

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

Christos Pliotas^{1,*} and James H Naismith^{1,2*}

1 Biomedical Sciences Research Complex, North Haugh, University of St Andrews,
KY16 9ST

2 State Key Laboratory of Biotherapy and Cancer Center, West China Hospital of
Sichuan University and Collaborative Innovation Center of Biotherapy and Cancer,
Chengdu 610041, China

* To whom correspondence should be addressed.

c.pliotas@st-andrews.ac.uk and naismith@st-andrews.ac.uk

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. Interestingly increased expression of MscL was recently found to increase streptomycin 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 obtaining 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 structures of an archeal MscL orthologue[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 90⁰) 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.

Acknowledgements

CP is a Royal Society of Edinburgh (RSE) Personal Research Fellow, funded by the Scottish Government. Research funds for this study were also partly covered by a Tenovus Scotland grant (Tenovus Grant Application T15/41), awarded to CP. JHN is supported by the Chinese National Thousand Talents Program, Wellcome Trust Senior Investigator Award (WT100209MA) and Royal Society Wolfson Merit Award.

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 the 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 response 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.

References

* *References of special interest*

** *References of outstanding interest*

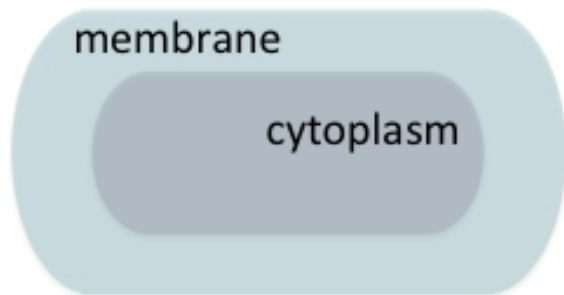
1. Huang CL, Feng S, Hilgemann DW: **Direct activation of inward rectifier potassium channels by PIP2 and its stabilization by Gbetagamma.** *Nature* 1998, **391**:803-806.
2. Bass RB, Strop P, Barclay M, Rees DC: **Crystal structure of Escherichia coli MscS, a voltage-modulated and mechanosensitive channel.** *Science* 2002, **298**:1582-1587.
3. Cox CD, Nakayama Y, Nomura T, Martinac B: **The evolutionary 'tinkering' of MscS-like channels: generation of structural and functional diversity.** *Pflugers Arch* 2015, **467**:3-13.
4. Chang G, Spencer RH, Lee AT, Barclay MT, Rees DC: **Structure of the MscL homolog from Mycobacterium tuberculosis: a gated mechanosensitive ion channel.** *Science* 1998, **282**:2220-2226.
5. Sukharev SI, Blount P, Martinac B, Blattner FR, Kung C: **A large-conductance mechanosensitive channel in E. coli encoded by mscL alone.** *Nature* 1994, **368**:265-268.
6. Teng J, Loukin S, Anishkin A, Kung C: **The force-from-lipid (FFL) principle of mechanosensitivity, at large and in elements.** *Pflugers Arch* 2015, **467**:27-37.
7. Dong YY, Pike AC, Mackenzie A, McClenaghan C, Aryal P, Dong L, Quigley A, Grieben M, Goubin S, Mukhopadhyay S, et al.: **K2P channel gating mechanisms revealed by structures of TREK-2 and a complex with Prozac.** *Science* 2015, **347**:1256-1259.
8. **Brohawn SG, Campbell EB, MacKinnon R: **Physical mechanism for gating and mechanosensitivity of the human TRAAK K⁺ channel.** *Nature* 2014, **516**:126-130.
This is the first study which demonstrates at a molecular detail how mechanosantion regulates eukaryotic ion channels and particularly potassium channels
9. Li J, Hou B, Tumova S, Muraki K, Bruns A, Ludlow MJ, Sedo A, Hyman AJ, McKeown L, Young RS, et al.: **Piezo1 integration of vascular architecture with physiological force.** *Nature* 2014, **515**:279-282.
10. *Cao E, Liao M, Cheng Y, Julius D: **TRPV1 structures in distinct conformations reveal activation mechanisms.** *Nature* 2013, **504**:113-118.
In this study the authors describe the first molecular-detail gating transitions undertaken by the TRPV1 mechanosensitive channel ,using high resolution cryo-EM
11. Levina N, Totemeyer S, Stokes NR, Louis P, A. JM, Booth IR: **Protection of Escherichia coli cells against extreme turgor by activation of MscS and**

