LO2a

Recap L01

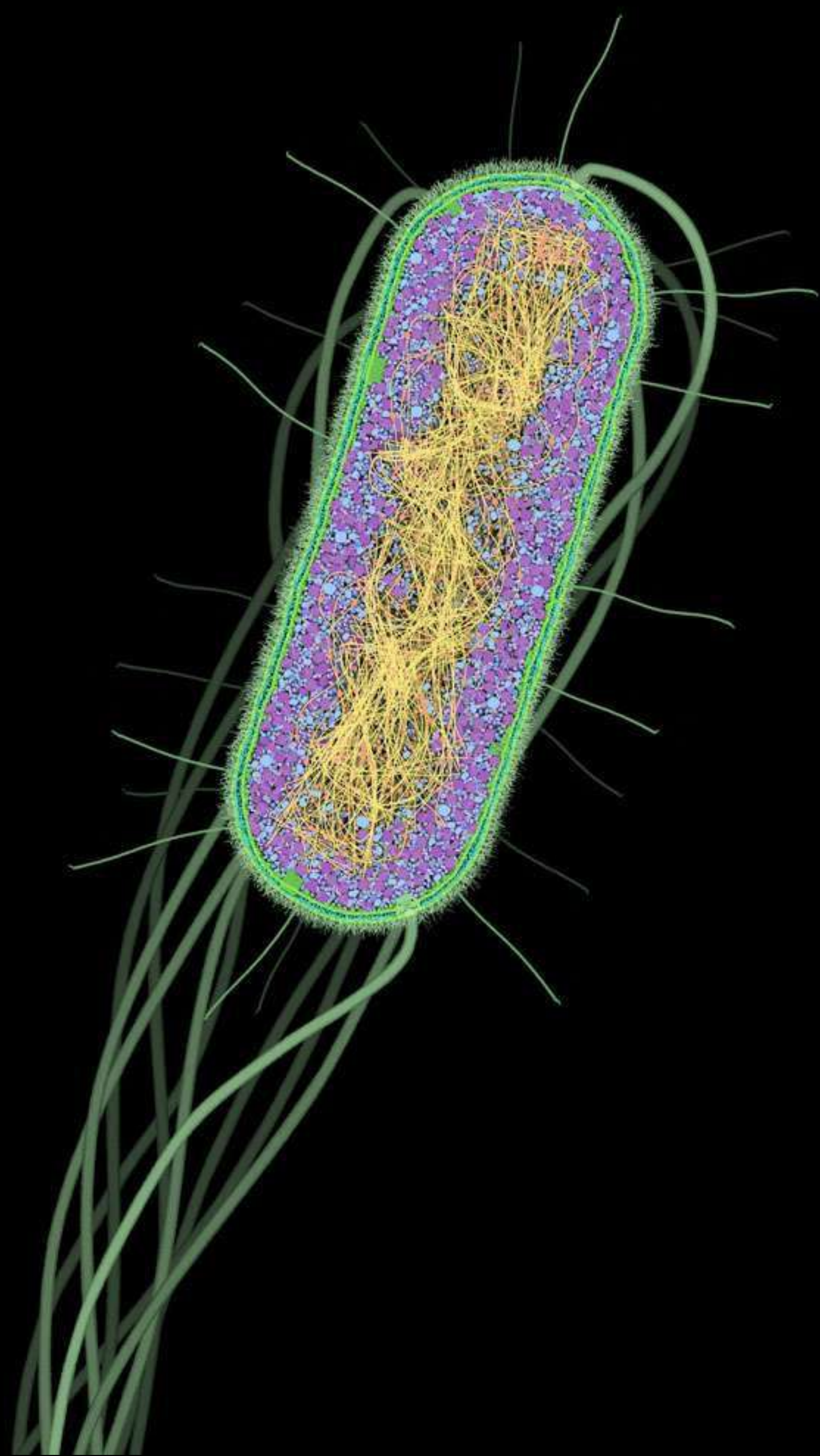
Volume of planet Earth
1.08321×10^{12} km^3

The Biosphere occupies about 0.00008 % of the mass of the Earth, $M_{\oplus} = 5.9722 \times 10^{24}$ kg (Knight and Schlager 2002) and or 0.0007 % of Earth volume

Prokaryote volume 1μm^3

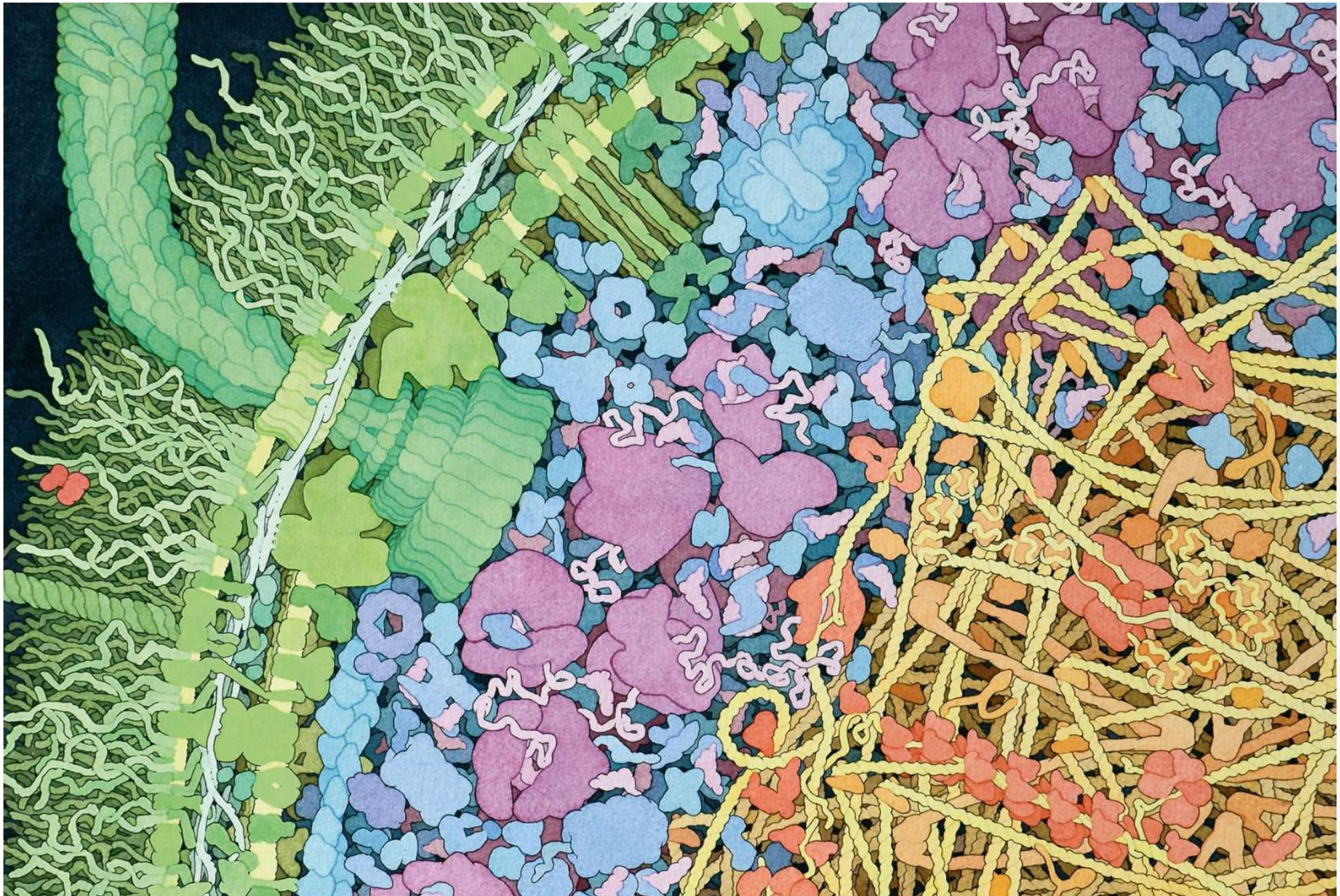
In a microbial cell the structure determine the function

The structures have evolved since the beginning to adapt



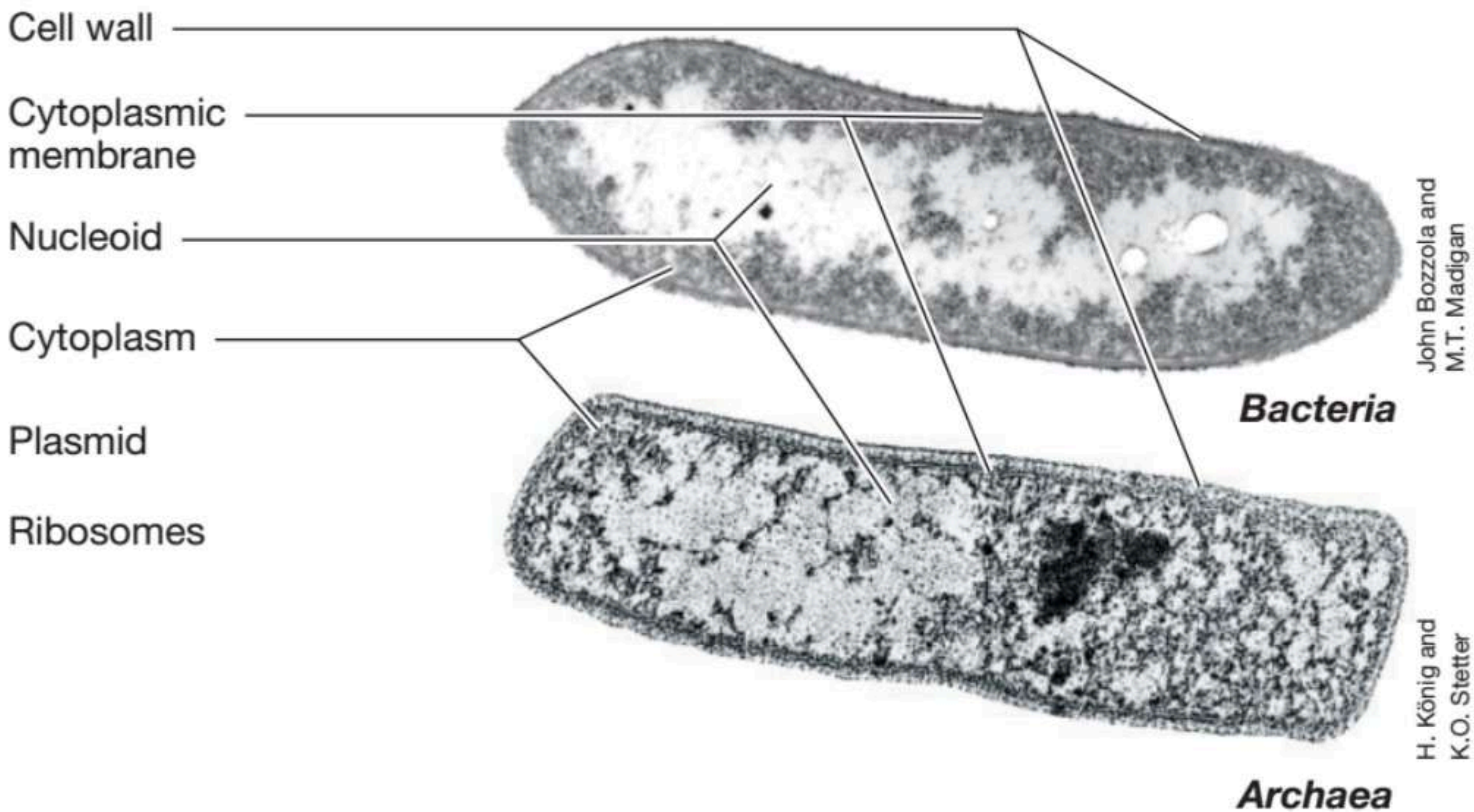
David S. Goodsell

Understanding structure and function, I



David S. Goodsell

Cell structure and function

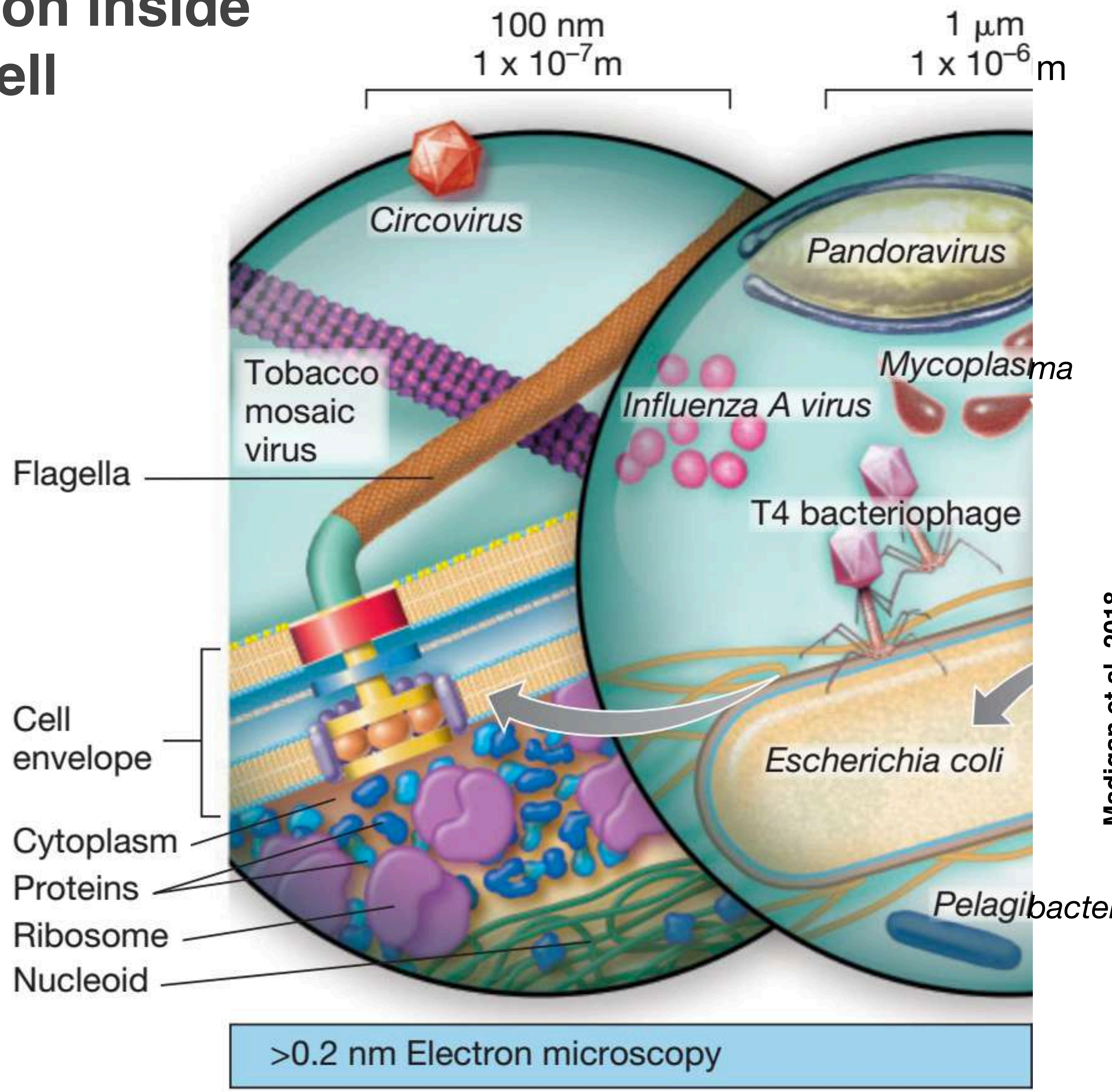


Madigan et al. 2018

As result of thermodynamic constrains, evolution and genomic information

Microscale exploration inside a microbial cell

- Cell wall - Peptidoglycan
- Gram positive- Gram negative membranes
- Flagella & Pili
- Capsule & slime layer & Inclusions & Vesicles
- Cytoplasm & components
- Diffusion (active/passive)

