HHS Public Access

Author manuscript

Trends Biochem Sci. Author manuscript; available in PMC 2020 January 01.

Published in final edited form as:

Trends Biochem Sci. 2019 January ; 44(1): 7–20. doi:10.1016/j.tibs.2018.09.013.

Monotopic Membrane Proteins Join the Fold

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Abstract

Monotopic membrane proteins, classified by topology, are proteins which embed into a single face of the membrane. These proteins are generally underrepresented in the protein data bank, but the last decade of research has revealed new examples that allow description of generalizable features. This review summarizes shared characteristics including oligomerization states, modes of membrane association, mechanisms of interactions with hydrophobic or amphiphilic substrates and homology to soluble folds. We also discuss how association of monotopic enzymes into pathways can be used to promote substrate specificity and product composition. These examples highlight the challenges in structure determination specific to this class of proteins, but also the promise of new understanding from future study of these proteins that reside at the interface.

Keywords

membrane-protein interaction; membrane interface; monotopic enzymes; membranome

Integral membrane proteins are implicated in a multitude of essential biological processes including metabolism, biosynthesis, and cellular signaling [1]. They comprise between 20 and 30% of all proteomes [2, 3]. Membrane proteins can be classified based on topology, which describes the physical arrangement of the structures with respect to the membrane (Box 1, Fig 1(A)). There are three topologically distinct classes of integral membrane proteins: polytopic, bitopic, and monotopic [4], all of which are irreversibly associated with the membrane and require addition of detergents for solubilization [5, 6]. Membrane proteins may adopt bitopic or polytopic geometries to satisfy functional properties. For example, cell-surface receptors such as receptor tyrosine kinases and G-protein-coupled receptors transduce information from the outside to the inside of cells. Similarly, channels and transporters move materials across membranes to perform critical tasks. In contrast, the

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relationships between topology and function are less clear-cut for monotopic membrane proteins, and they are the least well understood from a structural perspective. Additionally, some proteins, categorized as monotopic membrane proteins may only be peripherally associated thus adding to ambiguities that can only be resolved with detailed experimental analysis [7, 8]. Only a scant 0.06% of non-redundant structures exhibit the monotopic membrane topology, although integral membrane proteins as a whole make up 4.6% (Box 1, Fig 1(A)). The structures of full-length monotopic membrane proteins have proven to be more recalcitrant to structure determination, and computational methods to enable distinction between bitopic and monotopic membrane proteins are also needed.

Location, location, location

The structurally-characterized monotopic membrane proteins defined to date specialize in catalyzing reactions on membrane-resident substrates, often including a second hydrophilic substrate (Figure 1). Thus, information on the enzyme substrates and reaction mechanisms provides a valuable starting point for understanding how the structures are purposed for catalysis. There are two principle strategies for the interaction of monotopic enzymes with their substrates: enzymes may “extract” hydrophobic substrates from the lipid bilayer for catalysis at an active site positioned distal to the bilayer interface, or, they adopt an energetically-favorable “leave in place” strategy, whereby hydrophobic and amphiphilic substrates are not subject to translocation from the membrane for catalysis (Box 1, Fig. I(B)) [1]. Irrespective of the mode of substrate interaction, examination of monotopic proteins of known structure reveals common themes for membrane association (Box 1, Fig. I(C)). Membrane association may occur through hydrophobic interactions supported most commonly through amphipathic helices positioned parallel to the membrane plane [9]. Additional structural features enlisted include hydrophobic loops extending into one leaflet of the membrane and hydrophobic patches coupled with electrostatic interactions with phospholipid head groups [6, 10–13]. Alternatively, the protein structures may penetrate more deeply into the hydrophobic core of the membrane, for example through reentrant motifs that associate with both the membrane core and with domains of the protein that are positioned at the membrane interface [14, 15]. Molecular dynamics simulations have shown that the membrane association of monotopic proteins that extend deeper into the membrane result in local perturbations in the lipid bilayer [10], which can be functionally important.

In addition to these specific membrane-association modalities, the known structures of monotopic membrane proteins may share some global features (Box 1, Fig I(C)). They may comprise extensive soluble domains, with homology to known soluble proteins and minimal membrane-embedded domains, that have been repurposed for catalysis at the membrane interface (Figure 2, Table 1). Many are biologically active as dimers [16] and some feature long hydrophobic tunnels connecting the membrane interface to active sites located at a distance from the membrane, allowing access to hydrophobic substrates. The majority of monotopic membrane enzymes that have been structurally characterized to date share homology with existing soluble folds of known function [17, 18]. In some cases, the soluble and membrane-associated forms of enzymes are even isoforms of each other [19, 20]. In contrast, the structures of some monotopic membrane proteins are unique, indicating that

they may have evolved via membrane-dependent processes and reveal new folds not observed in soluble homologues [21, 22].

Double feature

As first noted for fatty acid amide hydrolase (FAAH) [6, 23] membrane association in monotopic membrane proteins is commonly enabled via insertion of membrane interacting segments and/or dimerization to increase the surface area of the protein-membrane interaction. This strategy is also exemplified by RPE65, an enzyme expressed in the retinal pigment epithelium that catalyzes the conversion of all-*trans*-retinyl esters to 11-*cis*-retinol (Figure 3A). An especially "physiologically relevant" crystal form was obtained by crystallizing RPE65 at high lipid/detergent ratios- resulting in RPE65 that is not separated from native phospholipids [24]. Thus, the isomerase packs as a dimer with the membrane-binding surface facing micelle sheets, mimicking the assembly at the membrane. Sequence- and structure-based alignments show a ~25 amino acid-long insert in RPE65 that affords the dimerization domain is not found in soluble, monomeric homologues (e.g. apocarotenoid oxygenase) [25]. Notably, orthologues of RPE65 from bacteria can bind membrane through a hydrophobic patch, but are monomeric and do not contain the dimer-mediating sequence. Thus, dimerization is not requisite for evolution of membrane binding and RPE65 and vertebrate family members may have elaborated a more extensive membrane-binding surface in concert with acquiring a dimer interface.