- MscL mechanosensitive channels: identification of genes required for MscS activity.** *EMBO J* 1999, **18**:1730-1737.
12. Wang W, Black SS, Edwards MD, Miller S, Morrison EL, Bartlett W, Dong C, Naismith JH, Booth IR: **The structure of an open form of an E. coli mechanosensitive channel at 3.45 Å resolution.** *Science* 2008, **321**:1179-1183.
 13. Malcolm HR, Blount P: **Mutations in a Conserved Domain of E. coli MscS to the Most Conserved Superfamily Residue Leads to Kinetic Changes.** *PLoS One* 2015, **10**:e0136756.
 14. Bottcher B, Prazak V, Rasmussen A, Black SS, Rasmussen T: **The Structure of YnaI Implies Structural and Mechanistic Conservation in the MscS Family of Mechanosensitive Channels.** *Structure* 2015, **23**:1705-1714.
 15. Iscla I, Wray R, Wei S, Posner B, Blount P: **Streptomycin potency is dependent on MscL channel expression.** *Nat Commun* 2014, **5**:4891.
 16. Wray R, Iscla I, Gao Y, Li H, Wang J, Blount P: **Dihydrostreptomycin Directly Binds to, Modulates, and Passes through the MscL Channel Pore.** *PLoS Biol* 2016, **14**:e1002473.
 17. Najem JS, Dunlap MD, Rowe ID, Freeman EC, Grant JW, Sukharev S, Leo DJ: **Activation of bacterial channel MscL in mechanically stimulated droplet interface bilayers.** *Sci Rep* 2015, **5**:13726.
 18. *Reading E, Walton TA, Liko I, Marty MT, Laganowsky A, Rees DC, Robinson CV: **The Effect of Detergent, Temperature, and Lipid on the Oligomeric State of MscL Constructs: Insights from Mass Spectrometry.** *Chem Biol* 2015, **22**:593-603.
- Using native mass spectrometry on MscL the authors demonstrate the variability of oligomeric states MscL could exist in vitro by altering the detergent of choice, temperature and orthologues construct used*
19. **Laganowsky A, Reading E, Allison TM, Ulmschneider MB, Degiacomi MT, Baldwin AJ, Robinson CV: **Membrane proteins bind lipids selectively to modulate their structure and function.** *Nature* 2014, **510**:172-175.
This study describes the application of native mass spectrometry to integral membrane proteins and demonstrates that they can exist folded in the gas phase
 20. Wang Y, Liu Y, Deberg HA, Nomura T, Hoffman MT, Rohde PR, Schulten K, Martinac B, Selvin PR: **Single molecule FRET reveals pore size and opening mechanism of a mechano-sensitive ion channel.** *Elife* 2014, **3**:e01834.
 21. Bavi N, Cortes DM, Cox CD, Rohde PR, Liu W, Deitmer JW, Bavi O, Strop P, Hill AP, Rees D, et al.: **The role of MscL amphipathic N terminus indicates a blueprint for bilayer-mediated gating of mechanosensitive channels.** *Nature Communications* 2016, **7**.
 22. Vanegas JM, Arroyo M: **Force transduction and lipid binding in MscL: a continuum-molecular approach.** *PLoS One* 2014, **9**:e113947.
 23. Li J, Guo J, Ou X, Zhang M, Li Y, Liu Z: **Mechanical coupling of the multiple structural elements of the large-conductance mechanosensitive channel during expansion.** *Proc Natl Acad Sci U S A* 2015, **112**:10726-10731.
 24. Schmidt D, del Marmol J, MacKinnon R: **Mechanistic basis for low threshold mechanosensitivity in voltage-dependent K⁺ channels.** *Proc Natl Acad Sci U S A* 2012, **109**:10352-10357.

25. *Brohawn SG, Su Z, MacKinnon R: **Mechanosensitivity is mediated directly by the lipid membrane in TRAAK and TREK1 K⁺ channels.** *Proc Natl Acad Sci U S A* 2014, **111**:3614-3619.
The authors in this study by using electrophysiology, first identify mechanosensitivity properties within eukaryotic potassium channels
26. **Tanaka K, Caaveiro JM, Morante K, Gonzalez-Manas JM, Tsumoto K: **Structural basis for self-assembly of a cytolytic pore lined by protein and lipid.** *Nat Commun* 2015, **6**:6337.
In this study the authors demonstrate at a molecular detail that the lipids constitute an integral structural and functional part of pore-forming toxins
27. Vasquez V, Sotomayor M, Cordero-Morales J, Schulten K, Perozo E: **A structural mechanism for MscS gating in lipid bilayers.** *Science* 2008, **321**:1210-1214.
28. Akitake B, Anishkin A, Liu N, Sukharev S: **Straightening and sequential buckling of the pore-lining helices define the gating cycle of MscS.** *Nat Struct Mol Biol* 2007, **14**:1141-1149.
29. Naismith JH, Booth IR: **Bacterial mechanosensitive channels--MscS: evolution's solution to creating sensitivity in function.** *Annu Rev Biophys* 2012, **41**:157-177.
30. Pliotas C, Ward R, Branigan E, Rasmussen A, Hagelueken G, Huang H, Black SS, Booth IR, Schiemann O, Naismith JH: **Conformational state of the MscS mechanosensitive channel in solution revealed by pulsed electron-electron double resonance (PELDOR) spectroscopy.** *Proc Natl Acad Sci U S A* 2012, **109**:E2675-2682.
31. *Ward R, Pliotas C, Branigan E, Hacker C, Rasmussen A, Hagelueken G, Booth IR, Miller S, Lucocq J, Naismith JH, et al.: **Probing the structure of the mechanosensitive channel of small conductance in lipid bilayers with pulsed electron-electron double resonance.** *Biophys J* 2014, **106**:834-842.
In this study the authors use PELDOR (or DEER) spectroscopy to show that the TM helical arrangement of MscS within lipids is in agreement with x-ray structures and thus voids constitute a real structural feature of MscS. This is a follow-up study of [30], where the TM helices were found to be in agreement with x-ray structure in solution as well
32. Lai JY, Poon YS, Kaiser JT, Rees DC: **Open and shut: crystal structures of the dodecylmaltoside solubilized mechanosensitive channel of small conductance from Escherichia coli and Helicobacter pylori at 4.4 Å and 4.1 Å resolutions.** *Protein Sci* 2013, **22**:502-509.
33. Zhang X, Wang J, Feng Y, Ge J, Li W, Sun W, Iscla I, Yu J, Blount P, Li Y, et al.: **Structure and molecular mechanism of an anion-selective mechanosensitive channel of small conductance.** *Proc Natl Acad Sci U S A* 2012, **109**:18180-18185.
34. *Pliotas C, Dahl AC, Rasmussen T, Mahendran KR, Smith TK, Marius P, Gault J, Banda T, Rasmussen A, Miller S, et al.: **The role of lipids in mechanosensation.** *Nat Struct Mol Biol* 2015, **22**:991-998.
In this paper the authors use a combination of structural, biophysical, computational and analytical methods to propose a new gating model of mechanosensation, using