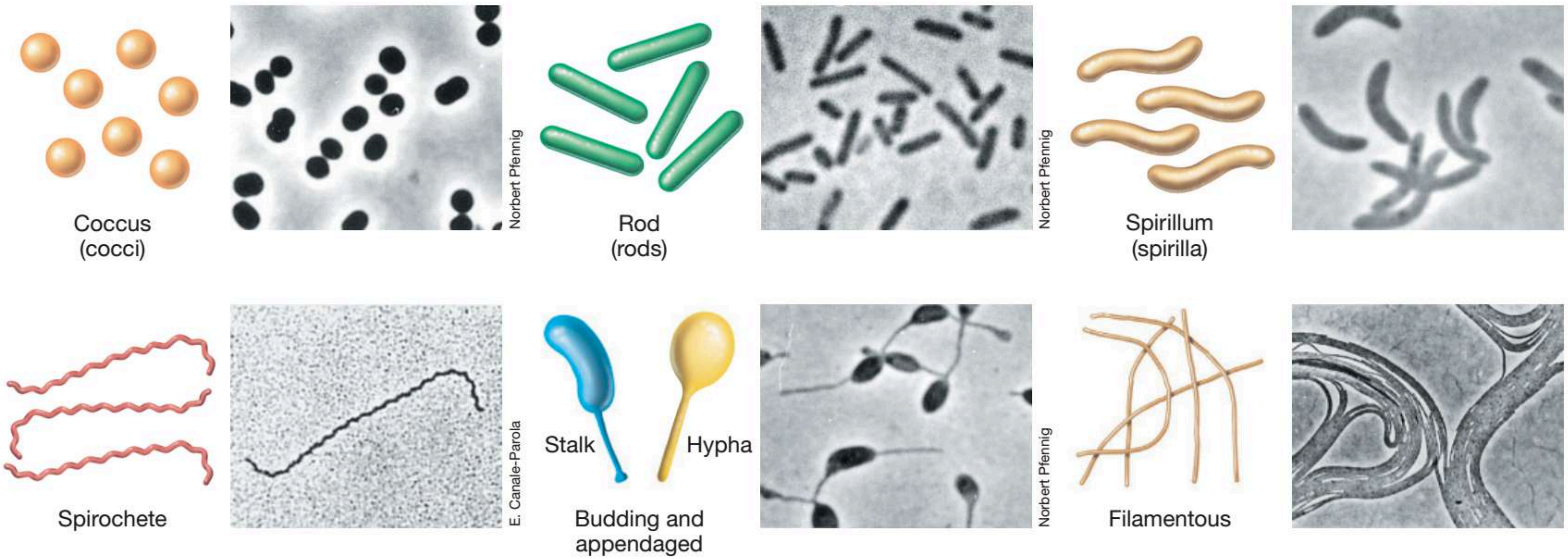


Madigan et al. 2018

Cell Shape and Size

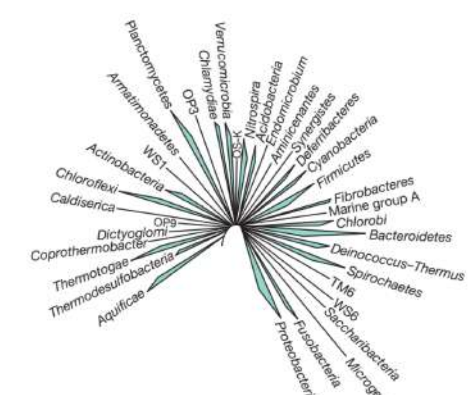
Madigan et al. 2018

cell diameter =1.5 μm **cell diameter =1 μm** **cell diameter =1 μm**



cell diameter =0.25 μm **cell diameter =1.2 μm** **cell diameter =0.8 μm**

- **6 broad categories for cell morphologies, not very informative for identify**
- **Highly diverse microbes share same shape (convergence as adaptive strategy)**



16S rRNA gene phylogenetic analysis

Cell Shape

Madigan et al. 2018

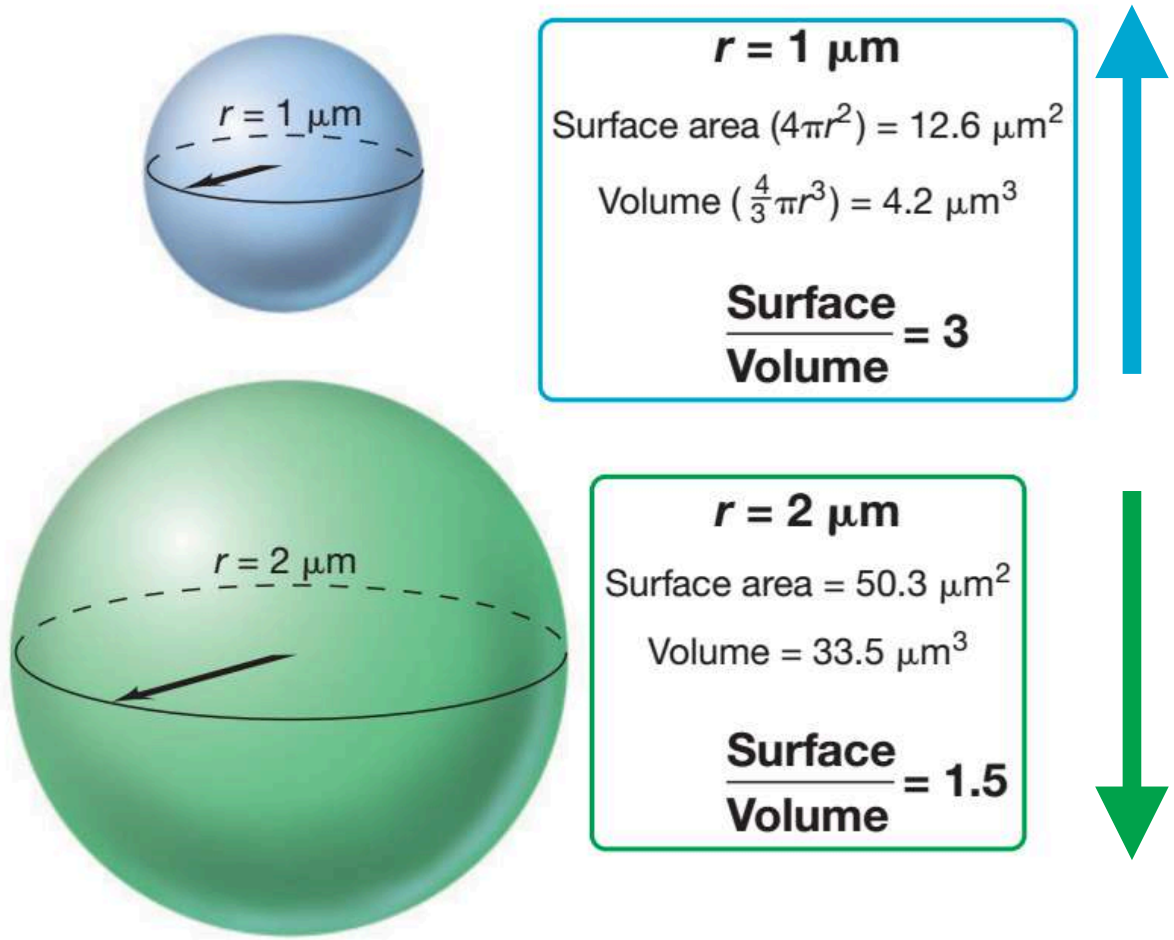


Figure 2.3 Surface area and volume relationships in cells. As a cell increases in size, its *S/V* ratio decreases.

Sphere
Ellipsoid $V = {}^{4}/_{3} \cdot \pi \cdot a \cdot b \cdot c$

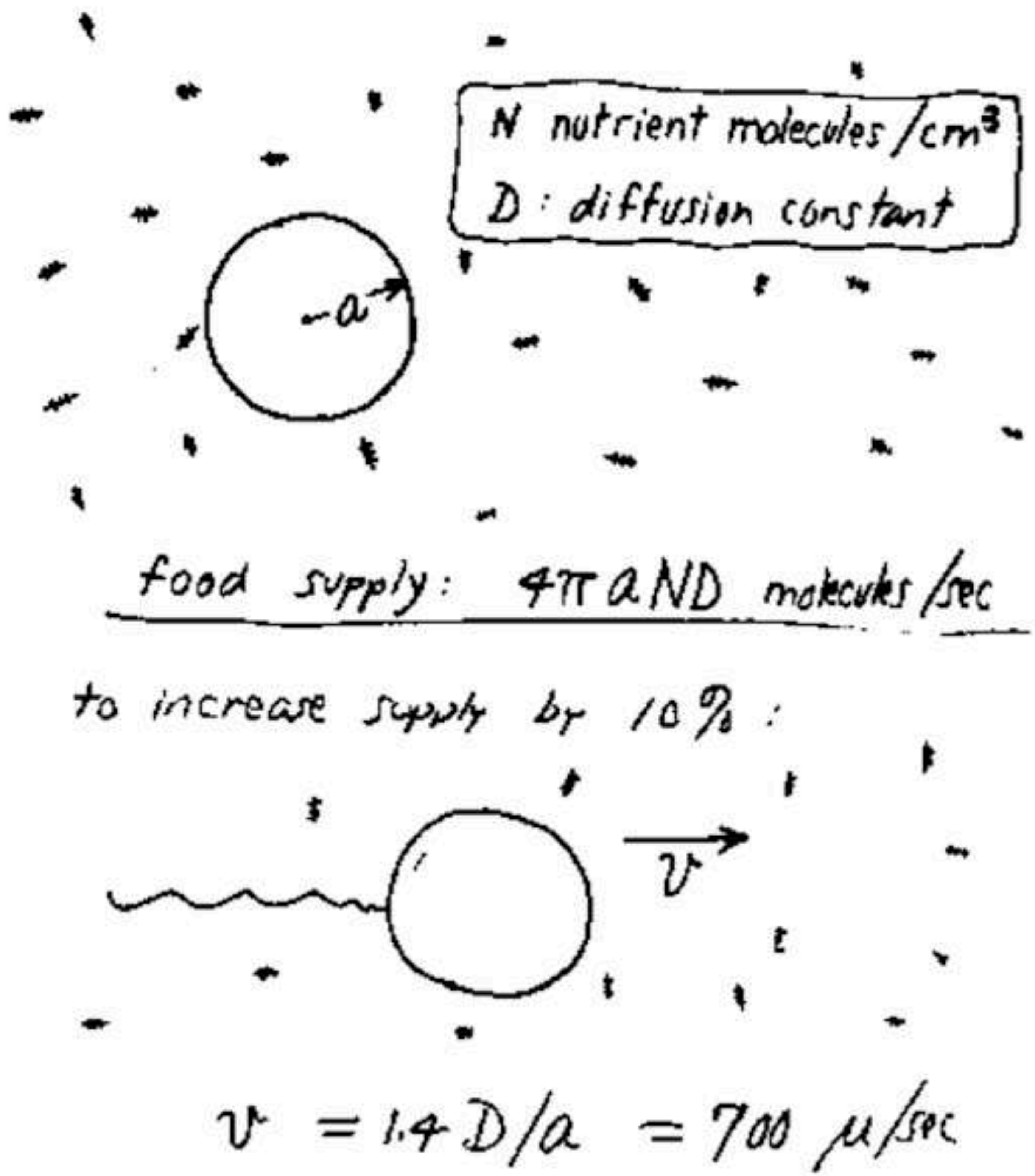
2 μm³ Escherichia coli

- **Microbes maximize surface to volume ratio thus more efficient nutrient uptake and waste expulsion (Swimming speed >> Diffusion)**
- **Motility increase nutrient uptake and maximize waste expulsion, but not motile cells how do they do it?**

Life at Low Reynolds Number, EM Purcell 1973.

Life at Low Reynolds Number

E.M. Purcell
Lyman Laboratory, Harvard University, Cambridge, Mass 02138 June 1976



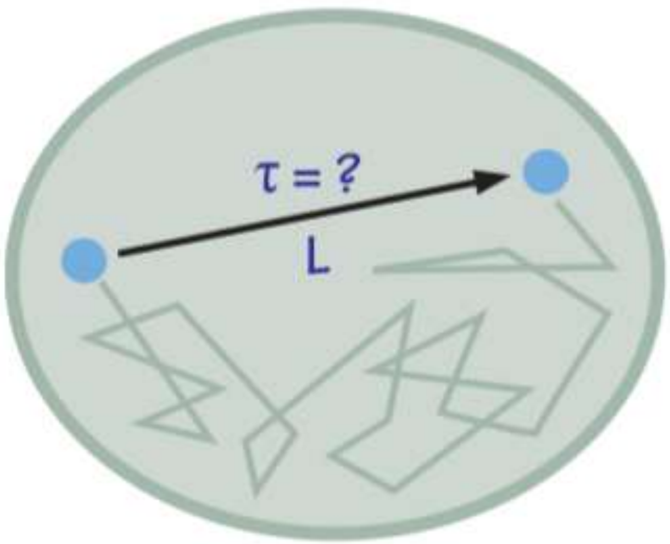
Molecular diffusion is the random motion of fluid molecules, so diffusion of solutes is a function of the fluid (solvent and solute)

Low Reynolds Number where viscous forces are more important than inertial forces

Humans live in high Reynolds Number

Temporal Dimension of Diffusion

time for protein diffusion across cell



time scale (τ) to traverse distance (L)
given diffusion coefficient (D)

$\tau = L^2/6D$

protein in cytoplasm $D \approx 10 \frac{\mu m^2}{s}$

E. coli, $L \approx 1\ \mu m \Longrightarrow \tau \approx 10\ ms$

HeLa cell, $L \approx 20\ \mu m \Longrightarrow \tau \approx 10\ s$

neuronal cell axon, $L \approx 1\ cm \Longrightarrow \tau \approx 10^6\ s \approx 10$ days!

Cell Biology by the Numbers, 2015

Busy busy at the molecular scale

Cell Biology by the Numbers, 2015

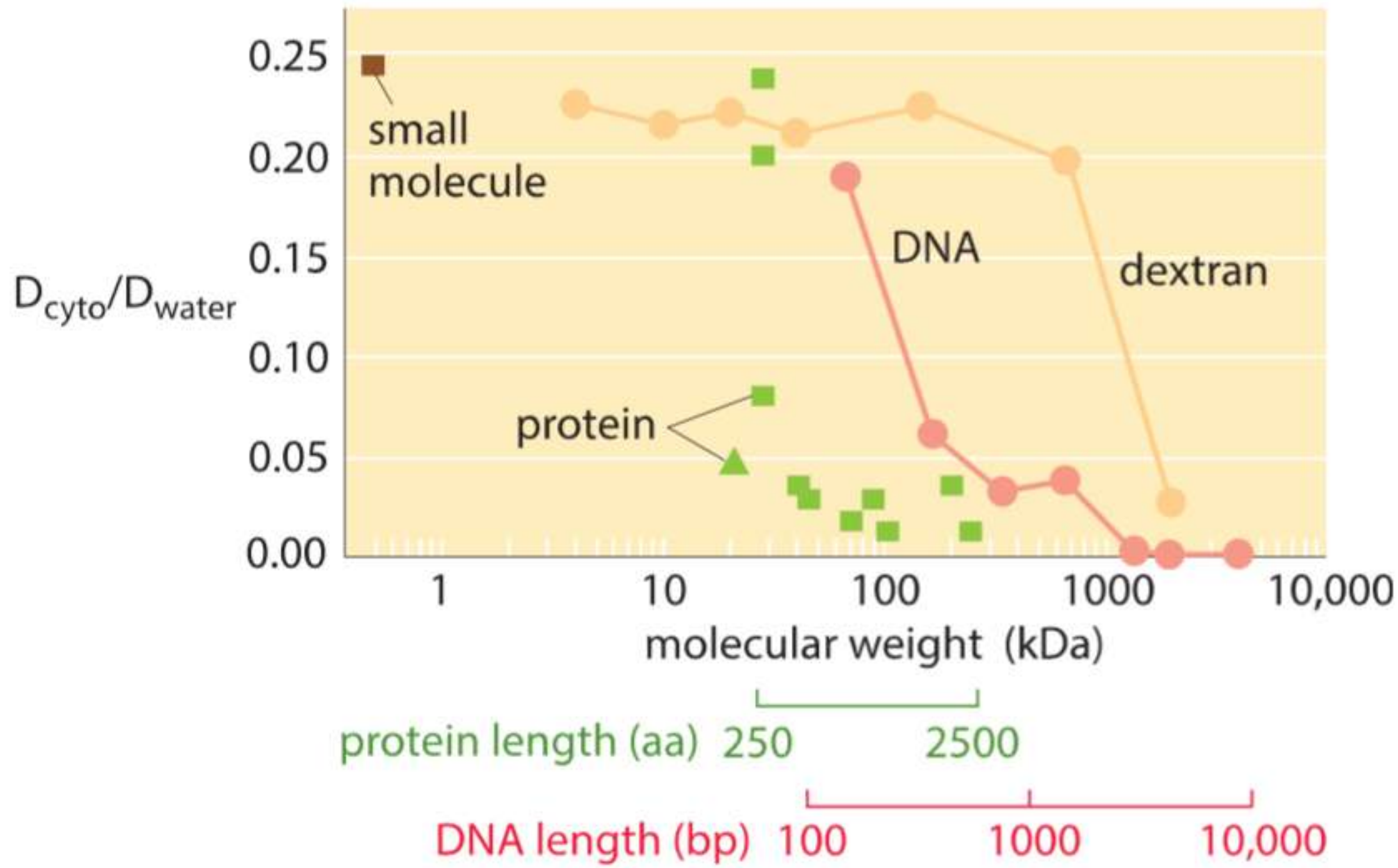


Figure 3: The decrease in the diffusion constant in the cytoplasm with respect to water as molecular weight increases. For the different proteins marked in green see Kumar et al 2010 and entries in the compilation table below. (Adapted from A. S. Verkman, Trends Biochem., 27:27, 2002; M. Kumar et al., Biophysical Journal, 98:552, 2010).