Another requirement of the dimerization strategy is met by FAAH, where a 180° rotation in the subunit orientations within the dimer, as compared to soluble dimeric family members, allows simultaneous access of both active sites to the membrane interface. Interface remodeling is not always needed, as is the case for the cyclooxygenase-2 (COX-2), also called prostaglandin H2 synthase (PGHS) [26], which catalyzes the first committed step of prostaglandin biosynthesis, converting arachidonic acid to prostaglandin H_2. (Figure 3B). The catalytic domain of COX-2 has low sequence identity (~20%) but high structural similarity (RMSD = 2.6 Å) to the soluble mammalian peroxidases (e.g. canine myelo-peroxidase) [27]. The subunit interface of the COX-2 dimer allows the same relative orientations of the catalytic domains as the subunit interface in the soluble mammalian peroxidases, with sequence inserts affording membrane interaction. Thus, nature has leveraged existing monomeric and dimeric soluble domains to allow direct access of these catalytic domains to the membrane interface, allowing extraction of substrate and release of product to the bilayer.

COX-2 [26] and α-dioxygenase (α-DOX) (Figure 3C) [28] illustrate a case where two enzymes from the same superfamily adopt distinct mechanisms of membrane interaction. α-DOX converts linoleic acid (LA, 18:2 ω−6) and related fatty acids into 2(R)-hydroperoxides, which undergo spontaneous decarboxylation to chain-shortened aldehydes [29] (Figure 3C). α-DOX has a two-domain structure with a membrane-interacting domain and a catalytic domain. There is significant structural similarity in the catalytic domains of α-DOX and COX-2 (RMSD = 1.7 Å). Four α-helices comprise a hydrophobic fatty-acid binding channel within the catalytic domain of α-DOX, which spans ~20 Å between the membrane domain opening and the catalytic site. The structural arrangement of the four helices and hence the

fatty-acid binding channel is similar to that of the substrate-binding channel of COX-2. In contrast, there is no sequence or structural similarity between the membrane-binding domains of the two folds, although both interact with the membrane via amphipathic helices. These α-helices tether α-DOX and COX-2 to one leaflet of the membrane and serve as the access point for entry of the substrate into the catalytic domain. Taken together, it seems that members of the cyclooxygenase-peroxidase family employ a diversity of modalities for membrane interaction. Why COX-2 forms a dimer whereas α-DOX exists as a monomer is not clear from the standpoint of energetics, as both interfaces bury similar surface area in the membrane.

A difficult extraction

Localization of monotopic proteins at the membrane interface positions them in proximity to lipid-soluble substrates, yet it is not uncommon for these enzymes to extract their substrates through hydrophobic channels into polar active sites distal to the interface for catalysis. Although energetically unfavorable, this strategy is favorable in terms of evolution, leveraging the recruitment of a soluble domain already optimized to enact catalysis proficiently. The nature of the connection between the catalytic site and membrane interface is critical in obviating the need to transfer substrate through aqueous cellular compartments, and thus has conserved features among monotopic membrane proteins that extract their membrane-soluble substrates. In RPE65, the side chains of residues lining a channel from the active-site to the membrane form a continuous aromatic surface that, by interacting with the polyene chain of retinal ester, allow uptake of substrate directly from the membrane (a distance of ~20 Å). In addition to the presence of a hydrophobic substrate channel, the features of the interface between the channel entrance and membrane-binding surface are similar in the monotopic membrane proteins FAAH, squalene-hopene cyclase (SHC) [30] (Figure 3D), and COX-2/α-DOX, despite all three examples originating from different fold families. These enzymes have basic amino acids surrounding the substrate channel entrance allowing binding of negatively-charged phospholipid head groups comprising the membrane. Monotopic folds with hydrophobic channels to the active site have the added advantage of protection from bulk solvent. For instance, the terpene cyclases squalene-hopene cyclase and oxidosqualene cyclase contain highly-conserved, hydrophobic active sites containing electron-rich amino acids that allow the generation, propagation, and shielding of carbocation intermediates from bulk solvent, preventing termination of the cyclization cascades by nucleophilic addition of water [30–32]. Similarly, the active site and heme prosthetic group of the cytochrome P450s are buried (e.g. at a distance of 28 Å from the membrane interface to the heme iron in CYP19A1 [33]). This feature prevents decomposition of the iron–oxygen intermediates by uncoupling reactions which consume NAD(P)H and result in formation of reactive oxygen species [34].

Membrane-bound cytochrome P450s are targeted to the interface by an N-terminal leader sequence that includes an amphipathic helix linked by a polar connector to the catalytic domain, which is structurally homologous to that of soluble cytochrome P450s [35]. As compared to these soluble homologs, in membrane-bound cytochrome P450s (except the CYP51 family) there is a shift in the location of the beta-domain and the A-propionate heme side chain towards the proximal side of the heme – a design feature that allows access of

substrates from the membrane interior [34]. To control reactivity, cytochrome P450s are structurally dynamic, with open and closed conformers for substrate association and product dissociation. Molecular dynamics studies support a model wherein interaction with the membrane promotes the open state of the substrate tunnel leading from the membrane interior to the buried active site [36]. In substrate-specific cytochrome P450s, lipophilic-substrate binding alters protein dynamics and increases catalytic specificity and efficiency. Additionally, consequences of the membrane-interaction mode on conformation were revealed in the structure of a full-length fungal cytochrome P450, which contained an extended N-terminal transmembrane helix (designated as bitopic) [35]. The structure shows a rigid interaction between the membrane-resident domain and catalytic domain suggesting that the transmembrane domain itself may promote the catalytically-competent pose [37].

As a final example, sn-glycerol-3-phosphate dehydrogenase (GlpD) is an essential FAD-dependent enzyme that catalyzes the oxidation of glycerol 3-phosphate (G-3-P) to dihydroxyacetone phosphate (DHAP) (Figure 3E). The enzyme is a dimer comprising a soluble extramembraneous C-terminal domain and a N-terminal FAD-binding domain that includes the membrane-resident region. GlpD similarly exemplifies both the adaptation of catalytic units to act at the membrane and extraction of a hydrophobic substrate from the membrane by both subunits of a dimer. Notably, GlpD can exist as a soluble isoform and a membrane-bound form with sequence identity >40% [19]. Based on the distribution of polar and hydrophobic residues it is estimated that GlpD embeds to depths of 12–15 Å into the lipid bilayer and the distance from the membrane surface to the isoalloxazine ring of FAD is ~18 Å. As in many of the dimeric monotopics, the molecular two-fold axis relating the subunits within the dimer is perpendicular to the membrane, allowing access from the membrane to both active sites.

