11 Bacterial glycosylation

Bacterial polysaccharides

Bacteria produce and secrete several types of oligo- and polysaccharides, which contribute to the formation of capsules and biofilms. Capsular polysaccharides increase the survival of bacteria in hostile environments, and are accordingly classified as virulence factors for pathogenic bacteria. Most of these polysaccharides are poorly immunogenic and further prevent the activation of complement-mediated lysis and phagocytosis. Some capsular polysaccharides, such as polysialic acid and hyaluronan, even mimic host glycans and thereby evade immune recognition.

The polysaccharide colanic acid, also known as M-antigen, was originally isolated from *Escherichia coli* but is also found covering other Enterobacteriaceae. The core of colanic acid consist of seven monosaccharides including Gal, GlcA, Glc, and Fuc. Colanic acid heptasaccharides are built at the cytoplasmic side of the inner membrane on the carrier undecaprenol-PP, then flipped to the periplasm, where multiple colanic acid units are polymerized and secreted in the extracellular space. Colanic acid is sometimes attached to the O-antigen of LPS. The synthesis of colanic acid is turned on in response to environmental stress related to damage of cell envelope.

The utilization of polyprenol-PP as a carrier in the synthesis of glycoconjugates is a recurrent feature across glycosylation pathways in prokaryotes and eukaryotes. Dolichol-PP is used in eukaryotes and Archaea, and bactoprenol-PP is used in bacteria for the synthesis of multiple structures including colanic acid, peptidoglycan, teichoic acid, lipopolysaccharide, lipoarabinomannan, N-linked glycans, and O-linked glycans. The translocation of lipid-linked oligosaccharides across membranes is also common to many glycosylation pathways.

The main types of large capsular polysaccharides are polysialic acid, hyaluronan, and poly β1-6 GlcNAc. Polysialic acid consists of polymers of α2-8 and α2-9 linked Sia and occurs on pathogenic *Escherichia coli*, *Neisseria meningitides* and *Pasteurella hemolytica*. As for all other types of sialylation in bacteria, only Neu5Ac is found in polysialic acid chains. The biosynthesis of polysialic acid takes

place at the cytoplasmic side of the inner membrane using CMP-Sia as donor substrate. The polysialic acid polymer transits then through a multiprotein channel across the periplasm to be secreted in the extracellular space. CMP-Sia is derived from endogenous UDP-GlcNAc or from Sia salvaged from the environment. Polysialic acid biosynthesis is maximal at 37°C and minimal below 20°C. It is essential for the proliferation of neuroinvasive infections of *Escherichia coli* and some strains of *Neisseria meningitides*. In addition to promoting immune evasion by mimicry, polysialic acid inhibits complement activation and phagocytosis.

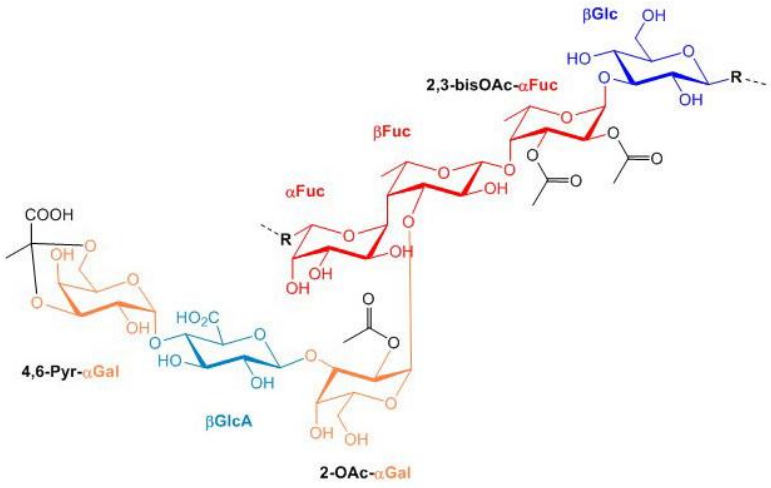

Figure 1 – Core structure of colanic acid from *Escherichia coli* K12. Connection points for the polymerization of colanic acid units are marked with **R**.

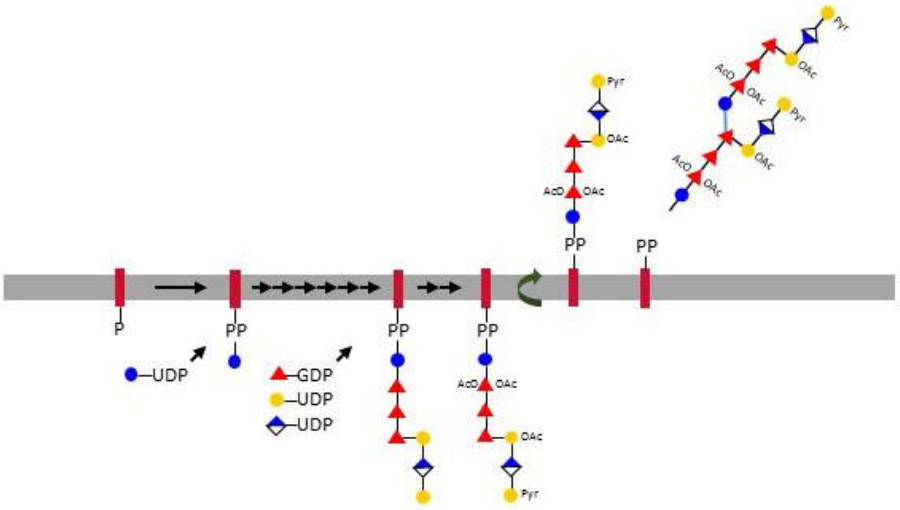

Figure 2 – Biosynthesis of colanic acid at the cytoplasmic leaflet of the inner membrane on the bactoprenol undecaprenyl-PP. Colanic acid units polymerize after flipping to the periplasmic space.

Hyaluronan is another virulence factor contributing to the pathogenicity of bacteria such group A *Streptococcus*. Bacterial hyaluronan is, like its counterpart

in animals, produced at the cell membrane by sequential elongation of a hyaluronan chain from the reducing end. Capsular hyaluronan shields bacteria from immune surveillance and confers resistance to phagocytosis. The last type of large polysaccharides found in bacteria is poly-N-acetylglucosamine, commonly abbreviated PNAG, which is produced by certain gram-positive and gram-negative bacteria. Such chains of poly β1-6 GlcNAc are for example deployed by pathogenic Escherichia coli for biofilm formation under limiting carbon availability.

