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Structural and mechanistic themes in glycoconjugate biosynthesis at membrane interfaces

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Abstract

Peripheral and integral membrane proteins feature in stepwise assembly of complex glycans and glycoconjugates. Catalysis on membrane-bound substrates features challenges with substrate solubility and active-site accessibility. However, advantages in enzyme and substrate orientation and control of lateral membrane diffusion provide order to the multistep processes. Recent glycosyltransferase (GT) studies show that substrate diversity is met by the selection of folds which do not converge upon a common mechanism. Examples of polyprenol phosphate phosphoglycosyl transferases (PGTs) highlight that divergent fold families catalyze the same reaction with different mechanisms. Lipid A biosynthesis enzymes illustrate that variations on the robust Rossmann fold allow substrate diversity. Improved understanding of GT and PGT structure and function holds promise for better function prediction and improvement of therapeutic inhibitory ligands.

Graphical abstract

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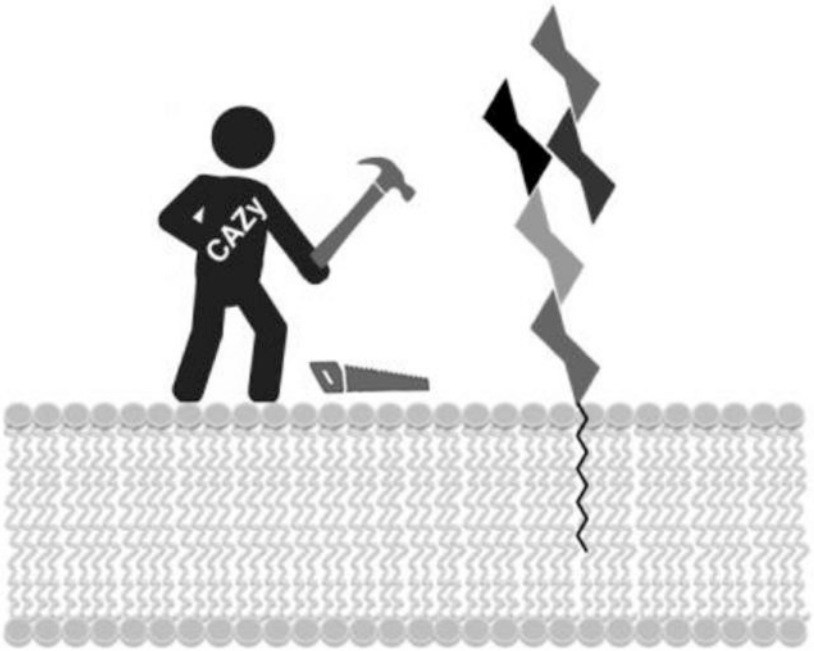

Introduction

Glycans, and the glycoconjugates to which they are appended, are some of the most abundant and complex organic materials in living organisms. Details of the assembly of these macromolecules have proven extremely challenging to dissect and define. This is, in part, because ordered, template-driven mechanisms common to nucleic acids and proteins are absent in glycan assembly and thus the biosynthesis and structure prediction of glycans is far more cryptic. To complicate matters, technical challenges arise from the membrane-associated transformations and/or translocations inherent to glycan assembly. For example, assembly of glycosylphosphatidylinositol (GPI) glycolipids [1] and dolicholdiphosphate-linked glycans [2] used for C-terminal modification and N-linked protein glycosylation respectively, takes place in steps that occur on each side of the endoplasmic reticulum (ER) membrane. Membrane association of these enzymes is also important because of the amphiphilic nature of the substrates and intermediates, and enzyme structure and function are adapted to accommodate and exploit this constraint [3].

The mainstays of glycan assembly are protein families collectively termed carbohydrate-active enzymes that feature in the expertly-curated CAZy database, which was launched online in 1998 [4]. This database includes a sequence-based classification of enzymes involved in synthesis (glycosyltransferases - GTs) and break-down (glycohydrolases - GHs) [5] of glycans. The database also includes other carbohydrate-modifying enzymes and carbohydrate-binding domains. The GTs carry out key steps, forming glycosidic bonds through glycosyl transfer from activated sugar donors to diverse acceptor substrates, including other carbohydrates, proteins, lipids, DNA and small molecules, and have been the subject of several valuable reviews [6–8]. These works illustrate the major progress made in defining the sheer scope of this abundant family of enzymes. In this opinion we turn

attention to recent developments in understanding the structural features and mechanistic logistics of selected transformations that are unique to glycan assembly at membsrane interfaces (Figure 1).

Polyprenol phosphate glycosyltransferases: Pren-P GTs

Glycosyl donor substrates for GTs are activated as nucleoside mono- or diphosphates (e.g. CMP-sialic acid or UDP-GlcNAc) or as amphiphilic polyprenol mono- or diphosphate derivatives (e.g. dolichol-P-Man or undecaprenol-PP-GlcNAc). This difference in donor chemistry relates to the availability of suitable sugar nucleotides relative to the location of the transferase active site. Nucleotide sugars are made in the cytoplasm and reach other subcellular compartments via active transport. The difference in the lipidic polyprenol carriers is discussed elsewhere [9].

The most extensively-studied family of Pren-P GTs is implicated in the biosynthesis of dolichol-P-Man (Dol-P-Man), which is an intermediate in the synthesis of essential eukaryotic glycoconjugates (Figure 1a) [10,11]. Dol-P-Man synthase (DPMS) transfers mannose from GDP-Man to Dol-P on the cytoplasmic face of the ER membrane. After catalysis, the Dol-P-Man is flipped across the membrane to the location where it is needed as a mannose donor - for example, the ER lumen in eukaryotes. The identity of the putative flippase has not yet been defined, however biochemical studies have provided evidence for an ATP-independent flippase that shows stereo- and polyprenol-specific translocation of Dol-P-Man in a liposome-based system [12].

DPMS enzymes share a common GT-A fold (ca. 230 residues) [6] that includes two non-contiguous amphipathic helices (Figure 1a). These interfacial (IF) or juxtamembrane (JM) helices may be components of the GT-A fold catalytic domain and play a key role in connecting the soluble domain to membrane-associated modules of the enzyme. DPMS enzymes are GT2 family (inverting) GTs, which include an Asp-Xaa-Asp sequence that is implicated in metal-ion and nucleoside-diphosphate binding. DPMS enzymes are currently classified into three types (I, II, and III) based on the presence of C-terminal sequence extensions or association with modules that include one or more transmembrane helices (TMHs) (Figure 2a). Recently the structure of the full-length type III DPMS from the archaeon *Pyrococcus furiosus*, including the catalytic domain and four C-terminal TMHs (Figure 2b), has been determined [13**]. This structure provides insight into the functional consequences of several mutations in the human DPMS in the respective catalytic domains that are associated with DPMS-related congenital disorders of glycosylation (designated as CDG1e disorders) [14]. For example, the structure shows how three of the single mutations in the human DPMS (R92G, S248P and G152V) destabilize protein tertiary structure and thus would be accompanied by major defects in function [15]. The structure also provides a consistent model of the mechanism of glycosyl transfer, which involves inversion of stereochemistry at the anomeric center. To investigate the role of the C-terminal TMHs, a truncation variant (Δ230–352) was prepared and found to be catalytically active and properly targeted to the membrane. Currently, the roles of the additional TMHs in the three enzyme types is not fully understood, although the human type II DPMS, which includes a soluble catalytic domain (DPM1), requires the auxiliary membrane-bound DPM3 protein for

targeting to the ER membrane [10]. It is also possible that the additional TMHs serve to mediate interactions with the flippase that delivers Dol-P-Man to enzymes in glycoconjugate assembly on the distal side of the membrane (Figure 1a).