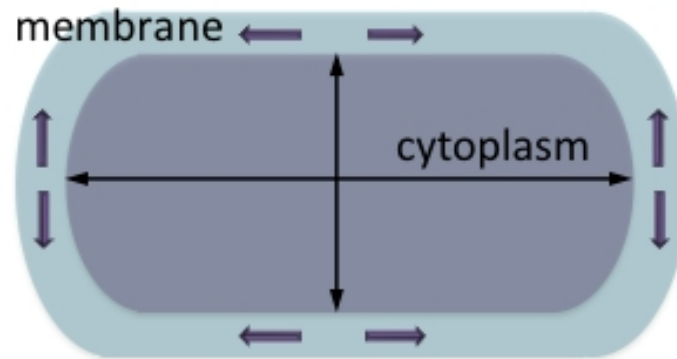
35. Shaikh S, Cox CD, Nomura T, Martinac B: **Energetics of gating MscS by membrane tension in azolectin liposomes and giant spheroplasts.** *Channels (Austin)* 2014, **8**:321-326.
36. Mukherjee N, Jose MD, Birkner JP, Walko M, Ingolfsson HI, Dimitrova A, Arnarez C, Marrink SJ, Kocer A: **The activation mode of the mechanosensitive ion channel, MscL, by lysophosphatidylcholine differs from tension-induced gating.** *Faseb j* 2014, **28**:4292-4302.
37. Gullingsrud J, Schulten K: **Lipid bilayer pressure profiles and mechanosensitive channel gating.** *Biophys J* 2004, **86**:3496-3509.
38. **Liao M, Cao E, Julius D, Cheng Y: **Structure of the TRPV1 ion channel determined by electron cryo-microscopy.** *Nature* 2013, **504**:107-112.
The authors of this study developed advanced cryo-EM methodology to solve the first TRPV1 channel structure at relatively high resolution
39. **Ge J, Li W, Zhao Q, Li N, Chen M, Zhi P, Li R, Gao N, Xiao B, Yang M: **Architecture of the mammalian mechanosensitive Piezo1 channel.** *Nature* 2015, **527**:64-69.
The authors used cryo-EM to resolve the first full length Piezo1 structure. Piezo1 was a homo-trimer with unique and very unusual structural features
40. Cox CD, Bae C, Ziegler L, Hartley S, Nikolova-Krstevski V, Rohde PR, Ng C-A, Sachs F, Gottlieb PA, Martinac B: **Removal of the mechanoprotective influence of the cytoskeleton reveals PIEZO1 is gated by bilayer tension.** *Nature Communications* 2016, **7**.
41. Yang F, Xiao X, Cheng W, Yang W, Yu P, Song Z, Yarov-Yarovoy V, Zheng J: **Structural mechanism underlying capsaicin binding and activation of the TRPV1 ion channel.** *Nat Chem Biol* 2015, **11**:518-524.
42. Syeda R, Xu J, Dubin AE, Coste B, Mathur J, Huynh T, Matzen J, Lao J, Tully DC, Engels IH, et al.: **Chemical activation of the mechanotransduction channel Piezo1.** *Elife* 2015, **4**.

- 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.

