TU02 – Bacteriology
Structure of the bacterial surface of Gram positive bacteria

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Courses organisation

Introduction

Gram negative cell wall

Gram positive cell wall

- Intro
- Peptidoglycan
  - Composition
  - Synthesis
  - Recycling
  - Host-pathogen interactions with PG
- Polysaccharides
- Surface proteins
- S-layer
- Capsules

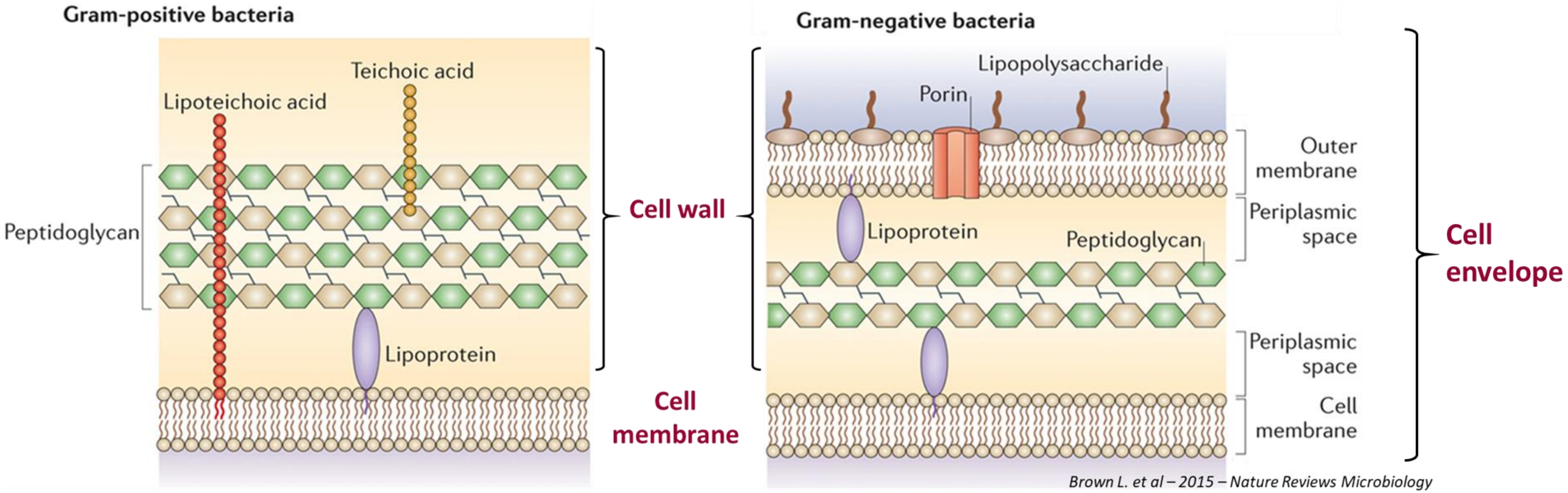
On Tuesday

Today

Why bacterial surface components are of interest ?

- In first line for **environment interactions**
- Allow bacteria to survive in **changing environments** → stressful for bacteria
  - ✓ Osmotic pressure
  - ✓ pH modifications
  - ✓ Oxygen level (for anaerobic or microaerophilic bacteria)
- Essential for bacteria to survive against different **aggressors** like
  - ✓ Chemical molecules
  - ✓ Antibiotics
  - ✓ Host immune response effectors

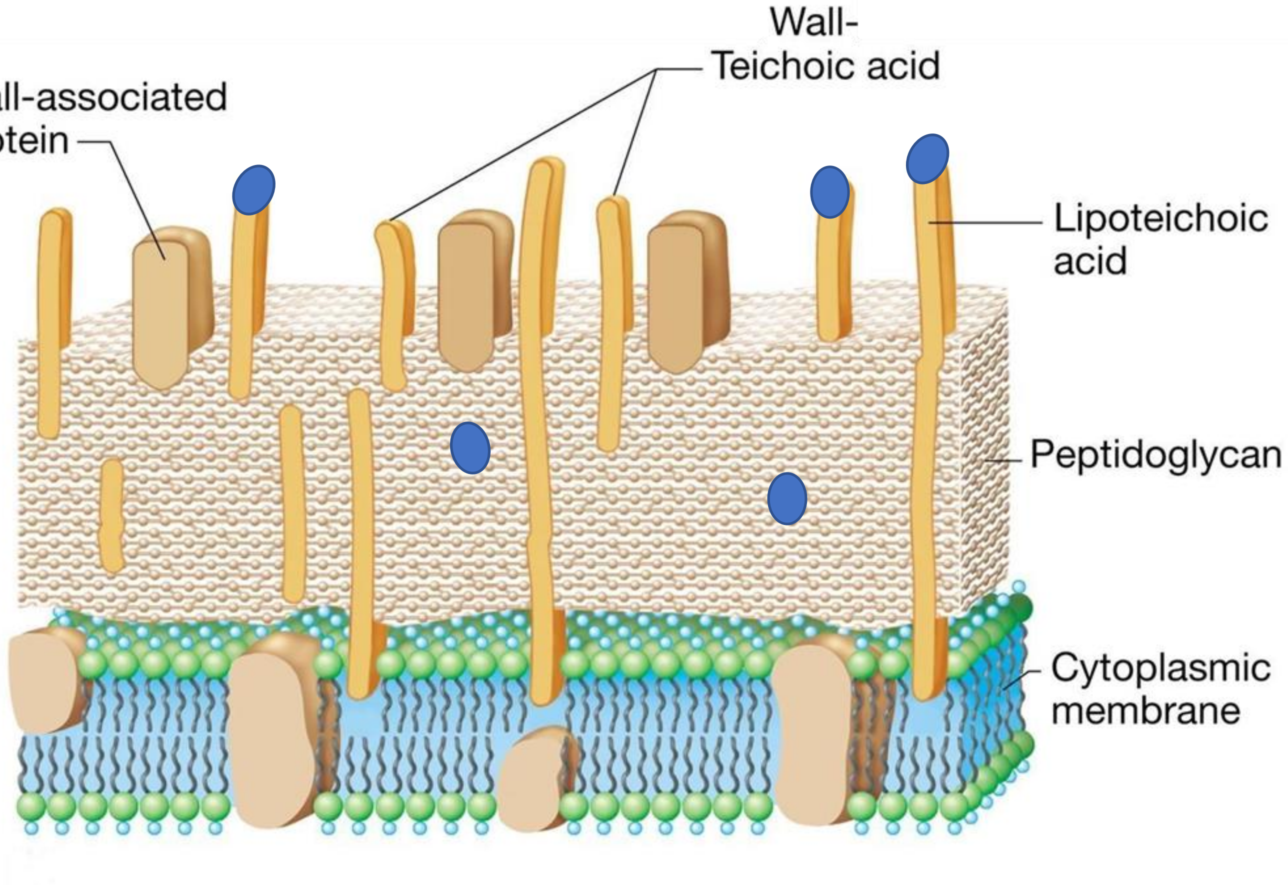
Gram + / Gram – envelopes (reminder)



Brown L. et al – 2015 – Nature Reviews Microbiology

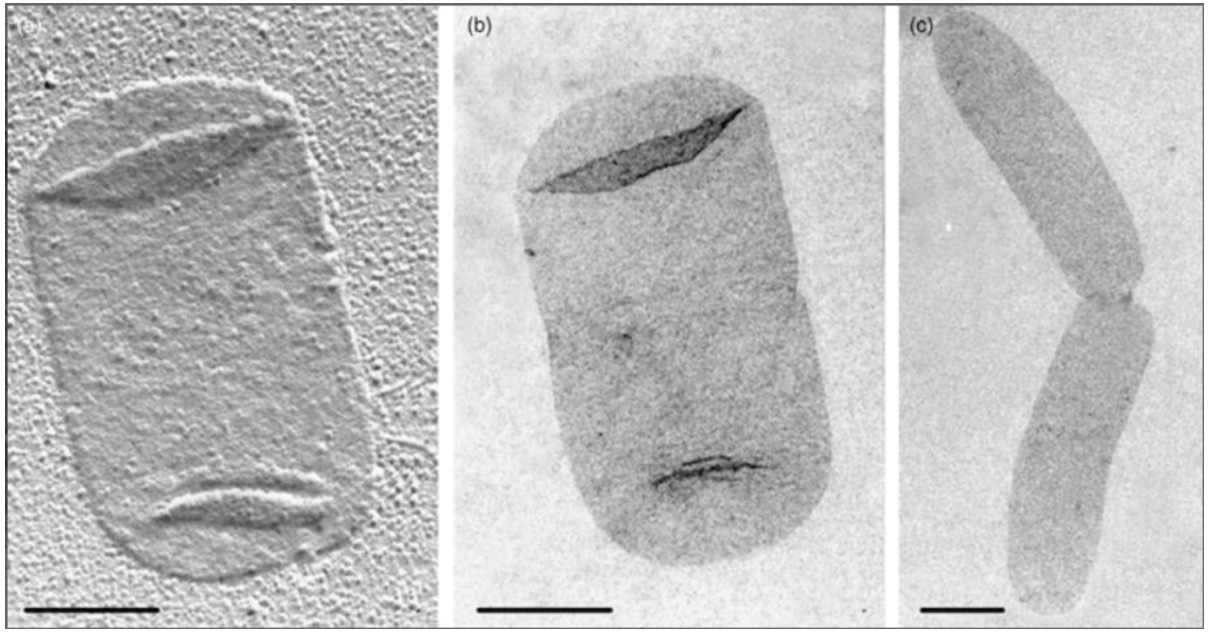
Gram positive surface

- **Cytoplasmic membrane**
- **Thick layer of peptidoglycan (PG)**
- **Polysaccharides**
  - ✓ **Teichoic acids** (TA) and TA-like polysaccharides
    → anchored in the PG
  - ✓ **Lipoteichoic acids** (LTA)
    → anchored in the membrane
- **Surface proteins**
  - ✓ Attached to the PG
  - ✓ Attached to polysaccharides filaments
- **S-layer**
- **Capsule**

