Published in final edited form as:

Curr Opin Struct Biol. 2012 August ; 22(4): 476–481. doi:10.1016/j.sbi.2012.03.005.

Highlights from recently determined structures of membrane proteins: a focus on channels and transporters

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Abstract

After decades of absent or lackluster growth, recent years have at long last witnessed an exponential growth in the number of novel membrane protein structures determined. Every single achievement has had a tremendous impact on the scientific community, typically providing an unprecedented wealth of information that only an atomic resolution structure can contribute to our molecular understanding of how a protein functions. Presented here is a review of some of the most exciting novel structures of channels and transporters determined by X-ray crystallography in the last two years, and a discussion of their analogies, differences and mechanistic implications.

Keywords

Membrane proteins; crystal structure; X-ray crystallography; transporters; channels

Introduction

Every biological, chemical, physical or nutritional exchange between cells, intracellular compartments, or between the cell and its proximal environment, is dependent for success on proteins residing in the cell membranes and endowed with the molecular capability of accurately fulfilling such a task. In prokaryotes and eukaryotes alike, 30% of all genes code for membrane-spanning proteins [1]. In humans, approximately 60% of all commercialized drugs are targeted to or exert their effect on a protein residing on the plasma membrane [2]. However, despite their importance, these hydrophobic molecules are but a fraction of a percent of proteins with a known structure. In fact, as of mid-February 2012 coordinates for only 320 unique membrane proteins had been deposited in the protein data bank (PDB; [3]), representing less than 1% of the total.

The field of membrane protein structure determination has progressed tremendously since the determination of the three-dimensional structures of bacteriorhodopsin at 7Å resolution by electron crystallography on two-dimensional crystals in 1975 [4], and the first structure by x-ray crystallography at 3Å resolution of the photosynthetic reaction center in 1985 [5].

While it took close to twenty years to reach 100 structures, the field appears to have at long last entered a phase of exponential growth (for details, see <http://blanco.biomol.uci.edu/mpstruc/listAll/list>). This increase in the rate of success is due

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to technological advances at every step of the procedures required to transition from gene to protein to structure, to the increase in the number of investigators willing to commit to the challenge, and to the advent of miniaturized high-throughput platforms allowing the efficient selection of membrane proteins with increased probability of crystallization [6]. Indeed, approximately one third of the total number of unique membrane protein structures deposited in the database as of February 2012 have been reported in the last two years. By far the majority of these structures have been determined by X-ray crystallography.

These recent times have been tremendously prolific and truly exciting for structural biology and for the scientific community at large. While G-protein coupled receptors and K^+ channels will be discussed separately in this issue, we will focus here on the structures of other channels and transporters. We will restrict our analysis to some of those that are self-standing and novel, representing a first report in their respective functional categories.

Channels

In the field of ion channels, an absolute novelty came from the 1.2Å resolution structure of TehA, a prokaryotic homologue of plant slow anion channel 1 (SLAC1) [7]. Plant leaves maintain a delicate balance of H_2O and CO_2 with their environment through opening and closing of guard cells on their stomatal pores [8]. SLAC1 plays a central role in this process as it responds to phosphorylation by opening its channel to allow the efflux of Cl^- , resulting in membrane depolarization and ultimately stomatal closure driven by exit of KCl and H_2O [9, 10]. The structure of TehA shows a tightly associated trimer. Each protomer consists of ten transmembrane (TM) helices (TMHs) arranged in a novel hairpin repeat with quasi-pentameric symmetry, resulting in an inner group of five helices, perpendicular to the membrane, directed outwardly and delineating a central pore, and surrounded by the second pentad of helices arranged in an inwardly directed outer layer (Figure 1a). The loops are short on the extracellular and long on the intracellular side maintaining the symmetry of this elegant arrangement. The pore has a 5Å and uniform diameter along five helical turns except for a position in the middle of the membrane, where it is occluded by the side chain of an absolutely conserved Phe. By coupling information derived from the structure to electrophysiological recordings on injected oocytes, the authors begin to decipher how SLAC1 works. They unveil the unique role of the conserved Phe in gating, they explain permeability of anions over cations, ion selectivity and differences with ClC channels. Understanding how phosphorylation leads to channel opening will require further work, but this beautiful structure sets the framework for this question to be answered.

