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Architectural and Functional Similarities between Trimeric ATP-gated P2X Receptors and Acid-Sensing Ion Channels

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ABSTRACT

ATP-gated P2X receptors and acid-sensing ion channels (ASICs) are two distinct ligand-gated ion channels that assemble into trimers. They are involved in many important physiological functions such as pain sensation and are recognized as important therapeutic targets. They have unrelated primary structures and respond to different ligands (ATP and protons), and are thus considered as two different ion channels. As a consequence, comparisons of the biophysical properties and underlying mechanisms have only rarely been made between these two channels. However, the recent determination of their molecular structures by X-ray crystallography has revealed unexpected parallels in the architecture of the two pores, providing a basis for possible functional analogies. In this review, we analyze the structural and functional similarities that are shared by these trimeric ion channels, and outline key unanswered questions that, if addressed experimentally, may help to elucidate how two unrelated ion channels have adopted a similar fold of the pore.

Keywords: ASIC; P2X receptors; trimeric ion channels; structure; function.

1. INTRODUCTION

P2X receptors and acid-sensing ion channels (ASICs) are ligand-gated ion channels. They are transmembrane allosteric proteins expressed in diverse organisms and mediate many different physiological functions ranging from taste to pain perception. Cloning of their genes in the mid 90s[1-5], followed by exhaustive sequence analyses, revealed that ASICs and P2X receptors belong to two distinct families of ion channels. There are no significant similarities in their amino acid sequences, and therefore it was concluded that these proteins are not homologous, and consequently have probably evolved from two distinct ancestral genes. However, early studies have revealed striking similarities in their molecular architectures. They are both trimeric ion channels and each subunit crosses the membrane twice with a similar topology: the two membrane-spanning segments of the transmembrane domain (TMD) are linked by a large, cysteine-rich extracellular domain (ECD) that protrudes outside the cell and contains the agonist-binding sites. Consequently, the amino and carboxy termini are intracellular. Despite these similarities, there was no evidence to indicate that P2X receptors and ASICs would share a common three-dimensional architecture of the pore.

Until recently, only few cross-connections were made between the gating mechanisms of ASICs and P2X receptors, and research was conducted rather independently in each family of ion channels. However, recent X-ray structures may change this view, as they have revealed unanticipated parallels in the architecture of the ASIC and P2X pore[6-8] (Figure 1a and b), raising the possibility that both channels may share more than a trimeric stoichiometry. In this review, we analyze the structural and functional similarities that are shared by P2X receptors and ASICs and outline key questions, such as whether or not the molecular mechanisms underlying channel gating, ion permeation, and pore dilation may be common for both channels, and consequently to what extent ASICs and P2X receptors have to be considered as two related or unrelated trimeric channels.

1. MOLECULAR ORGANISATION AND FUNCTIONAL PROPERTIES OF ATP-GATED P2X RECEPTORS

P2X receptors are gated by extracellular ATP and are composed of seven P2X subtypes (P2X1-7) that are expressed in virtually all mammalian tissues, including the nervous, cardiovascular and immune systems[9]. They are also found in various eukaryotic organisms, but to date no evidence supports their presence in *Drosophila*, *Caenorhabditis elegans*, yeast, or bacteria. There are multiple sources of extracellular ATP release ranging from neuronal and glial cells in response to physiological stimuli to damaged tissues in case

of pathological conditions[10-12]. In animals, a second type of purineroreceptors, the metabotropic G protein-coupled P2Y receptors, bind extracellular ATP. Very recently a new family of purineroreceptors, the P2K (K for kinase) receptors, has been identified in plants and harbors a very different molecular structure from the known animal counterparts[13]. These P2K receptors may represent the long-awaited candidate that senses ATP in plants.

The physiological and pathophysiological roles of P2X receptors comprise the modulation of synaptic transmission, taste, pain sensation, male fertility and inflammation[14-16], and accordingly, these receptors are considered to be attractive targets in many disorders, including neuropathic pain and stroke[9,17]. Early biochemical experiments and electrophysiological recordings, confirmed later by biophysical measurements, brought the first evidence for a trimeric quaternary structure of P2X channels[18-20]. A functional receptor is composed of three identical (all the seven subtypes, except for P2X6, form functional homotrimeric ion channels) or homologous subunits that assemble to form a central pore that is permeable to Na^+, K^+ and Ca^{2+}[21]. An exception to this is the homomeric P2X5 receptor, which is in addition permeable to chloride ions[22,23]. Calcium permeability also varies widely depending on the P2X isoform, with the human P2X4 receptor being the most permeable to calcium ions[24,25]. Agonist sensitivity and desensitization properties vary also considerably among subtypes. For instance, ATP at concentrations of tens of nanomolars activates P2X1 receptors and desensitizes very rapidly the channel, whereas P2X7 is activated by ATP concentrations of the order of hundred micromolar and does virtually not desensitize[14]. A recent study revealed that in addition to free ATP, distinct forms of ATP (ATP^{4-} and $MgATP^{2-}$) activate P2X receptors and do so with differential potency depending on the P2X subtype[26]. Furthermore, allosteric modulators, such as protons and Zn^{2+}, modulate the endogenous function of some P2X receptors, and contribute to the fine-tuning of function[27]. Heterooligomerization of P2X subtypes also adds a layer of diversity to the pharmacological and biophysical properties[28]. It has been shown that the ATP analogue αβmeATP activates P2X3 receptors with strong desensitization at concentrations that have no effect at P2X2 receptors, while the heteromeric P2X2/3 receptors are activated by αβmeATP with little desensitization[29,30]. Finally, the intracellular amino and carboxy termini, which vary in size depending on the subtype, are known to regulate the function and trafficking of the receptor[31-33]. All these distinct features likely contribute to the functional diversity of P2X signaling found in native tissues.

2. MOLECULAR ORGANISATION AND FUNCTIONAL PROPERTIES OF ACID-SENSING ION CHANNELS

ASICs are activated by extracellular acidification and constitute a sub-family within the Epithelial Na^+ channel (ENaC)/degenerin family of ion channels that comprises besides ASICs the mammalian ENaC involved in transepithelial Na^+ transport[34], the peptide-gated FaNaC of the mollusk nervous system[35], the degenerins being part of a mechanotransduction complex in *C. elegans* neurons[36], and related channels in *Drosophila*[37]. The four ASIC genes (ACCN1-4) code for at least 8 subunits, termed ASIC1a-b, ASIC2a-b, ASIC3a-c and ASIC4. ASICs are widely expressed in the nervous system[38]. ASIC1a, ASIC2a, ASIC2b and ASIC4 are found in both the peripheral (PNS) and central nervous systems (CNS), whereas ASIC1b and ASIC3 are expressed in the PNS but not or only at low levels in the CNS. The extracellular pH drops locally in synapses during synaptic activity due to the acidic pH of neurotransmitter-containing vesicles. Tissue acidification occurs in ischemia, inflammation and in tumors[38,39]. ASICs of the CNS are involved in learning, pain sensation and the expression of fear[38]. There is strong evidence for roles of ASICs of the PNS in pain sensation; a possible involvement of ASICs in mechanosensation is controversial. Due to their established physiological and pathological roles, ASICs are potential targets for anxiolytic and analgesic drugs.