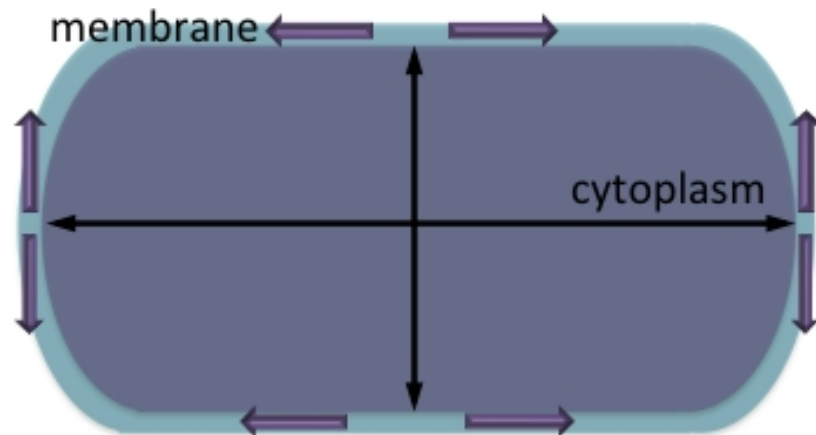


Equilibrium

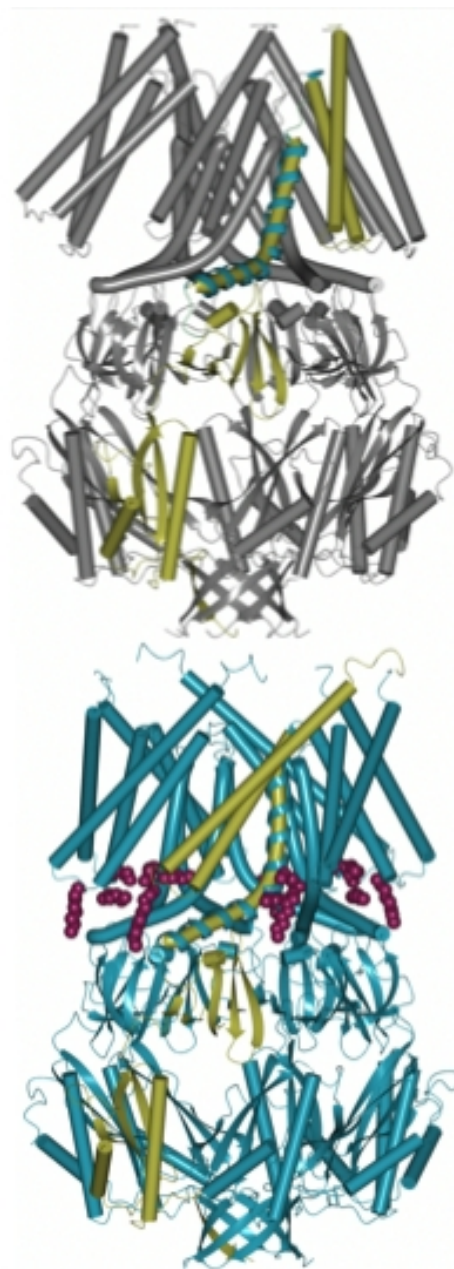
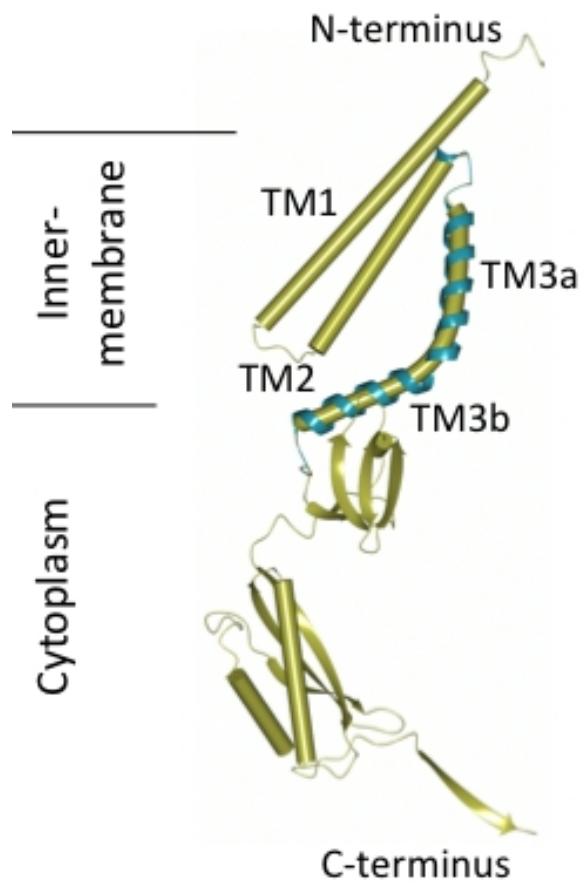
Cytoplasmic pressure
increase - Cell expansion

