Spectator no more, the role of the membrane in regulating ion channel function

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Abstract

A pressure gradient across a curved lipid bilayer leads to a lateral force within the bilayer. Following ground breaking work on eukaryotic ion channels, it is now known that many proteins sense this change in the lateral tension and alter their functions in response. It has been proposed that responding to pressure differentials may be one of the oldest signaling mechanisms in biology. The most well characterized mechanosensing ion channels are the bacterial ones which open when the pressure differential hits a threshold. Recent studies on one of these channels, MscS, have developed a simple molecular model for how they sense and adapt to pressure. Biochemical and structural studies on mechanosensitive channels from eukaryotes have disclosed pressure sensing mechanisms. In this review, we highlight these findings and discuss the potential for a general model for pressure sensing.

Introduction

Membrane proteins, by definition, function in combination with the lipid bilayer. The degree to which they are embedded within the lipid membrane ranges from fully embedded (spanning the entire bilayer) to associated (usually by an anchor such as a short peptide sequence or fatty acid modification). The region in contact with the bilayer is called transmembrane domain and the structure of this region must have co-evolved alongside the lipid bilayer, a very different environment from water. Ion channels are a key class of integral membrane proteins, which connect the cell's inner world with the wider world outside. Cell survival requires that nutrients enter but toxins are blocked and waste exits, whilst metabolites are retained. This in turn requires that the cell is able to control when channels are open or closed and what kind of molecules they allow to pass. The opening and closing of ion channels are known to be regulated by defined stimuli such as ions, ligands, peptides and pH. Often the membrane was treated like water to be little more than milieu in which the ion channel functioned, its role being to stabilize and localize the protein structure, with the lipid molecules often considered as bystanders. Although true in general, there were examples of lipid binding modifying protein behavior, for example phosphatidylinositol 4,5-bisphosphate binds K^+ channels and alters their function[1].

A change in the perception of lipids as actively regulating protein function came from the study of a pair of bacterial mechanosensitive channels, the heptameric mechanosensing channel of small conductance (MscS)[2,3] and the pentameric mechanosensing channel of large conductance (MscL)[4,5]. These proteins are controlled by changes in the lipid bilayer that arise from increased pressure inside the cell (known as turgor). In this review, we discuss recent progress in the structural

understanding of the role of lipids play, in regulating a wide range of ion channels. We highlight the differing molecular models that have emerged to rationalize this behavior and develop the suggestion by Kung and co-workers, that sensing the pressure differential across lipid bilayer is amongst the earliest evolutionary events in channel regulation[6].

Bacterial mechanosensors MscS and MScL: open and shut stories

Mechanosensitive channels open and close in response to changes in the cell turgor pressure. The family of mechanosensing channels are not confined to bacteria there are many examples from higher organisms, which do not share any sequence homology with the bacterial ones but fill important roles including K^+ transport[7,8] and mechanotransduction [9,10]. However, the bacterial systems are the most well studied.

In bacteria, the opening of the channels occurs at defined pressures and allows the rapid efflux of ions, solute and small molecules reducing the turgor pressure, thus protecting the cell against hypo-osmotic shock[11]. The increased turgor pressure manifests itself as lateral tension within the bilayer and it is for this reason the proteins are also known as stretch channels (Fig 1a). The gating of mechanosensitive channels is controlled by the lipid bilayer itself. Interestingly, both MscS and MscL proteins can also be opened by addition *lyso*-phospholipids (*lyso-PC*). The original open[12] and closed[2] crystal structures of the heptameric MscS protein revealed that the protein undergoes a very significant re-organisation of the transmembrane helices (Fig 1b) when it opens. Upon gating of MscS, the first two helices (TM1/2) pivot by a 1/7 of revolution and a central pore of over 14Å diameter is created by movement of

the seven TM3a (central pore) helices (Fig 1c). The pore helix is connected by a kinked region of structure to final transmembrane region, TM3b, which sits almost parallel to the membrane bilayer unlike TM1, 2 and 3a, which are approximately perpendicular to the membrane. Recent studies on MscS have shown that mutations on highly conserved residues on TM3b altered gating kinetics[13]. A cryo-EM structure of the presumed MscS homologue YnaI confirms structural conservation of the TM helices within the MscS family[14].

MscL gates at a higher pressure than MscS but as the its name implies (large conductance) it creates a larger pore. Interstingly increased expression of MscL was recently found to increase steptomycin effectiveness against bacterial cells[15] suggesting the protein maybe a future drug target. Indeed, it is now known, that streptomycin binds, opens and passes through MscL [16]. The activation of MscL in droplet interface bilayers[17] may herald novel nano-technological applications of the protein. The closed crystal structure of pentameric *M. tuberculosis* MscL [4] has been known for several years. Native mass spectrometry of MscL suggested the *in vitro* oligomerisation state (pentamer vs tetramer) is influenced by temperature, detergent and amino acid variability as well as by the precise construct used [18]. Protein stability has been shown to be influenced by the presence of particular lipids[19]. The variety of oligomeric states and their sensitivity to lipidation may explain the challenges in obtaing a structure of a fully open form. Consequently a variety of biophysical and spectroscopical tools were used to probe gating. FRET [20] measurements of *E. coli* MscL opened by addition of *lyso-PC* estimated a pore with over 25Å in diameter and favoured a helix-tilt gating model[20]. A combination of cwEPR, electrophysiology measurements and computational studies highlighted the

importance of the N-terminal amphipathic S1 helix on MscL gating[21] (Fig 2a), a region identified to interact with lipids[22]. Most recently two crystal stuctures of an archeal MscL orthlogue[23] revealed two states, one closed (pore diameter around 4Å) and the other at least partially opened (pore diameter around 8Å). The protein was crystallised as a fusion with another protein[23], however the two structures constitute a snapshot of the TM helices transit during gating (Fig 2a).