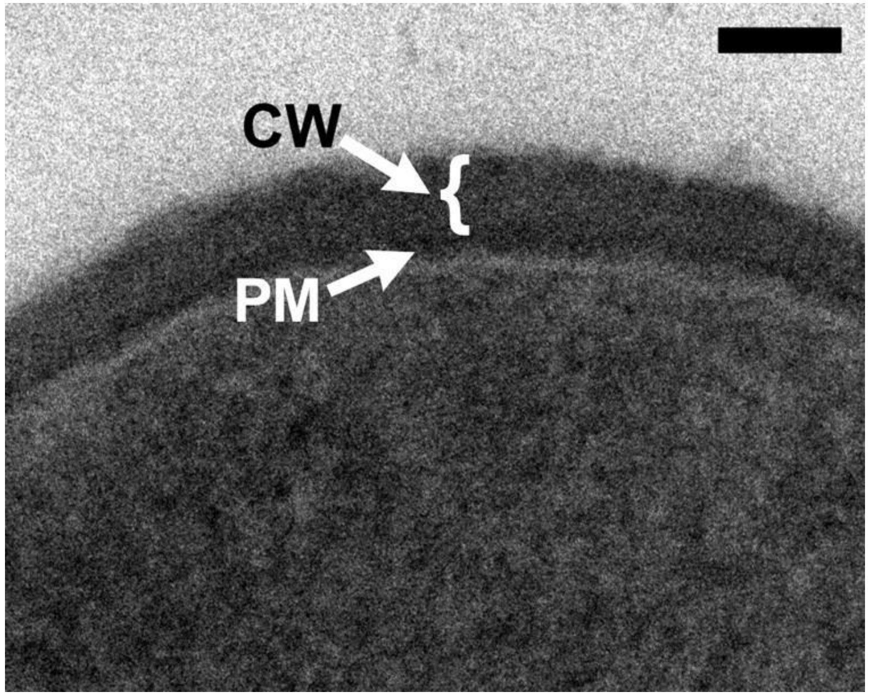


- **PG = rigid structure, determining the bacterial shape**

- **Confers protection against external aggressors**

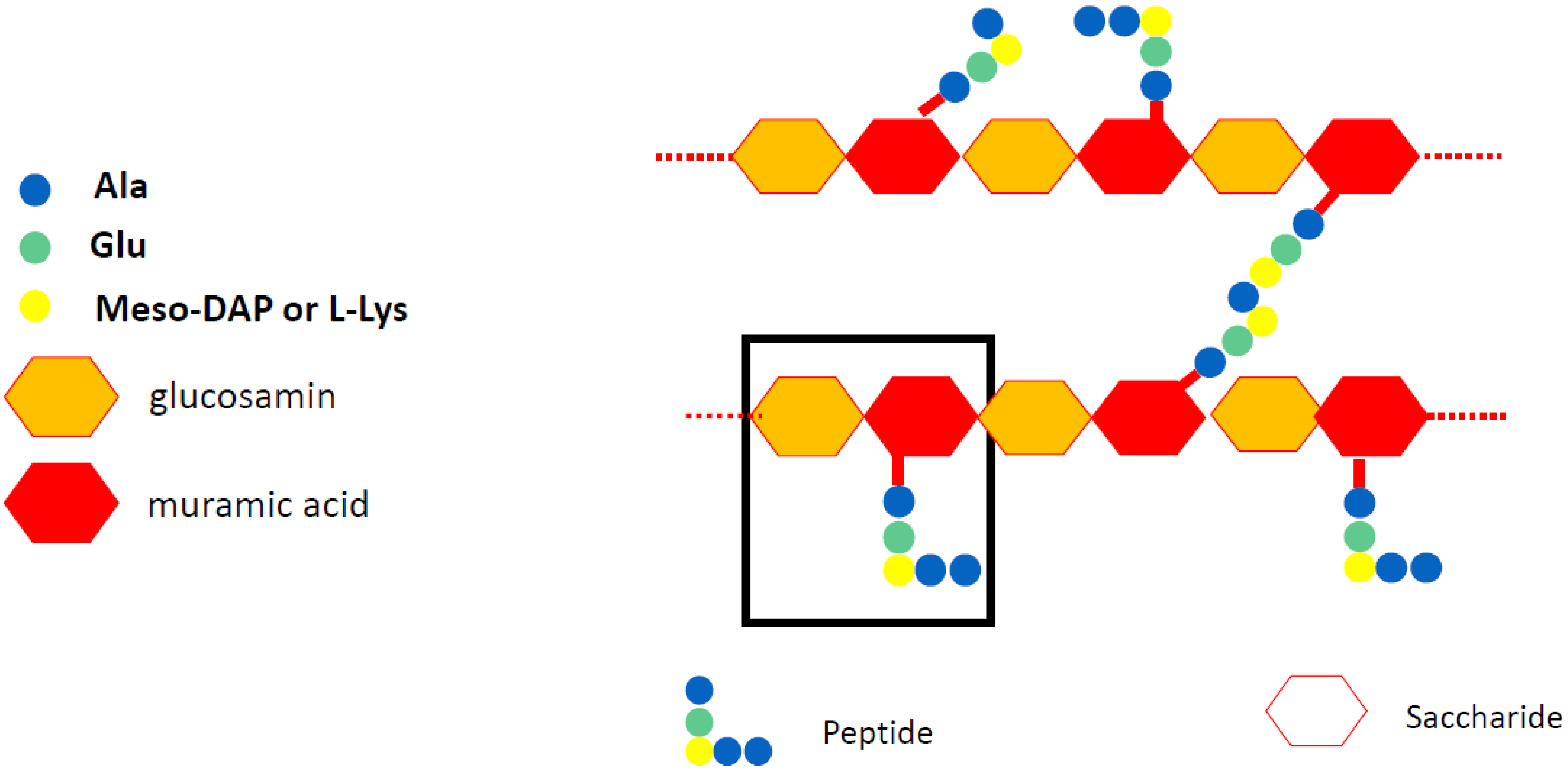


Vollmer W, Blanot D and Pedro MA, Microbiology reviews, 2008

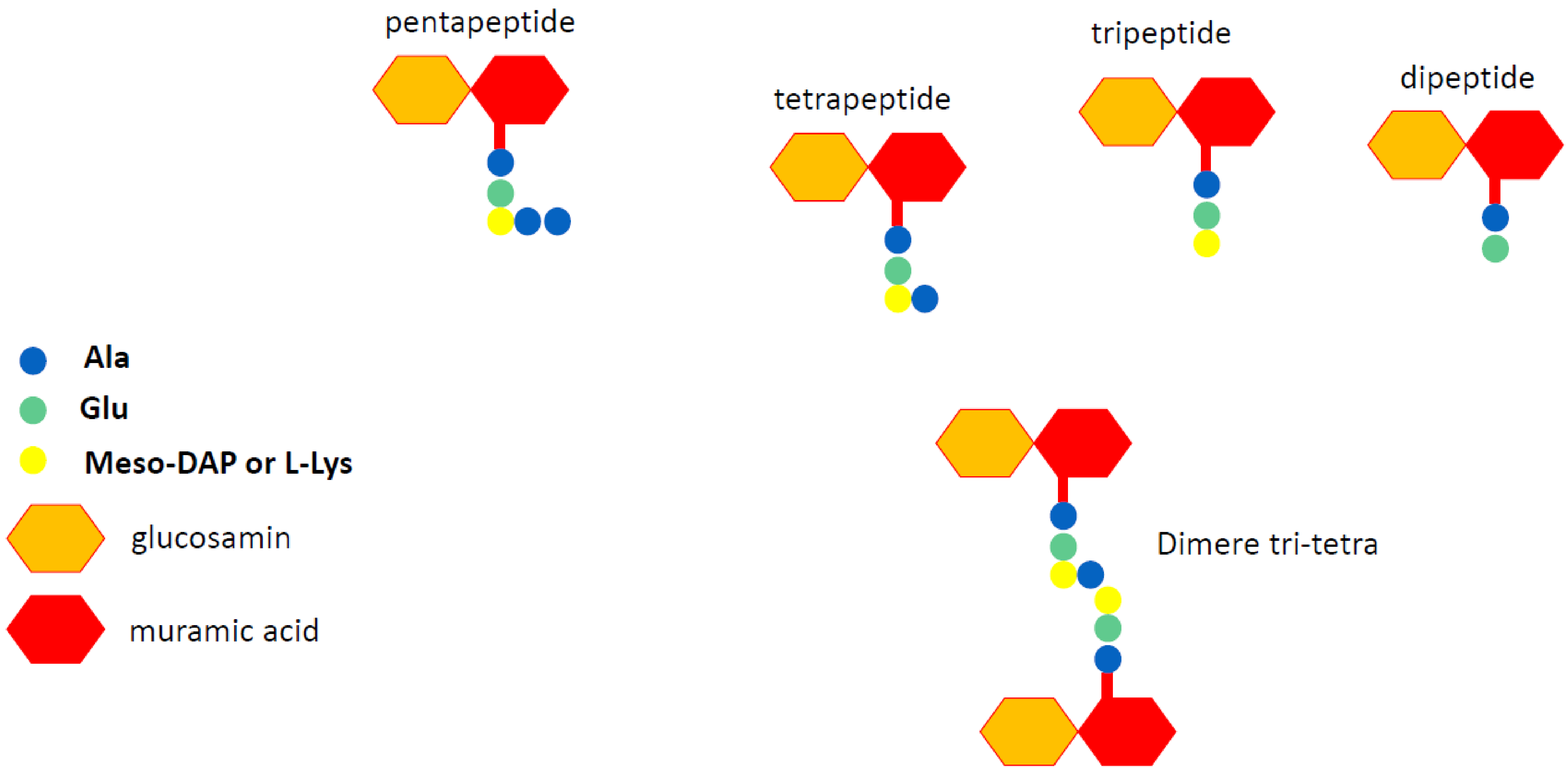


Matias VRF and Beveridge TJ, J. Bacteriol. 2006

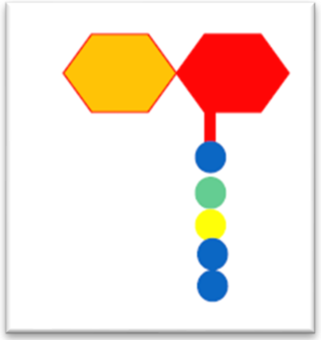
PG composition

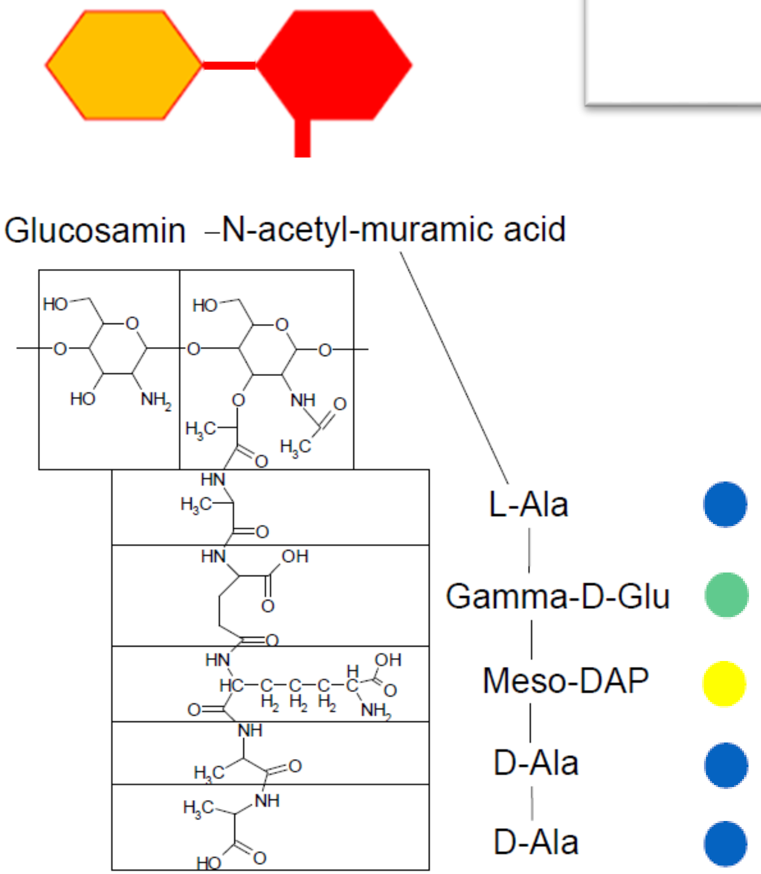


PG composition

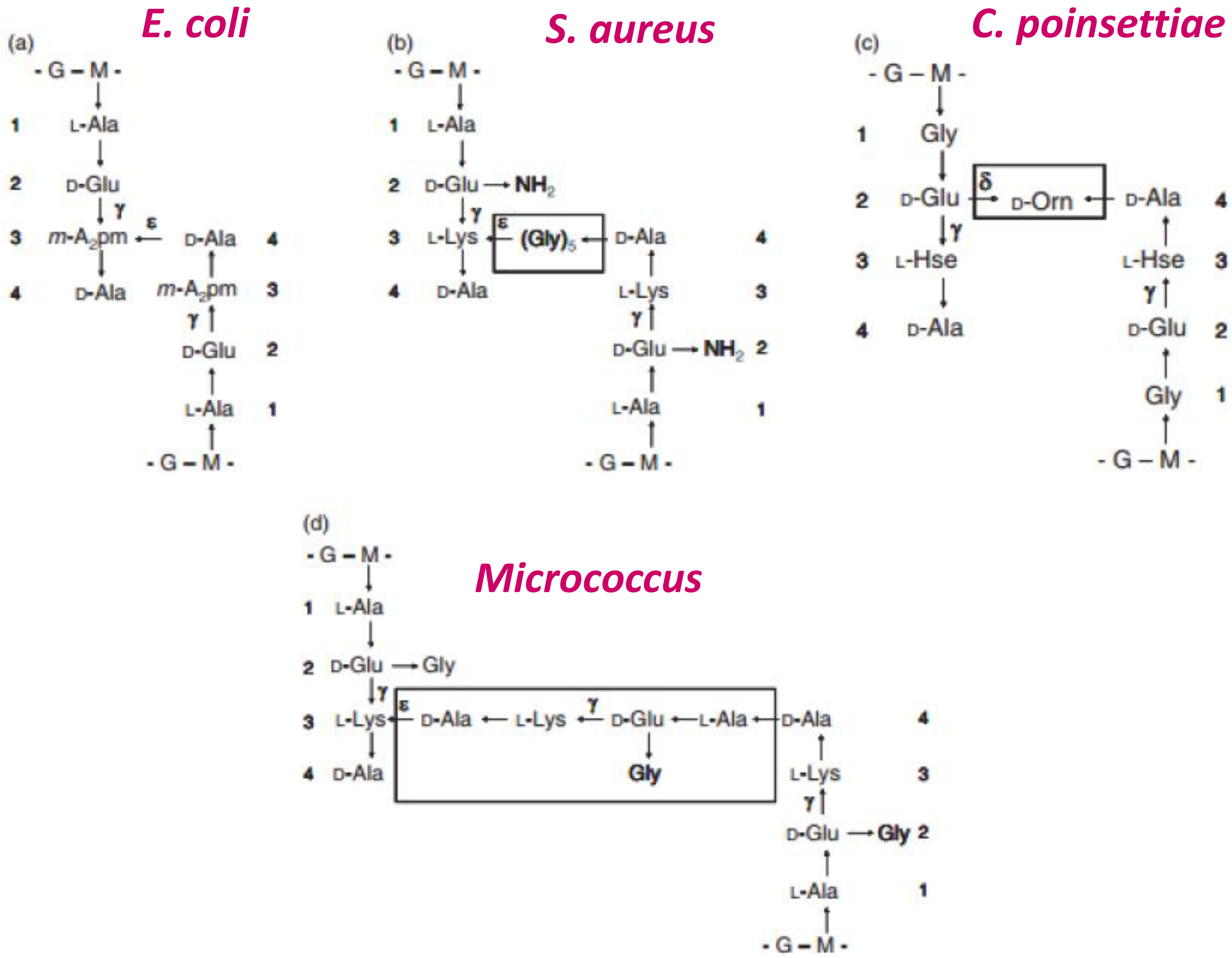


- **PG subunit**





- **PG cross-linkage**



Vollmer W, Blanot D and Pedro MA, Microbiology reviews, 2008

PG synthesis & cell division

- To devide, **PG synthesis and remodeling at the septum** is essential

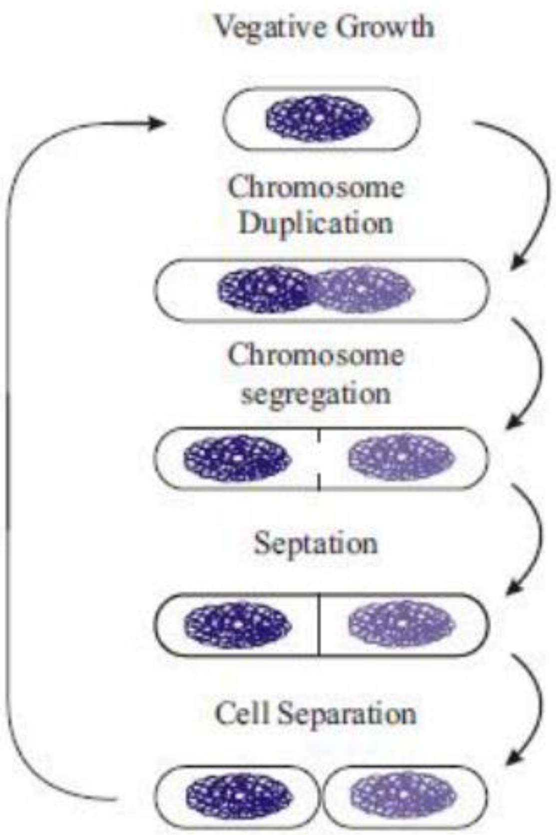
- Under the control of a multiprotein complex (**divisome**)

