

Minireview

Structural commonalities among integral membrane enzymes

Michael H. Bracey^a, Benjamin F. Cravatt^{a,b,c}, Raymond C. Stevens^{b,d,*}^aDepartment of Cell Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA^bDepartment of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA^cSkaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA^dDepartment of Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

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Abstract The X-ray crystal structures of five distinct enzymes (prostaglandin H₂ synthase, squalene cyclase, fatty acid amide hydrolase, microsomal cytochrome P450, and estrone sulfatase) challenge contemporary descriptions of integral membrane proteins. This structurally divergent group represents an important component of the integral membrane proteome that lies at the bilayer's aqueous interface. We summarize here what is collectively understood about the membrane insertion of these proteins, what roles they may play in lipid biology, and their relationship to soluble structural homologs.

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1. Introduction

As our collective understanding of protein structure and function becomes more advanced, we are able to identify commonalities among groups of polypeptides and use these trends as a means to categorize them. At the simplest level, the cellular proteome is divided according to solution behavior and comprises both soluble and membrane fractions. Members of the membrane population may be peripherally associated with the membrane surface by means of ionic forces, covalently attached by post-translational lipid modification, or stably reside in the bilayer itself by virtue of hydrophobic interactions [1,2].

Those proteins that fall in the first two categories of the membrane population can be stripped off the membrane with washes of high ionic strength or alkaline buffers while leaving the membrane itself intact. These proteins are classified as peripheral membrane proteins. Their proteinaceous contacts with the bilayer do not require interactions deeper than the head group and intermediate phases of the membrane [3], and these interactions may be reversibly regulated, as is the case for some phospholipases [4]. The third group, the integral membrane proteins (IMPs), displays large hydrophobic surfaces that interact with the acyl core of the lipid bilayer. These proteins cannot be separated from the membrane without

disruption of the bilayer itself and require detergents for solubilization.

At a more detailed level, the IMPs exhibit three basic modes of bilayer insertion, or topology. This distinction is made at the level of the membrane itself and how many times the polypeptide chain traverses it. The concept of monotopic membrane proteins was hinted at in the original works of Singer and Nicolson [5] and was explicitly put forward by Blobel [6], where he delineated proteins that cross the membrane once as bitopic, twice or more as polytopic, and not at all as monotopic. He went on to espouse the now widely recognized means by which topogenic sequences within the protein's primary structure may dictate these various insertion strategies.

Since the first detailed structural characterization of an integral membrane protein [7], we have further refined our classification schemes. Based on a survey of available X-ray and nuclear magnetic resonance (NMR) structures, structural biologists have divided the IMPs into an α class and a β class according to the polypeptide conformations of their lipid embedded domains [3,8,9] (Fig. 1). As these designations reflect membrane binding motifs and not protein folds per se, the α IMP and β IMP classes do not necessarily reflect the "all α " or "all β " folds described for soluble proteins (<http://scop.berkeley.edu>). Collectively, solubility, topology, and membrane binding motifs provide a hierarchy of independent parameters that may be combined to describe any single membrane protein.

Members of the α IMP class can internally satisfy all hydrogen bonding requirements within a single transmembrane segment [3]. This property gives rise to several known means of bitopic membrane insertion, which are defined by the polarity and location of the transmembrane domain within the protein's primary structure [6,10]. Alternately, α IMPs can traverse the bilayer two or more times to produce polytopic insertions of helix bundles [3,11]. When fully assembled in their native membranes, α IMPs may exist as monomers or oligomers, and examples have been described in which quaternary protein–protein interactions occur within the transmembrane helices themselves [3,12,13]. Outside the bilayer, the hydrophilic domains of these proteins may exhibit enzymatic activity, serve as anchors for soluble proteins, dictate organelle residency, transfer reductive equivalents in electron transport, or dock ligands for signal transduction. These extramembrane domains may arise from the concerted folding of several loops from a

* Corresponding author. Fax: +1-858-784-9483.

E-mail address: stevens@scripps.edu (R.C. Stevens).

