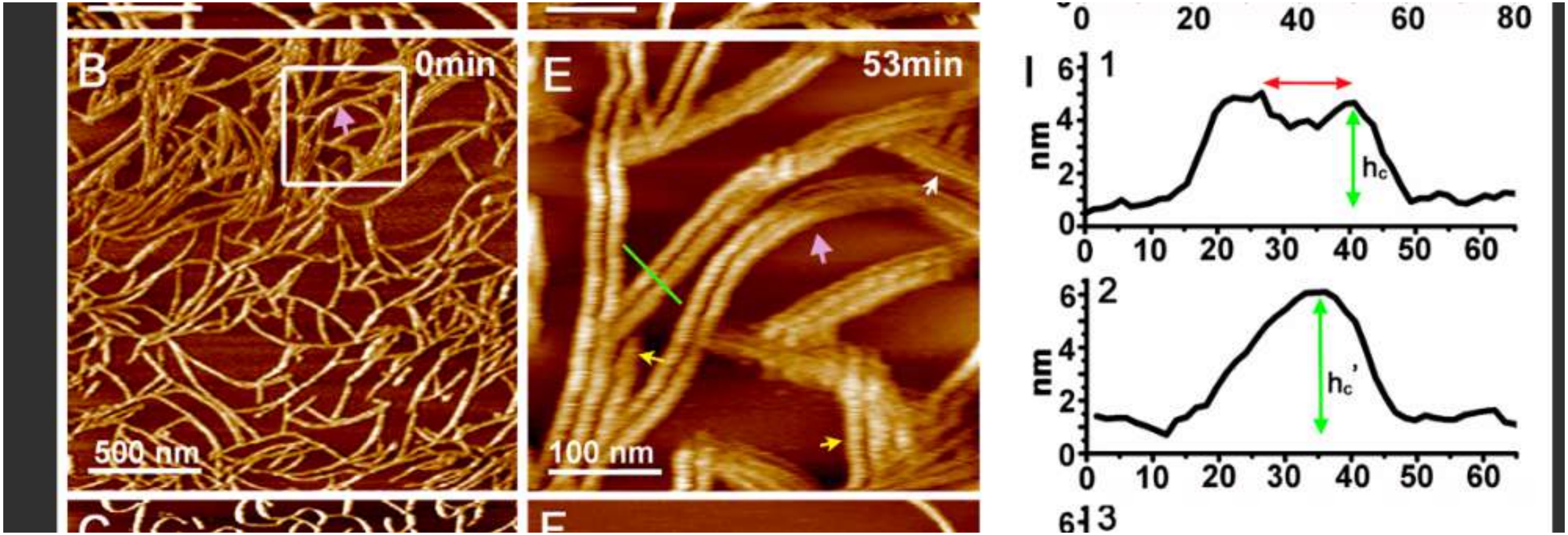
- **Type 4 secretion system (T4SS) mediates the translocation of DNA and proteins into bacterial or eukaryotic target cells**
- **T4SS in Bacteria and Archaea, conjugation of plasmid DNA, ATP based**
- **Type 6 secretion system (T6SS) cell envelopes panning machine that translocates toxic effector proteins into eukaryotic and prokaryotic cells**
- **T6SS, injection mechanisms similar to phage**



Costa et al. 2015

- **Type 5 secretion system (T5SS), chaperone-usher and curli OM only**
- **Sec traslocase mediates proteins into periplasmic space**
- **T5SS, substrate and its secretion pore are fused to form a single polypeptide —> a single polypeptide can drive its own secretion (autotransporter)**
- **Chaperone-usher, used to assemble and secrete multisubunit appendages pili or fimbriae, —> mediate host cell recognition and attachment pathogenicity and biofilm formation**
- **Curli biogenesis system, Curli are extracellular protein fibres, are functional amyloid**
- **Curli protect bacteria from hostile environments by contributing to biofilm formation and facilitating interactions with the host immune system**

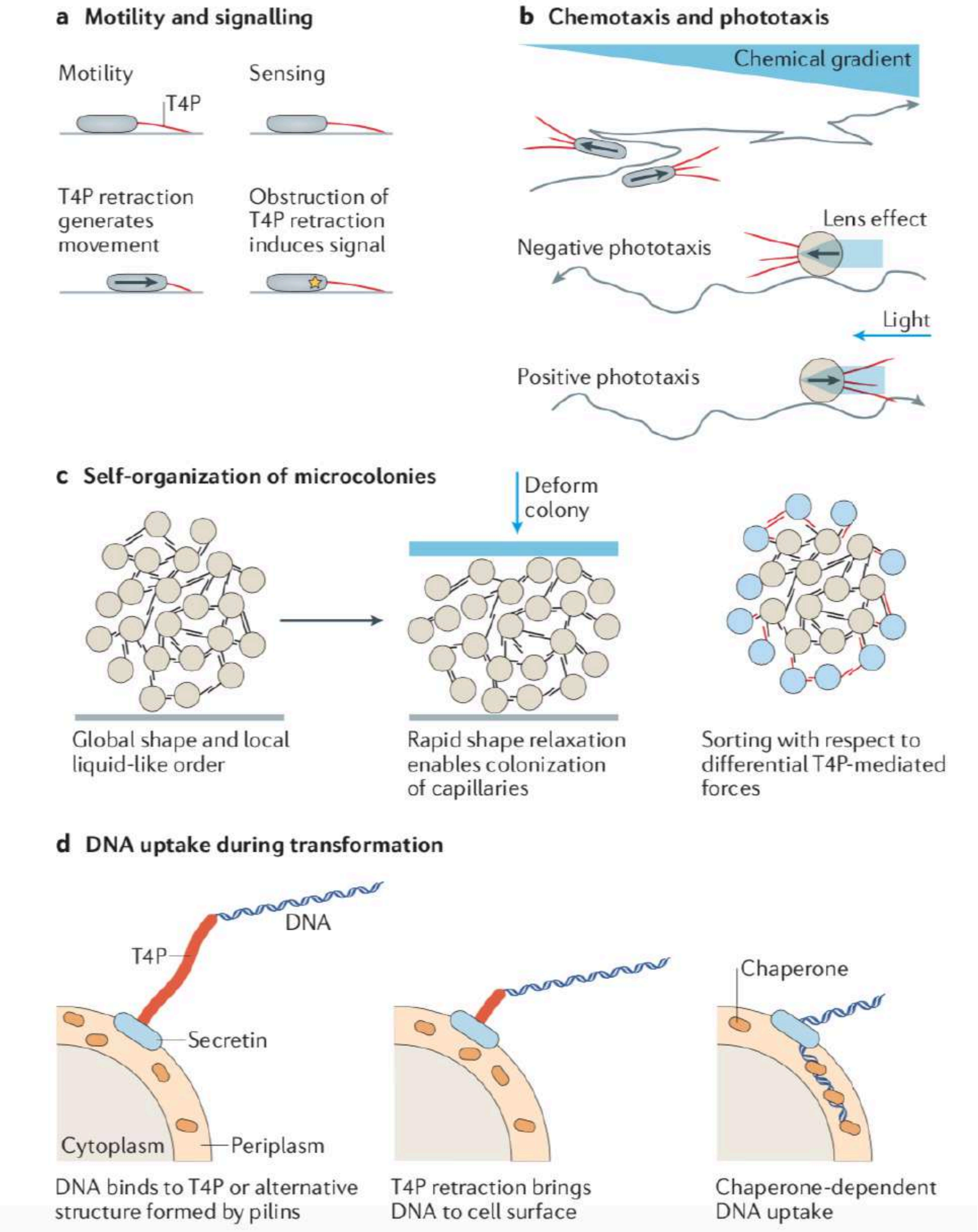
Self-assembling curli, by AFM



Amyloids A class of thread-like protein aggregates that self-assemble into insoluble toxic nanofibers. In bacteria, the accumulation of such fibers promotes the formation of a protective biofilm, whereas in humans they are involved in neurodegenerative diseases

Chang et al., 2019

Type IV pili in Gram negative & positive



Type IV pili are **dynamic**: filaments polymerize and depolymerize, leading to rapid cycles of extension and retraction that generate considerable mechanical force

Type IV pili

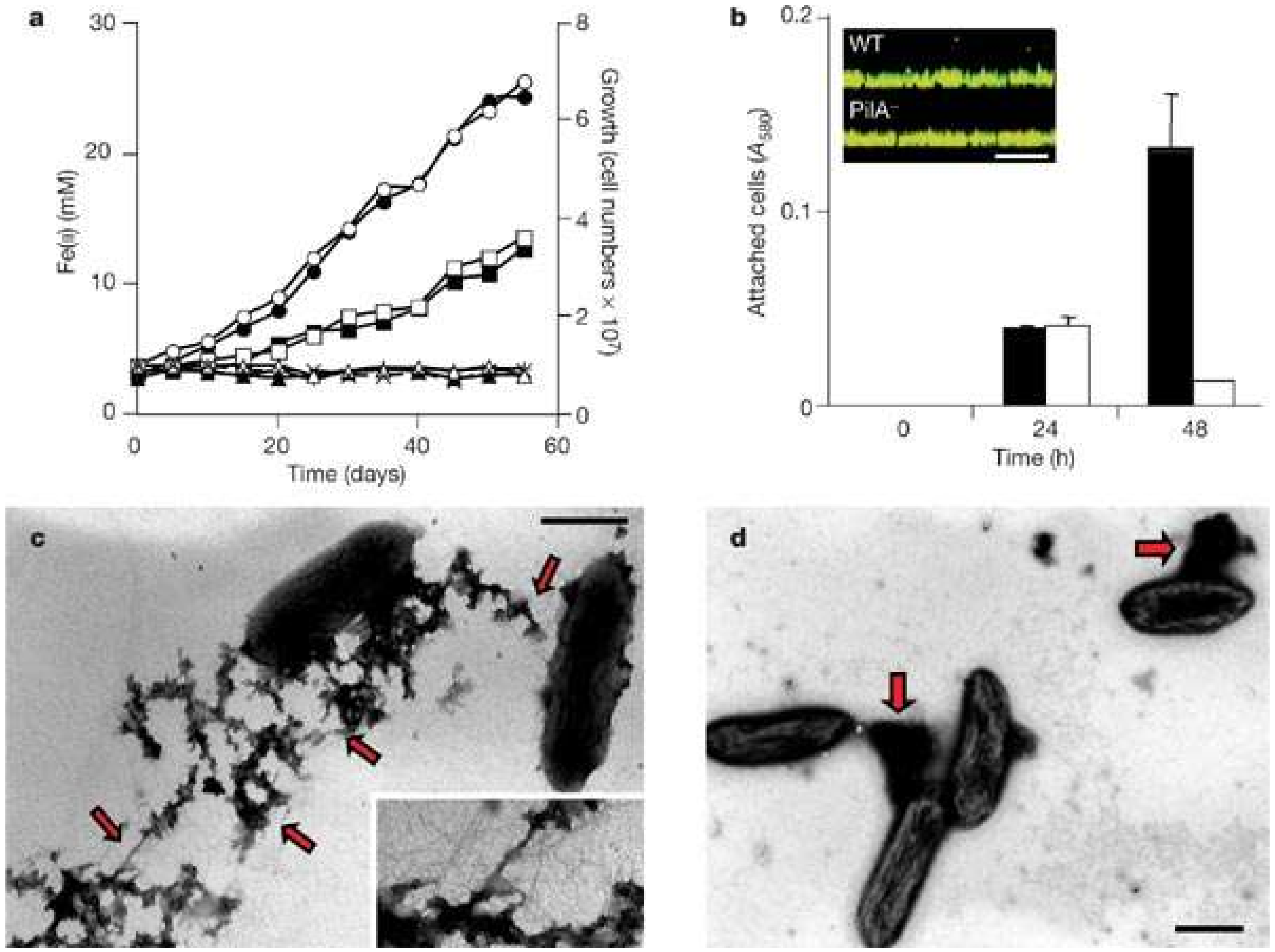
1. Pull adherent bacteria along mucosal surfaces into close association with host cells and other bacteria
2. Exert forces on host cells
3. Pull bound substrates like DNA and bacteriophages into the periplasm
4. Export exoproteins across the outer membrane

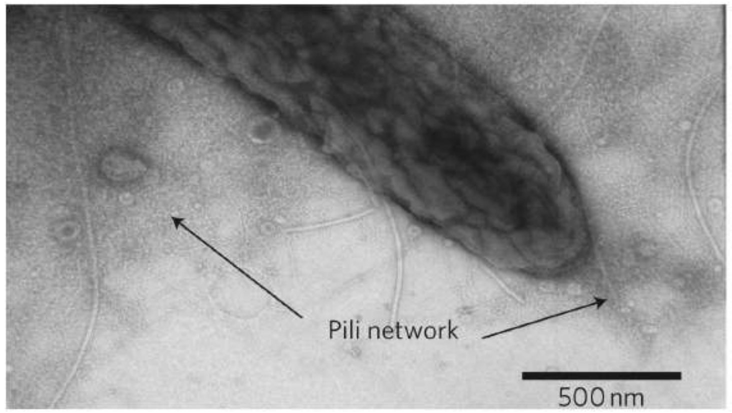
Type IV pili extend and retract at rates of ~1,000 subunits per second, requiring a complex protein machinery that spans both membranes of Gram-

Craig et al., 2019

Pili as conducting nanowires

Geobacter sulfurreducens





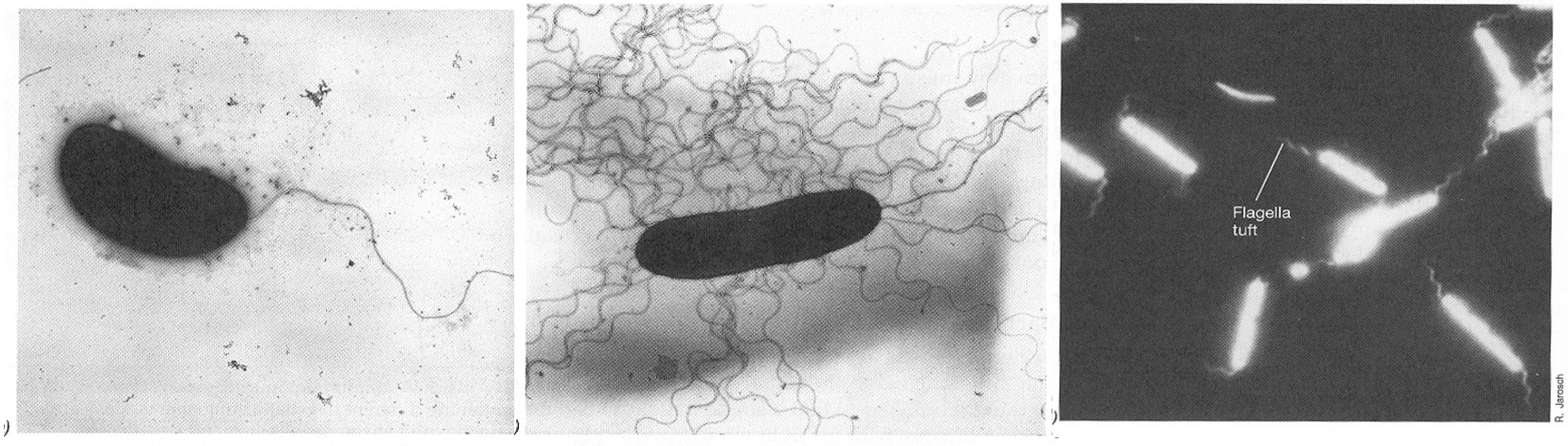
Shewanella oneidensis

Reguera et al., 2005
Malvankar et al., 2011
Gorby et al., 2006

- **Pili are made of proteins**
- Pili form networks
- γ-Proteobacteria, Cyanobacteria, Methanogens
- Reduction of ferric oxide by touching via pili
- Cytochromes are involved in electron conduction
- **Strictly anaerobic conditions/ low O_2:**

A. growth medium supplemented with fumarate (40 mM) as the electron acceptor and with acetate (10 mM) as the electron donor for G.s.
B. Fe(III) citrate (50 mM) as the electron acceptor and lactate (20 mM) as the electron donor

Bacteria Flagella (gross structure)

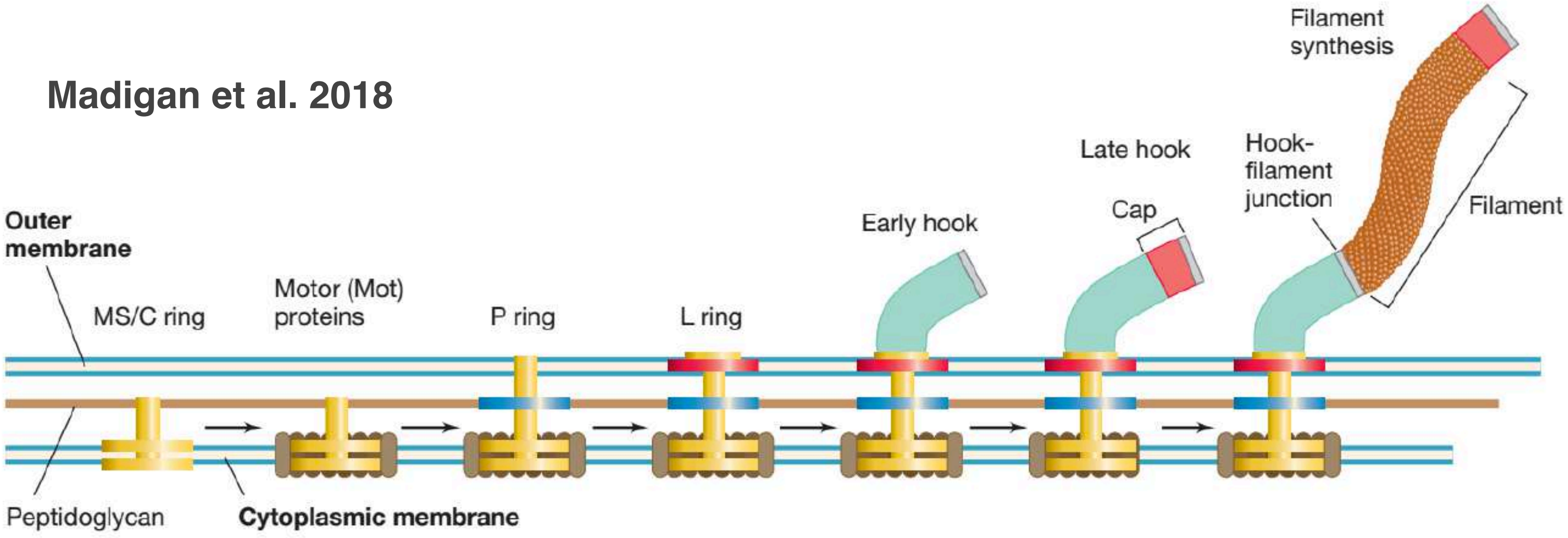


- For motility; Not always present
- 20 nm, hollow, very long (10-20 body lengths)
- Single protein, flagellin
- If broken they can regenerate
- Flagellar arrangement; polar, peritrichous
- Wavy; wavelength constant for a species
- Rigid, do not make wave-like motion like sperm

Madigan et al. 2018

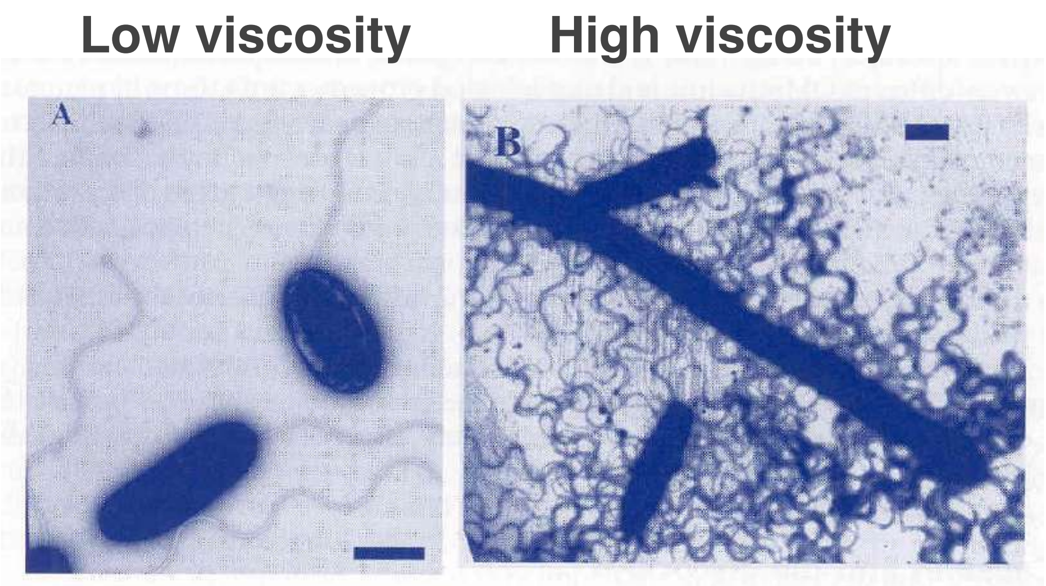
Bacteria Flagella (gross structure)