The first 20 years of membrane protein structure determination yielded only four examples of monotopic folds, all of which act by extracting substrates from the membrane and extending membrane interactions by dimerization [1]. This initially gave a limited view of membrane-association strategies, shown later with additional examples to be more generalizable. The past decade has now yielded new examples that afford a greater diversity of both structure and the associated mechanisms of substrate interaction (Box 1, Fig. I (C)).

Something old, something new

A critical step in the biosynthesis and remodeling of phospholipids is catalyzed by the extensive family of 1-acyl-*sn*-glycerol-3-phosphate acyltransferases (Figure 3F). These monotopic enzymes simultaneously bind to the membrane-committed lysophospholipid substrate and the soluble acyl-thioester donor. Despite the importance of these enzymes, an experimentally-determined structure has been elusive and attempts at structure prediction failed to yield a coherent solution that was consistent with a viable biochemical model [38]. The recent structure of PlsC from *Thermatoga maritima* now reveals that the functional domain of the enzyme is based on a known soluble αβ-acyltransferase fold with a signature HX_4D motif, including the catalytic dyad, that is tightly associated with one face of the membrane by an intriguing two-helix motif. An extended N-terminal helix includes a core of aromatic and other hydrophobic residues, delineated by basic residues, and is predicted to be

positioned into a single leaflet of the bilayer, running parallel to the membrane interface (Figure 2). An adjacent partner helix is rich in basic residues favoring placement at the membrane interface in the phospholipid head-group region. In contrast to the “extracting” folds, a considerably higher percentage of the protein (16% calculated using the PPM server, Figure 1) is membrane-embedded. Thus, this intriguing, yet simple, architecture enables simultaneous interaction with both of the target substrates, despite their very different properties, and sets the stage for ternary complex formation without requiring extraction of the amphipathic lysophospholipid from its membrane environment. The structure also provides insight into acyl chain specificity. The native specificity of PlsC is for a C16 palmitoyl group, which is shown to be perfectly accommodated in a hydrophobic pocket poised for transfer to the 2-position of the lysophospholipid substrate. The assignment of the acyl binding site is corroborated by mutational studies that successfully switch the substrate specificity from palmitoyl to myristoyl transfer.

A marriage of convenience

Monotopic membrane enzymes are prominent in biochemical pathways where localization of multiple enzymes, acting in sequence at the membrane interface, is highly advantageous. A common theme in the biogenesis of complex glycoconjugates is the stepwise assembly of glycans onto linear long-chain polyprenol phosphates [39]. For example, N-linked protein glycosylation in the pgl pathway of Gram-negative *Campylobacter* species involves assembly of glycan-linked polyprenol diphosphate, through the action of the phosphoglycosyl transferase (PGT) PglC, followed by glycosyl transferases (GTases) PglA, PglJ, PglH and PglI on the cytoplasmic face of the inner bacterial membrane [40, 41] (Figure 3G). These enzymes are predicted monotopic membrane enzymes that act directly and sequentially on amphiphilic polyprenol diphosphate-linked substrates, which remain resident in the membrane. The recently reported structure of PglC shows a novel membrane-association mode anchored by a reentrant membrane helix [15] that interacts with three short coplanar amphiphilic helices at the membrane interface [21]. The experimentally-determined structure deviates from predictions based on primary structure and hidden Markov model (TMHMM) [2], which suggested a bitopic architecture. PglC is the first characterized representative of a monotopic PGT superfamily and offers a unique structural view of a reentrant membrane helix supporting membrane association [21]. Based on extensive homology and covariance analysis the reentrant membrane helix is conserved across this monotopic superfamily [42]. Structural and mechanistic studies on PglC revealed an active site, featuring an Asp-Glu dyad, perfectly positioned to capture the soluble uridine diphosphate bacillosamine (UDP-Bac) substrate for transfer of Bac-P to polyprenol phosphate (Pren-P) via a covalent enzyme intermediate [43] to afford Pren-PP-Bac. The structure of PglC shows no homology to known soluble folds or to the other superfamily of PGTs, which is exemplified by MraY and WecA, and features a polytopic architecture with 11–12 transmembrane helical domains (TMDs) and an active site crafted from inter-TMD loops [44, 45].

Further assembly of the glycan-linked polyprenol diphosphate involves the action of a series of monotopic GTases based on a GT-B fold (Figure 3G) [46]. The GT-B-fold GTases are highly adaptable, and this soluble fold has evolved into peripheral, monotopic, and bitopic

variants to meet functional requirements [47]. The GT-B fold displays C- and N-terminal Rossmann-fold domains, which meet at a central cleft containing the enzyme active site. The C-terminal domain binds the nucleoside diphosphate-sugar donor and the N-terminal domain, which associates with the membrane, binds the glycosyl moiety of the glycan-linked polyprenol diphosphate acceptor substrate. Thus, the hydrophobic polyprenol moiety positions the latter substrate for catalysis without the need for extraction from the membrane. Early in the pgl pathway, PglA transfers GalNAc to Pren-PP-Bac. Although PglA has yet to be crystallized, a functional homologue, WbnH (*Escherichia coli*), which catalyzes transfer of GalNAc from UDP-GalNAc to Pren-PP-GalNAc to afford an α−1,3-GalNAc linkage has been characterized [48]. The membrane-associated structure of WbnH, predicted using the Xray structure and the PPM web server [49], shows that WbnH associates with the membrane through three hydrophobic loops on the surface of the N-terminal domain (Figure 4A). This membrane-association domain is consistent with the C-terminal domain “dipping” towards the membrane surface to accommodate transfer of GalNAc to Pren-PP-monosaccharide at the active site situated between the protein domains.