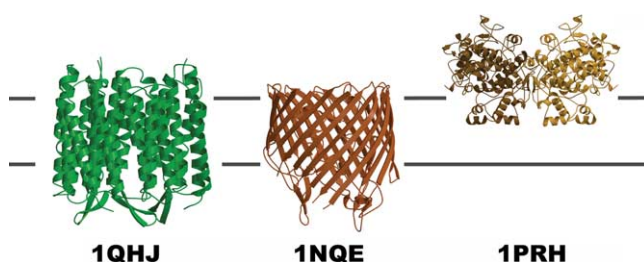


Fig. 1. Ribbon diagrams of representative proteins of the classes discussed here: α integral membrane protein (bacteriorhodopsin, 1QHJ; green); β integral membrane protein (outer membrane cobalamin transporter Btub, 1NQE; brown); and PGHS (1PRH; gold). The boundary of a cell membrane is approximated by gray lines.

polytopic protein or from a single domain of a bitopic protein [11]. In the case of the latter, bitopic membrane proteins, the transmembrane domain constitutes an anchor that can be proteolytically cleaved to free the soluble, globular extra-membrane domain. As a correlate to their diversity, the α IMPs collectively possess a rich heterogeneity of function, and examples of solved structures include photosystems (PDB entry 2PPS), receptors (1F88), respiratory proteins (1L0V), channels (1BL8), and pumps (1EUL).

In contrast, members of the β IMPs form membrane pores allowing the passage of various metabolites and small molecules [9], and at least two representatives of this class also possess enzymatic activity [14,15]. These proteins have only been observed in the outer membranes of Gram negative bacteria, mitochondria, and chloroplasts. The β IMPs form barrels of differing sizes and shapes, may exist as monomers or oligomers, and traverse the bilayer anywhere from 8 to 22 times [9]. They are strictly polytopic in nature. Several X-ray and NMR structures have been solved for this class and examples include outer membrane phospholipase A (1QD5), maltoporin (1MAL), OmpA (1G90), and TolC (1EK9).

Despite intense biological and pharmaceutical interest, the IMPs remain a relatively small population in the Protein Data Base compared to soluble proteins (<http://www.rcsb.org/pdb/>). As a result, our insights into structural motifs that engage the bilayer continue to rely heavily on inference from members of the α and β classes, homology modeling, and hydropathy algorithms. It follows, then, that our overall view of protein fold space in the context of the lipid bilayer is a narrow one. Either protein fold evolution has found only a few means to stably insert a polypeptide into a cellular membrane, or there exist new IMP motifs to discover. This latter possibility advocates the need for continued structural analysis of membrane proteins, which has the potential to reveal new, unanticipated means by which polypeptides might stably reside within cell bilayers. Indeed, such a structural revelation has occurred during the past decade and challenges us to expand our current view of membrane protein structure.

2. A new structural class of integral membrane proteins

Presently, the X-ray crystal structures of five distinct integral membrane proteins have been described that cannot strictly be placed into either the α IMP or β IMP classes (Fig. 2). They are prostaglandin H_2 synthase [16], squalene cyclase [17], fatty acid amide hydrolase [18], microsomal cytochrome P450 [19], and estrone sulfatase [20]. Despite the absence of fold or sequence homology among these proteins, many structural and biochemical features are held in common among them. First, they are all enzymes that are able to function on lipophilic substrates and with soluble homologs. Second, each protein engages the hydrophobic core of the bilayer with motifs that run parallel to the membrane surface. These motifs form apolar plateaus that are hypothesized to bury into one leaflet of the membrane only. Third, these enzymes do not adhere to the accepted notion that IMPs strictly utilize either α or β elements; they collectively exhibit a combination of α helices, β sheets, loops, and turns in their membrane binding motifs.

