Review

3D structure and allosteric modulation of the transmembrane domain of pentameric ligand-gated ion channels

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ABSTRACT

Pentameric ligand-gated ion channels mediate rapid chemo-electric signal transduction in animals. The active site of this family of proteins is their ion channel pore, which is located at the center of the transmembrane domain. The opening/closing motions of the channel pore are governed by the binding of neurotransmitter to the extracellular domain, but also by allosteric effectors acting within the transmembrane domain. Here, we review the structure of the transmembrane domain as well as its role in the allosteric modulation of pentameric ligand-gated ion channel function. We focus on two examples: the interactions of nicotinic ACh receptors with lipids, for which a novel "uncoupled" state has been proposed, and the interactions of GABA$_A$ and Glycine receptors with allosteric modulators, such as general anesthetics, ethanol and neurosteroids. We revisit these data in light of the recently solved X-ray structures of bacterial members of the family, which provide atomic-resolution insight into the structures of both the transmembrane domain and associated lipids.

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1. Introduction

Pentameric ligand-gated ion channels (pLGICs) are transmembrane protein complexes that mediate chemical communication between cells in both the central and peripheral nervous systems (Taly et al., 2009; Miller and Smart, 2010). These transmembrane receptors are grouped into distinct families, named according to neurotransmitter pharmacology. In vertebrates, nicotinic acetylcholine (ACh), serotonin (5HT$_3$R), and zinc (ZAC) receptors are linked to cation-selective channels, while γ-aminobutyric acid (GABA$_A$ and GABA$_A$-ρ) and Glycine receptors are linked to an anionic chloride ion-selective channel. In invertebrates, γ-aminobutyric acid, serotonin, glutamate, histamine and proton-gated channels have also been identified. More than 40 distinct pLGIC subunits exist in humans, including numerous subunit types within each of the neurotransmitter receptor families. Within a family, these subunits combine to form a variety of both homomeric and heteromeric complexes, each with different functional and pharmacological profiles.

The primary role of most pLGICs is to convert the binding of a neurotransmitter into the opening of a transmembrane ion channel leading either to intracellular excitation or inhibition, depending on the ionic selectivity of the channel. These two primary functions, ligand-binding and ion channel conductance, are located in distinct structural domains; an extracellular domain (ECD), which carries two to five neurotransmitter binding sites per receptor, and a transmembrane domain (TMD), which carries a single ion channel along the symmetry axis of the protein (Corringer et al., 2000; Smit et al., 2001; Brejc et al., 2001; Miyazawa et al., 2003; Unwin, 2005). X-ray structural data for homologs of the nicotinic ACh receptor ECD have been collected for nearly a decade (Kalamida et al., 2007; Rucktooa et al., 2009) thus providing atomic-level insight into the nature of pLGIC-ligand interactions. In contrast, X-ray structural data for the TMD has been lacking until recently, thus precluding atomic-level investigation of the structural features responsible for both channel conductance/gating and the mechanisms by which pLGICs interact with surrounding lipids, such as cholesterol, and other hydrophobic allosteric effectors, notably general anesthetics, alcohols, and neurosteroids. This short review presents our current knowledge of the structural architecture of the TMD taking into account recent

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advances derived from the discovery (Tasneem et al., 2005; Bocquet et al., 2007) and subsequent X-ray structure determination (Hilf and Dutzler, 2008, 2009; Bocquet et al., 2009) of bacterial members of the pLGICs super-family. In light of this new structural insight, we review and discuss the role of the TMD in the allosteric modulation of pLGICs, focusing on two examples; protein–lipid interactions at the nicotinic ACh receptor and anesthetic action at anion-selective pLGICs.

2. General structure of pentameric ligand-gated ion channels

To date, structural data have been collected for 1) the *Torpedo* nicotinic ACh receptor, which is easily purified in large quantities thus allowing detailed analysis using electron microscopy and chemical labeling approaches (Unwin, 2005), 2) the acetylcholine binding proteins (AChBPs), which are water-soluble pentamers homologous to the ECD of the nicotinic ACh receptor. Structures of AChBPs have been solved by X-ray crystallography in the presence and absence of a series of nicotinic agonists and antagonists (Brejc et al., 2001; Ulens et al., 2009; Rucktooa et al., 2009), 3) the monomeric ECD of the muscle-type α1 nicotinic ACh receptor subunit bound to α-bungarotoxin (Dellisanti et al., 2007), 4) the bacterial pLGICs from the proteobacteria *Erwinia chrysanthemi* (called ELIC, Hilf and Dutzler, 2008) and the cyanobacteria *Gloeobacter violaceus* (called GLIC, Bocquet et al., 2009; Hilf and Dutzler, 2009). The structures of the latter pLGICs were solved by X-ray crystallography at 3.3 and 2.9 Å resolution, respectively, and 5) the ECD of GLIC, which crystallizes in both pentameric and hexameric forms (Nury et al., 2010a).

The collected data establish a consistent picture for the structures of all pLGICs. In each case, the five subunits of each pentamer are organized symmetrically (homo-pentamers) or pseudo-symmetric (hetero-pentamers) around a central axis that functions as the ion channel (Fig. 1). Each subunit consists of an ~200 residue long N-terminal ECD with 10 β strands (β1-β10) folded into β-sandwich, as well as an ~100 residue long transmembrane domain (TMD) consisting of four transmembrane α-helices (M1 to M4) organized into a classic four α-helix bundle. The receptor's agonist-binding site and ion channel gate reside in the ECD and TMD, respectively, and are separated from each other by more than 60 Å. The extracellular and transmembrane domains are demarked by an abrupt change in secondary structure and meet at an interface located close to the bilayer surface. Contact between the two domains is mediated by the covalent link between the C-terminus of β10 in the ECD and the N-terminus of M1 in the TMD, as well as by non-covalent connections between the β1/β2 and β6/β7 loops (in eukaryotic pLGICs the latter is referred to as the Cys-loop) of the ECD and the M2-M3 loop of the TMD. An additional cytoplasmic domain is also present between transmembrane α-helices M3 and M4 in eukaryotic pLGICs and is characterized by a high variability in sequence and in length, with only one α-helix seen by electron microscopy (Unwin, 2005).

Extensive work has focused on understanding both the mechanisms of agonist-binding to the ECD and the mechanisms by which ligand-binding leads to the opening of the ion channel (i.e. channel gating). In terms of the gating mechanism, the muscle-type nicotinic ACh receptor has been the most extensively studied. Numerous artificial and natural mutations (the latter leading to congenital myasthenic syndromes), which alter the gating mechanism, have been identified (Lee et al., 2009; Purohit et al., 2007). These mutations are spread over the entire structure of the α-subunit, particularly at the interfaces between subunits, consistent with the idea that allosteric transitions result from global motions involving all five subunits within the protein. Recent studies highlight the importance of the β1–β2 and β6–β7 loops from the ECD and the M2–M3 linker from the TMD in coupling
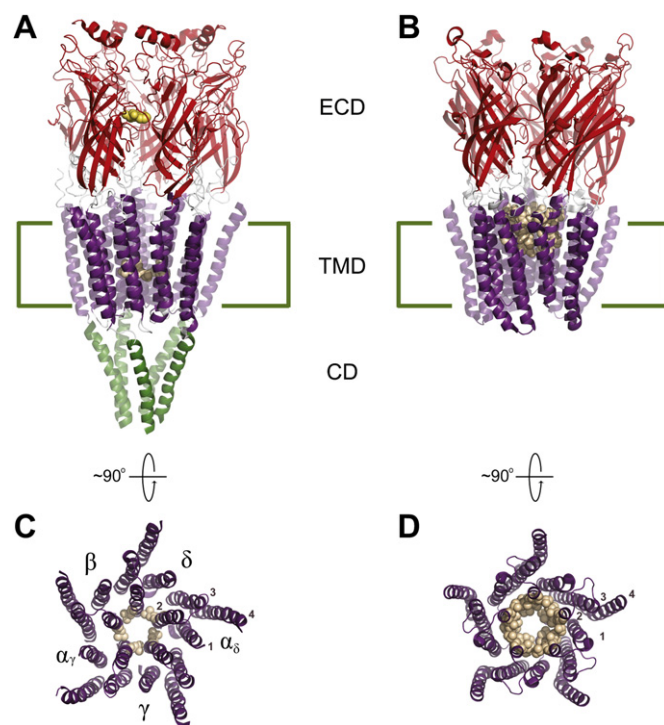

Fig. 1. 3D structure of pLGICs. Structures of A) the eukaryotic *Torpedo* nicotinic ACh receptor and B) the prokaryotic homolog, GLIC, highlighting the extracellular domain (ECD, red), the transmembrane domain (TMD, purple), and the cytoplasmic domain (CD, green). For the nicotinic ACh receptor, the agonist-binding site (αTrp-147) and the proposed channel gate (αLeu-251 and homologous residues in the β, γ, and δ subunits) are shown in orange and beige, respectively. For GLIC, residues forming the channel gate (Ile-233, Ile-236, Ala-237, Ile-240, Leu-241) are shown in beige. Views of the TMDs of the C) nicotinic ACh receptor and D) GLIC from the extracellular membrane surface highlight the M1 to M4 transmembrane α-helices in each subunit, as well as the heteromeric subunit organization of the nicotinic ACh receptor. (For the interpretation of the reference to color in this figure legend the reader is referred to the web version of this article.)

agonist-binding site to the channel gate (Lummis et al., 2005; Lee and Sine, 2005).