Enter the lipids

The scope of pressure sensing and thus the role of lipids in regulating membrane protein function was revolutionized by the discovery that voltage-dependent potassium (Kv) channels exhibit exquisite sensitivity to changes in the pressure differential across the cell membrane[24]. Pressure sensing behaviour has now been demonstrated for other very different eukaryotic channels TRAAK and TREK1[25]. It is important to note, that these proteins do not belong to the eukaryotic mechanosensors. Despite the lack of sequence conservation and structural homology, the biophysical property of mechanosensation represents a functional bridge between the two kingdoms of life. The structure of TRAAK revealed that the binding of lipid acyl chain resulted in structural changes which regulated the channel[8] (Fig 2b). Further evidence for lipid involvement in function came from the structure of the pore forming toxin[26]. The TM pore of the functional toxin was formed by a combination of protein and lipids, with lipids playing a cofactor role.

The structures of MscS (closed and open) were critiqued, as the transmembrane helices were not closely packed. Rather, large voids were observed amongst the helices, most notably between the outer face of TM3a and the inner face TM1/2 (Fig 1b,d). Since such voids are not physically plausible and in molecular dynamics calculations collapse, various explanations for their presence in the structures were advanced including artifacts due to detergent extraction or crystal packing. Notably such voids were absent in cwEPR[27] and molecular dynamics[28] models of gating, both of which proposed very different protein re-arrangements (from each other as well as the crystal structure)[29].

The structure of MscS was re-evaluated with PELDOR (DEER) spectroscopy, a pulsed-EPR methodology that allowed an independent test of the competing MscS structural models both in detergent[30] and in bilayer mimics[31]. These studies confirmed the arrangement of helices seen in the crystal structures and the "voids". The same helical arrangement and voids have now been seen in other crystal structures from other labs, including those from different organisms [32,33]. The "void conundrum" was resolved with significantly higher resolution x-ray study of *E. coli* MscS, which identified endogenous lipid acyl chains (or detergent alkyl chains) packing between the helices[34] (Fig 1d). This was the first experimental evidence that rather than voids, lipid binding pockets sit between the helices. Molecular dynamics showed that with lipids filling the pockets, the structure was stable but the open form of the channel needed less lipid than the closed form. Analysis by both native and lipid mass spectrometry showed PE 14:0/14:0 and PE 16:1/14:0 lipids from *E. coli* were associated with the purified protein[34]. Biophysics showed that the lipids were in rapid exchange with the bulk bilayer. Although *lyso-PC* did open the channel in single channel experiments [34], the open form had a different conductivity to that observed pressure (patch clamp) stimulus. *Lyso-PC* was proposed to fill the pockets less efficiently than *E. coli* lipids and thus destabilise the closed form (i.e. *lyso-PC* does not act to deform the membrane bilayer rather it acts by a direct interaction with the protein). Studies of MscS in different lipid environments (giant spheroplasts and liposomes) revealed difference in behaviour of the channel[35]. A study on MscL, has also observed that opening by *lyso-PC* differs from tension-induced gating[36], suggesting *lyso-PC* may interact with MscL.

How is pressure sensed?

MacKinnon *et al* have outlined a general mechanism for tension sensing, the increase in cross sectional area of the protein results in work done, a well-established and theoretically sound model, based on the expansion of a circle in a plane. Thus, the lateral tension of the membrane is a crucial component of the energy of gating. This simple model fits well with the TRAAK K^+ channel[8]. However, MscS does not conform to this simple model nor to a more advanced model which includes a three dimensional membrane[37]. The overall diameter of MscS does not change [34]; rather it is the volume of the lipid binding pockets that shrinks, upon opening, by an amount equal to approximately 1 lipid molecule per subunit. We proposed a model in which, the equilibrium position of lipids bound in the protein pockets against being in the bilayer, determines which conformation state is most stable (the closed state needs more lipid than the open state)[34].

Under tension, the equilibrium position of the lipid (bilayer or bound to protein) changes and this affects the relative stability of the open and closed structures. This model of pressure sensing is both simple and general; any protein which binds lipids in pockets, if the volume of the pockets (hence the amount of lipid) changes during protein function, then protein function will be sensitive to the membrane bilayer tension. In such a model lipids move first (or at the same time as protein), the protein adjusts its conformation in response to the lipid "availability" [34]. Such a model does not require specific lipid protein interactions, thus the residues that form the pockets do not need to be conserved; as is observed for the MscS superfamily. The model does not require the lipids to "pull" the structure into a new conformation, clearly unlikely for MscS, given the rotational nature of the opening. The specific

interactions observed in TRAAK[8] system can be seen as a special case of the general model (Fig 2b).

The role of a "horizontal" helix in mechanosensation

Although the pockets or binding grooves vary between structures there is an amphipathic helix common to all systems. The helix sits parallel to the membrane plane, lying at the interface between the lipid bilayer and the plasma membrane at the lower leaflet of lipid bilayer (Fig 3). The helix has been shown to be important to function in MscS[13]. The "horizontal" helix has no conservation in sequence or length (Fig 3). This helix radiates outwards (almost 90^0) to the central pore in MscS[34], MscL[4] and TRPV1[38] channels. In TRAAK[8] an acyl chain from a lipid obstructs the ion conduction pathway (Fig 3C). In the cartoon model of the EM structure of Piezo1 (adapted from [39]) (Fig 3D) a horizontal helix is seen between cytosol and TM region. We suggest the helix plays two roles. The first is a structural anchor around which the conformational change occurring on gating pivot. The second role is to form the lipid binding pockets that sense pressure[34].

A universal mechanism for pressure sensing

The general model of reversible binding of lipid and preferential structural stabilisation is potentially widely applicable. The model suggests that it is the packing of the headgroups that determines the efficiency of lipid filling of the inter-helical pockets (Fig 4A and B). From an evolutionary point of view, the lack of very specific protein lipid interactions would be beneficial as it avoids both protein and lipid having to change at the same time.

In eukaryotes, it seems likely that mechanosensitivity would also arise from bilayer tension as opposed to forces originating from the extracellular matrix or the cytoskeleton. The dependence on the bilayer tension has been demonstrated for Piezo1[40]. Therefore, the suggested model could constitute a potential unification mechanism for all ion channels found to mechanosense and perhaps not only limited to this class[34].