Biochemical and functional studies with the related ENaC had indicated that these channels are tetramers[40-42]. Surprisingly however, the crystal structures obtained from chicken ASIC1 (cASIC1), showing 90% amino acid sequence identity with human ASIC1a, were trimers in all five published studies, suggesting that most likely all ENaC/degenerin channels are trimers[6,8,43-45]. Functional ASICs are homotrimers formed by one subunit type, or heterotrimers, and have a ~10-fold higher permeability for Na^+ than for K^+. ASIC1a displays in addition a low Ca^{2+} permeability[4,46,47], which may be important for its physiological and pathological roles. Some functional properties depend on the ASIC subunit composition. ASIC1a and ASIC3 are the most pH-sensitive ASICs with pH of half-maximal activation (pH50) of ~6.5. The other homomeric ASICs require more acidic pH for activation, with pH50 of ~6 and ~4.5 for ASIC1b and ASIC2a, respectively. ASIC2b and ASIC4 cannot form functional channels by themselves. ASIC2b was shown to affect ASIC properties if co-expressed with other subunits[48]. The pH dependence of heteromeric ASICs is intermediate between the values of the involved subunits[49]. The low efficiency of protons in activating ASIC2a and some ASIC2-containing channels suggests that ASIC activators or modulators other than protons may exist. Indeed, Mit-Toxin of the Texas coral snake venom activates

several ASIC subtypes at pH 7.4 and increases the pH sensitivity of ASIC2a[50]. The compound 2-guanidine-4-methylquinazoline as well as the Arg metabolites agmatine and arcaine activate ASIC3[51]. Acidification activates ASICs transiently, because it rapidly induces desensitization, limiting the duration of the current peak to hundreds of milliseconds to seconds, depending on the subunit composition. Desensitized ASICs cannot be opened by further acidification. The extracellular pH needs to be returned to physiological values before a new opening is possible. In ASIC3 and some heteromeric ASICs, desensitization is not complete in certain pH ranges, leading to biphasic currents with a transient and a sustained current component. At pH values that are more acidic than pH 7.4 but not sufficient for channel opening, ASICs desensitize slowly without apparent opening. In analogy to a similar property of voltage-gated Na^+ channels this process is called steady-state desensitization (SSD). During a long-lasting slight lowering of the extracellular pH inducing SSD, the availability of ASICs for opening is reduced accordingly. ASIC function is modulated by many endogenous and exogenous substances[38,52]. Ca^{2+} is believed to compete with protons for common binding sites in ASIC1a and ASIC3, since increasing its concentration shifts the pH dependence of ASIC activity to more acidic values[53,54]. Many ASIC modulators are subunit-specific. As for the P2X receptors, the intracellular N- and C-termini of the related ENaC play important roles in the control of channel activity, as evidenced by mutations leading to human diseases[55,56]. Similarly, the cytoplasmic parts of ASIC subunits also contribute to channel gating[57], and a stretch of the intracellular N-terminus co-determines ion selectivity[46,58]. Finally, a recent study revealed a functional interplay between ASICs and P2X receptors during ischemic acidosis, where sensory neurons detect coincidently protons and ATP through a molecular complex that is formed between ASIC3 and the P2X5 receptor[59].

3. X-RAY STRUCTURES REVEALED UNFORESEEN ANALOGIES

The first X-ray structure determination in 2007 and 2009 of the cASIC1 channel[6,8] and the zebrafish P2X4 (zfP2X4) receptor[7] provided a significant achievement in the understanding of ion channel functioning, as it revealed the three-dimensional structure of both the channel pore and the ectodomain (Figure 1a and b). It definitively confirmed the trimeric stoichiometry of P2X receptors, and further provided evidence that ASICs are also trimeric channels. However, the intracellular domains were lacking due to extensive truncation of their amino and carboxy termini that was required for crystallization, and the crystal structure of the remaining cytoplasmic parts was not resolved either. Consequently, no

structural data are currently available for the intracellular domains. Yet, the truncated ion channels were still functional except for the first ASIC structure[6], and all engineered proteins were amenable to crystallization. For the ΔzfP2X4 receptor, the structure was solved at 3.1 Å in the absence of ATP in a closed, presumably resting state[7], while ΔcASIC1 was crystallized at pH 5.6 and 6.5 in a non-conducting, presumably desensitized state, and crystals diffracted to 1.9 and 3 Å resolution, respectively[8]. More recently, ATP-bound ΔzfP2X4 receptor and ΔcASIC-toxin complexes with the gating modifier Psalmotoxin 1 (PcTx1) and the ASIC activator Mit-Toxin (MitTx) provided crystal structures of open channels[43-45]. The structures confirmed the anticipated similar subunit topology of a large ectodomain, two transmembrane α helices and intracellular N- and C-termini, and showed that P2X and ASIC channels are trimers. In agreement with secondary structure predictions[60], the ectodomain is mainly folded into β-sheets and to a lesser extent into α-helices, but contrary to previous models[60], there is a poor structural similarity in the folding of the ectodomain between ASIC and P2X receptor (Figure 1c). However, there is an interesting parallel in the scaffolding of each subunit. The central core of each monomer is folded mainly into β-sheets that are decorated by autonomous structural domains folded for some into α-helices known to be involved in agonist binding and channel gating. For P2X receptors, for which the structure resembles the shape of a dolphin, the body domain composes the central β-sheet core (Figure 1a). Attached to this domain are four distinct structural elements, the head, right and left flippers, and dorsal fin, which contribute directly or indirectly to ATP binding, as recently shown by the X-ray structure solved in the presence of ATP at 2.8 Å resolution[61]. Similarly to P2X receptors, the ECD of ASIC subunits, which resembles an up-right clenched hand, is composed of a central β-sheet core, the palm domain, surrounded by four distinct elements, the finger, thumb, β-ball and knuckle[6] (Figure 1b). Another interesting parallel resides in the transmembrane region of the channel, where the arrangement of transmembrane helices is similar, indicating that ASIC and P2X receptor adopt a similar fold of the pore (Figure 1c).