Strikingly, the systematic investigation of hundreds of muscle-type nicotinic ACh receptor mutants by patch-clamp single channel electrophysiology has demonstrated that nicotinic ACh receptor channel gating follows the Monod-Wyman-Changeux (MWC) allosteric mechanism (Purohit and Auerbach, 2010; Jha et al., 2009; Bertrand and Gopalakrishnan, 2007), in agreement with previous work on neuronal nicotinic ACh receptors (Changeux and Edelstein, 2005). The nicotinic ACh receptor spontaneously isomerizes between the basal (also called resting) and open states. Agonist-binding shifts the equilibrium between these conformations in favor of the open state, for which the ligand displays the highest affinity. In addition, prolonged agonist occupancy promotes receptor desensitization through isomerization toward one or several high affinity agonist-binding states that is(are) refractory to activation by agonist. In this context, understanding the functional architecture of pLGICs will require solution of a 3D structure for each allosteric conformation. A comprehensive mechanistic framework will also require insight into the mechanisms by which each ligand/allosteric modulator preferentially interacts with and thus preferentially stabilizes one conformation over another. The currently available structures, while each coming from a different species, offer three different conformations of the protein each endowed with a different degree of opening of the channel: ELIC is clearly closed while GLIC and nicotinic ACh receptor structure are

much more open. These structures thus provide a framework to begin to understand the basis of pLGIC allosteric transitions.

3. Structure of the transmembrane domain

Accumulated biochemical and structural data provide a consistent picture of the TMD structure with the ion channel located along the central symmetry axis of the pentamer bordered by five M2 segments, one from each of the five subunits. In each case, M2 adopts an α-helical conformation, as shown by affinity labeling (Giraudat et al., 1986), the substituted cystein accessibility method (SCAM) (Akabas et al., 1994), electron microscopy (Unwin, 2005), and X-ray crystallography (Hilf and Dutzler, 2008, 2009; Bocquet et al., 2009). The five M2 segments generate rings of identical (homo-pentamers) or homologous (hetero-pentamers) residues lining the ion channel pore including, from the top extracellular to the bottom cytoplasmic side, a ring of charged/polar residues, three rings of hydrophobic residues, two rings of polar residues, and one ring of charged residues (Fig. 2). Extensive mutational analysis shows that residues at the cytoplasmic border of M2 contribute substantially to the selectivity filter of the channel, which discriminates ions mainly according to their charge (Galzi et al., 1992; Corringer et al., 1999, Keramidas et al., 2004).

The location of the channel gate, which both sterically and energetically occludes the movement of ions in the basal state, is still controversial. Some experiments suggest a location close to the cytoplasmic end of M2 (Paas et al., 2005), while others suggest a location closer to the extracellular surface at the level of the three

rings of hydrophobic residues. The structure of ELIC shows a narrow constriction of these hydrophobic residues, which clearly prohibits ion translocation by both size and the hydrophobic effect. The X-ray structures of GLIC and ELIC also provide atomic-resolution insight into the nature of the structural changes in the ion channel pore upon gating (Fig. 2). While ELIC channel is clearly closed, the pore in the crystal structure of GLIC is apparently open and is filled with six molecules of dodecylmaltoside (DDM). Control experiments performed to remove this tight bundle of detergent molecules, by using bulkier brominated detergent (Bocquet et al., 2009) and increasing the size of the acyl chain a position 13′ (Nury et al., 2010b), all confirm that the bound DDM does not alter the conformation of the ion channel pore.

The bundle of five M2 α-helices that line the ion channel is surrounded by a second ring of α-helices formed by the M1 and M3 transmembrane segments of each subunit, thereby shielding M2 from the membrane. The M4 transmembrane α-helix is located distal to M1 and M3 at the periphery of the protein and thus interacts extensively with lipids, in a manner consistent with chemical labeling of *Torpedo* nicotinic ACh receptors with hydrophobic membrane-inserted probes (Blanton and Cohen, 1992, 1994). Interestingly, electron densities are seen outside the protein in the crystal structure of GLIC revealing the presence of partially ordered lipids. Three lipids per subunits were constructed in this density with well-defined alkyl chains, but barely visible polar headgroups, thus indicating some structural mobility (Fig. 3). Two of the three lipid binding sites are found at the interfaces between transmembrane α-helices within the same subunit. An
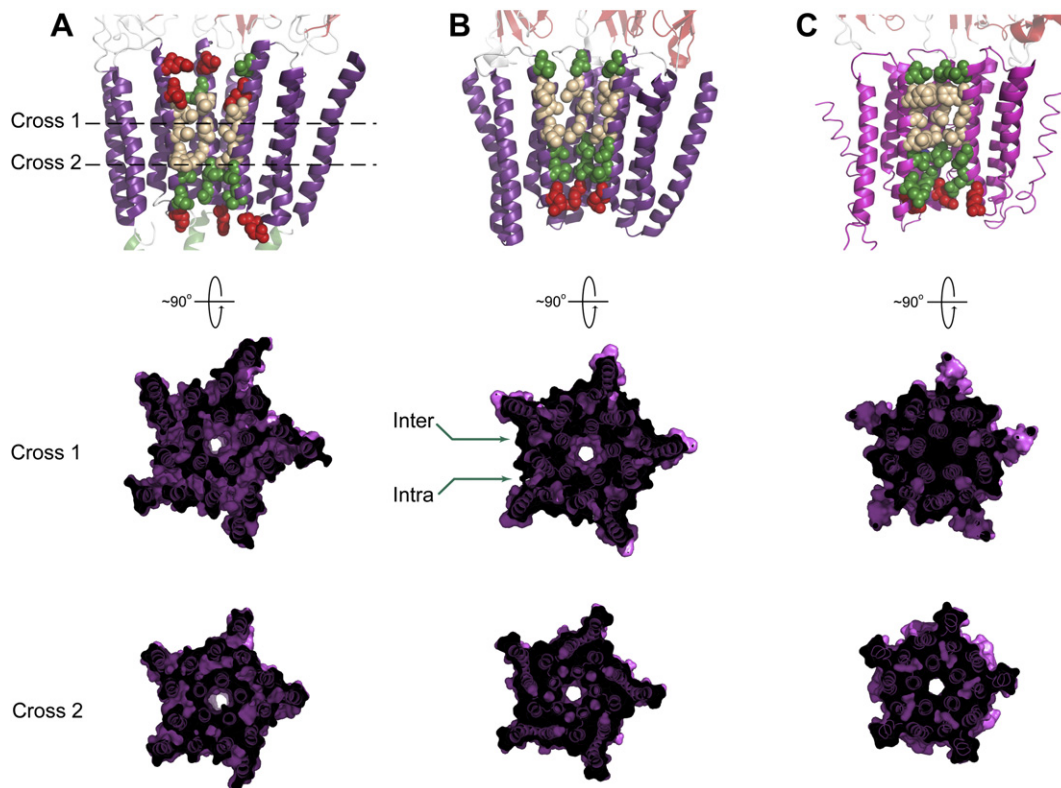

Fig. 2. The transmembrane domain of pLGICs. The TMD of A) the *Torpedo* nicotinic ACh receptor, B) GLIC, and C) ELIC highlighting residues that contribute to the channel pore (top row), as well as cavities located between TMD α-helices that may serve as sites for the allosteric effects of anesthetics and alcohols (middle and bottom row). Views of the channel pore are roughly from the same perspective as in Fig. 1A and B, except that the front two subunits (α_γ and γ, for the nicotinic ACh receptor) have been removed. Anionic, polar, and hydrophobic residues contributing to the channel pore are shown in red, green, and beige. The dashed lines in the top row of (A) represent the approximate locations of the two 12 Å thick slabs shown as *cross 1* and *cross 2* in the middle and bottom rows. In the cross sectional slabs, nicotinic ACh receptor, GLIC, and ELIC density is black, while cavities are shown in purple. "Inter" and "Intra" labels highlight inter- and intra-subunit cavities, respectively, located between transmembrane α-helices in GLIC. (For the interpretation of the reference to color in this figure legend the reader is referred to the web version of this article.)
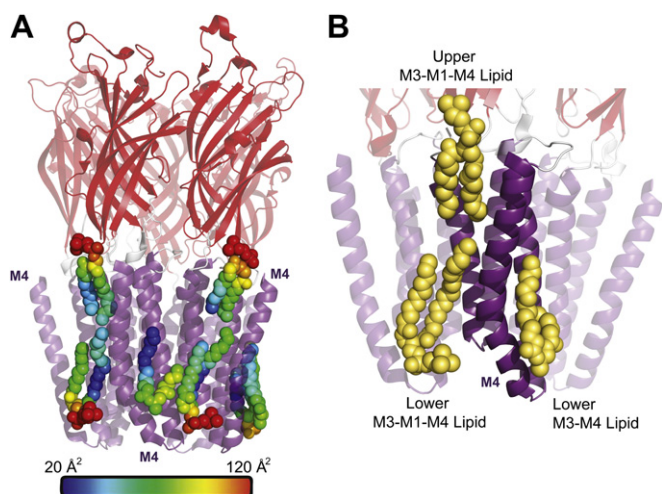
Fig. 3. Lipids bound to the GLIC structure. A) Lipids bound to the TMD in the crystal structure of GLIC colour-coded according to the B-factors of individual lipid atom. B) Close-up of lipids bound to the TMD M4 α-helix of one GLIC subunit. Only the front three subunits are shown.

upper (extracellular polar head) lipid binding site is located in a groove between M1 (Y194, I198, L203, L206), M3 (Y254, 258) and M4 (N307, A311, F315), while a lower lipid site is found in the groove between M3 (F265, V268, E272, V275) and M4 (I291, A294, S295, A298, F299, V302). The third lipid is bound to a lower site between the M3 (F267, I271, T274, L279, E282) α-helix of one subunit and the M1 (F210, W213, T214, W217) and M4 (R293, R296, P300, F303; L304) α-helices from the other subunit.