Linking mechanosensing and ligand-gating

The triggering of MscS by *lyso-PC* [34] (Fig 4C) can be seen a prototype of ligand (Fig 4D) gating. The vanilloid agonist (resiniferatoxin, RTX) [10] and capsaicin [41] both have lyso-PC like acyl chains that bind within the TM region of the TRPV1 channel (akin to MscS [34]) activating it [10,41]. Norfluoxetine, the active metabolite of Prozac is very hydrophobic and binds within the Tm region modulating TREK-2 channel [7]. Yoda 1(2-[5-[[(2,6-dichlorophenyl)methyl]thio]-1,3,4-thiadiazol-2-yl]pyrazine), a molecule, which acts as an agonist for the Piezo1 eukaryotic mechanosensitive ion channel[42], binds to an unknown location.

In a thought provoking perspective article, Kung *et al* suggested that sensing and responding to pressure may be the original stimulus for ion channel firing[6]. Inspired by this we speculate that MscS represents a starting point for ion channel regulation, essentially non-specific lipid filling of pockets regulated by membrane tension. TRAAK[8] represents the intermediate case with specific binding of a lipid to regulate function. The final stage in evolution of gated ion channels is the binding of non-lipid molecules to regulate function.

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Figure legends

Fig 1. Pressure sensing and mechanosensitive protein of small conductance (MscS).
(a) A pressure gradient across a lipid bilayer will lead to a lateral stretching force within the bilayer. Mechanosensitive channels open in response to a pressure differential across the lipid bilayer allowing ions and solvent to flow through them.
(b) MscS has three TM helices and these are identified in a cartoon diagram. TM1 and TM2 undergo a large rotational movement upon gating, TM3b moves outwards from the central axis and thereby creating an open channel. One monomer is shown in order to illustrate the movements that occur. All figures which contain structural models have been created using QtMG (CCP4 routine)
(c) A surface view of the closed and open states of MscS viewed from periplasm.
(d) The TM helices of MscS are not close packed and creating pockets between them, In a recent study these pockets were found to bind *E. coli* lipids. The figure shows tha alkyl chains identified in the crystal structure.

Fig 2.
(a) MscL has two TM helices, which are labeled. The closed and expanded structures of MscL are shown. The opening involves both tilting and rotation of the TM helices.
(b) The TRAAK ion channel is now known to be mechanosensitive. The closed and open states of the channel are shown with K^+ ions as dark cyan balls. And the acyl chain as purple spheres.

Fig 3. The effect of TM pocket filling on ion channel structural state.

(a) Lipids (two acyl chains per headgroup) efficiently fill the empty hydrophobic pockets and the channel adopts a closed state.

(b) Upon increase of lateral tension pocket lipids are moving into the bulk bilayer, the channel senses this change and adopts an open conformation as a respond to this.

Fig 4.
A helix perpendicular to the membrane linked to helix parallel to the membrane may be a common feature of pressure sensing proteins. Cartoon representation of TRPV1(a) and Piezo1(b) monomers depicted in golden colour with the helices shown in cyan. The cartoon model in (d) is adopted from [39].

Fig 5.

Lyso-PC (one acyl chain per headgroup) binds in a non-specific lipid pocket and destabilizes the closed MscS structure mimicking pressure. We speculate that many channels have evolved from this simple ‘lipid binding’ pressure sensing to specific ligand triggering.

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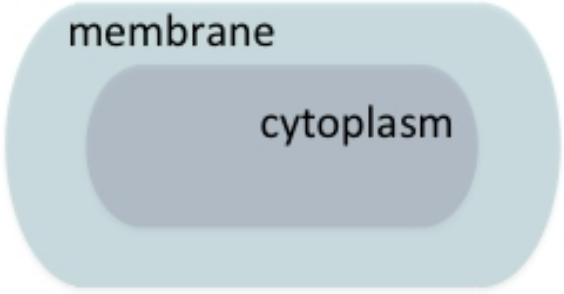
1 Ion channels are central to biology and are found in all domains of life.

2 With a few exceptions, the lipids that form the membrane were not thought to directly regulate the function of ion channels.

3 Mechanosensing ion channels are controlled by forces within the membrane bilayer. Recently these forces were shown to regulate many other ion channels.

3 Structural and functional data show that membranes, more accurately the lipid molecules that form the membrane, play key roles in the function of many eukaryotic and prokaryotic ion channels.

5 An evolutionary link between pressure sensing and ligand gating is proposed.



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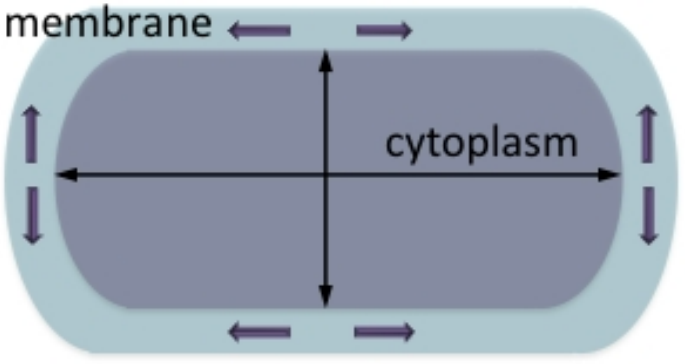

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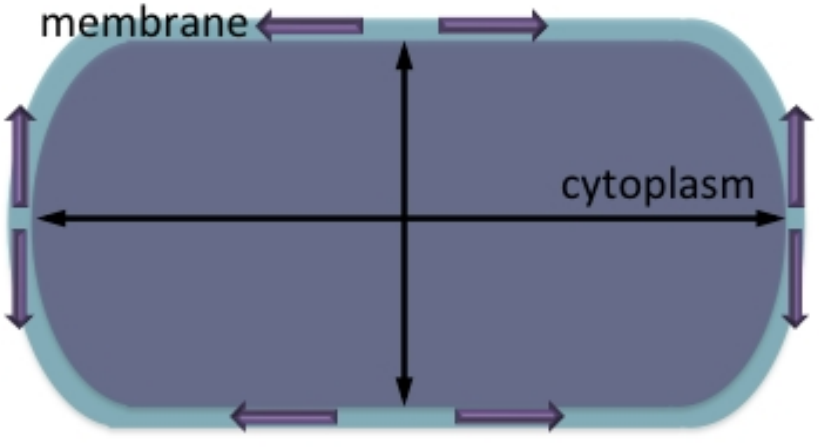