Peptidoglycan

Peptidoglycan is the main constituent of the bacterial cell wall and is essential for cell viability. Accordingly, several classical antibiotics such as penicillin and vancomycin impair peptidoglycan biosynthesis to kill bacteria. Polymeric chains of GlcNAc and N-acetylmuramic acid (MurNAc) constitute the backbone and invariant core of peptidoglycan. Each disaccharide unit of GlcNAc(β1-4) MurNAc comprise a peptidic side chain of five amino acids featuring rare D-amino acids. Whereas the carbohydrate moiety of peptidoglycan is invariant among bacteria, different peptide sequences occur across bacterial groups. For example in *Escherichia coli* the peptide is L-Ala~D-Glu~*m*A_2pm~D-Ala~D-Ala, where *m*A_2pm represents meso-diaminopimelic acid. In Gram-positive *Staphylococcus aureus*, the peptide includes a branch of five glycines linked to the middle lysine residue, yielding L-Ala~D-isoGlu~L-Lys[Gly]$_5$~D-Ala~D-Ala.

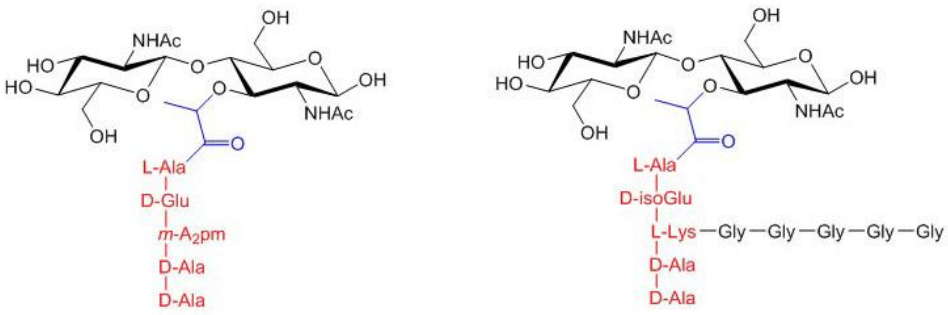

Figure 3 – Core structures of peptidoglycan from *Escherichia coli* (left) and *Staphylococcus aureus* (right). The lactyl group at C_3 of MurNAc is in blue and the pentapeptide is in red. The Gly_5 side-chain of *Staphylococcus aureus* is in black.

Although peptidoglycan is found in all bacteria containing a cell wall, its layout differs between Gram-negative and Gram-positive bacteria. Gram-negative bacteria have a double membrane and peptidoglycan is found as a thin layer on top of the inner cell membrane. Gram-positive bacteria lack an outer membrane and probably therefore require a much thicker layer of peptidoglycan to sustain the high internal osmotic pressure. Peptidoglycan is anchored to the inner membrane of Gram-negative bacteria through linkage to Braun's lipoproteins and to the membrane of Gram-positive bacteria through cross-links to lipoteichoic acid.

Peptidoglycan biosynthesis begins in the cytoplasm by generating UDP-MurNAc through addition of a lactyl group to the C3 atom of UDP-GlcNAc. UDP-MurNAc is then linked to the pentapetide side chain and the resulting glycoconjugate is transferred to undecaprenol-P, yielding MurNAc-(pentapeptide)-PP-undecaprenol, which is also called lipid I. As next step, GlcNAc is transferred in a β1-4 linkage to MurNAc, thereby yielding lipid II. Units of lipid II are then translocated across the membrane through a yet unknown mechanism and polymerized by a transglycosylase enzyme in a reaction that releases undecaprenol-PP. The length of the GlcNAc-MurNAc polymer varies between bacterial families, usually within a range of 5 to 100 disaccharide units. After separation from the membrane bound lipid carrier, chains of peptidoglycan are cross-linked by bridging peptidic side-chains from different polysaccharide strands through a transpeptidase reaction. The efficiency of peptide cross-linking depends on the bacteria and growth conditions. Most bacteria reach 40 to 60% cross-linking, but *Staphylococcus aureus* achieves up to 90% cross-linking thank to the additional pentaglycine secondary chain.

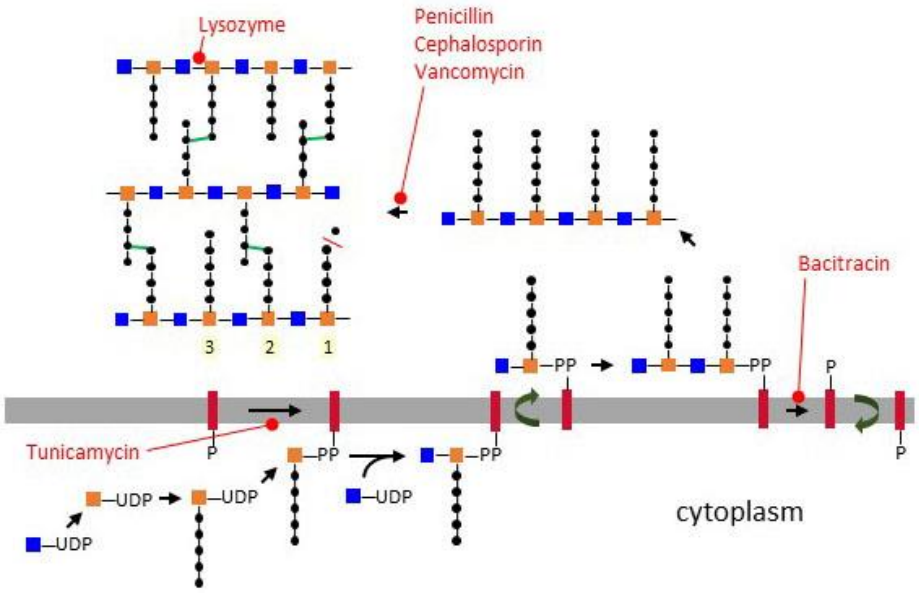

Figure 4 – Biosynthesis of peptidoglycan at the cell membrane and cross-linking in the periplasmic space. Reactions sensitive to antibiotics are marked in red.

Transglycosidases and transpeptidases belong to the class of penicillin-binding proteins (PBP). Bacterial genomes contain multiple PBP genes, which encode carboxypeptidases, transpeptidases, endopeptidases, and transglycosylases involved in peptidoglycan biosynthesis and remodeling at various locations of the cell wall. The cell wall is under constant remodeling to encompass changes of the cell architecture, such as during division. Peptidoglycan hydrolysis and biosynthesis are tightly coordinated to prevent any rupture of the cell wall. Several hypotheses have been proposed to explain the process. One hypothesis suggests that new layers are added closest to the cell membrane, whereas older

layers are hydrolyzed at the top facing the extracellular space. Another concept, referred to as the "three-for-one" hypothesis, states that hydrolysis and biosynthesis takes place through a multi-enzyme complex that synthesizes three new strands for one old strand being hydrolyzed. β-Lactam antibiotics like penicillin block peptidoglycan biosynthesis by inhibiting transpeptidases. Vancomycin also interferes with peptidoglycan formation by binding D-Ala~D-Ala of the peptidic side-chains, thus preventing cross-linking. The antibiotic bacitracin works by inhibiting the dephosphorylation of undecaprenol-PP, which abolishes the recycling of the lipid carrier to the internal leaflet of the membrane for further biosynthesis of lipid I units. Interestingly, tunicamycin, which inhibits the first step of N-linked glycosylation in eukaryotes, also interferes with peptidoglycan formation by inhibiting the transfer of MurNAc to undecaprenol-P. In addition to the multitude of microbial antibiotics targeting peptidoglycans, animals also express the antibacterial protein lysozyme, which cleaves the glycosidic bond between GlcNAc and MurNAc.