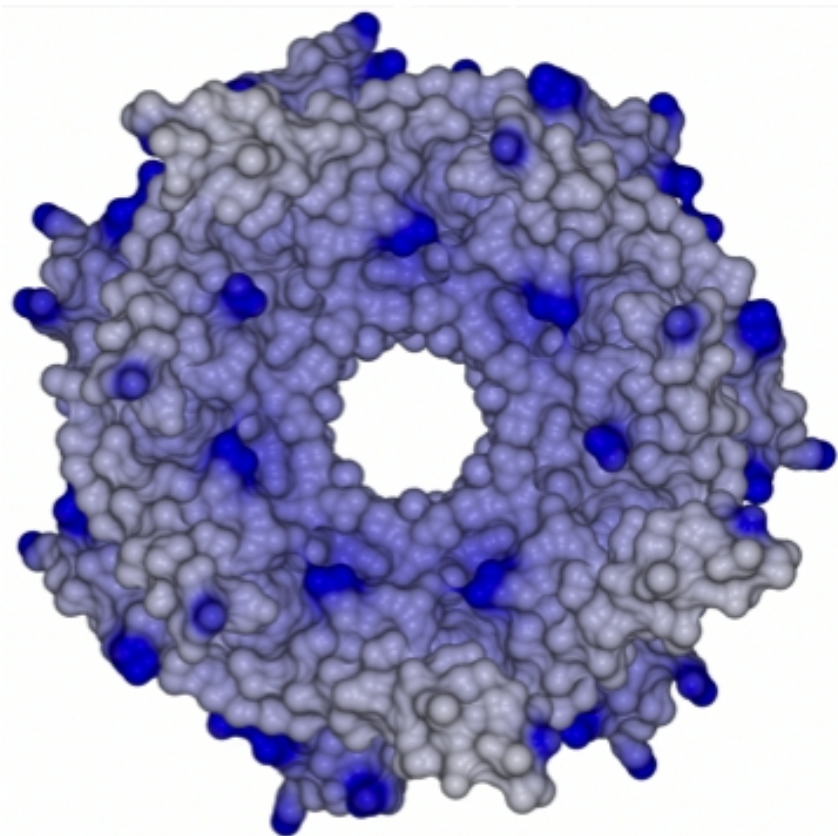


Lytic pressure threshold –
Membrane thinning

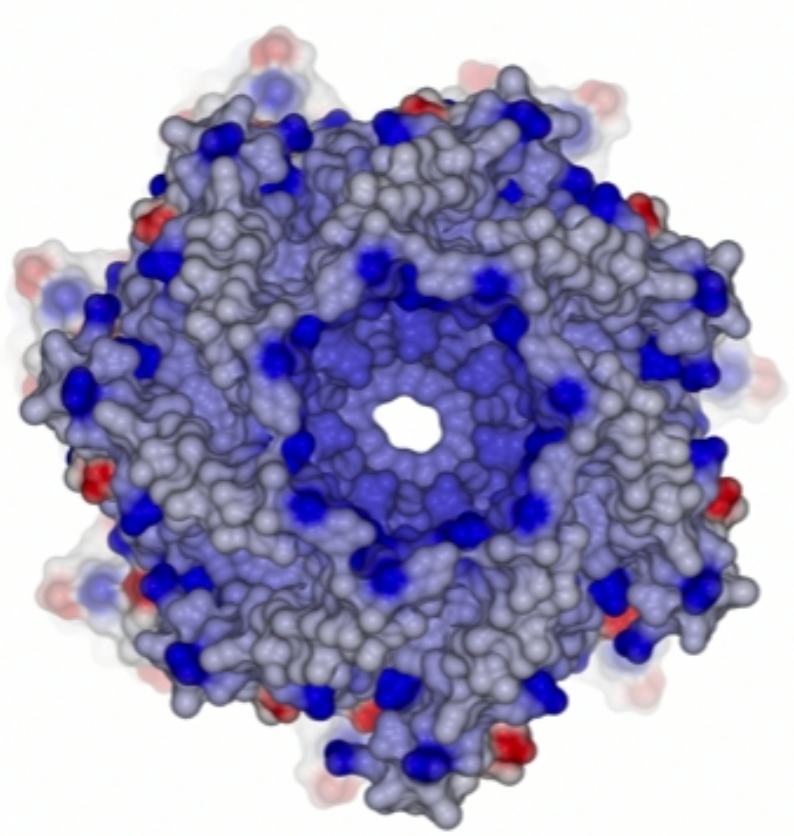


MscS monomer