Madigan et al. 2018

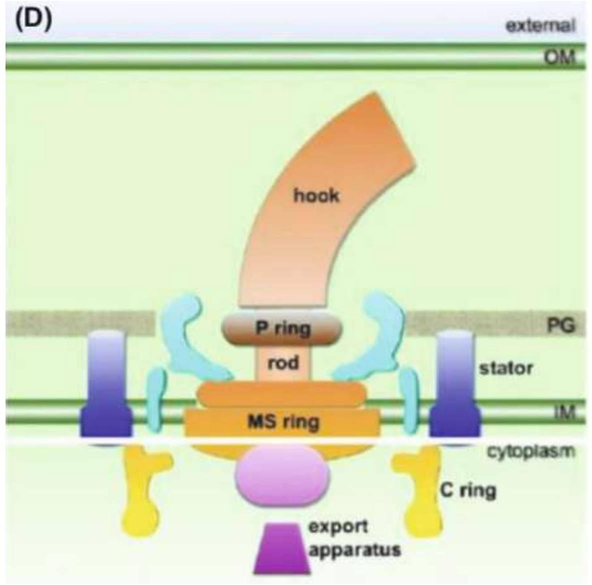


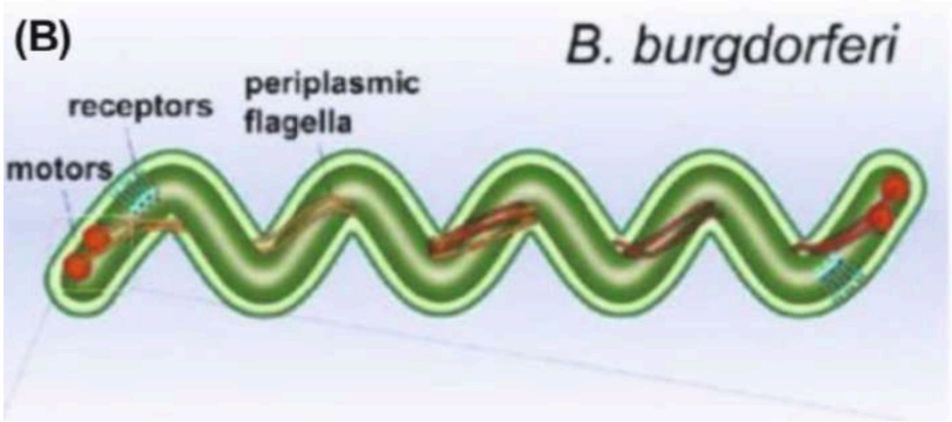
- Grow at free end
- Self assembly from transported flagellin

- Microenvironment viscosity modulate expression of swarmer cells (many lateral flagella)
- *Vibrio*s have sheathed polar flagellum


Low viscosity

High viscosity

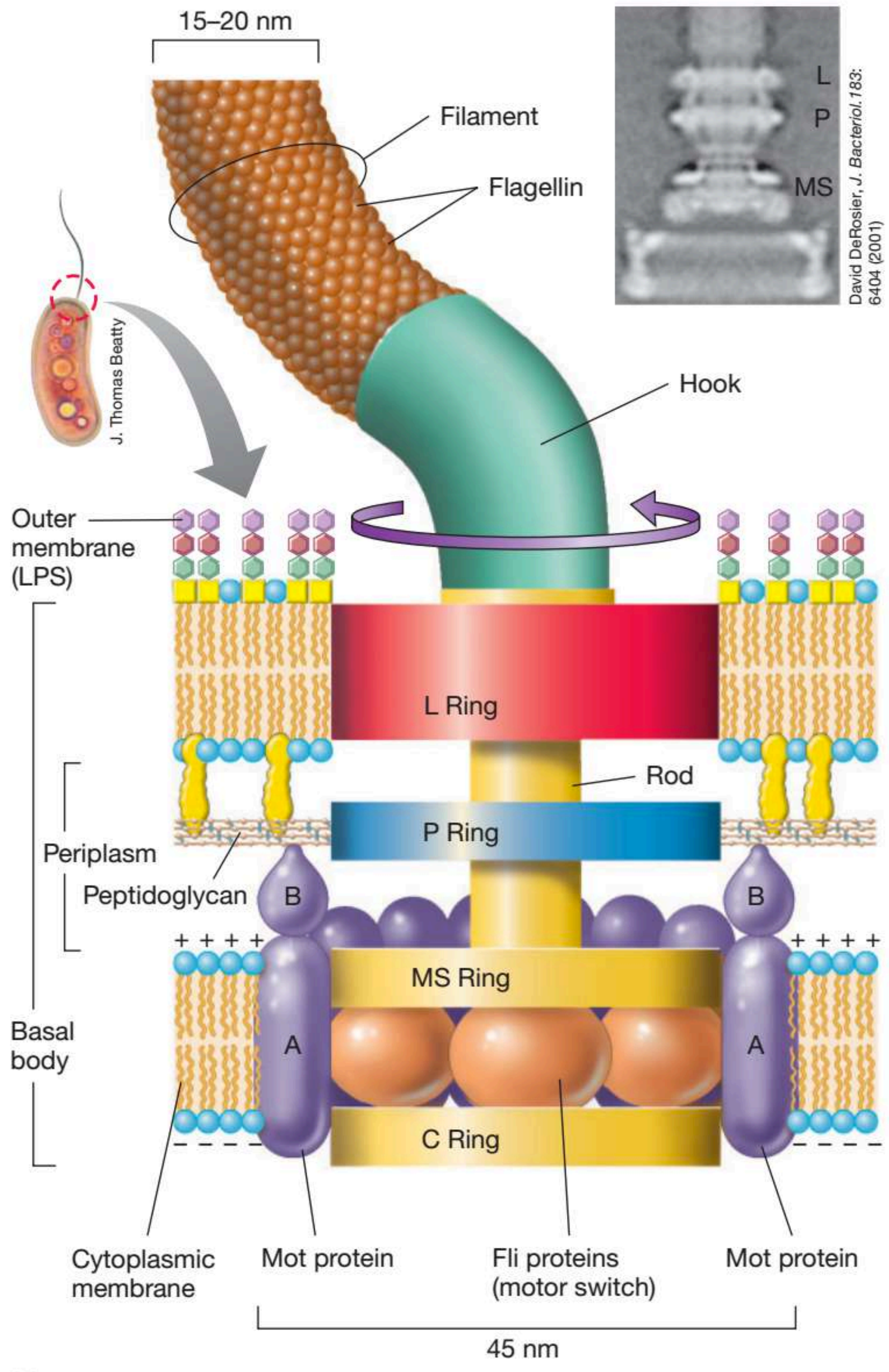




- Periplasmic flagella in *Borrelia burgdorferi*

Kim et al. 2017

Bacteria Flagella (ultra-structure and function)

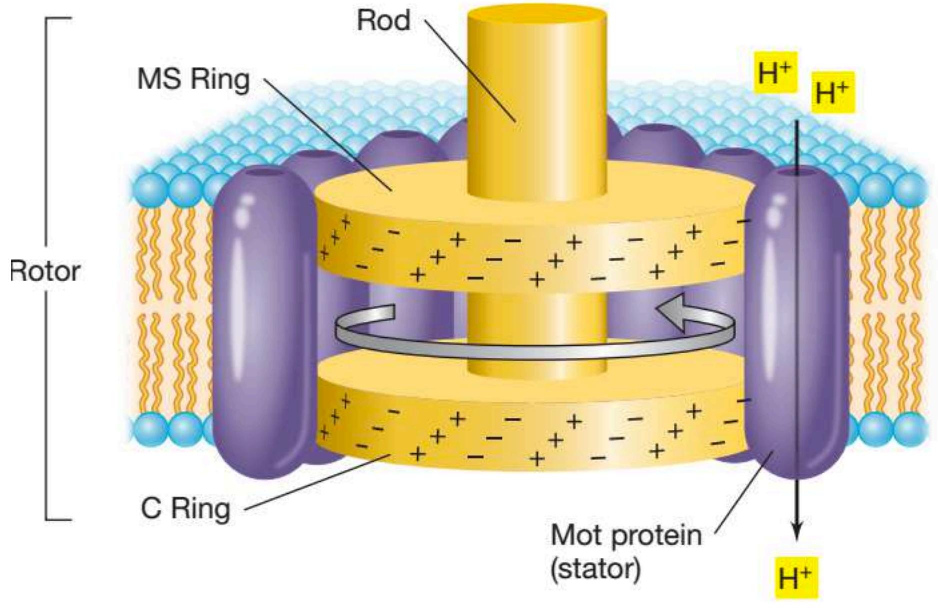


David DeRosier, J. Bacteriol. 183: 6404 (2001)

J. Thomas Beatty

- Filament
- Hook
- Basal body (rod and rings)

- Filament rotates at base like propeller
- Basal body acts like a motor
- Most studied marine bacteria have H^+/Na^+ driven flagella motors
- 1700 rps/400μm s^{-1}

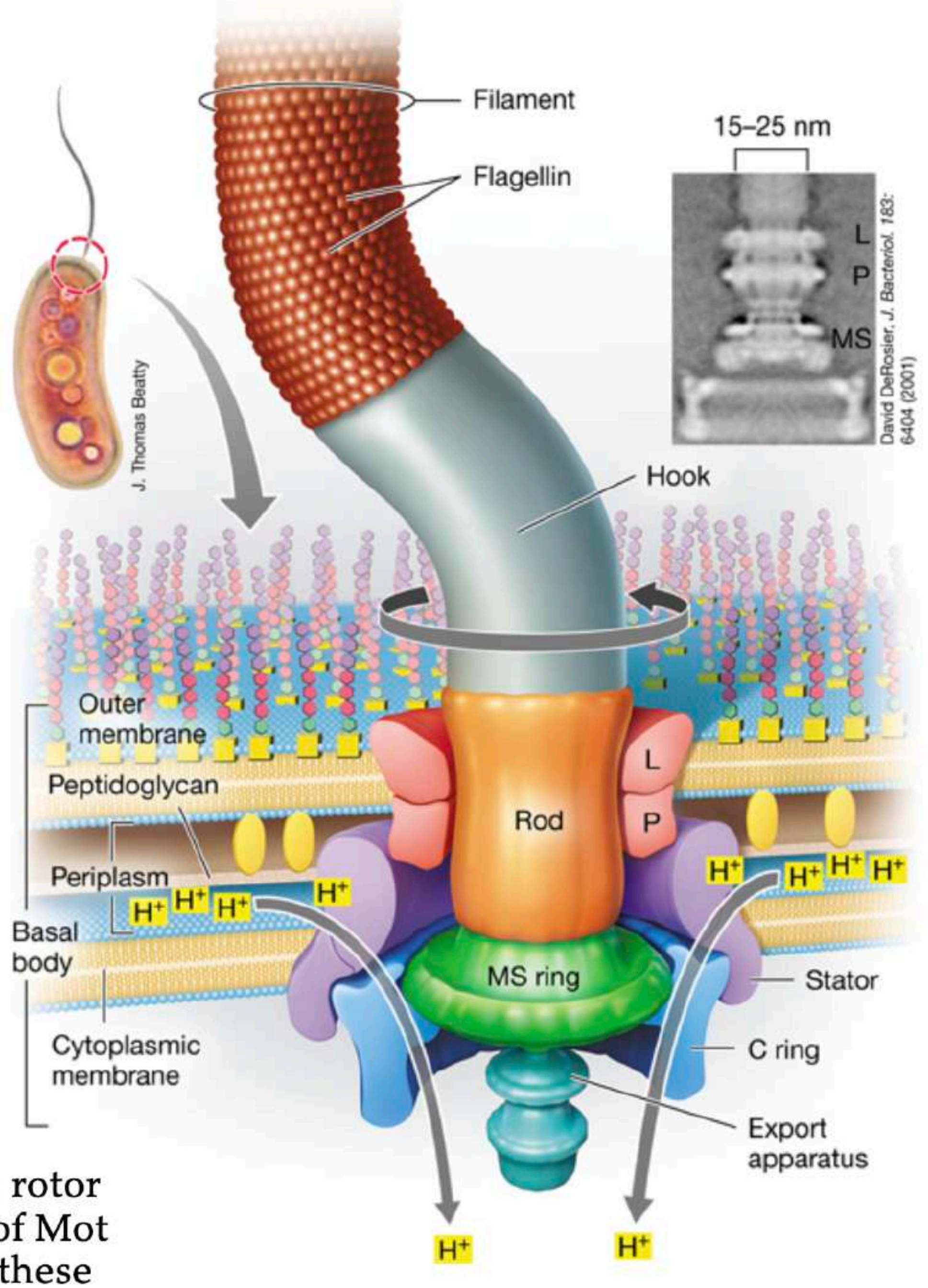


Proton turbine model of flagella movement

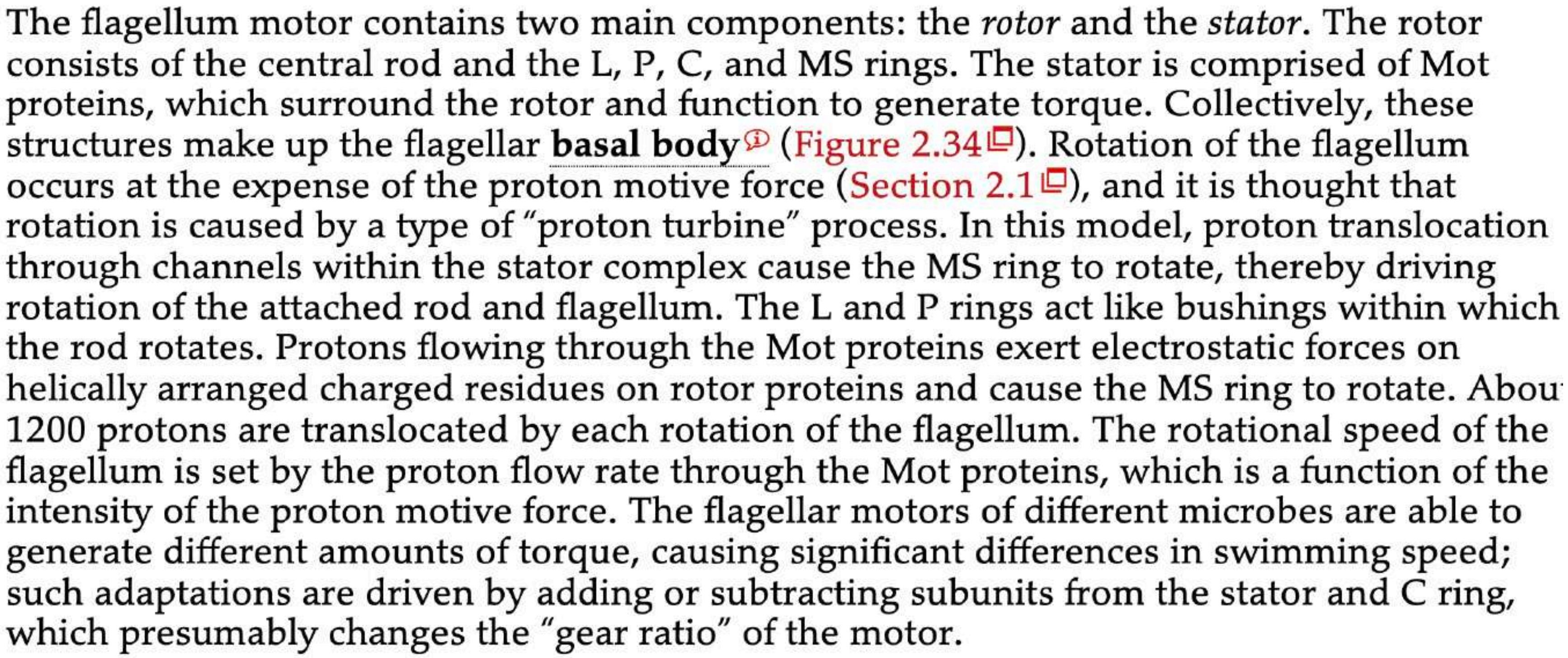
Madigan et al. 2018

Microbial Motion -> electrochemical potential does the job!