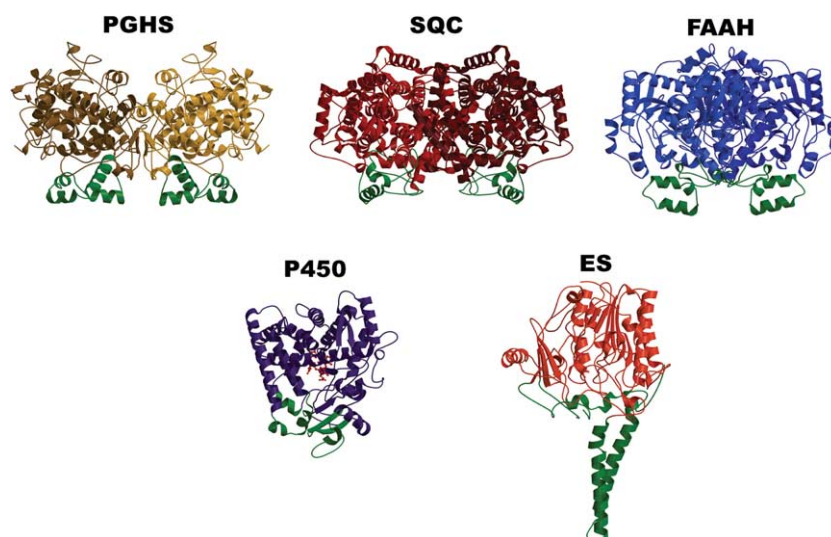


Fig. 2. The five known integral membrane proteins that do not conform to the α IMP or β IMP classes. Prostaglandin H_2 synthase, PGHS; squalene cyclase, SQC; fatty acid amide hydrolase, FAAH; cytochrome P450, P450; estrone sulfatase, ES. The hydrophobic domains that are believed to bury themselves within the lipid core of the cell membrane are colored in green.

2.1. Prostaglandin H₂ synthase

The prostaglandin H₂ synthases (PGHS, COX) are the pharmacological targets of analgesic non-steroidal anti-inflammatories (NSAIDs) [21–23]. These dual function enzymes convert arachidonic acid first to prostaglandin G₂ by a cyclooxygenase step and then to prostaglandin H₂ via a separate peroxidase activity. PGHS is known to behave as an integral membrane protein of the endoplasmic reticulum based on the inability to extract the enzyme from microsomes using perchlorate to strip away peripheral membrane proteins [24,25]. They are oriented to the luminal side of the membrane where they are glycosylated and form intramolecular disulfide bonds [16,21]. The PGHS proteins can be divided into three domains: an epidermal growth factor-like domain, a hydrophobic domain, and a domain resembling soluble mammalian peroxidases such as myeloperoxidase [16]. Accordingly, it has been suggested that PGHS evolved by the modular adaptation of a soluble precursor [26].

The X-ray crystal structure of PGHS-I purified from sheep seminal vesicles reveals a dimer with α helical content, simple twofold symmetry, and an overall ellipsoidal structure [16] (Fig. 2). The dimer is aligned such that the active site entrances of each constituent monomer fall on the same face of the holoenzyme in a “parallel” quaternary assembly. Conspicuously absent from the structure are any excursions from the body of the protein that might make up a transmembrane domain to explain the integral membrane behavior of the enzyme. However, surface hydrophobicity analysis reveals a concentration of apolar amino acids on one face of the dimer surrounding the active site entrances [16]. This hydrophobic “plateau”, is formed by four amphipathic helices and their joining turns, denoted A through D, from each monomer [16]. The concerted presentation of all eight helices on one face of the protein results from the dimer symmetry axis and is colocalized with the entrances to each monomer’s active site [26]. This membrane protein structure, therefore, not only represents the first deviation from the α and β classes but also demonstrates monotopic topology.

As the prototype of a potentially new class of integral membrane proteins, the PGHS-I structure illustrates several salient features (Table 1). Most striking, the simple rendering of the backbone trace as a ribbon drawing does not confer any

indication that this protein resides within the hydrophobic confines of a cellular membrane. Instead, the three-domain fold conceptually resembles the gross, overall shape of a globular soluble protein, something that is not easily said of the α or β membrane proteins. Additional features that have proven common to several subsequent IMP structures include structural homology with a soluble protein, the parallel quaternary orientation of individual subunits of the homooligomer, a hydrophobic prominence on one face of this oligomer, and the coincident presentation of this membrane-inserting domain with the active site entrance. Further, the functional significance of these structural elements of PGHS-I is supported by their total conservation in PGHS-II [22].