The structural data show that more than half of the M4 residues are engaged in binding specifically to lipid. Since only one "face" of M4 is bound to the protein, lipids likely play a significant role in maintaining M4 structure (Li et al., 1992). The fact that the lipid acyl chains are ordered and co-purify with the GLIC through one affinity column and two size-exclusion columns suggests that they bind tightly to the protein. These observations coupled with data showing that M4 in nicotinic ACh receptor plays an important role in channel gating, suggest that bound lipids modulate GLIC allosteric transitions.

The structures of the TMD of GLIC and ELIC also show several cavities, especially in the upper part of the TMD. The GLIC structure has two cavities between the M2 segment and both M1 and M3. One is located within each individual subunit and is open to the bulk membrane. The other is located at the interface between subunits (Fig. 3). These cavities rearrange in the ELIC structure, as a consequence of the M2–M3 segment tilting motion associated with channel closing.

For eukaryotic cation-selective pLGICs, the best available model of the TMD is derived from electron microscopy studies of the Torpedo nicotinic ACh receptor at 4 Å resolution (Unwin, 2005). The Torpedo nicotinic ACh receptor structure resembles that of GLIC in that the M2 α-helices create a funnel-shaped channel (Figs. 1 and 2). In contrast, the M1, M3 and M4 α-helices are located further away from M2 in the Torpedo nicotinic ACh receptor as compared to GLIC, thereby generating large cavities within the TMD (Fig. 2). In this context, recent molecular dynamic simulation shows that these cavities could accommodate cholesterol, cholesterol preventing collapse of the structure (Brannigan et al., 2008). Also, while the axes of the M2 α-helices match well between nicotinic ACh receptor and GLIC, there is a one α-helix turn difference between the two, with the M2 of GLIC starting at residue −4′ and that of Torpedo nicotinic ACh receptor at 0′.

No structural data is currently available for anionic-selective pLGICs, so one must currently rely on homology models. Construction of a GABA$_A$ receptor model based on the 4 Å resolution nicotinic ACh receptor structure was difficult due to the alignment uncertainty between the nicotinic ACh receptor and GABA$_A$ receptor M3 and M4 transmembrane α-helices (Bali et al., 2009) (Fig. 5). Homology models of anionic pLGICs based on nicotinic ACh receptor structure have indeed been proposed in the literature, but even a one residues difference in the alignment produces a reorganization of the side chains yielding different predictions, notably in relation to binding site identification at the amino acid level (Trudell and Bertaccini, 2004; Ernst et al., 2005; Jansen and Akabas, 2006). Interestingly, the TMDs of the anion-selective pLGICs align satisfactorily with that of bacterial pLGICs, especially at the level of the M3 and M4 segments, as illustrated by the alignment of GLIC with a few representative anion-selective channels (Fig. 5). GLIC thus appears to be a suitable template for homology modeling of anion-selective pLGICs.

4. Lipids as allosteric modulators of nicotinic ACh receptor function

Nicotinic ACh receptors are found throughout the central and peripheral nervous systems, as well as abundantly in the electric ray, Torpedo. In addition to their primordial role in neuromuscular and motor autonomous transmission, nicotinic ACh receptors are involved in central neurological functions, including control of voluntary motion, memory and attention, sleep and wakefulness, reward (nicotine) and pain. Seventeen genes encoding for nicotinic ACh receptor subunits have been identified in humans. The $(\alpha 1)_2 \beta 1 \varepsilon \delta$ nicotinic ACh receptor is found at synapses in adult muscle, while α4β2 nicotinic ACh receptors are most abundant within the human brain.

Nicotinic ACh receptor function is modulated by a wide range of compounds. Allosteric modulators that interact with the TMD include both general and local anesthetics, as well as a variety of other natural and synthetic ligands, such as neurosteroids. The nicotinic ACh receptor is also highly sensitive to the composition of its surrounding lipid membrane. The lipid sensitivity of the Torpedo nicotinic ACh receptor has been known since the earliest attempts to isolate and reconstitute nicotinic ACh receptor function in model membranes. To recover agonist-induced channel flux, the receptor must be solubilized/purified in the presence of lipid and then placed in a membrane with an appropriate lipid composition (Epstein and Racker, 1978; Heidmann et al., 1980; Criado et al., 1984; Fong and McNamee, 1986). Both anionic lipids, such as phosphatidylserine or phosphatidic acid, and neutral lipids, such as cholesterol, are important for activity.

Recent work has emphasized the role of lipids as classic allosteric modulators interacting preferentially with and thus preferentially stabilizing pre-existing conformational states. It was initially suggested that lipids modulate the natural equilibrium between activatable basal and non-activatable desensitized conformations (McCarthy and Moore, 1992; Baenziger et al., 2000; Hamouda et al., 2006b) with native Torpedo lipids strongly favoring the basal state (basal:desensitized ∼80:20) (Heidmann and Changeux, 1979; Boyd and Cohen, 1980). The nicotinic ACh receptor in reconstituted membranes also adopts one or several distinct non-responsive conformation(s) collectively referred to as the "uncoupled" state (daCosta and Baenziger, 2009) (Fig. 4A).

Lipids influence the proportion of agonist-responsive nicotinic ACh receptors in a reconstituted membrane by stabilizing varying proportions of basal versus desensitized and uncoupled conformations. Phosphatidylcholine (PC) membranes with a mixture of cholesterol and an anionic lipid (either phosphatidylserine or
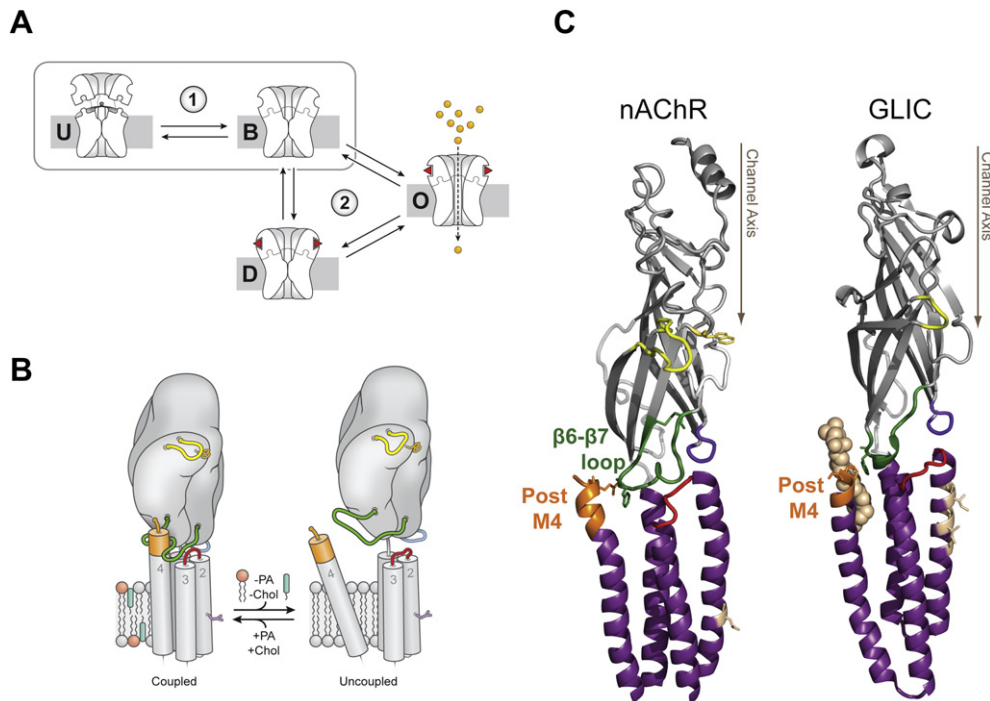
Fig. 4. A conformational scheme involving an uncoupled state. A) Nicotinic ACh receptor function is usually interpreted in terms of a conformational scheme involving basal (B), open (O), and desensitized (D) conformations (scheme 2). The *Torpedo* nicotinic ACh receptor in reconstituted membranes also adopts a non-responsive uncoupled (U) conformation (scheme 1). B) In the nicotinic ACh receptor, uncoupling may result from altered interactions between post-M4 and the β6–β7 loop, which may lead to both a functional and structural weakening of the ECD and TMD. C) Proximity of post-M4 (orange) to the β6-β7 loop (green). For the nicotinic ACh receptor, direct interactions occur between M4 (Q435) and the Cys-loop (F137), shown in ball and stick representations. For GLIC, a lipid molecule (beige space filling model) bridges interactions between post-M4 (F315) and the equivalent phenylalanine in β6-β7 loop (F121). The M2-M3, β1-β2, β9-β10 (part of the acetycholine binding site in the nicotinic ACh receptor) loops are highlighted in red, blue, and yellow, respectively. Pore-lining hydrophobic residues thought to be involved in the channel gate are highlighted as ball and stick models in beige. A) and B) are adapted from daCosta and Baenziger (2009).

phosphatidic acid) stabilize a large proportion of basal nicotinic ACh receptors. Mixtures of PC and phosphatidic acid are also quite effective. In contrast, mixtures of PC and phosphatidylserine stabilize primarily a mixture of uncoupled and desensitized nicotinic ACh receptors, whereas PC membranes lacking both cholesterol and anionic lipids favor the uncoupled state (daCosta et al., 2009). The ability of the nicotinic ACh receptor to undergo allosteric transitions is less sensitive to acyl chain unsaturation, with both *cis* and *trans* unsaturated lipids supporting function (Fong and McNamee, 1986).