A membrane subsystem for compartmentalizing the functional network components

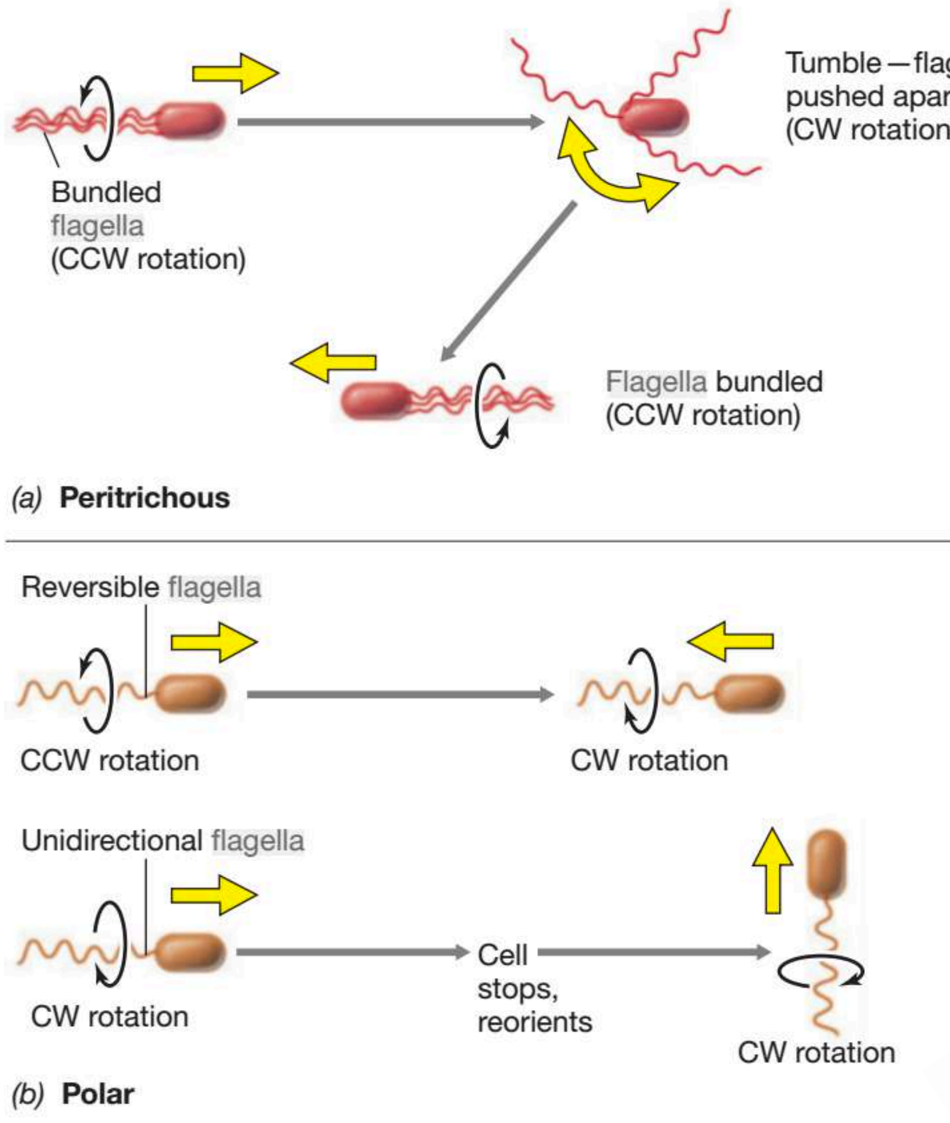


Madigan et al. 2020

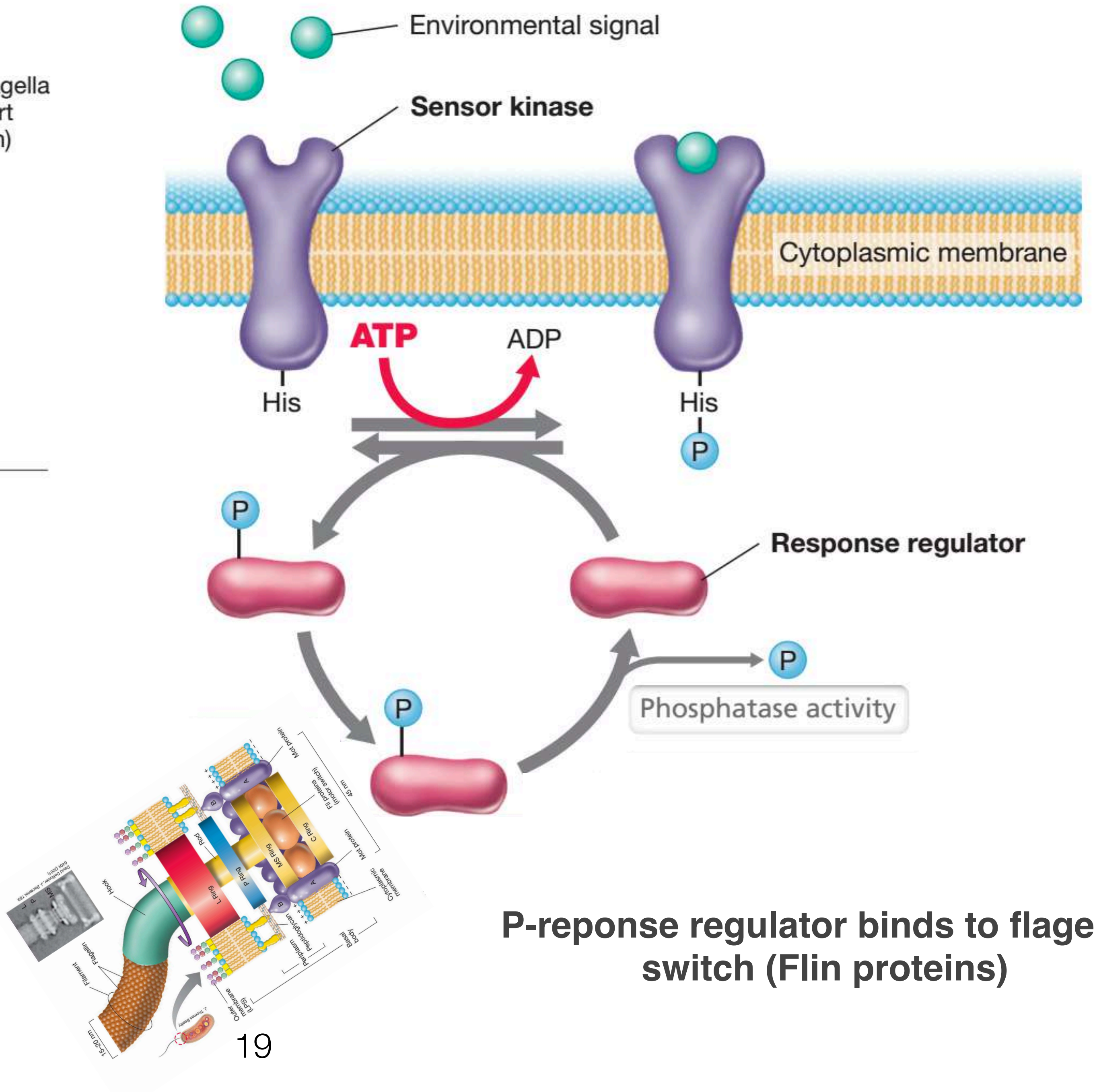

The flagellum motor contains two main components: the *rotor* and the *stator*. The rotor consists of the central rod and the L, P, C, and MS rings. The stator is comprised of Mot proteins, which surround the rotor and function to generate torque. Collectively, these structures make up the flagellar **basal body** (Figure 2.34). Rotation of the flagellum occurs at the expense of the proton motive force (Section 2.1), and it is thought that rotation is caused by a type of "proton turbine" process. In this model, proton translocation through channels within the stator complex cause the MS ring to rotate, thereby driving rotation of the attached rod and flagellum. The L and P rings act like bushings within which the rod rotates. Protons flowing through the Mot proteins exert electrostatic forces on helically arranged charged residues on rotor proteins and cause the MS ring to rotate. Abou 1200 protons are translocated by each rotation of the flagellum. The rotational speed of the flagellum is set by the proton flow rate through the Mot proteins, which is a function of the intensity of the proton motive force. The flagellar motors of different microbes are able to generate different amounts of torque, causing significant differences in swimming speed; such adaptations are driven by adding or subtracting subunits from the stator and C ring, which presumably changes the "gear ratio" of the motor.

Bacteria decision-making system for motility

Bacteria integrate environmental signal



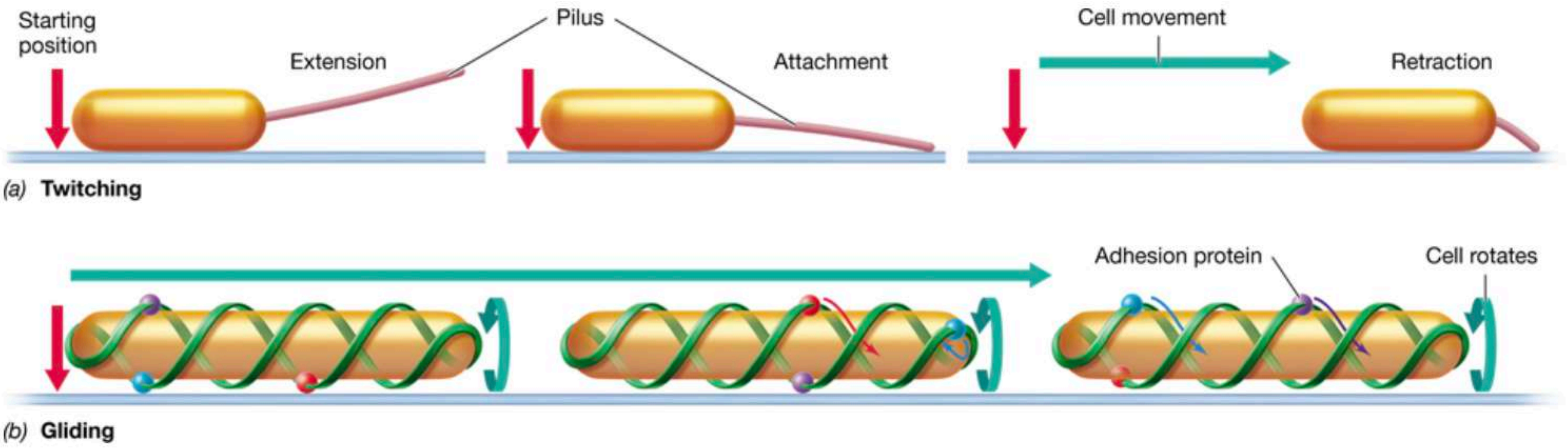
Two-component regulatory system send message to flagellum machinery to move either CCW or CW



P-reponse regulator binds to flagellar switch (FliN proteins)

Madigan et al. 2018

Figure 2.38 **Surface motility.**

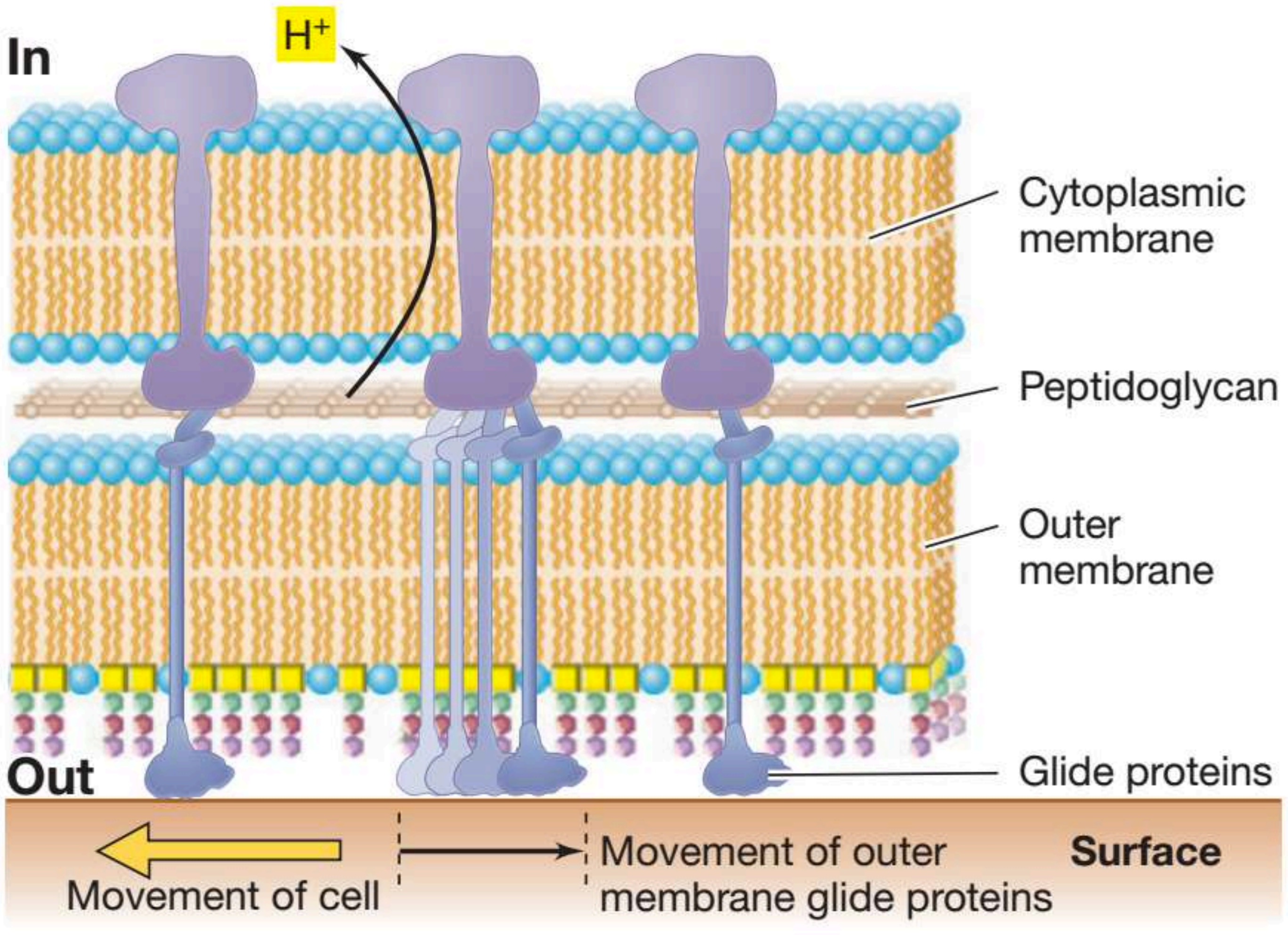


(a) Twitching motility employs type IV pili. These structures require ATP hydrolysis to extend (up to several micrometers) and then retract, causing the cell to move forward; movement occurs in discrete increments. *(b)* Gliding motility requires a helical intracellular protein track that interacts with gliding motors and extracellular adhesion proteins. The proton motive force drives rotation of gliding motors that translate this force to the helical track, causing adhesion proteins to move in a helical pattern; this results in continuous forward motion and clockwise rotation of the cell.

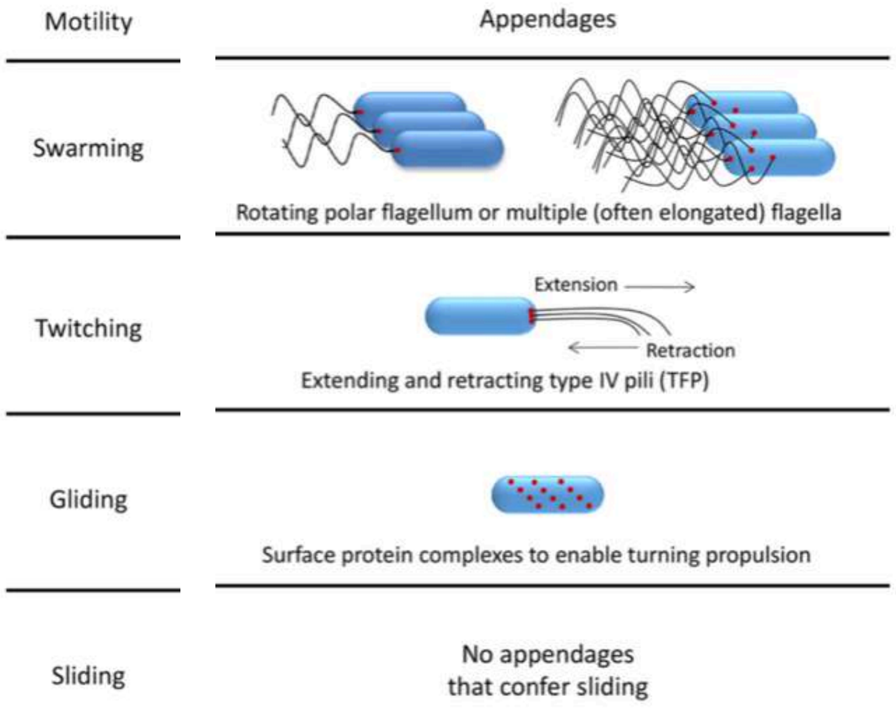
Madigan et al. 2020

Gliding Motility

- Movement of cytoplasmic membrane proteins (gliding protein)is driven by proton motive force
- This somehow transmits energy to move to outer membrane proteins
- This (hypothetically) pulls the cell against a **solid surface**
- 10 µm/sec



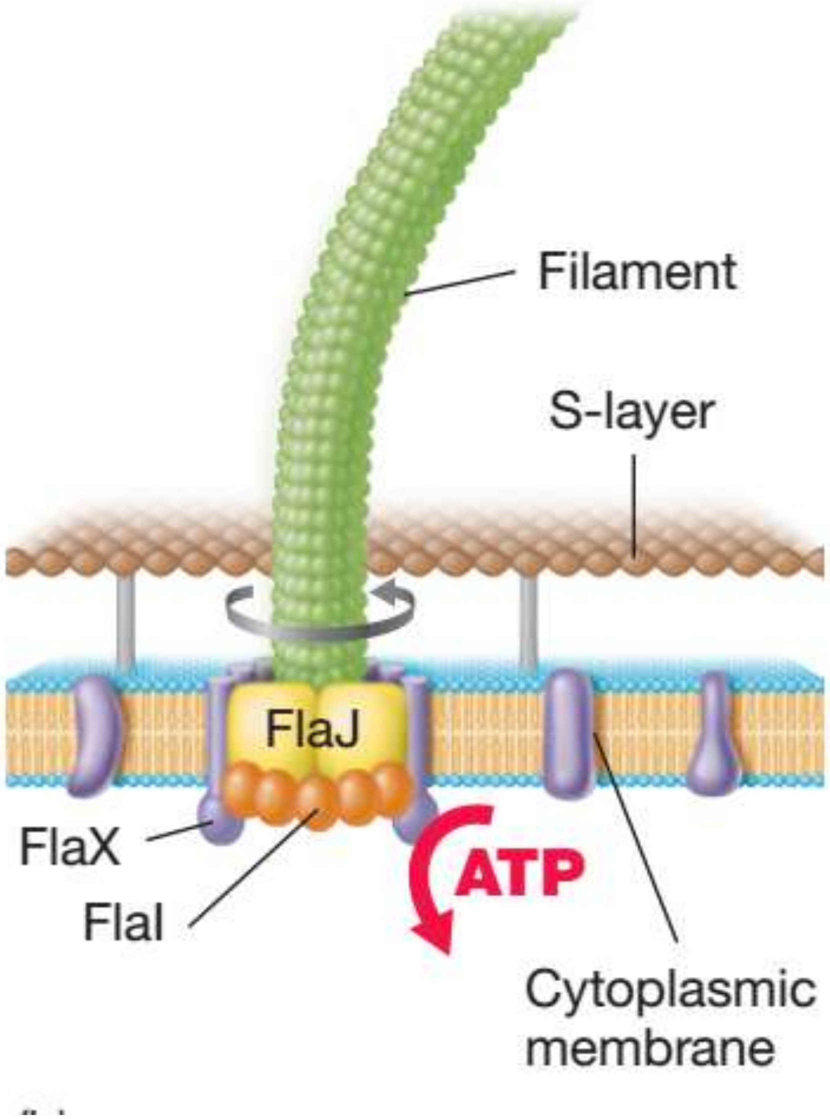
Madigan et al. 2018



Motility	Appendages
Swarming	Rotating polar flagellum or multiple (often elongated) flagella
Twitching	Extension Retraction Extending and retracting type IV pili (TFP)
Gliding	Surface protein complexes to enable turning propulsion
Sliding	No appendages that confer sliding

Mattingly et al., 2018

Archaea Flagella (ultra-structure and function)



- ~ half the diameter of flagella, measuring about 10–13 nm in width
- Archaellum can be considered a rotating type IV pilus capable of both CW and CCW rotation
- **In flagellum**, whose energy requirement is met by **dissipation of the proton motive force**, **achaellum**’s rotation is driven by **ATP hydrolysis**
- In Bacteria a single type of protein makes up the filament
- In Archaea several different filament proteins

Prokaryotic cytoskeleton

- Similar to eukaryotic cytoskeletal actin, tubulin and intermediate filaments
- Cytoskeletal proteins may dictate shape during peptidoglycan synthesis

Proteins involved

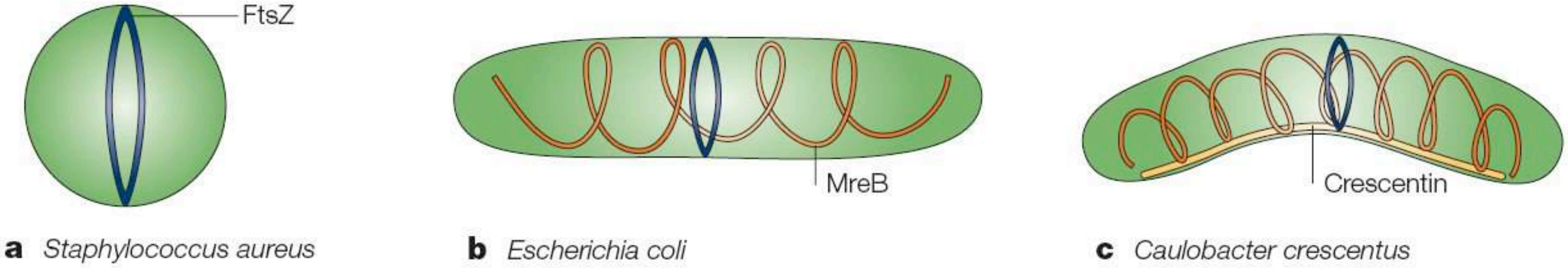
1. ***FtsZ:* Cell division protein; related to tubulin; assembles as ring at cell division site, recruits other proteins to form contractile septal ring constricting cell during cell division**

2. *MreB:* Member of actin superfamily; in rod-shape, filamentous and helical bacteria; encircles cell as spiral under cell membrane along longitudinal axis contributing to shape of non-spherical bacteria

- *Crescentin:* In *Caulobacter crescentus*; gives vibroid shape; helical filament along cell membrane

- *Spiroplasma:* Move in viscous media; single protein ribbon, stretch & release by conformational change

- *Mycoplasma*: Some motile on solid surface; internal cytoskeletal fibers for movement and attachment