2.2. Squalene-hopene cyclase

The determination of the X-ray crystal structure of squalene-hopene cyclase (SQC) from *Alicyclobacillus acidocaldarius* demonstrated that the unusual structural properties of PGHS are not restricted to higher eukaryotes [17]. Given the absence of sequence, structural, or enzymatic homology between SQC and PGHS, this observation suggests that the evolution of novel membrane insertion motifs was a convergent event. SQC represents a family catalyzing the cyclization of linear terpenoids to fused ring structures; in mammals, this process provides precursors for cholesterol and steroid hormones [27]. The enzyme cosediments with membrane fractions and cannot be liberated by variations in pH from 6 to 9, stripping with 1 M KCl, dialysis against distilled water, or washing with 0.1 M EDTA [28]. Furthermore, the yeast enzyme can be quantitatively extracted from membranes only with detergents [29].

The fold of SQC forms two domains, and one of these resembles soluble glucanases and a farnesyltransferase [17]. Like PGHS, SQC is a dimer with a concerted presentation of two hydrophobic plateaus on one face of the holoenzyme with a combined surface area of 1600 Å² (Fig. 2). And again like PGHS, these presumed membrane-binding domains are coincident with the entrances to the active sites of the SQC dimer. In contrast to PGHS, however, the hydrophobic plateau presented by SQC does not comprise a contiguous protein stretch, but rather three separate domains of primary structure [17,27]. This patch is also partly composed of regions lacking periodic

Table 1
Unifying characteristics of integral membrane proteins that fall outside the α IMP and β IMP classes

Protein	PDB code	Soluble homolog	Integral membrane	Hydrophobic plateau	Lipid embedded AS entrance	Parallel multimer	Topology	Enzyme	Hydrophobic substrates
PGHS (sheep)	IPRH	✓	✓	✓	✓	✓	M	✓	✓
SQC (<i>Alicyclobacillus</i>)	ISQC	✓	✓	✓	✓	✓	M	✓	✓
FAAH (rat)	1MT5	✓	✓	✓	✓	✓	M/B?	✓	✓
ES (human)	1P49	✓	✓	✓	✓	monomer	P	✓	✓
P450 (human)	1OG2	✓	✓	✓	✓	monomer	M/B?	✓	✓
MDH (<i>Pseudomonas</i>)	1HUV ^a	✓	✓	✓	✓	✓	M	✓	✓
SppA (<i>Arabidopsis</i>)	x		✓				M	✓	peptide bond
MgP _{1X} MT (<i>Arabidopsis</i>)	x		✓				M	✓	✓
Stomatin (human)	x		✓				M	x ^b	
MAO (human)	1GOS	✓	✓	x	x	✓	B	✓	x

Fields for which no entry appears are undetermined. Properties of those proteins without solved structures are based on biochemical data and sequence analysis. Topology is indicated as monotopic (M), bitopic (B), or polytopic (P). It is presently unknown if FAAH and P450 are monotopic or bitopic.

^a This structure was solved from a soluble chimera; the existence of the hydrophobic plateau and its coincidence with the entry to the active site is inferred from the soluble structure.

^b The exact function of stomatin is unknown.

structure as evidenced by the loops between helices $\alpha 6$ and $\alpha 7$ and between helices $\alpha 15$ and $\alpha 16$. Hence, in SQC we see again non-repetitive elements of protein structure that presumably interact with the hydrophobic core of the lipid bilayer. Since the protein shows no potential for traversing the thickness of the membrane, SQC, like PGHS, can also be described as monotopic.