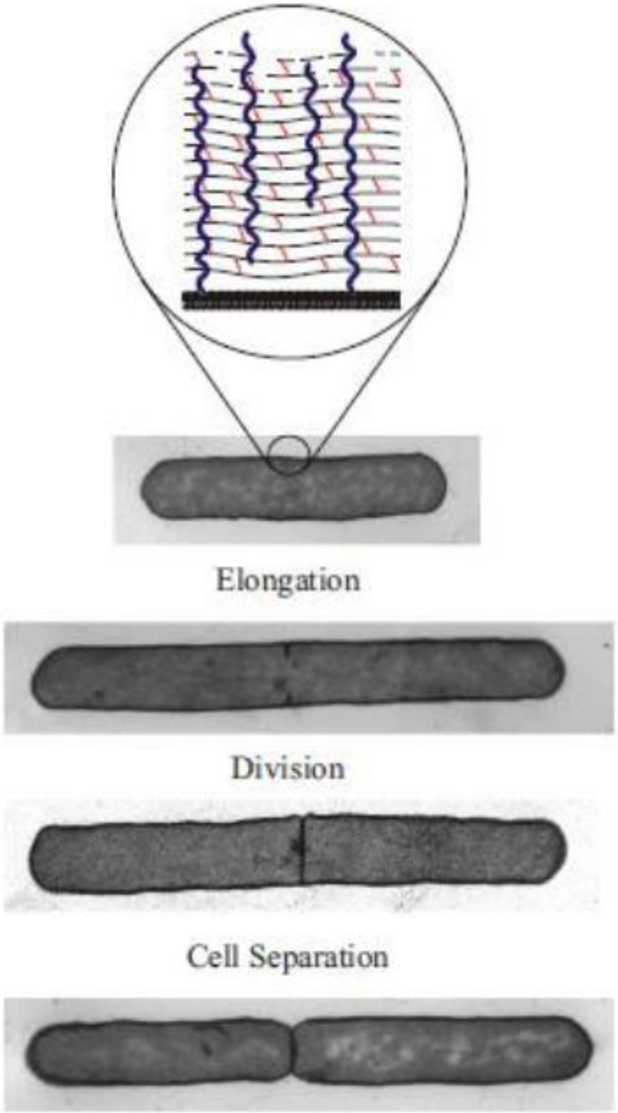
  → PG synthesis **concentrates at the mid-cell** (septum) driven by MreB (bacterial actin)