Cabeen & Jacob-Wagner, 2005 Shih & Rothfield, 2006

Intracytoplasmic membrane (ICM), I

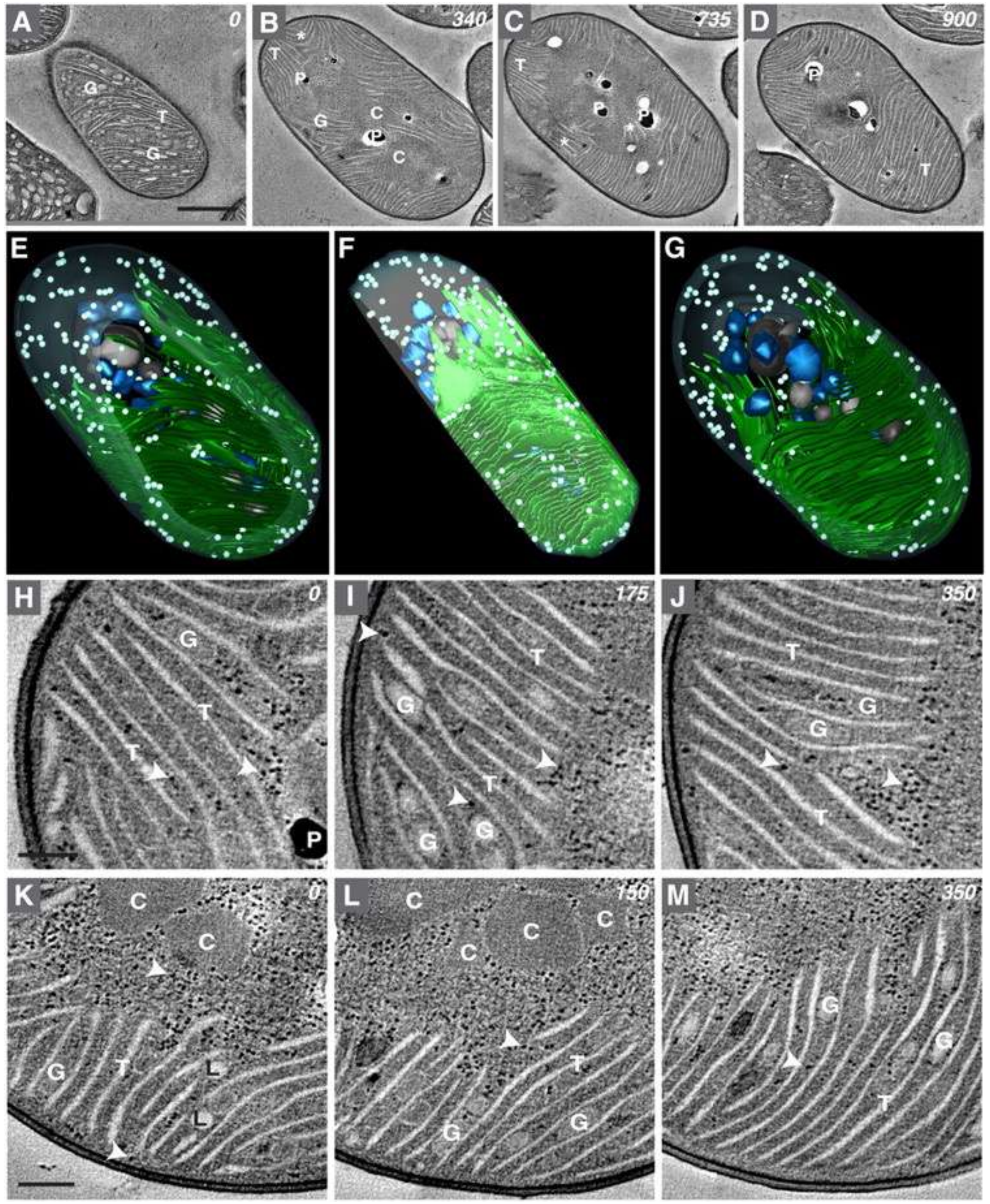
- ICM as a strategy to concentrate and stabilize functions within the cell
- ICM promote more efficient reactions
- ICM is a solution to the fight for diffusion
- **Phototrophs: site of photosynthetic apparatus**
- **Cyanobacteria**

Tomographic reconstruction of a
Cyanothece 51142 cell

T, Thylakoid membrane; C, carboxysome; G,
glycogen granule; P, polyphosphate body

Blue gray, Plasma membrane (rendered partially transparent for clarity);
white, lipid bodies;
blue, carboxysomes;
green, thylakoid membranes;
gray, polyphosphate bodies

Bar = 1,000 nm



Bar = 200 nm

Liberation et al., 2011

Intracytoplasmic membrane (ICM), II

Methylobacterium organophilum

Patt & Hanson 1978

Nitrifk et al., 2004

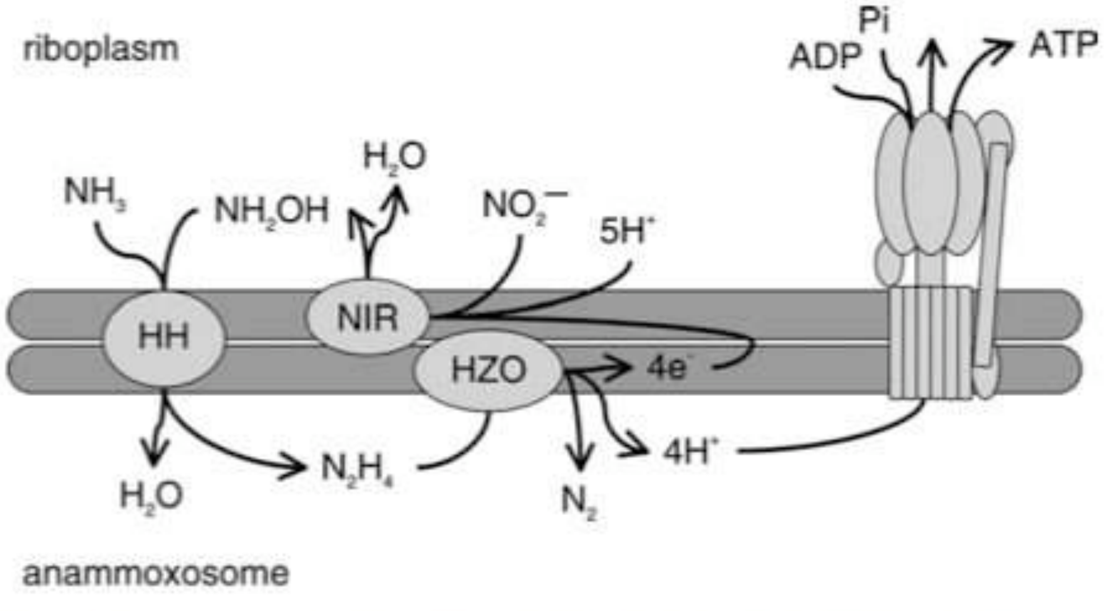


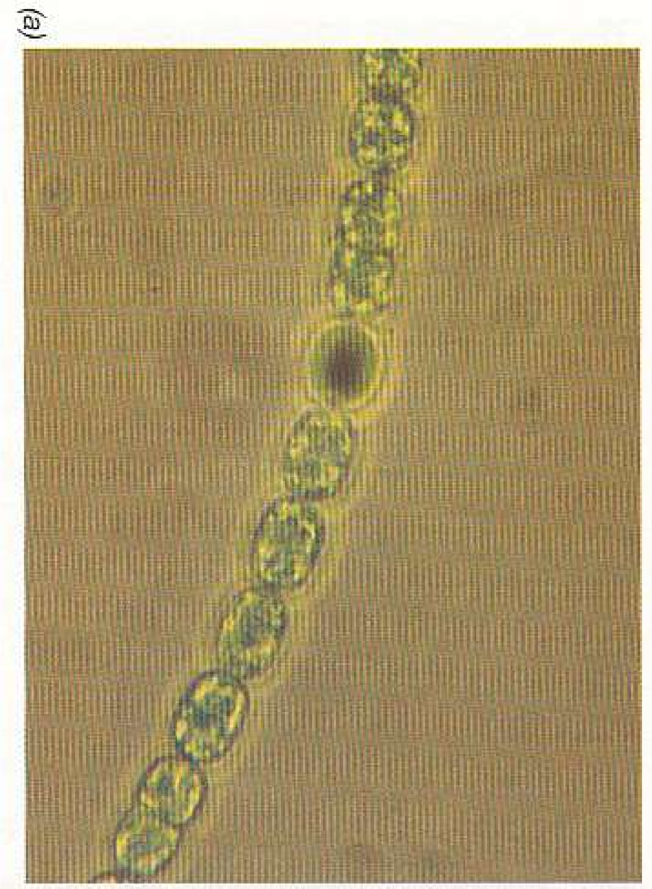
Fig. 4. Postulated anaerobic ammonium oxidation coupled to the anammoxosome membrane in anammox bacteria resulting in a proton motive force and subsequent ATP synthesis via membrane-bound ATPases. HH: hydrazine hydrolase; the hydrazine-forming enzyme, HZO: hydrazine-oxidizing enzyme, NIR: nitrite-reducing enzyme.

ICM present in methanotrophs, N_2 fixers, nitrifiers and phototrophs

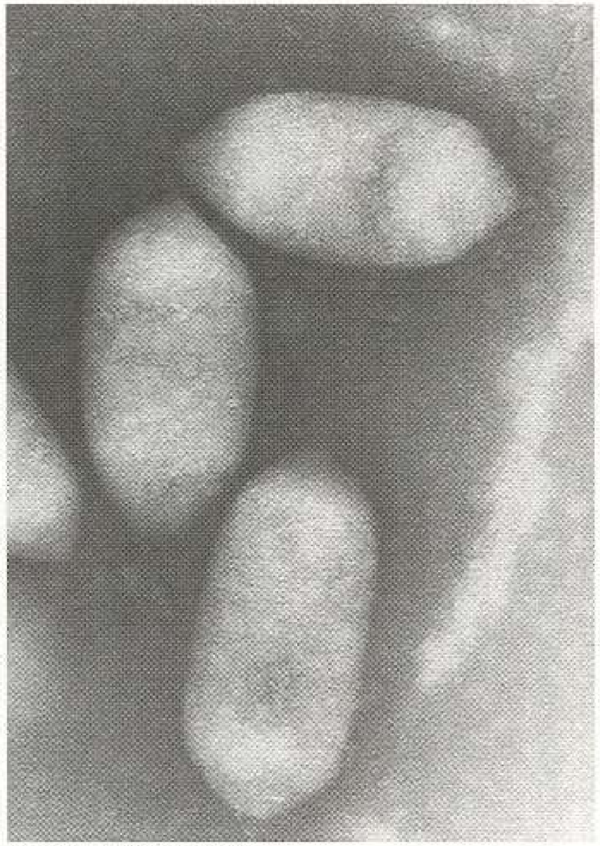
(see also: magnetosomes, gas vacoule, minicompartments, anammoxosome)

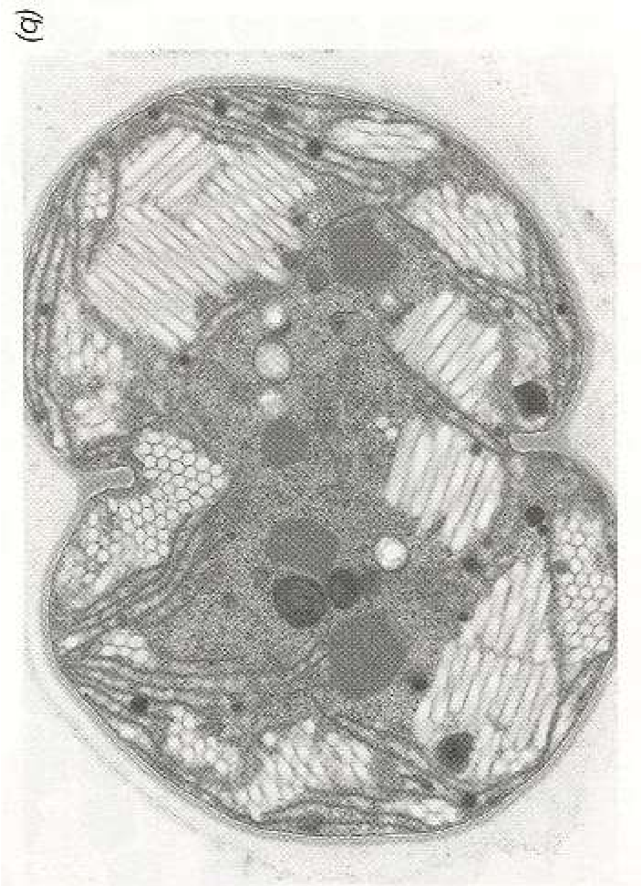
- *Methanotrophs*: ICM is the site of methane oxidation
- *N_2 fixers*:Increases respiratory activity to provide ATP for N_2 fixation and remove O_2 near nitrogenase
- *Nitrifiers*: Site of enzymes catalyzing ammonia and nitrate oxidation

Gas Vacuoles



A. E. Walsby


A. E. Konopka and J.T. Staley



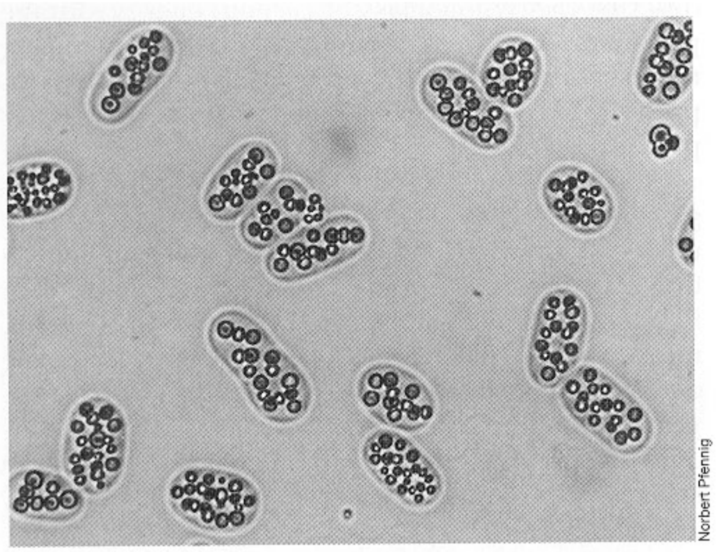
S. Pellegrini and M. Grilli Caiola

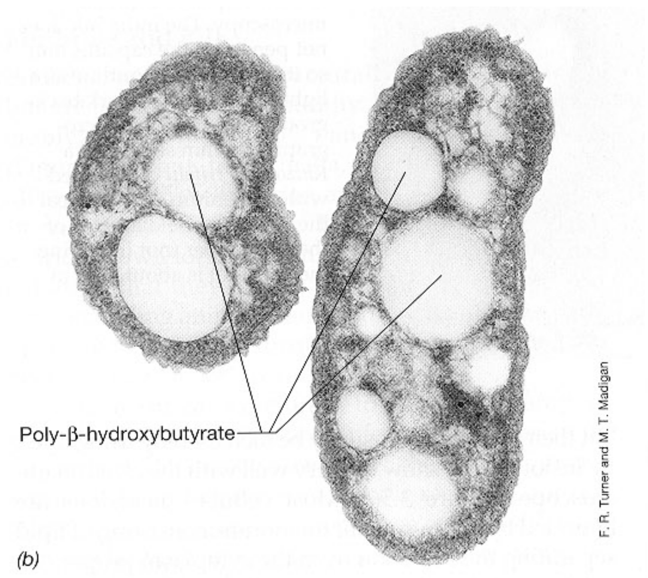
Madigan et al. 2018

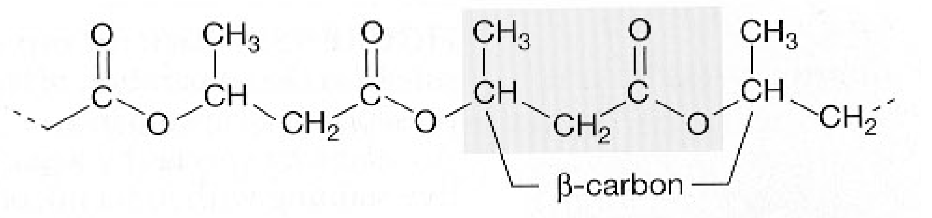
- In aquatic bacteria; for buoyancy
- Means of motility (float up and down)
- In Cyanobacteria, some purple and green phototrophic bacteria, some Archaea
- Spindle shaped hollow, rigid, 300-1000 nm, few to hundreds per cell
- Membrane only protein (rigid), 2 nm thick, impermeable to water, permeable to gases
- Gases same in and out; vesicle density 5-25% of cell density

Storage products

Madigan et al. 2018



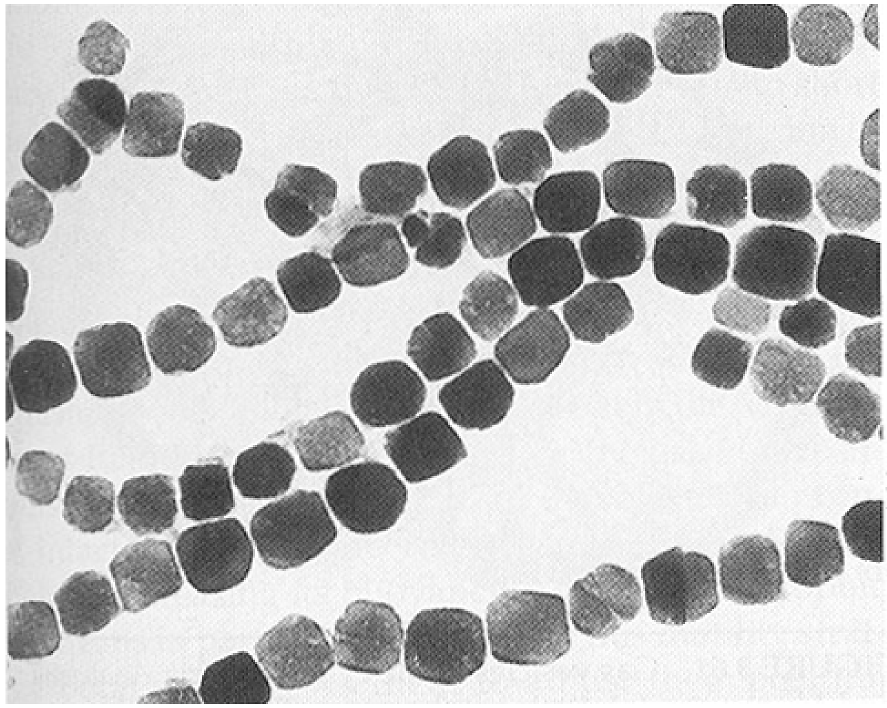




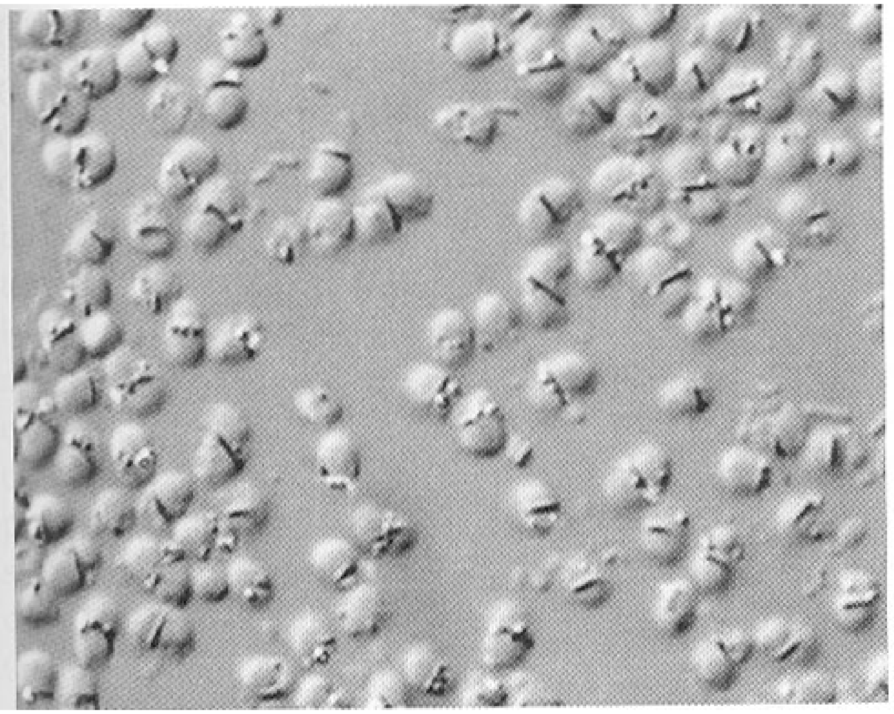
Common storage products- enclosed in thin lipid layer, also in periplasm:

- Poly-ß-hydroxybutyrate (PHB); sulfur globules in S oxidizing bacteria and archaea
- Glycogen
- Polyphosphate
- Nitrogen store?
- Carboxysomes (RuBP carboxylase in cyanobacteria); crystal-like, 120 nm; membrane bound
- Mobilized when needed
- Advantageous in nutritionally fluctuating environments

Magnetosomes



R. Blakemore and W. O'Brien



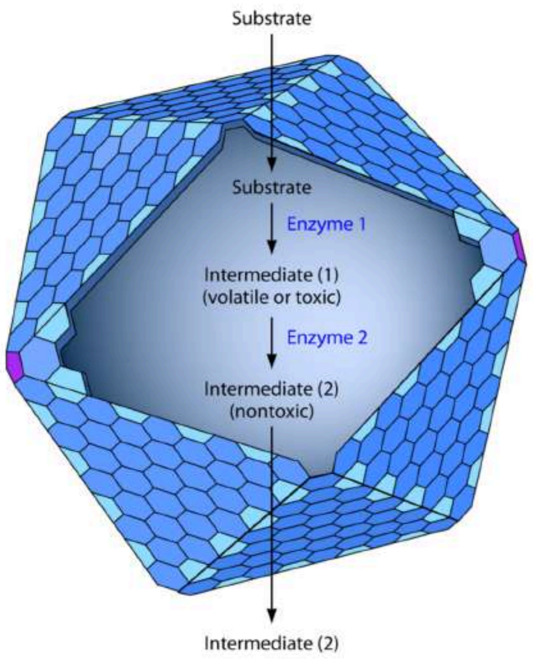
Stefan Spring

Madigan et al. 2018

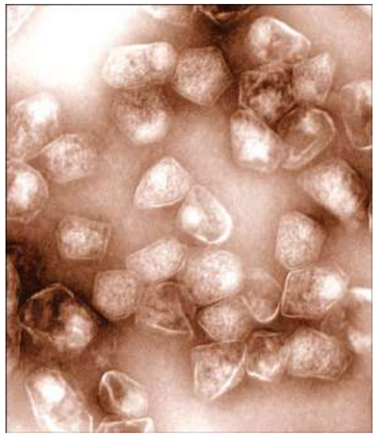
Ueber and Schueler, 2016

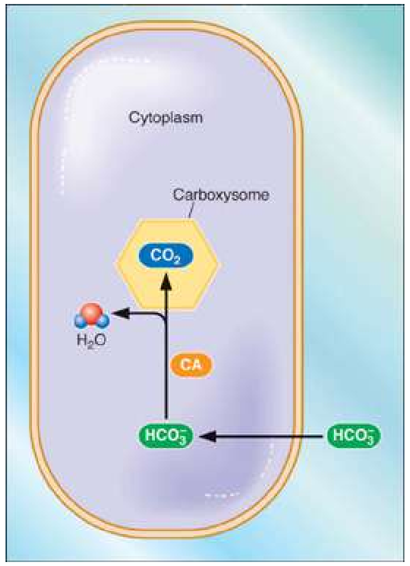
- **Cytoplasmic membranes** forms an internal invagination and **recruit proteins for Fe precipitation**
- Magnetotactic bacteria usually mineralize either **iron oxide** magnetosomes, which contain crystals of magnetite (Fe_3O_4), or **iron sulphide** magnetosomes, which contain crystals of greigite (Fe_3S_4)
- Mainly in aquatic bacteria; some algae
- Morphology species-specific
- **Permanent magnetic dipole to the cell, for N-S** *orientation* in environment ("**magnetotaxis-aerotaxis**") efficient swimming, passive cell alignment to geomagnetic field lines
- Microaerophilic bacteria may use them to **stay in low oxygen at oxic/anoxic interface**
- Magnetosome Fe_3O_4 and Fe_3S_4 crystals are typically **35–120 nm long**

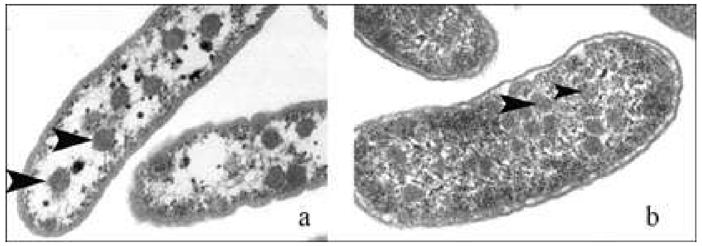
Bacterial microcompartments



Bobik, 2007
Chowdhury et la., 2014







- Sophisticated protein-based organelles used to **optimize** specific metabolic pathways

- **Metabolic enzymes encapsulated within a protein shell, increase specificity and yield**

- Widely distributed and functionally diverse

- **Compartmentalization creates an ideal environment for catalysis and facilitates the channeling of toxic/volatile intermediates to downstream enzymes**

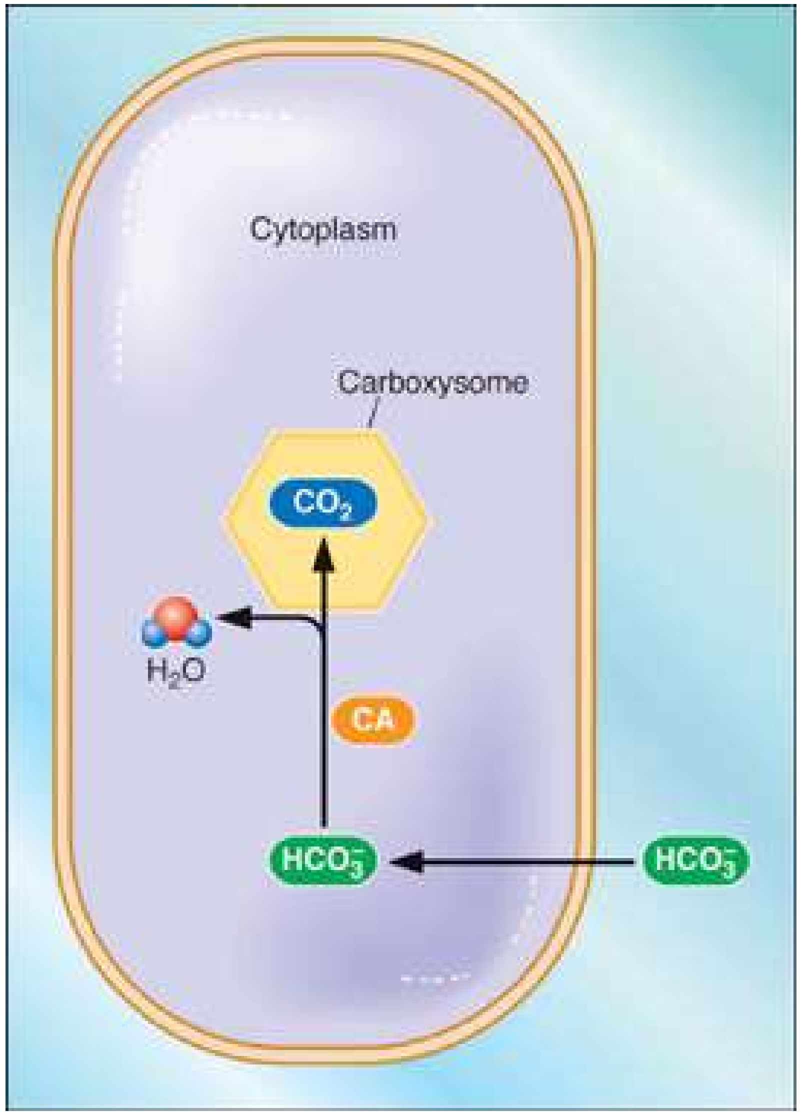
- Structurally resemble viral capsids

- Carboxysome channels CO_2

- Pdu microcompartment channels propionaldehyde, *Salmonella enterica*

- Eut microcompartment channels acetaldehyde

Bacterial microcompartments: carboxysome



RuBisCO is the CO_2-fixing enzyme of the Calvin-Benson-Basham Cycle, CBB

1. RuBisCO catalyses the conversion of CO_2 and ribulose bisphosphate into two 3-phosphoglycerate

2. RuBisCO reacts with O_2 in a nonproductive process known as **photorespiration**, which can drain away up to 50% of the carbon fixed by the CBB cycle—> competition with carboxylation

3. The carboxysome is essential part of a **carbon dioxide concentrating mechanism** (CCM) that improves the efficiency of CO_2 fixation by RuBisCO

4. CCM starts with the concentration of HCO_3^- in the cytoplasm of the cell by active transport

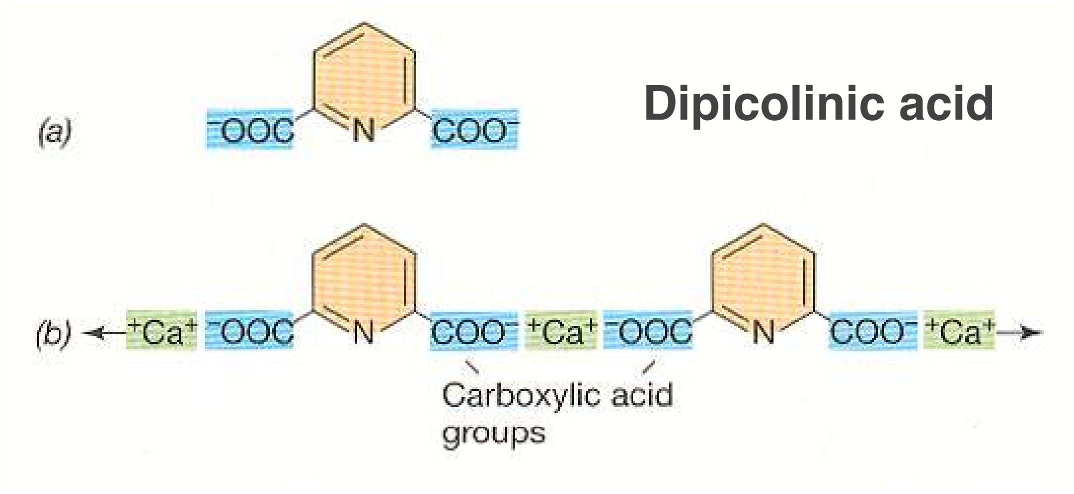
5. Equilibrium with CO_2 is not reached due to a lack of carbonic anhydrase (CA)

6. Carboxysomal CA converts HCO_3^- to CO_2 and releases it within the microcompartment

7. The protein shell of the microcompartment impedes CO_2 diffusion

8. **Consequently, CO_2 is concentrated in RuBisCO vicinity —> increase in CO_2 fixation and suppression of photorespiration**

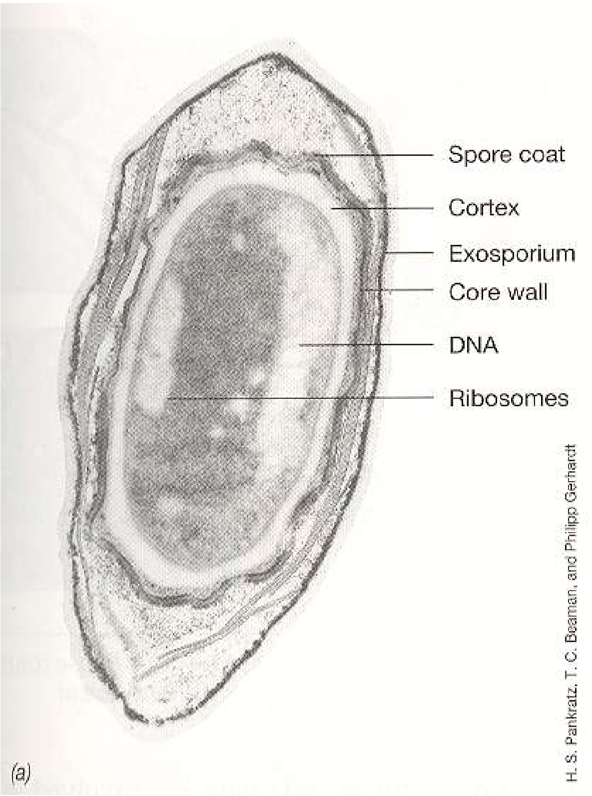
Spores



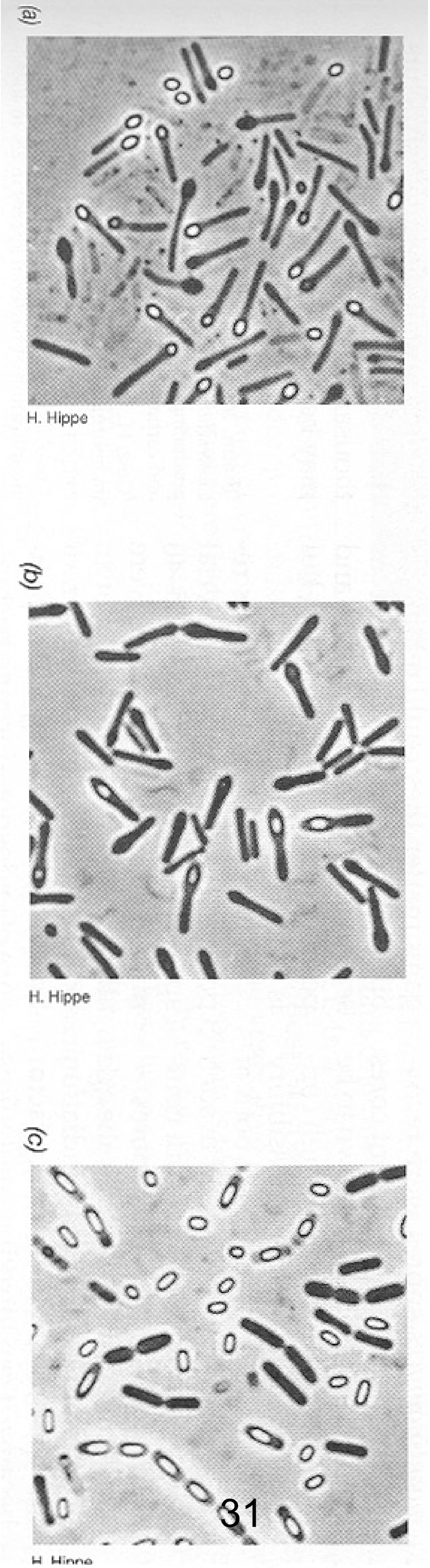
Dipicolinic acid

Ca^{2+} crosslinking dipicolinic acid

Madigan et al. 2018



H. S. Pankratz, T. C. Beaman, and Philipp Gerhardt



H. Hippe

H. Hippe

H. Hippe

- •Differentiated cells within bacteria (endo-)
- •Very resistant (heat, desiccation, chemicals); persist long
- •Gram Positive (e.g. Bacillus, Clostridium)

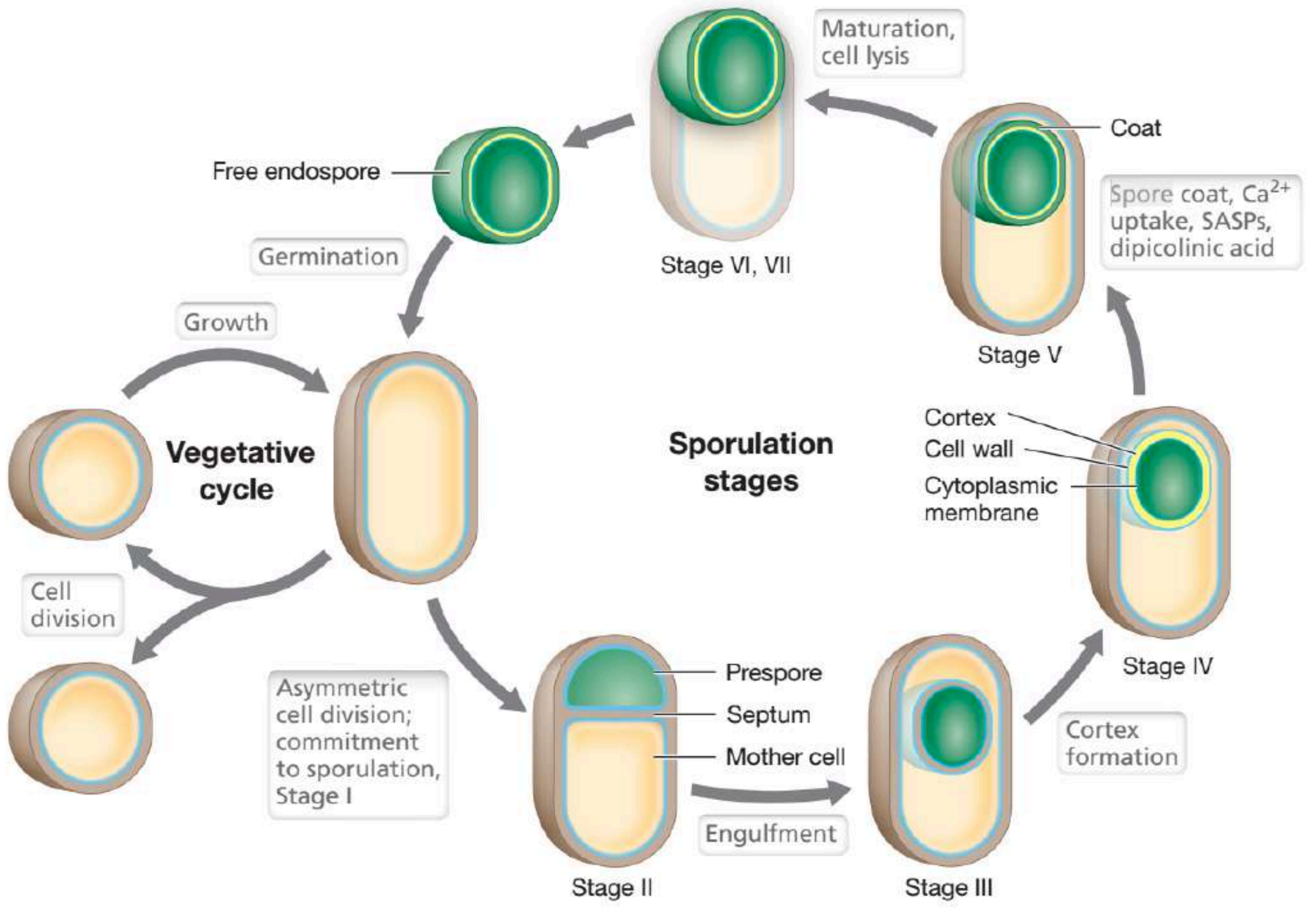
•LM: Seen as refractile bodies; TEM structure, very different from vegetative cell:

•Many layers: thin (protein) exosporium; spore coat (protein) cortex (PG), core (protoplast)

•Characteristic: Dipicolinic acid (in core); high Ca^{2+} (most in Ca-DPA) ~10 w/w endospore

•Core: 10-30% water; Ca-DPA; cytoplasm is a gel; enzyme inactive; lower pH;
SASPs (small Acid soluble proteins)- bind and protect DNA & C/e source during outgrowth