Pren-P GTs also provide Pren-P-sugars that are translocated to the bacterial periplasm and to the surface of Gram-positive bacteria and archaea for use as glycosyl donors in glycan assembly. GtrB from *Synechocystis* is homologous to the *P. furiosus* DPMS, but shows a different pattern of membrane association modules relative to the DPMSs (Figure 2a). GtrB catalyzes transfer of glucose from UDP-Glc to undecaprenol phosphate (Und-P) for use in O-antigen biosynthesis [16*]. The GtrB monomer includes two JM helices in the catalytic domain similar to the DPMSs; however, there are only two C-terminal TMHs. X-ray crystallographic analysis reveals a tetrameric quaternary structure with each monomer contributing two TMHs to a helical bundle. Inter-subunit interactions are presented to support the assignment of the tetramer as the physiologically-relevant arrangement. The structure with UDP bound was determined, however information about bound Und-P could not be derived directly, although the putative site of the phosphoryl group of Und-P could be inferred from the position of a bound tungstate ion. In support of the assignment, tungstate was observed in the same position as the acceptor phosphoryl moiety in the structures of soluble GT-A-fold enzymes. The most intriguing finding in the structure is that the active site in the soluble catalytic domain is located 15 Å from the membrane interface, raising the question of how the amphiphilic Und-P substrate is extracted from the membrane to position the allylic phosphate for catalysis. As it is not likely that the highly stabilized, tetrameric membrane-associated structure moves from the membrane towards the active site, it is suggested that, instead the Und-P moves towards the active site in the soluble domain. A compelling mechanism whereby the Und-P is translocated from the membrane to the active site, based on a series of loss-of-function mutations in a zebrafish model for CDG1e-related pathologies, is proposed [16*].

Pren-P GTs that have been structurally characterized proceed with inversion of stereochemistry and in each case ligand-bound structures provide a good basis for defining the roles of the active-site residues. In the long term, further advances will be needed to understand how the GT structures differ in enzymes that carry out glycosyl transfer to Pren-P with retention of stereochemistry. In many cases the stereochemical outcome of specific enzymes is not defined, although it is sometimes assumed based on homology. However, this approach is risky. A case in point is AglK, a Pren-P GT from *Methanococcus voltae* that is involved in archaeal N-linked protein glycosylation. AglK is annotated as a GT-A fold, GT2 family Pren-P GT with considerable sequence identity to the *Saccharomyces cerevisiae* Dol-P-glucose synthase known to proceed with inversion of stereochemistry. However, NMR analysis revealed that AglK proceeds with retention of stereochemistry [17] and there are a number of close homologs in other archaea that likely share this feature based on their parallel roles in N- and O-linked glycoprotein biosynthesis [18]. Ultimately these results underscore that biochemical studies are the best way to unambiguously assign stereochemical outcome. In the future, more structural and bioinformatics approaches will surely help to provide insight into predictive signature sequences to reduce ambiguity in assignments.

Polyprenol phosphate phosphoglycosyl transferases: PGTs

Pren-Ps are also acceptor substrates for Pren-P phosphoglycosyl transferases (PGTs), which catalyze transfer of a C1-phosphosugar moiety from a nucleotide sugar to afford a Pren-PP-sugar [19,20]. These enzymes act at the beginning of many glycoconjugate biosynthetic pathways and have been dubbed “initiating” or “priming” GTs. The enzymes have also been designated according to substrate preferences; however, as noted recently [21], this classification is less informative as there is now considerable divergence from such a definition. Recently, a classification of the PGT enzymes into two broad superfamilies based on the topology (monotopic or polytopic) of the minimal catalytic core structure (Figure 1b) and mechanism has been introduced [22**]. There has been tremendous progress in understanding the structures and mechanisms of representative PGTs from each superfamily. These insights will enable progress in the design and development of potential antibiotic agents, as these enzymes feature at the inception of glycoconjugate biosynthesis pathways including those of peptidoglycan, lipopolysaccharide and teichoic acid [20,21].

Polytopic PGT superfamily members are found in bacteria and eukarya. The enzymes include 10–11 predicted TMHs and subgroup members can be distinguished by conserved microdomains that are predicted to be located at or near the membrane interface [23,24]. MraY is the best-characterized polytopic PGT with its pivotal role in Und-PP-MurNAc-pentapeptide (lipid I) formation, which is the first membrane-committed step in peptidoglycan biosynthesis. The first structure of MraY was reported in 2013 [25] and since that time there has been major progress in the determination of inhibitor-bound complexes. The polytopic PGT superfamily also includes the GlcNAc-1-P transferases (GPTs), which act at the inception of the eukaryotic dolichol pathway [2,23]. Historically, the polytopic PGTs have been difficult to investigate due to challenges associated with expression, solubilization and purification. However, recent progress with MraY and related enzymes now provides insight into the mechanism [26*,27]. In one study, extensive kinetic analyses and isotopic-labeling were applied to detergent-solubilized MraY from both Gram-negative and Gram-positive bacteria [26*]. Ultimately, the data support a random bi-bi ternary complex mechanism involving attack by the Pren-P phosphate on the β-phosphate of the nucleotide substrate, leading to the formation of Pren-PP-linked product and release of UMP in a single step.

Concomitantly, there has been exciting progress in the determination of MraY structures from *Aquifex aeolicus* ($MraY_{AA}$) bound to nucleoside natural products including muraymycin D2 (MD2) [28**] and tunicamycin [29] and of the human GPT with tunicamycin [30]. $MraY_{AA}$ binds to MD2 with a K_D 17.2 nM (determined by isothermal titration calorimetry) in contrast to the K_m for the native UDP-MurNAcpentapeptide substrate, which is 190 μM. A comparison of free and MD2-bound MraY structures shows that after binding to MD2, MraY undergoes significant conformational rearrangements in the active-site region creating sites for nucleoside and peptide-binding determinants of MD2 (Figure 3a and b). Intriguingly, in contrast to substrate interactions for binding and catalysis, which involve Mg^{2+} and highly-conserved acidic residues, MD2 binding is independent of these factors [28**] (Figure 3c and d).[31]These efforts now provide insight into the design

of selective inhibitors as anti-infective agents as they reveal new high-affinity binding sites that are not apparent in the unbound or substrate-complexed structures.