Gating mechanism is a theme with a surprising twist from the recent structures of the formate channel FocA [11-13], the first representative models of the formate-nitrate transporter family. Formate is a major bacterial metabolite during anaerobic respiration [14]. Its cytoplasmic concentration needs to be controlled in a pH-dependent manner and its uptake and efflux were shown to require a protein named FocA [15]. The first two structures of FocA were obtained at pH above 7 and show that the protein, despite the absence of any sequence similarity has a fold which is remarkably similar to that of the water and glycerol channels of the aquaporin (AQP) and glycerol facilitator (GlpF) families [16-18]. FocA shares with AQP and GlpF the number and topology of TMHs, similar inverted two-fold symmetry, the twist of the helical bundle, and the presence of a central pore, but carries its own unique oligomeric assembly into a homopentamer as opposed to tetramer. One of the structures [12] shows a pore occupied by formate across the entire TM width, strongly suggestive of a channel and not a transporter as originally classified, thus making FocA the first example of an organic ion selective channel. The third FocA structure [13], determined at pH 4, provides another surprise (Figure 1b). The N-terminus of FocA, absent [11] and disordered [12] in the high pH forms, spans across the entrance of the pore, thus serving as a

gate. Finally, the authors hypothesize that the protonation of a histidine might be responsible for the movement of the gate, providing a plausible, although as yet untested mechanistic explanation for pH-dependent closing of FocA.

Pentameric arrangements are also a known signature for Cys-loop receptors, ligand-gated ion channels that play a crucial role in fast inhibitory neurotransmission [19, 20]. The first structure of a eukaryotic anion-selective Cys-loop receptor was recently determined, in complex with an allosteric agonist, and with the addition of its endogenous neurotransmitter L-glutamate, and of an open-channel blocker [21]. The fold is similar to that witnessed for the nicotinic acetylcholine receptor [22] and for bacterial homologues [23-25], with each protomer of the pentamer contributing one TMH to the ion conducting pore. The structure unveils a novel-binding site for the partial allosteric agonist ivermectin at the protein-lipid interface with structural implication both for the pore and the distant ligand-binding pocket. This site could be shared by other lipophilic modulators such as cholesterol and neurosteroids, and could have profound pharmacological implications. The structure also shows how selectivity is conserved by proline residues forming a constriction in the pore allowing the passage of dehydrated species only, and how, in a manner reminiscent of ClC channels [26], selectivity of anions over cations is achieved from the oriented electropositive dipoles of the pore-lining TMHs. This work provides unparalleled insight into the function of this class of ion channels of fundamental importance to human health.

Finally, we would like to comment the very recent structure of channelrhodopsin [27], a seven TMHs light-gated cation channel found in *algae* [28]. The protein is related to the light-driven pumps encoded by the bacterial opsin genes [29, 30]. The novelty of this structure is in the fact that it is the first of a light-switchable cation channel, while its importance is due to the central role of channelrhodopsin in optogenetics [31]. The structure of channelrhodopsin in the dark state provides insight into the environment of the bound retinal, and shows how, in respect to bacteriorhodopsin, helices are rearranged to form a negatively charged ion conduction pathway. It also allows the authors to propose a mechanism of activation upon retinal isomerization. This structure will be an invaluable guide to the rational design of variants with altered and defined spectral properties.

Transporters

Transitioning from channels to transporters, the structure of TrkH [32], a representative of the prokaryotic superfamily of K⁺ transporters SKT [33], is particularly relevant. It shows the diverse use of a protein fold attributed until now to K⁺ channels. Unlike K⁺ channels which are tetramers, TrkH is a dimer, perhaps related to allosteric control and interaction with intracellular partners [34, 35]. However, tetrameric symmetry is still present within each protomer, resulting in four quasi-symmetric repeats of a basic TM-(P-helix/P-loop)-TM motif, generating a pore lined by the P-loops. Besides differences in the pore geometry with K⁺ channels, an unprecedented gating mechanism relies on a flexible loop which can obstruct the pore on its cytosolic side by establishing salt bridges with conserved residues.