Endospore formation



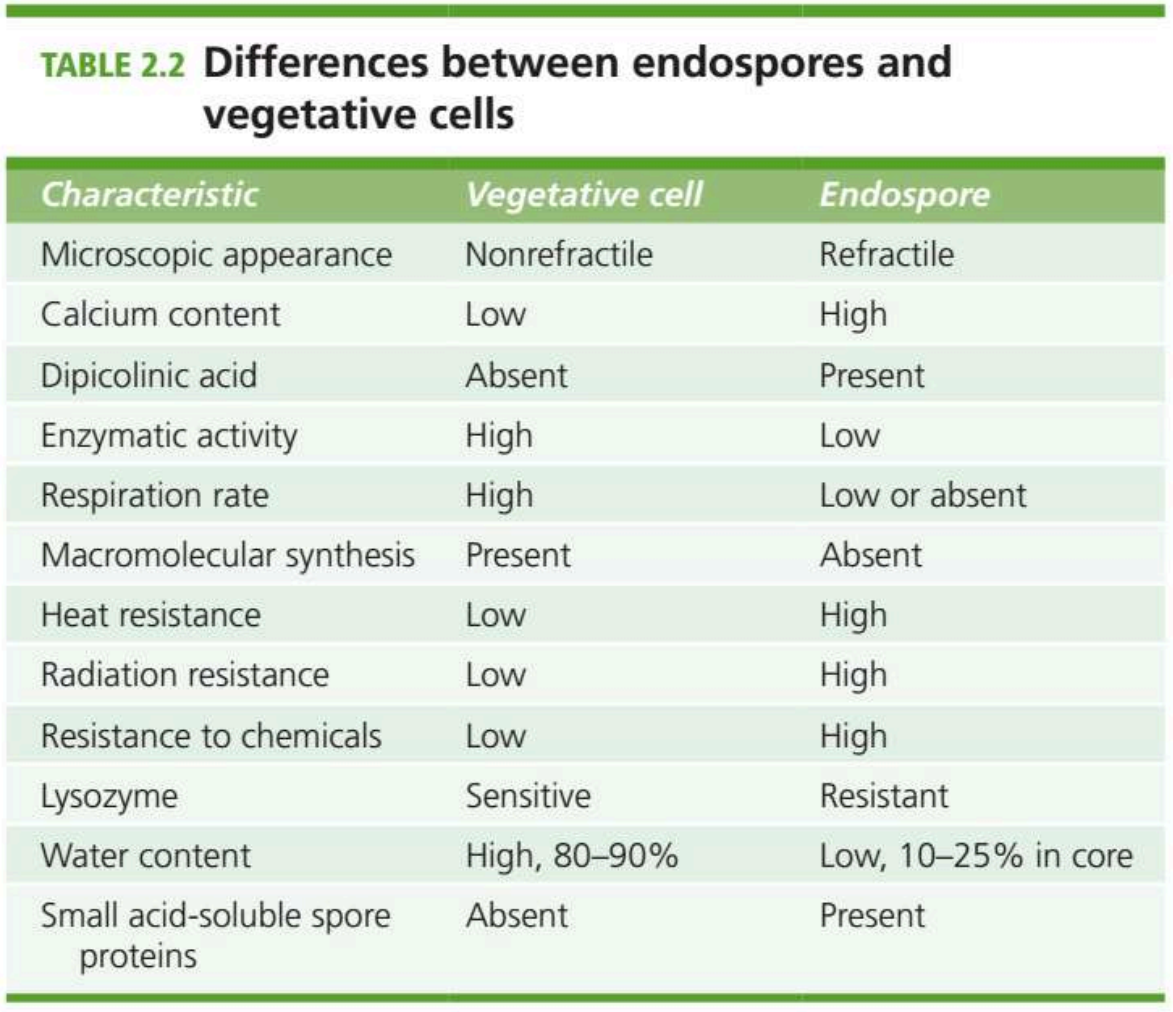


TABLE 2.2 Differences between endospores and vegetative cells

Characteristic	Vegetative cell	Endospore
Microscopic appearance	Nonrefractile	Refractile
Calcium content	Low	High
Dipicolinic acid	Absent	Present
Enzymatic activity	High	Low
Respiration rate	High	Low or absent
Macromolecular synthesis	Present	Absent
Heat resistance	Low	High
Radiation resistance	Low	High
Resistance to chemicals	Low	High
Lysozyme	Sensitive	Resistant
Water content	High, 80–90%	Low, 10–25% in core
Small acid-soluble spore proteins	Absent	Present

- Initiated in response to nutrient stress
- Many steps; in B. subtilis 8h; ~200 genes
- Activation of a number of spore specific genes (spo, ssp; encoding SASPs)
- Germination: Activation, germination, outgrowth
- Ca-DPA and cortex lost; SASPs degraded; spore swells (takes in water); new RNA, protein, DNA synthesis; cell emerges from the broken coat

Madigan et al. 2018

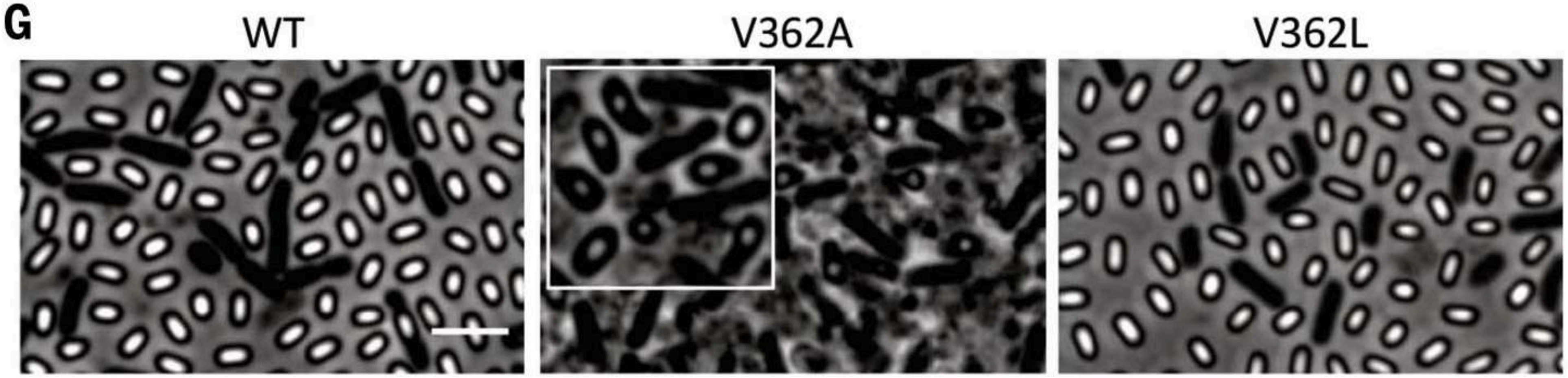
Resurrection! Germination program

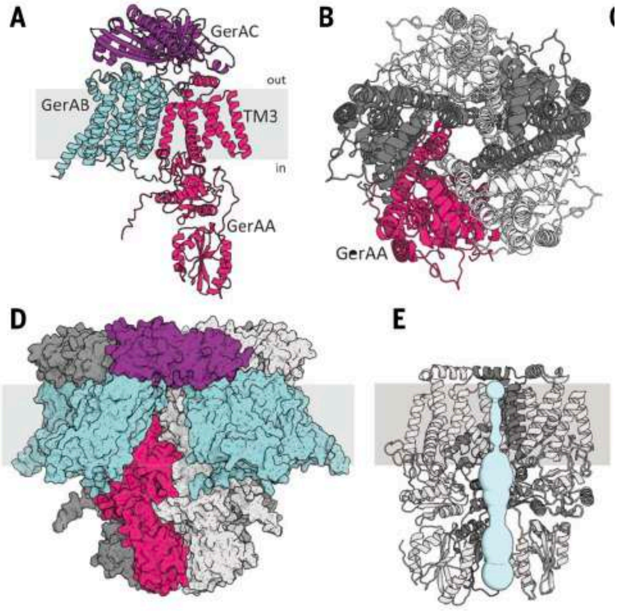
GerA family receptors embedded in the spore membrane are required for sensing amino acids, sugars, and/or nucleosides

Nutrient detection leads to the release of mono- and divalent cations from the spore core, which is rapidly followed by the expulsion of large stores of dipicolinic acid (DPA) through the SpoVA transport complex

DPA release activates cell wall hydrolases that degrade the specialized peptidoglycan that encases the spore, allowing core rehydration, macromolecular synthesis, and resumption of growth

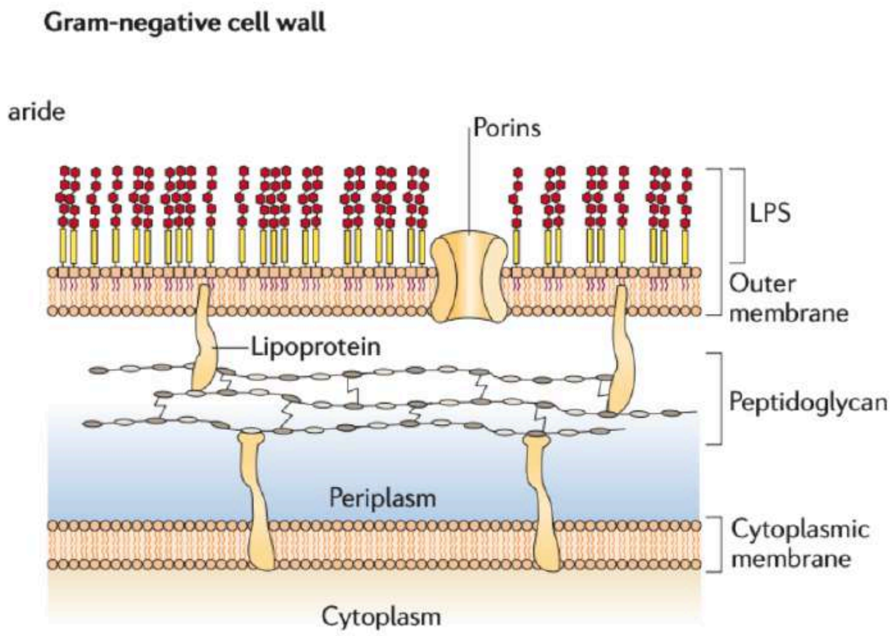
GerA family receptors assemble into a family of pentameric ligand-gated ion channels that transduce germinant signals by releasing cations, which activates SpoVA complexes to expel DPA from the spore core. DPA release triggers degradation of the spore cortex peptidoglycan and exit from dormancy.

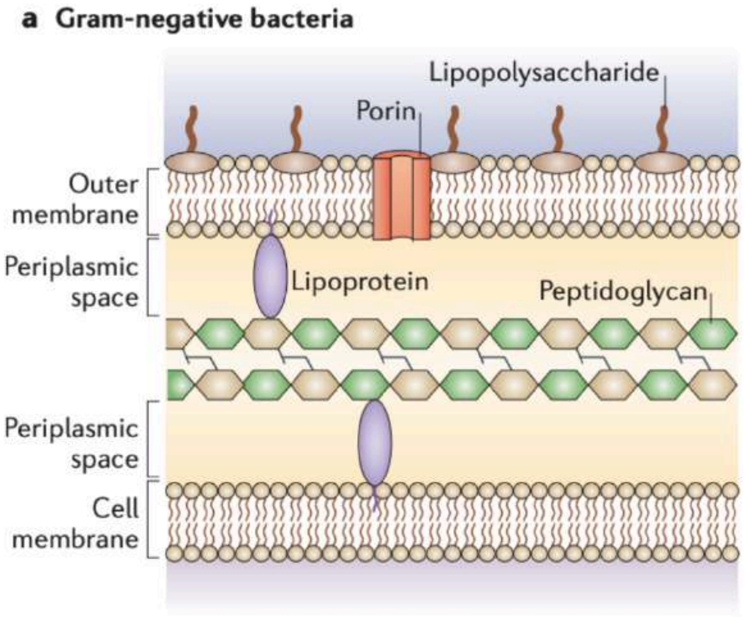




Gao et al. 2023

Periplasm-periplasmic space

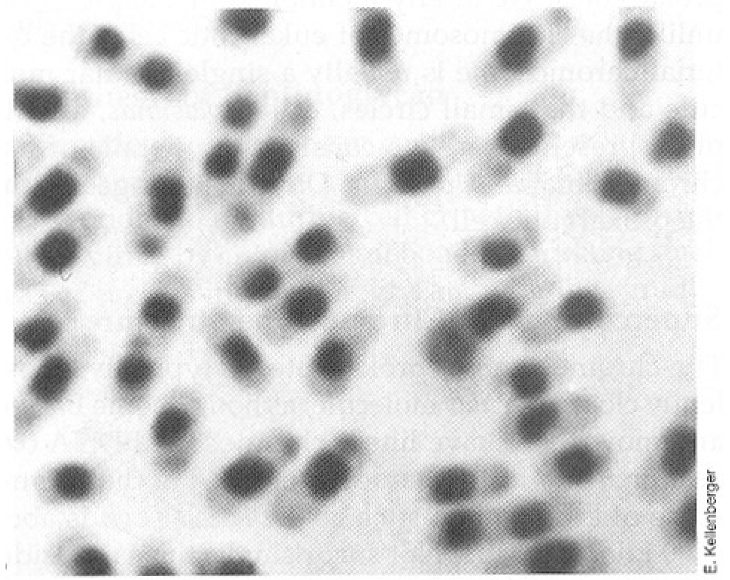




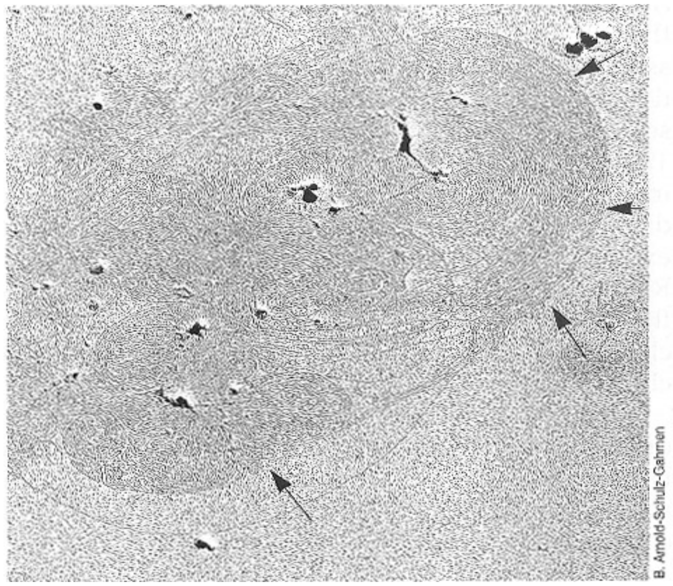
- Active metabolic site (reduced in Gram-positive)
- Very viscous —> high concentration of extracellular proteins (via cytoplasmatic protein-exporting system)

- Outer membrane is impermeable to proteins and very large molecules—> prevents extracellular proteins from diffusing away from the cell
- Width of periplasmic space 15 nm
- Major periplasmic proteins:
    - Hydrolytic enzymes (degradation of polymeric substances)
    - Binding proteins (transporting substrates)
    - Chemoreceptors (Chemotaxis response)
    - Structural proteins (peptidoglycan, outer & cytosolic membrane)

Nucleoid



E. Kellenberger

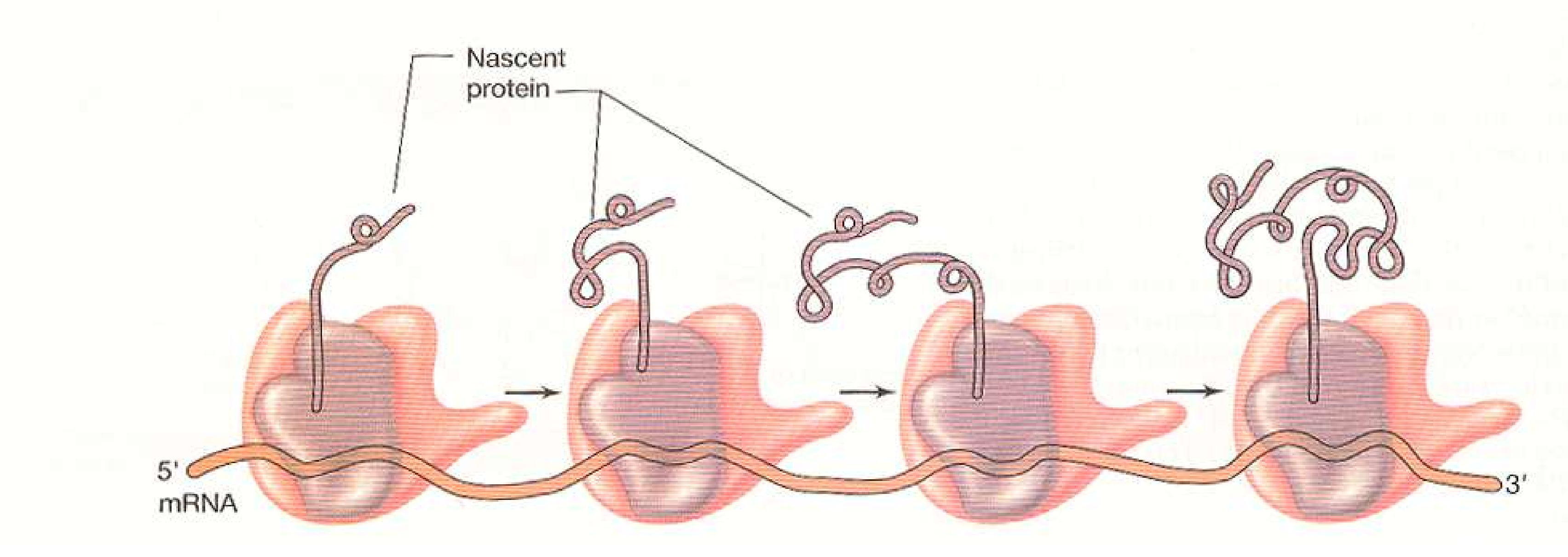


B. Arnold-Schulz-Gahmen

Madigan et al. 2018

- No nuclear membrane
- Naked DNA
- Generally 2 - 4 x 10^9 Daltons (but Vibrios--2 chromosomes; Myxobacteria)
- One mm long (1000 - 5000 body lengths) supercoiled (histone-like proteins)
- Plasmid, common carrier of antibiotic resistance and metal resistance genes
- *E. coli* nucleoid occupies 0.07 μm^3
- In diverse environments the degree of supercoiling is different and coupled with surface/volume ratio

Ribosomes



Madigan et al. 2018

- **Fill cytoplasm in fast growing cells (20 000 cell^{-1} in rapidly growing *E.coli)***
- **Number depends on the physiological state of the cell**
- **0.02 *µ*m diameter**
- **Site of protein synthesis**
- **Simpler body plan of procaryotes allows simultaneous transcription and translation as well as fine regulation of protein synthesis**
- **Perhaps only 20 -100 ribosomes in marine bacteria with growth rate <1 d**
- **RNAs are structural molecules**
- **Very dynamic micro machines**

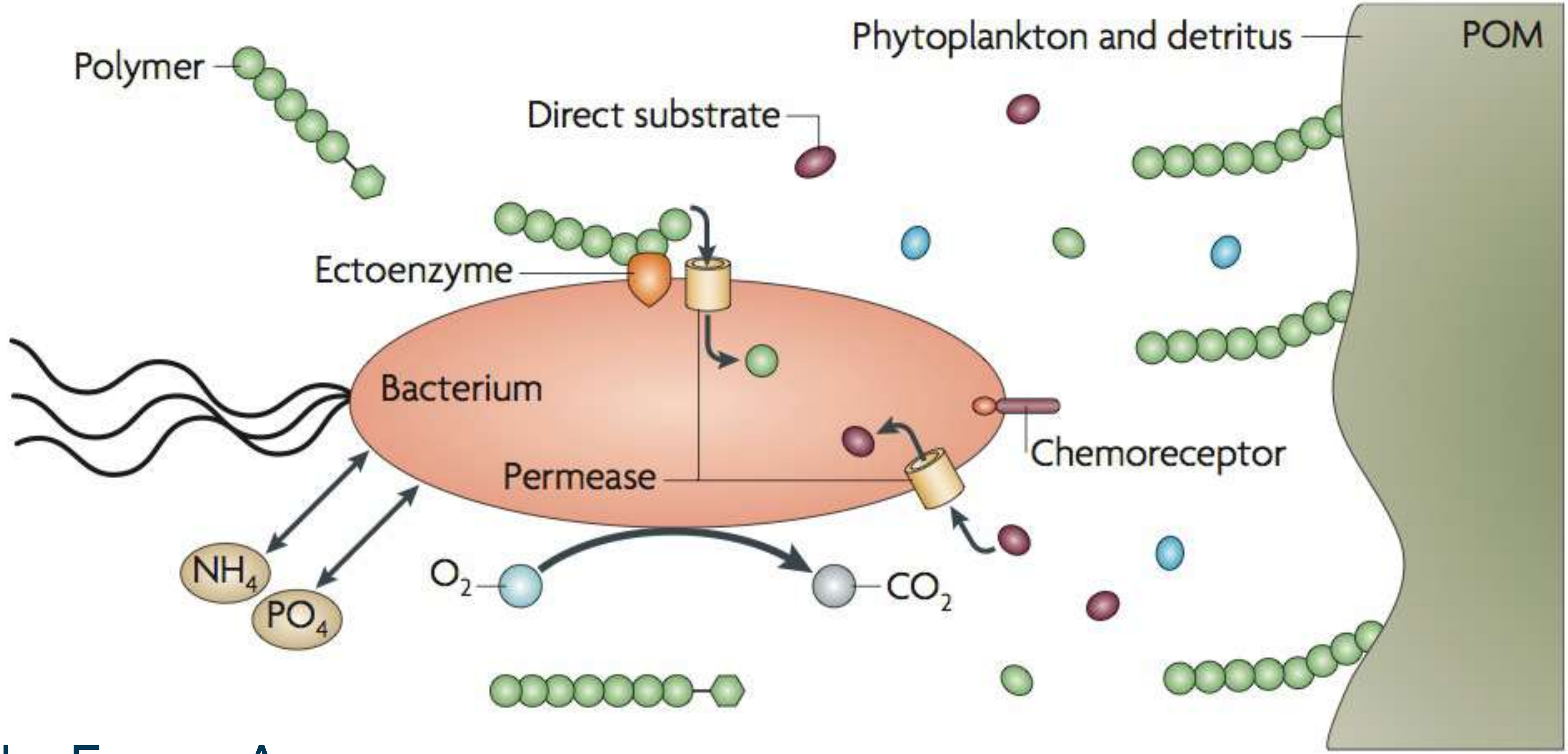
Protein

- 55% of dry weight in *E. coli* and in marine bacteria
- Together with DNA makes the cytoplasm a thick gel
- Occupies 13% of the cell volume in *E. coli*, but up to 50% in marine bacteria
- L and D aa