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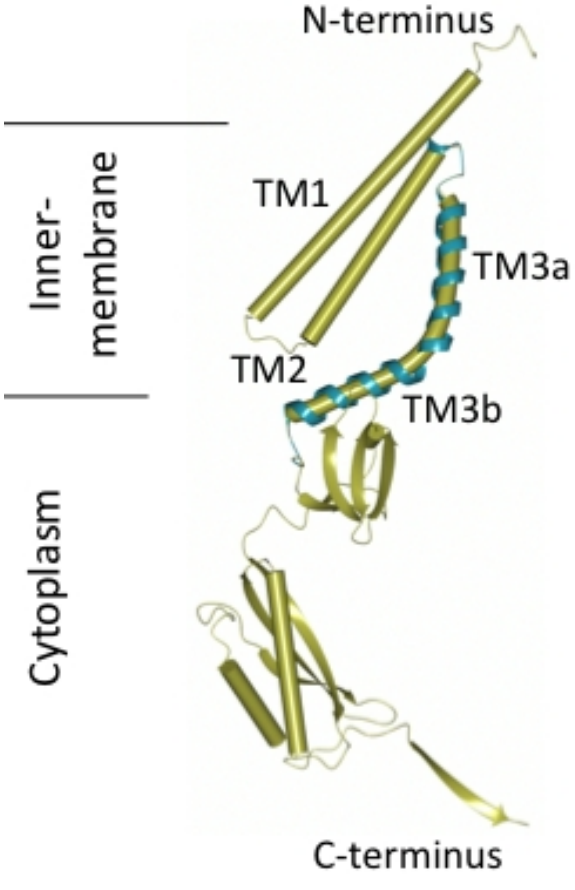
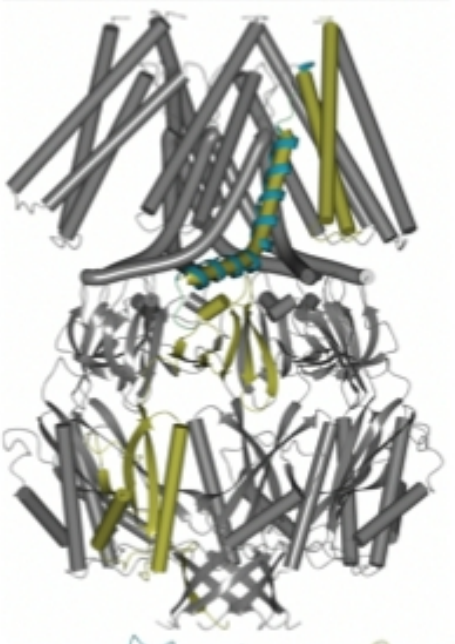
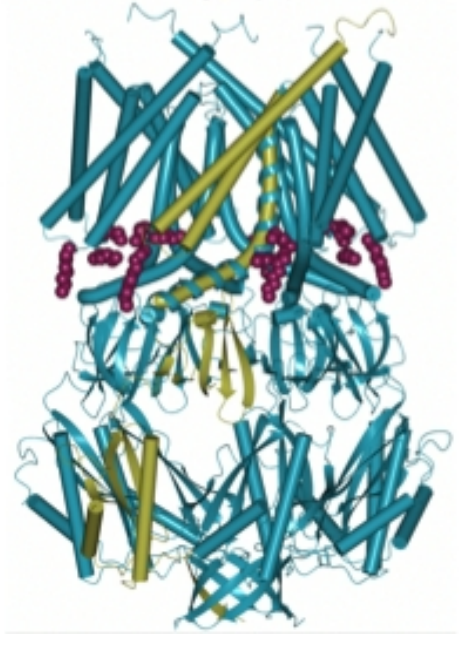
MscS monomer