Details of a PGT superfamily that features a monotopic catalytic domain have also emerged [32]. Identification of this superfamily was initially confounded by N- and C-terminal sequence extensions that obscured a common functional core, as well as ambiguity in defining the correct membrane topology. Biochemical analysis of the *E. coli* WcaJ, a PGT with four N-terminal TMHs beyond the conserved catalytic core, gave the first clear biochemical evidence for the presence of a conserved reentrant membrane helix (RMH) in the catalytic domain [33]. Bioinformatics analysis applied to the catalytic domain then provided an indication of the prevalence of the superfamily across bacteria [34]. A survey of the data shows that bacteria may express both PGT superfamily enzymes with different members featuring in diverse glycoconjugate assembly pathways. Structural analysis of detergent-solubilized PglC from *Campylobacter concisus*, bound to Mg^{2+} and phosphate [22**] (Figure 1b), shows that the conserved catalytic domain of the monotopic PGTs associates with the membrane via an RMH, which penetrates a single leaflet of the bilayer. The RMH is also associated with three coplanar amphiphilic helices, which are located at the membrane interface and include a conserved Asp-Glu dyad. The striking difference between the structures of monotopic and polytopic PGTs suggests that the protein folds have evolved independently to carry out the same transformation between amphiphilic and soluble substrates at the membrane interface. In addition to the structural dichotomy, detailed biochemical studies have provided evidence that the catalytic mechanisms are also very distinct [35]. The mechanism of PglC involves a two-step ping-pong mechanism, wherein the Asp (in the conserved Asp-Glu dyad) serves as the nucleophile forming a covalent phosphosugar intermediate. This catalytic strategy fundamentally differs from the ternary complex mechanism established for the polytopic PGT superfamily. Advantages of this dichotomy may be related to controlling flux through two pathways that use a common Pren-P substrate and the need to coordinate the use of this resource when the following steps are irreversible [36].

Cellulose Synthase: Catalytic and channel domains enforce processivity and polymer structure

Chitin, alginate, and cellulose synthases, transfer the glycosyl moiety from nucleotide-activated sugar to a hydroxyl group acceptor of an elongating polysaccharide chain. The newly-added sugar becomes the acceptor in the next reaction. These enzymes are processive, that is, the rate of bond formation and polymer translocation by one glycan unit (elongation) is greater than that of strand release/termination. In these processive GTs, the catalytic subunit is tightly associated with a transmembrane channel such that the translocation step of polymer formation brings about secretion. In cellulose synthase the BcsA subunit is composed of a GT domain and a helical transmembrane domain, to which the BcsB subunit contributes a single helix and is essential for polymer production (Figure 1c) [37]. Sequence alignment of selected family 2 GT members guided by the structure of cellulose synthase [38,39] shows retention of a bent helix lining the transmembrane channel [40], with a conserved Trp near the bend that forms the glycan acceptor-binding site at the channel mouth. A tour-de-force of *in crystallo* enzymology was used to generate a model of BscA-

catalyzed cellulose biosynthesis (see Figure 4) [41**]. The model reveals that in the Michaelis complex a gating loop is inserted into the catalytic pocket, positioning the glucose donor for transfer witsh the finger helix in an ‘up’ position. Notably, the N-terminus of the finger helix bears an Asp in an invariant Thr-Glu-Asp motif that deprotonates the acceptor C4-hydroxyl. After transfer, retraction of the gating loop and UDP release sets the finger helix to the ‘down’ position contacting the new terminal glucose of the polymer. Substrate binding and/or gating loop reinsertion induce the upward movement of the finger helix pushing the elongated polymer into the transmembrane channel. Molecular dynamics simulations and free-energy calculations [42] explain the origin of the alternating glucose conformations found in cellulose. A rotation of the newly-added glucosyl moiety as it enters the channel allows it to adopt a planar configuration stabilized by carbohydrate–π stacking interactions with aromatic residues lining the binding tunnel. There is little to no energetic barrier to this translocation process (0 −3.1 kcal/mol); indeed, kinetic experiments wherein product cellulose chains are solubilized in ionic liquids enabling gel permeation chromatography and multi-angle laser light scattering show that the rate of elongation/translocation exceeds that of steady-state turnover, and thus is not rate-limiting [37].

Enzymes in LPS Biosynthesis and Modification

Gram-negative bacteria feature a surface lipopolysaccharide (LPS) layer, which acts as a barrier to hydrophobic toxins and as a virulence factor [43]. LPS is composed of lipid A anchored in the outer membrane, and an inner and outer oligosaccharide core. The inner core includes a 3-deoxy-D-manno-oct-2-ulosonate (Kdo) disaccharide and two L-glycero-D-b-manno-heptose units, and the outer core is a variable glycan generally including hexoses and heptoses, which is attached to the O-antigen repeat. Recent studies reveal unique structural features of GTs that act on and build the LPS.

The ArnT GT confers polymyxin antibiotic resistance by transfer of the cationic sugar 4-amino-4-deoxy-L-α-arabinose (Ara4N) to lipid A phosphate groups, modifying the charge on the LPS. The ArnT donor substrate is Und-P-Ara4N and thus both substrates must be brought from the membrane to the active site. ArnT is composed of a transmembrane domain and a periplasmic domain, with a similar GT-C fold [44] to the bacterial and archeal oligosaccharyl transferases PglB [45] and AglB [46]. The structure of *Cupriavidus metallidurans* ArnT alone and in complex with Und-P [47*] reveals a long binding cavity for the glycosyl donor that is primarily hydrophilic near the membrane boundary proximal to the active site, and lined with hydrophobic residues distal to the active site (Figure 5a). Following a similar strategy to cellulose synthase, completion of the active site and access of the acceptor lipid A substrate to a large amphipathic site spanning the membrane boundary is governed by a conformational change enacted by Und-P-Ara4N binding, suggesting a ternary complex with sequential order of addition.

Recent GT-B structures show variations that highlight the robustness of the fold in terms of tolerating substrate substitutions. For example, in the heptosyl transferase WaaC, the means of accommodating the acceptor lipid A substrate was recently uncovered [48**]. WaaC catalyzes transfer of the heptosyl unit from ADP-L-glycero-β-D-manno-heptose onto the inner Kdo of lipid A (Figure 1d). The structure of a pseudo-ternary complex was

accomplished using an N-acetylated, O-deacylated lipid A (catalytically competent) acceptor, with a non-hydrolyzable C-glycoside analog. The donor binds in the C-terminal domain and the lipid A acceptor in the N-terminal domain. Significant conformational changes in the N-terminal domain and interdomain motions are observed upon acceptor binding by comparing unliganded and donor-bound binary complexes [49]. These conformational changes are corroborated by fluorescence studies [50]. In the ternary complex, lipid A is bent into a horseshoe conformation and positioned in the active site by conserved Arg and Lys residues and by an adjacent hydrophobic patch. This patch also orients the N- and O-acyl chains into the membrane bilayer. The catalytically-competent ternary complex is best modeled by superposition with the ADP-2F-gluco-heptose bound structure [49] showing proximity and orientation of the nucleophilic C5-OH of Kdo, with the proposed general acid/base (Asp13), and sugar donor (for a review on the mechanisms of heptosyltransferases see [51]).