  → **fortifies the daughter cell poles**

- Antibiotics targeting PG synthesis

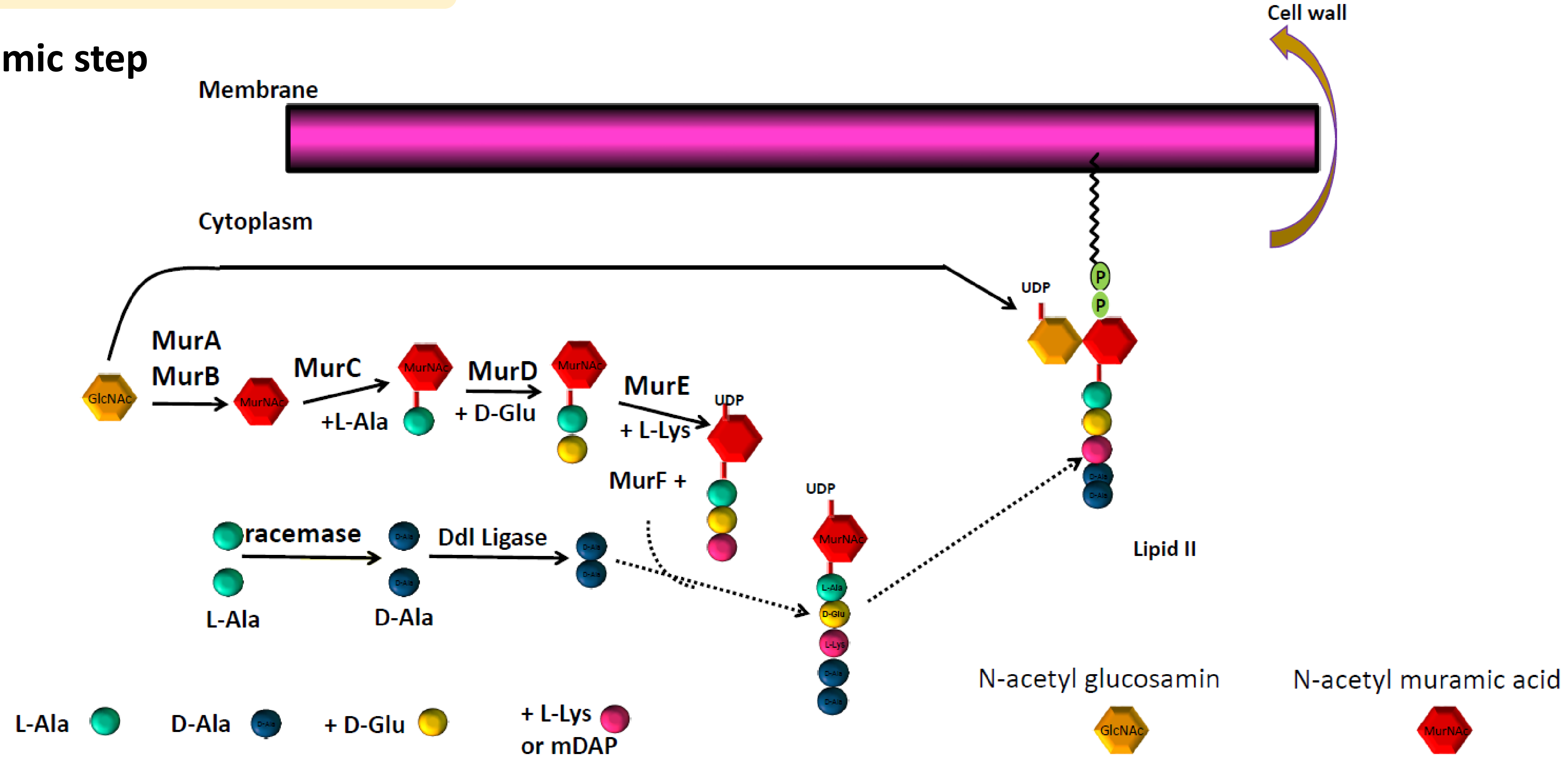
  → cell division stopped



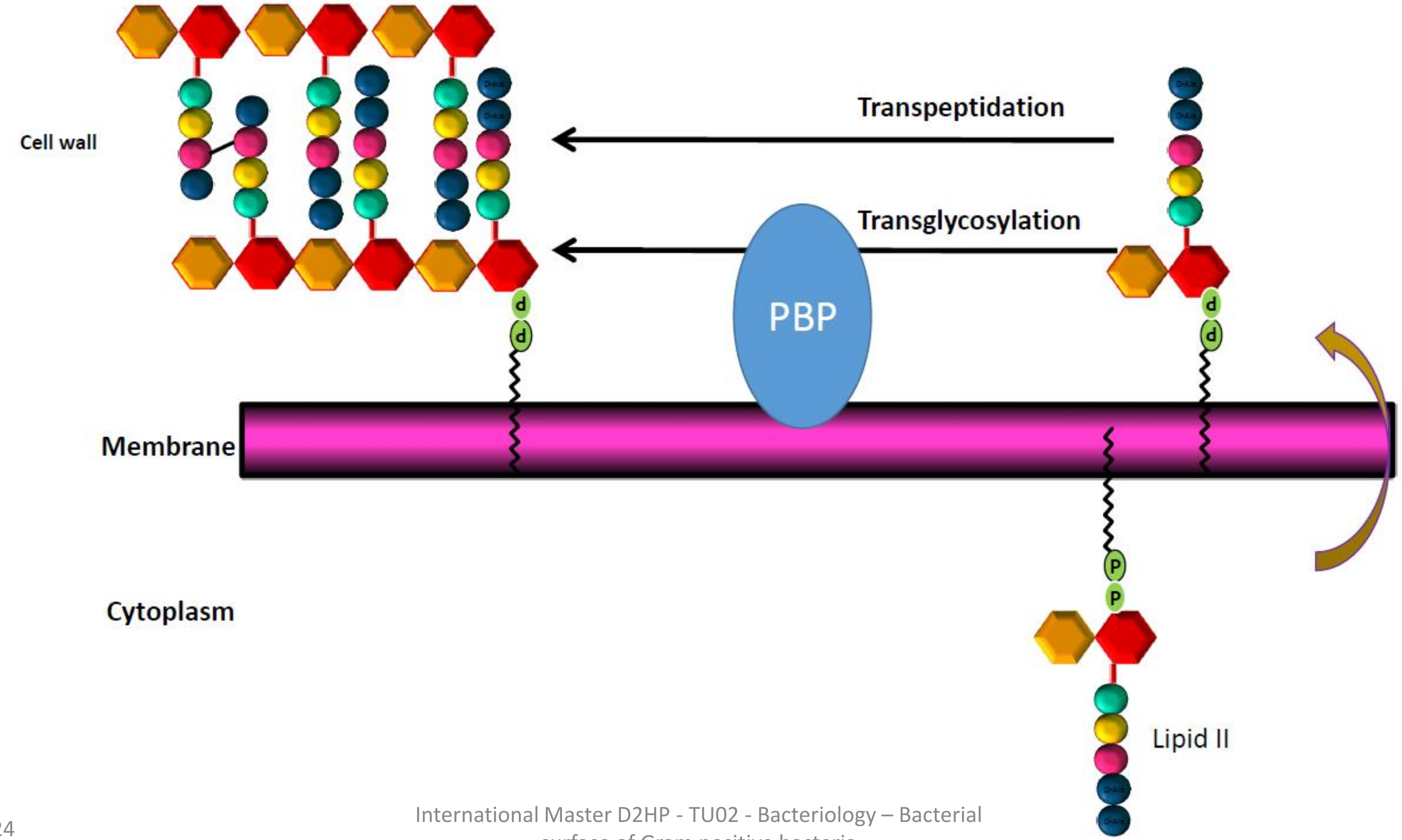


PG synthesis pathway

- **Cytoplasmic step**



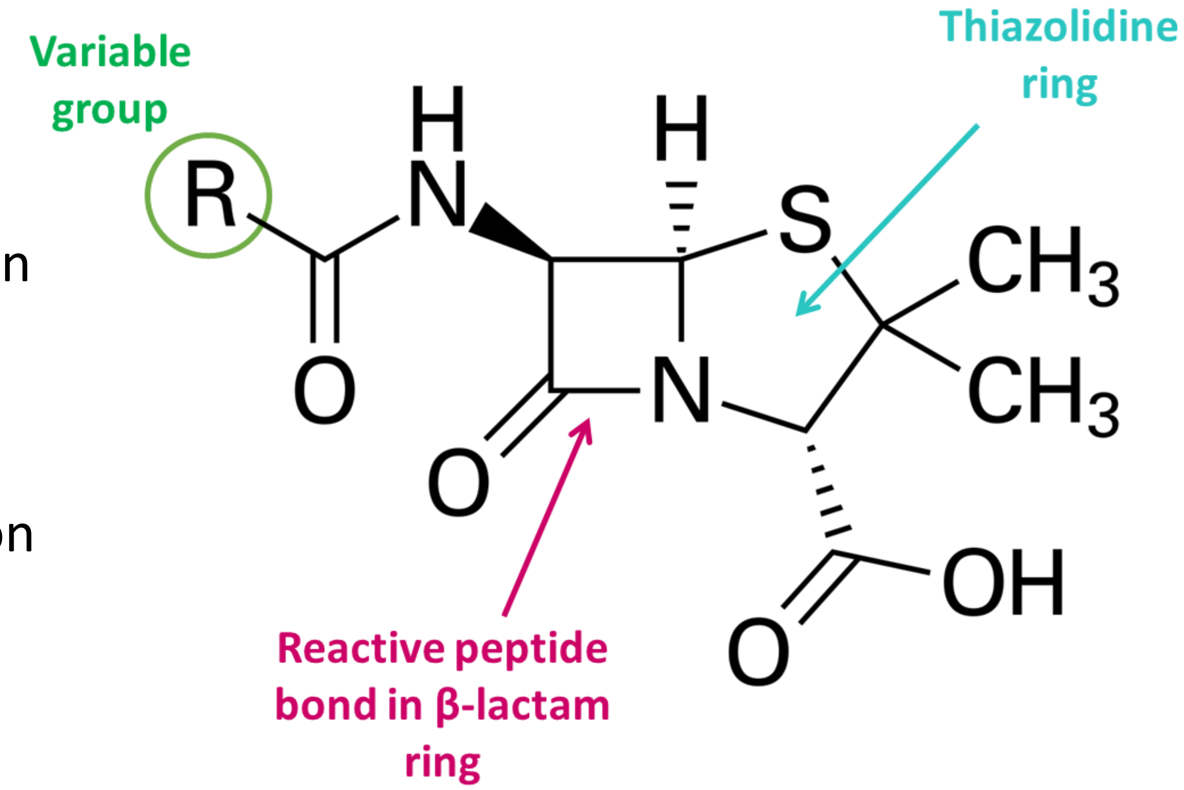
- **Extra-membrane step**



PG synthesis : target of antibiotics

1. Penicillins

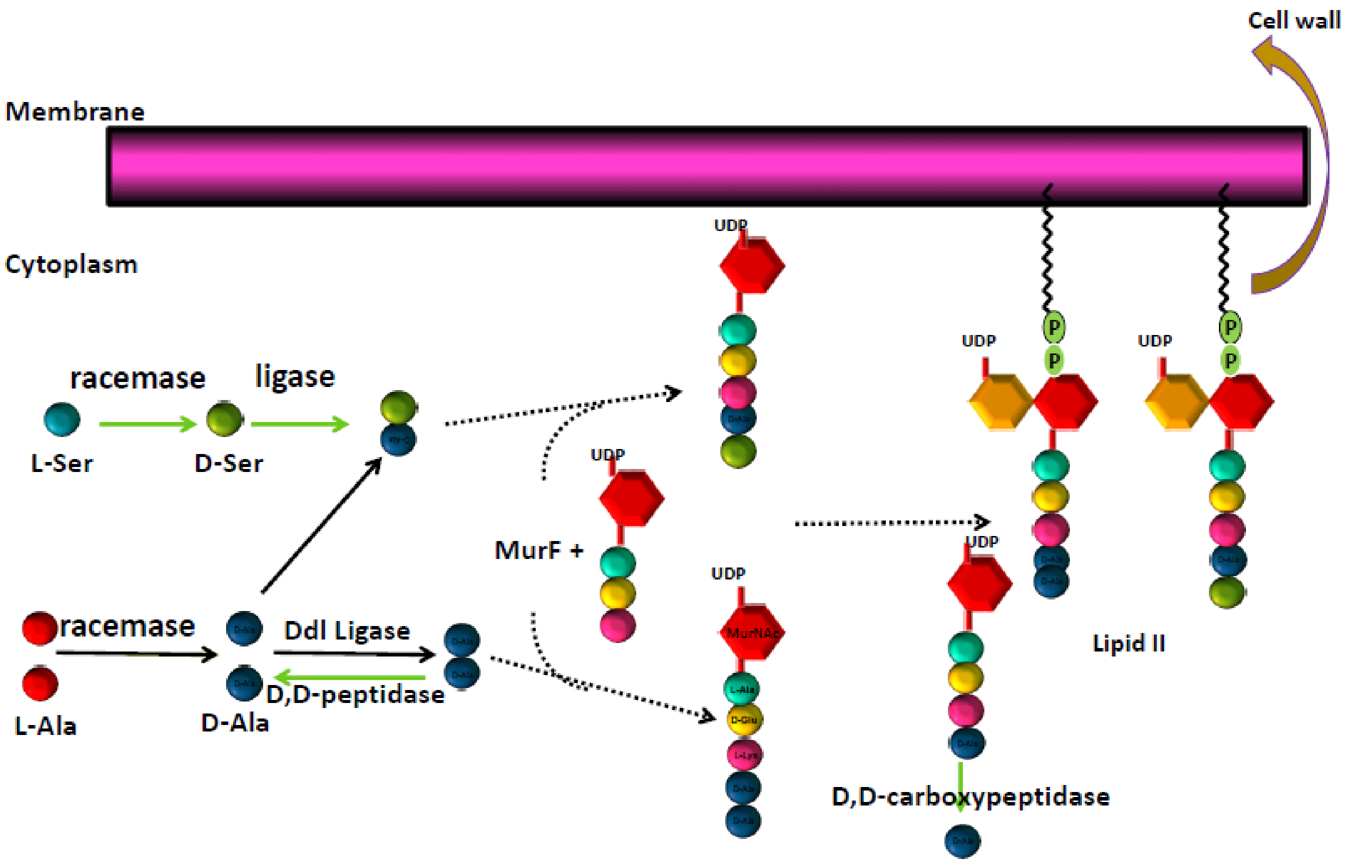
- **First discovered in 1928 by A. Fleming**
- **Structure** with two rings and a variable side chain
- **<u>Target</u> : Penicillin Binding Proteins**
  - ✓ Responsible for PG cross-linking / reticulation
- **Resistance mechanisms to penicillins**
  - ✓ β-lactamases synthesis
  - ✓ Selection of PBPs less sensitive to penicillins



PG synthesis : target of antibiotics

2. Glycopeptides

- **<u>Target</u> : D-Ala D-Ala peptide**
  - ✓Bind to the peptide
  - ✓Block cross-linking / reticulation of PG



- **Resistance mechanisms to glycopeptides**
  - ✓Target modification : affinity ↘

vanA & *vanB* : D-Ala D-Ala → D-Ala D-**lactate**

vanC : D-Ala D-Ala → D-Ala D-**Ser**

vanY (D,D-carboxypeptidase) : D-Ala D-Ala → **D-Ala**

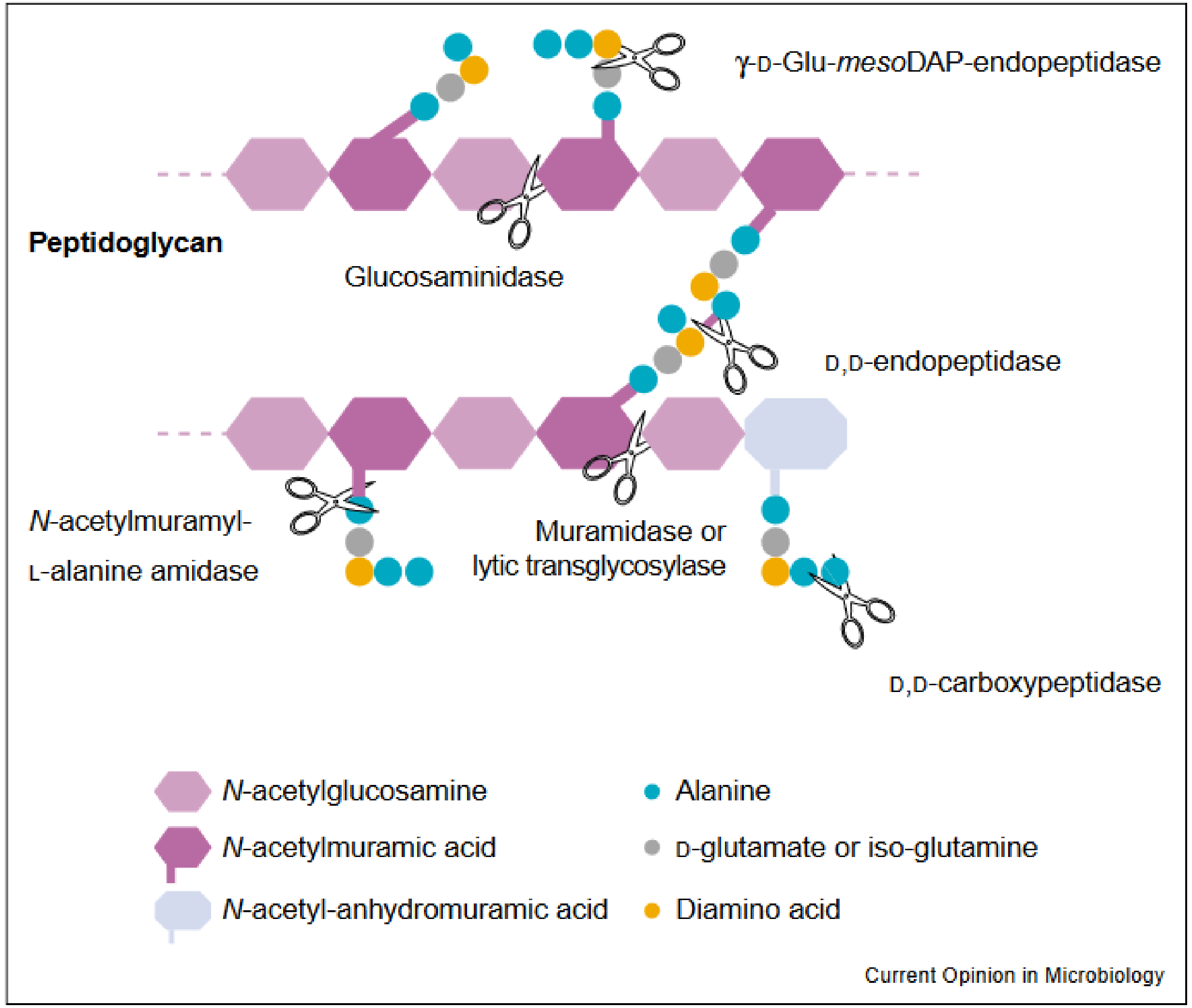
vanX (D,D-peptidase) : hydrolyzes peptidic link → ↘ **D-Ala D-Ala pool** → in favor of other dipeptides like D-Ala D-Ser or D-Ala D-lactate.

PG recycling

- **During growth and division**
  → PG layer is remodeled continuously

- **By lytic enzymes called PG hydrolases**

- **Each enzyme hydrolyzes one specific liaison**
  - ✓ Glucaminidase
  - ✓ γ-D-Glu-*meso*DAP-endopeptidase
  - ✓ D,D-endopeptidase
  - ✓ D,D-carboxypeptidase
  - ✓ Muramidase or lytic transglycosylase
  - ✓ N-acetylmuramyl-L-alanine amidase



Boneca I, Current Opinion in Microbiology, 2005

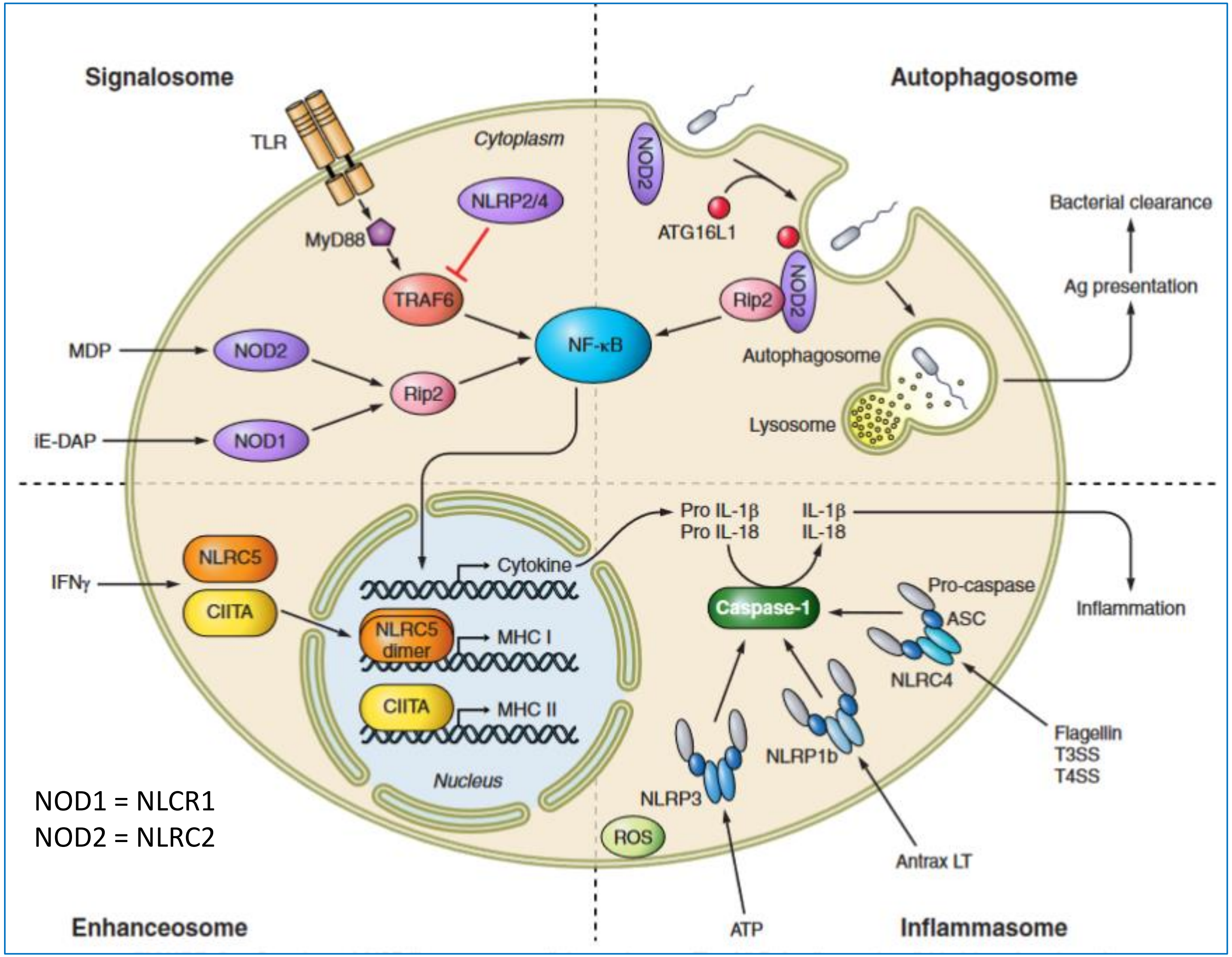
- **High number of PG fragments liberated**
  → Recycling involves their transport and reuse in other pathways

Host defenses against bacteria

1. Immune response to bacteria

- **Host detects bacteria via ≠ receptors from diverse cellular pathways**

- **Bacterial components stimulate ≠ receptors of NLR pathways**
    - ✓ Bacterial cell : NOD2
    - ✓ Flagellin : NLRC4
    - ✓ Secretion systems (T3/T4) : NLRC4
    - ✓ iE-DAP : NOD1
    - ✓ MDP : NOD2