PglH is a monotopic GT-B GTase, which adds the fourth, fifth and sixth sugars to the Pren-PP-trisaccharide (Figure 3G). PglH is intriguing as it adds three saccharide units in a processive fashion and reactions occur distal to the membrane surface. In this case, the predicted placement of PglH in the membrane calculated using PPM server [49] reveals how the tilt of the structure relative to the membrane surface may accommodate these reactions by placing the active site cleft further from the membrane surface [50] compared to WbnH (Figure 4A). Insight into how PglH transfers predominantly three carbohydrate units comes from both mechanistic and structural analyses. Kinetic analysis with native substrates shows that each progressive sugar addition is retarded due to product inhibition, ultimately disfavoring further elongation of the product [51]. Additionally, recent structural analysis with substrates and substrate analogs suggests that specific interactions between the diphosphate moiety of the Pren-PP-glycan and basic residues on an α-helix at the membrane surface acts as a molecular ruler that forms the basis for substrate specificity and determines the final product composition [50]. Ultimately, the placement of the extended polyprenol moiety of the substrate in the membrane, married to the distal active-site binding of the soluble glycan moiety, takes full advantage of the monotopic enzyme topology of PglH.

There are currently many aspects of monotopic GTase structure and function left to understand and a richer selection of structures in the fold family is needed. However, it is intriguing to speculate that the GT-B fold can be enlisted to transfer carbohydrates to specific positions within an elongating glycan chain based on the relative placement of the enzyme active site and the membrane surface.

In the neighborhood

Monotopic membrane enzymes also feature in early steps of the “Raetz pathway” [52] for lipopolysaccharide (LPS) biosynthesis (Figure 3H). The first five steps of Kdo-Lipid A assembly occur in the cytosol. At this stage in the pathway, the intermediate has accumulated a preponderance of long-chain β-hydroxyacyl moieties and thus would not be free in solution, thus, the process transitions to a new neighborhood: the cytosolic face of the

inner membrane. Tetraacyldisaccharide-1-phosphate 4'-kinase (LpxK) catalyzes ATP-dependent phosphorylation to afford the bis-phosphorylated Lipid IV_A. The structure of LpxK from *Aquifex aeolicus* (Figure 4B) reveals the first example of a P-loop kinase [53] adapted for function at the membrane interface through the addition of a membrane-associating N-terminal extension [54]. Thus, in this case, localization to the membrane occurs by sequence extension rather than by mutation within the fold, as in previously discussed examples. The *A. aeolicus* pathway is homologous to that from *E. coli* showing only minor differences in the lengths of the β-hydroxyl-acyl tails installed [54]. The N-terminal helix of LpxK was originally predicted to be membrane spanning [2]; however, like PglC, interactions between the N-terminus and the globular domain, support a native monotopic topology and a functional monomer. Structure analysis of the ADP-Mg^{2+}-bound enzyme shows engagement of the Walker A and B motif residues and supports a model wherein the tetraacylated disaccharide substrate is "partially" extracted from the membrane to bring the C-4-hydroxyl group into proximity with the γ-phosphate of bound ATP (Figure 4B). Kinetic analysis supports the intermediacy of a ternary complex.

The LpxK product is elaborated by the monotopic GTase WaaA, which transfers a single Kdo (3-deoxy-D-*manno*-oct-2-ulosonic acid) from an activated CMP-Kdo donor. WaaA, like WbnH and PglH, is also a GT-B fold GTase. In this case, structural and computational studies illustrate how large-scale conformational changes may promote activity at the membrane interface, without the need for extracting the amphiphilic substrate from its preferred environment: the N-terminal domain of WaaA associates with the membrane via a surface hydrophobic patch surrounded by a horseshoe-shaped ribbon of basic residues, which is proposed to facilitate access of the lipid A precursor to the active site at the interdomain cleft [55]. In WaaA, the cleft is large (23 Å x 20 Å) in the substrate-free state, but closes down to position the catalytic machinery via motion of two hinge regions (Box 1, Fig I(B)). Following WaaA are LpxL and LpxM, which are lauroyl and myristoyl acyl transferases belonging to a large superfamily of long-chain acyl transferases. The N-terminal hydrophobic domains of LpxL and LpxM had been previously predicted to form transmembrane helices, indicating a bitopic topology. Structures of full-length variants of this important superfamily have been very difficult to obtain: the structure of the *Acinetobacter baumannii* LpxM was only recently reported [56]. The mode of membrane association for the fold cannot be inferred from the sole reported structure, in which the N-terminal domain protrudes from the soluble domain as a helix-break-helix motif. However, recent biochemical and bioinformatics analyses of LpxM from *E. coli* now suggest a monotopic topology with the soluble domain anchored on the cytoplasmic face of the membrane by a reentrant helix [57].

Conclusions

Over the past decade the pace of membrane proteins structure determination has increased due to collective advances in protein expression strategies, including cell-free methods, dedicated membrane protein crystallization screens, technical advances such as crystallization with endogenous lipids and in lipidic cubic phases, structure analysis in membrane-like scaffolds such as nanodiscs and bicelles, and a recent upsurge in the capabilities of, and access to, cryo-electron microscopy. However, despite these

technological advances, structure determination of monotopic membrane proteins face challenges specific to this class. In particular, creation of truncation constructs often utilizes natural domain boundaries to truncate proteins for improved purification and structure determination properties. However, these constructs often omit entirely or perturb membrane-associated domains. Therefore, while this strategy may lead to favorable crystallization and structure determination, omission of these domains leads to a loss of structural insight. The many examples described herein, together with evolving methodologies in structural biology, now pave the way towards a deeper understanding of monotopic membrane proteins and their diversity of folds.