4.1. The lipid-dependent uncoupled state

The uncoupled nicotinic ACh receptor binds agonist, but does not undergo allosteric transitions upon agonist-binding (i.e. binding and gating are uncoupled). It is distinguished from the desensitized receptor by its relatively low affinity for ligands that characteristically bind with high affinity to both the ECD and the TMD in the desensitized state (Rankin et al., 1997; Baenziger et al., 2008, daCosta and Baenziger, 2009). The ECD of the nicotinic ACh receptor binds the agonist acetylcholine with an apparent dissociation constant of 3–10 μM (daCosta and Baenziger, 2009), which is similar to that of the basal, but ∼1000-fold lower than that of the desensitized state (800 nM and 2 nM, respectively; Boyd and Cohen, 1980). The TMD pore does not bind appreciably the fluorescent allosteric probe, ethidium bromide, at concentrations up to 30 μM (daCosta and Baenziger, 2009), the latter being ∼100 fold above the dissociation constant for desensitized, but well below the dissociation constant for the basal nicotinic ACh receptor (0.3 μM and ≥1 mM, respectively; Herz et al., 1987).

In addition, the uncoupled nicotinic ACh receptor is distinguished from the basal nicotinic ACh receptor by its labeling pattern with the hydrophobic photoactivatable probe, 3-trifluoromethyl-3-(m-[^{125}I]-iodophenyl)diazirine ([^{125}I]-TID). [^{125}I]-TID binds to the ion channel of the resting receptor affinity and labels preferentially pore-lining residues in the α and γ subunits (McCarthy and Moore, 1992; daCosta et al., 2002; Hamouda et al., 2006b). In contrast, only weak stoichiometric labeling of the subunits by [^{125}I]-TID, likely at the lipid-exposed periphery, is observed in the uncoupled state.

The uncoupled conformation is also distinguished from the basal and desensitized conformations by its biophysical properties. Although the secondary structures are similar (Méthot et al., 1995), the thermal denaturation of the uncoupled receptor is slightly less cooperative and occurs at a temperature slightly below (3–5 °C) that of basal and desensitized nicotinic ACh receptors. In addition, the peptide hydrogen exchange kinetics of the uncoupled nicotinic ACh receptor differ substantially from that of both basal and desensitized receptors (Méthot et al., 1995; daCosta and Baenziger, 2009). Roughly 40% of the peptide hydrogens in the basal and desensitized conformations are resistant to peptide N-^{1}H/N-^{2}H exchange after exposure of the nicotinic ACh receptor to ^{2}H$_2$O for 12 h, while only ∼20% of the peptide hydrogens are resistant to exchange in the uncoupled state. Significantly, the drop in the proportion of exchange-resistant peptide hydrogens in the uncoupled state is compensated for by an increase in the proportion of rapidly exchanging peptide hydrogens. This suggests that uncoupling is accompanied by an increase in the solvent accessibility of previously buried peptide hydrogens (daCosta and Baenziger, 2009).

4.2. Sites of lipid-nicotinic ACh receptor interactions

Lipids can allosterically influence protein conformational equilibria by binding directly to conformationally sensitive protein binding sites, by altering bulk membrane physical properties (which indirectly couple with different conformational states), or by a combination of both (Lee, 2004). In the case of the nicotinic ACh receptor, the M4 transmembrane α-helix is the most lipid-exposed of the transmembrane α-helices and is thus a likely site for mediating both specific and non-specific nicotinic ACh receptor−lipid interactions. Mutagenesis of lipid-exposed residues on M4 alters channel gating, highlighting the importance of M4 in nicotinic ACh receptor function (Li et al., 1992; Lee et al., 1994; Lasalde et al., 1996; Bouzat et al., 1998; Tamamizu et al., 2000; Shen et al., 2006; Mitra et al., 2004). In fact, mutation of a lipid facing residue on M4 (αC418W) in the muscle-type receptor leads effects on human health demonstrating that M4-lipid interactions are important in humans (Shen et al., 2006). M4 may act as a "lipid sensor" translating membrane properties into altered nicotinic ACh receptor function.

M4 is a potential site for the allosteric effects of both cholesterol and anionic lipids. Both lipids show a particular affinity for the annulus of lipids that surrounds the lipid-exposed surface of the nicotinic ACh receptor (Marsh and Barrantes, 1978; Ellena et al., 1983; Fernández-Nievas et al., 2008). At any given moment in time, both cholesterol and anionic lipids in this annulus are "bound" to the surface of the nicotinic ACh receptor, and thus interact extensively with M4. A photoactivatable cholesterol analog labels sites on the lipid-exposed surface of the nicotinic ACh receptor, including predominantly residues on M4 (Hamouda et al., 2006a). Another cholesterol analog covalently linked to phosphatidylcholine, which presumably resides within the bulk membrane environment, is as effective as cholesterol in supporting nicotinic ACh receptor function, consistent with a membrane-exposed surface-site for cholesterol action (Addona et al., 1998). In fact, the entire lipid-exposed surface of the nicotinic ACh receptor may serve as an "allosteric site" that is sensitive to bulk membrane physical properties.

In addition to annular sites of action, "non-annular" cholesterol binding sites located between transmembrane α-helices have been proposed based on fluorescence quenching studies with brominated lipids (Jones and McNamee, 1988). Molecular dynamics suggests that such non-annular cholesterol bound to cavities located between the nicotinic ACh receptor transmembrane α-helices stabilize the transmembrane domain structure thus facilitating interactions with the ECD (Brannigan et al., 2008).

4.3. A M4-lipid sensor model of uncoupling

M4 is the least conserved among the nicotinic ACh receptor transmembrane α-helices. While the hydrophobic transmembrane portion of M4 can be replaced with transmembrane α-helices from other proteins with minimal functional consequences, the extended length of M4 appears to be critical (Tobimatsu et al., 1987). The C-terminal of M4 (referred to as post-M4) is essential for trafficking of both Torpedo nicotinic ACh receptor and an α7 nicotinic ACh receptor-5HT$_{3A}$ chimera to the cell surface to form functional ion channels (Tobimatsu et al., 1987; Pons et al., 2004). Post-M4 is also a site for neurosteroid-induced potentiation of both neuronal α4β2 nicotinic ACh receptors and GABA$_A$ receptors (Paradiso et al., 2001; Hosie et al., 2006).

Interestingly, post-αM4 (Q435) extends beyond the lipid bilayer to interact with a conserved residue (F137) in the β6−β7 loop, which is located at the interface between the agonist-binding and transmembrane domains (Fig. 4). Given that the β6−β7 loop plays a central role at the interface between the ECD and TMD (Lummis et al., 2005; Xiu et al., 2005), interactions between post-M4 and the β6−β7 loop may be essential for coupling agonist-binding to channel gating. It has been suggested that lipids influence nicotinic ACh receptor function by modulating post-M4 interactions with the β6−β7 loop (daCosta, 2006). Altered interactions between post-M4 and the β6−β7 loop in membranes lacking neutral and anionic lipids may lead to weakened interactions between, and thus partial dissociation of the ECD from the TMD in the uncoupled state (daCosta and Baenziger, 2009). Partial dissociation of the two domains leading to increased solvent accessibility of loops at the coupling interface between the domains would account for the increased polypeptide backbone peptide hydrogen exchange kinetics in the uncoupled versus the resting and desensitized conformations. The structure of the coupling interface may also change in the desensitized state, thus leading to conformationally-specific interactions with different lipids.

4.4. Possible mechanisms of nicotinic ACh receptor-lipid action

The M4-lipid sensor model provides a framework for testing proposed models regarding the mechanisms of nicotinic ACh receptor-lipid interactions. A central feature of this model is that the ECD adopts an independent structure that can at least partially dissociate from the TMD to form the uncoupled conformation. The proposed structural independence of the ECD is supported by the existence of stable pentameric water-soluble AChBPs, which are homologous to the ECD of the nicotinic ACh receptor (Brejc et al., 2001; Ulens et al., 2009; Rucktooa et al., 2009; Hansen et al., 2005). Folded, stable, water-soluble ECDs of both the nicotinic ACh receptor α-subunit and GLIC can also be expressed independently of their respective TMDs (Dellisanti et al., 2007; Nury et al., 2010a). Both pentameric and hexameric structures of the GLIC ECD have been solved by X-ray crystallography.

The M4-lipid sensor model is also supported by the crystal structure of GLIC, which exhibits three lipids bound directly to each M4 α-helix (Bocquet et al., 2009). One of the three lipids is located near post-M4 where it bridges interactions between post-M4 (F315) and the β6-β7 loop (F121), the loop analogous to the β6−β7 loop/ "Cys-loop" in eukaryotic pLGICs (Figs. 3 and 4). The bridged F121 in the β6-β7 loop is analogous to the highly conserved αF137 in the β6-β7 loop of the Torpedo nicotinic ACh receptor, which in the latter structure contacts directly post-M4. The observed lipid bound to both the β6−β7 loop and post-M4 highlight this region as a potential site for lipid-dependent allosteric modulation of pLGICs.

