Selectivity mechanisms in MscS-like channels: from structure to function

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Running title: MscS subfamily selectivity

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Footnotes. Abbreviations: MS, mechanosensitive; TM, transmembrane domain; MscS, mechanosensitive channel of small conductance.

ABSTRACT

The *E. coli* mechanosensitive (MS) channel of small conductance (EcMscS) is the prototype of a diverse family of channels present in all domains of life. While EcMscS has been extensively studied, recent developments show that MscS may display some characteristics not widely conserved in this protein subfamily. With numerous members now electrophysiologically characterised, this subfamily of channels displays a breadth of ion selectivity with both anion and cation selective members. The selectivity of these channels may be relatively weak in comparison to voltage-gated channels but their selectivity mechanisms represent great novelty. Recent studies have identified unexpected residues important for selectivity in these homologues revealing different selectivity mechanisms than those employed by voltage gated K^+, Na^+, Ca^{2+} and Cl^- channels whose selectivity filters are housed within their transmembrane pores. This review looks at what is currently known about the MscS subfamily selectivity and begins to unravel the potential physiological relevance of these differences.

[150 words] no more than 150 words

Key words: *Escherichia coli* | selectivity | mechanosensitive | patch clamp | MscS-like

Introduction

The *E. coli* mechanosensitive channel of small conductance (EcMscS) is often thought of as the archetypal member of the MscS channel subfamily. Characteristic stretch-activated activity of this family of channels was indeed the first report of bacterial mechanosensitive channels more than 25 years ago.[1] This diverse family can be broadly subdivided into two groups; the smaller proteins such as EcMscS (circa 250 - 500 aa) and the larger proteins such as MscK (circa 500 – 1000^{+} aa).[2-4] These channels, in the main, play an integral role in osmoprotection acting as emergency release valves in the face of large reductions in environmental osmolarity (Fig. 1).[3, 5] However, due to the seeming 'redundancy' of some paralogues, alternative, as yet undiscovered, physiological functions must be carried out by these channels (See Malcolm *et al.,* 2012 for review)[6]. Examples of electrophysiologically characterised channels that potentially fulfil alternate or additional functions include MSC1 (*Chlamydomonas reinhardtii*), Msy1/2 (*Saccharomyces pombe*) and MSL10 (*Arabidopsis thaliana*).[7-9] It is important to note that the seeming redundancy of some MscS-like channels may well be simply a function of their cellular expression levels and does not imply an inherent lack of mechanosensitivity or preclude roles in processes other than osmoprotection.[10]

MscS family structure is characterised by multiple N-terminal transmembrane domains followed by a large water-filled C-terminal cytoplasmic vestibule.[11, 12] The number of N-terminal transmembrane helices varies greatly throughout the MscS family with channels like EcMscS and MscSP possessing three and larger proteins such as MscK likely possessing 11.[13-15] The ubiquitous nature of the cytoplasmic domain is hinted at from hydropathy plots[16-18] from MscS-like channels and is clearly illustrated in each of the three MscS homologues to have been crystallised thus far (*Escherichia coli* MscS, *Helicobacter pylori* MscS and *Thermoanerobacter tengcongensis* MscS) (Fig. 2).[19-21] In addition to this, all crystal structures we currently possess illustrate homoheptameric assembly. A notable feature of EcMscS is the seven lateral ~12 Å portals that perforate the cytoplasmic vestibule.[11, 12, 22] Whether the vestibular perforations seen in *E. coli* MscS are a widely distributed structural characteristic of this family of channels is however unknown. This is further complicated by the fact that the C-terminal vestibule is an area in which large insertions and/or deletions can be found in MscS homologues.[2] As an example, these perforations seem to be greatly restricted and almost absent in an MscS-like homologue found in the anaerobic thermophile *T. tengcongensis* which leads to the possibility that larger conducting homologues possess these perforations, in various sizes, whereas they are largely constricted or absent in lower

conducting homologues.[20] In all likelihood the presence of cytoplasmic perforations will impact greatly on both conductance and the location of the structural determinants of selectivity in MscS-like channels.