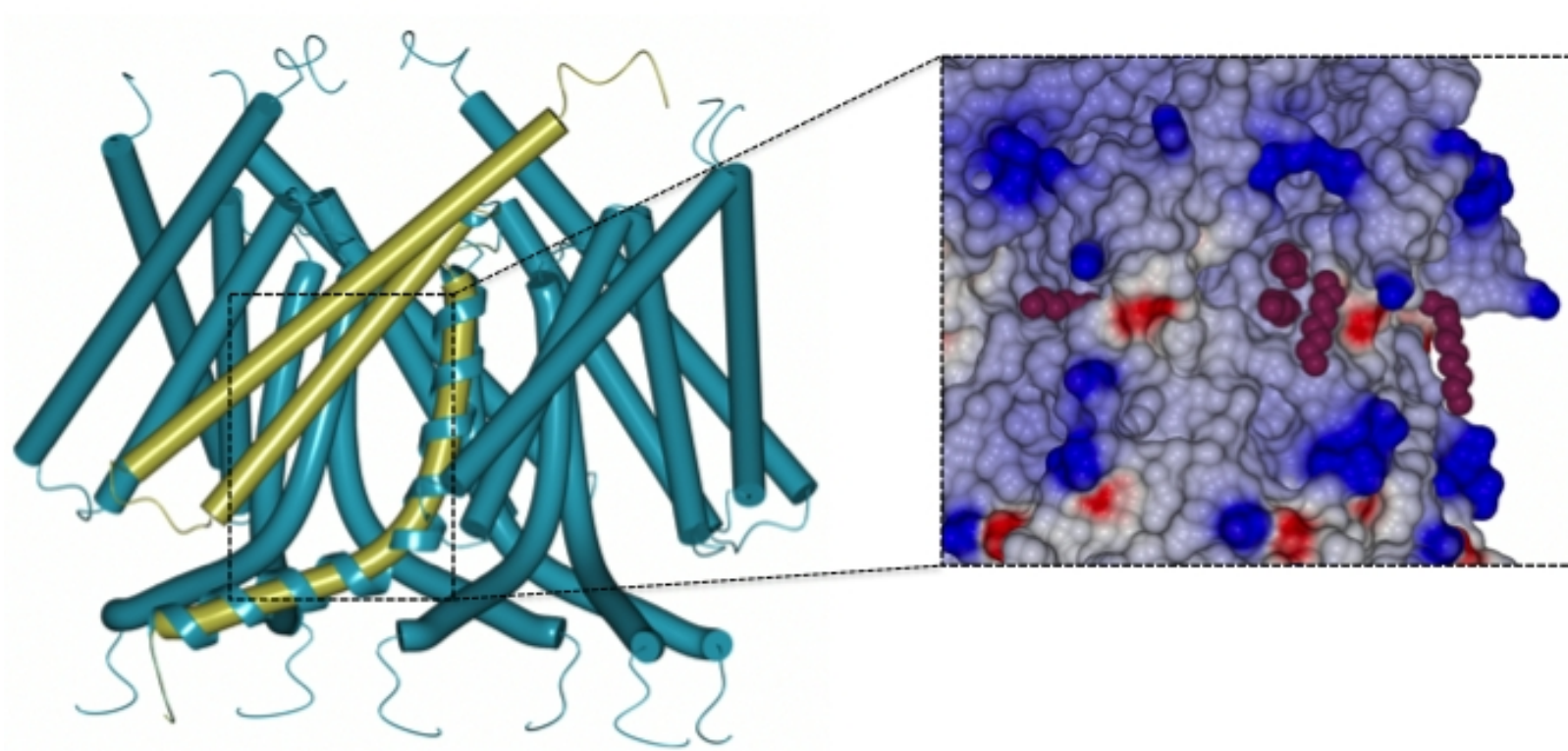


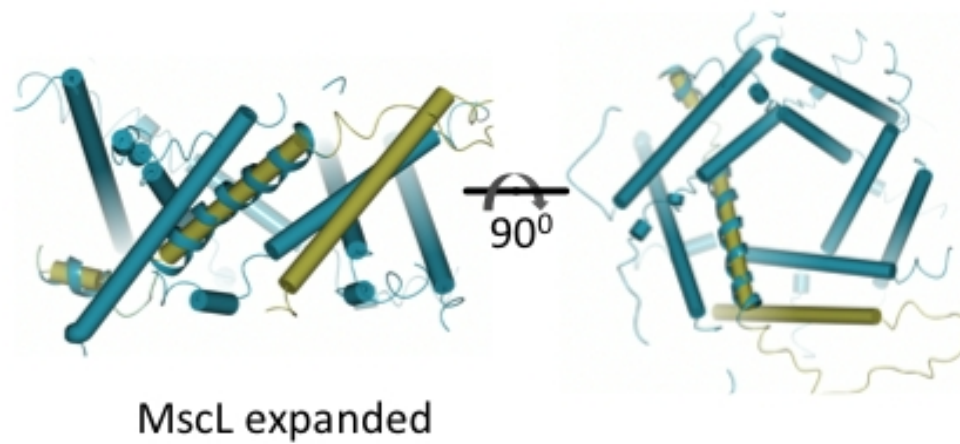
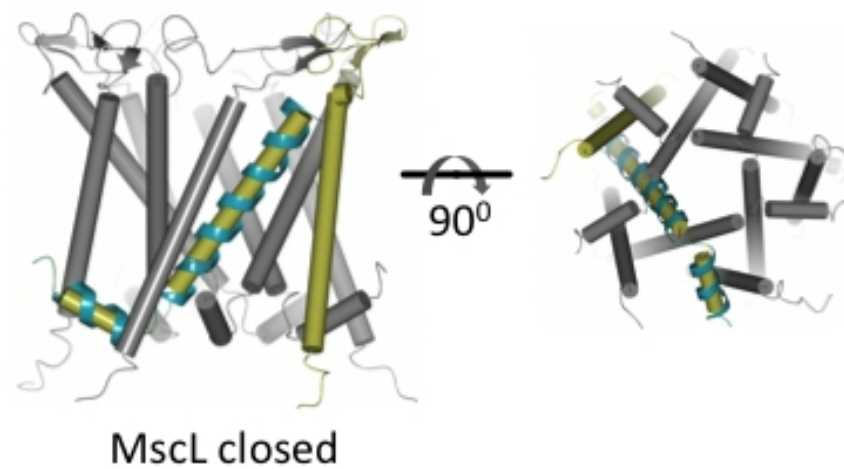
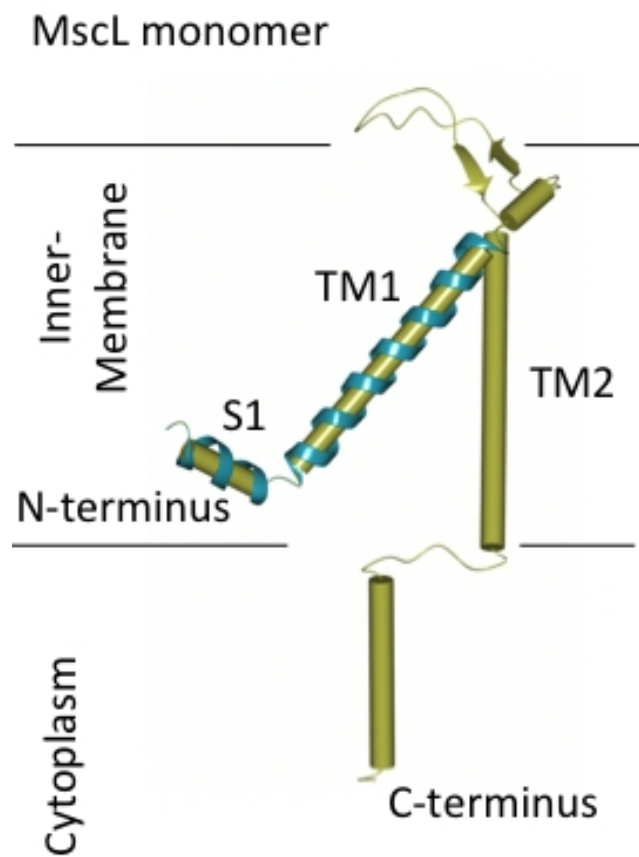