2.3. Fatty acid amide hydrolase

The X-ray crystal structure of a recombinant, N-terminal truncated form of rat fatty acid amide hydrolase (FAAH) recapitulates many of the features seen in PGHS and SQC despite an absence of homology [18]. The overall shape of the protein is globular, with no apparent motifs to function as a transmembrane domain. The enzyme crystallized as a dimer such that the active site entrances of each monomer are oriented in the same direction, and this face of the dimer is again highly hydrophobic and a likely means by which the enzyme inserts into the bilayer (Fig. 2). Like the previous two membrane proteins, FAAH also acts upon lipid substrates and probably gains access to these bilayer-embedded molecules by the intimate relationship shared between its membrane-binding domain and active site. Notably, FAAH also possesses a second, lateral access channel leading from the cytoplasm to the active site that may facilitate the simultaneous transport of water and hydrophilic reaction products.

Much like PGHS and SQC, FAAH also retains a protein fold that essentially mimics soluble relatives [18]. In the same year that the FAAH structure was determined, independent groups solved the structures of two soluble homologs [30,31]. This coincidence allowed the direct comparison of three variants of the same protein fold. One soluble relative, malonamidase MAE2, completely lacks the amino acid stretch that composes FAAH's membrane-binding domain [31]. In this sense, the insertion of this structural element suggests a modular adaptation of a basic fold to direct the new protein to the cell membrane and recapitulates observations made for PGHS. Further, when FAAH's structure is compared to the peptide amidase PAM, one sees that these two enzymes share the presence of this domain, but the one present in PAM is made up of hydrophilic residues [30]. Thus, PAM may conceptually represent a structural transition leading to the integral membrane association seen in FAAH. The case for these arguments, at least from a protein engineering point of view, could be made in the future by constructing soluble variants of FAAH based upon these observations.

In comparison to soluble homologs, it is interesting to note that FAAH bears an amino terminal extension of roughly 35 amino acids that is predicted by primary sequence analysis to form a transmembrane helix [32]. However, the crystallized FAAH truncation lacking this domain still behaves as an IMP, presumably due to the presence of its hydrophobic plateau domain. Both wild-type and truncated forms of the enzyme remain bound to microsomes after stripping with alkaline carbonate [33]. A conclusive demonstration of the role served by the amino terminus, and therefore the protein's true topology, remains lacking. As a result, clarity on this issue will likely require the solution of the full-length protein structure or a demonstration that the amino and carboxyl termini of the protein are mutually inaccessible in native membranes.

2.4. Microsomal cytochrome P450

Structures of mammalian P450s have been solved recently from both rabbit [19] and man [34]. In contrast to the soluble P450s from bacteria, these IMPs are localized to the endoplasmic reticulum and, like other microsomal P450s, bear a hydrophobic amino terminus thought to mediate membrane binding as a transmembrane helix [35]. However, collections of truncation and mutation studies among various P450 isozymes show that this domain is not required for irreversible membrane insertion; both wild-type and truncated forms of the P450 isozymes 2E1 and 2B4 remain bound to microsomes following alkaline carbonate washes [36,37]. Additionally, NMR spectra of the would-be transmembrane helix support a model in which this domain does not traverse the bilayer but instead resides within only one leaflet [38]. In fact, the homologous mitochondrial P450s, though membrane proteins also, do not even bear this amino terminal domain [39]. Experiments relying on epitope accessibility assays demonstrate that domains believed to be associated with the entrance to the active site are occluded by the membrane itself [40], and NMR data implicate additional regions in penetration of the membrane as well [41].

The X-ray crystal structures of soluble mutant P450s CYP2C5 and CYP2C9 provide a structural basis for explaining this rich collection of biochemical data [19,34]. As described, this enzyme is globular with a hydrophobic face (Fig. 2). Like PGHS, SQC, and FAAH, this face also provides access to the active site, and the authors speculate that "the substrate access channel... is buried in the lipid core" [19]. This arrangement likely facilitates the recruitment of this enzyme's hydrophobic substrates, a property that seems common to the unconventional IMPs described here. These structural elements correspond to the F/G loop, B' helix, and the first two strands of beta sheet 1, and these domains all correspond to the same sequences implicated by biochemical data as membrane embedded [34]. Additionally, like FAAH, this protein also shows a second, cytoplasmic approach to the active site that is likely the means by which reductive equivalents are transferred from cytochrome P450 reductase. These observations support the conclusion that members of the microsomal, and presumably the mitochondrial, P450s bind their respective membranes with structural motifs first observed in PGHS.