Many studies have focused on the role of the cytoplasmic vestibule in MscS channel function[23-25]. In particular the possibility that the cytoplasmic vestibule is a dynamic structure which plays an active role in channel gating [23, 26, 27]. For example interactions between the amphiphilic TM3b helices and the roof of the cytoplasmic domain have been shown to be important in EcMscS adaptation/inactivation[26]. Interestingly inactivation in this channel family seems not to be wide spread with only two of the nine currently characterised homologues exhibiting this attribute (Fig 3.). Although there is a recent report that suggests an MscS homologue in *Vibrio cholerae* may also exhibit inactivation[28]. The suggestion that the cytoplasmic domain is involved in channel selectivity is not a new hypothesis[29] and was strengthened greatly by the fact that mutations within the pore region of MscS do not affect its selectivity profile[30]. In fact, looking at the putative pore forming helices of all electrophysiologically characterised MscS homologues shows they are devoid of any potential descriptors of ionic selectivity, being in the main hydrophobic[16]. In addition, a molecular dynamics study also demonstrated the fact the cytoplasmic domain may play an ion filtering role[25]. Specific structural information about the cytoplasmic selectivity function of MscS-like channels has been provided by two recent publications. These have provided the first insights into the potential basis of ionic selectivity mechanism or mechanisms of MscS-like channels [16, 20]. By going beyond the current insight[31] this review aims to address what is currently known regarding MscS-like channel ionic selectivity with particular reference to the role of the C-terminal domain.

Reports of MscS selectivity date back to the very first description of mechanosensitive channels in bacteria and range between P_{Cl}/P_K 1.2 - 3[30, 32, 33]. Since that report 8 other members of this diverse family have been electrophysiologically characterised. Their selectivity ranges from a P_{Cl}/P_K – 0.1 – 9 (Fig. 3). These anion-cation permeability ratios are, in the main, relatively modest especially when compared to chloride channels like the glycine receptor chloride channel (P_{Cl}/P_{Na} = ~25)[34] and cannot compare with the exquisite selectivity shown by other channels such as voltage-gated K^+ channels (P_{Cl} = ~0).

In addition to these homologues, 4 other members of the MscS family from *E. coli* have been electrophysiologically characterised[17, 35]. These are the gene products of *ybdG*, *ybiO*, *yjeP* and *ynaI*. Initially thought to be a product of *ybdG* MscM activity is now thought to be related to the gene *yjeP*[17]. This is due to the fact that MscM-like activity was still seen in

a *ΔybdG* mutant and that MscM-like activity in spheroplasts over expressing YbdG could only be identified via mutation (V229A)[35]. The selectivity is only known of an MscM-like channel identified by Berrier *et al.*, 1996 ($P_{Cl}/P_K - 0.4$)[36] with the likely genetic origin of this activity being *yjeP* and not *ybdG* as previously suggested[10, 17, 31].

Structural determinants of MscS-like channel selectivity

In *E. coli* MscS two negatively charged residues (E187 and E227) situated in the cytoplasmic domain (Fig. 4, top right panel) in relatively close proximity to the lateral vestibular portals have been implicated in selectivity[16]. This is proposed to occur via cation 'trapping' creating a more favourable path for anions (Fig. 5). This is supported by the larger transit times of cations through MscS compared with anions demonstrated in a molecular dynamic simulation study[25]. In addition, Zhang *et al.,* 2012[18] convincingly implicate a charged residue (E278) on the outside of the β-Barrel in the anion selectivity of an MscS homologue from *T. tengcongensis*. These authors suggest that this residue may also act by cation 'trapping' resulting in a more thermodynamically favourable path for the transit of anions (Fig. 4, bottom left panel, Fig. 5). This residue however, is not well conserved throughout anion selective MscS homologues. In addition, neutralisation of this charge (E278A) resulted in a mutant that still displayed anion selectivity. A possible explanation of such a result is that while E278 plays an integral role in the selectivity of TtMscS this is in addition to other charged residues within its cytoplasmic domain (Fig. 4, top left panel, Fig. 5)[16]. A region of high electronegativity is identifiable in the cytoplasmic domain of TtMscS (D226 and D229) akin to that seen in EcMscS (Fig. 4, top left and right panels). As a result the cytoplasmic domain in this MscS homologue may well 'trap' cations much in the same way as illustrated for EcMscS. Another interesting finding from this study relates to ion permeation via the β-barrel of *E. coli* MscS. A number of *in silico* studies utilizing molecular dynamics indicate that permeation via this narrow hydrophobic pore is unlikely[37]. However, a chimera of the β-barrel region of *E. coli* MscS with the *T. tengcongensis* MscS protein creates a functional protein hinting that permeation may in fact be possible[20].