Glossary

Amphipathic
A molecule, protein segment or protein having both hydophobic and hydrophilic components

Bitopic protein
A membrane protein that spans the membrane bilayer a single time; sometimes referred to as a “single-pass” transmembrane protein

Glycosyl transferase (GTase)
An enzyme that catalyzes transfer of a sugar from an activated sugar donor, commonly a nucleoside diphosphate derivative, to an acceptor such as another sugar or a lipid

Homologue
A member of the same protein family

Integral membrane protein
A protein that is inserted into the membrane and can only be solubilized by detergent

Isoform
Two structurally and funtionally similar proteins with a non-identical sequence due to encoding by different genes or splicing variations

Monotopic protein
A membrane protein that does not span the membrane bilayer, but rather enters and exits on a single face of the membrane bilayer

OPM database
Orientations of Proteins in Membranes database is a curated web resource providing information on the position of proteins in membranes. Included in the database is output from the PPM server

Orthologue
A member of the same protein family having the same function but occuring in a different organism

Paralogue

A member of the same protein family having a different function

Phosphoglycosyl transferase (PGT)
An enzyme that catalyzes transfer of a phosphosugar from an activated sugar donor, commonly a nucleoside diphosphate derivative, to an acceptor substrate such as a polyprenol phosphate

Peripheral membrane protein
A protein associated with the membrane surface in a manner that can be dissociated by the addition of high salt

Polyprenol
A linear long-chain alcohol featuring greater than six isoprene units (branched 5 carbon units) of either *cis* or *trans* configuration

Polytopic protein
A membrane protein that spans the membrane bilayer multiple times

PPM server
This server calculates rotational and translational positions of transmembrane and peripheral proteins in the membrane using the PDB coordinate files as input

Reentrant helix
A discontinuous hydrophobic helix that enters and exits on a single face of the membrane bilayer

Rossmann fold
A fold characterized by repeating motifs of α-helix-β-strand-α-helix secondary structure elements where the β-strands together form a parallel β-sheet

TMHMM
Algorithm to predict transmembrane helices in proteins using a hidden Markov model

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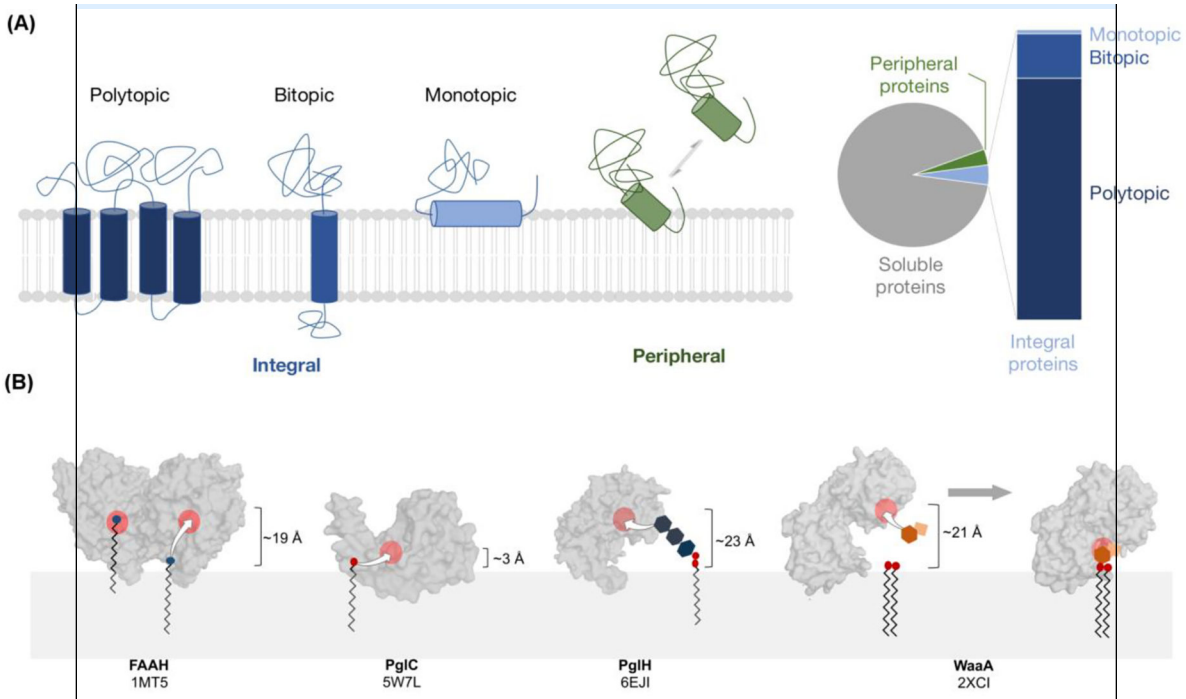
BOX 1. Classification, characteristics and structural representation of membrane proteins.

Integral membrane proteins are classified as polytopic, bitopic, or monotopic dependent upon their membrane-associated topology and domain distribution across the membrane. Peripheral proteins associate reversibly with the lipid bilayer (Figure IA, left). The distribution of membrane protein structures within the PDB shows a paucity of monotopic membrane protein structures (Figure IA, right). Numbers for each class are compiled from non-redundant (70% sequence ID) PDB, OPM, and MPStruct databases: soluble proteins – 34,987; peripheral proteins – 1,350; integral proteins – 1,679; polytopic – 1,399; bitopic – 256; monotopic – 25. For a curated database of membrane protein structures see: http://blanco.biomol.uci.edu/mpstruc/. The modes of interaction of monotopic enzymes with membrane and substrates are diagrammed in Figure IB. FAAH exemplifies enzymes that extract the membrane soluble substrate into the active site above the membrane interface. PglC exemplifies enzymes where the active site is at the interface, obviating extraction. In enzymes like PglH, active sites are distal to the membrane surface, allowing the correct spacing for membrane-resident substrates with hydrophilic moieties. WaaA undergoes a conformational change bringing the active site to the membrane interface. Comparison of the monotopic membrane proteins highlights shared features including oligomerization states, modes of interaction with membrane and substrate(s), and homology to soluble folds (Figure IC).



(C) Comparison of features of monotopic membrane proteins.

CHARACTERISTIC	MONOTOPIC ENZYME
Functional dimer	NAPE-PLD, COX-2, AOX, FAAH, GlpD, SHC, RPE65, NDH-2, TagF
Amphipathic helices	CPT-II, COX-2, AOX, CYP19A1, α-DOX, FAAH, ETF-QO, PlsC, PglC, SHC, PglH, SQR, LpxK, NDH-2, TagF
Long hydrophobic loop	NAPE-PLD, RPE65, NDH-2
Hydrophobic "channel" to active site	CPT-II, COX-2, CYP19A1, α-DOX, FAAH, SHC, GlpD, RPE65
Hydrophobic patch surrounded by positive charges	COX-2, α-DOX, FAAH, GlpD, WaaA, SHC
Reentrant membrane helix	LpxM, PlsC, PglC
Homology to soluble folds	All except AOX and PglC

Abbreviations, protein data bank accesion code, and references as in Table 1.