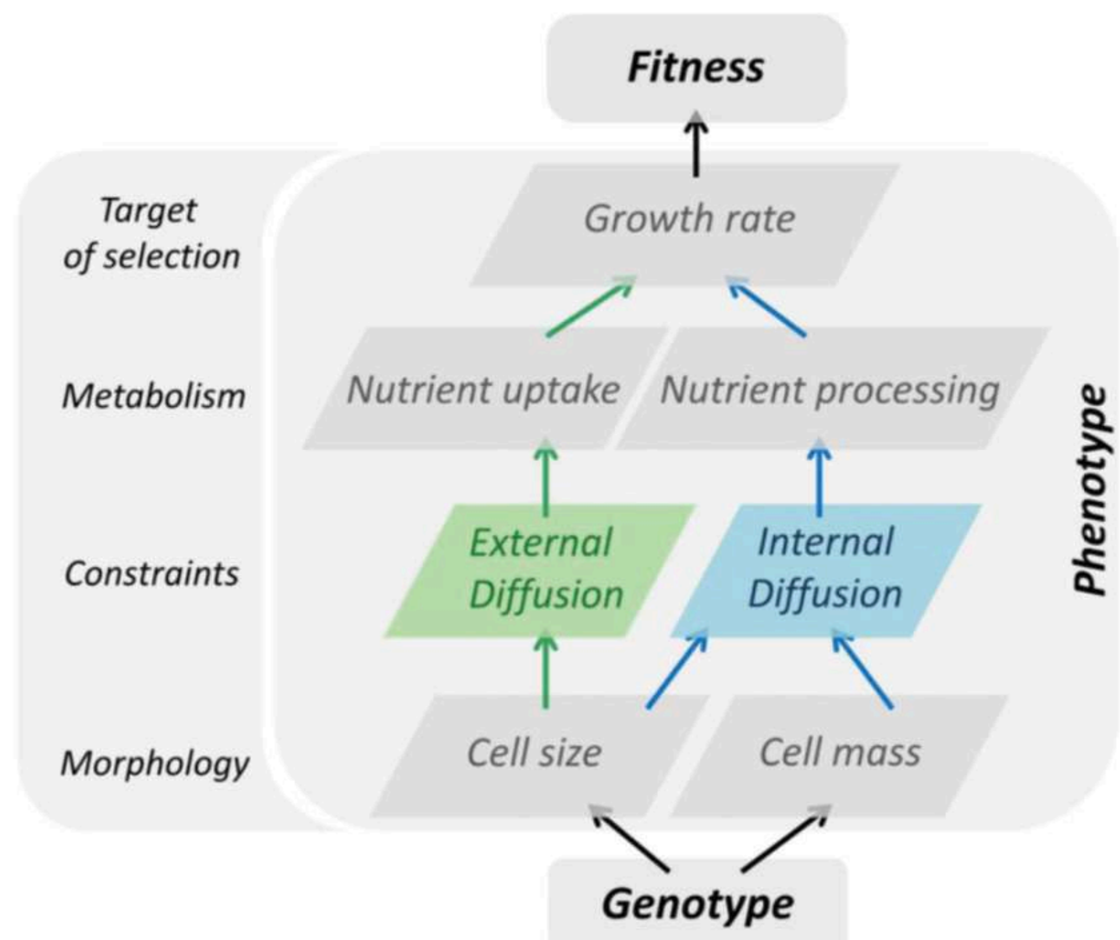
Cell Size (small or big)

External diffusion' theory (EDC) predicting that cell size should have evolved toward smaller cell

Internal diffusion-constraint' (IDC) but Lenski's LTEE (long-term evolution experiment, started 1988) *E. coli* adapts to a simple glucose medium increase over time growth rate, fitness (reproductive success) and its cell size

A change in cell volume affects metabolite concentrations in the cytoplasm

Higher metabolism can be achieved by a reduction in the molecular traffic time inside of the cell, by increasing its volume (lower mass-to-volume ratio)

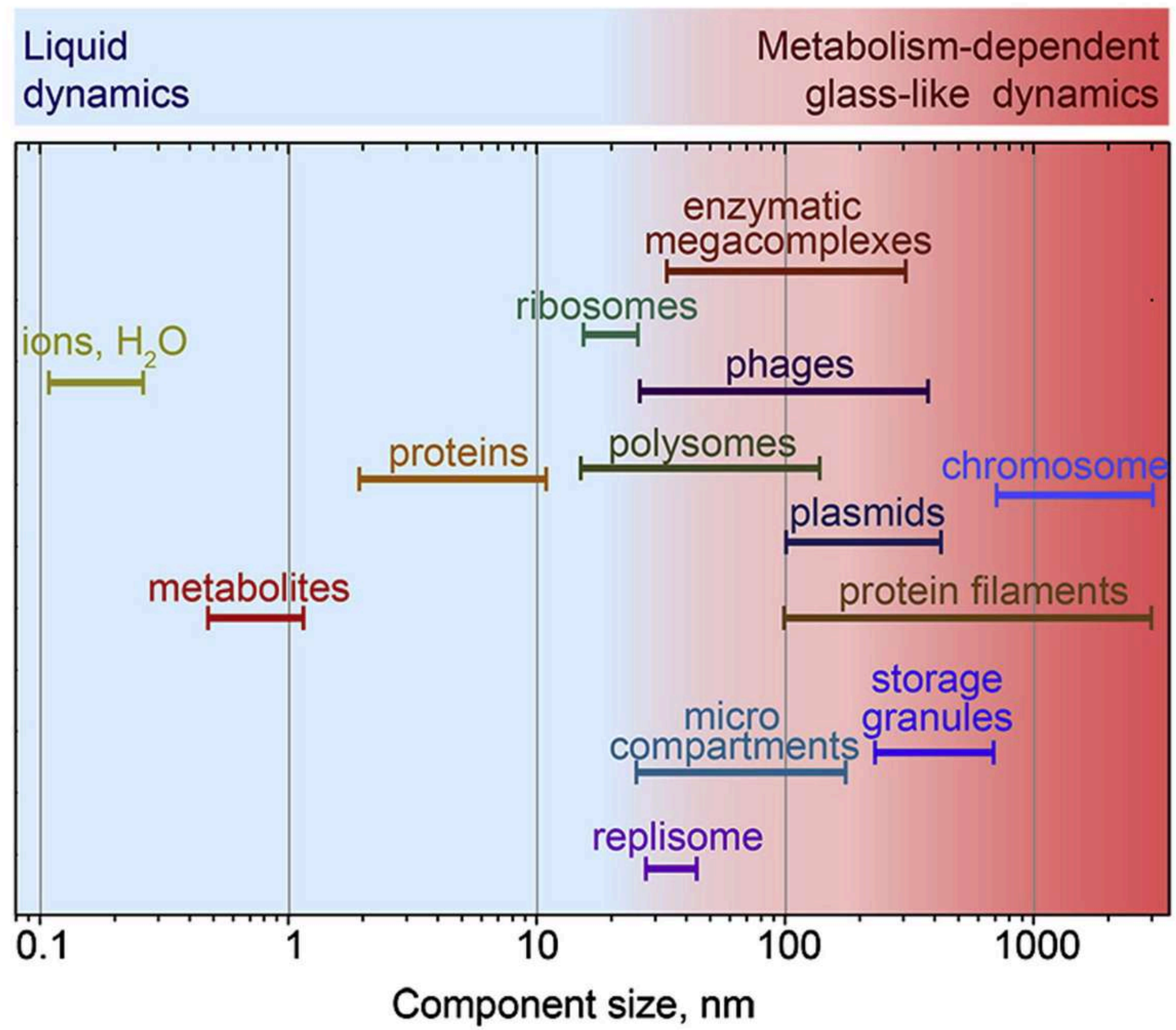


http://myxo.css.msu.edu/ecoli/index.html

Gallet et al. 2017

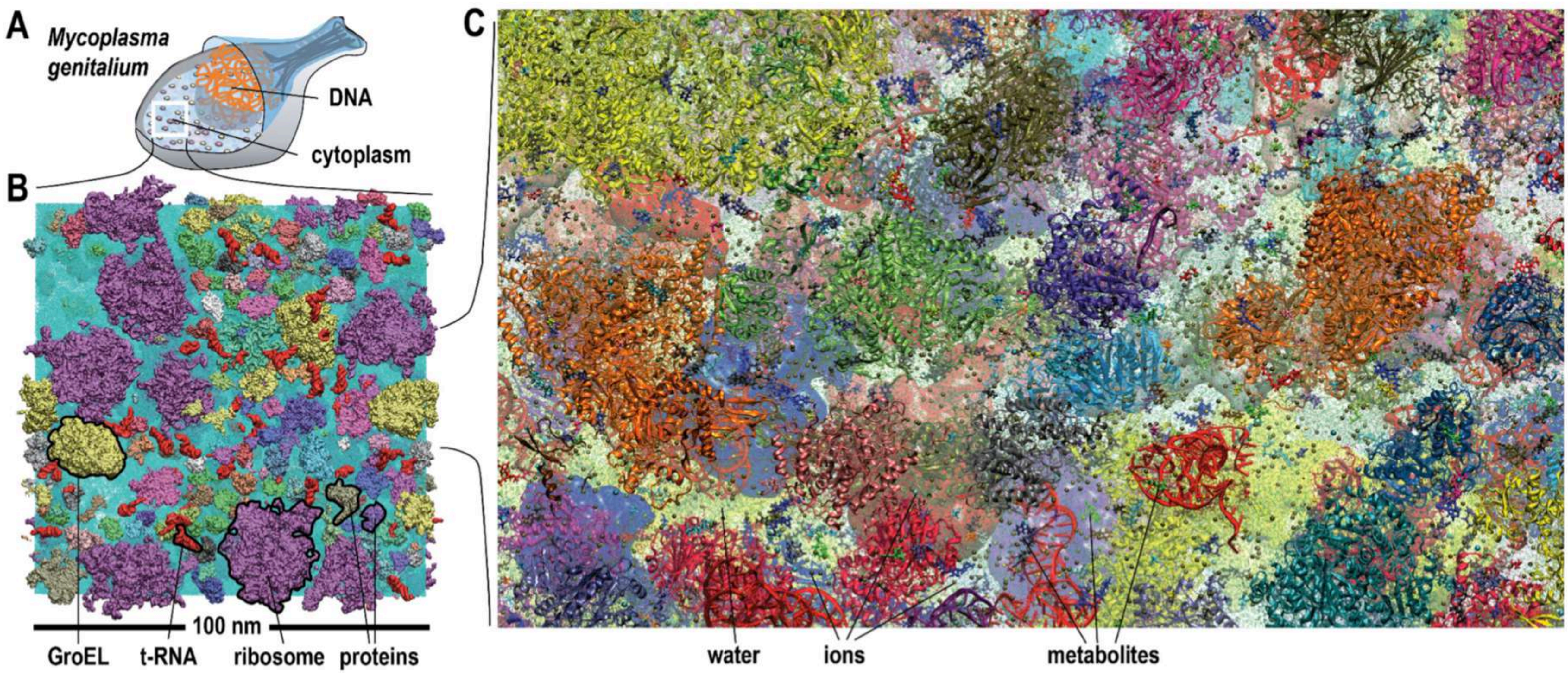
Cytoplasm

- Properties of glass-forming liquids and changes from liquid-like to solid-like in a component size- dependent fashion
- **Motion** of cytoplasmic components becomes **disproportionally constrained with increasing size**
- **Cellular metabolism fluidizes the cytoplasm,** allowing larger components to escape their local environment and explore larger regions of the cytoplasm
- Cytoplasmic fluidity and dynamics **change** as cells shift between metabolically active and dormant states in **response to fluctuating environments**



Parry et al. 2014

Cytoplasm, molecular modelling



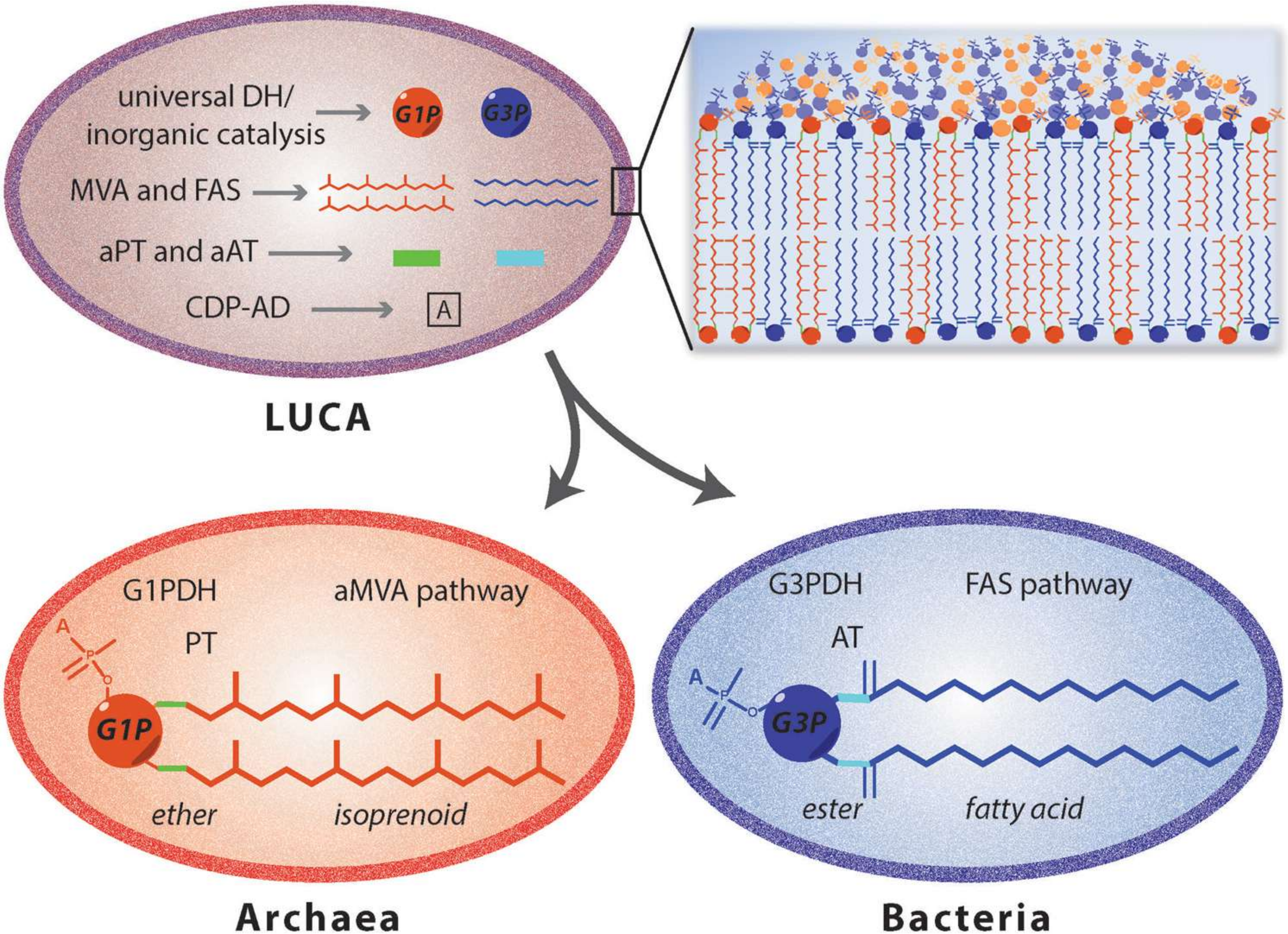
Biological macromolecules function in highly crowded cellular environments