These slightly different mechanisms may reveal an interesting feature of this family whereby selectivity mechanisms are unique to individual homologues. But a unifying fact is that the structural descriptors of ion selectivity are found in the cytoplasmic domain and not in the transmembrane pore region.

The MscS-like family contains a number of unique members one of the currently most intriguing being MscCG[38-40]. This channel possesses an extra transmembrane domain after the

large cytoplasmic vestibule and exhibits a slight cationic preference (P_{Cl}/P_K – 0.3-0.8)[39, 41]. This protein has been found to be responsible for glutamate efflux under certain conditions (i.e. penicillin or biotin treatment) and is utilised industrially for the production of glutamate [40, 41]. This raises the questions: (i) can the selectivity of this channel be modified to increase glutamate efflux, and (ii) can the selectivity be modified to increase the efflux of other overproduced compounds? Intriguingly, the putative β-barrel structure of MscCG has a lysine at the equivalent position to M273 in EcMscS, which is a major constriction point in the β-barrel of EcMscS. It is possible that rearrangement of the cytoplasmic domain under certain conditions results in permeation via the β-barrel alone and this ring of lysines increases anion (glutamate) selectivity. A recent study indicates that a loop which forms part of the cytoplasmic cage between residues 221-232 containing three negatively charged residues (IAP**E**ILG**E**L**D**VH) is essential for glutamate efflux as ablation of this loop abolishes glutamate efflux[42]. The exact role this loop plays in facilitating glutamate efflux is thus far unknown.

Another interesting development is the reporting of the crystal structure of the MscS homologue from *Helicobacter pylori*[43]. The crystal structure illustrates a homoheptameric assembly of HpMscS thought to correspond to the channel closed state (Fig. 2). Many similar functional residues can be identified including a potentially conserved interaction with a phenylalanine (TM2) and two leucine residues (TM3) shown to be important in MscS mechanosensing[43, 44] (Fig. 6). This is in addition to hydrophobic residues which represent the channel gate (Fig 2. & Fig 6)[45-47]. This channel is yet to be electrophysiologically characterised but has conserved acidic residues in positions shown to be important for EcMscS anion selectivity, namely E187 and E227.

MSL10, an anion selective (P_{Cl}/P_{Na} – 5.9) MscS-like channel homologue expressed in the plant *Arabidopsis thaliana* shows little homology with EcMscS (< 5%) and displays vastly different gating kinetics (Haswell 2012). This makes structural comparisons from sequence alone difficult. There is at least no conservation of the residues proposed to be important for TtMscS and EcMscS selectivity, E278 and E187/E227 respectively. However, this does not preclude a similar selectivity mechanism in this homologue. The proposal has been made that the selectivity profile of this channel is intimately linked with its physiological function. An exciting paradigm is that this channel allows Cl^- efflux potentially culminating in membrane depolarisation and the propagation of an electrical signal[48]. While such a proposition is attractive, assumptions about effects on membrane potential subsequent to

channel gating are difficult when the ionic environment and resting membrane potential are to a large extent unknown.

The MscS-like channel MSC1 expressed in the chloroplasts of the green alga *Chlamydomonas reinhardtii* displays a similar selectivity profile to MSL10 ($P_{Cl}/P_K - 7$)[49]. In addition much like MSL10, there is no conservation of residues purported to be important for selectivity in TtMscS and EcMscS. Interestingly, this channel seems to be intimately involved in chloroplast organisation, which is allegedly dependent on its anion preference. This channel has an N-terminal cleavable sequence, and failure to cleave this sequence results in an inability to incorporate in the *E. coli* membrane meaning this sequence would likely be a targeting sequence for the chloroplast membrane.