An example of the structural innovations to accommodate amphiphilic substrates is seen in the catalytic domains of *Raoultella terrigena* WbbB (residues 2–401), which transfers Kdo to rhamnose in LPS O-antigen synthesis. WbbB shows a dual Rossmann-fold GT-B motif modified by extensive deletions and insertions [52*] (Figure 5b and c). A C-terminal extension, together with an elongated linker between the Rossmann fold domains makes up an additional α-helical domain; together these elements displace and rotate the Rossmann-fold domains from the prototypical positions seen in the GT-B-fold WaaA characterized previously [53]. Moreover, the extensive interactions between these elements suggest that the induced-fit mechanism seen in WaaA, where nucleotide binding closes the catalytic cleft placing donor and acceptor in proximity, is not adopted in this more rigid platform. This modification accommodates an active site that extends from the donor binding site into a 20-Å long groove that can bind the long O-antigen chain acceptor.

Concluding remarks

As structures of glycoconjugate biosynthesis enzymes including their membrane-association domains become defined, important principles emerge. A striking feature is the widespread occurrence of the prototypical GT-A and GT-B GT folds. These folds are robust to variation and can be adapted to function on amphiphilic substrates at membrane interfaces through evolution of hydrophobic-interaction surfaces on existing folds or through added sequences that connect soluble catalytic domains to one or more TMHs. These adaptations accommodate interactions with amphiphilic substrates and position enzyme active sites at or above the membrane interface. Importantly, interfacial positioning of the catalytic sites allows reactions without the energetically-costly removal of substrate from the native membrane environment. Also intriguing is the divergent evolution of PGTs that catalyze the first membrane-committed step in glycan biosynthesis. The PGT superfamily members employ distinct polytopic and monotopic folds, which are tailored to accommodate and exploit membrane association for function through different mechanistic strategies. Future studies to understand the dichotomy of these “gatekeeper” enzymes will certainly reveal important paradigms. Studies on cellulose synthase also highlight the coupling of substrate processing to product translocation – adeptly secreting polymeric products to the extracellular milieu. The use of developing techniques in material science will allow

quantitative approaches critical to the understanding of the mechanisms of these glycan-based polymers.

In the future biochemical and biophysical studies in native-like membrane environments will enrich our understanding of the chemistry and biology of glycan and glycoconjugates. Here, high-resolution structures of individual enzymes and enzyme complexes via X-ray crystallography and cryo-EM will certainly afford new insight into how glycan biosynthesis enzymes in pathways interact and the preferred modes of substrate transfer.

Acknowledgements

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References and recommended reading

Papers of particular interest published with the period of review, have been highlighted as:

• of special interest

•• of outstanding interest

References

1. Kinoshita T, Fujita M: Biosynthesis of GPI-anchored proteins: special emphasis on GPI lipid remodeling. J Lipid Res 2016, 57:6–24. [PubMed: 26563290]
2. Burda P, Aebi M: The dolichol pathway of N-linked glycosylation. Biochim Biophys Acta 1999, 1426:239–257. [PubMed: 9878760]
3. Dufrisne MB, Petrou VI, Clarke OB, Mancia F: Structural basis for catalysis at the membrane-water interface. Biochim Biophys Acta Mol Cell Biol Lipids 2017, 1862:1368–1385. [PubMed: 27913292]
4. Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B: The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res 2014, 42:D490–495. [PubMed: 24270786]
5. Bourne Y, Henrissat B: Glycoside hydrolases and glycosyltransferases: families and functional modules. Curr Opin Struct Biol 2001, 11:593–600. [PubMed: 11785761]
6. Lairson LL, Henrissat B, Davies GJ, Withers SG: Glycosyltransferases: structures, functions, and mechanisms. Annu Rev Biochem 2008, 77:521–555. [PubMed: 18518825]
7. Breton C, Fournel-Gigleux S, Palcic MM: Recent structures, evolution and mechanisms of glycosyltransferases. Curr Opin Struct Biol 2012, 22:540–549. [PubMed: 22819665]
8. Gloster TM: Advances in understanding glycosyltransferases from a structural perspective. Curr Opin Struct Biol 2014, 28:131–141. [PubMed: 25240227]
9. Hartley MD, Imperiali B: At the membrane frontier: a prospectus on the remarkable evolutionary conservation of polyprenols and polyprenyl-phosphates. Arch Biochem Biophys 2012, 517:83–97. [PubMed: 22093697]
10. Maeda Y, Kinoshita T: Dolichol-phosphate mannose synthase: structure, function and regulation. Biochim Biophys Acta 2008, 1780:861–868. [PubMed: 18387370]
11. Banerjee DK, Zhang Z, Baksi K, Serrano-Negron JE: Dolichol phosphate mannose synthase: a Glycosyltransferase with Unity in molecular diversities. Glycoconj J 2017, 34:467–479. [PubMed: 28616799]
12. Sanyal S, Frank CG, Menon AK: Distinct flippases translocate glycerophospholipids and oligosaccharide diphosphate dolichols across the endoplasmic reticulum. Biochemistry 2008, 47:7937–7946. [PubMed: 18597486]

13 ••. Gandini R, Reichenbach T, Tan TC, Divne C: Structural basis for dolichylphosphate mannose biosynthesis. Nat Commun 2017, 8:120. [PubMed: 28743912] This first structure of a full-length DPM synthase provides important insight into loss-of-funtion mutations in congenital defects of glycosylation. The structural study also provides a unified view of the various DPMS class members.

14. Freeze HH: Understanding human glycosylation disorders: biochemistry leads the charge. J Biol Chem 2013, 288:6936–6945. [PubMed: 23329837]

15. Yang AC, Ng BG, Moore SA, Rush J, Waechter CJ, Raymond KM, Willer T, Campbell KP, Freeze HH, Mehta L: Congenital disorder of glycosylation due to DPM1 mutations presenting with dystroglycanopathy-type congenital muscular dystrophy. Mol Genet Metab 2013, 110:345–351. [PubMed: 23856421]

16 •. Ardiccioni C, Clarke OB, Tomasek D, Issa HA, von Alpen DC, Pond HL, Banerjee S, Rajashankar KR, Liu Q, Guan Z, et al.: Structure of the polyisoprenyl-phosphate glycosyltransferase GtrB and insights into the mechanism of catalysis. Nat Commun 2016, 7:10175. [PubMed: 26729507] The structure of GtrB highlights the structural elements that allow the movement of Pren-P substrate into the active site in the soluble domain.

17. Larkin A, Chang MM, Whitworth GE, Imperiali B: Biochemical evidence for an alternate pathway in N-linked glycoprotein biosynthesis. Nat Chem Biol 2013, 9:367–373. [PubMed: 23624439]

18. Eichler J, Imperiali B: Stereochemical divergence of polyprenol phosphate glycosyltransferases. Trends Biochem Sci 2018, 43:10–17. [PubMed: 29183665]

19. Price NP, Momany FA: Modeling bacterial UDP-HexNAc: polyprenol-P HexNAc-1-P transferases. Glycobiology 2005, 15:29R–42R.

20. Lukose V, Walvoort MTC, Imperiali B: Bacterial phosphoglycosyl transferases: initiators of glycan biosynthesis at the membrane interface. Glycobiology 2017, 27:820–833. [PubMed: 28810664]

21. Hug I, Feldman MF: Analogies and homologies in lipopolysaccharide and glycoprotein biosynthesis in bacteria. Glycobiology 2011, 21:138–151. [PubMed: 20871101]

22 ••. Ray LC, Das D, Entova S, Lukose V, Lynch AJ, Imperiali B, Allen KN: Membrane association of monotopic phosphoglycosyl transferase underpins function. Nat Chem Biol 2018, 14:538–541. [PubMed: 29769739] The first structure of PglC a PGT with a monotopic catalytic core shows a novel fold with a reentrant membrane helix stabilized by three amphipathic helices, which form the active site platform at the membrane interface.