Molecules are competing to diffuse away according to the metabolic state

GroEL, chaperon protein for correct folding of other proteins

Yu et al. 2016

LUCA, structural diversity in cellular membrane

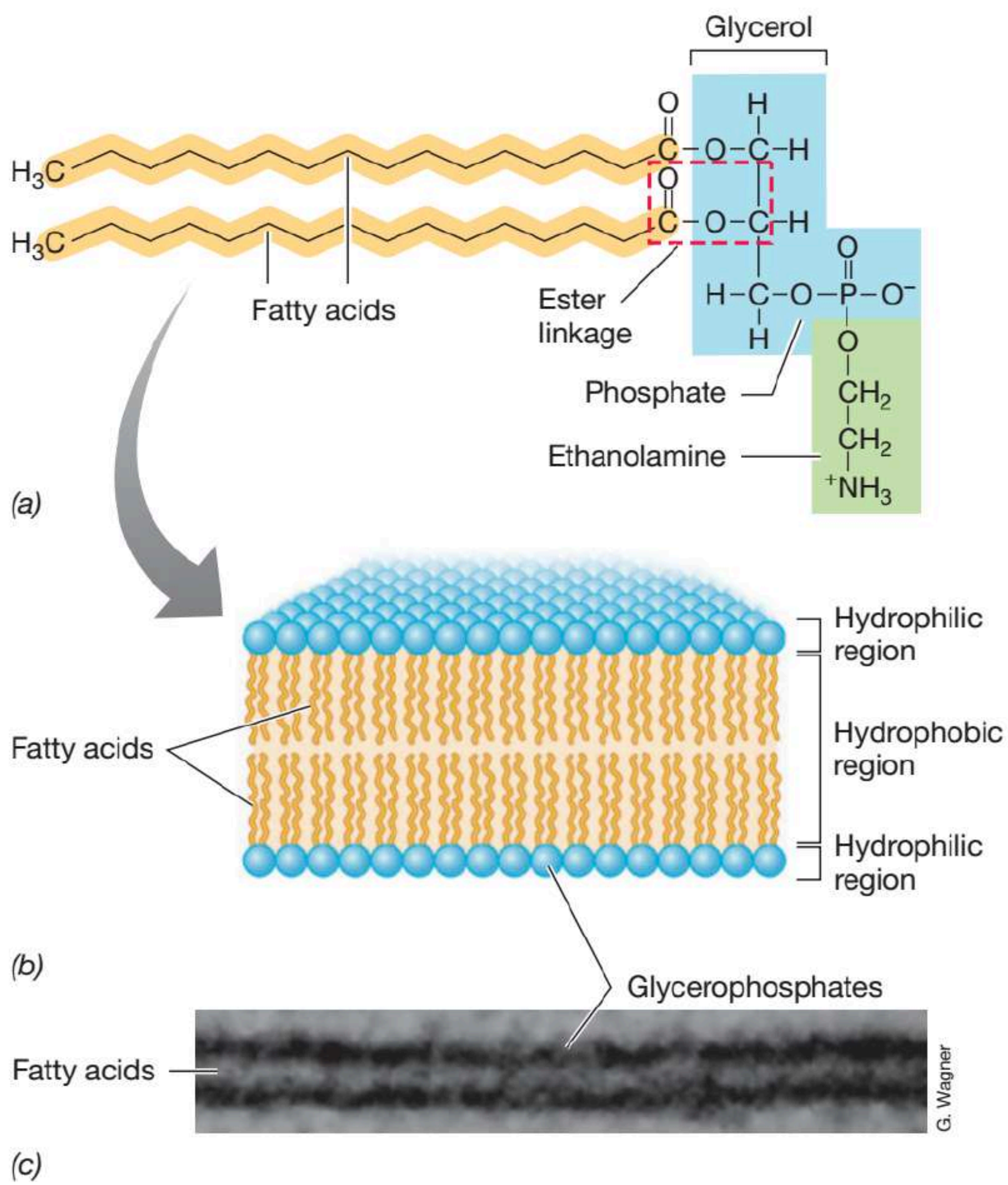


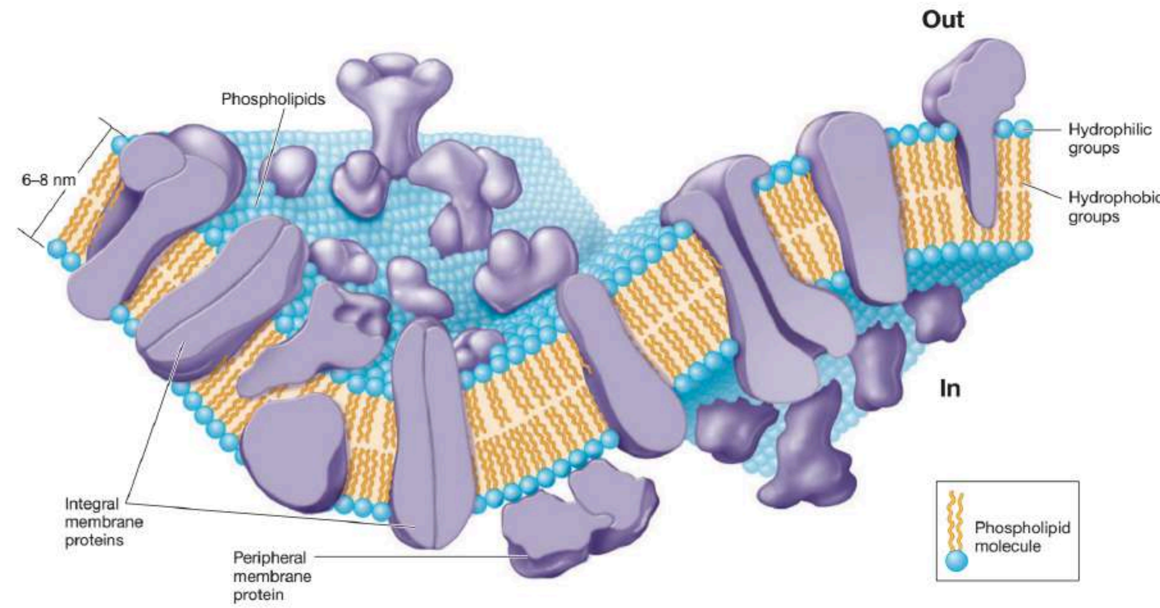
Jain et al. 2014

Structure of cytoplasmic membrane

Bacteria

Madigan et al. 2018





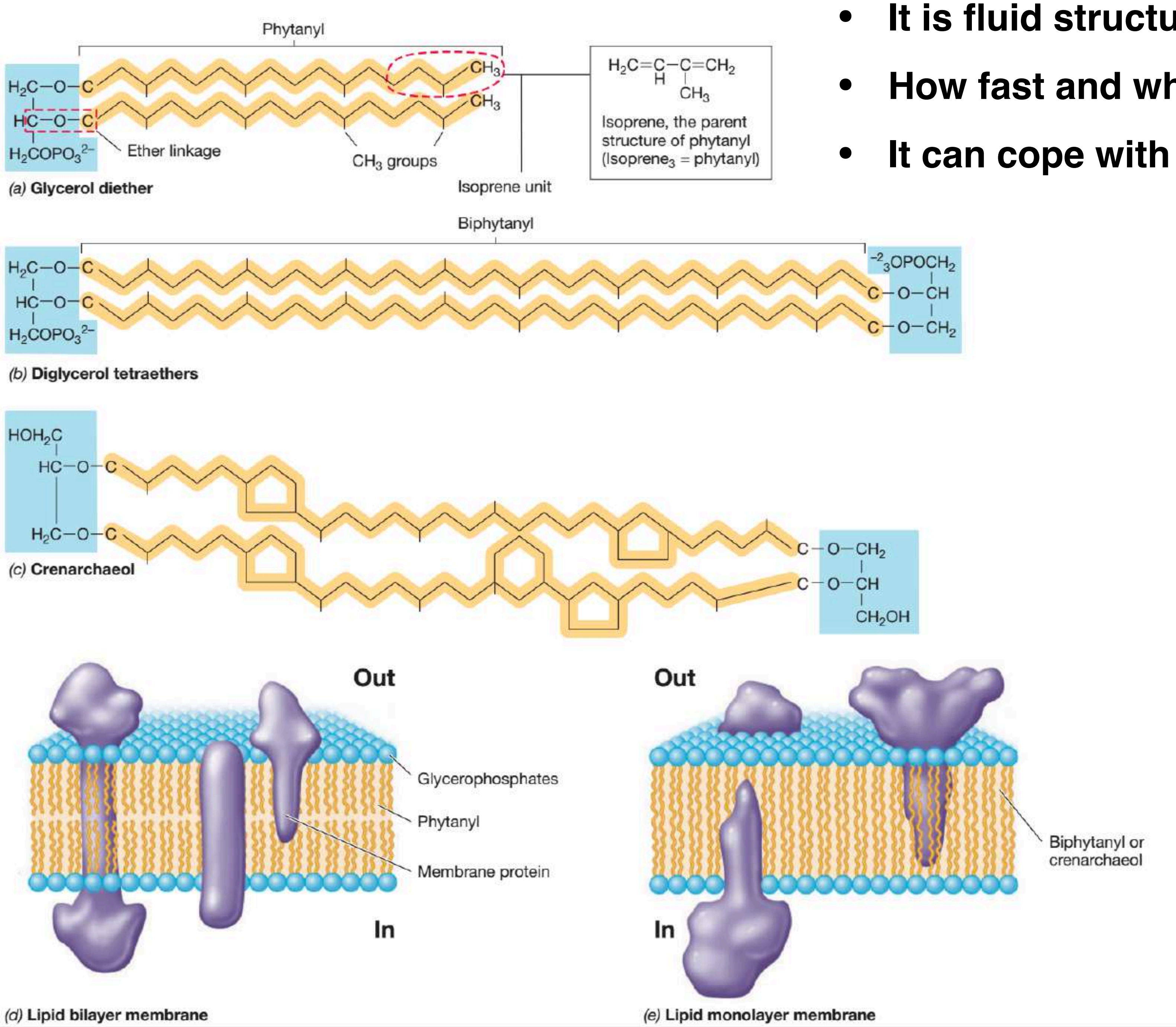
- **It is fluid structure that changes,**
- **How fast and what %?**
- **It can cope with temperature, pH and pressure**

8–10 nanometers wide, bilayer

Structure of cytoplasmic membrane

Archaea

Madigan et al. 2018



- **It is fluid structure that changes,**
- **How fast and what %?**
- **It can cope with temperature, pH and pressure**

Bacteria	Temperature				pH				Pressure	
	T_{min} <15 °C		T_{max} >75 °C		pH_{min} <3		pH_{max} >10		>70 MPa	
Level of chain length		Ref		Ref		Ref		Ref		Ref
shorter chain ≤C14	+	(7,8)			+	(31, 33)	+	(42, 43)		
longer chain ≥C18										
Level of unsaturation										
PUFA	+	(1–3)							+	(39)
MUFA-cis	+	(7,8, 40)	+	(21)	+	(33)	+	(44)		
MUFA-trans	+	(8)								
Level of branching										
BCFA-iso			+	(4,15,41)	+	(29)	+	(38, 42–44)		
BCFA-*anteiso*			+	(4)	+	(29, 32)	+	(44)		
Diabolic acid			+	(18, 45)	+	(35)				
(β)-hydroxy FA	+	(8)			+	(30, 33)				
Level of cyclization										
Ω-Cyclohexyl					+	(29, 32)				
Cyclopropyl	+	(7)	+	(21)	+	(30, 33)				
Level of tetraester and etherlipids										
Tetraesters			+	(22, 46)						
Mono- di- tetraethers			+	(18–21)	+	(34, 35)				
Level of terpenes										
Polar carotenoid	+	(5,6)	+	(16, 17)						
Non-polar terpenes							+	(44)		
Other modifications										
Cardiolipins	+	(7)					+	(44)		
Glycolipids	+	(7)	+	(16)						
BMP							+	(44)		

PUFA polyunsaturated fatty acids, *MUFA-cis* cis-monounsaturated fatty acids, *MUFA-trans* trans-monounsaturated fatty acids, *BCFA-iso* iso-branched chain fatty acids, *BCFA-anteiso* anteiso-branched chain fatty acids, *BMP* bis-mono-acylglycero-phosphate, *TE* tetraethers, + increased production, − decreased production

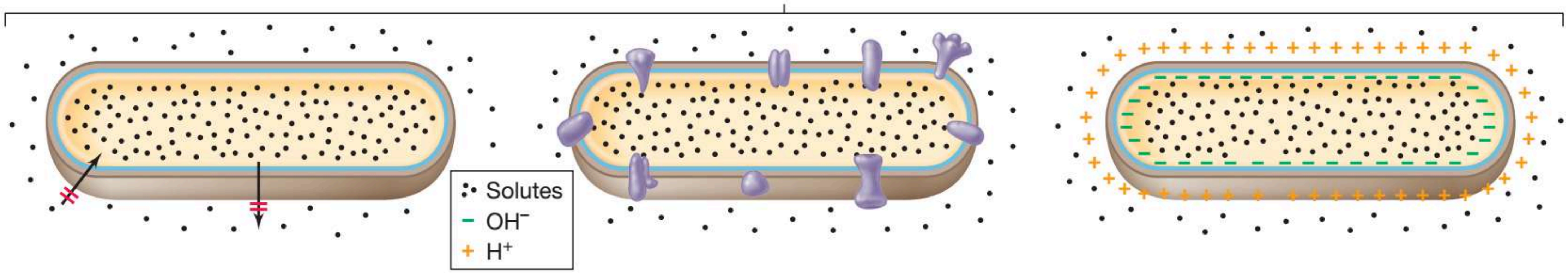
Siliakus et al. 2017

Archaea	Temperature				pH				Pressure	
	T_{min} <15 °C		T_{max} >75 °C		pH_{min} <3		pH_{max} >10		>40 MPa	
Level of chain length		Ref		Ref		Ref		Ref		Ref
C20-chain	+	(9)	+	(24–26)			+	(47–53)	+	(28, 54–55)
C25-chain			+	(56)			+	(47–53)		
Level of saturation										
Unsaturated diethers	+	(9, 10)	+	(11)						
Level of branching										
Hydroxyarchaeol	+	(9)								
Level of cyclization										
Pentacyclic TE			+	(13, 27)	+	(13,27, 36, 37)				
Macrocyclic			+	(57)					+	(28, 57)
Level of tetraether lipids										
Tetraethers	–	(9)	+	(12,23)	+	(14,36, 60)	–	(61, 62, 63)	–	(28)
Other modifications										
Glycolipids			+	(11)	+	(27, 37)	–	(48, 50, 53, 58, 59)		

PUFA polyunsaturated fatty acids, *MUFA-cis* cis-monounsaturated fatty acids, *MUFA-trans* trans-monounsaturated fatty acids, *BCFA-iso* iso-branched chain fatty acids, *BCFA-anteiso* anteiso-branched chain fatty acids, *BMP* bis-mono-acylglycero-phosphate, *TE* tetraethers, + increased production, − decreased production

Siliakus et al. 2017

Functions of cytoplasmic membrane



(a) **Permeability barrier:** Prevents leakage and functions as a gateway for transport of nutrients into, and wastes out of, the cell

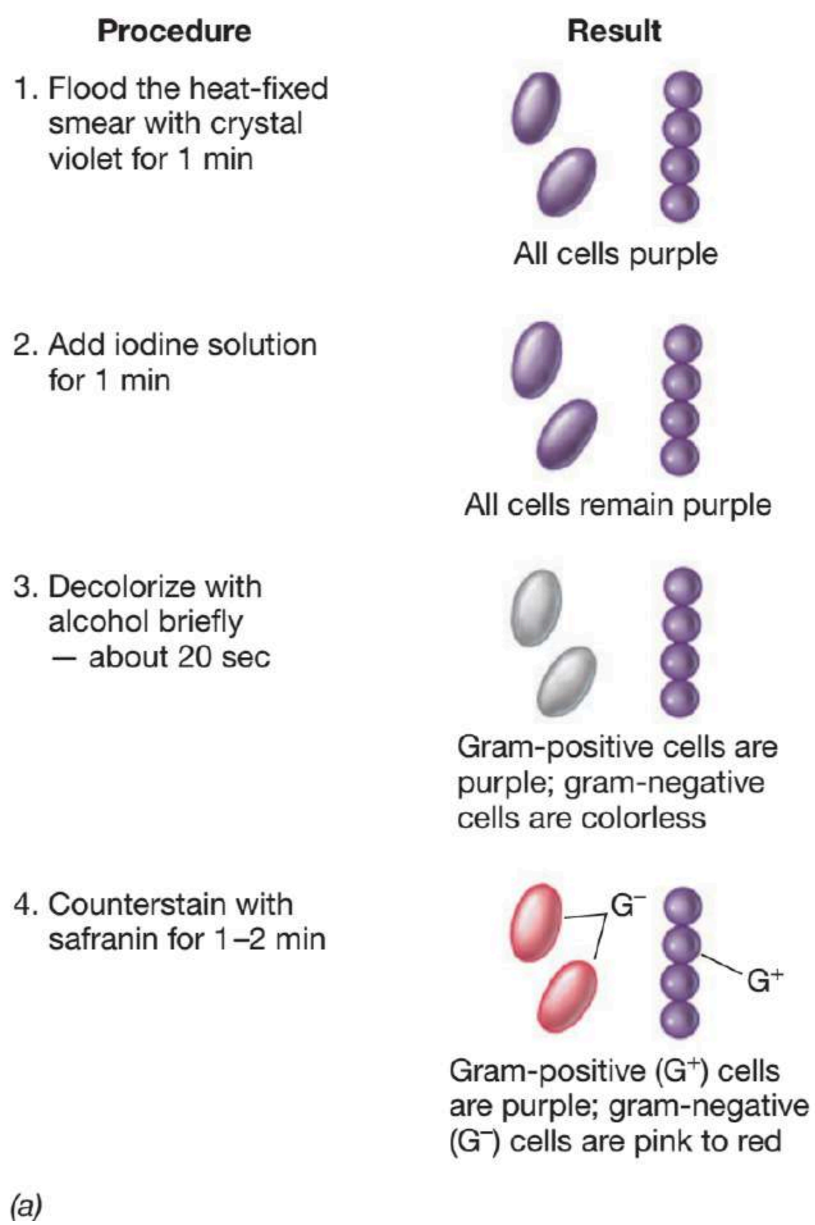
(b) **Protein anchor:** Site of proteins that participate in transport, bioenergetics, and chemotaxis

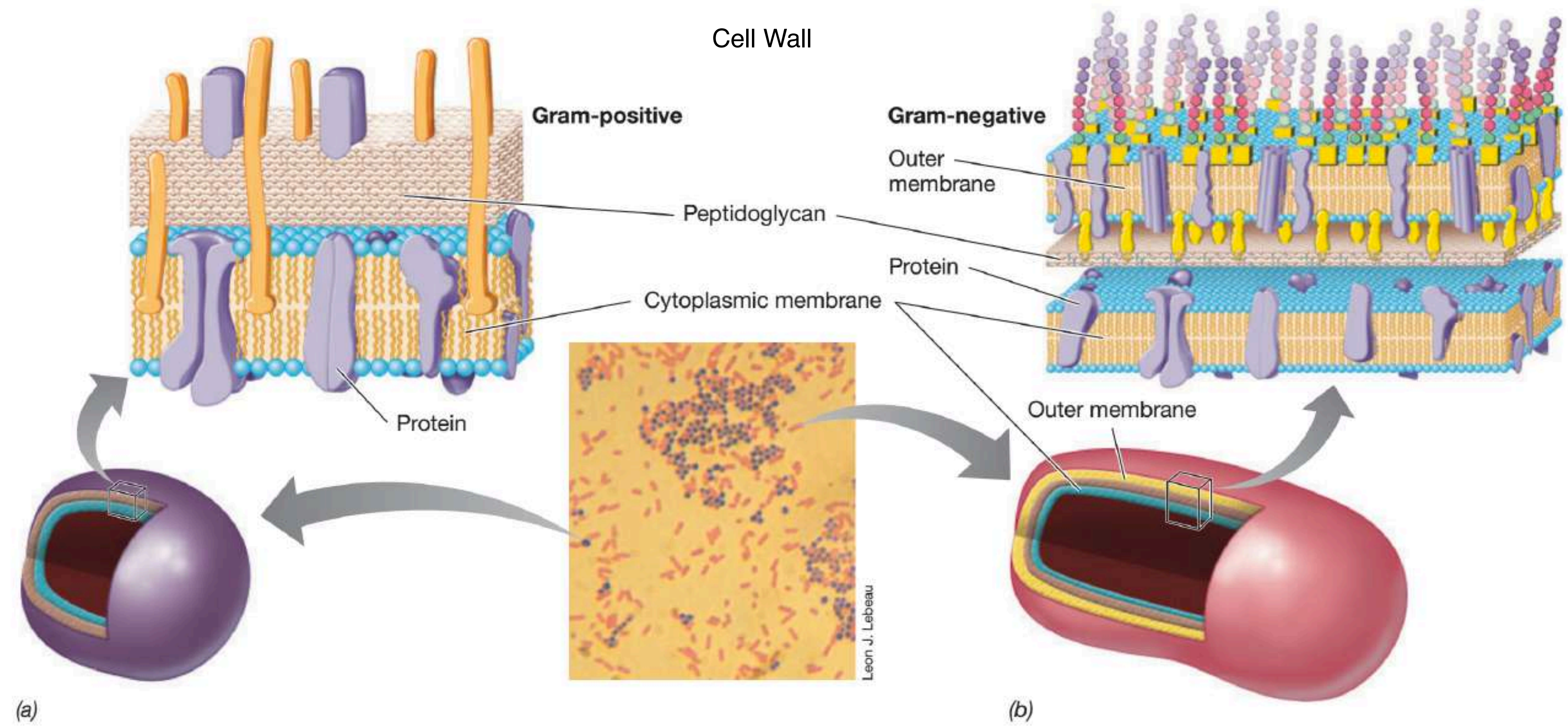
(c) **Energy conservation:** Site of generation and dissipation of the proton motive force