How do cholesterol and anionic lipids allosterically influence nicotinic ACh receptor function? Cholesterol, via effects on bulk membrane properties, could drive interactions between M4 and the remaining nicotinic ACh receptor transmembrane α-helices, M1-M3, thus positioning post-M4 at the coupling interface to interact with the β6−β7 loop (Antollini et al., 2005). In the absence of the appropriate membrane physical properties in membranes lacking cholesterol, post-M4 may dissociate from the β6−β7 loop leading to formation of the uncoupled state. Cholesterol, could also interact directly with post-M4 in a manner similar to that by which neurosteroids bind to post-M4 and potentiate both nicotinic ACh receptor and GABA$_A$ receptor activity. Alternatively or in addition, non-annular cholesterol sites could play a key role in stabilizing the structure of the entire TMD thus modulating the position of post-M4 relative to the β6−β7 loop and/or altering the conformation of the M2-M3 linker.

Anionic lipids could influence nicotinic ACh receptor activity via similar mechanisms. The net pattern of electrostatic residues at the interface between the agonist-binding and transmembrane-pore domains plays an important role in channel gating (Xiu et al., 2005). As suggested, the structure at this coupling interface may change

between resting, desensitized, and uncoupled conformations possibly leading to alterations in the net charge distribution. Changes in this charge distribution could enhance conformationally-specific interactions with anionic lipids. Anionic lipids could also interact specifically with post-M4 leading to either attractive (R429) or repulsive (E432, E426) forces that modulate the position of post-M4 relative to the $\beta 6$–$\beta 7$ loop.

Anionic lipids exhibit dramatically different efficacies in their abilities to stabilize a functional nicotinic ACh receptor. For example, mixtures of PC and phosphatidic acid are effective at stabilizing an activatable basal nicotinic ACh receptor, while mixtures of PC and phosphatidylserine are not (daCosta et al., 2004). PA may be more efficacious because of its relatively small headgroup, which influences lipid packing and thus bulk membrane properties (Sturgeon and Baenziger, 2010). The nicotinic ACh receptor may require both an appropriate membrane physical environment (possibly created by cholesterol) and an anionic lipid to drive interactions between M4 and the Cys-loop to preferentially stabilize the activatable basal conformation.

5. Allosteric modulation of anionic pLGICs

GABA, the major inhibitory neurotransmitter in the brain, mediates both phasic (synaptic) and tonic (extra-synaptic) inhibition via GABA$_A$ receptors (Olsen and Sieghart, 2009), while Glycine receptors are expressed predominantly at the periphery (Breitinger and Becker, 2002; Betz and Laube, 2006). Due to their widespread expression in the brain, GABA$_A$ receptors play a major role in virtually all brain physiological functions and are the targets of numerous classes of drugs. Nineteen genes coding for GABA$_A$ receptor subunits have been found in humans. The most abundant GABA$_A$ receptor subtype is formed from a combination of $\alpha 1$, $\beta 2$, and $\gamma 2$ subunits ($\alpha 1 \beta 2 \gamma 2$ receptors). Five genes encode different Glycine receptor subunits, with the adult form of the Glycine receptor formed from α_1 and β subunits.

The chloride ion-selective GABA$_A$ receptors are potentiated by a wide range of clinically important central nervous system depressants including benzodiazepines and barbiturates. GABA$_A$ receptors are also potentiated by general anesthetics, ethanol, and neuroactive steroids, compounds which potentiate Glycine receptors. Because of their central role in general anesthesia and ethanol action, the effects of anesthetics and ethanol on both GABA$_A$ receptor and Glycine receptor function have been studied extensively using a combination of biochemical, biophysical, and site-directed mutagenesis approaches (Franks, 2008).

5.1. Sites for anesthetic- and alcohol action at GABA$_A$ and glycine receptors

Although both the Glycine-$\alpha 1$ and GABA$_A$ receptor activities are potentiated by general anesthetics and alcohols, activity of the GABA$_A$-$\rho 1$ receptor subtype, in contrast, is reduced. Chimeras of Glycine-$\alpha 1$ and the GABA$_A$-$\rho 1$ receptors show that two residues, S267 and A288, located in the extracellular portion of the M2 and M3 transmembrane α-helices, respectively, in the Glycine-$\alpha 1$ receptor are critical for allosteric potentiation by both general anesthetics and alcohols (Mihic et al., 1997). Changing both residues in the Glycine-$\alpha 1$ receptor to the corresponding residues in the GABA$_A$-$\rho 1$ (S267I and A288W) leads to mutant Glycine-$\alpha 1$ receptors that respond normally to glycine, but are inhibited, as opposed to potentiated, by both general anesthetics and alcohols. Additional mutagenesis studies highlight a similar role for homologous residues in the potentiation of GABA$_A$ receptors, and also identify a third important residue (Glycine-$\alpha 1$ receptor I229) located in M1 (Lobo et al., 2004; Lobo and Harris 2005).

A photoactivatable analog of the anesthetic, etomidate, labels brain GABA$_A$ receptors at positions M236 and M286 of the M1 and M3 transmembrane α-helices (Li et al., 2006). The latter of the two residues is located at a position in M3 that is homologous to the position of A288 in M3 of the Glycine-$\alpha 1$ receptor. As noted above, A288 of the Glycine-$\alpha 1$ receptor is involved in both general anesthetic- and alcohol-induced potentiation of activity. The etomidate affinity labeling thus identifies a common site for general anesthetic- and alcohol-induced potentiation of the Glycine-$\alpha 1$ and GABA$_A$ receptors.

The labeling of GABA$_A$ receptors by photoactivatable etomidate, however, is only partially inhibited by the anesthetics, propofol, and neurosteroids (Li et al., 2009, 2010). The partial inhibition of etomidate labeling by these allosteric modulators suggests that etomidate may bind and thus act at additional sites. There may be other sites within the TMD by which general anesthetics and alcohols influence the activity of both GABA$_A$ receptors and the Glycine-$\alpha 1$ receptor.

5.2. Sites for neurosteroid-induced potentiation and activation at GABA$_A$ and glycine receptor receptors

The activities of all major isoforms of GABA$_A$ receptors are modulated by the endogenous neurosteroids, allopregnanolone and tetrahydro-deoxycorticosterone. At low nanomolar concentrations, these two neurosteroids potentiate the GABA$_A$ response, while at higher sub-micromolar to micromolar concentrations they lead to direct activation. The two distinct functional consequences of neurosteroid action at GABA$_A$ receptors (potentiation and activation) and their distinct pharmacological profiles suggest that there are two distinct sites of action, each with a different binding affinity for neurosteroids. In contrast, the *Drosophila melanogaster* resistance-to-dieldrin GABA receptor (RDL) has low sensitivity to potentiation by neurosteroids and lacks direct activation at the sub-micromolar concentrations that typically activate GABA$_A$ receptors.

To identify the sites of action, the sequences of GABA$_A$ receptors and RDL in the TMD were compared and the roles of variant residues in both neurosteroid-induced potentiation and activation studied using site-directed mutagenesis. While indirect allosteric effects of the studied mutants may account for the some of the observed phenotypes (see below), the data suggest that neurosteroid-induced potentiation of the GABA$_A$ receptor response occurs via a binding site formed by residues Q241, N407 and Y410. This site of potentiation was proposed, based on homology modeling with the *Torpedo* nicotinic ACh receptor transmembrane domain, to be located within the $\alpha 1$-subunit between the transmembrane α-helices M1 and M4. Mutagenesis of all three residues to eliminate their hydrogen bonding capabilities eliminates neurosteroid-induced potentiation (Hosie et al., 2006, 2009).

Two residues located at the interface between α and β subunits (αT236 and βY284) play a key role in neurosteroid-induced activation of GABA$_A$ receptors (Hosie et al., 2006, 2009). Mutation of either threonine to isoleucine (αT236I) or tyrosine to phenylalanine (βY284F), to remove the hydrogen bonding capacity of each residue, completely eliminates neurosteroid-induced activation of GABA$_A$ receptors with no effects on potentiation. Surprisingly, glutamine 241, which as noted above is a residue that plays a role in neurosteroid-induced potentiation, was also found to influence channel activation. It was concluded that relatively high-affinity binding of neurosteroids to the site of potentiation allosterically enhances neurosteroid-induced activation of GABA$_A$ receptors.

5.3. New insight derived from structures of GLIC

The mutagenesis and affinity labeling studies of anesthetic- and alcohol action at GABA$_A$ and the Glycine-$\alpha 1$ receptor have been

interpreted in terms of homology models of GABA$_A$ receptors and the Glycine-α1 receptor created using the *Torpedo* nicotinic ACh receptor as a template. Such models are dependent on an accurate sequence alignment of the TMDs of both GABA$_A$ and the Glycine-α1 receptors with the TMD of the nicotinic ACh receptor, but as noted above the alignments are uncertain particularly in the M3 and M4 transmembrane α-helices. In contrast, the TMD of GABA$_A$ receptors and the glycine-α1 receptor align satisfactorily with the TMDs of prokaryotic pLGICs, particularly at the level of the M3 and M4 transmembrane segments, suggesting that GLIC may represent a better template for homology modeling. The structure of GLICs lead to different homology models for both GABA$_A$ and the Glycine-α1 receptors.

Based on the improved sequence alignments of GABA$_A$ and the Glycine-α1 receptors with GLIC, we propose an alternative template for locating mutations within the structures of both GABA$_A$ and the Glycine-α1 receptors, and thus for interpreting the data on anesthetic-, alcohol-, and neurosteroid-induced potentiation (Fig. 5). In this new model, the key residues thought to contribute to anesthetic- and alcohol-binding sites are spread across the primary sequence in M1, M2, and M3, but are in close proximity in the 3D structure. These mutations point toward the subunit interface at the level of a cavity named "inter" (for inter-subunit) in Fig. 2. The residues involved in neurosteroid-induced potentiation are grouped within a single subunit closely associated with M1 and M4. In contrast, residues

involved in direct neurosteroid-induced activation are distal to each other, a feature that may suggest the effects of neurosteroid action caused by some mutations are complicated due to indirect allosteric effects. This new homology model, supported by clear sequence alignments, provides a different interpretation to that proposed using the *Torpedo* nicotinic ACh receptor as a structural template.