Water

- ~90% of the cell volume in *E. coli*
- ~50-90% of cell volume in marine/aquatic bacteria depending on the cell size
- Dryness may be an adaptation for rapid response to nutrient supply
- Just add water to become bigger

Adaptive strategies of Bacteria for nutrient uptake



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**

Transport Mechanisms

- Passive diffusion

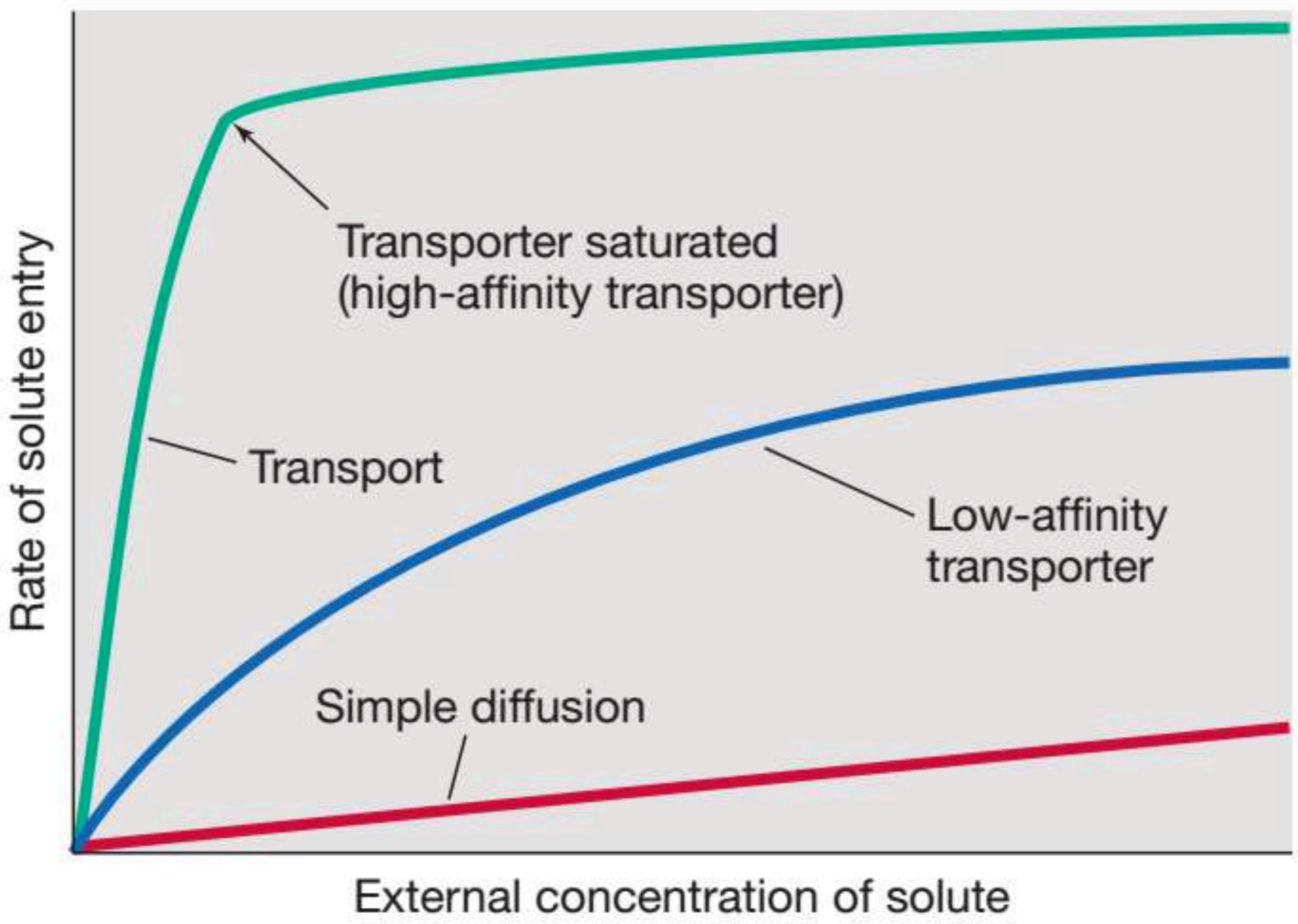
- Facilitated diffusion

- Active transport
  - Energy coupling for active transport
  - Primary and secondary active transport
  - Binding protein dependent active transport

- Group translocation

Microbial adaptations to increase uptake of molecules

Microbial interfaces, the membranes as hotspots of activities



Low-concentration

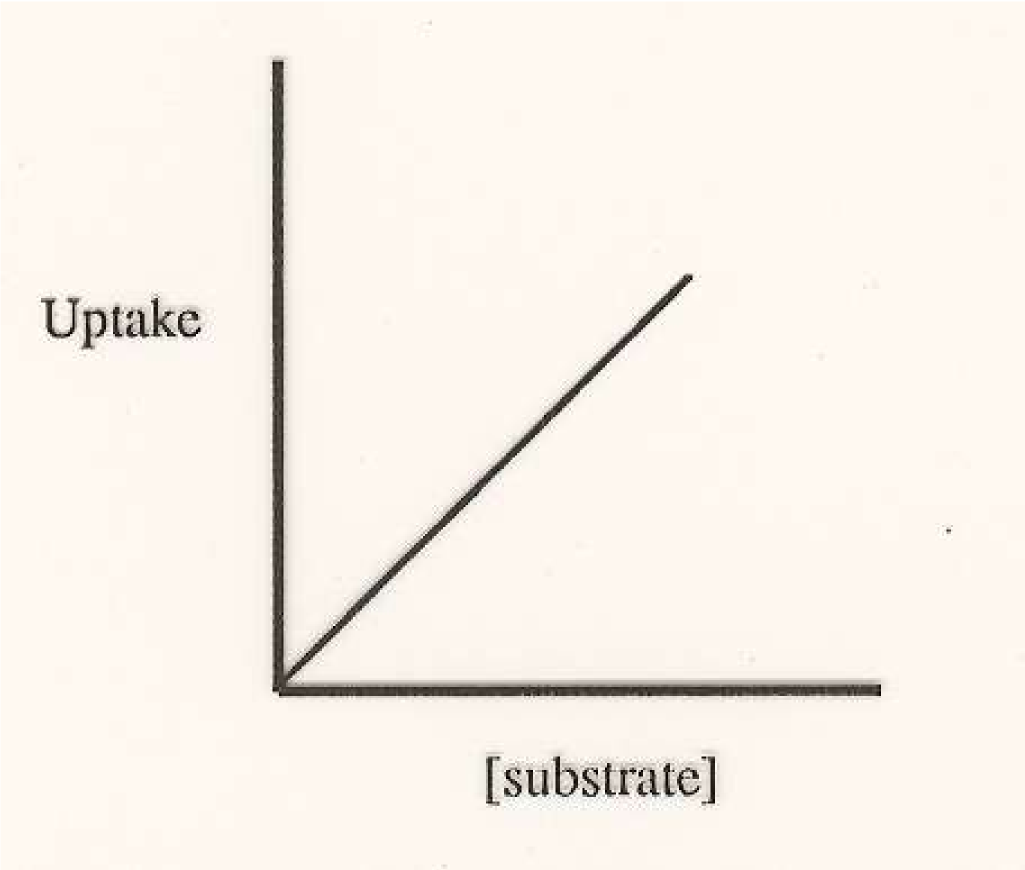
High-concentration

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

Passive Diffusion

Neidhardt et al 1990

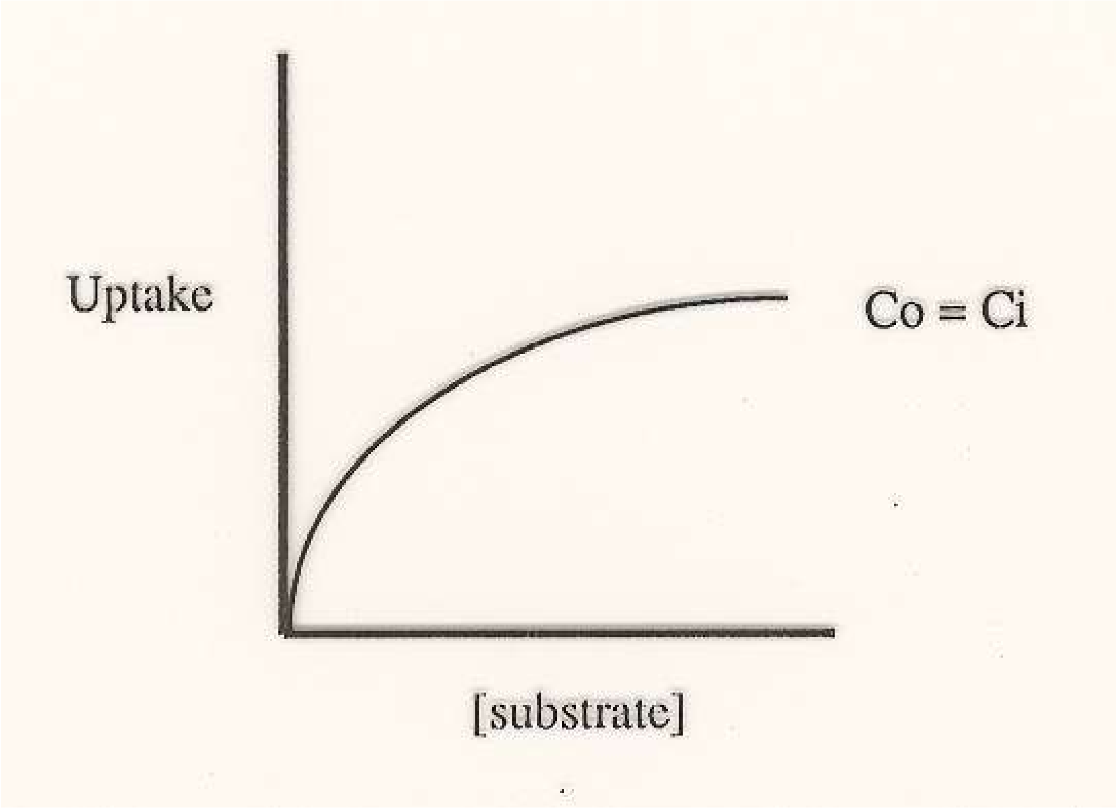


Concentration dependence of uptake by passive diffusion

- Net flux only until $C_{inside} = C_{outside}$ (no accumulation)
- No metabolic energy required
- No specific interaction with cell membrane component
- If environment, $C_{outside} << C_{inside}$, not useful for nutrient uptake
- Used for the uptake of O_2, CO_2 and H_2O
- Through the phospholipid bilayers, small and non-polar

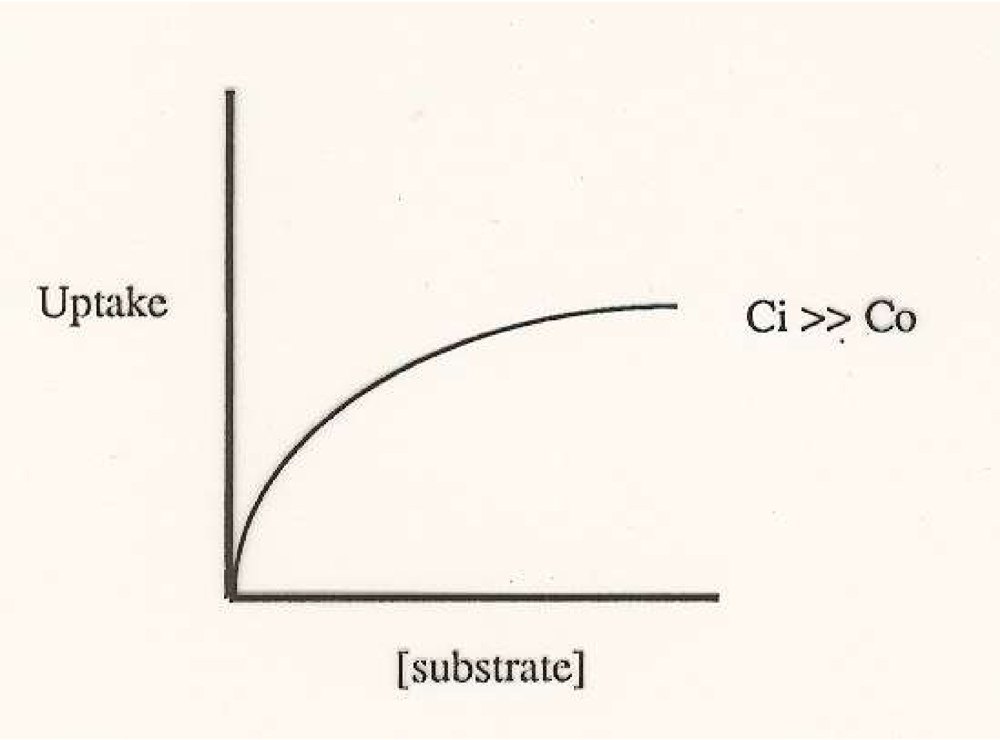
Facilitated Diffusion

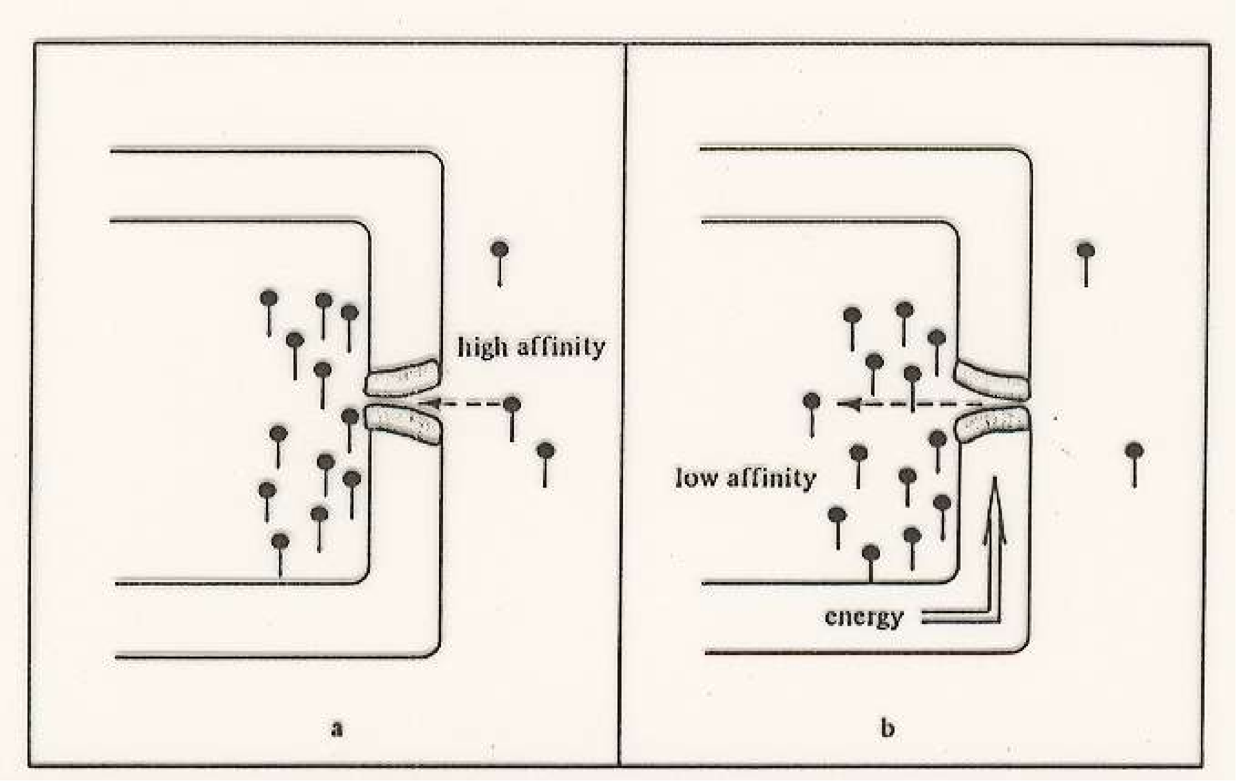
Neidhardt et al 1990



- **Large or polar molecules**
- No metabolic energy required
- Substrate specific interaction with a **membrane permease or carrier**
- **Stereospecific** (D and L amino acids completely discriminated)
- Substrate binds to carrier outside cell and is released inside the cell
- Not effective in dilute solutions unless C_i is kept low by utilization

Active Transport

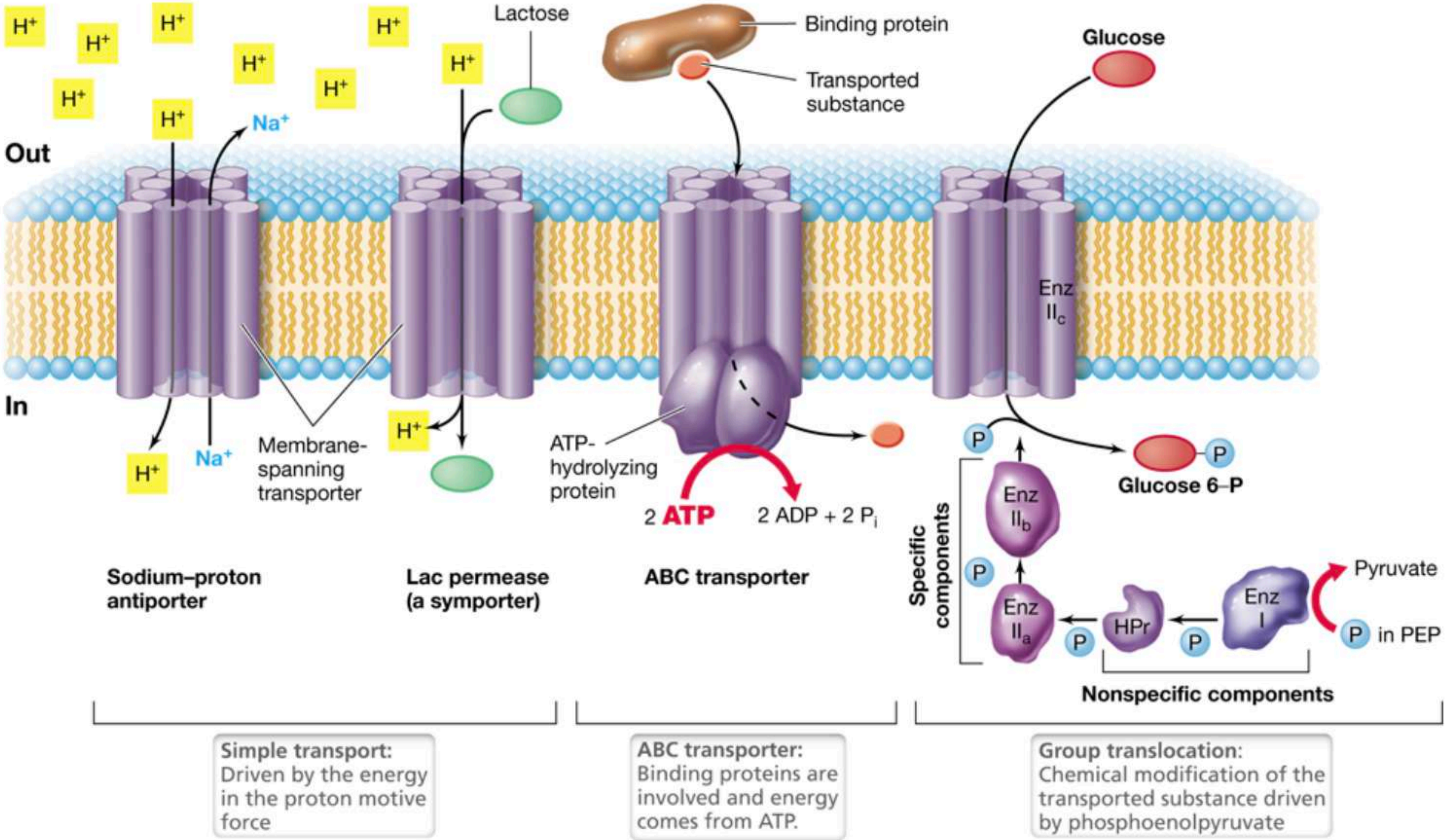




Neidhardt et al 1990

- **Accumulation against a concentration gradient**
- **Requires metabolic energy**
- Carrier-substrate complex formed outside of the membrane
- Structural specificity and stereospecificity
- Substrate is released into the cell unmodified (unlike PTS)

Figure 2.6 The three classes of transport systems.