23. Lehrman MA: Biosynthesis of N-acetylglucosamine-P-P-dolichol, the committed step of asparagine-linked oligosaccharide assembly. Glycobiology 1991, 1:553–562. [PubMed: 1668306]

24. Anderson MS, Eveland SS, Price NP: Conserved cytoplasmic motifs that distinguish sub-groups of the polyprenol phosphate:N-acetylhexosamine-1-phosphate transferase family. FEMS Microbiol Lett 2000, 191:169–175. [PubMed: 11024259]

25. Chung BC, Zhao J, Gillespie RA, Kwon DY, Guan Z, Hong J, Zhou P, Lee SY: Crystal structure of MraY, an essential membrane enzyme for bacterial cell wall synthesis. Science 2013, 341:1012–1016. [PubMed: 23990562]

26 •. Liu Y, Rodrigues JP, Bonvin AM, Zaal EA, Berkers CR, Heger M, Gawarecka K, Swiezewska E, Breukink E, Egmond MR: New insight into the catalytic mechanism of bacterial MraY from enzyme kinetics and docking studies. J Biol Chem 2016, 291:15057–15068. [PubMed: 27226570] This study presents a quantitative kinetic analysis and isotope-exchange studies that resolve a long standing question as to the mode of catalysis showing a random bi-bi mechanism.

27. Al-Dabbagh B, Olatunji S, Crouvoisier M, El Ghachi M, Blanot D, Mengin-Lecreulx D, Bouhss A: Catalytic mechanism of MraY and WecA, two paralogues of the polyprenyl-phosphate N-acetylhexosamine 1-phosphate transferase superfamily. Biochimie 2016, 127:249–257. [PubMed: 27312048]

28 ••. Chung BC, Mashalidis EH, Tanino T, Kim M, Matsuda A, Hong J, Ichikawa S, Lee SY: Structural insights into inhibition of lipid I production in bacterial cell wall synthesis. Nature 2016, 533:557–560. [PubMed: 27088606] The structure of muraymycin D2 bound to MraY highlights conformational rearrangements near the active site with interactions distinct from those involving the essential Mg2+ cofactor. Thus the study shows that potent inhibitors can be developed without recourse to highly-charged phosphate mimics.

29. Hakulinen JK, Hering J, Branden G, Chen H, Snijder A, Ek M, Johansson P: MraY-antibiotic complex reveals details of tunicamycin mode of action. Nat Chem Biol 2017, 13:265–267. [PubMed: 28068312]

30. Yoo J, Mashalidis EH, Kuk ACY, Yamamoto K, Kaeser B, Ichikawa S, Lee SY: GlcNAc-1-P-transferase tunicamycin complex structure reveals basis for inhibition of N-glycosylation. Nat Struct Mol Biol 2018, 25:217–224. [PubMed: 29459785]

31. Laskowski RA, Swindells MB: LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. J Chem Inf Model 2011, 51:2778–2786. [PubMed: 21919503]

32. Allen KN, Entova S, Ray LC, Imperiali B: Monotopic membrane proteins join the fold. Trends Biochem Sci 2019, 44:7–20. [PubMed: 30337134]

33. Furlong SE, Ford A, Albarnez-Rodriguez L, Valvano MA: Topological analysis of the Escherichia coli WcaJ protein reveals a new conserved configuration for the polyisoprenyl-phosphate hexose-1-phosphate transferase family. Sci Rep 2015, 5:9178. [PubMed: 25776537]

34. Lukose V, Luo L, Kozakov D, Vajda S, Allen KN, Imperiali B: Conservation and Covariance in Small Bacterial Phosphoglycosyltransferases Identify the Functional Catalytic Core. Biochemistry 2015, 54:7326–7334. [PubMed: 26600273]

35. Das D, Kuzmic P, Imperiali B: Analysis of a dual domain phosphoglycosyl transferase reveals a ping-pong mechanism with a covalent enzyme intermediate. Proc Natl Acad Sci U S A 2017, 114:7019–7024. [PubMed: 28630348]

36. Jorgenson MA, Kannan S, Laubacher ME, Young KD: Dead-end intermediates in the enterobacterial common antigen pathway induce morphological defects in Escherichia coli by competing for undecaprenyl phosphate. Mol Microbiol 2016, 100:1–14. [PubMed: 26593043]

37. McManus JB, Yang H, Wilson L, Kubicki JD, Tien M: Initiation, elongation, and termination of bacterial cellulose synthesis. ACS Omega 2018, 3:2690–2698. [PubMed: 30023847]

38. Morgan JL, McNamara JT, Zimmer J: Mechanism of activation of bacterial cellulose synthase by cyclic di-GMP. Nat Struct Mol Biol 2014, 21:489–496. [PubMed: 24704788]

39. Morgan JLW, Strumillo J, Zimmer J: Crystallographic snapshot of cellulose synthesis and membrane translocation. Nature 2013, 493:181–U192. [PubMed: 23222542]

40. Bi Y, Hubbard C, Purushotham P, Zimmer J: Insights into the structure and function of membrane-integrated processive glycosyltransferases. Curr Opin Struct Biol 2015, 34:78–86. [PubMed: 26342143]

41 ••. Morgan JL, MscNamara JT, Fischer M, Rich J, Chen HM, Withers SG, Zimmer J: Observing cellulose biosynthesis and membrane translocation in crystallo. Nature 2016, 531:329–334. [PubMed: 26958837] An impressive use of in situ crystallography with substrate analogs allows the development of a working model of cellulose elongation and translocation.

42. Knott BC, Crowley MF, Himmel ME, Zimmer J, Beckham GT: Simulations of cellulose translocation in the bacterial cellulose synthase suggest a regulatory mechanism for the dimeric structure of cellulose. Chem Sci 2016, 7:3108–3116. [PubMed: 27143998]

43. Whitfield C, Trent MS: Biosynthesis and export of bacterial lipopolysaccharides. Annu Rev Biochem 2014, 83:99–128. [PubMed: 24580642]

44. Tavares-Carreon F, Fathy Mohamed Y, Andrade A, Valvano MA: ArnT proteins that catalyze the glycosylation of lipopolysaccharide share common features with bacterial N-oligosaccharyltransferases. Glycobiology 2016, 26:286–300. [PubMed: 26515403]

45. Lizak C, Gerber S, Numao S, Aebi M, Locher KP: X-ray structure of a bacterial oligosaccharyltransferase. Nature 2011, 474:350–355. [PubMed: 21677752]

46. Matsumoto S, Shimada A, Nyirenda J, Igura M, Kawano Y, Kohda D: Crystal structures of an archaeal oligosaccharyltransferase provide insights into the catalytic cycle of N-linked protein glycosylation. Proc Natl Acad Sci U S A 2013, 110:17868–17873. [PubMed: 24127570]

47 •. Petrou VI, Herrera CM, Schultz KM, Clarke OB, Vendome J, Tomasek D, Banerjee S, Rajashankar KR, Belcher Dufrisne M, Kloss B, et al.: Structures of aminoarabinose transferase ArnT suggest a molecular basis for lipid A glycosylation. Science 2016, 351:608–612. [PubMed: 26912703] The structure of ArnT from crystals grown in lipidic cubic phase supplemented with Und-P reveals how cavities for both amphiphilic substrates converge at the active site for catalysis.