Peptidoglycan remodeling liberates fragments into the environment, which are strong activators of the innate immune system. Peptidoglycan-recognition proteins are widely distributed among animals. For example, insects express a battery of at least 20 proteins involved in the cleavage, recognition, and presentation of peptidoglycan to immune cells. In mammals, the toll-like receptor-2 (TLR2)/CD14 complex recognizes polymeric peptidoglycan in the extracellular space. Peptidoglycan fragments are also recognized inside cells by the cytoplasmic NOD1 and NOD2 proteins. NOD1 recognizes L-Ala~D-Glu~mesoA_2pm and NOD2 recognizes MurNAc~L-Ala~D-Glu. The mechanisms involved in the transfer of peptidoglycan fragments to the cytoplasm are however currently unknown. In addition to membrane-bound and cytoplasmic proteins, several secreted lectins, such as mannose-binding lectin and mouse RegIIIγ, recognize GlcNAc-MurNAc polymers and contribute to bacterial elimination.

Teichoic acid

The cell wall of Gram-positive bacteria contain teichoic acids, which are anionic glycoconjugates contributing to the stability and permeability of the cell surface. Teichoic acids come either as lipo-teichoic acid anchored in the cell membrane, or wall teichoic acid, which are covalently bound to the peptidoglycan network. Both types of teichoic acid extend through the peptidoglycan layers and provide points of anchor for extracellular and cell wall proteins. The biosynthesis of teichoic acid follows the same pattern as colonic acid and peptidoglycan biosynthesis with initiation and elongation on a polyprenol carrier at the cytoplasmic side of the plasma membrane. GlcNAc-P is first transferred to undecaprenol-P from UDP-GlcNAc, yielding undecaprenol-PP-GlcNAc. This reaction is analogous to the formation of dolichol-PP-GlcNAc formation in eukaryotes, which initiates N-linked glycosylation. Remarkably, tunicamycin also inhibits this first step of teichoic acid biosynthesis. Elongation proceeds on

undecaprenol-PP by addition of a ManNAc residue followed by about 50 units of glycerol-P or ribitol-P.

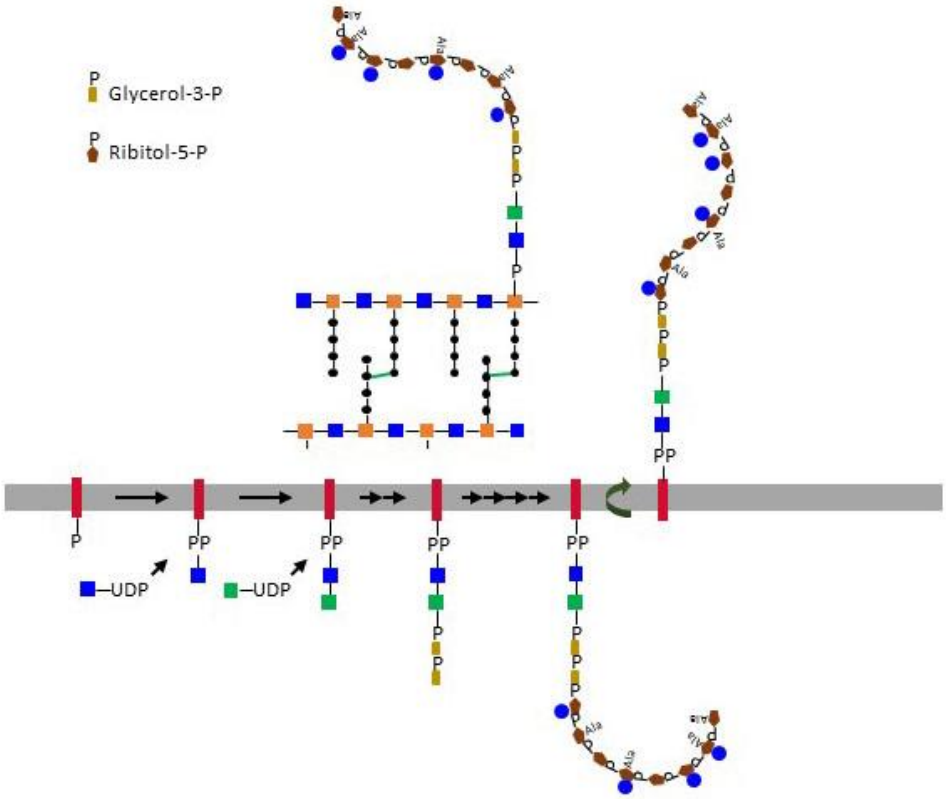

Figure 5 – Biosynthesis of teichoic acid. Some bacterial strains (e.g. *Bacillus subtilis* 168) only synthesize glycerol polymers. The glycosylation of ribitol varies greatly between bacterial stains. Glc usually decorates ribitol in *Bacillus subtilis and* GlcNAc in *Staphylococcus aureus*.

Whereas the main function of teichoic acids is to ensure structural rigidity by attracting cations to the cell wall, the glycoconjugate also contributes to the adhesion of bacteria to biological surfaces. For example, the adhesion of *Staphylococcus aureus* to epithelial cells require teichoic acid. The functional significance of the Glc and GlcNAc on teichoic acid chains are still unclear. Teichoic acids, like peptidoglycan, activate innate immunity through binding to the TLR2/CD14 complex.

Lipopolysaccharide

Teichoic acids only occur in Gram-positive bacteria and lipopolysaccharide (LPS) only occur in Gram-negative bacteria. LPS is synthesized at the cytoplasmic and periplasmic leaflets of the inner membrane, and transferred to the outer membrane where it builds up a dense coat of polysaccharide chains at the surface of bacteria. Although the basic architecture of LPS is shared by most Gram-negative bacteria, a formidable structural heterogeneity is encountered across and even within bacterial strains under different growth conditions. LPS is essential

for the growth of most Gram-negative bacteria by providing a barrier against environmental stress. In addition, the dense carbohydrate extensions of LPS protect bacteria from antibiotics and the immune system by blocking complement activation and antibody binding. The constant variation of terminal carbohydrate sequences, called O-antigens, also contribute to the evasion of bacteria from immune surveillance.