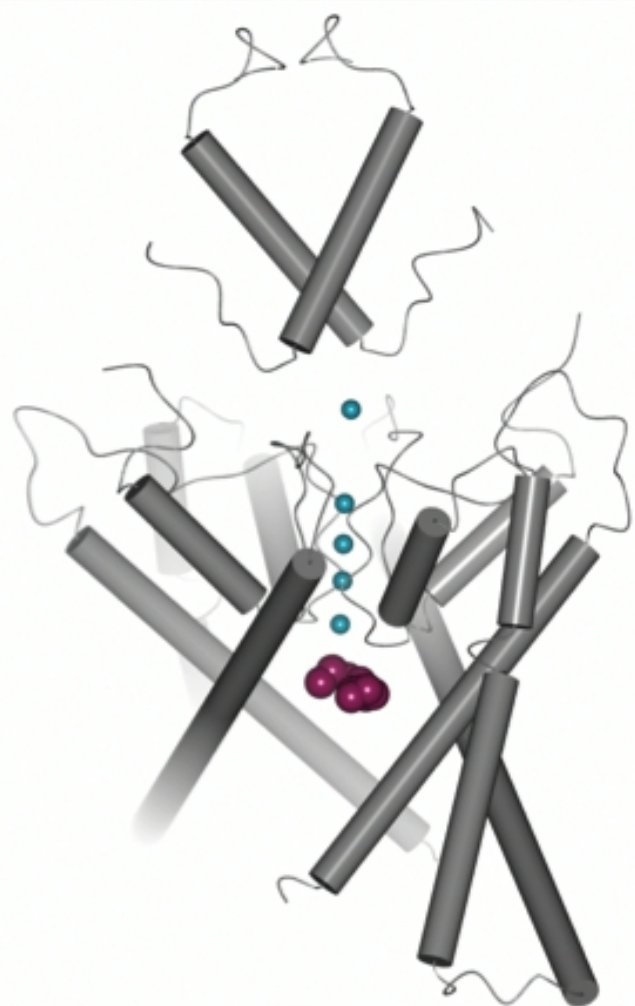
MscS open



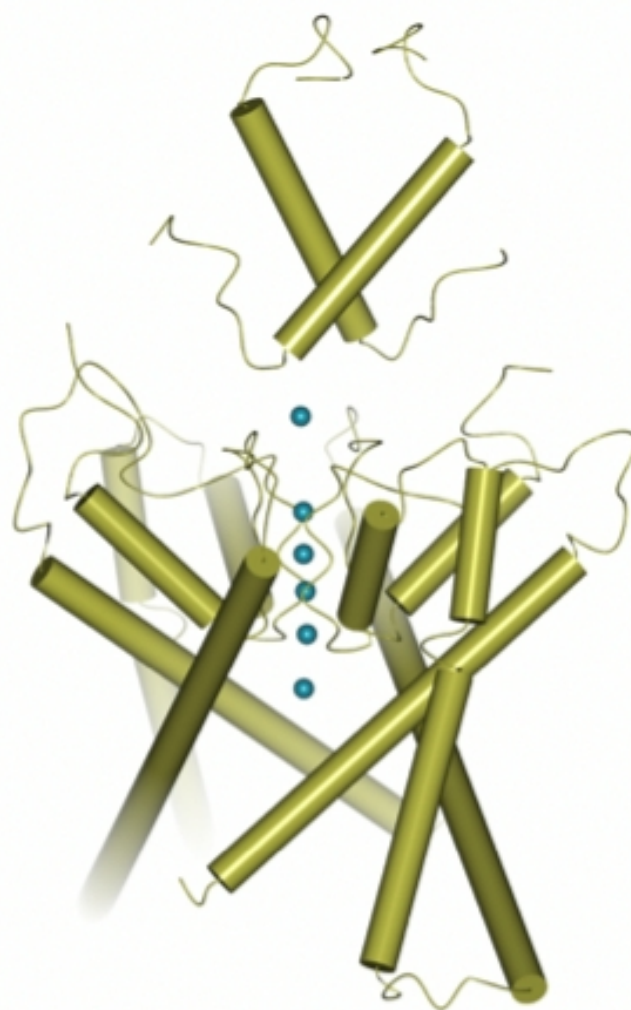
MscS closed



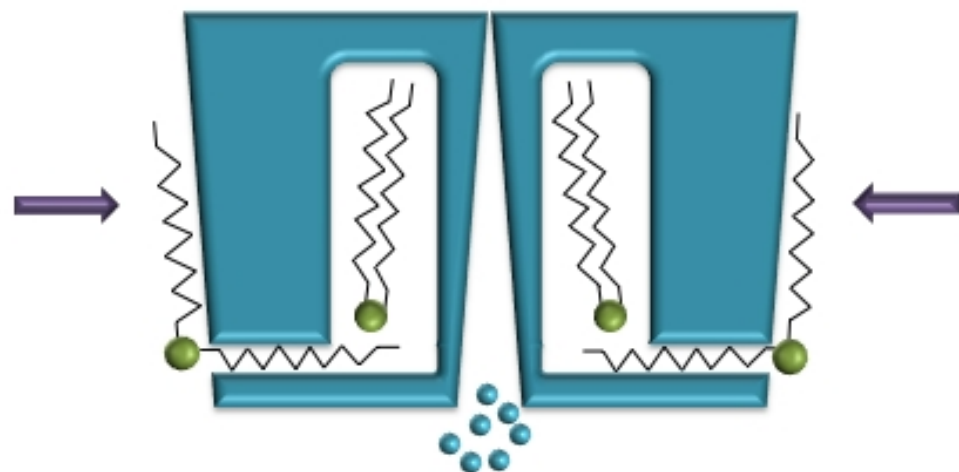


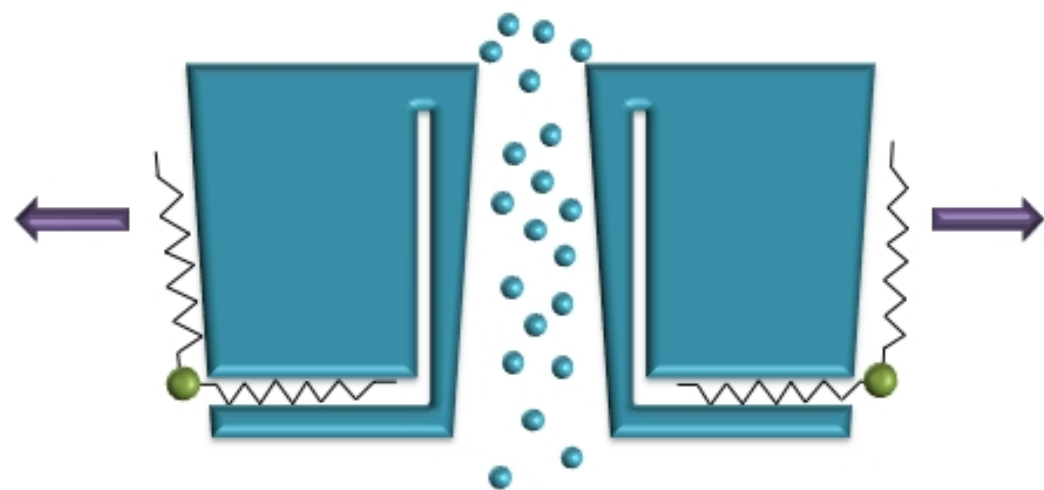