48 ••. Blaukopf M, Worrall L, Kosma P, Strynadka NCJ, Withers SG: Insights into Heptosyltransferase I Catalysis and Inhibition through the Structure of Its Ternary Complex. Structure 2018, 26:1399–407 e1395. [PubMed: 30122450] The structure of WaaC shows a pseudo ternary complex with a modified Kdo2-lipid A and a non hydrolyzable glycosyl donor. The study highlights the mode of interaction of the substrates and illustrates how the GT-B fold accommodates the amphipathic lipid A substrate for catalysis.

49. Grizot S, Salem M, Vongsouthi V, Durand L, Moreau F, Dohi H, Vincent S, Escaich S, Ducruix A: Structure of the Escherichia coli heptosyltransferase WaaC: binary complexes with ADP and ADP-2-deoxy-2-fluoro heptose. J Mol Biol 2006, 363:383–394. [PubMed: 16963083]

50. Cote JM, Ramirez-Mondragon CA, Siegel ZS, Czyzyk DJ, Gao J, Sham YY, Mukerji I, Taylor EA: The stories tryptophans tell: Exploring protein dynamics of heptosyltransferase I from Escherichia coli. Biochemistry 2017, 56:886–895. [PubMed: 28098447]

51. Cote JM, Taylor EA: The glycosyltransferases of LPS core: A review of four heptosyltransferase enzymes in context. Int. J. Mol. Sci 2017, 18:2256–2273.

52 •. Ovchinnikova OG, Mallette E, Koizumi A, Lowary TL, Kimber MS, Whitfield C: Bacterial beta-Kdoglycosyltransferases represent a new glycosyltransferase family (GT99). Proc Natl Acad Sci U S A 2016, 113:E3120–3129. [PubMed: 27199480] This study introduces a novel GT (GT99) highlighting the plasticity of the core fold to major insertions that afford a channel with new specificity for long-chain glycan substrates.

53. Schmidt H, Hansen G, Singh S, Hanuszkiewicz A, Lindner B, Fukase K, Woodard RW, Holst O, Hilgenfeld R, Mamat U, et al.: Structural and mechanistic analysis of the membrane-embedded glycosyltransferase WaaA required for lipopolysaccharide synthesis. Proc Natl Acad Sci U S A 2012, 109:6253–6258. [PubMed: 22474366]

Highlights

- Peripheral and integral membrane enzymes drive glycan and glycoconjugate assembly
- Amphiphilic substrates in glycan assembly feature acyl and polyprenyl groups
- Recent studies highlight the mechanisms of diverse transformations at the membrane
- Glycosyltransferases are adapted to accommodate and exploit membrane association
- Structure determination of interacting enzymes will enrich glycan biosynthesis studies

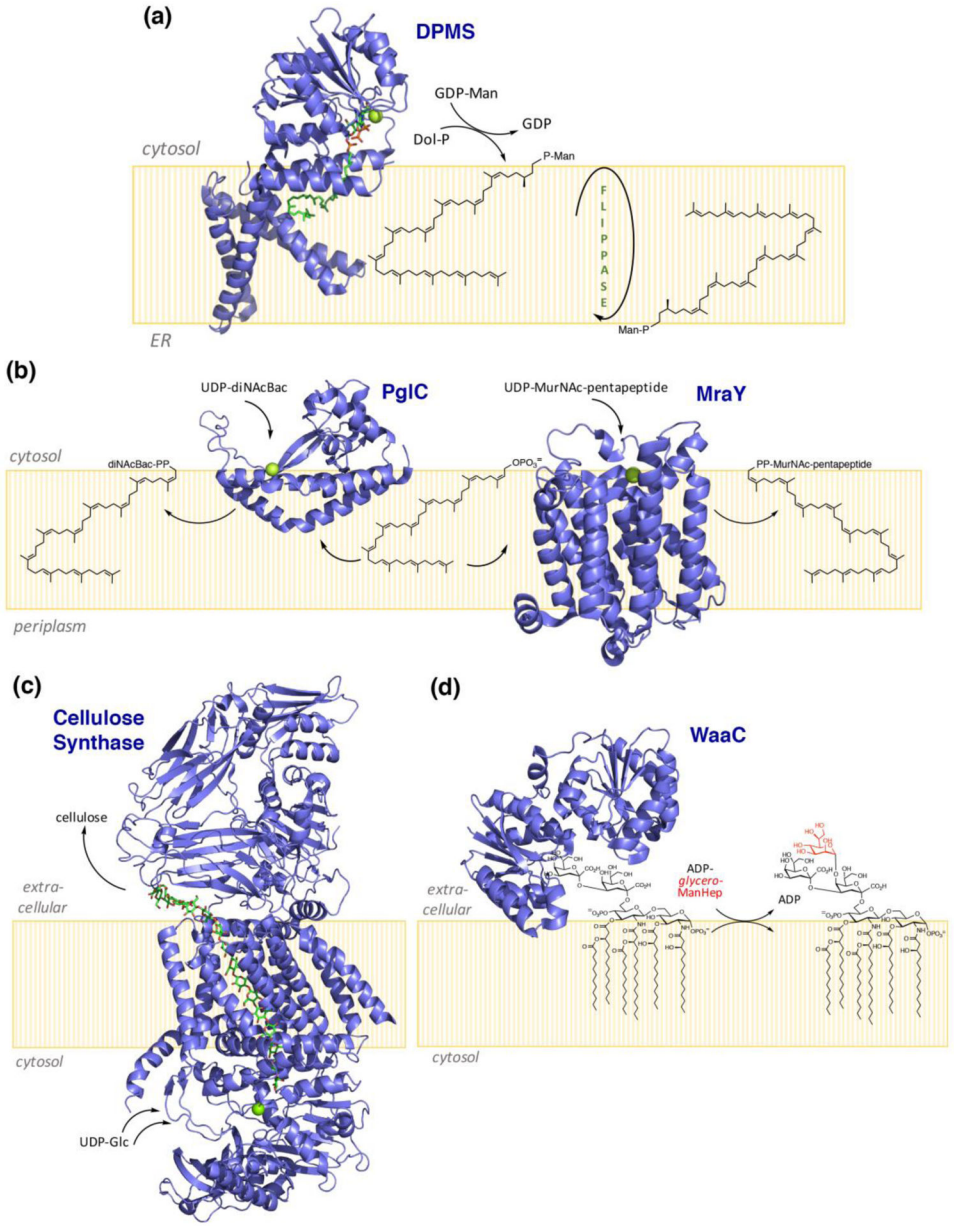

Figure 1.