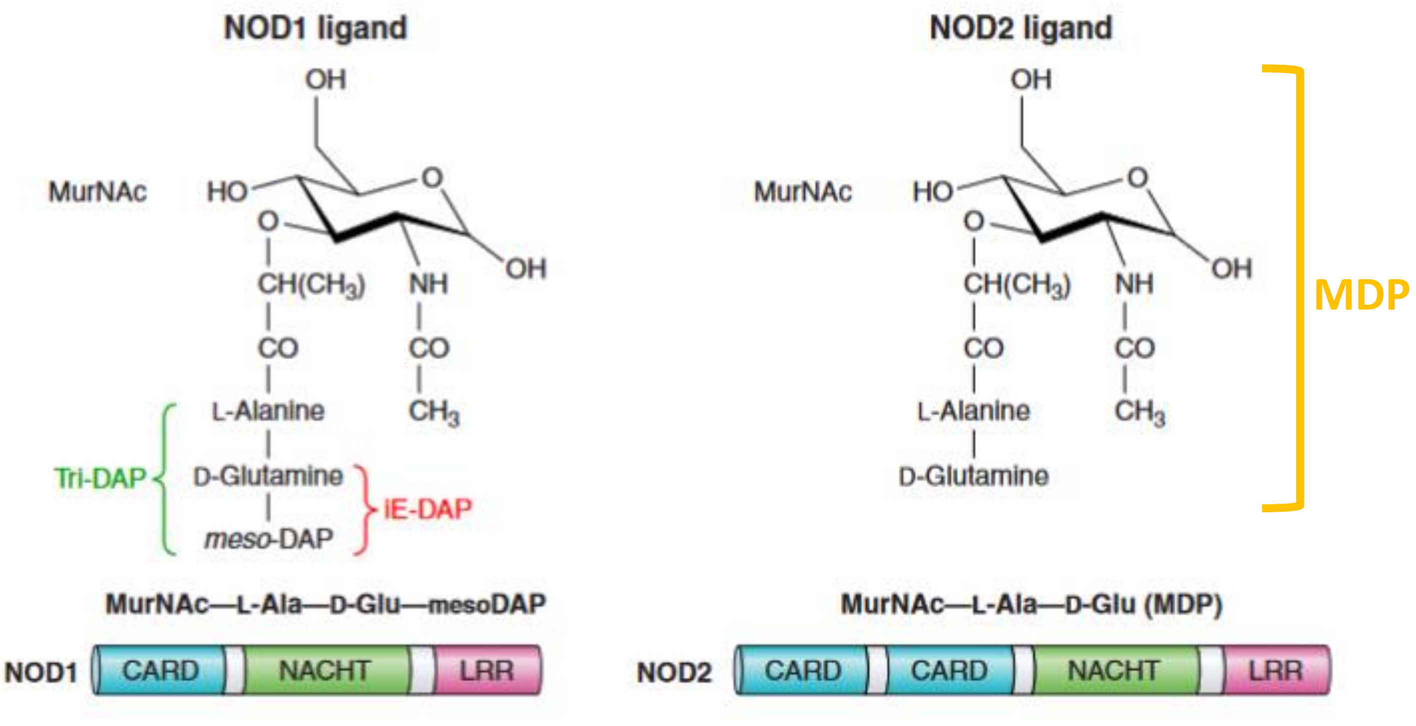
Motta V et al, Physiol Rev, 2015

Host defenses against bacteria

2. Immune response to Gram + PG

- **iE-DAP**
  - ✓ D-Glu-mesodiaminopimelic acid
  - ✓ Ligand of NOD1 receptor
- **MDP**
  - ✓ Muramyl dipeptide
  - ✓ Ligand of NOD2 receptor

Both lead to induction of NF-κB pathway
→ **inflammation**

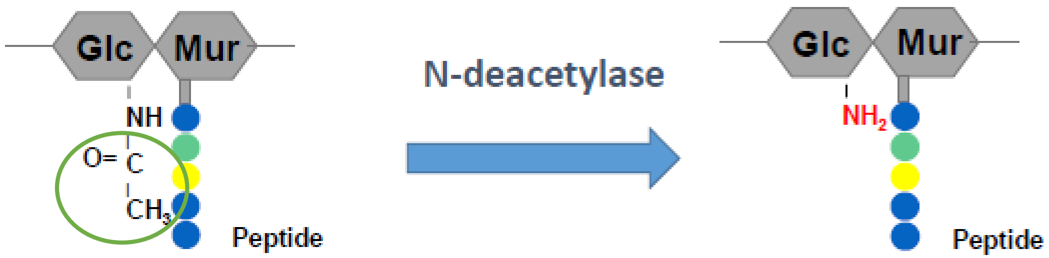


Motta V et al, Physiol Rev, 2015

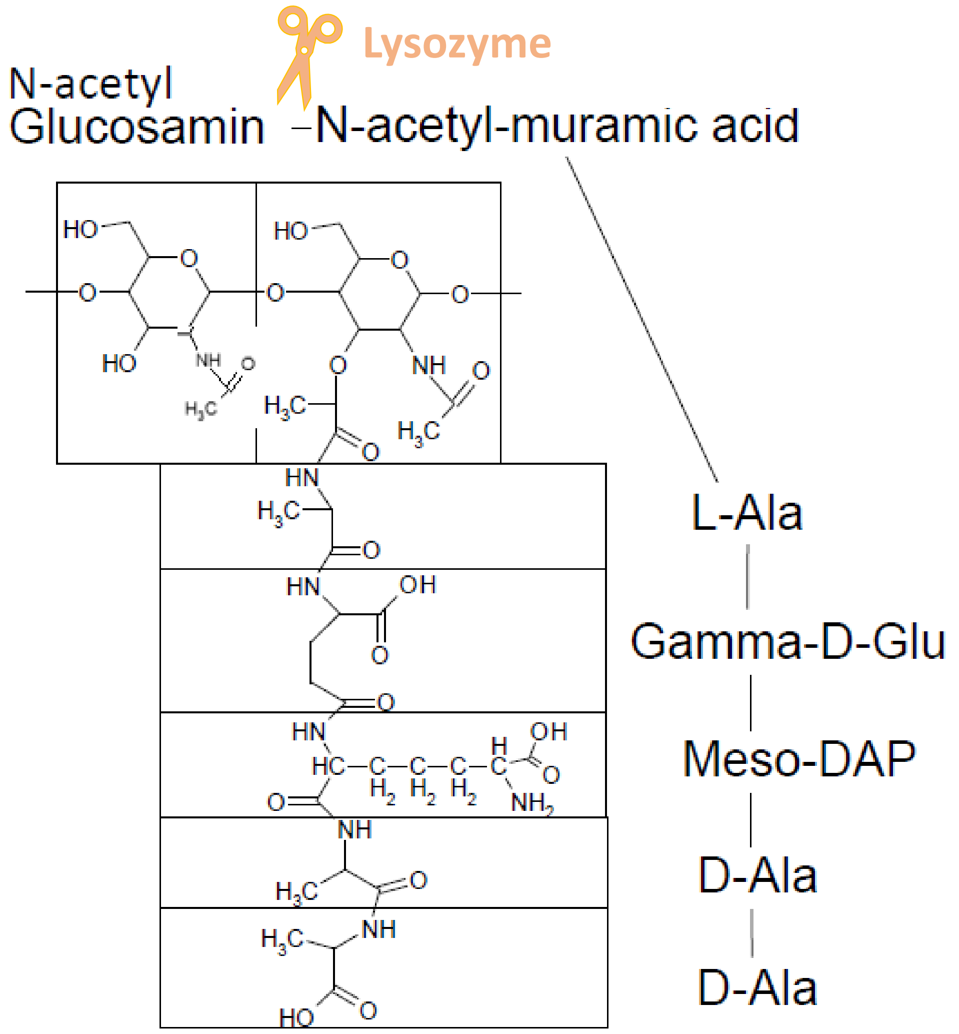
Host defenses against bacteria

3. Production of lysozyme

- **Eucaryote cells produce lysozyme that cleaves peptidoglycan**
  - ✓ Between the N-acetyl-glucosamin and the N-acetyl-muramic acid
- **Bacteria produce enzymes can modify PG to block lysozyme action**
  - ✓ N-deacetylases remove acetyl group



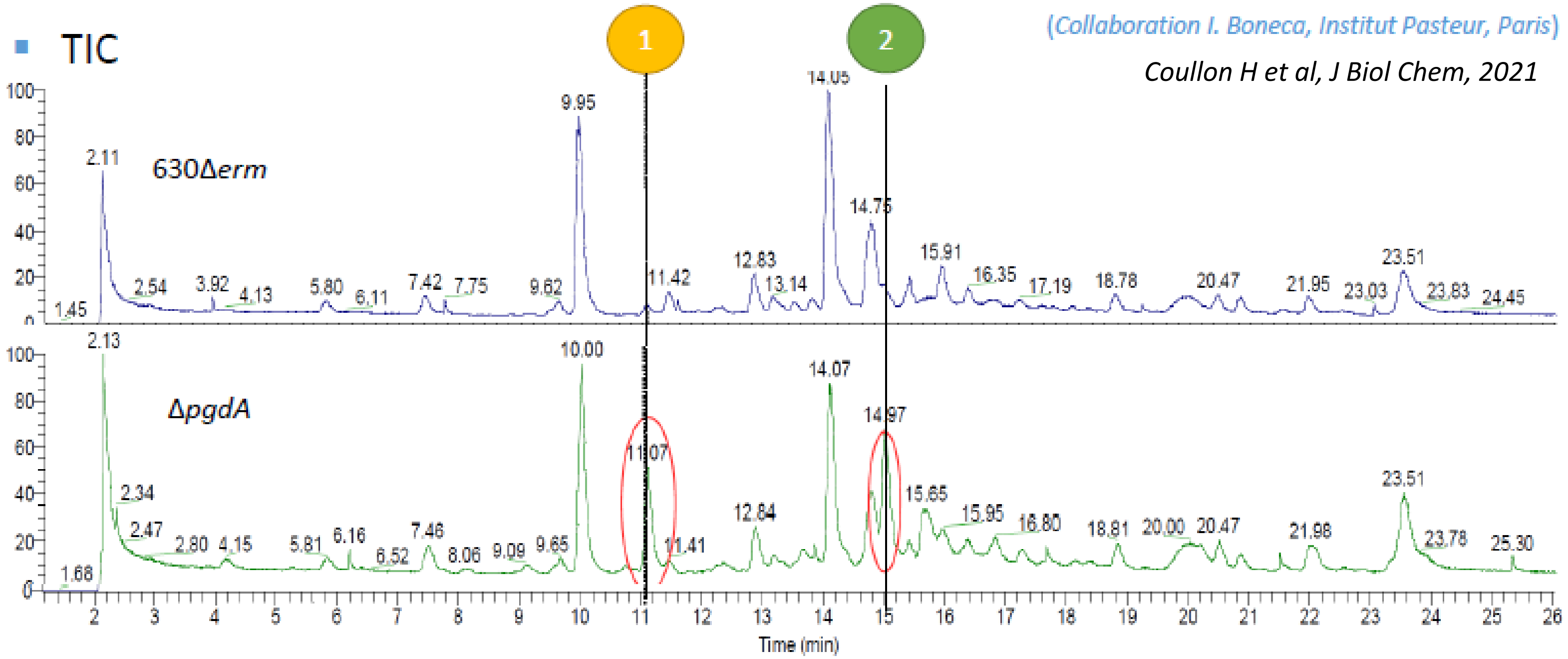
  - ✓ Modulation of host-pathogen interactions : immune system evasion, virulence, intracellular survival



- **Example of *C. difficile* defense against lysozyme : the PgdA N-deacetylase**

(Collaboration I. Boneca, Institut Pasteur, Paris)

Coullon H et al, J Biol Chem, 2021



	Structure	Variation
1	N-acetyl-glucosamine-tetrapeptide monomer	6 fold increase in *ΔpgdA*
2	tripeptide-tetrapeptide, mono-deacetylated dimer	10 fold increase in *ΔpgdA*

N-deacetylation	
630*Δerm*	97,45%
ΔpgdA	79,65%

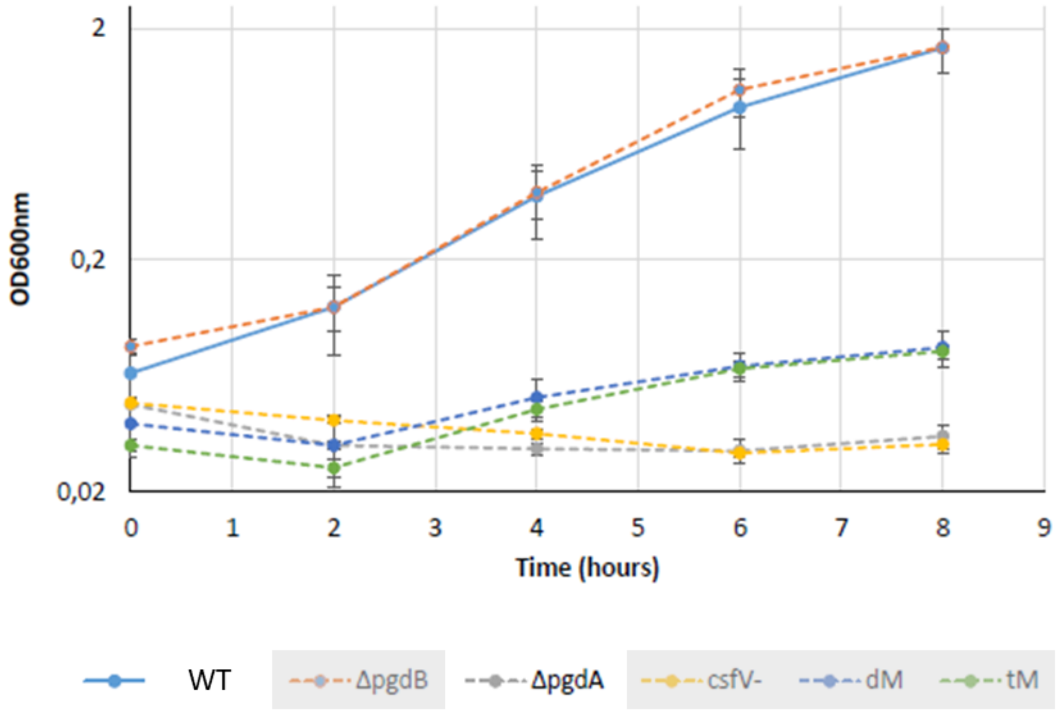
↘ 17,8%

- ✓ Increase quantity of N-acetyl-glucosamine monomers
- ✓ Major **decrease in PG N-deacetylation** in *ΔpgdA* mutant

→ Impact on lysozyme sensitivity ?