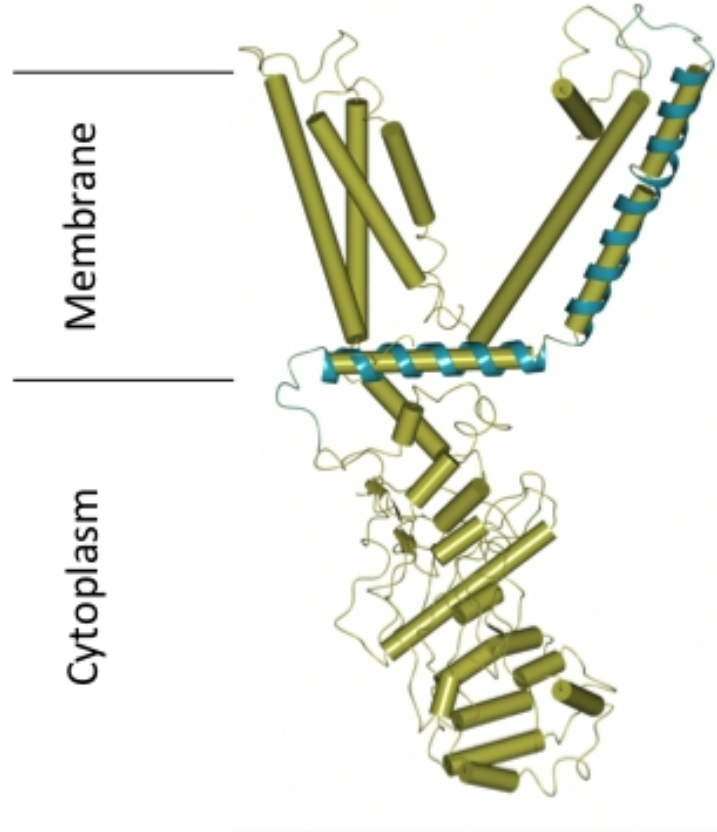
TRAAK closed



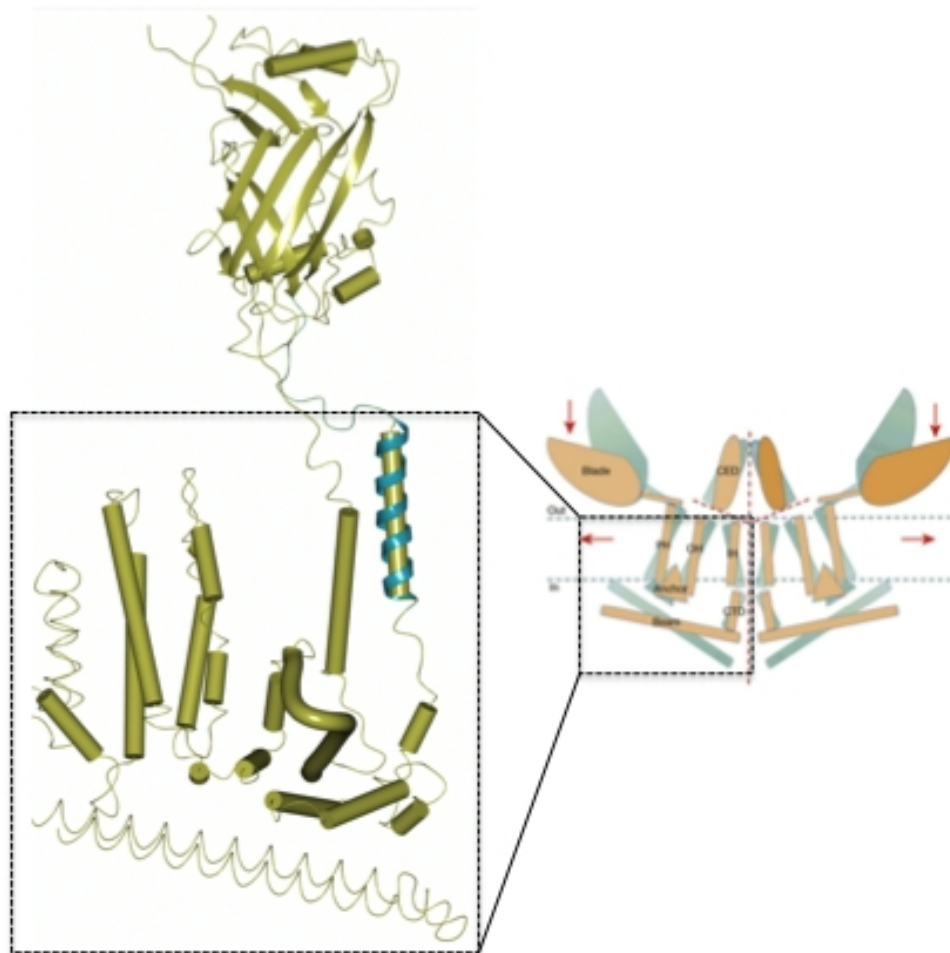
TRAAK open



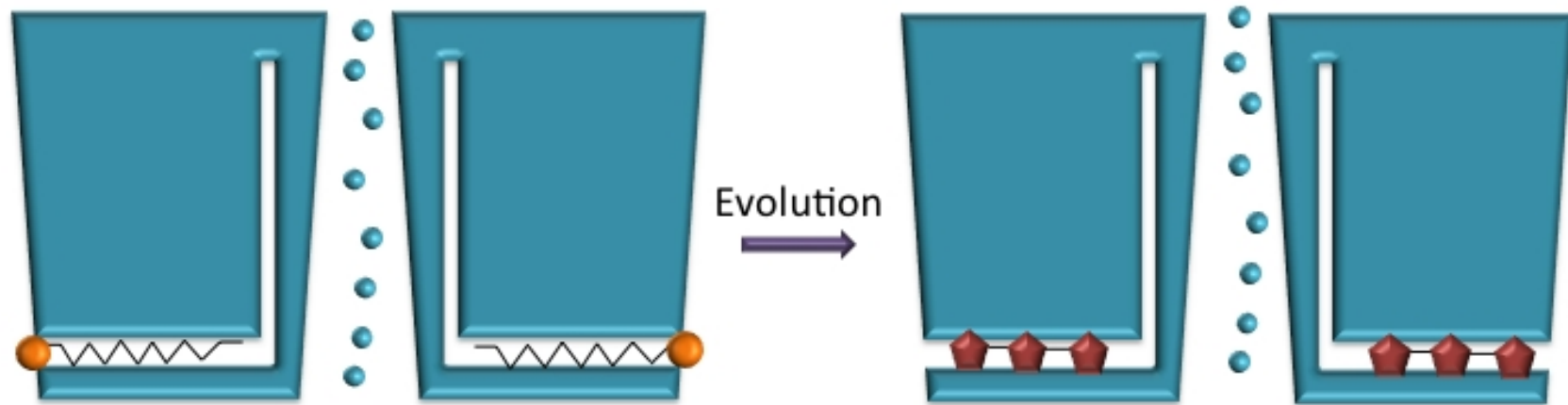




TRPV1 monomer



Piezo 1 monomer



Lipid-like gated

Ligand gated