2.5. Estrone sulfatase

Human estrone sulfatase (steroid sulfatase, ES) removes sulfate groups from sterols such as dehydroepiandrosteron sulfate and cholesterol sulfate to ultimately produce cholesterol, androgens, and estrogens [42]. The protein cannot be extracted from microsomes following washes with 0.5 M KCl or 0.1 M Na_2CO_3 , and it partitions with the detergent during Triton X-114 phase separation [42]. Therefore, ES is classified as an integral membrane protein.

The structure of this enzyme was recently solved and is essentially superimposable with the soluble aryl sulfatases A and B [20]. Additionally, ES displays many of the hallmarks described for the above four IMPs: it is an enzyme that acts on hydrophobic, albeit sulfated, substrates; the entrance to the active site is proposed to be associated with the bilayer; and two hydrophobic strand regions of 20 and 32 amino acids each are positioned at the active site opening to penetrate the lipid core [20] (Fig. 2). It thus seems reasonable to assume that ES also gains access to its hydrophobic substrates by direct recruit-

ment from the bilayer, and previous authors have speculated that the enzyme's active site itself is buried in the cell membrane [43].

The structure also confirms prior biochemical evidence that this enzyme is anchored in the membrane by an additional structural motif, a transmembrane hairpin formed by helices 8 and 9 [42]. These helices, as well as the two strand segments mentioned above, are absent in soluble homologs of this protein, and it remains unclear if either of these domains is necessary or sufficient for membrane binding. Since this enzyme is monomeric, it is possible that the presence of one set of the hydrophobic strands is not sufficient to maintain membrane integration. Accordingly, it would be interesting to learn if deletion of this hairpin abolished membrane binding. It has been reported that the non-translocated *in vitro* translation product of a deletion mutant that lacks the transmembrane hairpin and/or one of the hydrophobic strands “strongly adhered to microsomes” [42]. However, the translation product was not shown to be folded or enzymatically active. Overall, ES possesses the attributes of a polytopic transmembrane protein combined with the membrane association strategies of PGHS.

3. Other speculatively similar IMPs currently under investigation

3.1. Monoamine oxidase B

The X-ray crystal structure of the integral membrane protein monoamine oxidase B (MAO) satisfies some of the trends observed in the above proteins including a parallel dimer arrangement, a dimerization axis perpendicular to the plane of the membrane, and the existence of soluble homologs [44]. However, many other properties of this enzyme counter its comparison to the above described enzymes (Table 1). For instance, MAO's biological substrates, including dopamine and epinephrine, are not particularly hydrophobic and partition quite easily into the aqueous phase. It therefore seems unlikely that the MAO active site entrance would be buried in the hydrophobic lipid core. In fact, in comparison with the related polyamine oxidase, observations suggest that the protein interacts with the anionic surface of the mitochondrion membrane and not the acyl portion [45].

It is generally accepted that MAO B associates with membranes through a carboxy-terminal transmembrane helix that is absent in the soluble homologs polyamine oxidase [46] and L-amino acid oxidase [47]. MAO mutants lacking this domain display up to 48% release into the soluble fraction [48]. Further, since this partitioning was not measured after harsh washes of the membranes (e.g., chaotropes, high salt, and alkaline carbonate), it remains possible that the truncations with residual bilayer associations behave merely as peripheral membrane proteins. If one were to make predictions for MAO based on the five proteins described above, the two turn regions situated about residues 110 and 157 should each exhibit extensive hydrophobicity and bury themselves in the cellular bilayer. However, these domains do not seem suited to this function. Though the authors note that Pro109 and Ile110 are positioned so that they could interact with the membrane [44], the equivalent surface in the soluble homolog L-amino acid oxidase displays similar properties with numerous proline, valine, alanine, leucine, and isoleucine residues [47]. Without

further functional characterization of these structural elements in MAO, it currently seems unwarranted to regard this enzyme in the same class as PGHS, SQC, FAAH, P450, and ES. Rather, MAO more readily favors inclusion as a bitopic member of the α class.