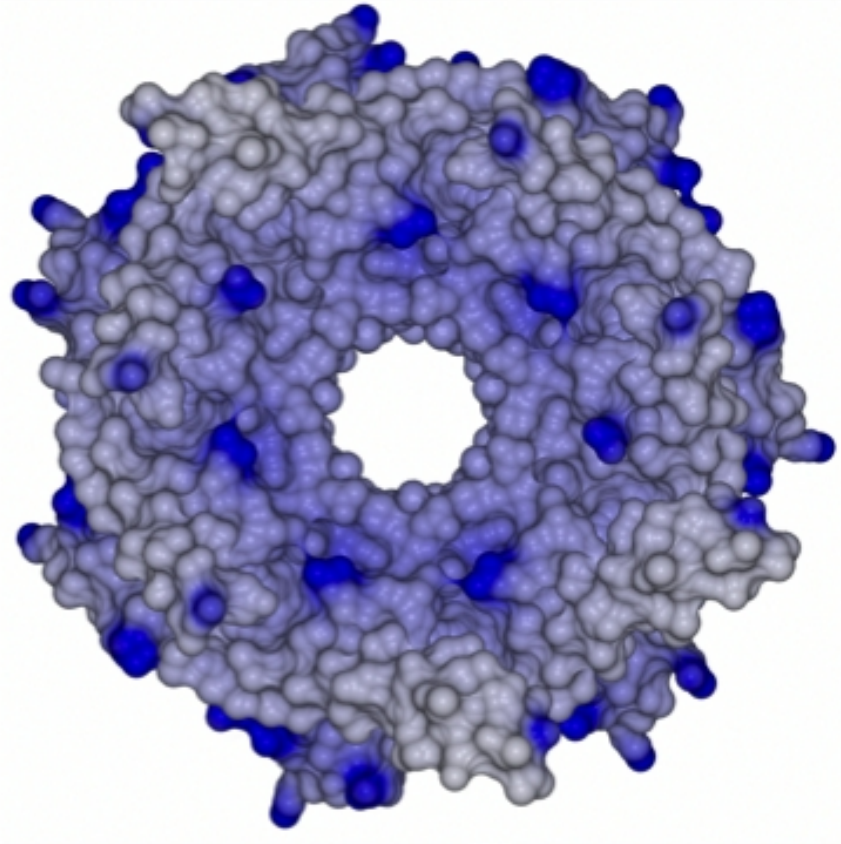




MscS closed



MscS open



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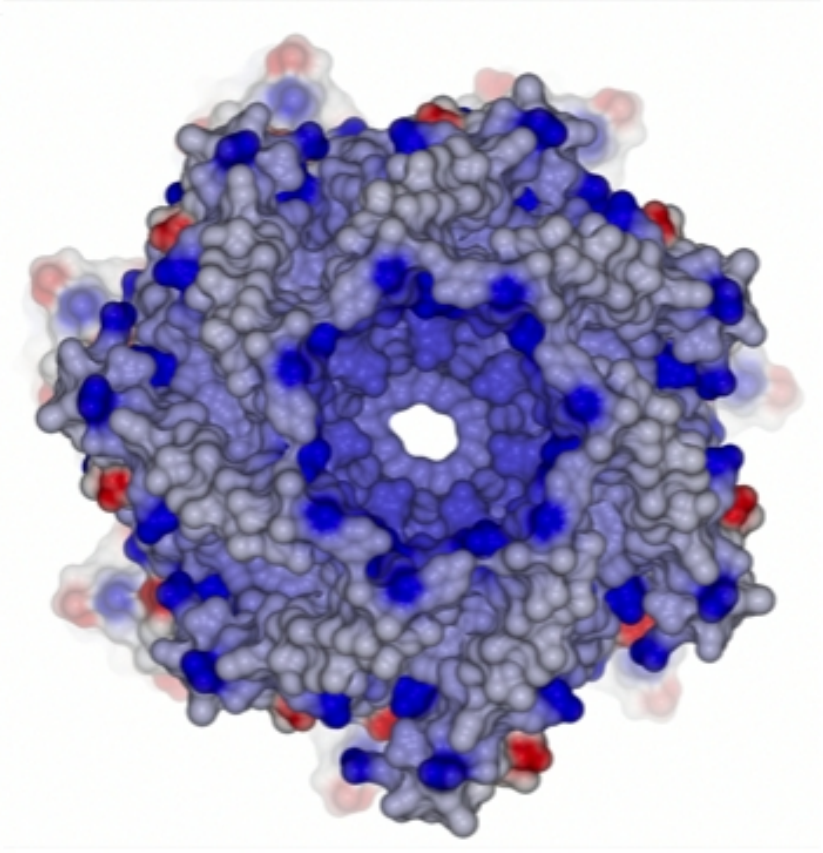