Madigan et al. 2018

- **Selective chemical barrier**
- **Defines cell shape**
- **Allow cell to sustain large mechanical loads (turgor pressure)**
- **Stiffness and strength of *E. coli* cells due to the outer membrane (Rojas et al. 2018, not only cell wall)**
- **Respiratory chain**
- **Sensing the environment and metabolic hotspot (enzymes)**

Gram Staining: defining diversity based on structural differences

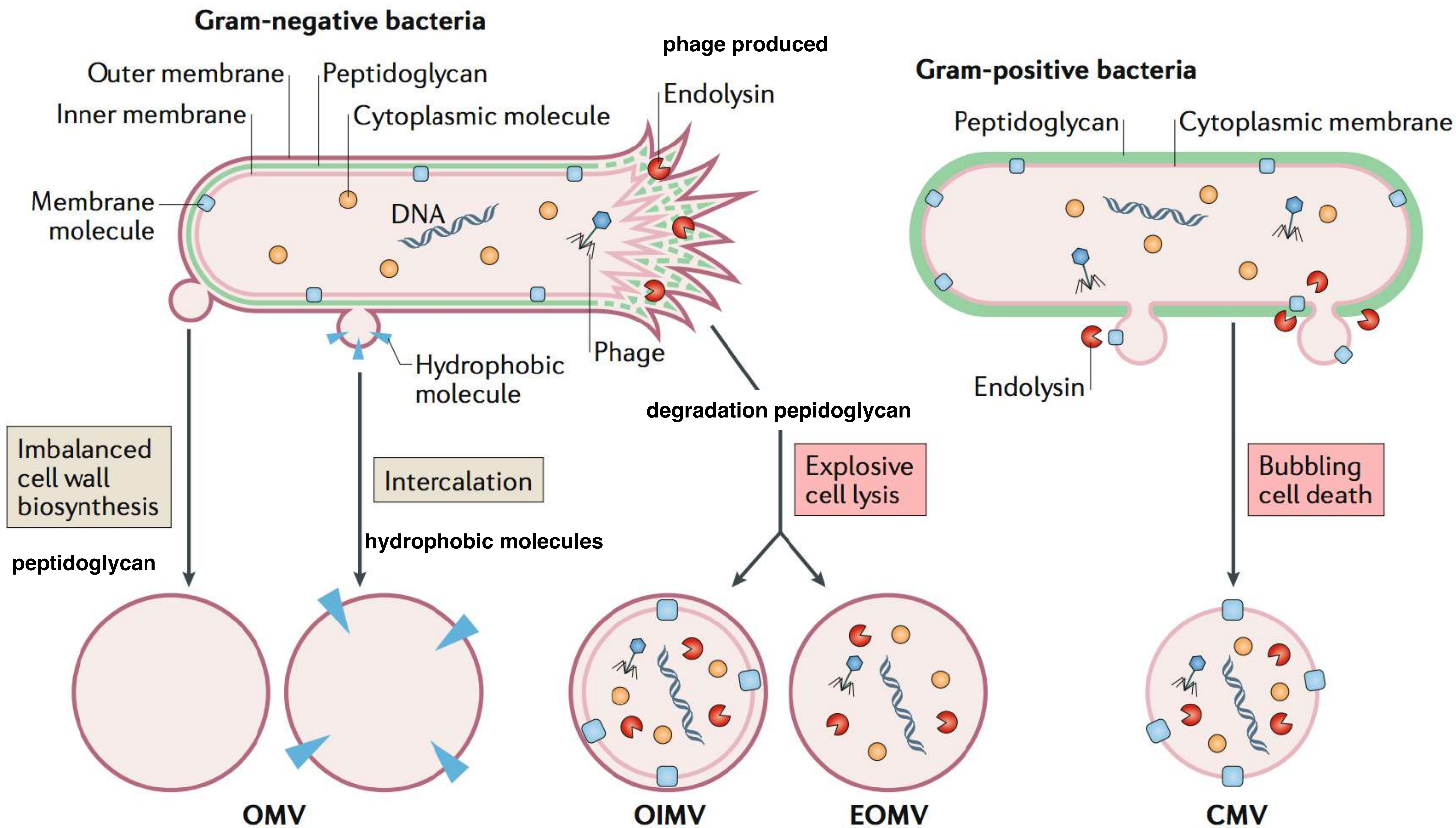




Madigan et al. 2018

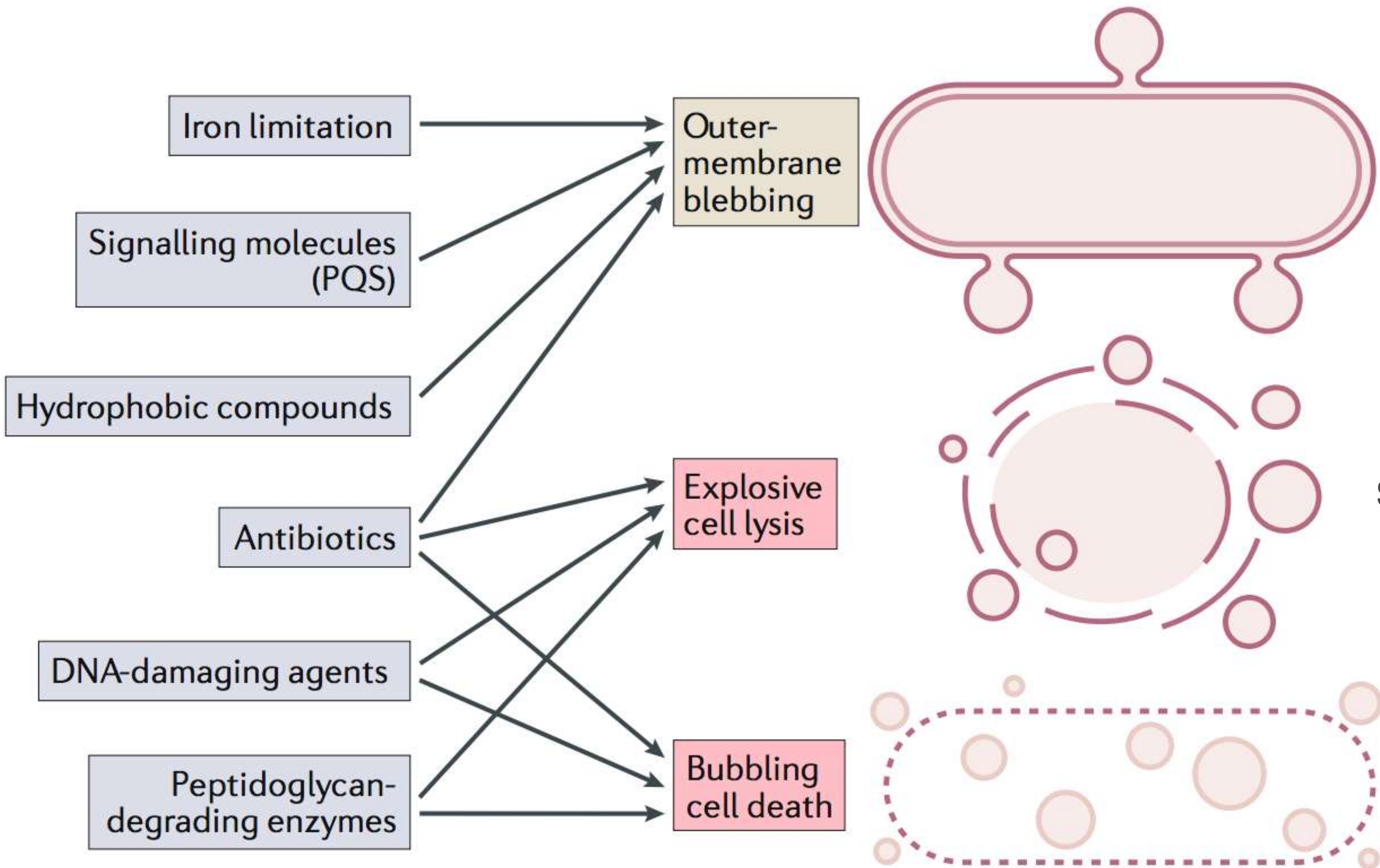
Distinct membrane vesicle types and formation mechanisms

Toyofuku et al., 2018



- Outer-membrane vesicles (OMVs)
- Outer-inner membrane vesicles (OIMVs)
- Explosive outer-membrane vesicles (EOMVs)
- Cytoplasmic membrane vesicles (CMVs)

Different triggers inducing membrane vesicle formation



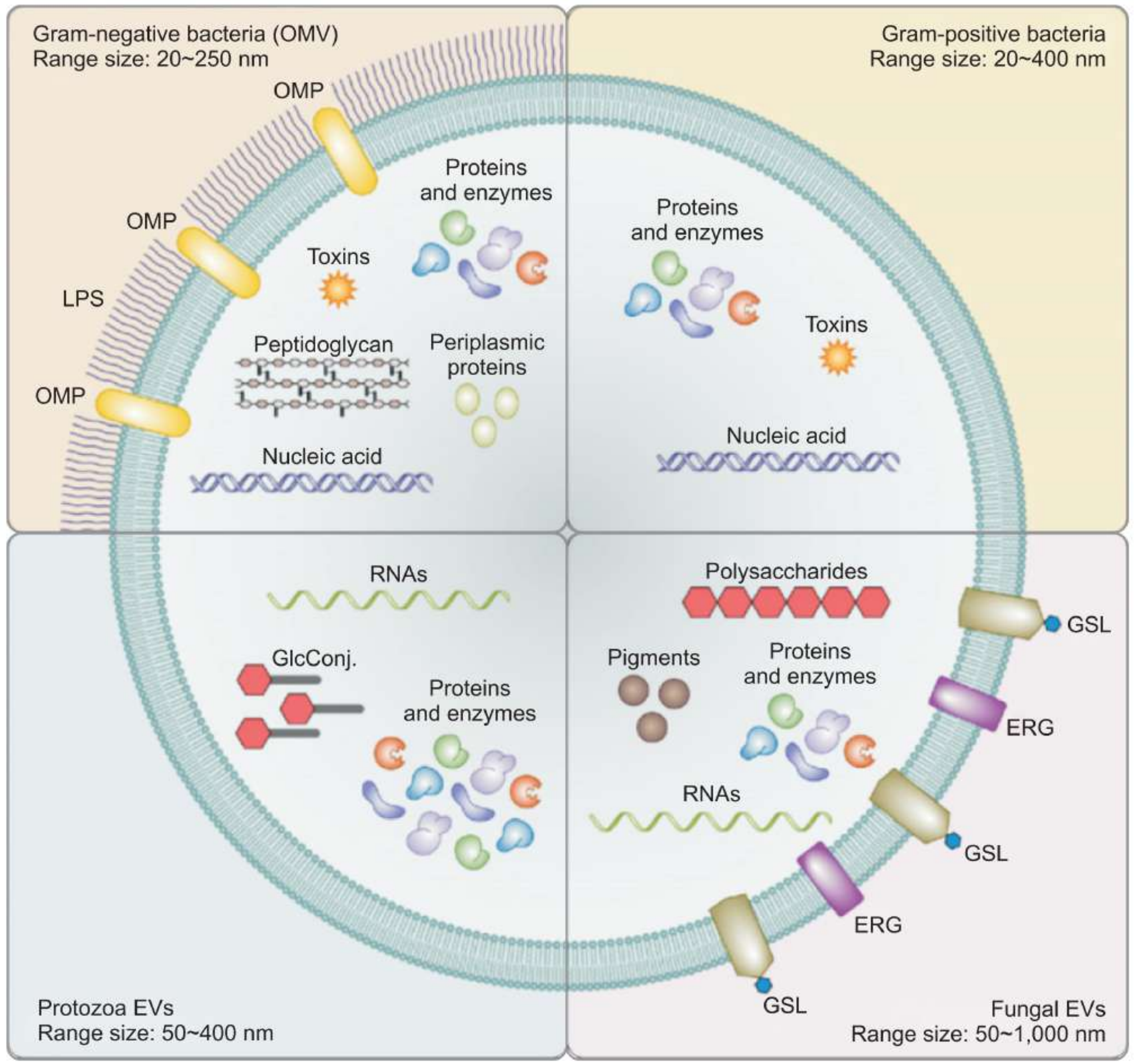
Influence lipid homeostasis

SOS response

β-lactam antibiotics

Toyofuku et al., 2018

Extracellular vesicle



Joffe et al., 2016

- Spherical portions (~ 20–250 nm in diameter) of the outer membrane of Gram-negative bacteria
- Containing outer-membrane lipids and proteins, and soluble periplasmic content
- OMVs are not the products of cell lysis
- Diverse strategies from defense/offense to nutrient acquisition and scavenging

Outer-Membrane Vesicles, I
Gram Negative

Spherical portions (~ 20–250 nm in diameter) of the outer membrane of Gram-negative bacteria, containing outer-membrane lipids and proteins, and soluble periplasmic content

OMVs are not the products of cell lysis.

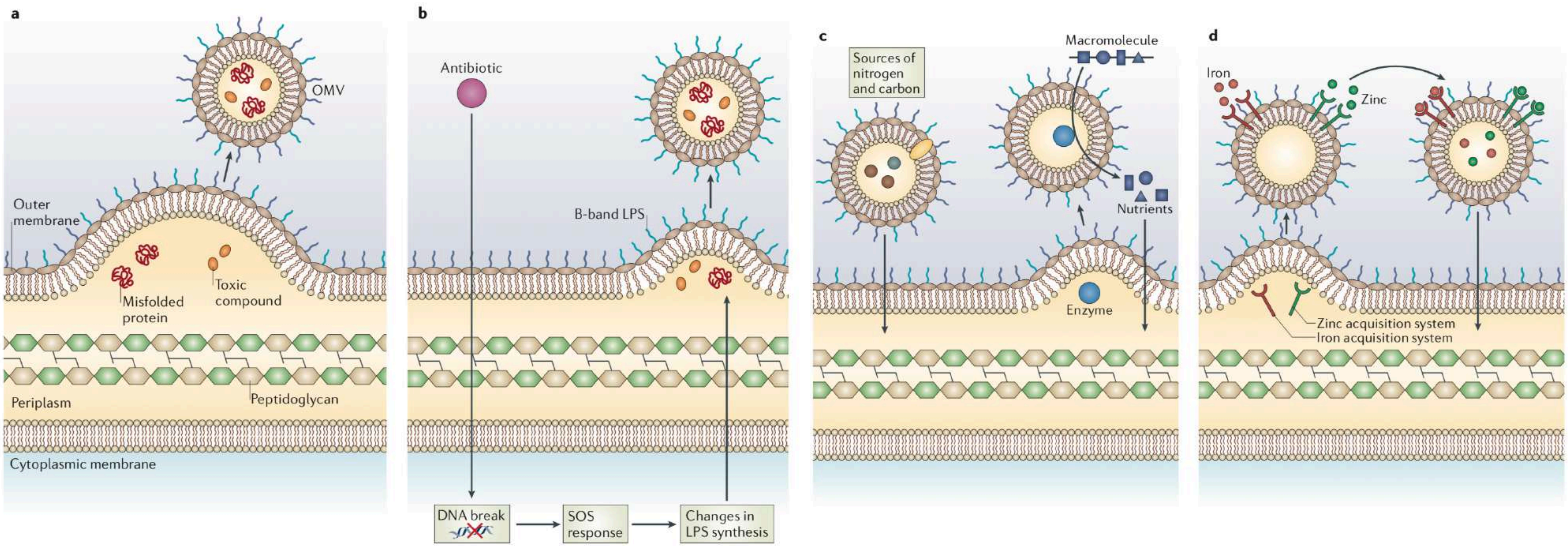


Figure 3 | **Functions of outer-membrane vesicles in bacterial physiology.** Outer-membrane vesicles (OMVs) function in multiple pathways that promote bacterial survival. **a** | OMVs can serve as a mechanism to remove toxic compounds, such as misfolded proteins, from bacterial cells under stress conditions. **b** | Stress conditions can increase OMV production. For example, exposure to antibiotics can induce DNA breaks, which triggers an SOS response. As part of the SOS response, changes in the synthesis of lipopolysaccharide (LPS) can alter the composition of the outer membrane and increase the production of OMVs. **c** | OMVs can serve as sources of carbon and nitrogen, and can carry and disseminate enzymes that break down complex macromolecules to provide the cell with essential nutrients. **d** | OMVs can also carry iron and zinc acquisition systems that are able to bind these metals in the environment, providing the bacteria with access to these essential compounds.