Structures and transformations of enzymes in glycan and glycoconjugate biosynthesis (approximate location with respect to membrane shown with yellow lines). (a) Polyprenol phosphate glycosyl transferase exemplified by Dol-P-Man synthase. Glycosyl transfer by from a GDP-Man donor to Dol-P by a GT-A fold transferase on the cytoplasmic face of the ER membrane, followed the action of a flippase to translocate the Dol-P-Man to the ER lumen. Ribbon diagram of Dol-PMan and GDP bound to *P. furiosus* DPMS (5MM1) with Mg^{2+} superimposed from a related structure (5MLZ). (b) Monotopic and polytopic phosphoglycosyl transferases. Left: The monotopic PGT PglC (5W7L) from *C. concisus* transfers a C1-phosphosugar from UDP-diNAcBac to Und-P at the inception of the pgl pathway for N-linked protein glycosylation in *Campylobacter* species. Right: The polytopic

PGT MraY from *A. aeolicus* (4J72) transfers MurNAc-pentapeptide to Und-P. All PGTs use a common pool of Und-P. (c) Cellulose synthase bound to nascent cellulose polymer and Mg^{2+}. Cellulose synthase from *Rhodobacter sphaeroides* (5EJZ) includes a BcsA subunit with both a cytosolic GT-A fold domain and a transmembrane domain supporting channel function. The essential periplasmic BcsB domain caps and contributes a single helix to the transmembrane domain. (d) Elaboration of lipid A intermediate by WaaC. WaaC from *Escherichia coli* (6DFE) is a GT-B fold GT that catalyzes heptose transfer to the Kdo_2-lipid A intermediate. The transfer occurs at a distance above the membrane to accommodate the Kdo_2-lipid A structure.

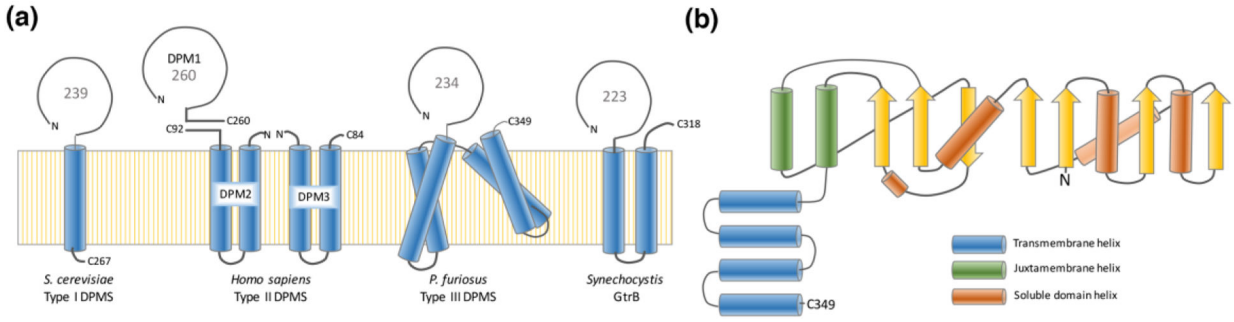

Figure 2.

Topologies of Pren-P glycosyltransferases. (a) Topologies of Type I, Type II and Type III DPMS enzymes from diverse organisms and GtrB from *Synechocystis*. The number of amino acids in each soluble domain is denoted. (b) Secondary structure schematic of the *P. furiosus* Type III DPMS highlighting transmembrane, juxtamembrane, and soluble domain helices (5MM1).

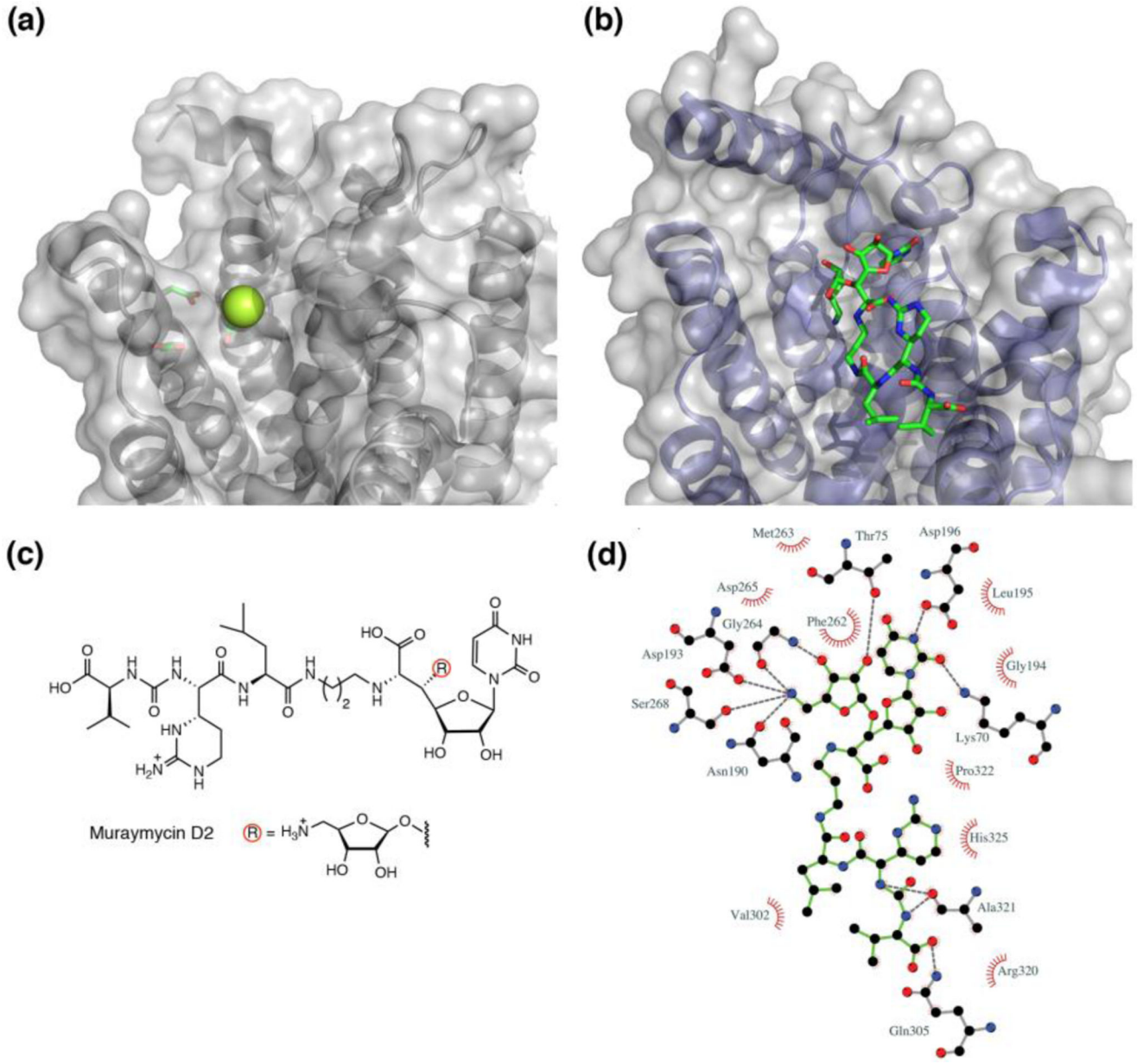

Figure 3. Comparison of MraY with Mg^{2+} cofactor and complexed to muraymycin D2 (MD2) shows plasticity of the binding site.