Two recent novel structures have also emerged amongst primary transporters from the energy-coupling factor (ECF) family, ATP-dependent transporters involved in micronutrient uptake in prokaryotes. Whereas the prototypical ABC transporter is composed of two cytoplasmic ATP binding components and two symmetrical or quasi-symmetrical TM domains (A and T components, respectively) [36], in ECF transporters a common platform of two A components and one T component can interact with several S subunits, membrane proteins that harbor substrate specificity. The structures of two S subunits [37, 38] show a six TMHs fold never before associated with transport, but previously found in a particulate methane monooxygenase [39]. Both structures explain substrate binding to the S subunit,

but do not solve the debate about the possibility of transport through the S subunit only [37], or at the interface between S and T [38].

Novel structures representing two more families of secondary active transporters (SATs) have recently emerged. A prokaryotic homologue provides the reference structure for the eukaryotic apical sodium-dependent bile acid transporter (ASBT) family. The structure of ASBT from *Neisseria meningitidis* (ASBT_{NM}) with substrate bound [40] in an inward-facing hydrophobic cavity shows a ten TMHs fold (Figure 2a), unexpectedly similar to the twelve TMHs fold of Na/H⁺ antiporter NhaA [41]. As is common amongst SATs [42, 43], the overall structure results from internal, pseudo-symmetric repeats, oppositely oriented in the membrane plane, with a “panel” domain that embraces the “core” domain through two so-called “arm” TMHs. Two TMHs in the core domain are discontinuous, as often found in SATs [43, 44], and, unique feature of this fold, they cross inside the lipid bilayer. Binding of Na⁺ at the crossover point is postulated to neutralize the partial negative dipoles of these discontinuous helices, thus stabilizing their quasi-alignment. This was also described for NhaA, where the conformational change of these broken helices would allow transition between outward and inward facing state of the transporter. As ASBT_{NM} transports a bulkier substrate, dipole-mediated changes probably underlie a larger rearrangement between the core and panel domains, culminating in the alternate opening of the intracellular and extracellular binding sites at their interface.

The structure of bacterial UraA [45] is a template for the nucleobase/cation symporter 2 (NCS2) family, ubiquitously responsible for mediating the uptake of nucleobases, and in mammals of vitamin C [46, 47]. While NCS1 family members share the LeuT fold [42, 43], UraA exhibits a novel fourteen TMHs fold, with an unprecedented core composed of four TMHs (Figure 2b). Furthermore, unlike other SATs, the “arm” helices of the panel domain do not embrace the core domain. These arms are instead provided by the C-terminal helices of the core domain. The structurally equivalent TMHs 3 and 10 in the core domain are both broken and preceded by two β -strands that form an intramembrane antiparallel β -sheet flanking the uracil-binding site. In this way, TMHs 3 and 10 cross each other, as do TMHs 4 and 9 in ASBT_{TN}. This arrangement seems to provide a solution for the instability that would arise from partial dipoles of α -helices crossing each other in the core domain. Finally, key glutamate and histidine residues are the likely main players for remodeling the core/panel interface, through which substrate translocation occurs.

One of the most outstanding recent discoveries is the 3Å resolution structure of the membrane component of respiratory complex I [48]. Besides several novel structural features, the three proton-pumping subunits NuoL, M and N, contain fourteen TMHs, arranged in two inverted repeats reminiscent of the NhaA fold [41] (Figure 2c). However, these repeats are packed as four layers in a face-to-back instead of a face-to-face manner, from the N- to the C-terminal one. Thus, panel and core domains do not form, nor can arm structures be observed. NuoL, M and N account for three completely unprecedented H⁺ hemi-channels, running from the cytoplasm to the membrane core in the N-terminal inverted repeat, and continuing to the periplasm in the C-terminal one. Opening and closing of the eight hemi-channels is amazingly synchronized in a mechanical manner, by interactions with horizontal structural elements spanning the entire complex on both sides of the membrane.