6. Conclusions

The above discussion highlights the complex and diverse effects of allosteric modulators that act within the TMD of pLGICs. Despite the diversity of effects and sites of action, the data also reveal two distinct, but related themes, with regards to the sites of allosteric modulation within the TMD:

First, many of hydrophobic/amphipathic allosteric regulators that act on the TMD bind to pockets located at interfaces between subunits. Some act at interfaces located between the subunits within the TMD, while others likely act at the interface between the ECD and TMD. These sites of action are reminiscent of the binding sites for allosteric agonists, which are located at interfaces between subunits in the ECD. Inter-subunit movement is central to allosteric transitions in pLGICs. Inter-subunit binding sites are thus ideal for the allosteric modulation of pLGICs.

Second, sites not located at subunit interfaces are typically found within individual subunits, but at the interfaces between different transmembrane α-helices. Although individual transmembrane α-helices cannot strictly speaking be regarded as individual "domains", in many instances these secondary structural elements may function as distinct structural units. The relative movements of transmembrane α-helices within individual subunits may also be important to pLGIC allosteric transitions. Such reorganizations are clearly seen when comparing the relative orientations of the transmembrane α-helices in the bacterial GLIC and ELIC structures. The potential intra-subunit movement of M4 relative to M1 and M3 to mediate lipid- and neurosteroid-induced potentiation, possibly by positioning post-M4 to interact with the Cys-loop at the interface between the ECD and TMD, is also a prime example.

We also discuss evidence for the existence of a lipid-dependent uncoupled conformation that may form as a result of an M4-dependent loss of allosteric contact between the ECD and the TMD. While this conformation has only been conclusively demonstrated for *Torpedo* nicotinic ACh receptors in reconstituted membranes, it may also exist for other pLGICs in native membranes. The structural independence of the ECDs of both the nicotinic ACh receptor and GLIC — i.e. both exist as stable water-soluble structures in the absence of the TMD — supports this hypothesis. Lipids and other allosteric modulators in native membranes may enhance the ability of pLGICs to transit from uncoupled to coupled conformations. If so, the uncoupled state may play a broader role in the potentiation of pLGICs that is observed with a variety of natural and exogenous effectors.

There is considerable interest in the use of allosteric modulators of pLGICs for the treatment of diseases, such as Alzheimer's disease (Taly et al., 2009; Bertrand et al., 2008), with many of the currently known modulators interacting within the TMD. Although we have focused here on the role of lipids as allosteric modulators of the *Torpedo* nicotinic ACh receptor and the role of general anesthetics, alcohols, and neurosteroids as potentiators of both GAGA$_A$ and Glycine receptor function, many of the fundamental principles illustrated by these examples have broader implications. For example, the amino acid residues facilitating allosteric potentiation of α7 nicotinic ACh receptors (PNU-120596 and LY-2087101) have been located within the TM1 (S222 and A225), TM2 (M253), and TM4 (F455 and C459) transmembrane α-helices (Young et al., 2008). All five amino acids point toward an intra-subunit cavity
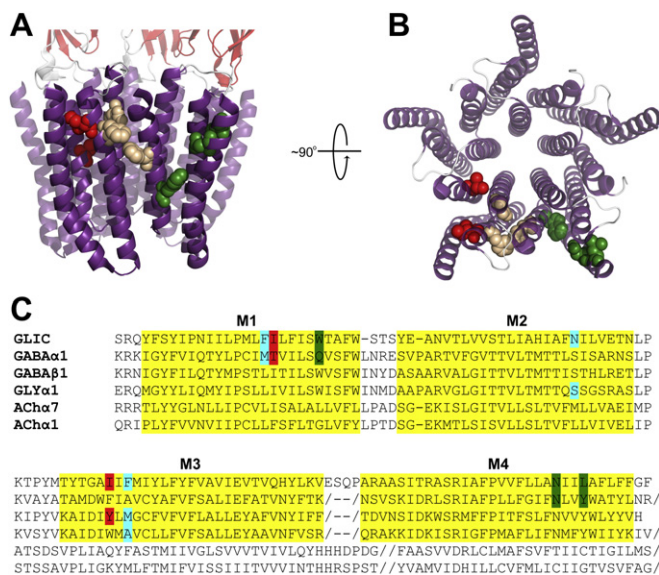

Fig. 5. Allosteric effector binding sites superimposed on the GLIC structure. Anesthetic, alcohol, and neurosteroid sites of action at anionic receptors, as modeled using the GLIC structure as a template. The upper panel shows the TMD of GLIC subunits A) seen from within the membrane and B) seen from the membrane surface. Equivalent amino acids whose mutation alters or which are affinity labeled (in the case of etomidate) by allosteric effectors of the Glycine and GABA$_A$ receptors are superimposed on the GLIC structure as spheres in two of the subunits. Sites of general anesthetic, ethanol, and etomidate action are beige (highlighted GLIC resicues correspond to Glycine-α1 receptor Ser267, Ala288, and GABA$_A$-α1 receptor M236, M286) sites of steroid potentiation are in green (GABA$_A$-α1 receptor Q241, N407, Y410), and sites of steroid activation are in red (GABA$_A$-α1 receptor T236, GABA$_A$-β1 receptor Y284). For visual simplicity and because of uncertainty regarding subunit quaternary arrangement in the GABA$_A$ structure, residues involved in neurosteroid activation are superimposed on a single subunit in the GLIC structure. C) Alignment of the TMD sequence of GLIC with the TMD sequences of selected human GABA$_A$-α1, GABA$_A$-β1, Glycine-α1, nicotinic ACh-α7, and *Torpedo* nicotinic ACh-α1 subunits (the cytoplasmic loops between M3 and M4 have been removed). Yellow boxes show transmembrane segments that can be satisfactorily aligned. Amino acids highlighted in the GLIC structure as beige, green, and red spheres are highlighted in the sequence as aqua, green, and red, respectively. (For the interpretation of the reference to color in this figure legend the reader is referred to the web version of this article.)

located between the four α-helices, in agreement with docking studies (see also Sattelle et al., 2009). Fundamental knowledge of pLGIC structures thus paves the way for understanding the mechanisms of action of important therapeutic compounds.

References

Addona, G.H., Sandermann, H., Kloczewiak, M.A., Husain, S.S., Miller, K.W., 1998. Where does cholesterol act during activation of the nicotinic acetylcholine receptor? Biochim. Biophys. Acta 1370, 299–309.

Akabas, M.H., Kaufmann, C., Archdeacon, P., Karlin, A., 1994. Identification of acetylcholine receptor channel-lining residues in the entire M2 segment of the alpha subunit. Neuron 13, 919–927.

Antollini, S.S., Xu, Y., Jiang, H., Barrantes, F.J., 2005. Fluorescence and molecular dynamics studies of the acetylcholine receptor γM4 transmembrane peptide in reconstituted systems. Mol. Membr. Biol. 22, 471–483.

Baenziger, J.E., Morris, M.L., Darsaut, T.E., Ryan, S.E., 2000. Effect of membrane lipid composition on the conformational equilibria of the nicotinic acetylcholine receptor. J. Biol. Chem. 275, 777–784.

Baenziger, J.E., Ryan, S.E., Goodreid, M.M., Vuong, N.Q., Sturgeon, R.M., daCosta, C.J.B., 2008. Lipid composition alters drug action at the nicotinic acetylcholine receptor. Mol. Pharmacol. 73, 880–890.

Bali, M., Jansen, M., Akabas, M.H., 2009. GABA-induced intersubunit conformational movement in the GABAA receptor alpha1M1-beta2M3 transmembrane subunit interface: experimental basis for homology modeling of an intravenous anesthetic binding site. J. Neurosci. 29, 3083–3092.

Bertrand, D., Bertrand, S., Cassar, S., Gubbins, E., Li, J., Gopalakrishnan, M., 2008. Positive allosteric modulation of the alpha7 nicotinic acetylcholine receptor: ligand interactions with distinct binding sites and evidence for a prominent role of the M2-M3 segment. Mol. Pharmacol. 74, 1407–1416.

Bertrand, D., Gopalakrishnan, M., 2007. Allosteric modulation of nicotinic acetylcholine receptors. Biochem. Pharmacol. 74, 1155–1163.

Betz, H., Laube, B., 2006. Glycine receptors: recent insights into their structural organization and functional diversity. J. Neurochem. 97, 1600–1610.

Blanton, M.P., Cohen, J.B., 1992. Mapping the lipid-exposed regions in the Torpedo californica nicotinic acetylcholine receptor. Biochemistry 31, 3738–3750.

Blanton, M.P., Cohen, J.B., 1994. Identifying the lipid-protein interface of the Torpedo nicotinic acetylcholine receptor: secondary structure implications. Biochemistry 33, 2859–2872.

Bocquet, N., de Carvalho, L.P., Cartaud, J., Neyton, J., Poupon, C.L., Taly, A., Grutter, T., Changeux, J.P., Corringer, P.J., 2007. A prokaryotic proton-gated ion channel from the nicotinic acetylcholine receptor family. Nature 445, 116–119.

Bocquet, N., Nury, H., Baaden, M., Poupon, C.L., Changeux, J.P., Delarue, M., Corringer, P.J., 2009. X-ray structure of a pentameric ligand-gated ion channel in an apparently open conformation. Nature 457, 111–114.