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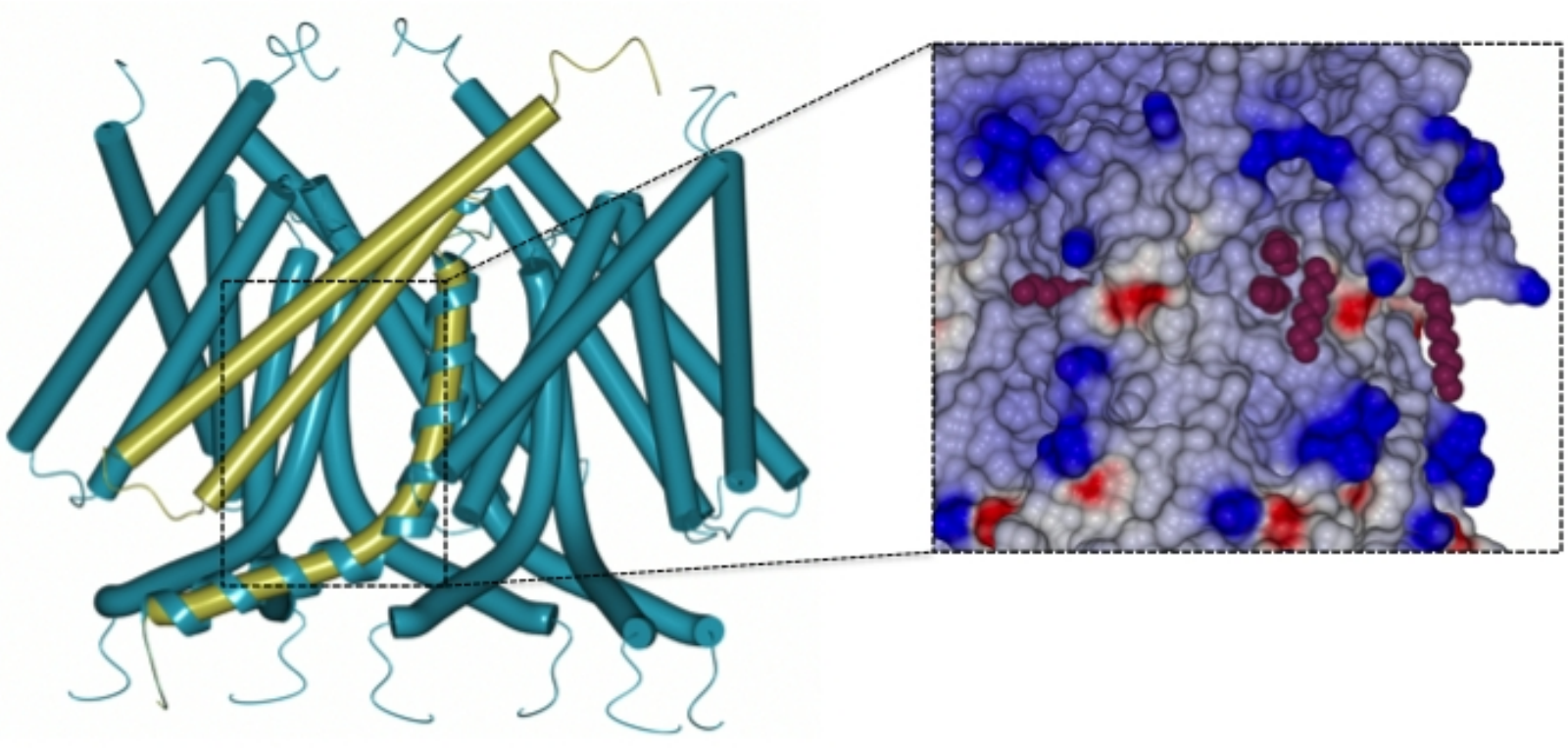
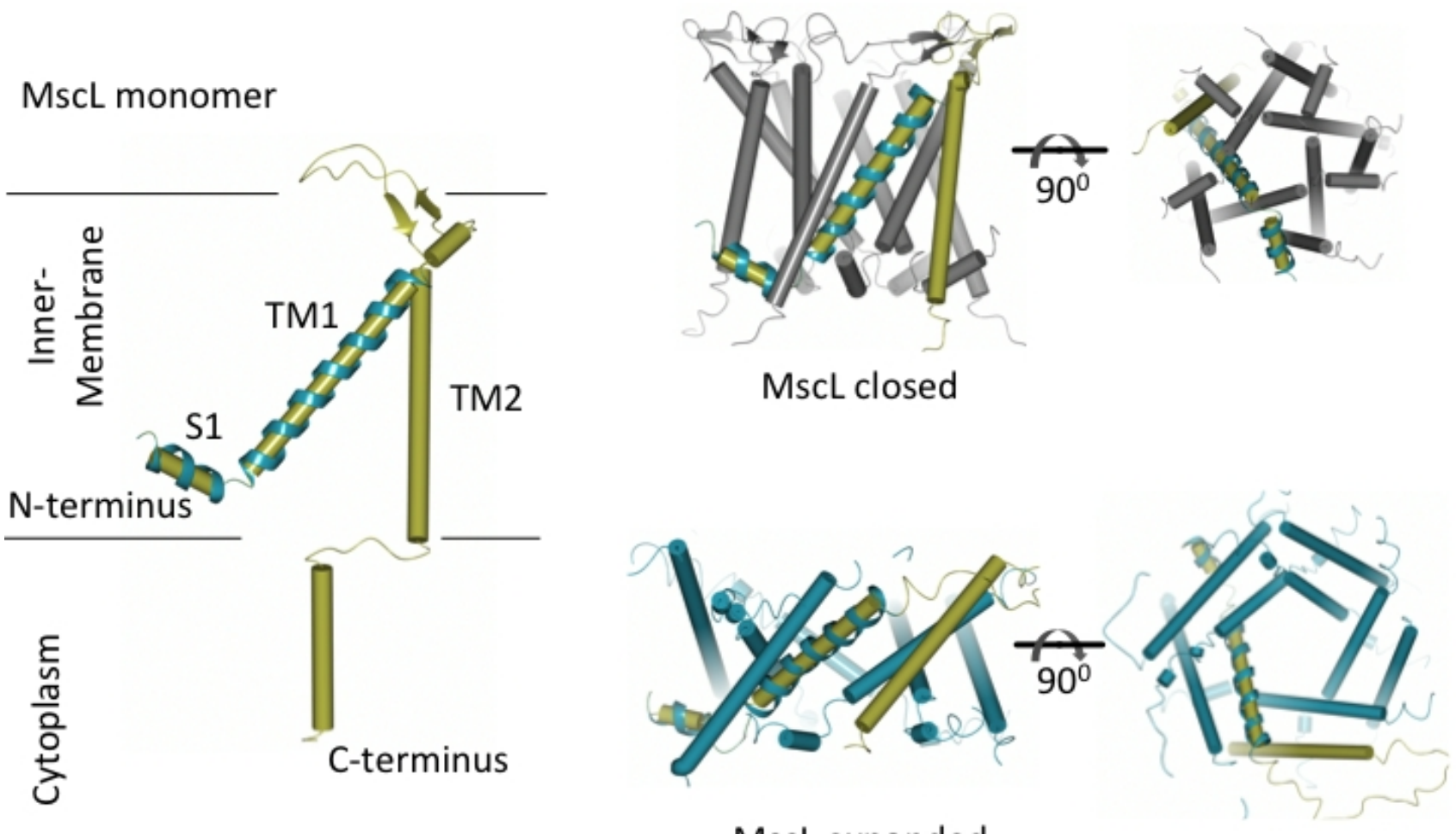