Structurally, LPS is subdivided in three components: lipid A, core oligosaccharide, and O-polysaccharide (O-antigen). Lipid A shows the highest structural conservation of the three LPS components across Gram-negative bacteria, although the number and type of acyl chains varies as well as the occurrence of multiple side-groups. To simplify the discussion, a generic LPS structure of *Escherichia coli* will be outlined here as example. LPS biosynthesis begins at the cytoplasmic side of the inner membrane by attaching two acyl chains to the C3 and to the de-acetylated amino group at C2 of UDP-GlcNAc. Two of such diacylated UDP-GlcNAc are combined through a β1-6 linkage and phosphorylated, thereby yielding the intermediate lipid IVa. Synthesis proceeds by addition of two units of the carbohydrate 3-deoxy-D-manno-octulosonic acid (abbreviated KDO). Lipid A is completed by the attachment of two or three additional acyl chains. Lipid A including the KDO disaccharide represent the minimal LPS backbone enabling the survival of *Escherichia coli* under laboratory conditions. Lipid A can be further modified by addition of phosphoethanolamine and 4-amino-4-deoxy-arabinose (L-Ara4N) side chains, which decrease the susceptibility of *Escherichia coli* to antibiotics of type polymyxin-B. The donor substrate for the transfer of L-Ara4N is not a nucleotide-activated sugar but undecaprenol-P-L-Ara4N. This donor substrate is analogous to dolichol-P-Man used in various eukaryotic glycosylation pathways.

The glycosylation of lipid A continues at the cytoplasmic leaflet of the inner membrane through the action of multiple glycosyltransferases using nucleotide-activated sugars as substrates. In Escherichia coli, the inner core includes L-glycero-D-mannoheptose (Hep), Gal, GlcN, GlcNAc, and Rha. The next group of oligosaccharides is heterogeneous and build at least five different “outer core” structures in *Escherichia coli*. The LPS core is then flipped to the periplasmic leaflet of the inner membrane, where subsequent elongations take place. Some Gram-negative bacteria further glycosylate LPS by addition of large O-polysaccharides, whereas other bacteria, such as *Neisseria meningitidis* and *Campylobacter jejuni*, have longer core oligosaccharides often including Sia but without O-polysaccharide addition. The resulting structures are then called lipooligosaccharides (LOS) to differentiate them from true LPS. Bacteria lacking outer core oligosaccharides show membrane instability and decreased pili expression. Such cells induce colanic acid expression, which is then cross-linked to LOS and favors the formation of biofilms.

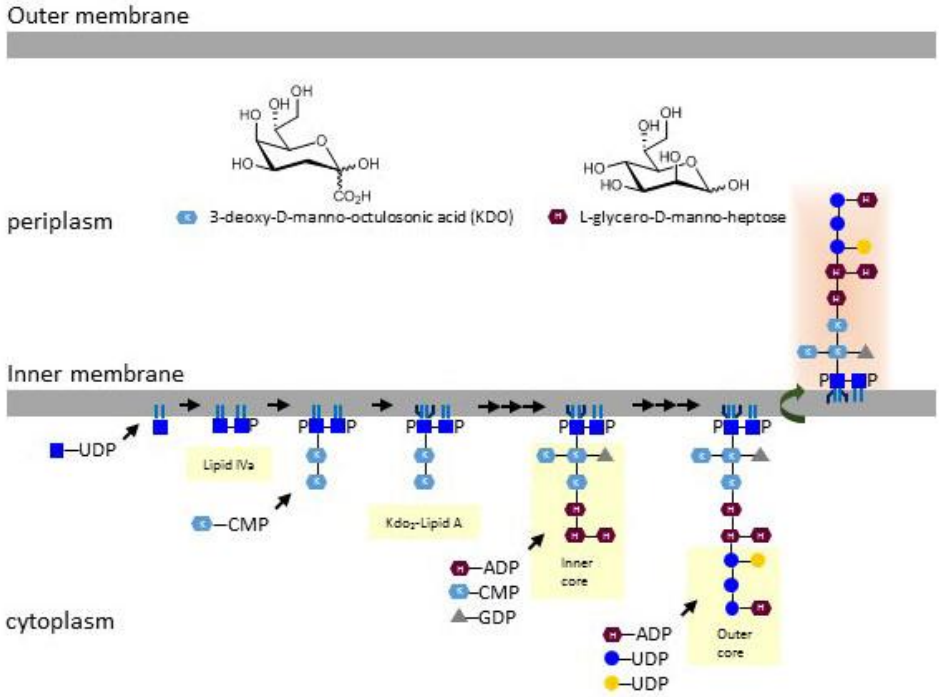

Figure 6 – Biosynthesis of lipid A and LPS core oligosaccharides (of *Escherichia coli* K12) at the inner cell membrane of Gram-negative bacteria.

The biosynthesis O-polysaccharide units takes place on undecaprenol-PP at the cytoplasmic side of the inner membrane. About three to five monosaccharides are added by glycosyltransferases using nucleotide-activated sugar substrates. At this stage, the undecaprenol-PP-oligosaccharide is translocated to the periplasmic side of the inner membrane, where oligosaccharide units are ligated to a growing polysaccharide of repeating units. The typical complex controlling translocation, ligation and chain elongation are proteins of the WecA-Wzx-Wzy-Wzz family. Upon termination of chain extension, the O-polysaccharide is cleaved from undecaprenol-PP and transferred to the LPS core. The carbohydrate sequences of O-polysaccharides vary extensively; at least 60 different monosaccharides and 30 non-carbohydrate modifications have been identified to date. The structural complexity of O-polysaccharides is further increased by the multiple glycosidic linkages connecting monosaccharides. O-polysaccharides define the antigenic properties of Gram-negative bacteria. In Escherichia coli, about 170 O-serotypes have been described. After ligation of O-polysaccharide chains, LPS is transported by Lpt factors (LptBCFG, LptA, LptDE) through peptidoglycan and across the outer membrane, in which it decorates the external leaflet.

LPS is of tremendous medical importance as it is the main component of endotoxin. Even low doses of LPS in the range of 10 pg/ml activate leukocytes and induce a strong inflammatory response. LPS binds to the CD14/TLR4 complex, which is mainly expressed on leukocytes of the myeloid lineage. The endotoxic potency of LPS and its derivatives largely depend on the exposed organisms. For

example, the phosphate groups on lipid IVa potentiate endotoxin activity on mouse leukocytes but prevent TLR4 activation on human leukocytes. The acyl chains of lipid A also influence the intensity of TLR4 activation. The LPS from the photosynthetic bacteria *Rhodobacter sphaeroides* even acts an endotoxin antagonist by competing for binding to the MD-2 protein, which is required for TLR4 activation by LPS. *Rhodobacter sphaeroides* LPS has only five acyl chains, which appears to be the key factor preventing TLR4 activation.