Bouzat, C., Roccamo, A.M., Garbus, I., Barrantes, F.J., 1998. Mutations at lipid-exposed residues of the acetylcholine receptor affect its gating kinetics. Mol. Pharmacol. 54, 146–153.

Boyd, N.D., Cohen, J.B., 1980. Kinetics of binding of [3H]acetylcholine and [3H]carbamoylcholine to Torpedo postsynaptic membranes: slow conformational transitions of the cholinergic receptor. Biochemistry 19, 5344–5353.

Brannigan, G., Hénin, J., Law, R., Eckenhoff, R., Klein, M.L., 2008. Embedded cholesterol in the nicotinic acetylcholine receptor. Proc. Natl. Acad. Sci. USA 105, 14418–14423.

Breitinger, H., Becker, C., 2002. The inhibitory glycine receptor-simple views of a complicated channel. Chembiochem 3, 1042–1052.

Brejc, K., van Dijk, W.J., Klaassen, R.V., Schuurmans, M., van Der Oost, J., Smit, A.B., Sixma, T.K., 2001. Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. Nature 411, 269–276.

Changeux, J.P., Edelstein, S.J., 2005. Allosteric mechanisms of signal transduction. Science 308, 1424–1428.

Corringer, P.J., Bertrand, S., Galzi, J.L., Devillers-Thiéry, A., Changeux, J.P., Bertrand, D., 1999. Mutational analysis of the charge selectivity filter of the alpha7 nicotinic acetylcholine receptor. Neuron 22, 831–843.

Corringer, P.J., Novère, N.L., Changeux, J.P., 2000. Nicotinic receptors at the amino acid level. Annu. Rev. Pharmacol. Toxicol. 40, 431–458.

Criado, M., Eibl, H., Barrantes, F.J., 1984. Functional properties of the acetylcholine receptor incorporated in model lipid membranes. Differential effects of chain length and head group of phospholipids on receptor affinity states and receptor-mediated ion translocation. J. Biol. Chem. 259, 9188–9198.

daCosta, C.J.B., 2006. A structural role for lipids in coupling ligand binding to channel gating in a neurotransmitter receptor. Ph.D Thesis University of Ottawa, Ottawa.

daCosta, C.J.B., Baenziger, J.E., 2009. A lipid-dependent uncoupled conformation of the acetylcholine receptor. J. Biol. Chem. 284, 17819–17825.

daCosta, C.J.B., Ogrel, A.A., McCardy, E.A., Blanton, M.P., Baenziger, J.E., 2002. Lipid-protein interactions at the nicotinic acetylcholine receptor. A functional coupling between nicotinic receptors and phosphatidic acid-containing lipid bilayers. J. Biol. Chem. 277, 201–208.

daCosta, C.J.B., Medaglia, S.A., Lavigne, N., Wang, S., Carswell, C.L., Baenziger, J.E., 2009. Anionic lipids allosterically modulate multiple nicotinic acetylcholine receptor conformational equilibria. J. Biol. Chem. 284, 33841–33849.

daCosta, C.J.B., Wagg, I.D., McKay, M.E., Baenziger, J.E., 2004. Phosphatidic acid and phosphatidylserine have distinct structural and functional interactions with the nicotinic acetylcholine receptor. J. Biol. Chem. 279, 14967–14974.

Dellisanti, C.D., Yao, Y., Stroud, J.C., Wang, Z., Chen, L., 2007. Crystal structure of the extracellular domain of nicotinic ACh receptor alpha1 bound to alpha-bungarotoxin at 1.94 A resolution. Nat. Neurosci. 10, 953–962.

Ellena, J.F., Blazing, M.A., McNamee, M.G., 1983. Lipid-protein interactions in reconstituted membranes containing acetylcholine receptor. Biochemistry 22, 5523–5535.

Epstein, M., Racker, E., 1978. Reconstitution of carbamylcholine-dependent sodium ion flux and desensitization of the acetylcholine receptor from Torpedo californica. J. Biol. Chem. 253, 6660–6662.

Ernst, M., Bruckner, S., Boresch, S., Sieghart, W., 2005. Comparative models of GABAA receptor extracellular and transmembrane domains: important insights in pharmacology and function. Mol. Pharmacol. 68, 1291–1300.

Fernández-Nievas, G.A., Barrantes, F.J., Antollini, S.S., 2008. Modulation of nicotinic acetylcholine receptor conformational state by free fatty acids and sterols. J. Biol. Chem. 283, 21478–21486.

Fong, T.M., McNamee, M.G., 1986. Correlation between acetylcholine receptor function and structural properties of membranes. Biochemistry 25, 830–840.

Franks, N.P., 2008. General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. Nat. Rev. Neurosci. 9, 370–386.

Galzi, J.L., Devillers-Thiéry, A., Hussy, N., Bertrand, S., Changeux, J.P., Bertrand, D., 1992. Mutations in the channel domain of a neuronal nicotinic receptor convert ion selectivity from cationic to anionic. Nature 359, 500–505.

Giraudat, J., Dennis, M., Heidmann, T., Chang, J.Y., Changeux, J.P., 1986. Structure of the high-affinity binding site for noncompetitive blockers of the acetylcholine receptor: serine-262 of the delta subunit is labeled by [3H]chlorpromazine. Proc. Natl. Acad. Sci. USA 83, 2719–2723.

Hamouda, A.K., Chiara, D.C., Sauls, D., Cohen, J.B., Blanton, M.P., 2006a. Cholesterol interacts with transmembrane alpha-helices M1, M3, and M4 of the Torpedo nicotinic acetylcholine receptor: photolabeling studies using [3H]Azicholesterol. Biochemistry 45, 976–986.

Hamouda, A.K., Sanghvi, M., Sauls, D., Machu, T.K., Blanton, M.P., 2006b. Assessing the lipid requirements of the Torpedo californica nicotinic acetylcholine receptor. Biochemistry 45, 4327–4337.

Hansen, S.B., Sulzenbacher, G., Huxford, T., Marchot, P., Taylor, P., Bourne, Y., 2005. Structures of Aplysia AChBP complexes with nicotinic agonists and antagonists reveal distinctive binding interfaces and conformations. EMBO J. 24, 3635–3646.

Heidmann, T., Changeux, J.P., 1979. Fast kinetic studies on the allosteric interactions between acetylcholine receptor and local anesthetic binding sites. Eur. J. Biochem. 94, 281–296.

Heidmann, T., Sobel, A., Popot, J.L., Changeux, J.P., 1980. Reconstitution of a functional acetylcholine receptor. Conservation of the conformational and allosteric transitions and recovery of the permeability response; role of lipids. Eur. J. Biochem. 110, 35–55.

Herz, J.M., Johnson, D.A., Taylor, P., 1987. Interaction of non-competitive inhibitors with the acetylcholine receptor. J. Biol. Chem. 262, 7238–7247.

Hilf, R.J.C., Dutzler, R., 2008. X-ray structure of a prokaryotic pentameric ligand-gated ion channel. Nature 452, 375–379.

Hilf, R.J.C., Dutzler, R., 2009. Structure of a potentially open state of a proton-activated pentameric ligand-gated ion channel. Nature 457, 115–118.

Hosie, A.M., Wilkins, M.E., da Silva, H.M.A., Smart, T.G., 2006. Endogenous neurosteroids regulate GABAA receptors through two discrete transmembrane sites. Nature 444, 486–489.

Hosie, A.M., Clarke, L., da Silva, H., Smart, T.G., 2009. Conserved site for neurosteroid modulation of GABA A receptors. Neuropharmacology 56, 149–154.

Jansen, M., Akabas, M.H., 2006. State-dependent cross-linking of the M2 and M3 segments: functional basis for the alignment of GABAA and acetylcholine receptor M3 segments. J. Neurosci. 26, 4492–4499.

Jha, A., Purohit, P., Auerbach, A., 2009. Energy and structure of the M2 helix in acetylcholine receptor-channel gating. Biophys. J. 96, 4075–4084.

Jones, O.T., McNamee, M.G., 1988. Annular and nonannular binding sites for cholesterol associated with the nicotinic acetylcholine receptor. Biochemistry 27, 2364–2374.

Kalamida, D., Poulas, K., Avramopoulou, V., Fostieri, E., Lagoumintzis, G., Lazaridis, K., Sideri, A., Zouridakis, M., Tzartos, S.J., 2007. Muscle and neuronal nicotinic acetylcholine receptors. Structure, function and pathogenicity. FEBS J. 274, 3799–3845.

Keramidas, A., Moorhouse, A.J., Schofield, P.R., Barry, P.H., 2004. Ligand-gated ion channels: mechanisms underlying ion selectivity. Prog. Biophys. Mol. Biol. 86, 161–204.

Lasalde, J.A., Tamamizu, S., Butler, D.H., Vibat, C.R., Hung, B., McNamee, M.G., 1996. Tryptophan substitutions at the lipid-exposed transmembrane segment M4 of Torpedo californica acetylcholine receptor govern channel gating. Biochemistry 35, 14139–14148.

Lee, A.G., 2004. How lipids affect the activities of integral membrane proteins. Biochim. Biophys. Acta 1666, 62–87.

Lee, Y.H., Li, L., Lasalde, J., Rojas, L., McNamee, M., Ortiz-Miranda, S.I., Pappone, P., 1994. Mutations in the M4 domain of Torpedo californica acetylcholine receptor dramatically alter ion channel function. Biophys. J. 66, 646–653.