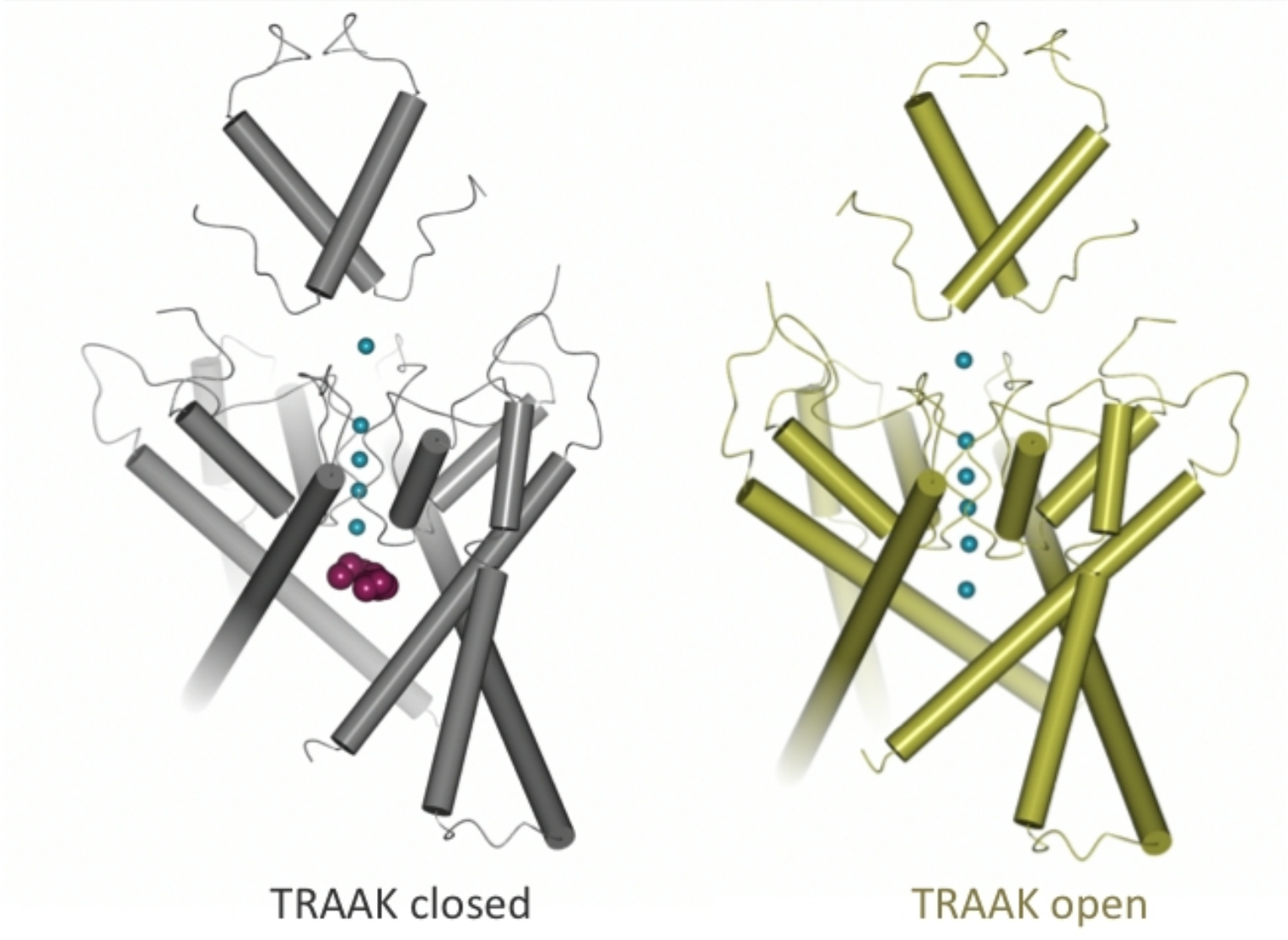
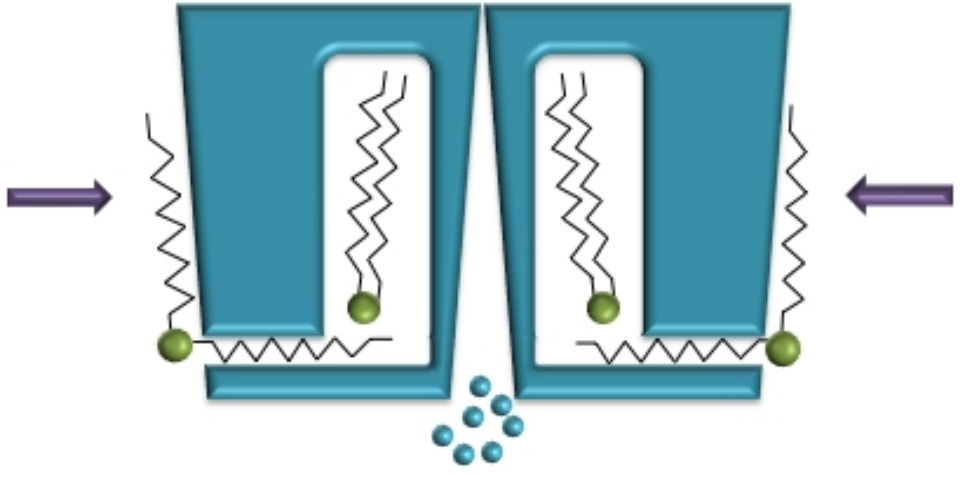
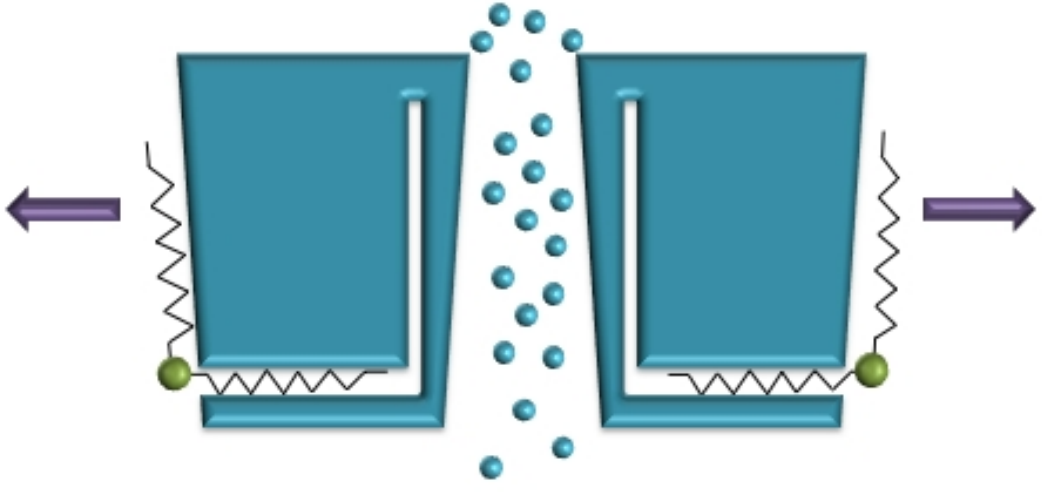
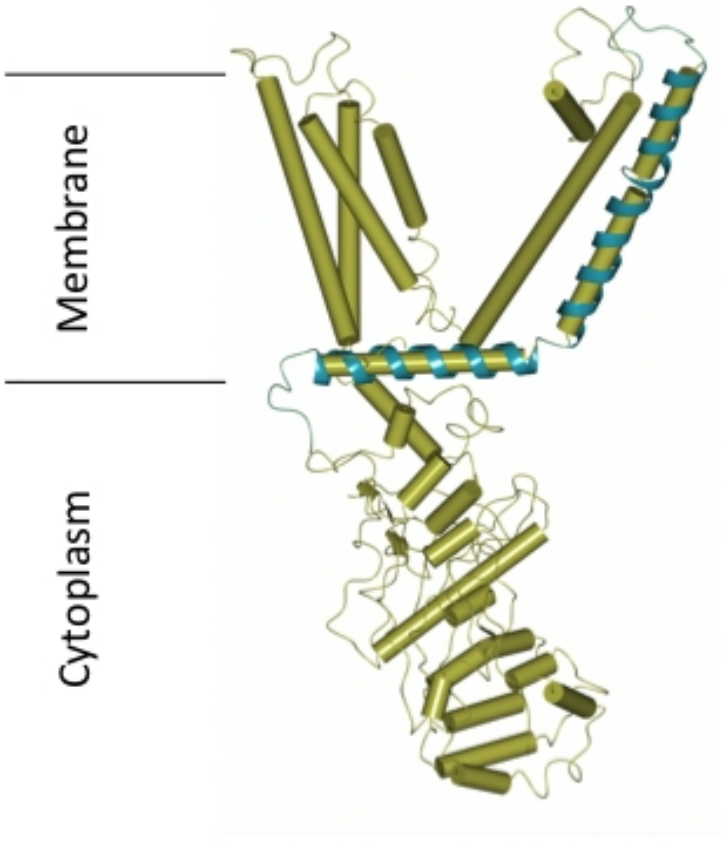