3.2. Mandelate dehydrogenase

A search of the current literature yields clues to the identities of other potential IMPs with structural features initially described for PGHS even where structure is not currently available (Table 1). The enzyme mandelate dehydrogenase (MDH) is an integral membrane protein with soluble homologs. Though an X-ray structure is unavailable for MDH, structures are available for its soluble relative, glycolate oxidase (GOX), from spinach [49]. Using sequence comparison and homology modeling, soluble MDH chimeras were engineered by replacing a hydrophobic stretch unique to MDH with the corresponding domain of GOX [50]. And the X-ray crystal structure of one of these chimeras reveals a tetrameric enzyme with many of the features outlined for the unusual IMPs here, including a predicted hydrophobic face coincident with the active site entrances in a parallel tetramer [51]. MDH, then, may represent the first member of this group to be engineered into a soluble form.

3.3. Monotopics

Since monotopic topology seems inaccessible to members of the accepted α IMP and β IMP classes, this feature might be exploited as a means to identify potential new proteins that adopt the membrane binding characteristics of PGHS. In plants, investigators have proposed that both the chloroplast protease SppA [52] and methyltransferase MgP_{1X}MT [53] are monotopics based on their primary structure, subcellular localizations, protease sensitivities, and resistance to ionic or chaotropic stripping. Curiously, the methyltransferase can be stripped by 0.1 N NaOH but not by 0.1 M Na₂CO₃ at pH 11, and the authors offer this as evidence that the protein is not transmembrane [53]. In mammals, sequence analysis provides evidence that stomatin, or erythrocyte band 7.2b, contains a single transmembrane helix. However, this protein is phosphorylated on both the amino and carboxy termini, suggesting that it either has an additional transmembrane helix or is also monotopic [54]. Overall, these divergent proteins suggest that the novel mode of membrane insertion adopted by PGHS and the above described proteins may be a common, perhaps even preferred means of addressing enzymatic needs within membranes since it structurally allows protein active sites to engage the lipid core without the constraints of pure α or pure β folds. This approach further provides the cell an adaptive means of recruiting virtually any enzymatic chemistry from the soluble proteome for use in lipid metabolism without the challenge of *de novo* protein design.

4. Conclusions

Many of the IMPs described here are the targets of drugs and drug development programs, and the solution of their structures in many instances is probably the result of their medical relevance. For example, PGHS has long been targeted by aspirin and countless other NSAIDs [21–23,55]. Likewise, the *Candida* homolog of SQC has been suggested as a target

for anti-fungals using substrate-mimicking inhibitors [56]. FAAH inhibition is currently under pursuit as a means to intersect the endogenous cannabinoid system for the treatment of pain and other neurological disorders [57,58]. Estrone sulfatase activity is correlated with the proliferation of breast carcinomas, and the heritable X-linked mutation of this gene results in ichthyosis [20]. The P450 prostacyclin synthase produces the platelet anticoagulator and strong vasodilator prostaglandin I₂ [59], while other isozymes are responsible for various modes of detoxification and drug metabolism [60]. Together, these enzymes and their substrates point to the powerful roles that lipids play in physiology and hint at an emerging division of integral membrane proteins so rich in potential drug targets that it could represent the enzymological equivalent of the G-protein coupled receptors [61].

Clearly, we still have much to learn about structure–function relationships at the protein–lipid interface. We have described here a newly emerged understanding of a third means by which polypeptides may stably reside within cellular bilayers without the constraints of the α IMP and β IMP paradigms. These five IMPs, PGHS, SQC, FAAH, P450, and ES may comprise a new grouping of lipid-active enzymes that have seemingly evolved from soluble precursors. In at least two instances, they defy the once accepted convention that IMPs must traverse both leaflets of the bilayer. They collectively also display a combinatorial appropriation of peptide backbone geometries within their membrane-embedded domains, and this appears in stark contrast with the all α and all β means by which other membrane proteins engage the hydrophobic core. With the accumulation of more structures and a heightened awareness of the importance of these integral membrane enzymes, this group is sure to provide yet another fascinating field for investigation by structural biologists and may eventually warrant a class of its own.

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