- **Example of *C. difficile* defense against lysozyme : the PgdA N-deacetylase**

Lysozyme sensitivity (1mg/mL)



MIC (mg/mL)	
WT	3,125
ΔpgdA	0,78
ΔpgdB	3,125
csfV-	0,78
csfV-ΔpgdA	< 0,024
csfV-ΔpgdAΔpgdB	<0,024

✓ Major **decrease in PG N-deacetylation** in *ΔpgdA* mutant

→ Major increase in lysozyme sensitivity in *ΔpgdA* mutant

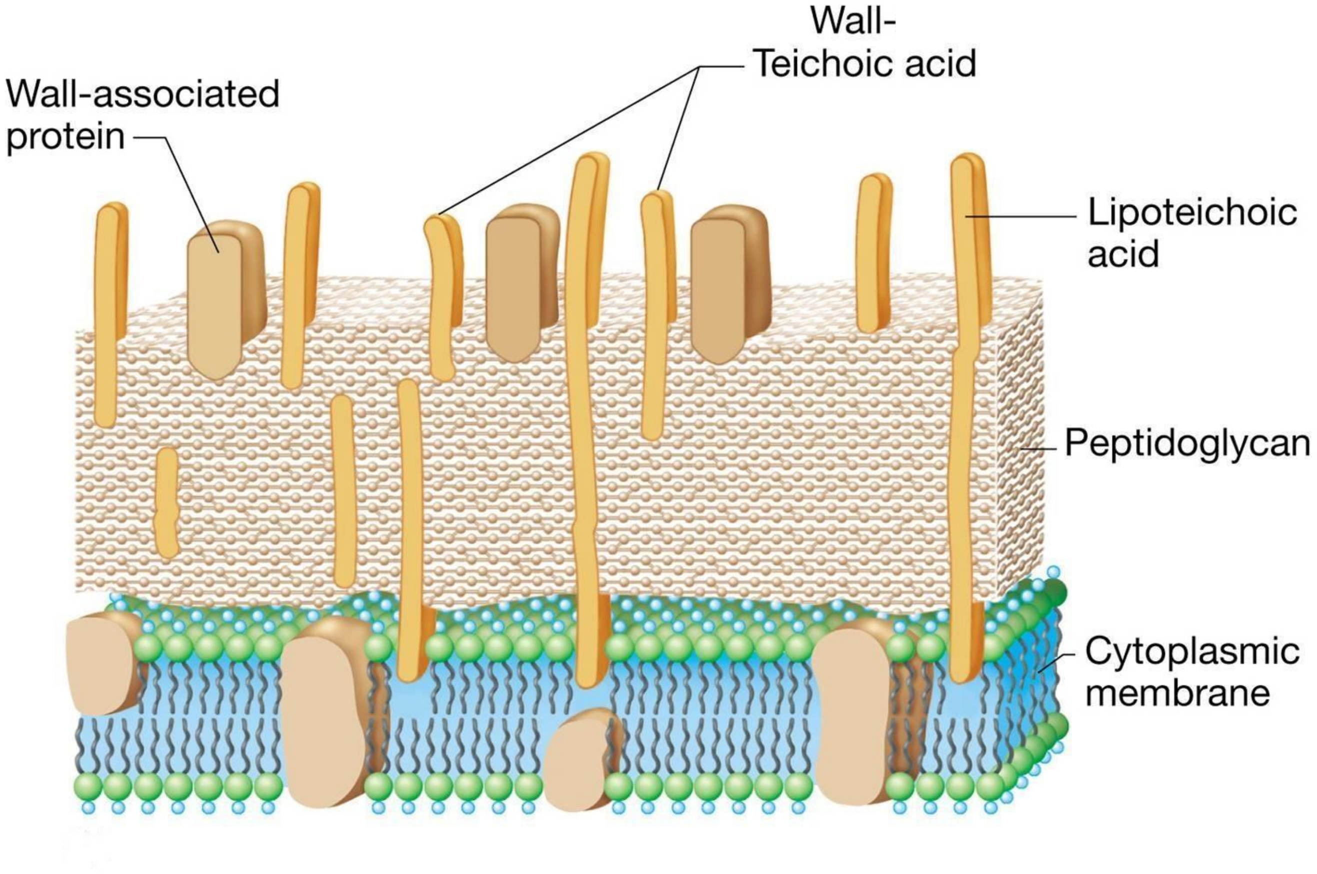
Coullon H et al, J Biol Chem, 2021

N-deacetylases are involved in **bacterial resistance to host defenses** (lysozyme)

PS in Gram positive bacteria

- **Teichoic acids**
  - ✓ **Wall teichoic acids (WTA)**
    - ❑ Anionic polymers
    - ❑ Polyol repeat units linked via a phosphodiester bond
    - ❑ Covalently bound in the PG
  - ✓ **Lipoteichoic acids (LTA)**
    - ❑ Zwiterrionic polymers
    - ❑ Anchored in the cytoplasmic membrane
    - ❑ Via a glycolipid anchor
- **Capsular polysaccharides (CPS)**

For capsulated bacteria



Tankeshwar A, Microbe online

Nature and functions

Array of proteins on the cell surface

- **Mediate interactions with host : colonisation / virulence factors**
  - ✓ Adhesins
  - ✓ Proteases
- **Involved in peptidoglycan modification : PG hydrolases (autolysins)**
  - ✓ Glucosaminidases
  - ✓ Transpeptidases
  - ✓ Glycohydrolases
- **Part of cell-wall components**
  - ✓ S-layer proteins

Surface proteins anchoring

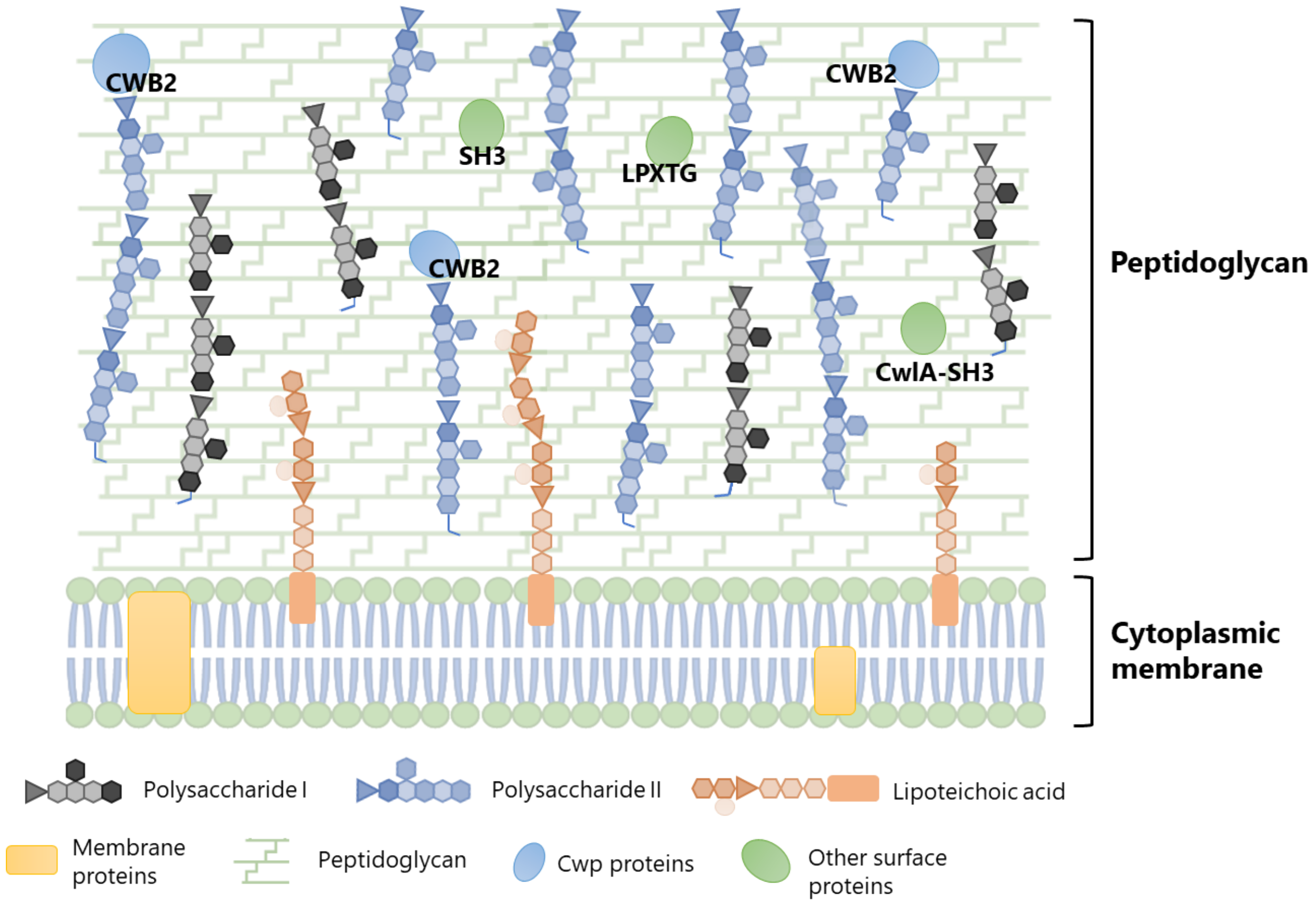
- **Anchored in the PG**
  - ✓ Covalently
    - ❑ Via LPXTG motif recognised by sortases
  - ✓ Non-covalently
    - ❑ Via LysM motifs, SPOR domains, WXL domains, SH3b domains (notably GW)

- **Anchored to the surface polysaccharides**
  - ✓ Covalently
    - ❑ Via SLH motifs
  - ✓ Non-covalently
    - ❑ Via CWB1 or CWB2 domains

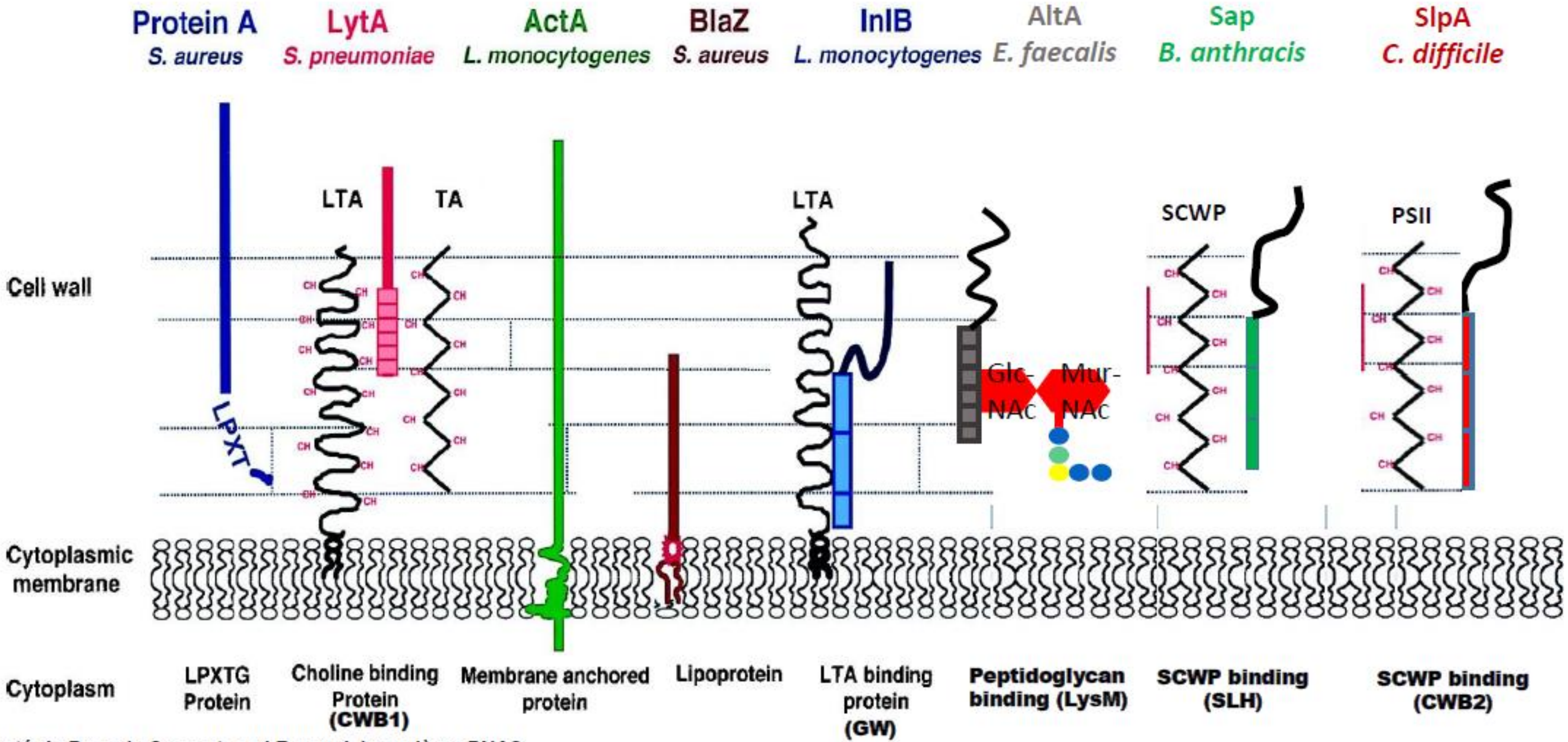
- **Anchored directly in the membrane**

Membrane anchored proteins, lipoproteins

Example in *C. difficile*



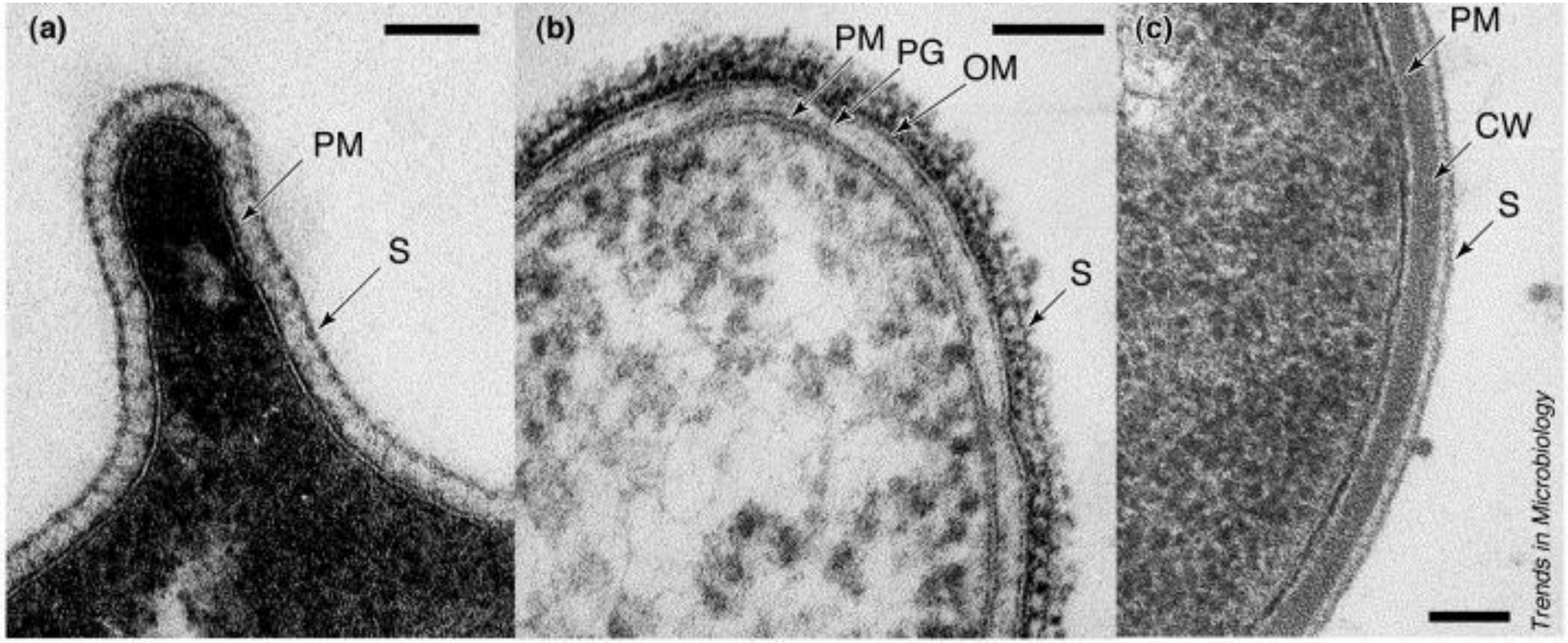
Surface proteins anchoring : examples



Adapté de Pascale Cossart, and Renaud Jonquières PNAS 2000;97:5013-5015

S-layer in the micro-organisms world

- S-layer = **most external layer** of the **cell envelope** of micro-organisms
- Can be found in some archaea, gram positive and gram negative bacteria