Schwechheimer and Meta J. Kuehn, 2016

Outer-Membrane Vesicles, II
Gram Negative

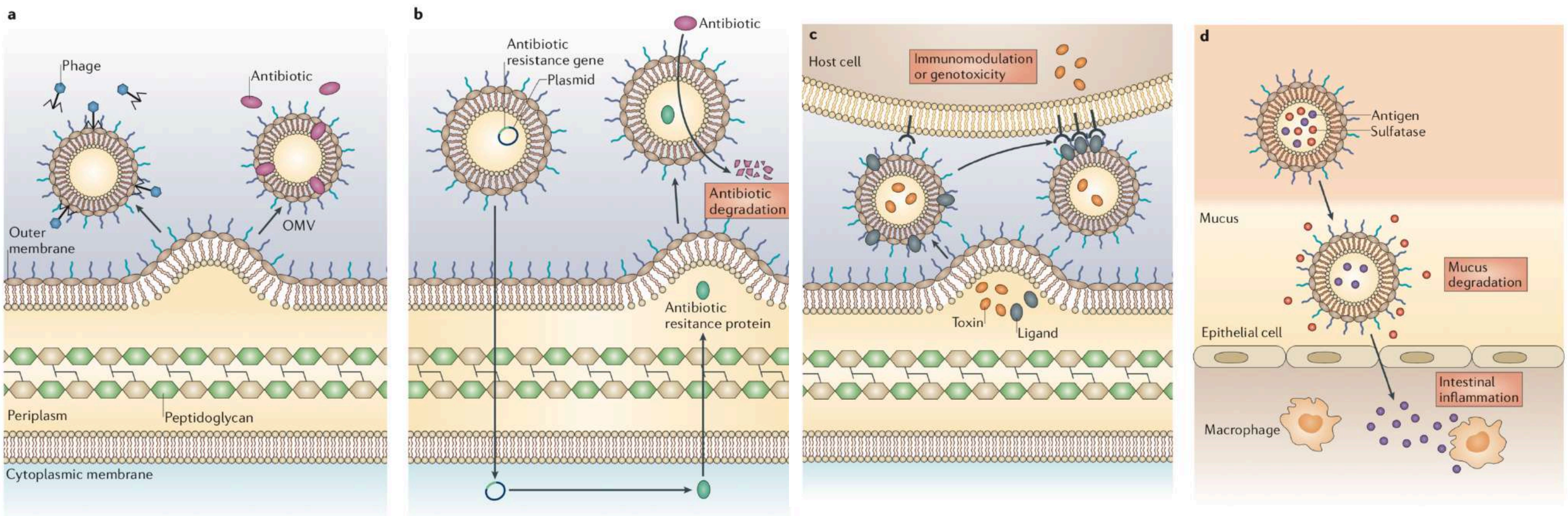
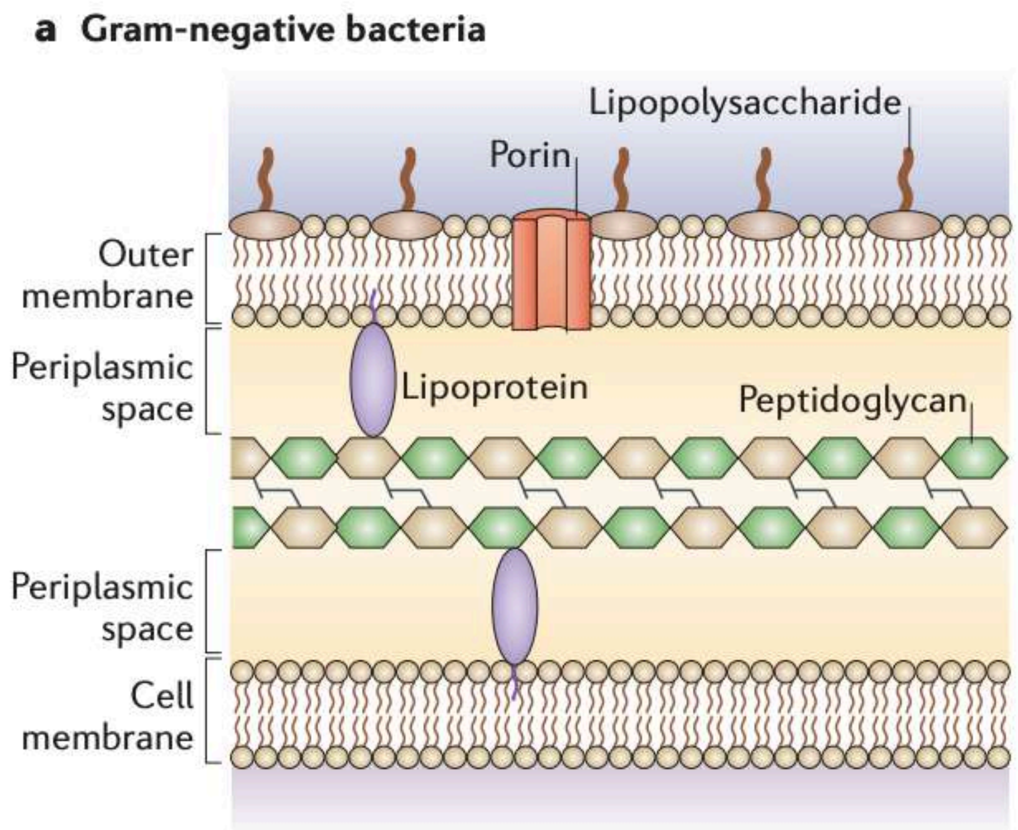
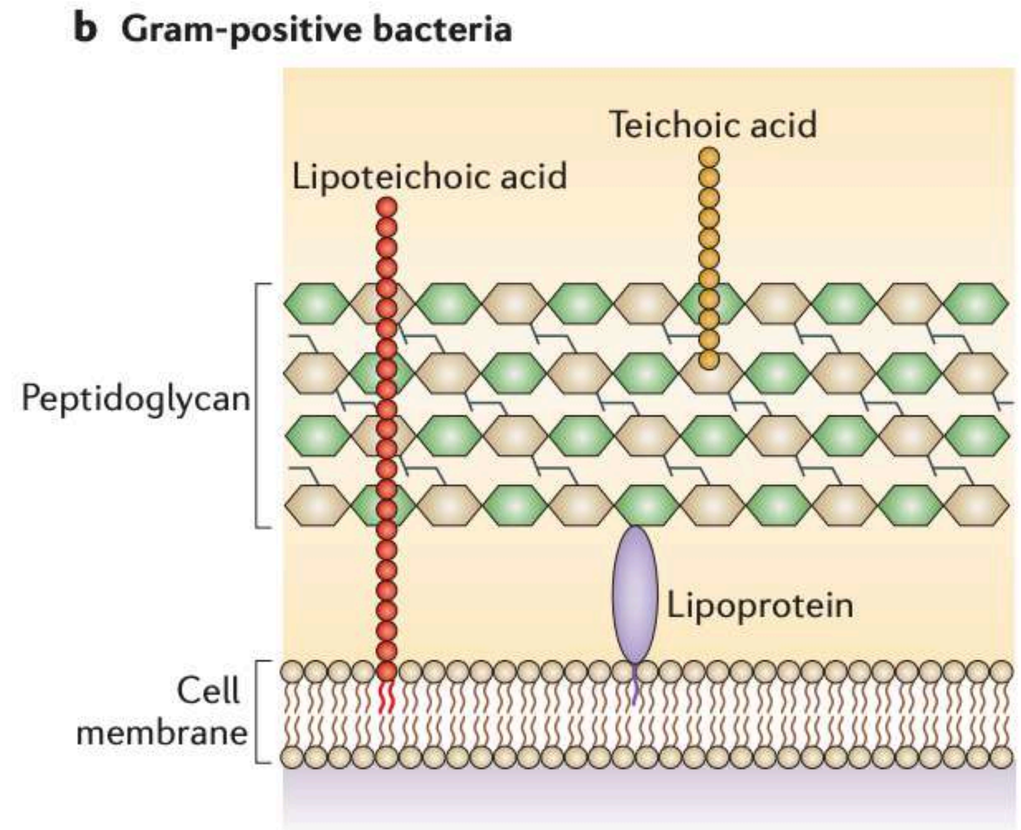


Figure 4 | **Functions of outer-membrane vesicles in pathogenesis.** Outer-membrane vesicles (OMVs) can increase bacterial pathogenicity via multiple mechanisms. **a** | OMVs can increase bacterial resistance to antibiotics and phages by serving as decoy targets for these molecules, thus protecting the bacteria cell. **b** | OMVs can also transfer DNA between cells, including antibiotic-resistance genes, and can carry enzymes that degrade antibiotics. **c** | Pathogenic Gram-negative bacteria are thought to utilize OMVs to interact with host cells during infection. For example, bacteria can use OMVs to mediate the delivery of virulence factors, such as toxins, into host cells, including immune cells. **d** | OMVs can also cross the mucus barrier in the gut and reach the intestinal epithelium, delivering bacterial antigens to the underlying macrophages, which triggers intestinal inflammation.

Schwechheimer and Meta J. Kuehn, 2016

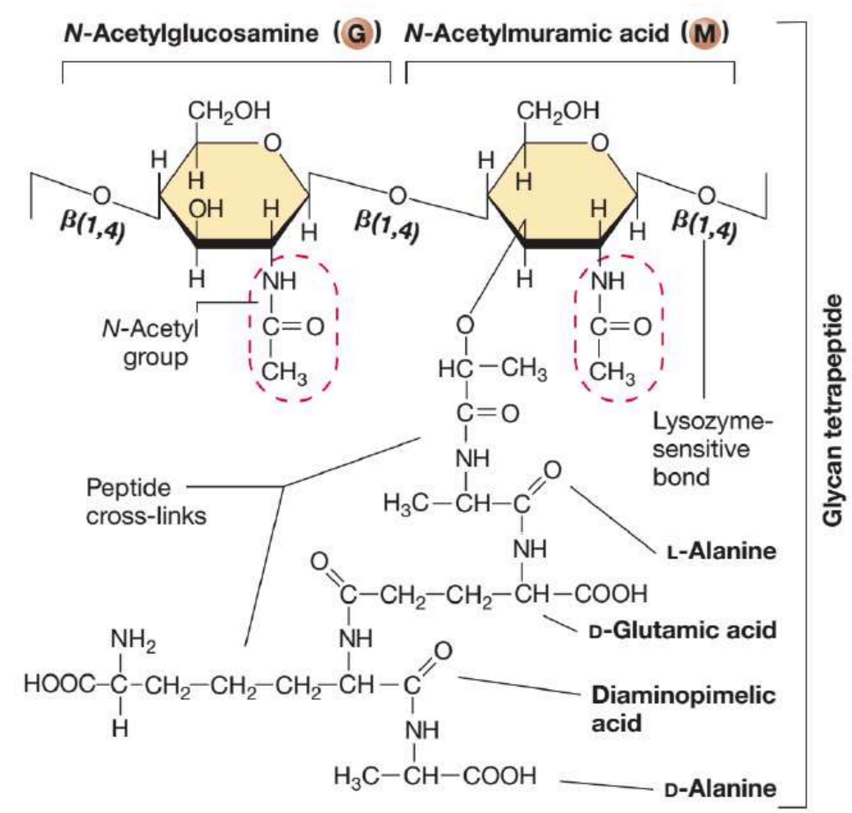
Cell Wall, 1





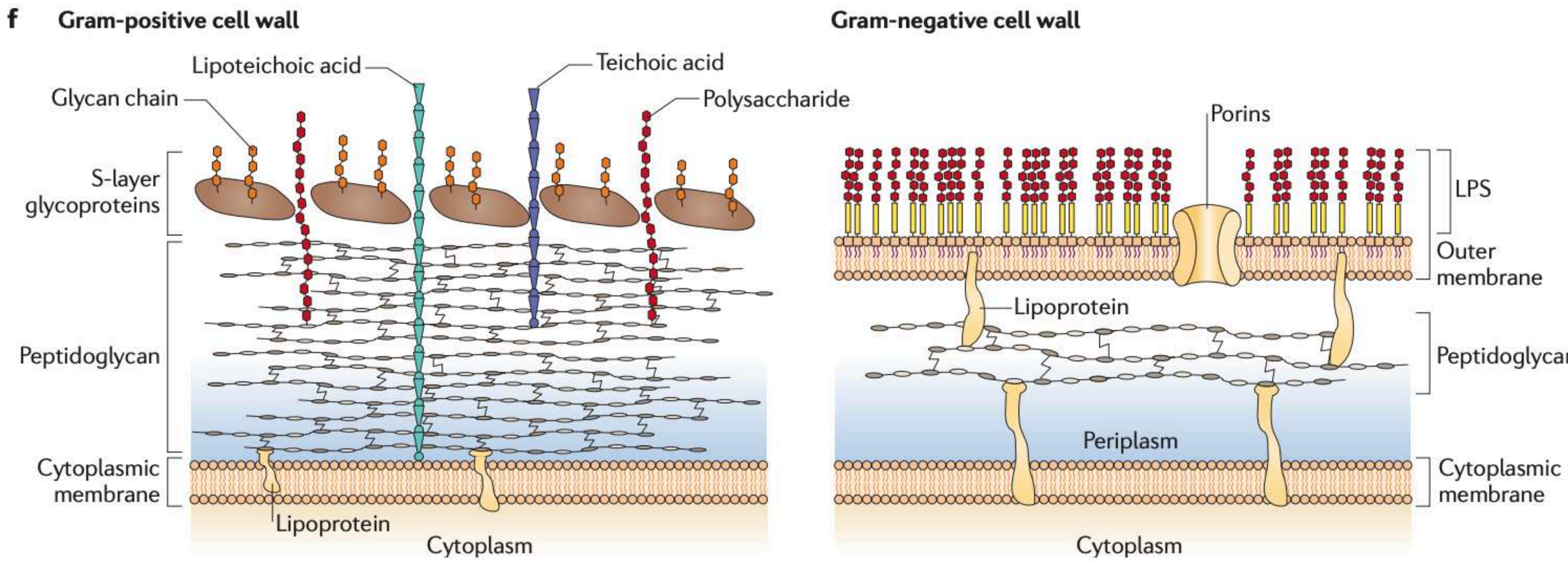
Brown et al. 2015

Peptidoglycan is composed of alternating repeats of two modified glucose residues called N-acetylglucosamine and N-acetylmuramic acid along with the amino acids l-alanine, d-alanine, d-glutamic acid, and either l-lysine or diaminopimelic acid (DAP). These constituents are connected in an ordered way to form the glycan tetrapeptide and long chains of this basic unit form peptidoglycan.