The crystal structures also revealed the presence of four highly electronegative cavities or vestibules along the three-fold axis of symmetry in both channels: the “upper”, “central”, “extracellular” and “intracellular” vestibules[62] (Figure 1d). The extracellular and intracellular vestibules are located immediately above and below the ion channel pore and represent most likely the main access route for conducting ions. The role of the upper and central vestibule is unclear, but the presence of cation-binding sites revealed in X-ray structures suggests that these cavities may contain regulatory sites that modulate channel function[7,44,45,63]. The

structures also reveal the presence for both channels of three wide windows or fenestrations at the interface of adjoining subunits just above the membrane that most likely constitute the main access route for ions to the extracellular vestibule (indicated by the asterisks in Figure 1d).

4. COUPLING OF EXTRACELLULAR DOMAIN TO CHANNEL GATES DURING ACTIVATION/DESENSITIZATION

Early studies identified critical amino acid residues of the ectodomain as important determinants for agonist action. From these studies, a general mechanism emerged, suggesting that ligand binding —ATP or protons— to extracellular sites induces the rapid opening of the channel. It should be borne in mind, however, that the fold of the ECDs differs significantly between ASICs and P2X receptors, and that the activating ligands are very different. ATP has one binding site per subunit, whereas pH changes are expected to affect the protonation status of several residues per subunit. Consequently, one may expect to find little conservation in the mechanism of activation. At the level of the agonist-binding sites, this seems to be case, but functional data revealed however that, unexpectedly, channel opening may share some common mechanisms.

For P2X receptors, ATP binds to three extracellular binding pockets shaped like open 'jaws" that are framed in part by the head and dorsal fin domains of two adjacent subunits[61] (Figure 1a). There is now functional[64,65] and structural evidence[61], supported by modeling[64,66], that ATP binding induces the closure of the open 'jaws'. By a flexing movement of the lower part of the body domain due to jaw tightening, the TM helices at the extracellular ends move outwardly. This outward motion, in turn, opens the channel (Figure 2a and b). Concomitantly, there is a significant separation of adjacent subunits at the lower part of the body domain, which considerably enlarges the fenestrations. Recent experiments have convincingly shown that ions gain access to the P2X pore through these extracellular lateral portals[63,67-69].

At variance to P2X receptors, there are multiple agonist binding sites in ASIC spread out over inter- and intra-subunit locations. The first crystal structure identified the "acidic pocket", a highly electronegative region that is formed by the thumb, β-ball and finger of each subunit and contains a network of acidic residues (Figure 1b). It was concluded that these acidic residues can only be so close to each other if some of them are protonated, and it was hypothesized that the acidic pocket is wider open at pH 7.4 when the channel pore is closed, and that the finger, thumb and β-ball move towards each other upon acidification, closing

thereby the acidic pocket. This conformational change in the acidic pocket would then be transmitted to the transmembrane domain, leading to opening of the pore[6]. However, the crystal structure of the closed state of ASIC is still unsolved, and consequently molecular motions of the acidic pocket during the closed-to-open transition are not known. Yet, available crystal structures of the open and desensitized states revealed no conformational change of the acidic pocket. Fluorescence approaches provided evidence for rapid conformational changes in several domains including the acidic pocket during ASIC activity[70,71]. Studies combining site-directed mutagenesis and functional analysis have clearly shown that several acidic residues of the acidic pocket are critical for the ASIC pH dependence of activation and desensitization[6,72,73]. These and other studies identified in addition Asp, Glu or His residues of the finger, the lower palm lining the central vestibule, and the "hinge" region just above the plasma membrane as critical for the pH dependence of activation[72-74]. This indicates that protonable residues in several domains contribute to ASIC activation and desensitization. Estimation of the pKa of such candidate protonation sites showed that the pKa values of several of these residues in the acidic pocket and the lower palm are in the range of ASIC gating by pH, suggesting therefore that these sites are proton sensors[73]. In addition, an intersubunit Asp-His pair in the extracellular vestibule (His73-Asp78) appears to be critical for ASIC activation[72]. Several studies have used modification by MTS reagents and functional analysis to show that the lower palm domain undergoes a closing movement around the central vestibule during desensitization[73,75-77]. This conformational change has been confirmed by the crystal structures[43,45] (Figure 2c and d). Likely this movement is transmitted to the TMDs where it induces pore closure during desensitization.

All these functional and structural data gathered from ASIC and P2X receptors suggest that during ASIC desensitization or P2X opening, there is a similar inward/outward movement of the TM domains that enlarges/constricts both the three fenestrations and central pore (Figure 2)[43,45,61].

5. A COMMON MECHANISM OF CHANNEL GATING?

The recent X-ray structures of non-conductive and open states revealed unexpected parallels in pore architecture of ASICs and P2X receptors (Figure 3a and b). Very recently, a study reported a new crystal structure of cASIC1 in the open state, solved at 2.1 Å and bound to the toxin MitTx, a pain-inducing toxin from the Texas coral snake[45]. Surprisingly, the structure revealed that TM2, initially interpreted as a continuous α-helix, is actually

interrupted at about two thirds of its length by the sequence Gly-Ala-Ser that consequently splits TM2 into TM2a and TM2b (Figure 3d). The Gly-Ala-Ser motif forms an extended horizontal structure that reaches over to an adjacent subunit and continues the TM2 of the adjacent subunit as a α helix. Re-analysis and refinement of the cASIC1 desensitized state structure[8] based on this new information indicated that a similar discontinuity of TM2 was also present. In contrast to these two structures, the TM2 helices were continuous in the first, desensitized[6] and the open PcTx1-complexed structures[43]. Currently it is not clear which of these structures comes closer to the real ASIC pore, and these new data clearly point out the limitations of crystallographic studies. Since the MitTx-ASIC1 structure has a high resolution, is symmetrical and compatible with known ASIC functions, the pore with discontinuous TM2 may be favored. The α-helical structure is not much changed by the observed swap, since the interrupted TM2 in two of the structures has a very similar conformation as the continuous TM2 in the other structures. The Gly-Ala-Ser motif forms in all open ASIC structures the narrowest part of the pore. In the structures with discontinuous TM2, the extended Gly-Ala-Ser motifs of the three subunits form however as a new structural element a horizontal belt lining the pore.