Lee, W.Y., Free, C.R., Sine, S.M., 2009. Binding to gating transduction in nicotinic receptors: cys-loop energetically couples to pre-M1 and M2-M3 regions. J. Neurosci. 29, 3189–3199.

Lee, W.Y., Sine, S.M., 2005. Principal pathway coupling agonist binding to channel gating in nicotinic receptors. Nature 438, 243–247.

Li, L., Lee, Y.H., Pappone, P., Palma, A., McNamee, M.G., 1992. Site-specific mutations of nicotinic acetylcholine receptor at the lipid-protein interface dramatically alter ion channel function. Biophys. J. 62, 61–63.

Li, G., Chiara, D.C., Sawyer, G.W., Husain, S.S., Olsen, R.W., Cohen, J.B., 2006. Identification of a GABAA receptor anesthetic binding site at subunit interfaces by photolabeling with an etomidate analog. J. Neurosci. 26, 11599–11605.

Li, G., Chiara, D.C., Cohen, J.B., Olsen, R.W., 2009. Neurosteroids allosterically modulate binding of the anesthetic etomidate to gamma-aminobutyric acid type A receptors. J. Biol. Chem. 284, 11771–11775.

Li, G., Chiara, D.C., Cohen, J.B., Olsen, R.W., 2010. Numerous classes of general anesthetics inhibit etomidate binding to gamma-aminobutyric acid type A (GABAA) receptors. J. Biol. Chem. 285, 8615–8620.

Lobo, I.A., Mascia, M.P., Trudell, J.R., Harris, R.A., 2004. Channel gating of the Glycine receptor changes accessibility to residues implicated in receptor potentiation by alcohols and anesthetics. J. Biol. Chem. 279, 33919–33927.

Lobo, I.A., Harris, R.A., 2005. Sites of alcohol and volatile anesthetic action on Glycine receptors. Int. Rev. Neurobiol. 65, 53–87.

Lummis, S.C.R., Beene, D.L., Lee, L.W., Lester, H.A., Broadhurst, R.W., Dougherty, D.A., 2005. Cis-trans isomerization at a proline opens the pore of a neurotransmitter-gated ion channel. Nature 438, 248–252.

Marsh, D., Barrantes, F.J., 1978. Immobilized lipid in acetylcholine receptor-rich membranes from Torpedo marmorata. Proc. Natl. Acad. Sci. USA 75, 4329–4333.

McCarthy, M.P., Moore, M.A., 1992. Effects of lipids and detergents on the conformation of the nicotinic acetylcholine receptor from Torpedo californica. J. Biol. Chem. 267, 7655–7663.

Méthot, N., Demers, C.N., Baenziger, J.E., 1995. Structure of both the ligand- and lipid-dependent channel-inactive states of the nicotinic acetylcholine receptor probed by FTIR spectroscopy and hydrogen exchange. Biochemistry 34, 15142–15149.

Mihic, S.J., Ye, Q., Wick, M.J., Koltchine, V.V., Krasowski, M.D., Finn, S.E., Mascia, M.P., Valenzuela, C.F., Hanson, K.K., Greenblatt, E.P., Harris, R.A., Harrison, N.L., 1997. Sites of alcohol and volatile anaesthetic action on GABA(A) and Glycine receptors. Nature 389, 385–389.

Miller, P.S., Smart, T.G., 2010. Binding, activation and modulation of Cys-loop receptors. Trends Pharmacol. Sci. 31, 161–174.

Mitra, A., Bailey, T.D., Auerbach, A.L., 2004. Structural dynamics of the M4 transmembrane segment during acetylcholine receptor gating. Structure 12, 1909–1918.

Miyazawa, A., Fujiyoshi, Y., Unwin, N., 2003. Structure and gating mechanism of the acetylcholine receptor pore. Nature 423, 949–955.

Nury, H., Bocquet, N., Poupon, C.L., Raynal, B., Haouz, A., Corringer, P.J., Delarue, M., 2010a. Crystal structure of the extracellular domain of a bacterial ligand-gated ion channel. J. Mol. Biol. 395, 1114–1127.

Nury, H., Poitevin, F., Van Renterghem, C., Changeux, J.P., Corringer, P.J., Delarue, M., Baaden, M., 2010b. One-microsecond molecular dynamics simulation of channel gating in a nicotinic receptor homologue. Proc. Natl. Acad. Sci. USA 107, 6275–6280.

Olsen, R.W., Sieghart, W., 2009. GABA A receptors: subtypes provide diversity of function and pharmacology. Neuropharmacology 56, 141–148.

Paas, Y., Gibor, G., Grailhe, R., Savatier-Duclert, N., Dufresne, V., Sunesen, M., de Carvalho, L.P., Changeux, J.P., Attali, B., 2005. Pore conformations and gating mechanism of a Cys-loop receptor. Proc. Natl. Acad. Sci. USA 102, 15877–15882.

Paradiso, K., Zhang, J., Steinbach, J.H., 2001. The C terminus of the human nicotinic alpha4beta2 receptor forms a binding site required for potentiation by an estrogenic steroid. J. Neurosci. 21, 6561–6568.

Pons, S., Sallette, J., Bourgeois, J.P., Taly, A., Changeux, J.P., Devillers-Thiéry, A., 2004. Critical role of the C-terminal segment in the maturation and export to the cell surface of the homopentameric alpha 7-5HT3A receptor. Eur. J. Neurosci. 20, 2022–2030.

Purohit, P., Auerbach, A., 2010. Energetics of gating at the apo-acetylcholine receptor transmitter binding site. J. Gen. Physiol. 135, 321–331.

Purohit, P., Mitra, A., Auerbach, A., 2007. A stepwise mechanism for acetylcholine receptor channel gating. Nature 446, 930–933.

Rankin, S.E., Addona, G.H., Kloczewiak, M.A., Bugge, B., Miller, K.W., 1997. The cholesterol dependence of activation and fast desensitization of the nicotinic acetylcholine receptor. Biophys. J. 73, 2446–2455.

Rucktooa, P., Smit, A.B., Sixma, T.K., 2009. Insight in nicotinic ACh receptor subtype selectivity from AChBP crystal structures. Biochem. Pharmacol. 78, 777–787.

Sattelle, D.B., Buckingham, S.D., Akamatsu, M., Matsuda, K., Pienaar, I., Jones, A.K., Sattelle, B.M., Almond, A., Blundell, C.D., 2009. Comparative pharmacology and computational modelling yield insights into allosteric modulation of human alpha7 nicotinic acetylcholine receptors. Biochem. Pharmacol. 78, 836–843.

Shen, X., Deymeer, F., Sine, S.M., Engel, A.G., 2006. Slow-channel mutation in acetylcholine receptor alphaM4 domain and its efficient knockdown. Ann. Neurol. 60, 128–136.

Smit, A.B., Syed, N.I., Schaap, D., van Minnen, J., Klumperman, J., Kits, K.S., Lodder, H., van der Schors, R.C., van Elk, R., Sorgedrager, B., Brejc, K., Sixma, T.K., Geraerts, W.P., 2001. A glia-derived acetylcholine-binding protein that modulates synaptic transmission. Nature 411, 261–268.

Sturgeon, R.M., Baenziger, J.E., 2010. Cations mediate interactions between the nicotinic acetylcholine receptor and anionic lipids. Biophys. J. 98, 989–998.

Taly, A., Corringer, P., Guedin, D., Lestage, P., Changeux, J.P., 2009. Nicotinic receptors: allosteric transitions and therapeutic targets in the nervous system. Nat. Rev. Drug Discov. 8, 733–750.

Tamamizu, S., Guzmán, G.R., Santiago, J., Rojas, L.V., McNamee, M.G., Lasalde-Dominicci, J.A., 2000. Functional effects of periodic tryptophan substitutions in the alpha M4 transmembrane domain of the Torpedo californica nicotinic acetylcholine receptor. Biochemistry 39, 4666–4673.

Tasneem, A., Iyer, L.M., Jakobsson, E., Aravind, L., 2005. Identification of the prokaryotic ligand-gated ion channels and their implications for the mechanisms and origins of animal Cys-loop ion channels. Genome Biol. 6, R4.

Tobimatsu, T., Fujita, Y., Fukuda, K., Tanaka, K., Mori, Y., Konno, T., Mishina, M., Numa, S., 1987. Effects of substitution of putative transmembrane segments on nicotinic acetylcholine receptor function. FEBS Lett. 222, 56–62.

Trudell, J.R., Bertaccini, E., 2004. Comparative modeling of a GABAA alpha1 receptor using three crystal structures as templates. J. Mol. Graph Model. 23, 39–49.

Ulens, C., Akdemir, A., Jongejan, A., van Elk, R., Bertrand, S., Perrakis, A., Leurs, R., Smit, A.B., Sixma, T.K., Bertrand, D., de Esch, I.J.P., 2009. Use of acetylcholine binding protein in the search for novel alpha7 nicotinic receptor ligands. In silico docking, pharmacological screening, and X-ray analysis. J. Med. Chem. 52, 2372–2383.

Unwin, N., 2005. Refined structure of the nicotinic acetylcholine receptor at 4Å resolution. J. Mol. Biol. 346, 967–989.

Xiu, X., Hanek, A.P., Wang, J., Lester, H.A., Dougherty, D.A., 2005. A unified view of the role of electrostatic interactions in modulating the gating of Cys loop receptors. J. Biol. Chem. 280, 41655–41666.

Young, G.T., Zwart, R., Walker, A.S., Sher, E., Millar, N.S., 2008. Potentiation of alpha7 nicotinic acetylcholine receptors via an allosteric transmembrane site. Proc. Natl. Acad. Sci. USA 105, 14686–14691.