(a) Close up of MraY with Mg^{2+} cofactor shown as green sphere with essential acidic residues shown as sticks (4J72). Image shows van der Waals surfaces on cartoon rendition. (b) Close up of MraY complexed with muraymycin D2 (5CKR) in exact alignment with (a). Image shows van der Waals surfaces on cartoon rendition and illustrates conformity of MraY to antibiotic shape and lack of Mg^{2+} occupancy. (c) Chemical structure of MD2. (d) Analysis of MD2 binding to MraY (prepared using LigPlot^{+} [31])

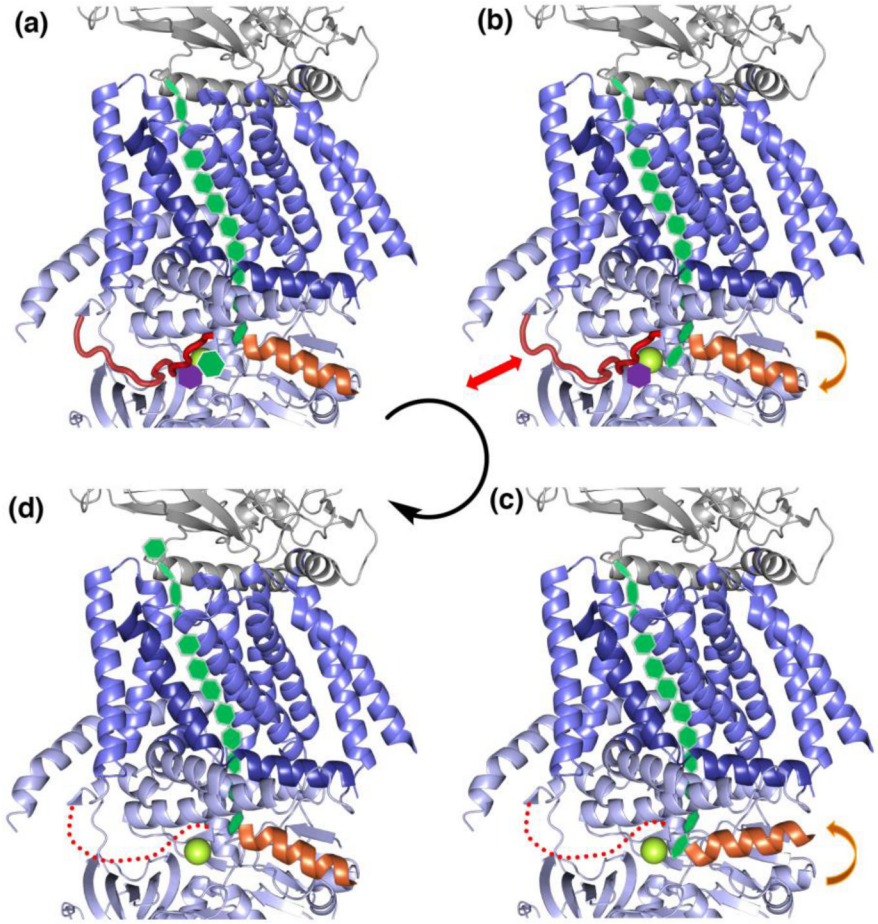

Figure 4. Model of cellulose biosynthesis

Rhodobacter sphaeroides BscA and BscB subunits (5EIY) are shown as ribbon diagrams (periplasmic domain, grey; transmembrane domain, blue; glycosyltransferase domain, light blue; bent helix, dark blue). The motion of a gating loop (red) and finger helix (orange) allow coordination of bond formation (elongation) and translocation, effecting polymer secretion (a-d). [Note that in all panels the gating loop and helix are schematized to emphasize the conformational cycle and shown with respect to the 5EIY structure]. Cellulose synthase from crystals incubated with non-hydrolyzable substrate analogue ($UDPCH_2$-Glc) plus a galactose-capped polymer yielded the substrate-bound complex with finger helix up and gating loop contacting substrate UDP-glucose (depicted a purple and green hexagons) (a). The product complex after glycosyl transfer obtained by first elongating with 2-fluoro-glucose then incubating with UDP/Mg^{2+} shows the finger helix and gating loop remain in position (b). The pre-translocation complex observed from crystals incubated with UDP-Glc in the absence of Mg^{2+} allowing addition of a single glycan unit shows UDP release and retraction of the gating loop (c). The finger helix moves to the up position in its post translocation conformation (d) and the gating loop moves in either concomitant with or following UDP-Glc binding. During translocation, hydrophobic residues lining the channel enforce planarity, causing rotation of the newly added glycan,

creating the “dimeric” chemical structure of the repeating unit of cellulose (interactions analyzed in [42]).

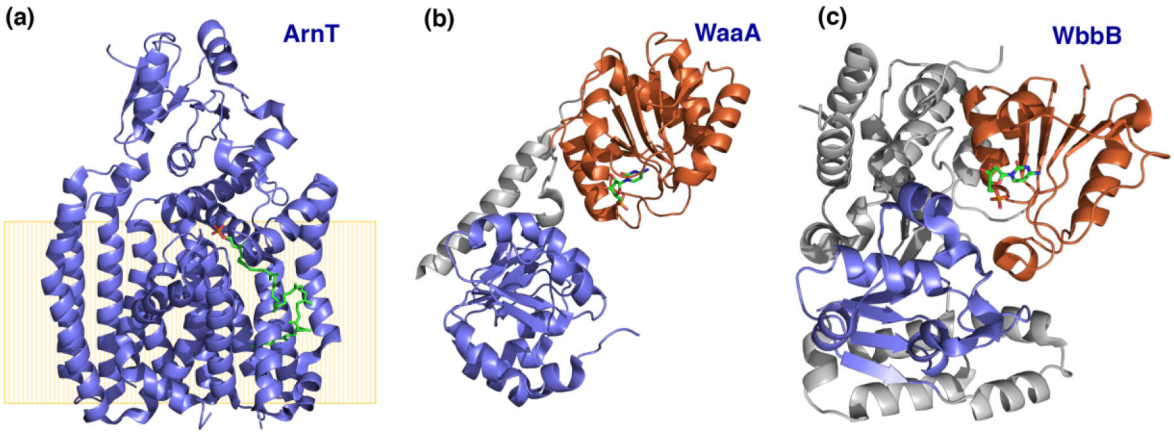

Figure 5. Enzymes of lipid A modification and biosynthesis

(a) Ribbon depiction of *Cupriavidus metallidurans* ArnT (5F15) with bound Und-P (green sticks). The approximate location of the UndP phosphoryl group with respect to membrane (shown in yellow lines) highlights the active-site location at the membrane interface. Comparison of ribbon diagrams of (b) *Aquifex aeolicus* WaaA (2XCU) and (c) *Raoultella terrigena* WbbB (5FA1) in complex with CMP (green sticks) shows the insertions into the two GT-B fold Rossmann domains (blue and orange) in WbbB that afford distinct substrate specificity.