Despite the presence of the swap in ASIC, there is still a high similarity between ASIC and P2X channels in the architectural organization of the channel pore, in both non-conducting and open states (Figure 3a and b). In both channels, the pore is lined primarily by residues contributed by the TM2, and the channel gate is located above the narrowest part of the pore[43,45,61], in agreement with gated accessibility studies[78-81]. In the ATP-bound P2X structure, the pore is expanded and there are practically no intersubunit interactions within the membrane, leading to the formation of large crevices between the subunits[61]. The physiological relevance of these crevices is unclear, but a recent molecular modeling study suggested that lipid molecules can diffuse through these intersubunit crevices to occupy and dehydrate the ion permeation pathway, raising the possibility that they are not present in native membrane-embedded receptors[82]. A similar organization of the transmembrane domain is observed in the PcTx1-ASIC1 structure obtained at pH 7.25. In contrast, a narrower open pore with the presence of intersubunit interactions was observed in the PcTx1-ASIC1 structure obtained at pH 5.5, and in the MitTx-ASIC1 structure[43,45], suggesting that these crevices are much less pronounced in ASICs as compared to P2X.

Regardless of the presence of these membrane crevices, the structural similarity between ASICs and P2X receptors is further supported by recent functional data showing that the gating mechanism might be similar, at least in some part of the channel. First, bridging

experiments with a light-sensitive azobenzene molecule covalently attached at two adjacent cysteine residues (P329C in the P2X2 receptor or G430 in hASIC1) mimicked agonist action and provided evidence that pore opening requires substantial lateral movements of the outer parts of the transmembrane domains (Figure 3a and b). Second, introduction of methanethiosulfonate (MTS) or azobenzene-containing reagents in the same region of the pore in both ASIC and P2X receptors, directly opens the channel without agonist binding[83-86].

In the deeper transmembrane part of the channel, however, the functional and structural similarities seem limited (Figure 3c and d). For P2X receptors, there is no evidence suggesting a swap between TM2 helices, although current X-ray data were solved at best at 2.8 Å, and it remains possible that data acquired at higher resolution will refine the structure of the inner part of the channel. However, if it were so, this would not be compatible with a recent study showing interactions between TM1 and TM2 residues from the same subunit[82] at the level of the inner part of the channel pore (intra-subunit interactions, see Figure 3c), since a swap conformation would predict rather interaction between TM1 and TM2 from adjacent subunits as observed for ASIC (inter-subunit interactions, see Figure 3d).

Taken together, these data suggest that there is, at least at the apex of the transmembrane helices, a functional and structural parallel in the gating mechanism between ASICs and P2X receptors (Figure 4). But for the following reasons, these parallels are limited. First, as already stated, the functional transitions from which comparison is made differ from both channels (closed-to-open for P2X and open-to-desensitized for ASIC). Second, the presence of the swap in ASICs that seems absent, so far, in P2X receptors suggests that ion selectivity mechanisms and conformational changes in the intracellular part of the transmembrane helices may be different for both channels. Additional experiments, including new crystal structures of desensitized and closed states for P2X receptor and ASIC, respectively, are needed to challenge the idea that P2X receptors and ASICs do share a real common gating mechanism.

6. MECHANSIM OF ION PERMEATION

Do the crystal structures of open states that are available for both channels provide a plausible mechanism for ion conduction in trimeric ion channels? The most narrow part of the ion pore is in both channels located at approximately two thirds down into the membrane with a pore diameter of ~7 Å for P2X and ~4 Å for ASIC, consistent with their different ion selectivities (Figure 5)[43,45,61]. There is still a lack of understanding of the mechanisms by

which P2X receptors discriminate between cations and anions, but functional data accumulated over two decades have identified several amino acid side chains that are important for permeation of Na^+, K^+ and Ca^{2+}[87]. As these residues are located throughout the permeation pathway, there is apparently no single narrow locus that controls ion permeation, but instead, ion selectivity is achieved by contributions of different determinants along the channel pore[78-80,87]. In contrast, the Gly-Ala-Ser motif that forms in all ASIC structures the narrowest part of the pore is critical for ASIC ion selectivity and conductance[81,88]. The homologous Gly/Ser-x-Ser motif had previously been shown to form the selectivity filter in ENaC and to distinguish among different ions by molecular sieving[89-91]. This indicates that ASICs and ENaC select ions at the narrowest part of their pore, although residues at other levels of the pore can also affect ion selectivity[46,58]. The pore diameter at the selectivity filter of the PcTx1-ASIC1 structure obtained at pH 5.5 showing a continuous TM2, as well as that of the MitTx-ASIC1 structure with discontinuous TM2 is compatible with a Na^+-selective or Na^+-preferring pore and indicates that ASICs transport hydrated Na^+ ions. The presence of coordinating residues in the Gly-Ala-Ser sequence, which adopts a belt-like conformation in the MitTx-ASIC1 structure, nicely explains the hypothesized permeation mechanism. ENaC has interestingly a 10- to 100-fold higher Na^+/K^+ selectivity than ASICs in spite of the conserved selectivity filter motif, indicating that ENaC transports completely dehydrated Na^+ ions and consequently the ENaC pore must be narrower.

The crystal structures of the open state in ASIC were solved in the presence of bound toxins, and it still remains unknown whether they correspond to the conformation of the pore that is opened by H^+ binding. There is no sequence similar to the Gly-Ala-Ser motif in the TM domains of P2X receptors, and it would be interesting to challenge the idea that the occurrence of such a sequence may swap both conformation and selectivity properties.

7. SIMILARITY IN PORE DILATION PROPERTIES

In some circumstances, some ASICs and P2X receptors display dynamic selectivity of their pores. For P2X receptors, this process, known as pore dilation, takes place only for P2X2, P2X2/5, P2X4 and P2X7 receptors, and is defined by a progressive increase in permeability to large organic cations, such as NMDG and propidium dyes[92-94]. The underlying mechanism remains unclear, but several lines of evidence, including data obtained with atomic force microscopy, support the idea that pore dilation is an intrinsic property of P2X channels[94] and may be related to pain signaling[95]. However, pore dilation was never observed at the single channel level[21,96] and, so far, there is no available X-ray structure of a

dilated P2X pore. ASICs also show dynamic behavior of their ion selectivity. In some ASICs displaying a sustained current, the transient current component is Na^+-selective, whereas the sustained current component is carried by both Na^+ and K^+[48]. Amiloride is a known pore blocker of ENaC and binds in ENaC and ASICs to a residue of the pore-forming TM2[97,98]. Amiloride inhibits the transient component of ASIC currents, but not the sustained component of ASIC3[99,100], suggesting that the conformation of the pore is different between transient and sustained currents. In the presence of high PcTx1 concentrations the cASIC1 current at pH 5.5 was Na^+-selective and inhibited by amiloride, while at pH 7.25 a non-selective and amiloride-insensitive cation current was observed[43]. When crystals were grown in these two conditions, the structure obtained at pH 5.5 showed a narrow, asymmetrical but clearly open pore, whose diameter at the selectivity filter was close to that of the MitTx-ASIC1 structure. In contrast, the pore in the pH 7.25 structure was considerably wider (pore diameter of ~10 Å)[43] (Figure 4). This suggests that a pore dilation may occur in the transition of the transient to the sustained current in certain ASICs, although the relevance of the dilated PcTx1-ASIC1 structure is uncertain in the light of the MitTx-ASIC1 structure that shows a different organization of the inner half of the TM2. As ASICs and P2X share some functional and structural parallels in channel gating, it remains possible that the molecular mechanism underlying the transition from the regular open state to the dilated state is similar in both channels (Figure 4). Further experiments are needed to clarify this issue.