Finally, a novel fold also emerges for the EIIC component of the prokaryotic phosphoenolpyruvate-dependent phosphotransferase system (PTS) [51]. This system relies on the phosphorylation of the imported sugars as a means to prevent their gradient-driven back diffusion. Each protomer in the EIIC homodimer is composed of ten TMHs, two oppositely oriented membrane re-entrant helical hairpins (HP1 and HP2), and two horizontal

amphipathic α -helices at the bilayer surface. In this inward occluded-state structure, the physiological substrate is found in a cytoplasmic electronegative pocket where its phosphorylated form could not bind. This site is occluded by the loop between TMHs 4 and 5, and lined by HP1 and HP2. This latter feature is reminiscent of the unrelated Glt_{Ph} fold [52, 53], and suggests a similar mechanism of transport, with the C-terminal domain of EIIc sliding the HP1 and HP2, and thus the binding site and the substrate along the N-terminal domain, and hence through the membrane.

Conclusions

These are exciting times for membrane proteins, in which structures are finally emerging at an accelerated pace. We have briefly commented on some of the most recent achievements in the fields of channels and transporters, surely without doing enough justice to these landmark studies, and we also apologize to the authors of the many contributions we have not had the opportunity to mention. We hope to have succeeded in highlighting some of the surprises, and novel structural features that have emerged from these works, and we look forward to a continuing and even greater expansion of the field.

Acknowledgments

The author's contributions to the field are supported by NIH grants U54GM095315 and R01GM068671, and by a Marie Curie International outgoing fellowship within the 7th European Community Framework Program (to G.S.).

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Highlights

- Slow anion channel 1 and formate transporter FocA have novel gating mechanisms.
- Structures of an anionic Cys-loop receptor and of channelrhodopsin were determined.
- K⁺ transporter TrkH has intriguing similarities and differences with K⁺ channels.
- There are novel and interesting folds of primary and secondary active transporters.
- We discuss EIIc of the phosphoenolpyruvate-dependent phosphotransferase system.

Figure 1. Fold and gating mechanism of the bacterial homologue of SLAC1 and of the formate channel FocA

(a) Cartoon representation of the TehA monomer. The five quasi-symmetric repeats that constitute the pentameric fold are colored individually, from blue (N-terminal) to red (C-terminal). The side chain for the absolutely conserved Phe 262, responsible for the gating of SLAC1, is shown, protruding into the middle of the channel in a high-energy conformation. **(b)** Cartoon representation of the FocA pentamer in its low pH form. Each protomer is colored differently. The “gating” N-terminus is highlighted with a white and black contour. It adopts three different conformations: a disordered open form (not visible) in the red subunit, an intermediate in the green and blue subunit and a closed form, spanning across the entrance of the pore, in the orange and cyan subunits. PDB accession codes used to generate this figure are 3M71 (TehA) and 3Q7K (FocA).

(a) ASBT_{NM} **(b) UraA** **(c) NuoM**

Figure 2. Topology and inverted repeat diagrams of ASBT_{NM}, UraA and NuoM, and comparison with previously reported SATs

TMHs are numbered according to the original references. The N- and C-terminal repeats are highlighted with grey and white trapezoids respectively. Structural elements classically described for SATs are shown in solid colors. The v-motifs of the panel domain are in blue, “arm” helices in green, and the core doublets in red. Elements that despite their position in the repeats do not comply with function and/or domain location previously described, are shown in striped colors. **(a)** ASBT_{NM} has a fold similar to NhaA but lacks two TMHs (indicated with an asterisk) that participate in the formation of the NhaA panel domain. As typically observed in all SATs, the v-motifs interlock creating a stable platform (panel

domain) from which “arm” helices protrude and embrace the four TMHs core domain. **(b)** UraA has a repeat structure which is similar to the one found in LeuT, aside from two extra TMHs (colored in orange, and labeled pre-core) preceding the classical core elements. $\square$ -strand/helix motif, instead of the classical broken helix. The arrangement of the UraA repeats is novel. The arm helices that embrace the core domain in all SATs described to date, in UraA (shown in striped green) participate in the helix-bundle of the panel domain. The connections between the two domains are provided by the C-terminal helices of the core doublets (TMHs 4 and 11). **(c)** The inverted-repeat scheme for NuoM resembles the one for ASBT_{NM}. However, in NuoM the v-motifs do not interlock, there are no arms that protrude from the panel to the core domain, and indeed there are neither panel nor core domains. The helices shown in each trapezoid lie approximately on a plane. Each of the two plains within a repeat is “folded” on to the other by a 180° rotation (rotation axes are shown as black arrows).