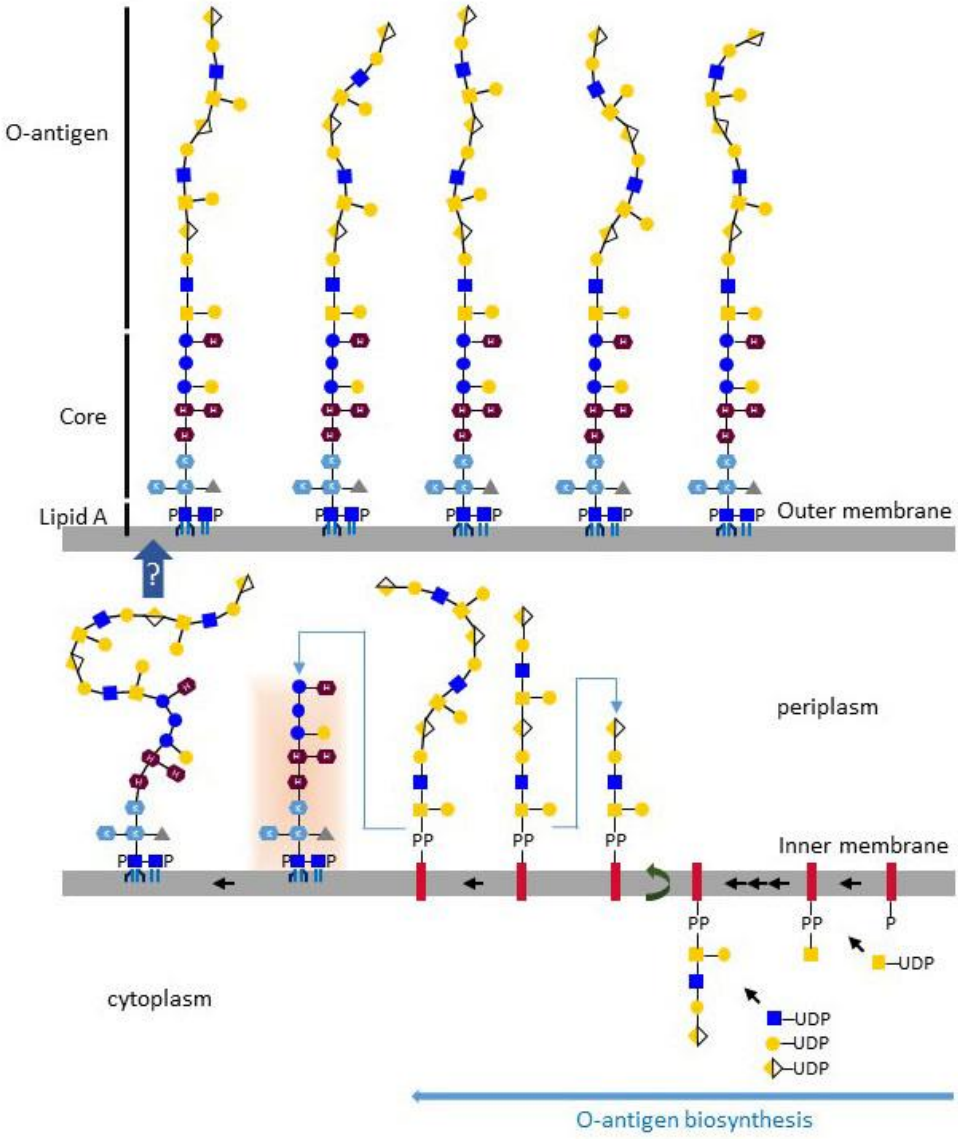

Figure 7 – Biosynthesis of LPS O-antigen (*E. coli* O113) on undecaprenol-PP and transfer to the LPS core. Complete LPS molecules are transported to the outer membrane by the Lpt multiprotein complex.

The antibiotic polymyxin B kills Gram-negative bacteria by binding to LPS and destabilizing the outer membrane. The affinity of polymyxin B for LPS is applied in laboratory conditions to decontaminate reagents from LPS. The biosynthesis of LPS itself is not the target of known antibiotics, but several drug

candidates have been shown to interfere with LPS biosynthetic enzymes, such as the GlcNAc deacetylase LpxC. The antibiotics tunicamycin and bacitracin inhibit the biosynthesis of O-polysaccharide through their interference with undecaprenol-PP formation and recycling.

Arabinogalactan and lipoarabinomannan

Mycobacteria are commonly classified as Gram-positive, although their cell wall remains impermeable to crystal violet without heating or phenol pre-treatment. This resistance to the penetration of dyes results from a dense barrier of fatty acids called mycolic acids, which are covalently bound to the polysaccharide arabinogalactan. The cell wall of mycobacteria contains a complex network of glycoconjugates, some of them being specific to the mycolata taxon. These include arabinogalactan, phosphatidylinositol-mannosides (PIM), lipomannan, and lipoarabinomannan (LAM). Besides Man, GlcNAc and MurNAc, the dominant monosaccharides found in the mycobacterial cell wall are the furanoside forms of Gal and Ara, abbreviated Gal*f* and Ara*f*.