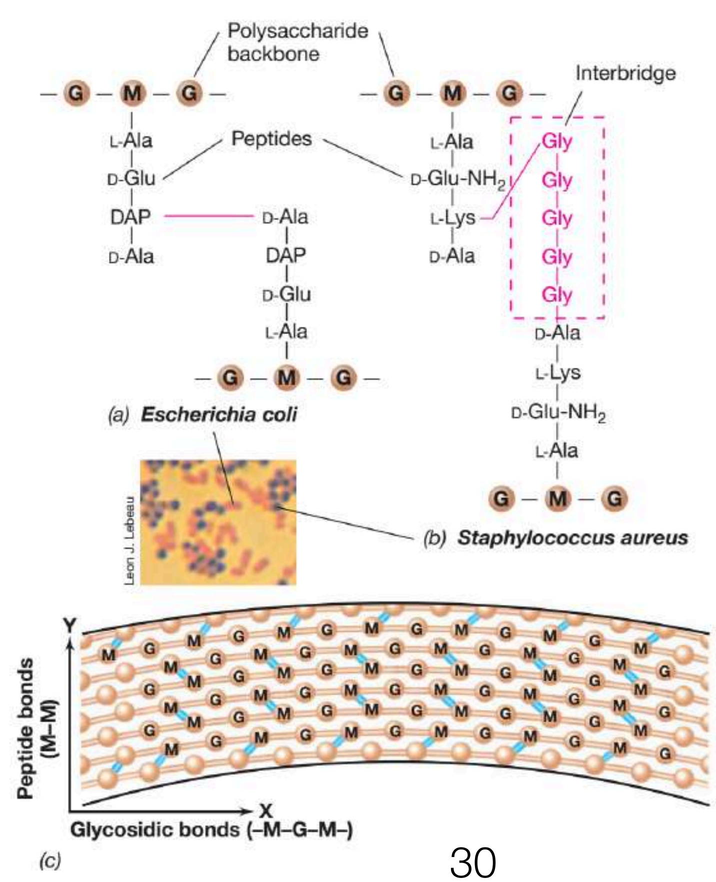


Madigan et al. 2018

Cell Wall, 2

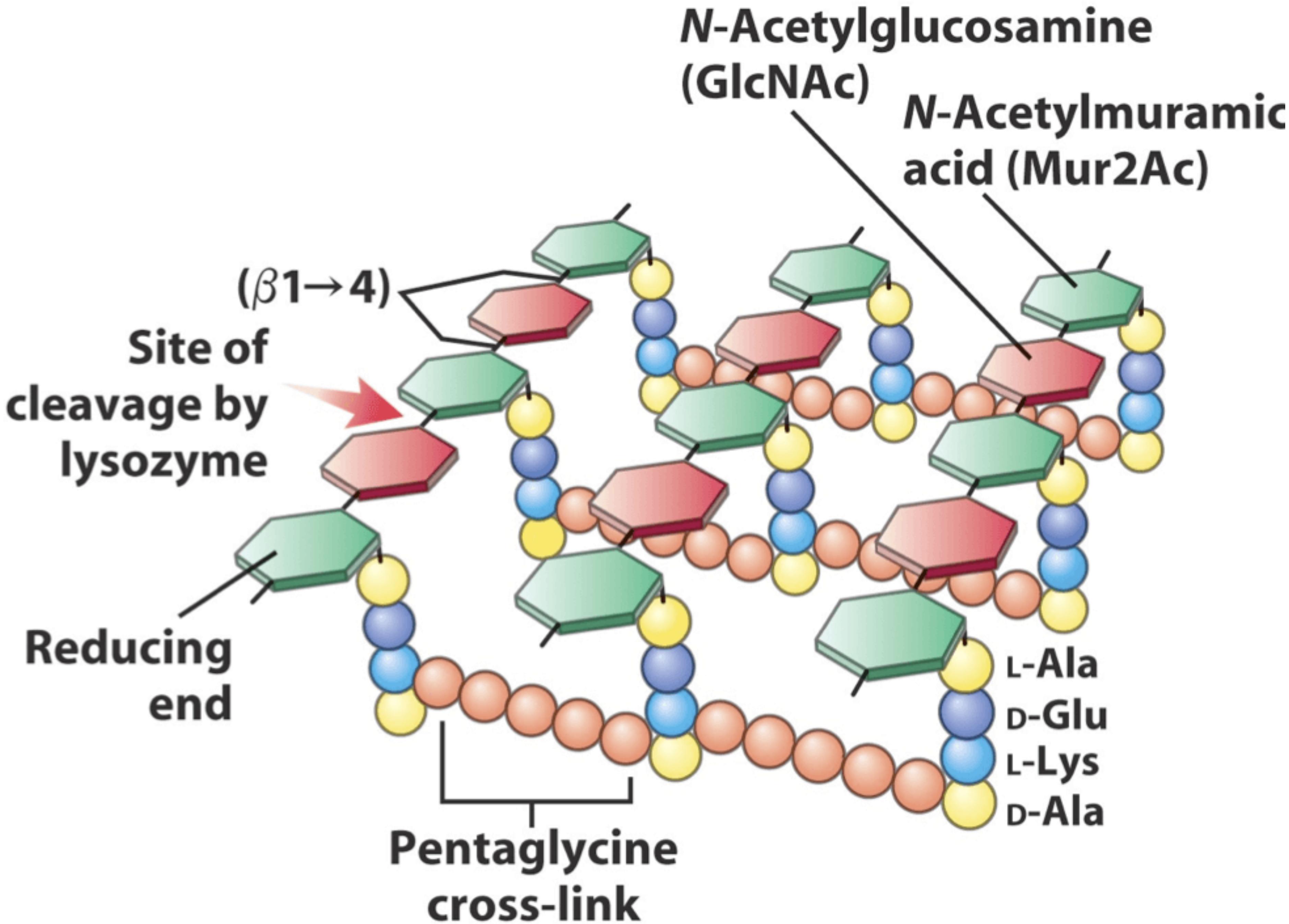


Peptidoglycan can be destroyed by lysozyme, an enzyme that cleaves the glycosidic bond between N-acetylglucosamine and N-acetylmuramic acid



Madigan et al. 2018

Peptidoglycan interaction site with lysozyme



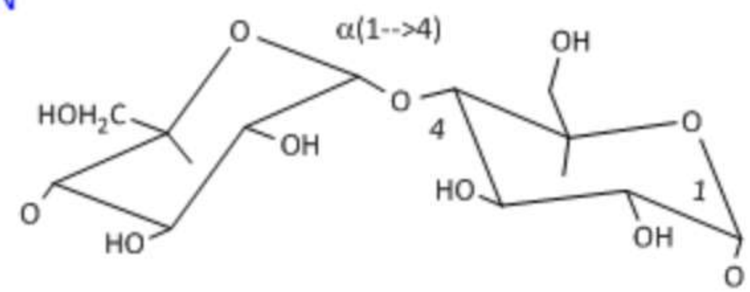
https://www.creative-proteomics.com/services/peptidoglycan-structure-analysis.htm

The importance of being 1-4

https://employees.csbsju.edu/hjakubowski/classes/ch331/cho/PS_3B1_Polysacch.htm

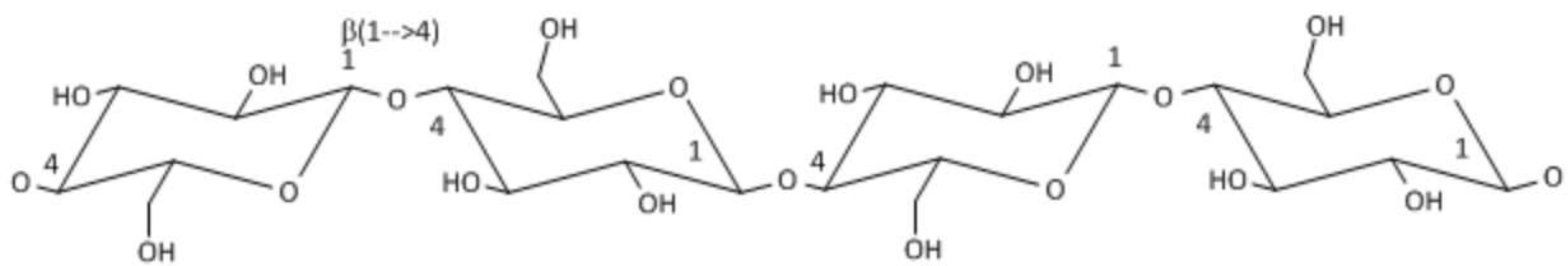
STARCH
GLYCOGEN

α(1-4) glycosidic links in main chain with α(1-->6) branches



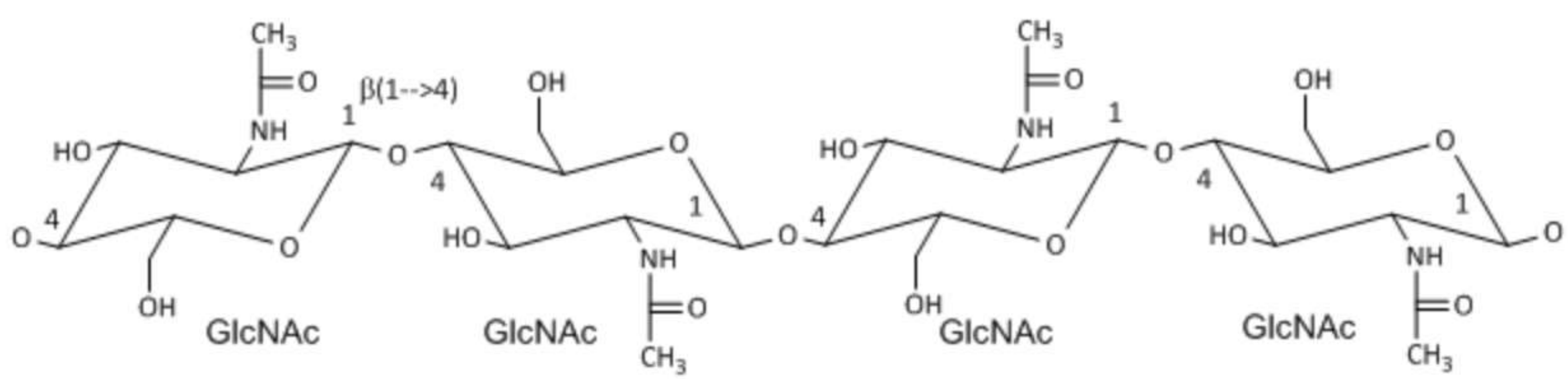
CELLULOSE

β(1-4) glycosidic links; multiple chains held together by intra/inter chain H-bonds



CHITIN

β(1-4) glycosidic links main chain; major substance in exoskeletons antropods/moll.

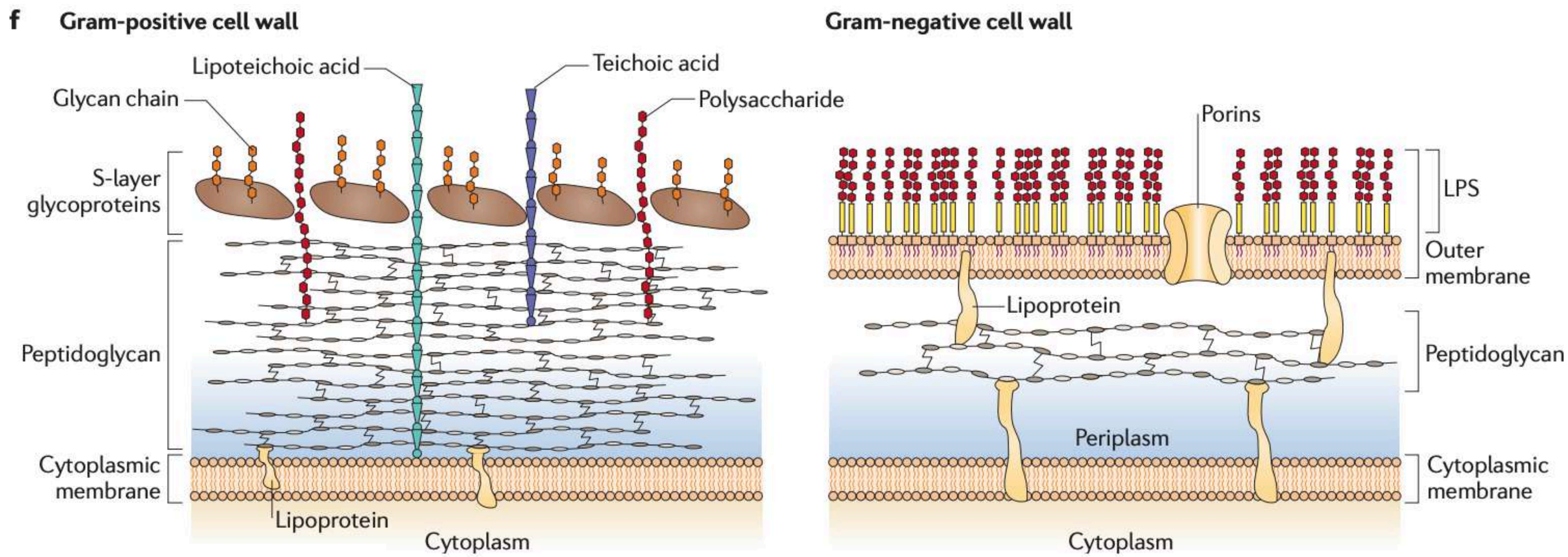


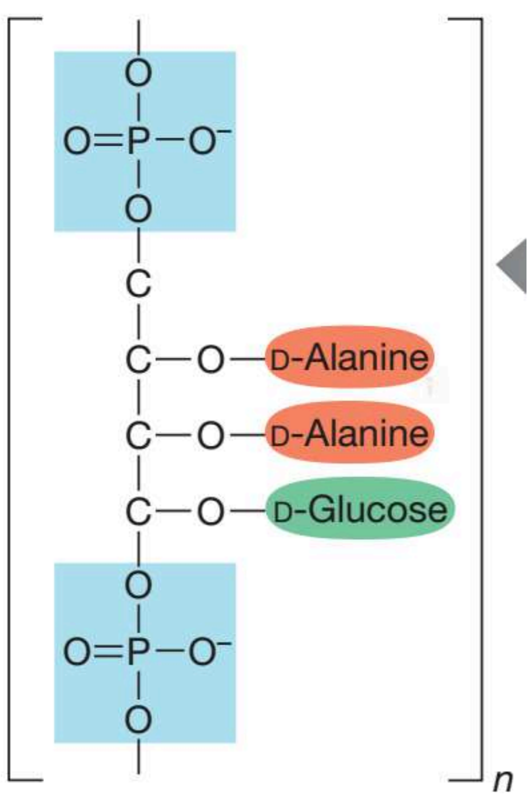
D-amino acids

- **Proteins are composed of L-amino** acids except for glycine, which bears no asymmetric carbon atom
- D-enantiomers
- D-serine and D-aspartate act as **neurotransmitters** and hormone-like substances, respectively, in humans
- Some D-amino acids act as a **biofilm disassembly** factor in bacteria
- D-amino acids can be used as **C-source in ocean water**
- D/L increase with depth and also utilization (source peptidoglycan)

Kobayashi, 2019; Perez et al., 2003

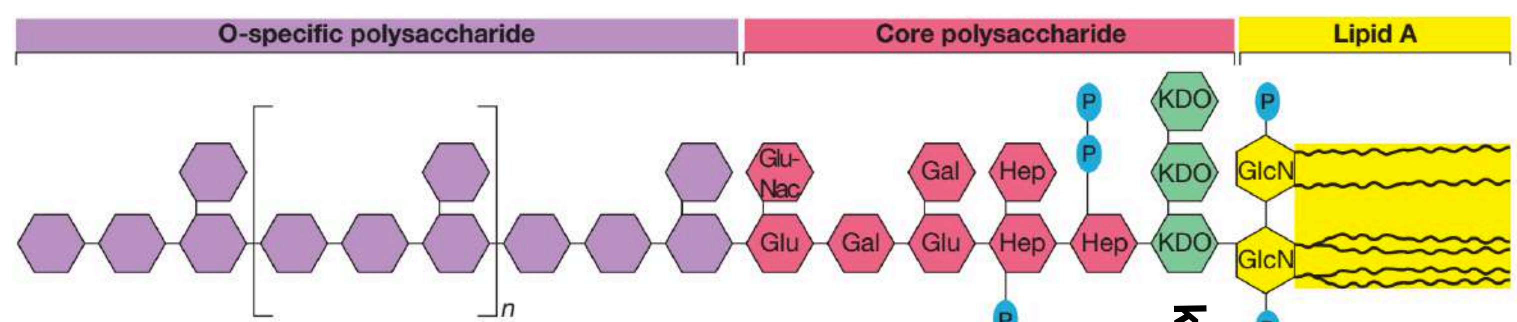
Cell Wall, 3





Ribitol teichoic acid

Lipopolysaccharide, LPS

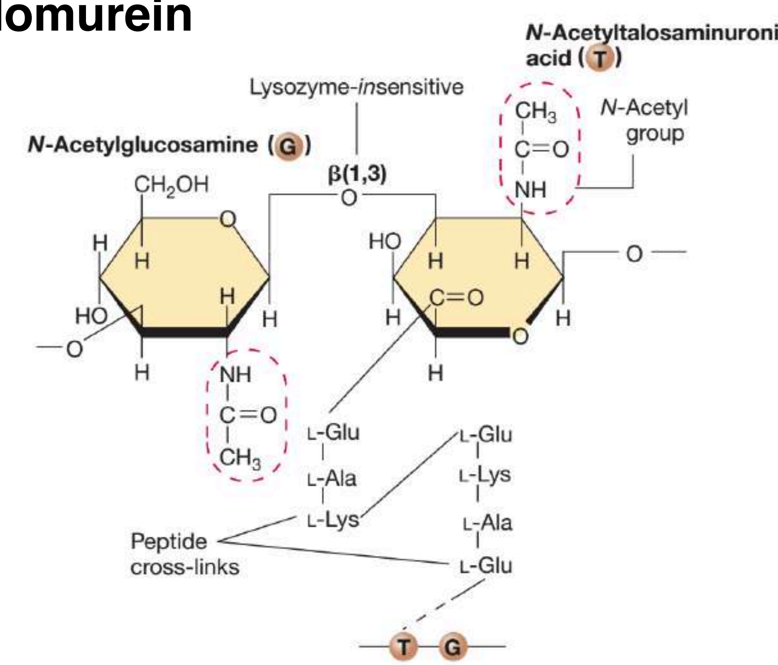


ketodeoxyoctonate

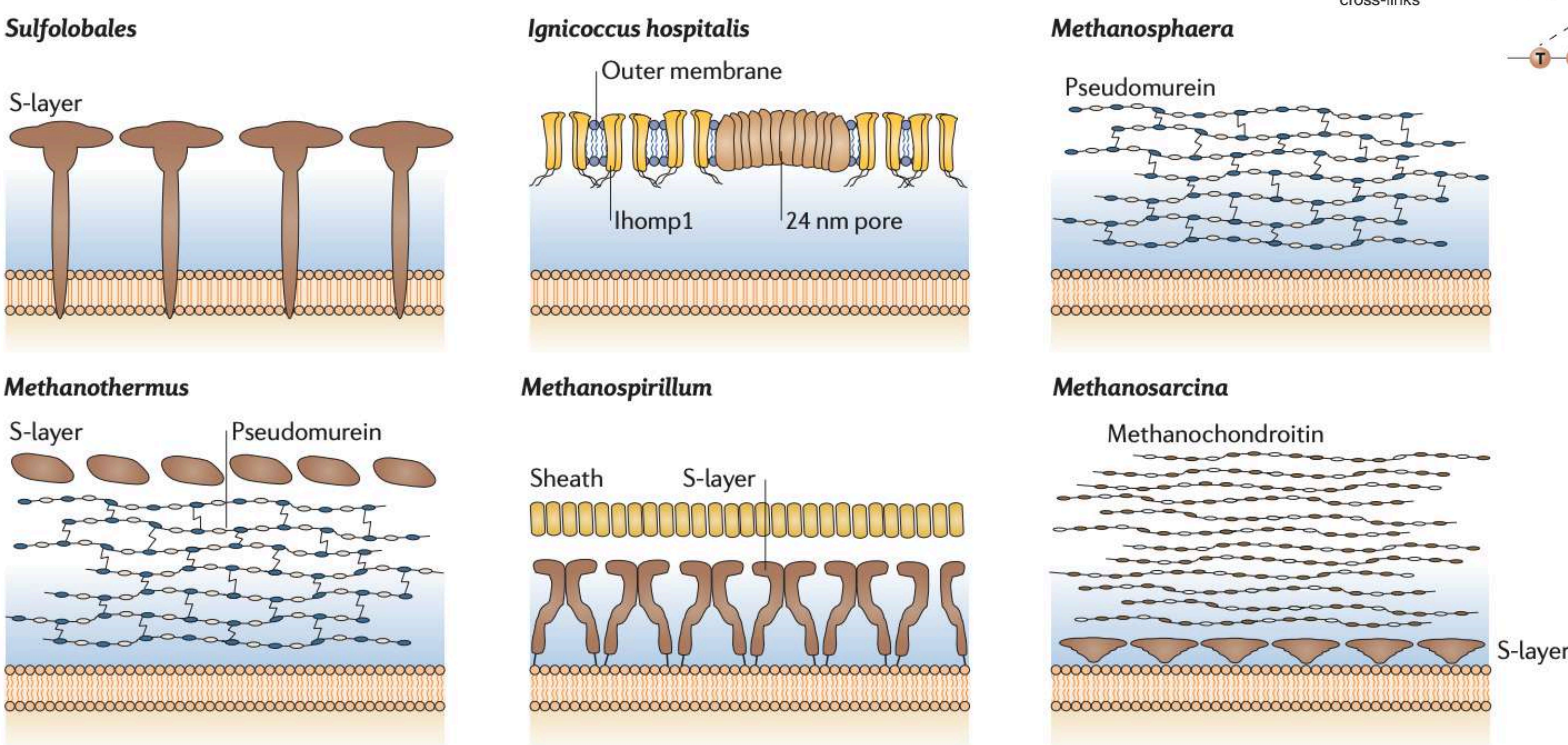
LPS replaces much of the phospholipid in the outer half of the outer membrane, and although the outer membrane is technically a lipid bilayer, its many unique components distinguish it from the cytoplasmic membrane.