8. FUTURE DIRECTIONS

Structural comparison between ASICs and P2X receptors reveals common aspects in their architecture, such as a similarity of the helix arrangement in the TMD, a common organization of the scaffold core in the ECD, the presence of vestibules along the three-fold axis of symmetry, and an enlargement of the three large fenestrations in the open channel states. Several important questions remain, but one that is of particular interest is why P2X receptors and ASICs, which are genetically unrelated, adopt a similar fold in the TMD and inner scaffold of the ECD. Given that there are no significant sequence similarities, it seems likely that P2X receptors and ASICs originated from two distinct ancestral genes and consequently their sequences have evolved independently, but that the structures converged in the TMD to create efficient, functional trimeric ion channels. This hypothesis is further supported by recent experiments using MTS[84,85] or light-sensitive azobenzene reagents[83,86] showing that the functioning of these channels seems quite similar, at least at the apex of the TMD. A relevant question to these data is whether or not the movement induced by agonist

binding to the ECD, for which the fold is different between P2X receptor and ASIC, converges at the level of the extracellular ends of TM2, which in turn induces similar motions to open the pore. One way to challenge this idea would be to create functional chimeras made between the ECD of one channel genetically fused to the TMD domain of another. Another interesting question is related to the principle of ion selectivity in trimeric ion channels. Why do some channels discriminate between cations, whereas others do not? What is the contribution to ion selectivity of local residues of the pore vs. that of residues determining the overall scaffold of the TMD? Additional experiments, including for example swapping of short sequences, may help elucidate this intriguing question. Another important question is related to the dynamic properties of ion permeation that are shared by both channels. Further experiments, including new crystal structures of dilated states, will certainly provide new insight into the molecular mechanism of ion selection in the multiple open states that harbor distinct permeation properties. Finally, it is known that the cytoplasmic parts of P2X receptors and ASICs play important functional roles, however, their 3D structure, as for the cytoplasmic parts of many other transmembrane protein crystals, is not known. Recently a high resolution structure of the TRPV1 channel, including its cytoplasmic parts was obtained by cryo-electron microscopy[101]. The identification of the 3D structure of full-length P2X receptors and ASICs would obviously be extremely useful.

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Figure legends

Figure 1. Crystal structures of ΔzfP2X4 receptor in a closed state (PDB: 4DW0) and of ΔcASIC1 in a desensitized state (PDB: 4NYK). Lateral view of the trimers, depicted in cartoon representation, where one subunit is highlighted in blue for P2X (a) and green for ASIC (b). Also represented are the different domains and location of the agonist-binding sites (ATP for P2X receptor and H^+ for ASIC). For clarity, only one of the three ATP sites is shown. Arrowhead in (a) indicates the dorsal fin from an adjacent subunit. (c) Overlay of the structures based on superposition of the respective TM2 helices. (d) Location of the different vestibules for both channels. Vertical section across the center of ΔzfP2X4 receptor (blue) and ΔcASIC1 (green) represented by surface model. Asterisks indicate one of the three lateral fenestrations. Approximate position of the membrane is also shown. ECD: extracellular domain; TMD: transmembrane domain.

Figure 2. Lateral views of the crystal structures of ΔzfP2X4 receptor (in blue) in a closed (a) (PDB: 4DW0) and ATP-bound (red spheres), open state (b) (PDB: 4DW1) and of ΔcASIC1 (in green) in an open (c) (PDB: 4NTW) and desensitized state (d) (PDB: 4NYK). Black arrows indicate protein motions relevant to the transition. Also indicated is the location of one of the three lateral fenestrations and of the proton binding sites in ΔcASIC1 open state (residues H74/D79; E80/E417; D238/E239/D346/E354 in the wrist, the palm and the acidic pocket are colored in red).

Figure 3. The transmembrane region of ΔzfP2X4 receptor (blue) and ΔcASIC1 (green). (a) Superimposed structures viewed from the extracellular side in non-conducting states (resting, closed state for P2X receptor (4DW0) and desensitized state for ASIC1 (4NYK)). Yellow doubled arrow indicates the distance separating two adjacent P337 (homologous to P329 in rP2X2) and G429 (homologous to G430 in hASIC1) residues in ΔzfP2X4 and ΔcASIC1,

respectively. (b) Same view as in panel A of ΔzfP2X4 receptor (PDB: 4DW1, blue) and ΔcASIC1 (PBD: 4NTW, green) in open states. Lateral view of ΔzfP2X4 receptor (c) and ΔcASIC1 (d) in open states. Blue spheres indicate positions in which a Cd^{2+} binding site has been engineered between TM1 and TM2 from the same subunit in the P2X2 receptor[82].

Figure 4. Cartoon summarizing the architecture and functional parallels between P2X receptors (within blue box) and ASICs (within green box). View of the TMD from the extracellular side of each channel in different functional states. a: +ATP, pH 8.0; b: -ATP, pH 6.5; c: +MitTx, pH 5.6; d:–MitTx, pH 5.6; e: +PcTx1, pH 7.25; f:–PcTx1, pH 5.5. Transitions e and f also involve unbinding and binding, respectively, of MitTx. Interrogation mark indicates putative transitions. Note that "GAS" sequence in ASIC appears as "SAG" when viewed from the extracellular side. Note that this figure is not a kinetic scheme of P2X and ASIC gating and that other transitions than those shown in this figure also occur.

Figure 5. View from the intracellular side of crystal structures of (a) ΔcASIC1 (PBD: 4NTW) and (b) ΔzfP2X4 receptor (PDB: 4DW1) in open states. Note the difference in the size of the open pore.