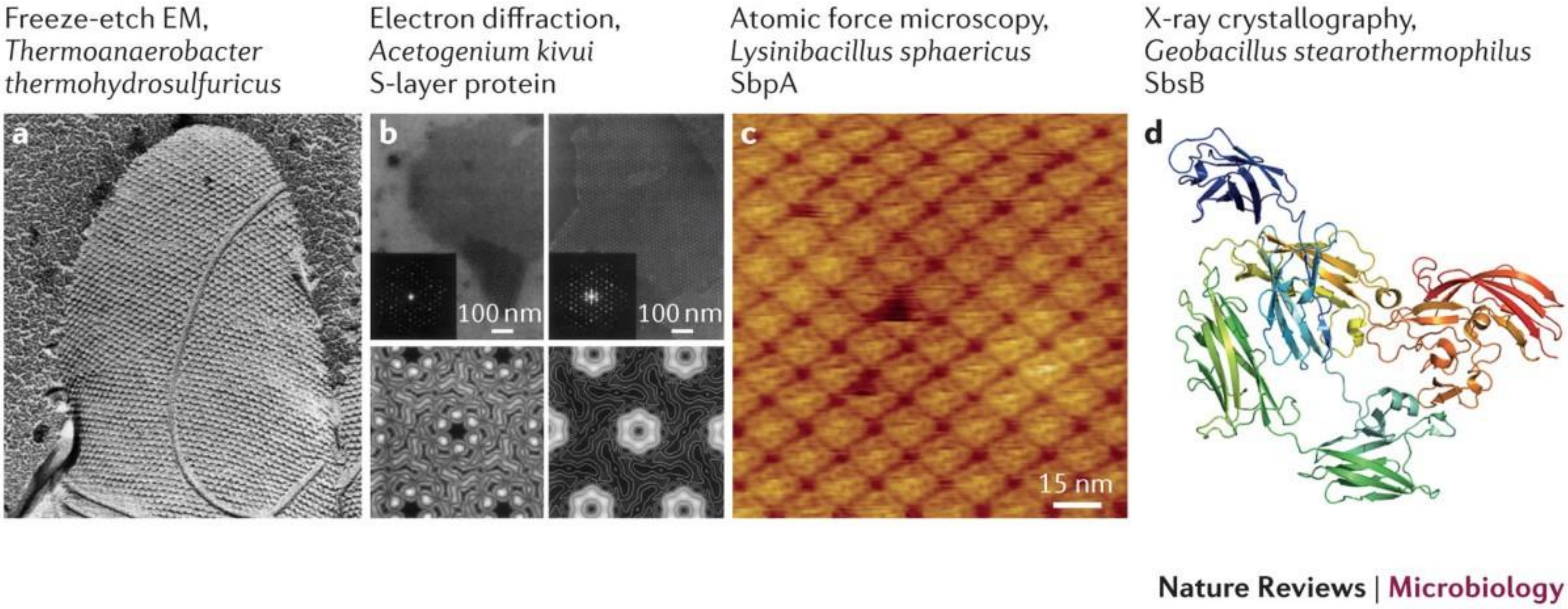


Archaea | *Gram negative bacteria* | *Gram positive bacteria*

Sleytr UB, Beveridge TJ, 1999

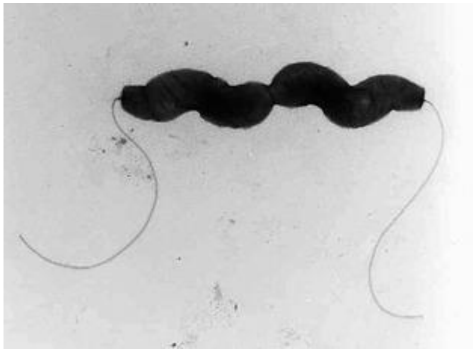
S-layer : structure

- S-layer = **self-assembled bidimensional crystalline protein network**

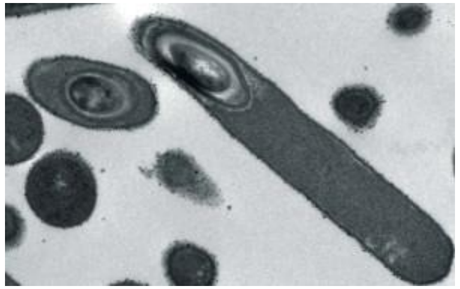


Fagan and Fairweather, 2014

S-layer : genetic diversity



- Genetic variation in SLPs expression
- Examples :
  - ✓ *Campylobacter fetus* serotype A
    - ❑ Genome contains **8 homologues for *sapA* gene** and 1 promotor element
    - ❑ High frequency of **chromosomal rearrangements** (DNA inversion & recombination)
      - → **Phenotypic switching** and expression of different *sap* homologues
      - → Antigenically **distinct S-layer**
  - ✓ *Clostridioides difficile*
    - ❑ S-layer composed of 2 proteins : **HMW-SLP** and **LMW-SLP**, resulting of proteolytic cleavage of SlpA precursor
    - ❑ LMW-SLP → considerable **sequence variability** among strains
    - ❑ 12 divergent cassettes encoding SlpA found in ≠ strains
    - ❑ Recombinational switching in populations → antigenic diversity



S-layer : from proteins to functional S-layers

- To be functional, **SLPs** need to :
  - be **transported to cell surface**
  - **autoassemble** in S-layer structures
  - be **anchored** to cell wall

E.g : in *C. difficile*, S-layer estimated to contain 500,000 subunits → requires **secretion of 140 subunits/sec/cell** during exponential growth !

- Secretion of SLPs

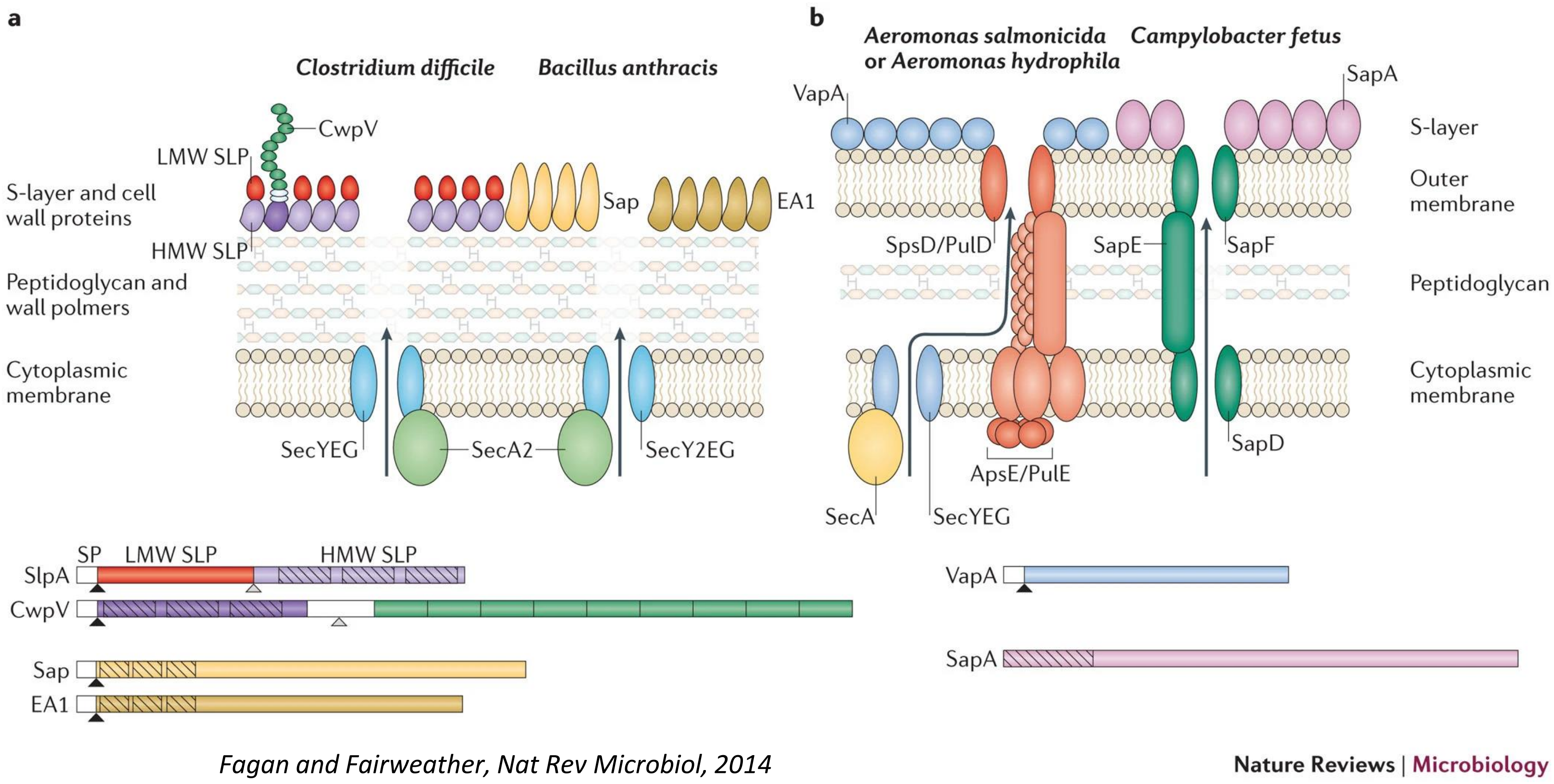
→ **huge challenge** in bacteria because **large quantity** of SLPs needed to form contiguous S-layer

→ **Secretion mechanisms have evolved** to cope with this high protein flux

→ In many Gram-negative species, S-layer secretion relies on **dedicated secretion system**

S-layer : from proteins to functional S-layers

Examples of secretion machineries



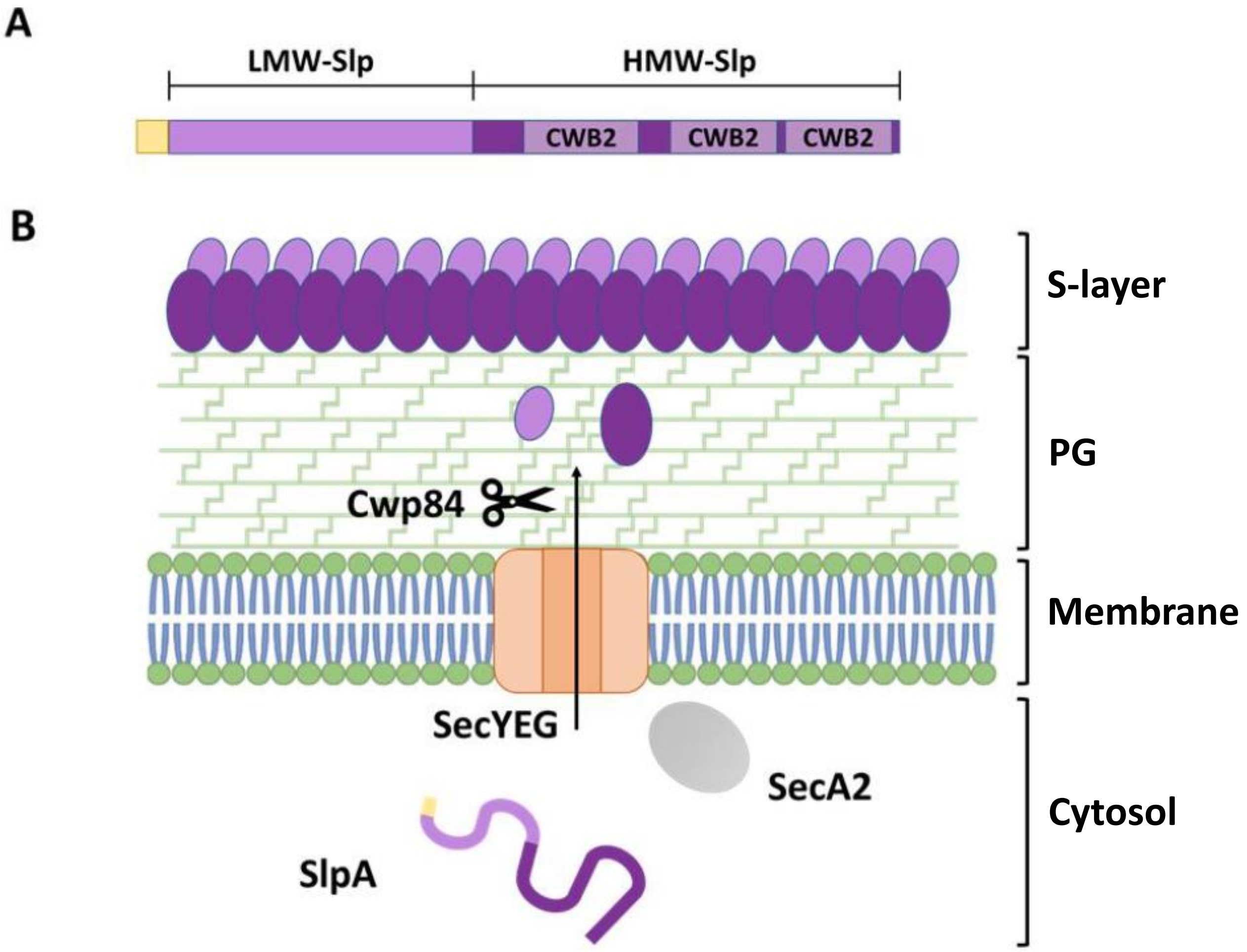
Fagan and Fairweather, Nat Rev Microbiol, 2014