Outstanding Questions

- What mechanisms might allow the evolution of monotopic membrane proteins apart from the mere recruitment of soluble folds to the membrane?
- How does the evolution of shallowly-embedded monotopic proteins differ from that of proteins that penetrate more deeply into the hydrophobic core of the membrane?
- Why have monotopic membrane proteins with reentrant helical domains proved to be so intractable to structure determination in the past? How can recent advances in the field be applied to expand the diversity of monotopic membrane proteins in the PDB?
- How can recent advances in techniques such as cryo-electron microscopy and use of model membrane scaffolds, such as nanodiscs and lipidic cubic phases, be leveraged to yield the most relevant structures of membrane-embedded proteins in native-like environments?
- Are there specific motifs driving monotopic membrane association via reentrant helices? Can the knowledge of these motifs be leveraged to predict membrane association modes for structurally- and functionally-uncharacterized proteins?
- How does the composition of the lipid bilayer, with respect to the phospholipid head groups, acyl chains and lipid additives, affect enzyme activity?
- Can the current understanding of the function of monotopic enzymes inform bioengineering efforts to apply enzymes as catalysts for transformations on hydrophobic or amphiphilic substrates?

Highlights

Monotopic proteins are underrepresented in the PDB, with only 25 non-redundant structures currently comprising ~0.06% of known structures.

- Many monotopic membrane proteins are homologous to soluble counterparts and use common structural features to embed shallowly in the membrane.
- Selected monotopic proteins engage more deeply in the membrane, e.g. associating via reentrant-helical domains.
- Monotopic enzymes are purposed for catalysis of reactions involving hydrophobic or amphiphilic substrates not readily soluble in water.
- The active sites of monotopic enzymes may be at the membrane surface or distal to it, and the requirement for hydrophobic substrate extraction is dictated by the substrate and the relative orientations of active site and membrane.
- Association of multiple monotopic enzymes in pathways can be advantageously applied in the assembly of complex glycoconjugates.

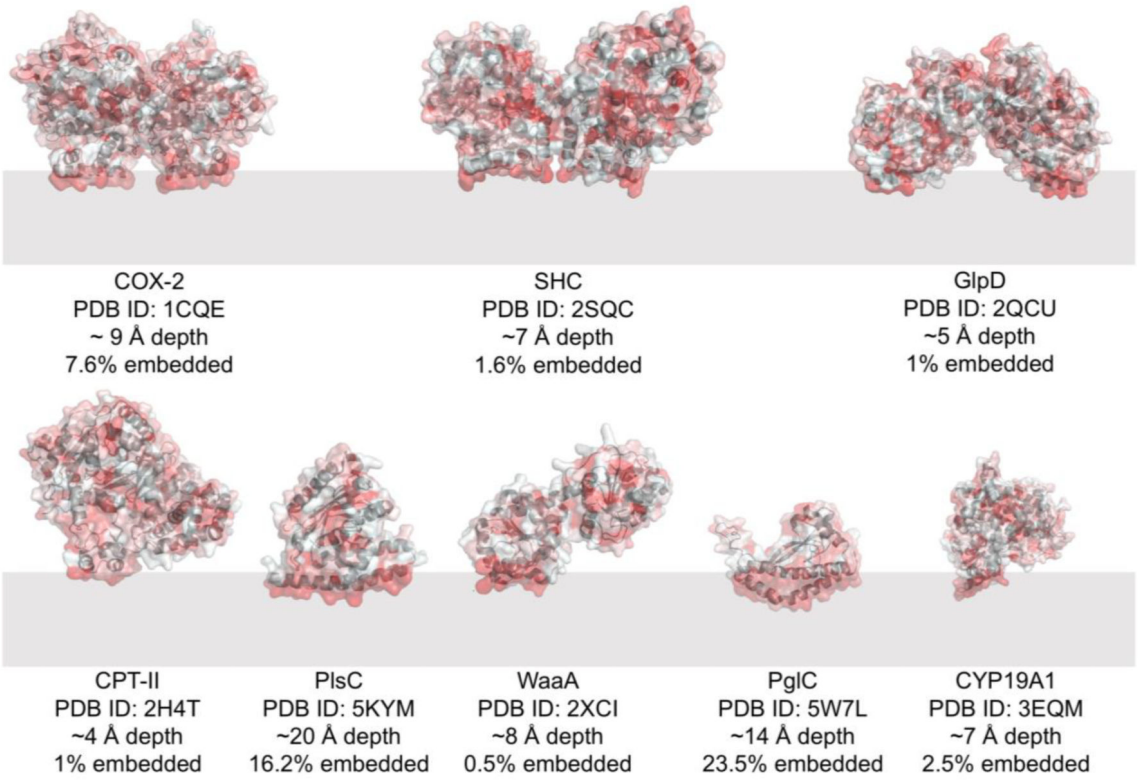


Figure 1.

Structures of representative monotopic membrane enzymes. Protein surface colored by normalized consensus hydrophobicity from hydrophobic (red) to hydrophilic (white). Membrane represented by gray box. Location of membrane plane calculated with PPM server (http://opm.phar.umich.edu/server.php) for 1CQE (COX-2), 2SQC (SHC), 2QCU (GlpD), 2H4T (CPT-II), 5KYM (PlsC), 2XC1 (WaaA), 5W7L (PglC), and 3EQM (CYP19A1).