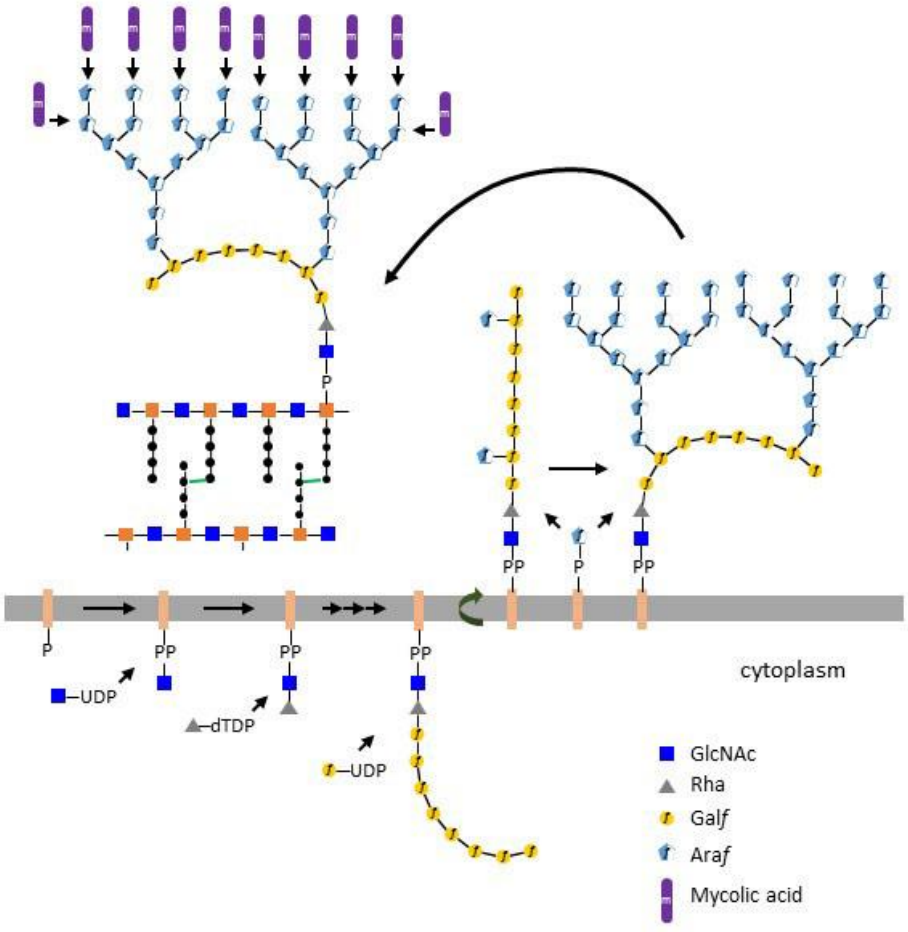

Figure 8 – Biosynthesis of arabinogalactan on decaprenol-PP and transfer to peptidoglycan in *Mycobacterium spp*.

Arabinogalactan is a large polysaccharide that is linked to peptidoglycan at its reducing end and carries multiple units of mycolic acid at its termini. The core of arabinogalactan is assembled at the cytoplasmic side of the cell membrane by first transferring GlcNAc to decaprenol-PP. This initial step is identical to the beginning of teichoic acid biosynthesis and dolichol-PP-oligosaccharide assembly in eukaryotes. The isoprenoid carrier in mycobacteria is however decaprenol (C_{50}) and not undecaprenol (C_{55}) like in other bacteria. After addition of α1-3 Rha to decaprenol-PP-GlcNAc from the donor dTDP-Rha, a chain of about 30 Gal*f* is built up at the cytoplasmic leaflet by at least three Gal*f*-transferases. The first enzyme transfers β1-4 Gal*f* to Rha and the following ones transfer β1-5 Gal*f* and β1-6 Gal*f* alternately. The galactan polymer then translocates to the extracellular side of the membrane in a reaction probably mediated by an ABC-transporter. At the outer leaflet of the membrane, a series of Ara*f*-transferases add two to three branched Ara*f* trees each consisting of about 20 monosaccharides to each galactan chain. These Ara*f*-transferases are embedded in the cell membrane and use decaprenol-P-Ara*f* as donor substrate. Completed arabinogalactan units are then conjugated at their reducing end to MurNAc residues on peptidoglycan. Finally, several mycolic acid molecules are attached covalently to the Ara*f* termini of arabinogalactan. The biosynthesis of mycobacterial peptidoglycan proceeds as in other bacteria but decaprenol-PP is used as carrier instead of undecaprenol-PP.

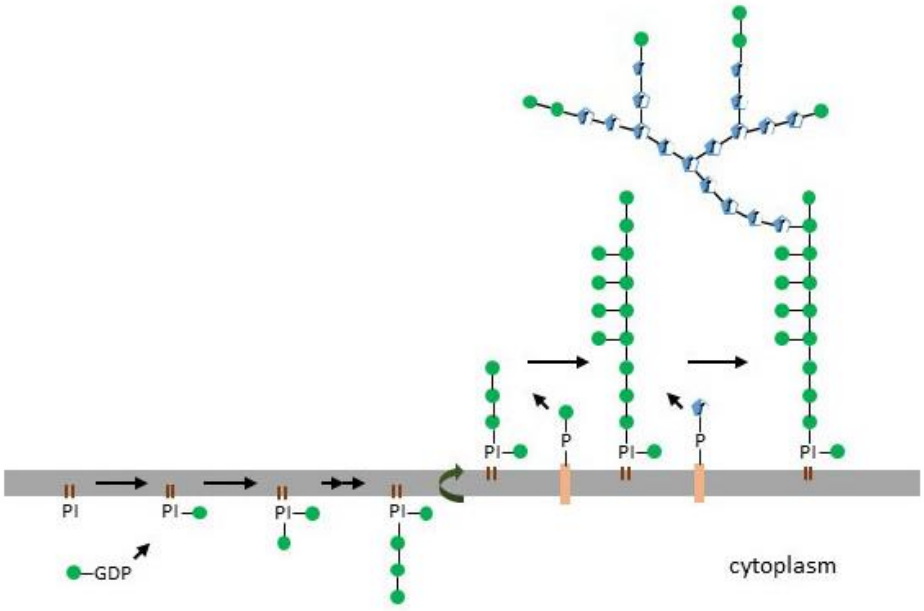

Figure 9 – Biosynthesis of lipoarabinomannan on phosphatidylinositol.

Whereas arabinogalactan covalently connects peptidoglycan and the mycolic acid layers, lipoarabinomannan is anchored in the cell membrane and in the mycolic acid layer through phosphatidylinositol. Phosphatidylinositol-mannosides, lipomannan, and lipoarabinomannan share the same initial biosynthetic steps, but only the biosynthesis of the more complex lipoarabinomannan will be

addressed here. The first four to six Man units are added on phosphatidylinositol by cytoplasmic α1-2 and α1-6 Man-transferases using GDP-Man as substrate. The resulting glycoconjugate then flips to the outer leaflet of the cell membrane, where other Man-transferases using decaprenol-P-Man add about 20 monosaccharides. The resulting lipomannan is further extended by transfer of a branched Ara*f* structure of about 50 to 80 monosaccharides. The lipoarabinomannan of pathogenic mycobacteria, such as *Mycobacterium tuberculosis* and *Mycobacterium leprae*, is capped by Man mono- or disaccharides, which are important for the binding and invasion of the host cells through Man-binding lectins. Arabinogalactan and lipoarabinomannan are essential for Mycobacterium viability. Truncated polysaccharides destabilize the barrier function of the cell wall and render bacteria more susceptible to antibiotics and environmental stress. In the context of pathogenic mycobacteria, lipoarabinomannan also contributes to immune evasion by suppressing the release of pro-inflammatory cytokines by dendritic cells and macrophages.