Figure1

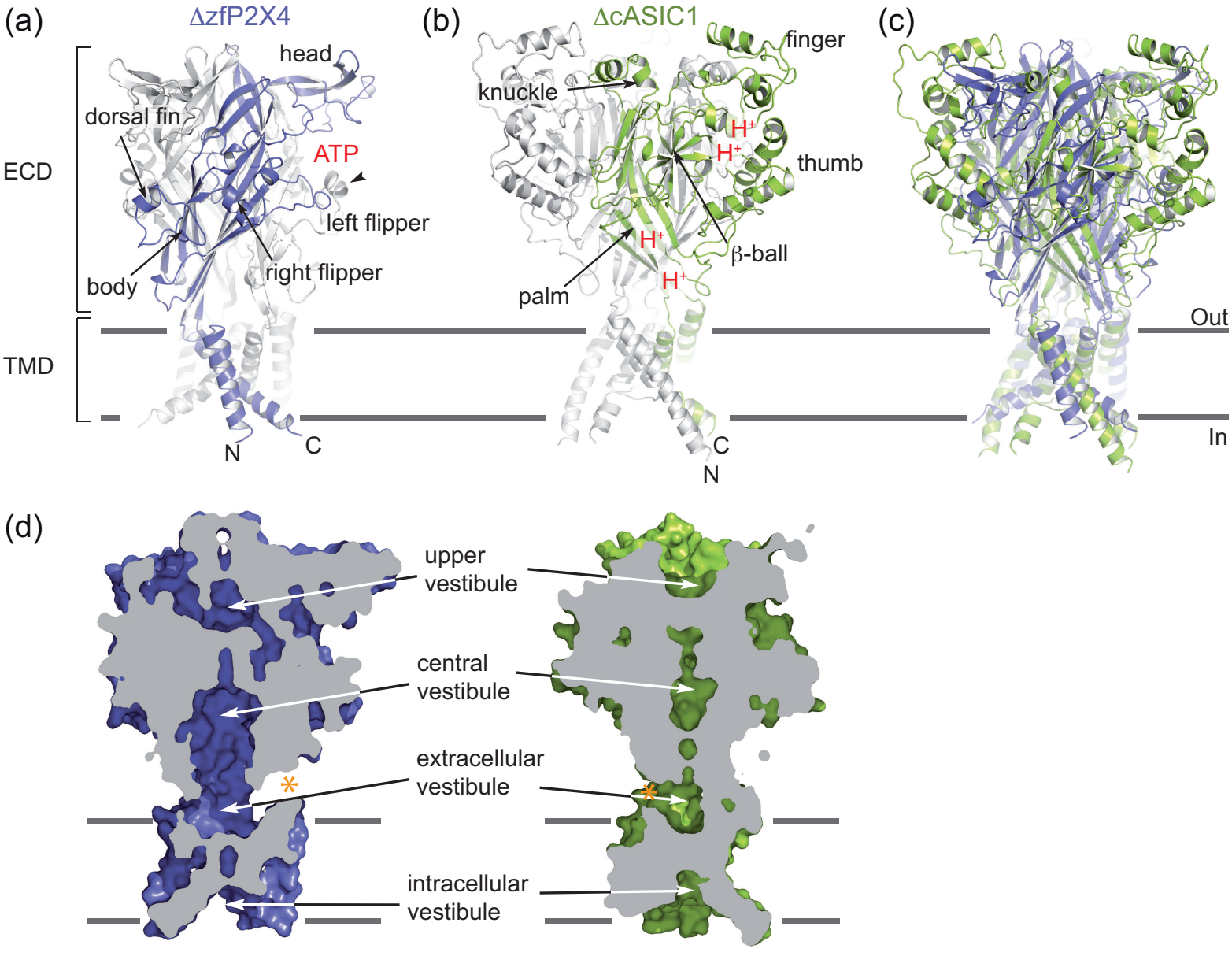


Figure 1

Figure2

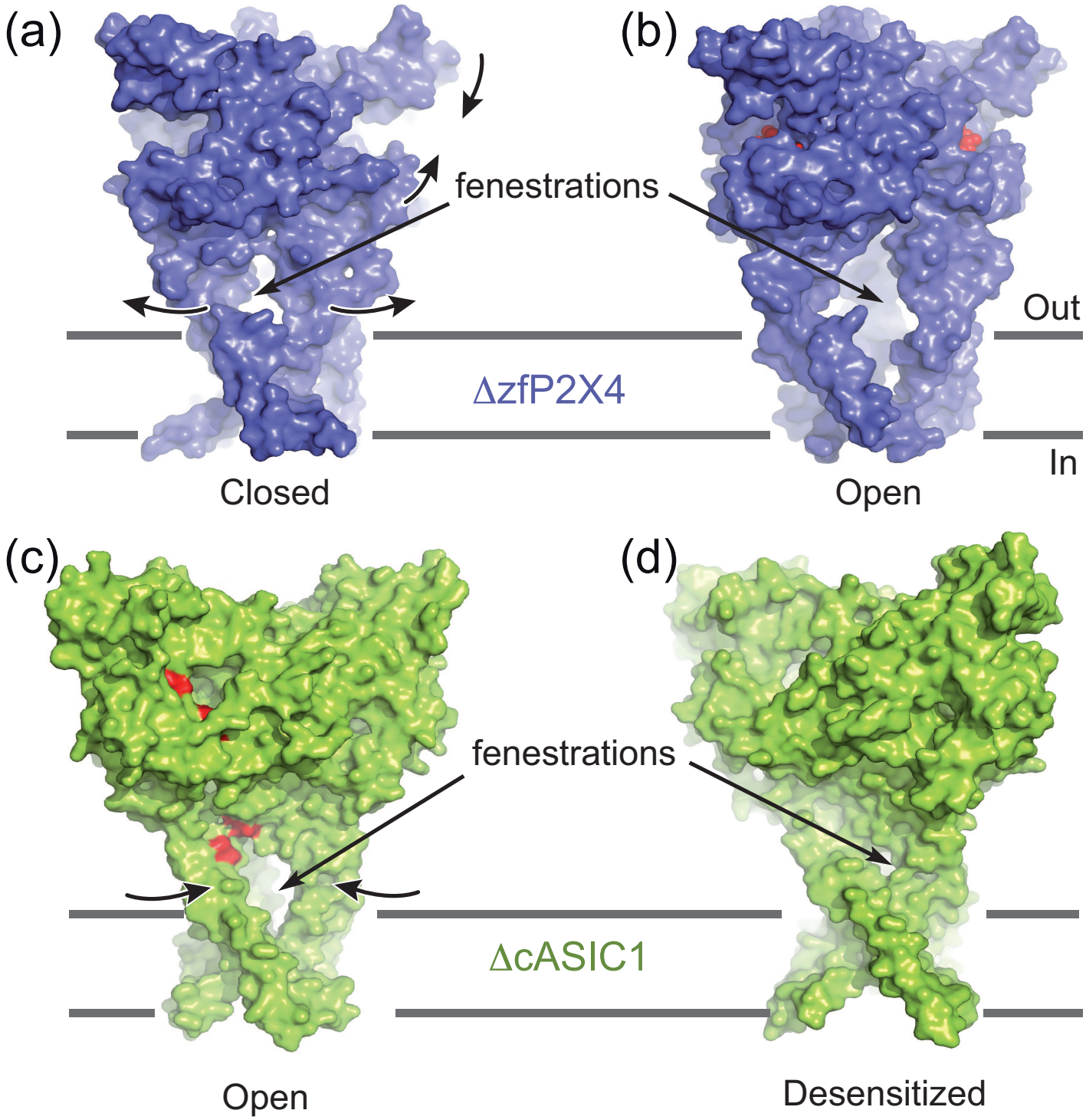