Biophysical characterisation provides functional insight

All this structural information and in depth biophysical characterisation of these channels is interesting, however the question becomes are these differences in selectivity physiologically relevant. One attractive proposal for EcMscS is that rather than being an arbitrary biophysical characteristic, its selectivity is as important to its function as K^+ selectivity is to K channels. By honing the selectivity of EcMscS and other MscS-like channels, whose main role is in osmoprotection, over many evolutionary years each organism has matched its MscS-like channel selectivity with the electrochemical gradient for efflux of major internal osmolytes to reduce the impact channel gating has on the resting membrane potential. As previously suggested for EcMscS, this ability to allow neutral efflux of positive and negatively charged solutes prevents any change in membrane potential conserving the ability of H^+-driven ATPases to generate ATP[25]. Any MscS-like channel whose major role is in osmoprotection would need to be able to balance anion and cation efflux in order to have as limited an effect on membrane potential as possible. Other MscS-like channels (MSC1 and MSL10) exhibit higher levels of selectivity that may have evolved over time and may indeed be indicative of their alternate functions within their host organism or may simply be a result of the different electrochemical environment that these respective channels find themselves in. In contrast, the lack of selectivity in MscL is indicative of its imperative role in osmolyte efflux. Its large pore diameter combined with a pressure threshold of activation lying directly below the lytic limit of the cell membrane makes this channel a perfect fit for its physiological role.

Conclusions

While initial inspection of the modest selectivity differences in the MscS famly of channels seems unimportant it is in fact far more interesting. Selectivity studies from these channels have revealed numerous potential selectivity mechanisms within a single channel family. These reports compound the important function of the large cytoplasmic vestibulum and confirm its role in selectivity. In addition, far more than being just a biophysical characteristic, the selectivity of these channels is likely to have been refined over millions of years of evolution to provide channels that can balance osmolyte efflux with minimal effect on membrane potential. Knowledge regarding MscS-like channel selectivity may well be commercially and industrially important particularly in the case of amino acid production (glutamate) using channels such as MscCG.

Recent work suggests that this family of channels, which contains anion and cation selective members, has a diverse set of structural motifs that dictate their selectivity. However, a unifying property is that the structural determinants of selectivity are not housed within the transmembrane region of these channels, as seen for all voltage gated Ca^{2+}, K^{+}, Na^{+} and Cl^{-} channels.

Methods

Bioinformatics

Phylogenetic analysis was carried out using Geneious software. Initial global alignment was carried using a Blosum matrix with the corresponding phylogenetic tree being produced using the Jukes-Cantor genetic distance model. Boot strap proportions are omitted for clarity

Molecular modelling

The MscS crystal structures ([*E. coli*: PDB:2VV5 & 2OAU] [H. pylori: PDB:4HW9] [T. tengcongensis: PDB: 3T9N]) were viewed in Chimera UCSF 1.6 and used to generate Coulombic charge maps at pH 7.2. Coulombic charge maps were generated using standard histidine protonation.

Author contributions

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FIGURE LEGENDS

Figure 1. Mechanosensitive channel function in *E. coli*. In response to a reduction in external osmolarity H_2O floods into bacterial cells resulting in swelling and a corresponding rise in cellular turgor and membrane tension. This rise in membrane tension first gates MscS and then MscL (immediately below the lytic limit of the cell membrane) allowing the efflux of intracellular osmolytes thus relieving this pressure and preventing cellular lysis (left panel). In the absence of MS channels (MscS and MscL) this rise in membrane tension is left unchecked and results in cell lysis (right panel).

Figure 2. Structure of three crystallised MscS family members. Crystal structures of *E. coli* MscS (EcMscS), *Helicobacter pylori* MscS (HpMscS) and *Thermoanaerobacter tengcongensis* (TtMscS). Upper panel is a side view illustrating characteristic MscS family structure including a large water-filled C-terminal domain. The lower panel provides a periplasmic view of the respective channels with the residues coloured in grey likely forming the vapour lock gates (EcMscS – L105 & L109, HpMscS – I94 & L98, TtMscS - L104, F108).