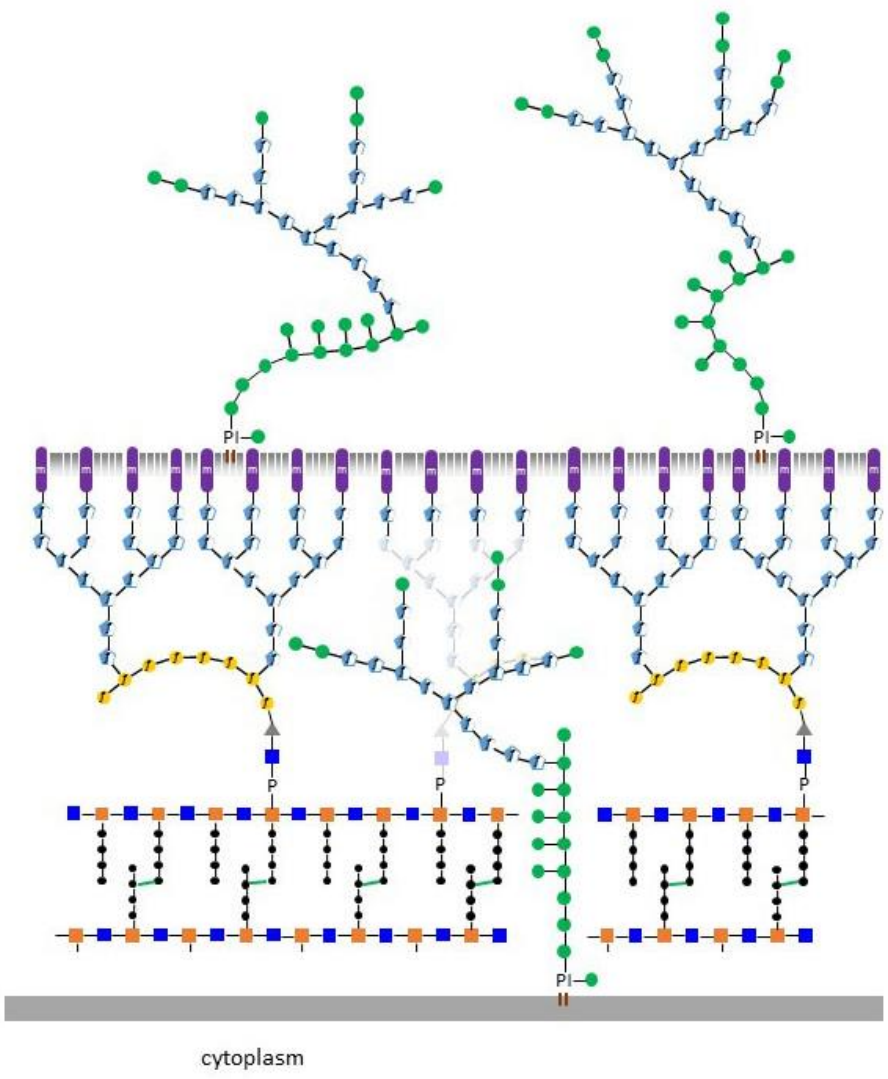

Figure 10 – Representation of the *Mycobacterium* cell wall with phospholipids and lipoarabinomannan embedded in the mycolic acid layer.

Various antibiotics target the mycobacterial cell wall, although the dense mycolic acid layer prevents the penetration of hydrophilic large molecules. Amphomycin blocks decaprenol-P-Man formation and prevents the elongation of phosphatidylinositol-mannosides after translocation to the extracellular side of the cell membrane. The action of amphomycin is not restricted to mycobacteria since this antibiotics also inhibits undecaprenol-PP-MurNAc-peptide translocation for peptidoglycan formation and also impairs dolichol-P-Man biosynthesis in eukaryotes. Other antibiotics, such as ethambutol, isoniazid and delamanid, are more specific to the mycobacterial cell wall. Ethambutol inhibits Araf-transferases, while isoniazid and delamanid inhibits mycolic acid biosynthesis.

Bacterial O-glycosylation

Beyond polysaccharides, bacteria harbor complex glycosylation machineries modifying proteins. Some of these glycosylation pathways are specific to few target proteins, whereas others generate a broad range of glycoproteins. As in eukaryotes the amino acids Ser and Thr are the main carriers of O-glycans in bacteria, although Tyr has also been described as carrier in some Gram-positive mesophilic bacteria. Flagellum and pilus proteins are the most frequent targets of O-glycosylation pathways. The glycosylation of flagella takes place in the cytoplasm by direct glycosylation of Ser/Thr residues on the target proteins. By contrast, pilus O-glycosylation proceeds in two steps through pathways similar to O-antigen biosynthesis.

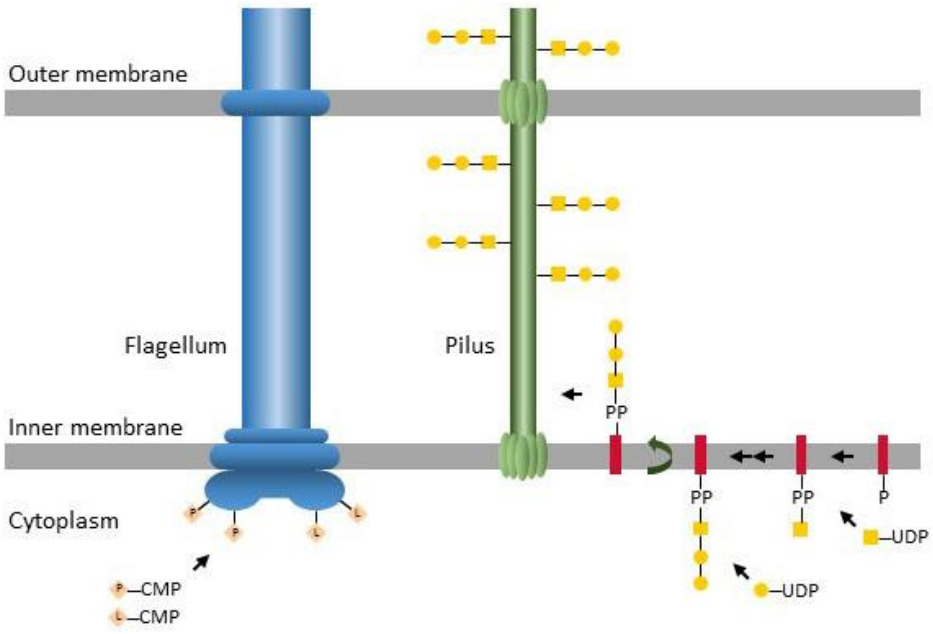

Figure 11 – O-glycosylation of flagellar proteins by cytoplasmic glycosyltransferases and *en bloc* transfer of oligosaccharides to Ser/Thr of pilus proteins. P: pseudaminic acid, L: legionaminic acid.