- ✓ *Clostridioides difficile* and *Bacillus anthracis* : **SecA1 and SecA2 ATPases, SecYEG complex (accessory Sec secretion system)**
- ✓ *Aeromonas hydrophila / Aeromonas salmonicida* : **SpsD/PulD and ApsE/PulE (homolgues to T2SS)**
- ✓ *Campylobacter fetus* : **SapDEF (homologues to T1SS) + SapC (unique)**

S-layer : from proteins to functional S-layers

Example of *C. difficile*

- **Recognition** of SlpA (S-layer precursor)
  → by **SecA2**, part of Sec protein translocase system
- **Translocation** through cytoplasmic membrane
  → via **SecYEG** canal
- **Cleavage** of SlpA = SLP-HMW + SLP-LMW subunits
  → by **Cwp84** protease
- **Autoassembly** in heterodimer
- **Incorporation** to S-layer



S-layer : roles

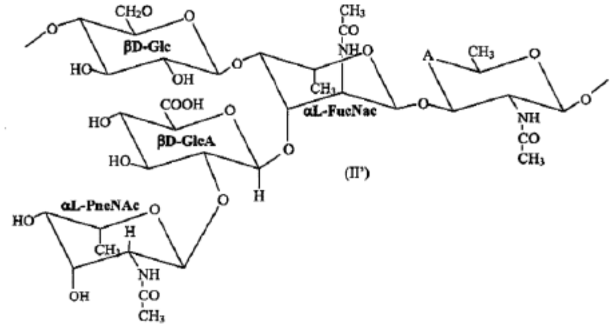
- S-layers structures and functions remain a **land of wonder**
  → no demonstrated roles, only hypotheses

- **Probable roles** of S-layer proteins :
  - ✓ In bacterial physiology :
    - ❑ **Maintenance of cell integrity** : abnormal S-layer → shedding of surface proteins
    - ❑ **Permeability barrier** : demonstrated in *B. coagulans*
    - ❑ **Cell division** : shown in *B. coagulans* and *B. anthracis*
  - ✓ In infection : interaction with host and its immune system
    - ❑ **Adhesin activity** : BslA of *B. anthracis*, Slp of *C. difficile*
    - ❑ **Aggregation and biofilm formation** : in *C. difficile* (aggregation), in *Tanerella forsythia* (biofilm)
    - ❑ **Protection from phagocytosis** : in *C. difficile* SlpA interacts with TLR4, in *C. fetus* Slp prevents binding of C3b complement factor
      → in both cases, blocks phagocytosis and killing

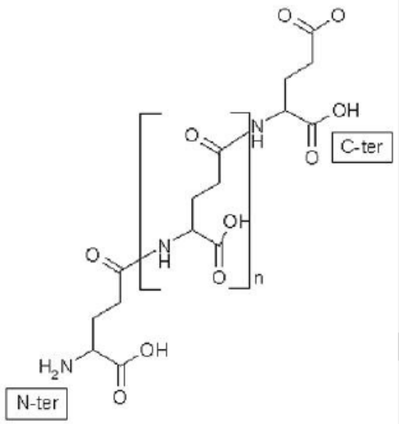
Capsule : composition and anchoring

- **Composition**

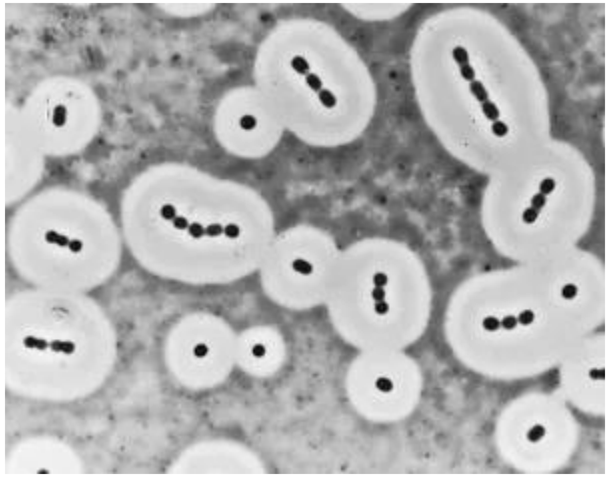
  - **Polysaccharides** (glycanes sometimes called polyosides)
    - ✓ <u>Example</u> : capsule of *Streptococcus pneumoniae*
    - ✓ Long chains of one ose and uronic acids (galacturonic or glucuronic acids)



  - **Polypeptides**
    - ✓ Example of *Bacillus anthracis*
    - ✓ Poly-γ-D-glutamic acid



poly- γ D-glutamic acid



Capsule of Acinetobacter calcoacetius, Lederberg J

- **Anchoring**

  In gram +, anchored in the peptidoglycan

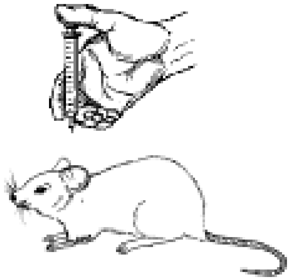
Roles

- **Escape from host immune system**
  - ✓ By blocking the phagocytosis
- **Masks the bacterial epitopes**
- **Used for vaccine production → purified capsular polysaccharides as antigens**
  - ✓ *Streptococcus pneumoniae* : Prevenar 13 vaccine, Pneumovax 23
  - ✓ *Haemophilus influenzae* : ActHib, InfanrixHexa, Vaxelis..

Example of *B. anthracis* capsule

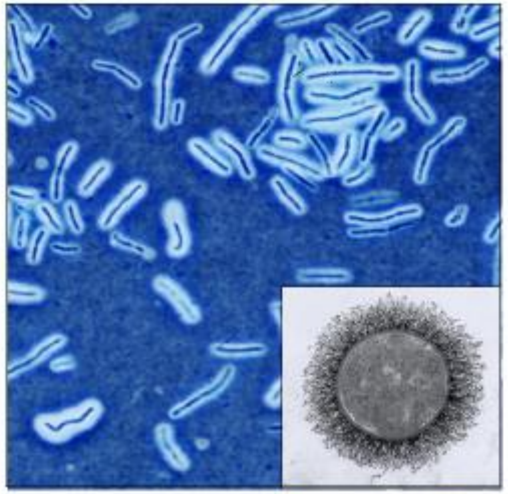
- **Composed of polyglutamate, not immunogenic**

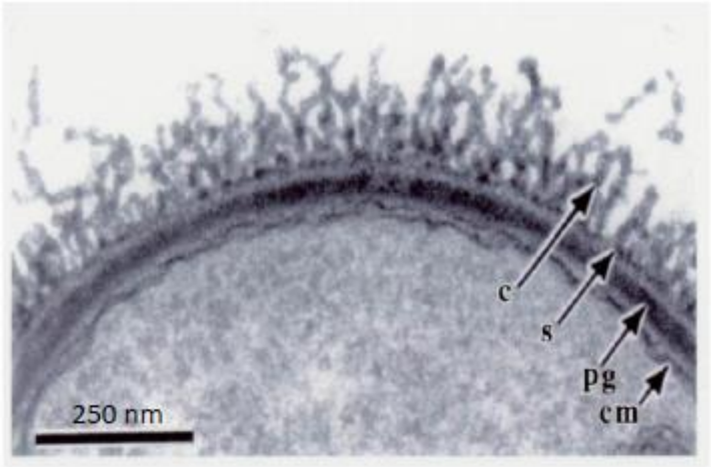
polyglutamate alone → No response directed against polyglutamate



polyglutamate linked to BSA → Antibodies specifically directed against polyglutamate

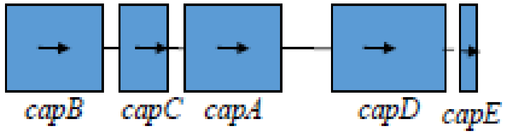
- **Synthetized thanks to *capABCDE* genes**





Example of *B. anthracis* capsule

- **Polyglutamate capsule presence and anchoring**



	capB	*capC*	*capA*	*capD*	*capE*	Polyglutamate	
Wild type						+	
capB mutant	-	+	+	+	+	-	
capC mutant	+	-	+	+	+	-	
capA mutant	+	+	-	+	+	-	
capD mutant	+	+	+	-	+	+	not anchored
capE mutant	+	+	+	+	-	-	

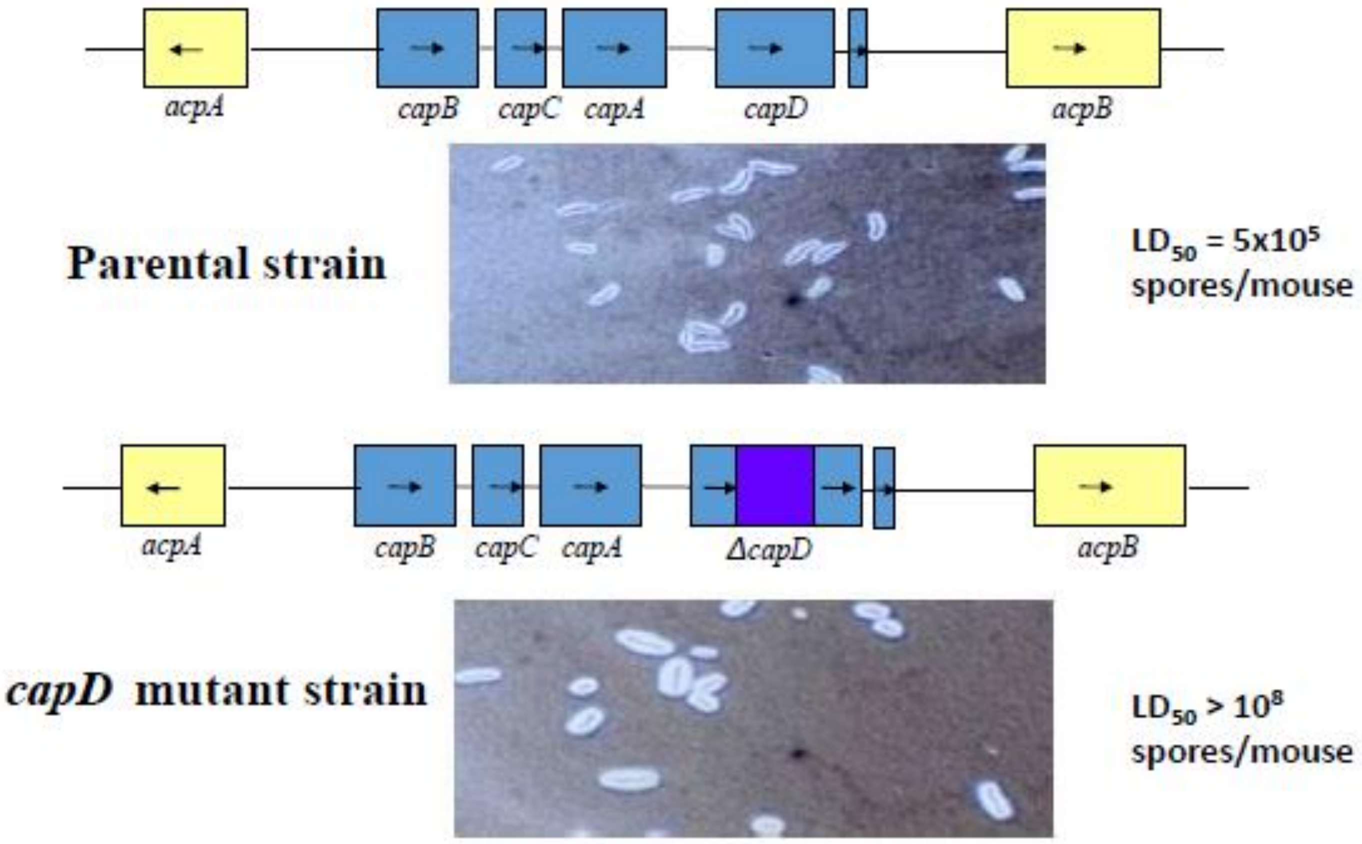
CapA, CapB, CapC, CapE → involved in polyglutamate **synthesis**
CapD → involved in polyglutamate **surface anchoring**

Example of *B. anthracis* capsule

- **Role of the capsule anchoring in the virulence of *B. anthracis***

LD50 = dose required to kill half the members of a tested population

capD mutant → polyglutamate capsule not anchored → **LD50 is much higher**



CapD → decreased virulence in mouse model

Take home messages

- **Bacterial surface structures**
  - ✓Gram negative: see precedent course
  - ✓Gram positive
    - ❑ **PG** : roles, composition, synthesis, antibiotics targeting PG, PG recycling, host defenses
    - ❑ **Polysaccharides** : WTA and TA
    - ❑ **Surface proteins** : nature, functions, anchoring
    - ❑ **S-layer** : definition
    - ❑ **Capsule** : composition, roles and examples