Figure 3. MscS subfamily phylogenetic tree displaying nine electrophysiologically charactertisedhomologues. The reported anion-cation permeability ratios expressed as P_{Cl}/P_K are illustrated along with whether these channels display inactivation. Bar represents 0.1 substitutions per site. (Green = archaea, Black = bacteria, Red = eukaryotes)

Figure 4. Comparison between EcMscS and Tt MscS cytoplasmic domains. Upper panel shows a Coulombic charge distribution of a transverse section as viewed from the periplasmic side of TtMscS (PDB: 3T9N; left side) and EcMscS (PDB: 2VV5; right side). Regions of electronegativity (denoted as red) can be identified centred around D226/D229 and E187/E227, respectively. The lower panel shows the underside of the cytoplasmic domain of TtMscS (right side) EcMscS (left side) highlighting the β-barrel residue implicated in anion selectivity in TtMscS ((kcal/(mol·*e*) at 298 K).

Figure 5. Graphic illustration of the proposed selectivity mechanisms of *E. coli* MscS and *T. tengcongensis* MscS. (A) An electronegative region centred around E187 and E227 on the floor of the cytoplasmic domain 'traps' cations resulting in easier transit for anions. **(B)** A residue on the outside of the β-barrel 'E278' likely traps cations making an environment conducive to anion conduction. This is in addition to an electronegative region on the floor of the cytoplasmic domain that likely 'traps' permeating cations in a similar manner to EcMscS, which results in higher anion selectivity of TtMscS compared to EcMscS (Fig. 4).

Figure 6. Conserved tension transmitting residues in EcMscS, HpMscS and TtMscS. (A) Open structure of EcMscS showing close association of F68 and L111/115. **(B)** Closed/inactivated structure of EcMscS showing dissociation of tension transmitting residues. **(C)** Illustration of similar position of a phenylalanine residue and leucine residues in closed structure of HpMscS. **(D)** In the closed structure of TtMscS the location of these residues may be switched. However multiple alternative hydrophobic residues are present that may interact in a similar way to F68 and L111/115 in EcMscS.

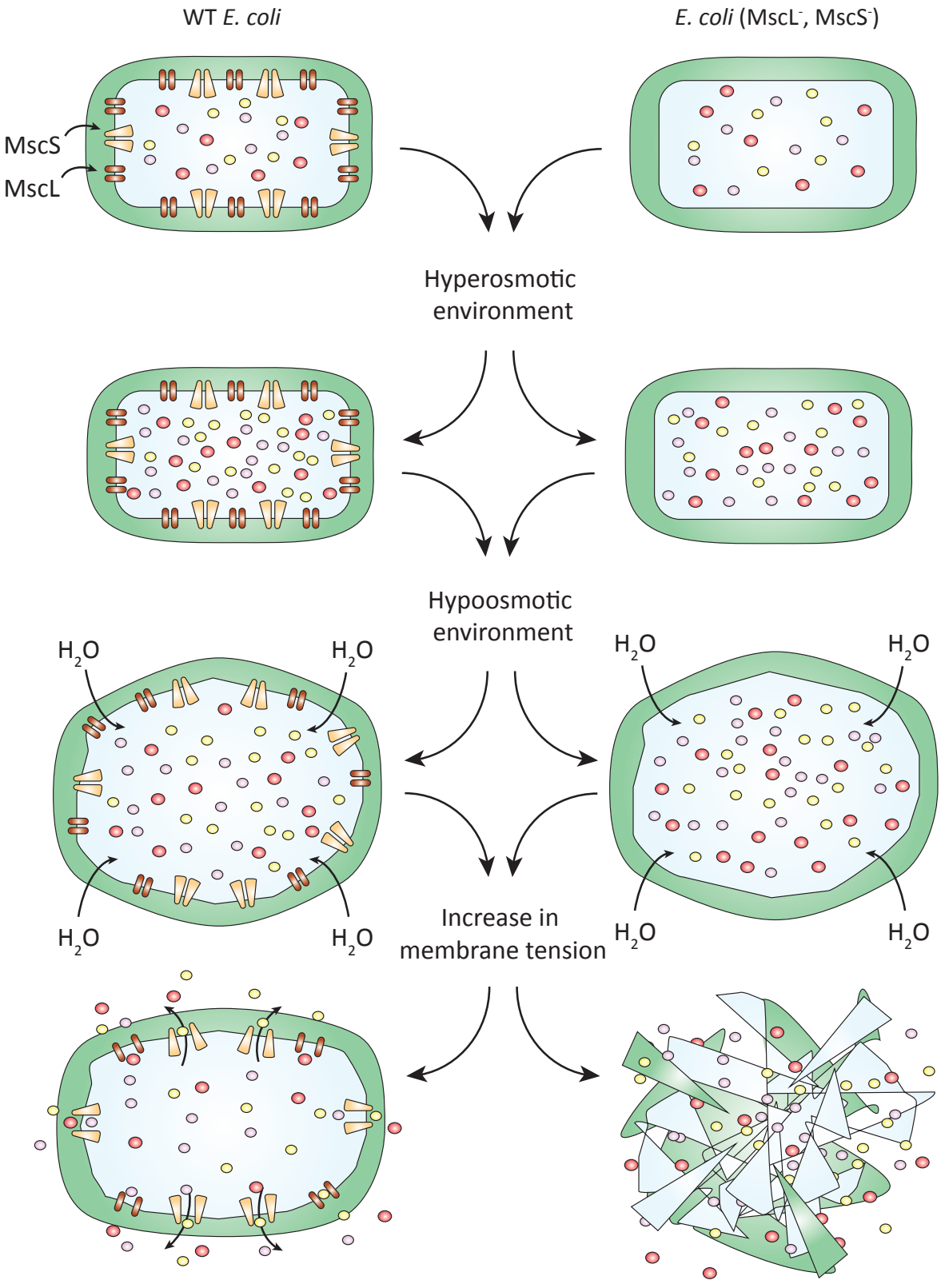
References (Max 150)