Cell Wall, 4-Archaea

Pseudomurein

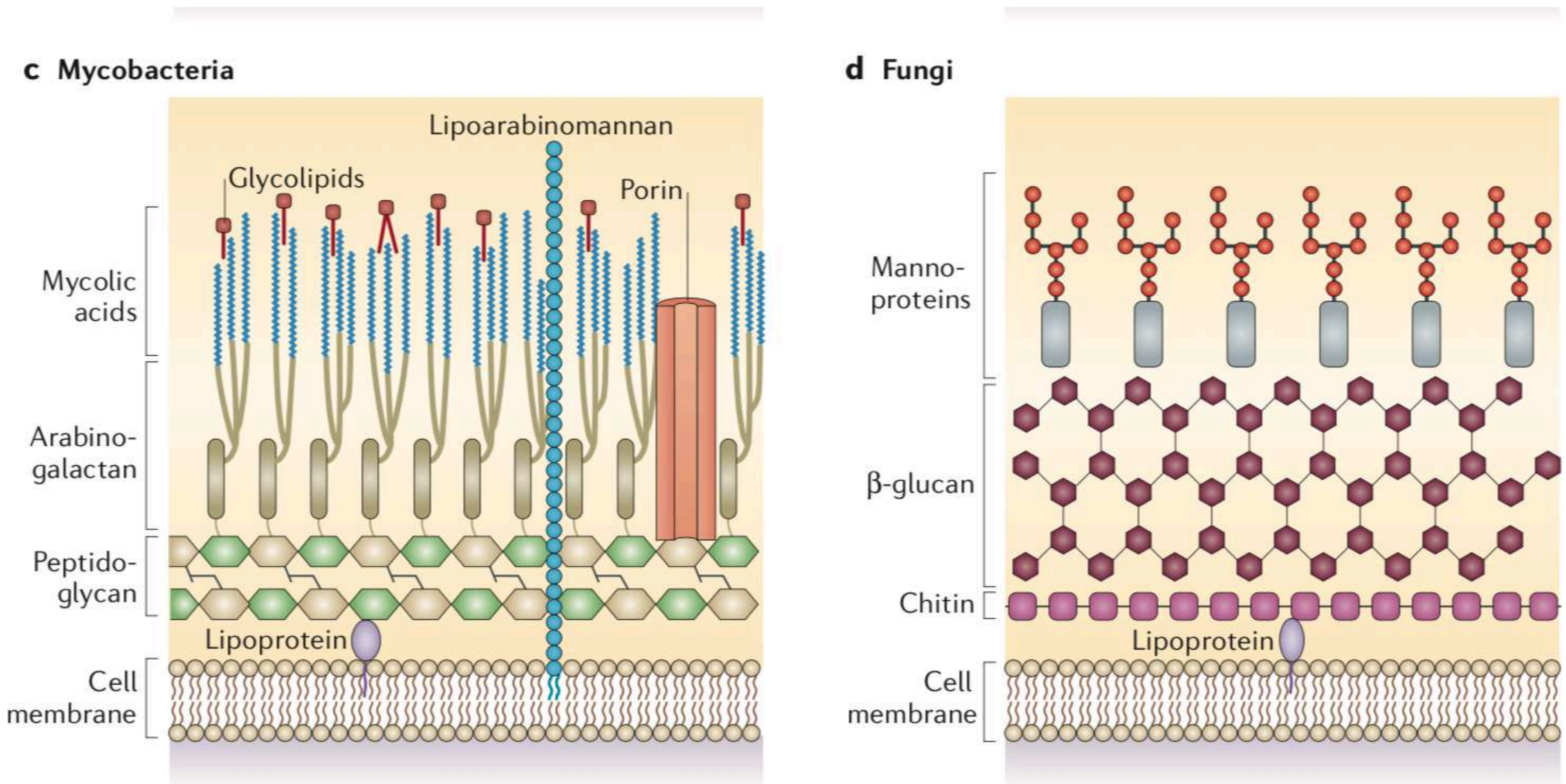


S-layer: interlocking molecules of protein or glycoprotein



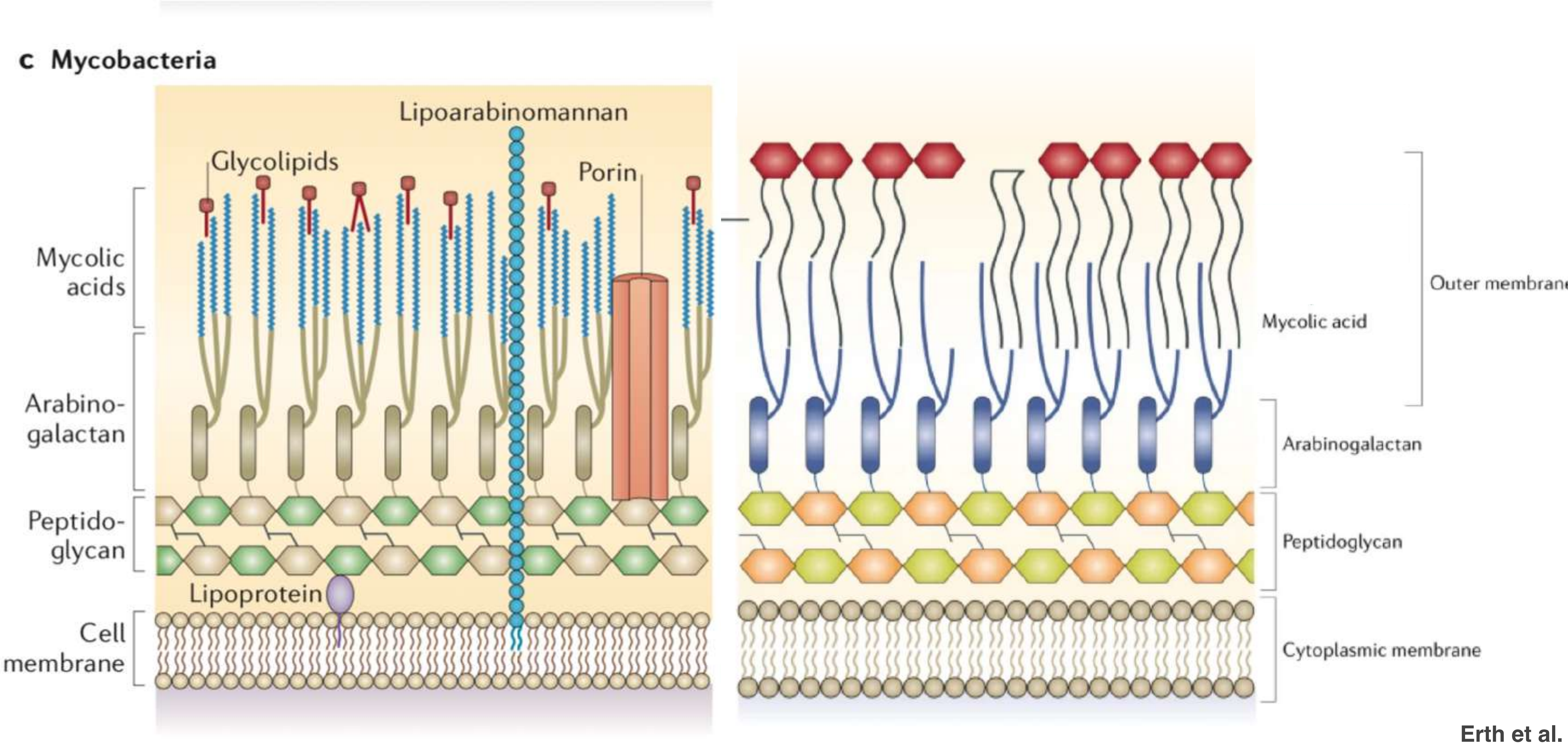
Polymers of glucose, glucuronic acid, galactosamine uronic acid, and acetate

Cell Wall, 5-Mycobacteria & Fungi



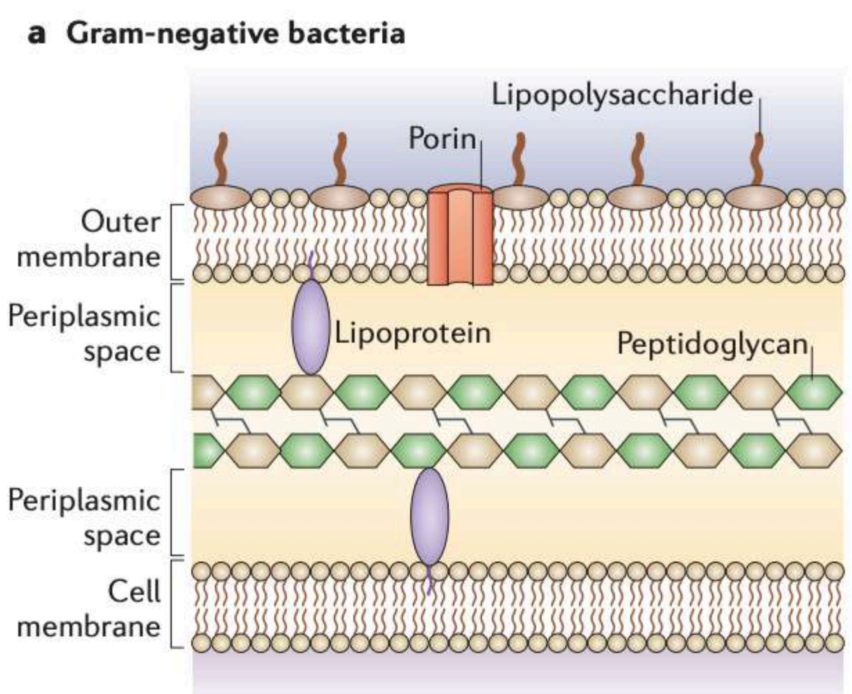
c | Cell walls of mycobacteria consist of thin layers of peptidoglycan and arabinogalactan, and a thick layer of mycolic acids[33]. Glycolipids and porins are also found in these cell walls, as is lipoarabinomannan, which is anchored to the cell membrane by diacylglycerol. This cell wall surrounds a single lipid membrane. **d** | A single plasma membrane is also present in fungi, surrounded by a cell wall consisting of various layers of the polysaccharides chitin, β-glucan and mannan (in the form of mannoproteins)[34].

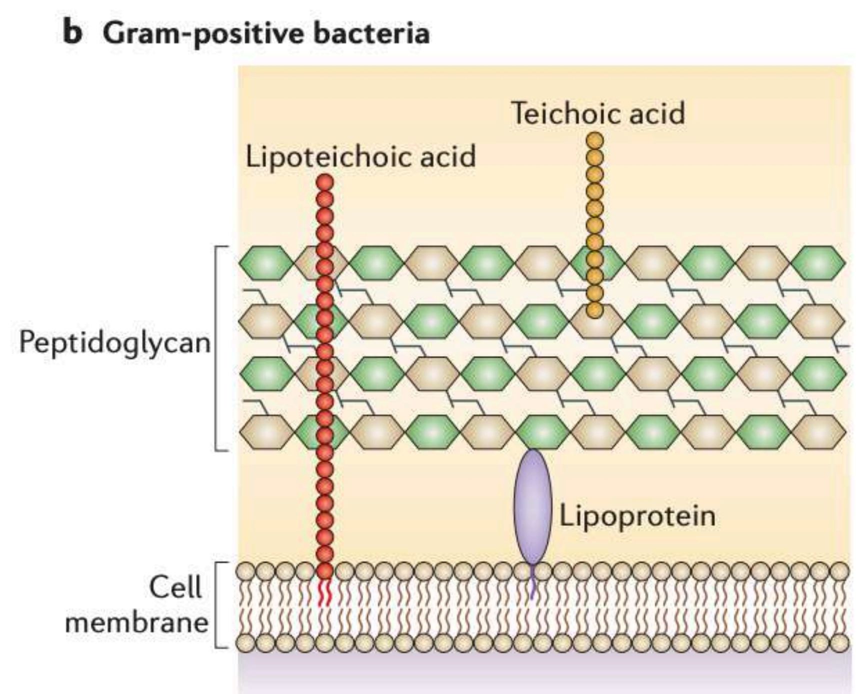
Brown et al. 2015



Erth et al. 2018

Something in the middle

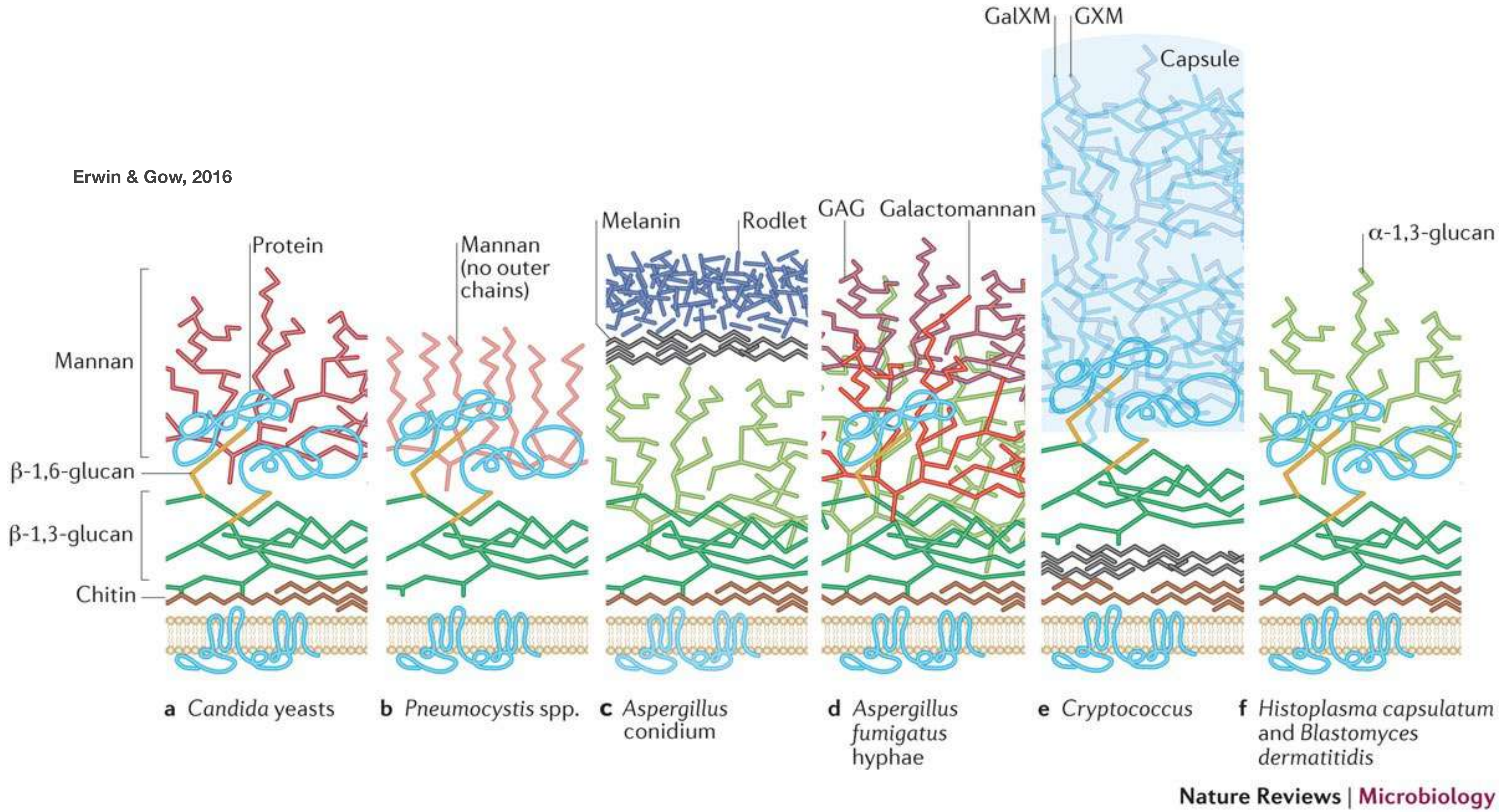




Brown et al. 2015

Fungal cell wall structure

Erwin & Gow, 2016



- Polysaccharides and other components of the cell wall are usually arranged in **distinct layers** and carry out **specific architectural and physiological roles** at different locations in the cell wall
- The layered nature of the fungal cell wall is highly relevant to immune detection