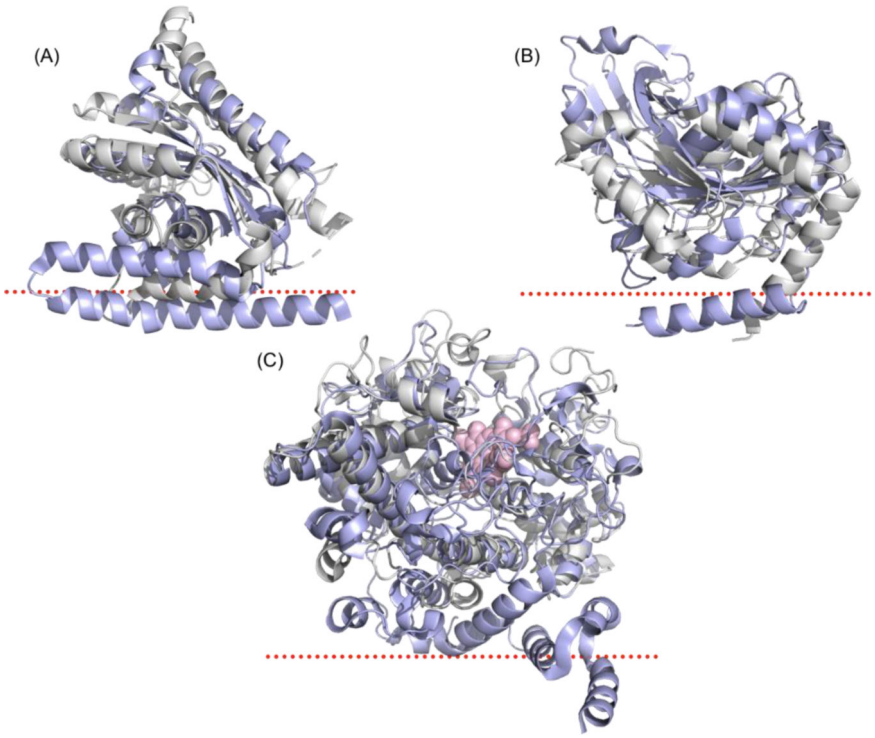


Figure 2.

Comparison of membrane proteins and their soluble homologues. Ribbon diagram of membrane proteins (purple) superimposed with the fold core of a prototypical soluble homologue (gray). Red dashed line indicates approximate membrane location calculated using the PPM server [49]. (A) lysophosphatidic acid acyltransferase, PlsC (purple, 5KYM) and glycerol-3-phosphate (1)-acyltransferase (gray, 1K30); (B) tetraacyldisaccharide-1-phosphate 4'-kinase, LpxK (purple, 4EHW) and GTP-binding protein HypB (gray, 2HF9); (C) fatty acid alpha-deoxygenase, α-DOX (purple, 4HHS) and myeloperoxidase (gray, 1CXP) with heme group in pink space-filling.

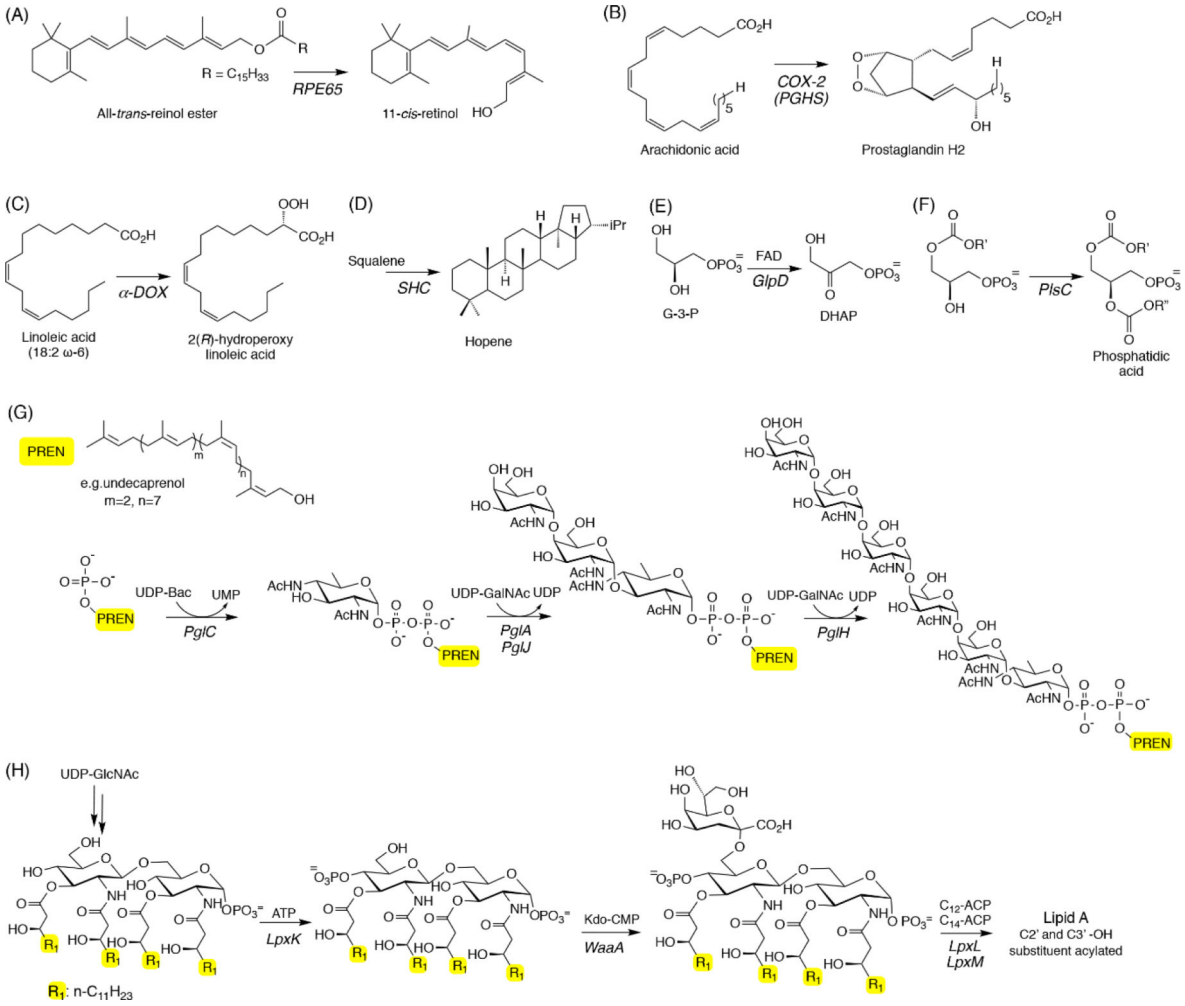


Figure 3.