Simple transport: Driven by the energy in the proton motive force

ABC transporter: Binding proteins are involved and energy comes from ATP.

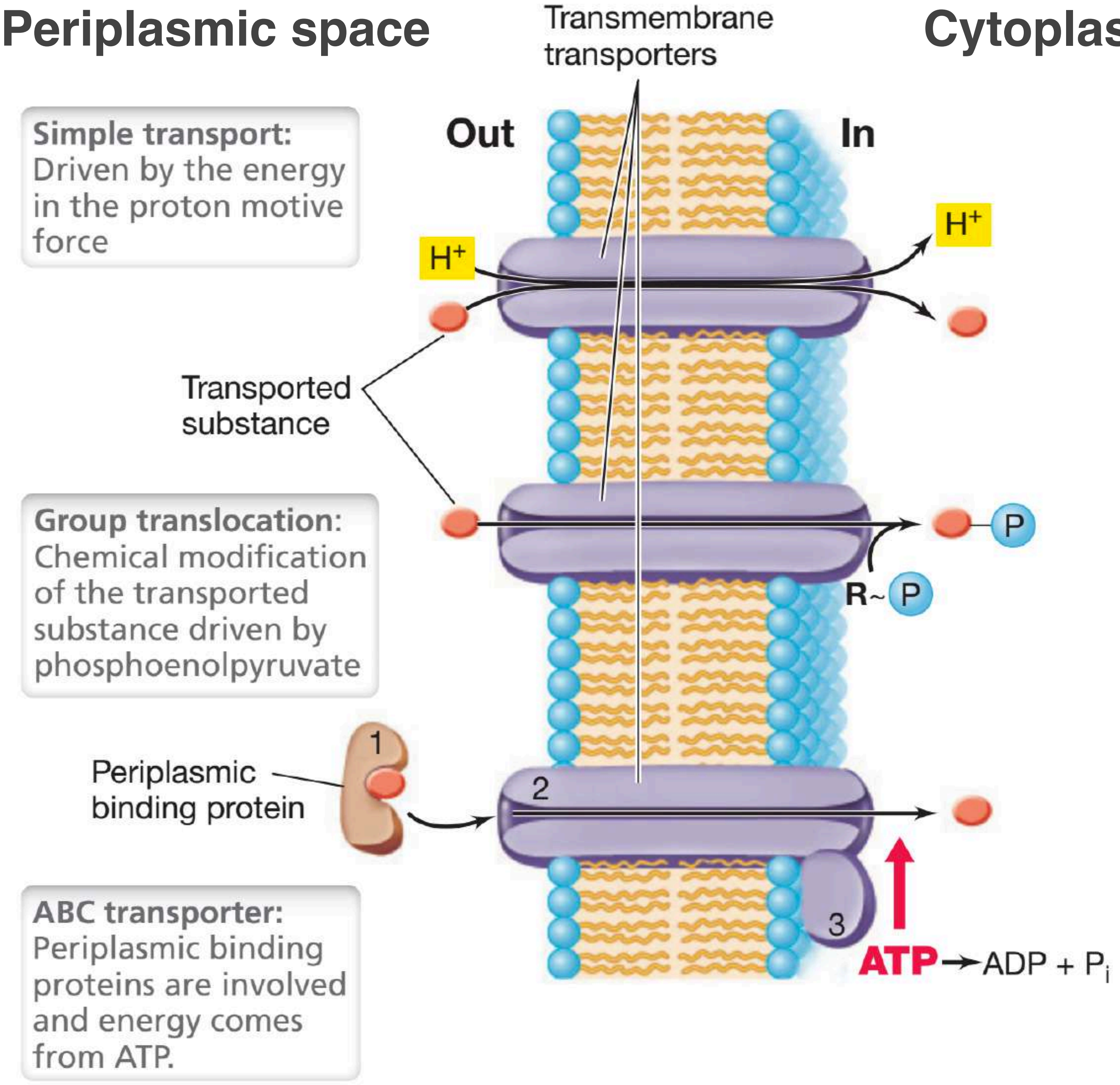
Group translocation: Chemical modification of the transported substance driven by phosphoenolpyruvate

Madigan et al. 2020

Uniporters, symporters and antiporters

Periplasmic space

Cytoplasm



Simple transport: Driven by the energy in the proton motive force

Group translocation: Chemical modification of the transported substance driven by phosphoenolpyruvate

ABC transporter: Periplasmic binding proteins are involved and energy comes from ATP.

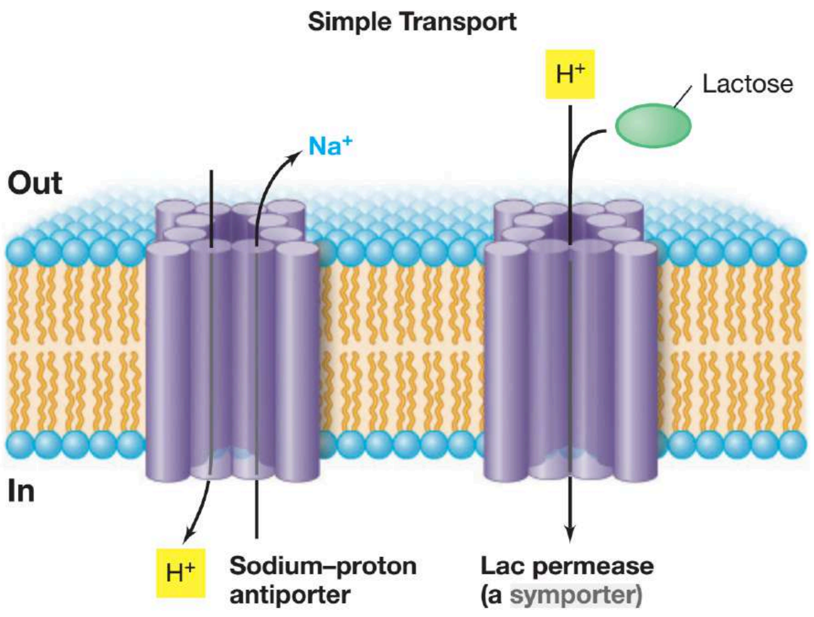
Madigan et al. 2018

Uniporter: Cause unidirectional transport (through membrane spanning protein)

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

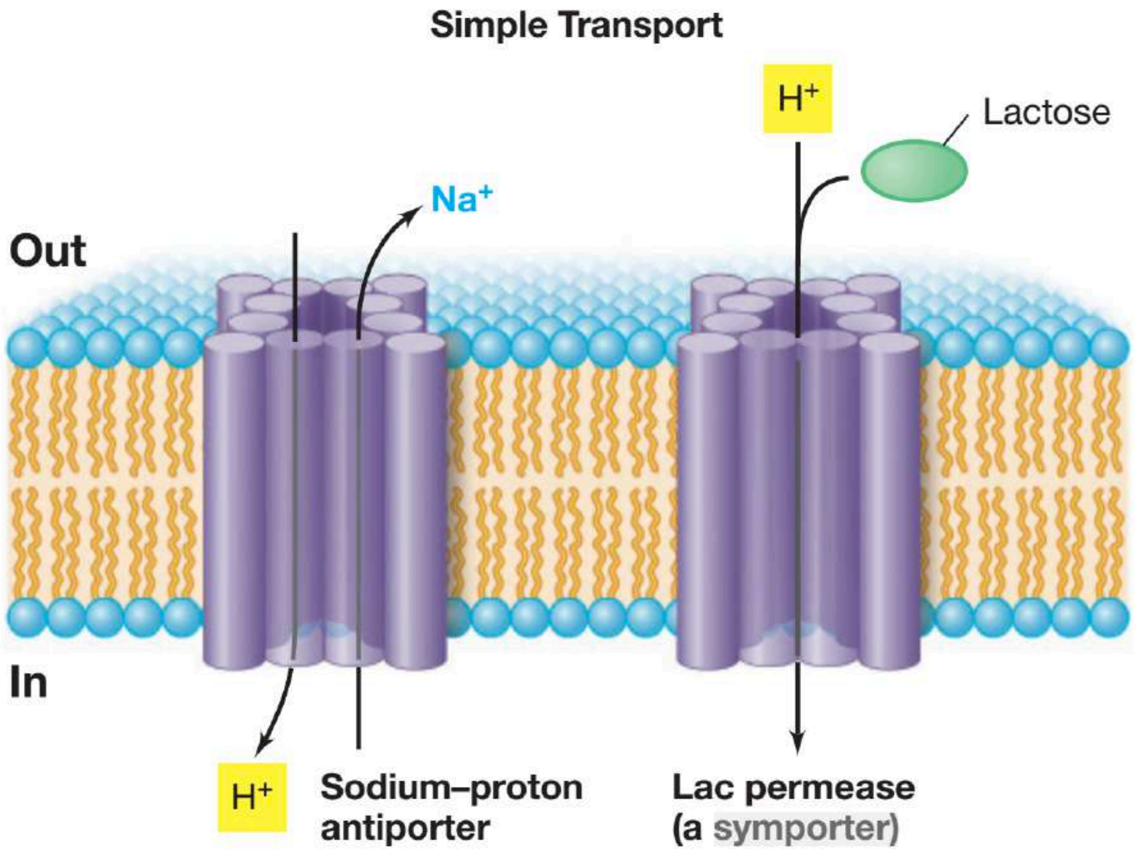
Antiporter: Substrate and H^+ (or Na^+) transported in opposite directions

[Require PMF, Proton Motive Force]



Simple Transport

Periplasmic space



Cytoplasm

Madigan et al. 2018

Transport is linked to dissipation of the proton motive force (PMF)

H^+ goes into cell

Same or opposite direction

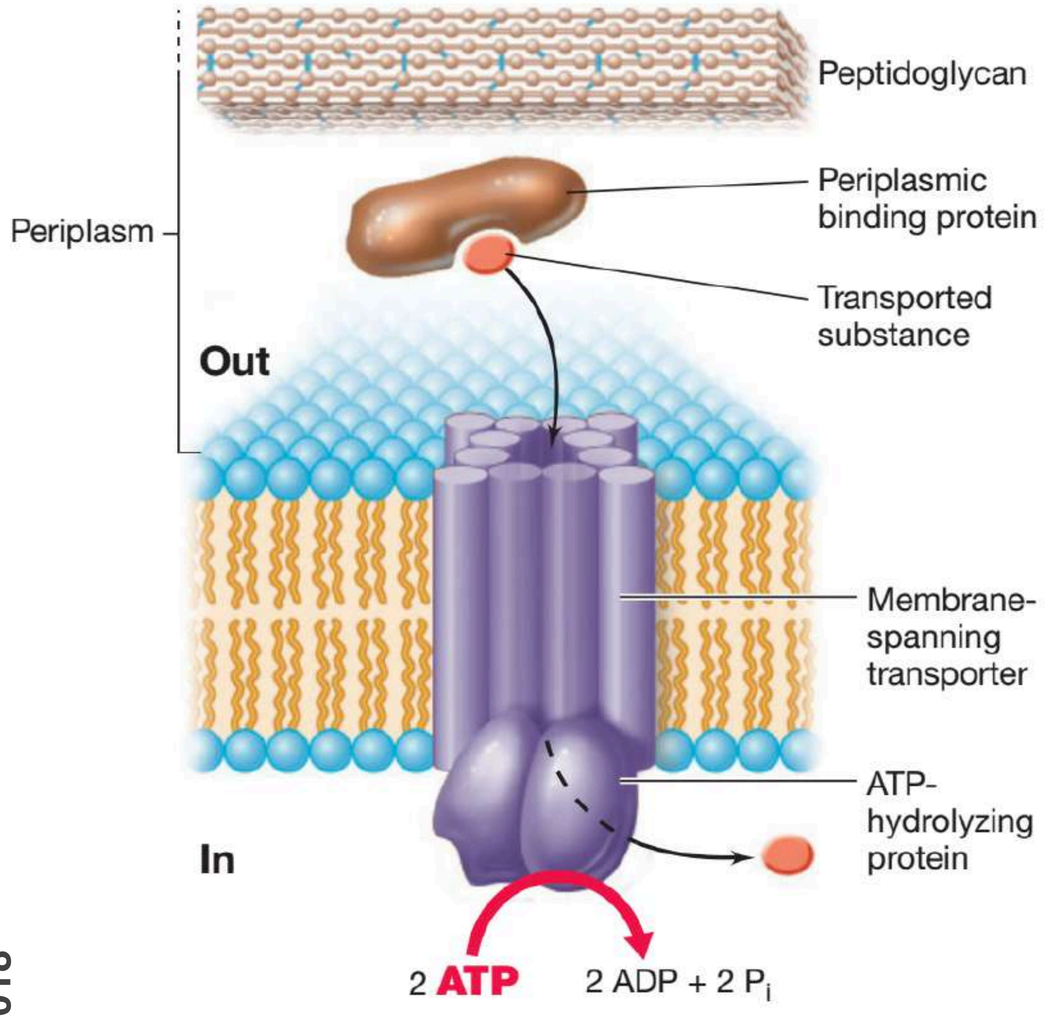
Transmembrane transporters are composed of a polypeptide that forms 12 a-helices —> a channel

PMF is generated by e^- transport, H^+ ions are extruded to the outer surface of the membrane

Inside of cell has net - charge and outside net +

ABC Transporters

Periplasmic space



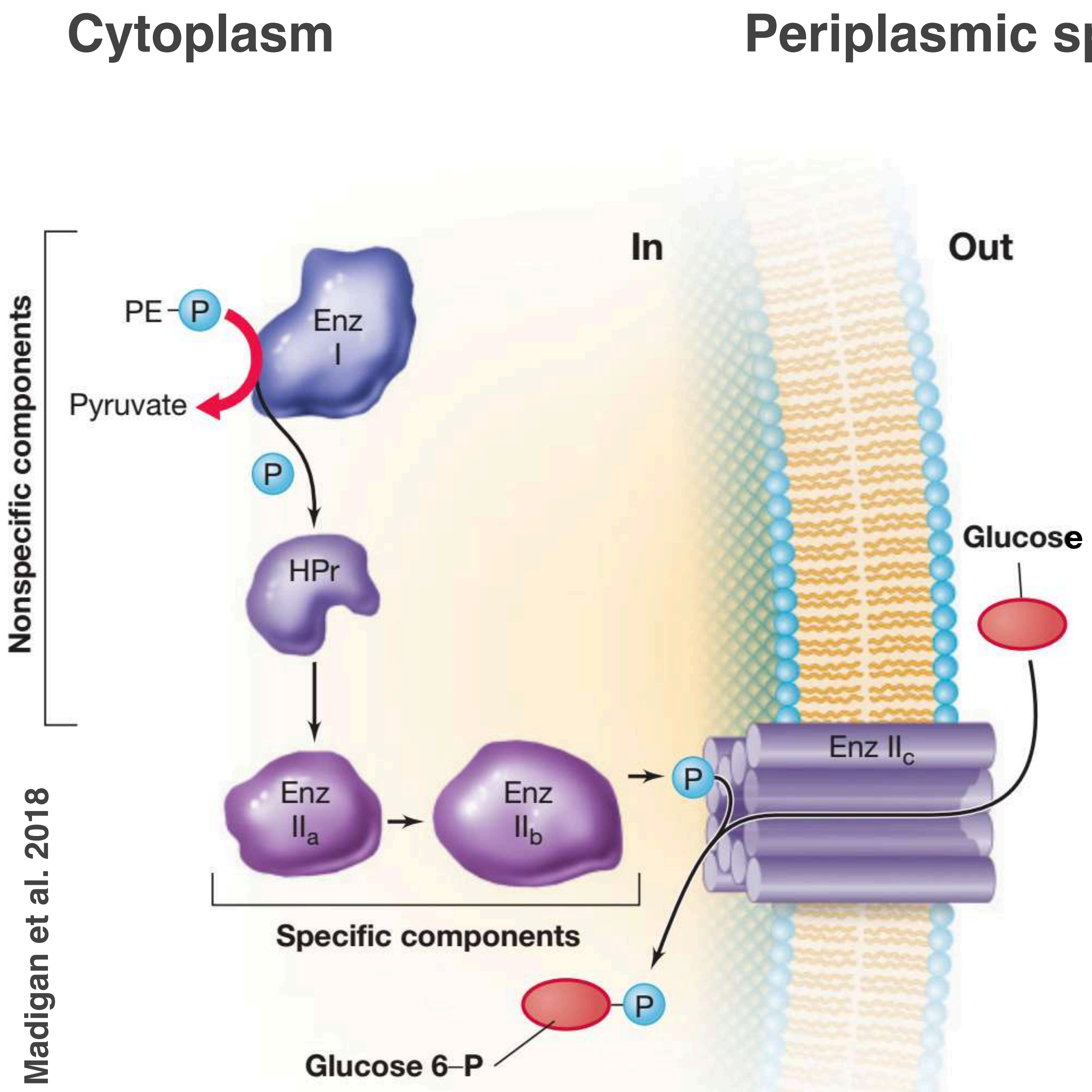
Cytoplasm

Madigan et al. 2018

- ABC (ATP Binding Cassette) systems: Gram- (periplasm); G+ & Archaea BP membrane-anchored
- Used for some amino acids, peptides, sugars, organic acids, sulfate, other ions
- Requires metabolic energy as **ATP** *(not PMF)*
- Mechanism:
  - Substrate binds to a high-affinity binding protein in periplasm
  - Complex interacts with a membrane-embedded multimeric carrier
  - ATP hydrolysis changes subunit interactions; creates a transport channel
- Multiple systems w/different K_m, V_{max} (3 for glucose in *E. coli*; scavenging system)

Group Translocation

(e.g. Phosphoenolpyruvate-PEP- phosphotransferase system)



Madigan et al. 2018

- **Substrate modified** during transport —> phosphorylated
- **Energy derived from metabolic compound PEP (glycolysis)**
- PEP donates ~P for phosphorylation (PEP PTS system)
- **Sugar-phosphate is 'trapped**' (membrane is impermeable to it)
- Conserves energy. Transport and phosphorylation with a single ~P
- EII are sugar specific; EIIb lies @inner membrane face; EIIc: integral
- Examples: sugars (glucose, fructose, mannose), NAG
- **Generally found in facultative anaerobes and anaerobes**