1. Martinac B, Buechner M, Delcour AH, Adler J, Kung C. Pressure-sensitive ion channel in Escherichia coli. Proc Natl Acad Sci U S A. 1987 Apr;84(8):2297-301.
2. Pivetti CD, Yen MR, Miller S, Busch W, Tseng YH, Booth IR, et al. Two families of mechanosensitive channel proteins. Microbiol Mol Biol Rev. 2003 Mar;67(1):66-85, table of contents.
3. Levina N, Totemeyer S, Stokes NR, Louis P, Jones MA, 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 Apr 1;18(7):1730-7.
4. Li Y, Moe PC, Chandrasekaran S, Booth IR, Blount P. Ionic regulation of MscK, a mechanosensitive channel from Escherichia coli. EMBO J. 2002 Oct 15;21(20):5323-30.
5. Booth IR, Blount P. The MscS and MscL families of mechanosensitive channels act as emergency release valves. Journal of Bacteriology. 2012;194:4802-9.
6. Malcolm HR, Maurer JA. The mechanosensitive channel of small conductance (MscS) superfamily: not just mechanosensitive channels anymore. . Chembiochem 2012;13:2037-43.
7. Nakayama Y, Fujiu K, Sokabe M, Yoshimura K. Molecular and electrophysiological characterization of a mechanosensitive channel expressed in the chloroplasts of Chlamydomonas. Proc Natl Acad Sci U S A. 2007 Apr 3;104(14):5883-8.
8. Maksaev G, Haswell ES. MscS-Like10 is a stretch-activated ion channel from Arabidopsis thaliana with a preference for anions. Proc Natl Acad Sci U S A. 2012 Nov 13;109(46):19015-20.
9. Nakayama Y, Yoshimura K, Iida H. Organellar mechanosensitive channels in fission yeast regulate the hypo-osmotic shock response Nature Comms. 2012;3.