Figure 2

Figure3

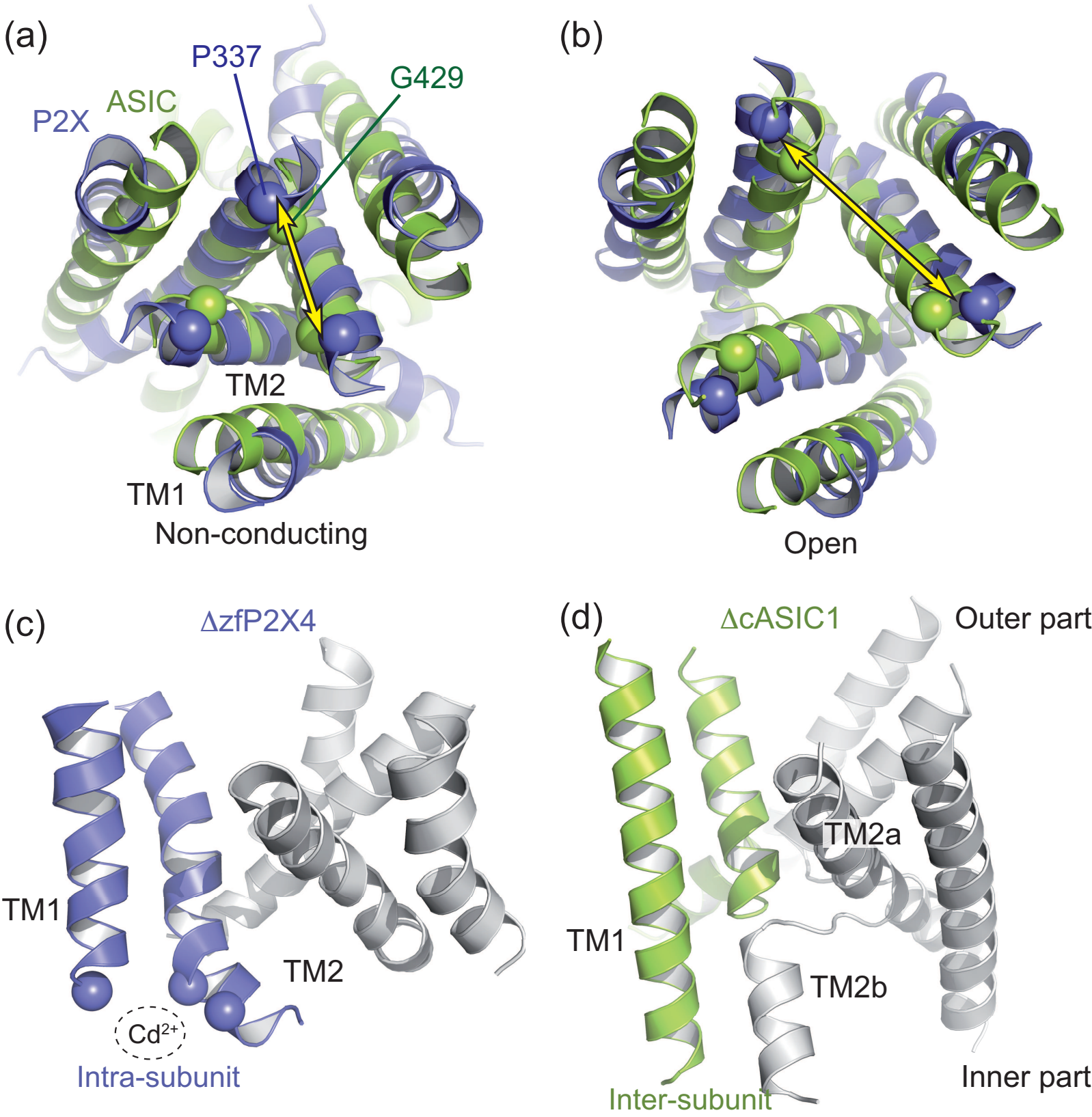


Figure 3

Figure4

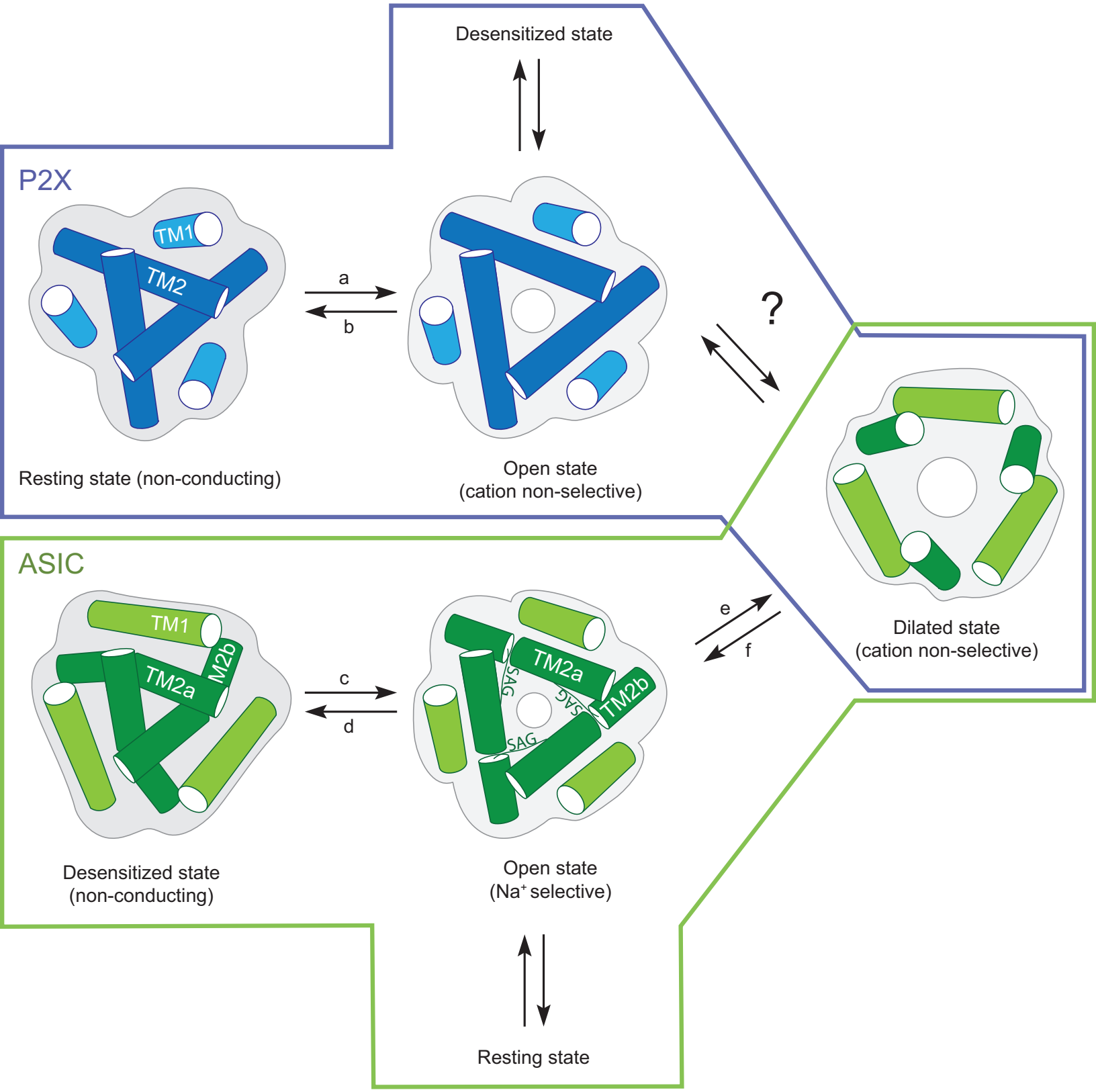