MscL monomer
Inner-Membrane
TM1
TM2
S1
N-terminus
Cytoplasm
C-terminus
90⁰
MscL closed
90⁰
MscL expanded

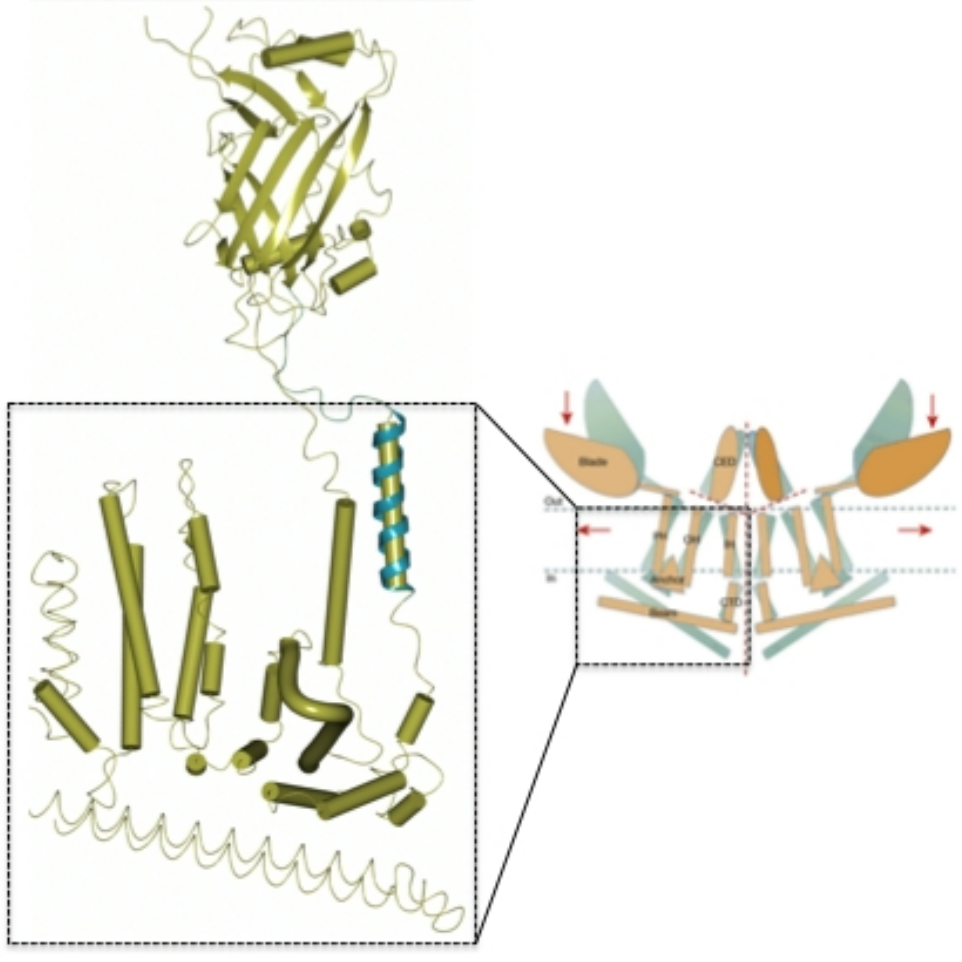

TRAAK closed
TRAAK open



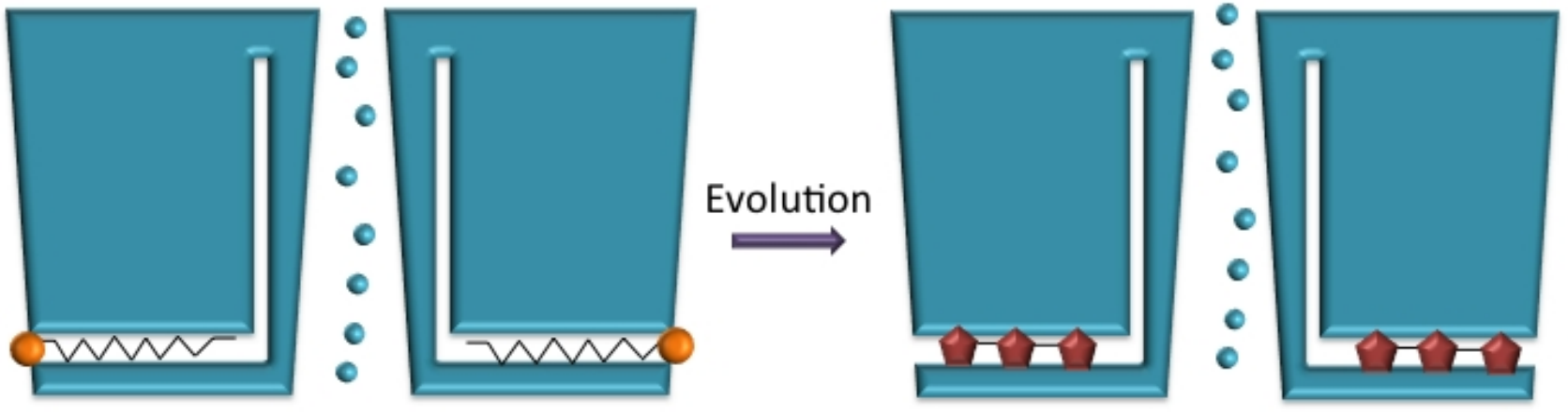




TRPV1 monomer



Piezo 1 monomer



Lipid-like gated

Ligand gated