10. Naismith JH, Booth IR. Bacterial mechanosensitive channels - MscS: Evolution's solution to creating sensitivity in function. Annual Review of Biophysics. 2012;41:157-77.
11. Bass R, Strop P, Barclay MT, Rees DC. Crystal structure of Escherichia coli, MscS, a voltage-modulated and mechanosensitive channel. Science. 2002;298:1582-7.
12. Wang W, Black SS, Edwards MD, Miller S, Morrison EL, Bartlett W, et al. The structure of an open form of an E. coli mechanosensitive channel at 3.45 A resolution. Science. 2008;321(5893):1179-83.
13. Li C, Edwards MD, Jeong H, Roth J, Booth IR. Identification of mutations that alter the gating of the Escherichia coli mechanosensitive channel protein, MscK. Molecular Microbiology. 2007 Apr;64(2):560-74.
14. Li YZ, Moe PC, Chandrasekaran S, Booth IR, Blount P. Ionic regulation of MscK, a mechanosensitive channel from Escherichia coli. EMBO J. 2002 Oct 15;21(20):5323-30.
15. Petrov E, Palanivelu D, Rohde PR, Cox C.D, Constantine M, Nomura T, et al. Patch-Clamp characterization of the MscS-Like mechanosensitive channel from Silicobacter Pomeroyi
Biophys J. 2013;104:1426-34.
16. Cox CD, Nomura T, Ziegler CS, Campbell AK, Wann KT, Martinac B. Selectivity mechanism of the mechanosensitive channel MscS revealed by probing channel subconducting states. Nat Comms. 2013 Jul;4.
17. Edwards MD, Black S, Rasmussen T, Rasmussen A, Stokes NR, Stephen T-L, et al. Characterization of three novel mechanosensitive channel activities in Escherichia coli. Channels. 2012 Jul-Aug;6(4):272-81.
18. Levina N, Totemeyer S, Stokes NR, Louis P, Jones MA, 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 Apr 1;18(7):1730-7.
19. 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 angstrom and 4.1 angstrom resolutions. Protein Sci. 2013 Apr;22(4):502-9.
20. Zhang X, Wang J, Feng Y, Ge J, Li W, Sun W, et al. Structure and molecular mechanism of an anion-selective mechanosensitive channel of small conductance. Proc Natl Acad Sci U S A. 2012 Oct 30;109(44):18180-5.
21. Bass RB, Strop, P., Barclay, M., and Rees, D. . Crystal structure of Escherichia coli MscS, a voltage-modulated and mechanosensitive channel. Science. 2002;298:1582-7.
22. Steinbacher S, Bass R, Strop P, Rees DC. Structures of the prokaryotic mechanosensitive channels MscL and MscS. Current Topics in Mechanosensitive Ion Channels. Current Topics in Mechanosensitive Ion Channels
2007;Part A. 58:1-24.
23. Nomura T, Sokabe M, Yoshimura K. Interaction between the cytoplasmic and transmembrane domains of the mechanosensitive channel MscS. Biophys J. 2008 Mar 1;94(5):1638-45.
24. Koprowski P, Kubalski A. C termini of the escherichia coli mechanosensitive ion cahnnel (MscS) move apart upon the channel opening J Biol Chem. 2003;88:3050-9.
25. Gamini R, Sotomayor M, Chipot C, Schulten K. Cytoplasmic Domain Filter Function in the Mechanosensitive Channel ofÂ Small Conductance. Biophys J. 2011;101(1):80-9.
26. Koprowski P, Grajkowski W, Isacoff EY, Kubalski A. Genetic Screen for Potassium Leaky Small Mechanosensitive Channels (MscS) in Escherichia coli: Recognition of the cytoplasmic beta domain as a new gating element. J Biol Chem. 2011 Jan 7;286(1):877-88.