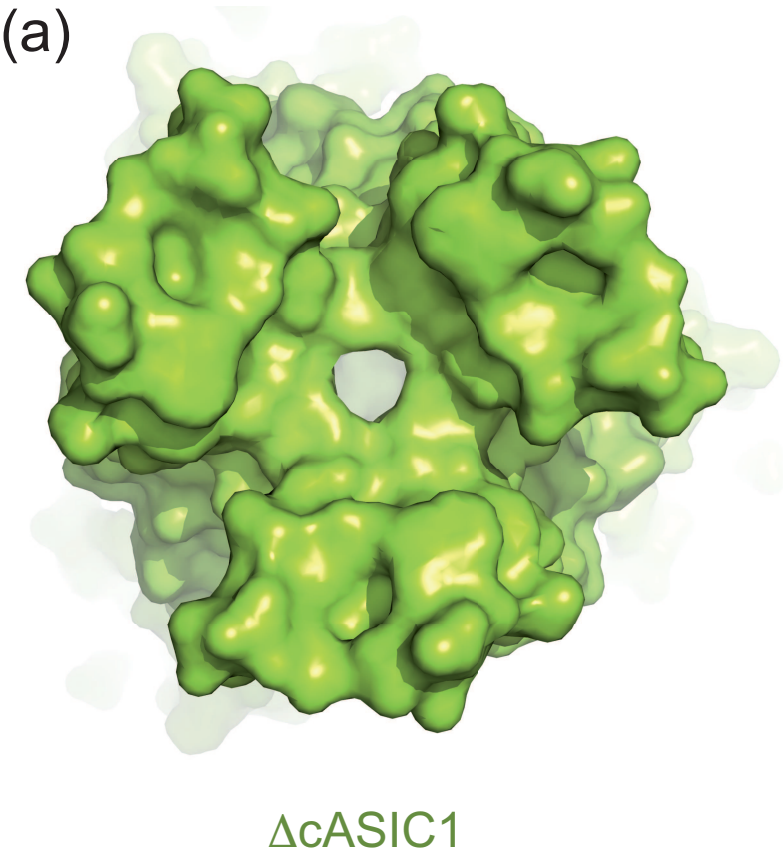


Figure 4

Figure5



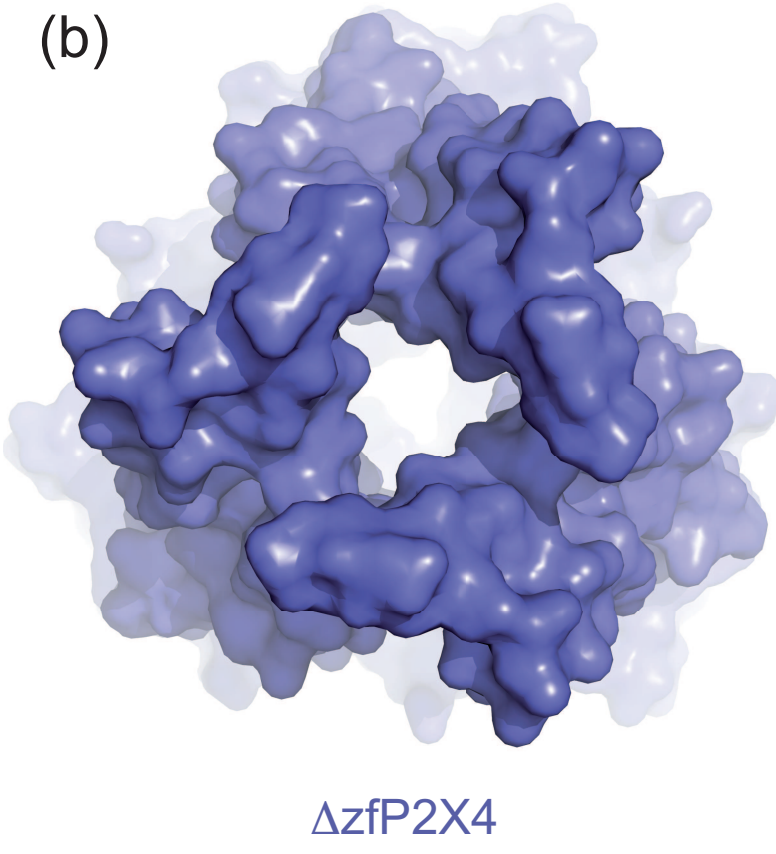


Figure 5