Reactions of structurally-characterized monotopic membrane enzymes. (A) Hydrolysis and alkene isomerization by RPE65; (B) COX-2 – prostaglandin endoperoxide synthase; (C) α–DOX, linoleic acid α-hydroperoxidase; (D) SHC Squalene-hopene cyclase; (E) GlpD – FAD-dependent oxidation of glycerol-3-phosphate; (F) PlsC lysophosphatidic acid acyltransferase; (G) Steps in the *Campylobacter jejuni* protein glycosylation pathway – phosphoglycosyl transferase PglC and glycosyl transferases PglA, PglJ and PglH; (H) Steps in the *E. coli* pathway for Lipid A biosynthesis, focusing on kinase LpxK and glycosyl transferase WaaA. Yellow highlighting in (G) and (H) emphasizes moieties with significant hydrophobic character.

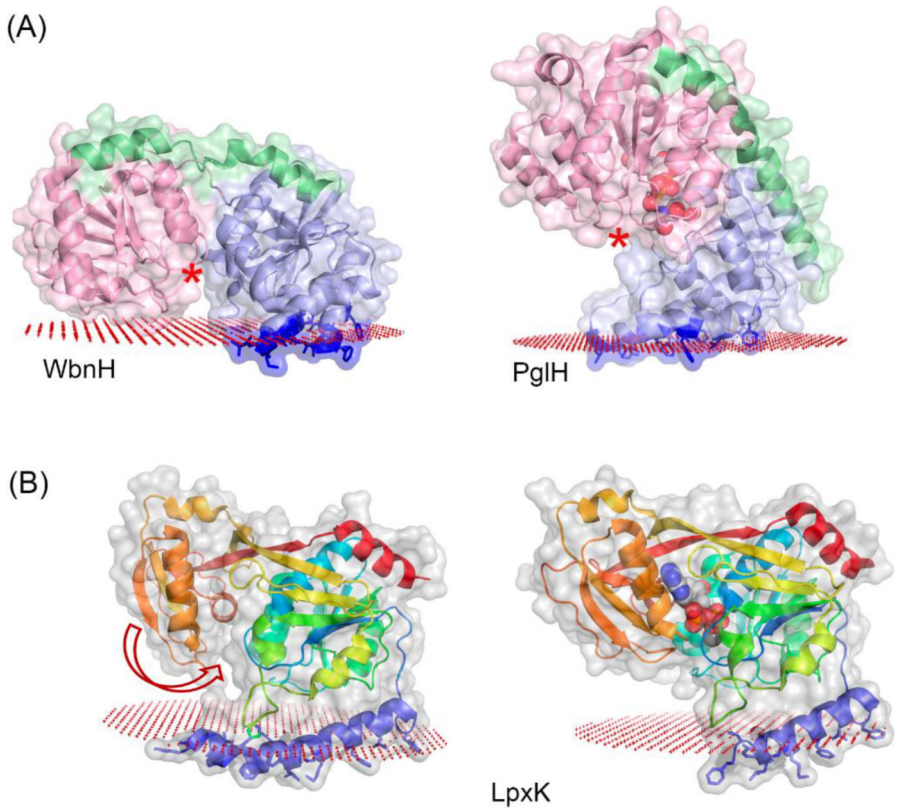


Figure 4.

Insight into GTase and kinase interactions with membrane calculated using the PPM server. (A) GTase interaction with the membrane changes based on glycosyl acceptor substrate: (Left) WbnH (4XYW) transfers GalNAc to membrane-resident Pren-PP-GalNAc, (right) PglH (6EJK) shown bound to non-hydrolyzable UDP-CH_2-GalNAc (spheres) sequentially transfers three GalNAcs to membrane-resident Pren-PP-trisaccharide. C-terminal GT-B domain (pink), N-terminal GT-B domain (pale blue), bracing helix (pale green), membrane-interacting residues (slate blue), membrane surface (red), active-site cleft red asterix; (B) LpxK kinase changes conformation on binding to ADP-Mg^{2+} Protein colored N- to C-terminus (rainbow), membrane-associated residues (slate blue). (Left) Open form of LpxK. (Right) Closed form bound to ADP-Mg^{2+}. ADP (space filling colored by atom), Mg^{2+} gray sphere. Red arrow shows movement of the C-terminal domain.

Table 1.

Monotopic membrane proteins with corresponding protein data bank coordinate accession code (PDB ID) of a representative orthologue, accession code of a soluble homologue, and oligomeric state.

Monotopics	PDB ID	Soluble Homologue	Oligomeric State
N-Acylphosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD)	4QN9[58]	1BMC[59]	dimer
Carnitine-palmitoyl transferase (CPT-II)	2H4T[60]	1NDB[61]	monomer
COX-2/Prostaglandin H2 synthase (COX-2/PGHS)	1CQE[26]	1CXP[62]	dimer
Cyanide-insensitive alternative oxidase (AOX)	3VV9[22]	none	dimer
Cytochrome P450 19A1 (CYP19A1)	3EQM[33]	2NZA[63]	monomer
Fatty acid alpha-deoxygenase (α-DOX)	4HHS[28]	1CXP[62]	monomer
Fatty acid amide hydrolase (FAAH)	1MT5[23]	1OCL[64]	dimer
Flavoprotein-ubiquinone oxidoreductase (ETF-QO)	2GMH[65]	1PBE[66]	monomer
Glycerol-3-phosphate dehydrogenase (GlpD)	2QCU[20]	4X9M[67]	dimer
Inverting GT-B glycosyltransferase (WaaA)	2XCI[55]	1JG7[68]	monomer
Lysophosphatidic acid acyltransferase (PlsC)	5KYM[38]	1K30[69]	monomer
Phosphoglycosyl transferase (PglC)	5W7L[21]	none	monomer
Squalene-hopene cyclase (SHC)	2SQC[31]	3DPY[70]	dimer
Retaining GT-B glycosyltransferase (PglH)	6EJI[50]	1JG7[68]	dimer
Retaining GT-B glycosyltransferase (WbnH)	4XYW[48]	1JG7[68]	monomer
Retinoid isomerohydrolase P65 (RPE65)	4F2Z[24]	2BIW[25]	dimer
Sulfide:quinone oxidoreductase (SQR)	3HYW[71]	1FCD[72]	trimer
Tetraacyldisaccharide 4’-kinase (LpxK)	4EHW[54]	2HF9[73]	monomer
Type II NADH dehydrogenase (NDH-2)	4NWZ[74]	3GRS[75]	dimer
Wall teichoic acid polymerase (TagF)	3L7I[76]	2IYA[77]	dimer