A large variety of monosaccharides are transferred to flagellar proteins across bacteria. For example, *Campylobacter jejuni* heavily glycosylates flagella with the ulosonic acids (C_9 sugars) pseudaminic acid (Pse), legionaminic acid (Leg) and derivatives. *Pseudomonas aeruginosa* glycosylates its flagella with Rha-based oligosaccharides. O-Glycosylation is required for the assembly of flagella and hence for bacterial motility. The oligosaccharide chains transferred *en bloc* to pili proteins also vary in composition and size across bacterial groups. Some strains of *Pseudomonas aeruginosa* transfer an oligosaccharide derived from the LPS O-antigen pathway, whereas other strains have developed a specialized pathway for pili glycosylation. The O-glycosylation machinery of some bacteria, such as *Neisseria* and *Bacteroides spp.*, is not restricted to flagella and pili. Several surface proteins have been identified as carrying O-glycans, although the general significance of such modifications remain unclear. Among other effects, O-glycosylation has been postulated to improve bacterial viability and decrease recognition by host immune cells. Some bacterial O-glycosyltransferases are structurally and functionally similar to eukaryotic counterparts. Man-transferases found in *Streptomyces* and Mycobacterium spp. use (un)decaprenol-P-Man as donor substrate to glycosylate proteins with O-Man. These bacterial Man-transferases share structural similarity to eukaryotic PMT enzymes. A paralog to eukaryotic O-GlcNAc-transferase (OGT) has also been identified in *Listeria monocytogenes* and shown to be involved in O-GlcNAc-ylation of flagellar proteins.

Bacterial N-glycosylation

N-glycosylation has been best characterized in *Campylobacter jejuni*, in which it was also first described. The similarity between *Campylobacter* and eukaryotic N-glycosylation pathways is striking. In both cases, the pathway begins at the cytoplasmic leaflet of the cell membrane with the assembly of an oligosaccharide on a polyprenol-PP carrier. This polyprenol-PP-oligosaccharide flips across the membrane and the oligosaccharide is transferred *en bloc* to Asn residues in the context of the sequon Asn-X-Ser/Thr. The true consensus site for N-glycosylation in *Campylobacter jejuni* is in fact more stringent as it requires an acidic amino acid, i.e. Glu or Asp, at position -2 of the acceptor Asn, thus yielding Glu/Asp-X-Asn-X-Ser/Thr where X cannot be Pro. The oligosaccharide synthesized in *Campylobacter* on undecaprenol-PP is an heptasaccharide featuring the GlcNAc derivative 2,4-diacetamido-2,4,6-trideoxy-a-D-Glc (diNAcBac), GalNAc and Glc. After translocation to the periplasmic side of the membrane, the oligosaccharide is cleaved from undecaprenol-PP and transferred to acceptor proteins by the membrane-embedded oligosaccharyltransferase PglB. In contrast to eukaryotic oligosaccharyltransferases, PglB acts on folded proteins and shows a relaxed specificity for the oligosaccharide transferred as long as the monosaccharide at the reducing end contains an acetamido group at C2. Furthermore, the majority of the oligosaccharides cleaved by PglB are not transferred to proteins but remain as free oligosaccharides in the periplasm. The general significance of N-glycosylation in *Campylobacter jejuni* is unclear. Mutants lacking N-glycosylation show various phenotypes, such as impaired proliferation in animals and decreased

adhesion to host tissues. It is likely that functional impairments are related to some of the 100 N-glycoproteins identified in *Campylobacter jejuni*. Oligosaccharyltransferase-like proteins similar to PglB are found in different bacterial families including *Desulfovibrio* and *Helicobacter spp.*, although not in *Helicobacter pylori*. N-glycosylation also occurs in archaea, in which various oligosaccharides are transferred to acceptor S-layer glycoproteins after assembly on dolichol-PP as in eukaryotes. The archeal oligosaccharyltransferase AglB recognizes the sequon Asn-X-Ser/Thr but does not required an N-acetylated aminosugar at the reducing end of the oligosaccharide. N-Glycosylation of S-layer proteins contribute to the resistance of archaea to hostile conditions. Interestingly, the halophile *Haloferax volcanii* changes the N-glycosylation pattern of its S-layer proteins based on the salinity of the environment.

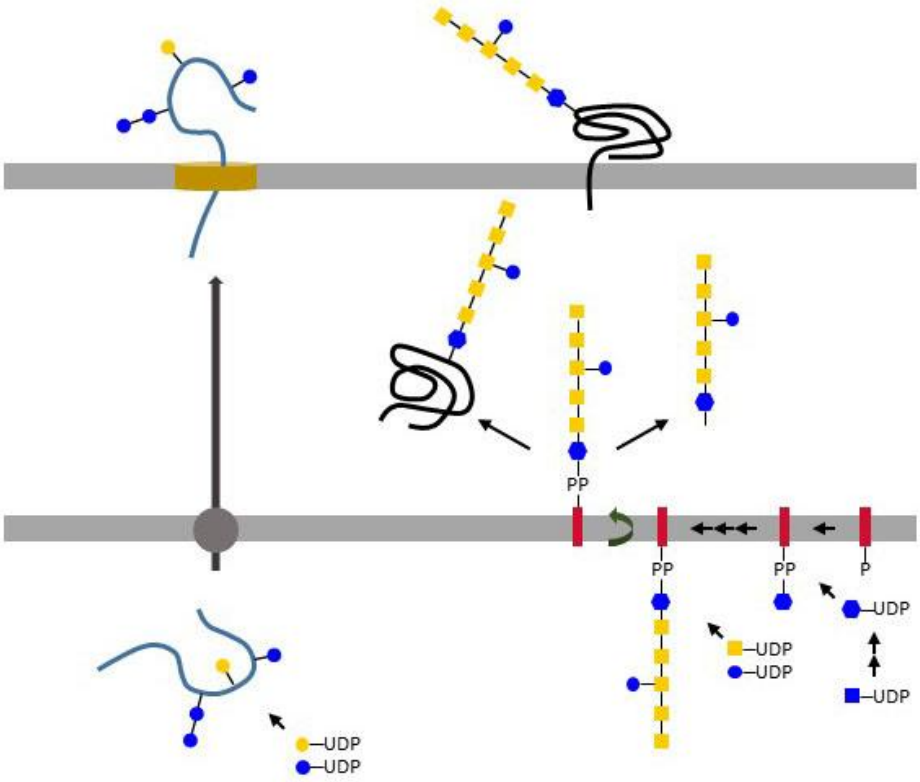

Figure 12 – Direct N-glycosylation of proteins in *Haemophilus influenzae* and *Actinobacillus pleuropneumoniae* (left side) and block-wise transfer of oligosaccharides by PglB oligosaccharyltransferase in *Campylobacter jejuni* (right side).

Another type of N-glycosylation involving the sequential transfer of monosaccharides on proteins has been recently described in *Haemophilus influenzae* and *Actinobacillus pleuropneumoniae*. there, cytoplasmic glycosyltransferases attach Glc or Gal from UDP-activated substrates to Asn in the typical Asn-X-Ser/Thr context. The few examples outlined here are probably the tip of the iceberg and future work will certainly further expand the complex chapter of bacterial glycosylation.