27. Machiyama H, Tatsumi H, Sokabe M. Structural Changes in the Cytoplasmic Domain of the Mechanosensitive Channel MscS During Opening. Biophysical Journal. 2009 Aug 19;97(4):1048-57.
28. Rowe I, Elahi M, Huq A, Sukharev S. The mechanoelectrical response of the cytoplasmic membrane of Vibrio cholerae. J Gen Physiol. 2013;142(1):75-85.
29. Sotomayor M, van der Straaten TA, Ravaioli U, Schulten K. Electrostatic Properties of the Mechanosensitive Channel of Small Conductance MscS. Biophys J. 2006;90(10):3496-510.
30. Edwards MD, Bartlett W, Booth IR. Pore mutations of the Escherichia coli MscS channel affect desensitization but not ionic preference. Biophys J. 2008 Apr 15;94(8):3003-13.
31. Maksaev G, Haswell ES. Recent characterizations of MscS and its homologs provide insight into the basis of ion selectivity in mechanosensitive channels. Channels. 2013 May 1;7(3):215-20.
32. Li Y, Moe PC, Chandrasekaran S, Booth IR and Blount P. Ionic regulation of MscK, a mechanosensitive channel from Escherichia coli. EMBO J Oct;21:5323-5330.
33. Sukharev S. Purification of the small mechanosensitive channel of Escherichia coli (MscS): the subunit structure, conduction, and gating characteristics in liposomes. Biophys J. 2002 Jul;83(1):290-8.
34. Sugiharto S, Lewis TM, Moorhouse AJ, Schofield PR, Barry PH. Anion-Cation Permeability Correlates with Hydrated Counterion Size in Glycine Receptor Channels. Biophys J. 2008;95(10):4698-715.
35. Schumann U, Edwards MD, Rasmussen T, Bartlett W, van West P, Booth IR. YbdG in Escherichia coli is a threshold-setting mechanosensitive channel with MscM activity. Proc Natl Acad Sci U S A. 2010 Jul 13;107(28):12664-9.
36. Berrier C, Besnard M, Ajouz B, Coulombe A, Ghazi A. Multiple mechanosensitive ion channels from Escherichia coli, activated at different thresholds of applied pressure. J Membr Biol. 1996 May;151(2):175-87.
37. Vora T, Corry B, Chung SH. Brownian dynamics investigation into the conductance state of the MscS channel crystal structure. Biochim Biophys Acta. 2006;1758:730-7.
38. Hashimoto K-i, Nakamura K, Kuroda T, Yabe I, Nakamatsu T, Kawasaki H. The Protein Encoded by NCgl1221 in Corynebacterium glutamicum Functions as a Mechanosensitive Channel. Biosci Biotech Biochem. 2010 Dec;74(12):2546-9.
39. Nakayama Y, Yoshimura K, Iida H. Electrophysiological Characterization of the Mechanosensitive Channel MscCG in Corynebacterium glutamicum. Biophys J. 2013 2013-Sep-17;105(6):1366-75.
40. Becker M, Boerngen K, Nomura T, Battle AR, Marin K, Martinac B, et al. Glutamate efflux mediated by Corynebacterium glutamicum MscCG, Escherichia coli MscS, and their derivatives. Biochimica Et Biophysica Acta-Biomembranes. 2013 Apr;1828(4):1230-40.
41. Borngen K, Battle AR, Moker N, Morbach S, Marin K, Martinac B, et al. The properties and contribution of the Corynebacterium glutamicum MscS variant to fine-tuning of osmotic adaptation. Biochimica et Biophysica Acta (BBA) - Biomembranes. 2010;1798(11):2141-9.
42. Yamashita C, Hashimoto K-i, Kumagai K, Maeda T, Takada A, Yabe I, et al. L-Glutamate Secretion by the N-Terminal Domain of the Corynebacterium glutamicum NCgl1221 Mechanosensitive Channel. Biosci Biotech and Biochem. 2013 May;77(5):1008-13.
43. Lai JY, Poon YS, Kaiser JT, Reed DC. Open and shut: crystal structures of the dodecylmaltoside solubilized mechanosensitive channel of small conductance from

Escherichia coli and Helicobacter pylori at 4.4 A and 4.1 A resolutions. Protein Sci. 2013;22(502-509).
44. Belyy V, Anishkin A, Kamaraju K, Liu N, Sukharev S. The tension-transmitting 'clutch' in the mechanosensitive channel MscS. Nat Struct Mol Biol. 2010;17:451-9.
45. Anishkin A, Sukharev S. Water dynamics and dewetting transitions in the small mechanosensitive channel MscS. Biophys J. 2004;86:2883-95.
46. Beckstein O, Biggin PC, Sansom MSP. A hydrophobic gating mechanism for nanopores. J Phys Chem. 2001 Dec 27;105(51):12902-5.
47. Beckstein O, Sansom MSP. Liquid-vapor oscillations of water in hydrophobic nanopores. Proc Natl Acad Sci U S A. 2003 Jun 10;100(12):7063-8.
48. Maksaev G, Haswell ES. MscS-like10 is a stretch-activated ion channel from Arabidopsis thaliana with a preference for anions. Proc Natl Acad Sci U S A. 2012;109:19015-20.
49. Nakayama Y, Fujiu K, Sokabe M, Yoshimura K. Molecular and electrophysiological characterization of a mechanosensitive channel expressed in the chloroplasts of Chlamydomonas. Proc Natl Acad Sci U S A. 2007 Apr;104(14):5883-8.


WT E. coli
E. coli (MscL-, MscS-)
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H2O
H2O
H2O
H2O
H2O
H2O
Increase in membrane tension

FIGURE 1

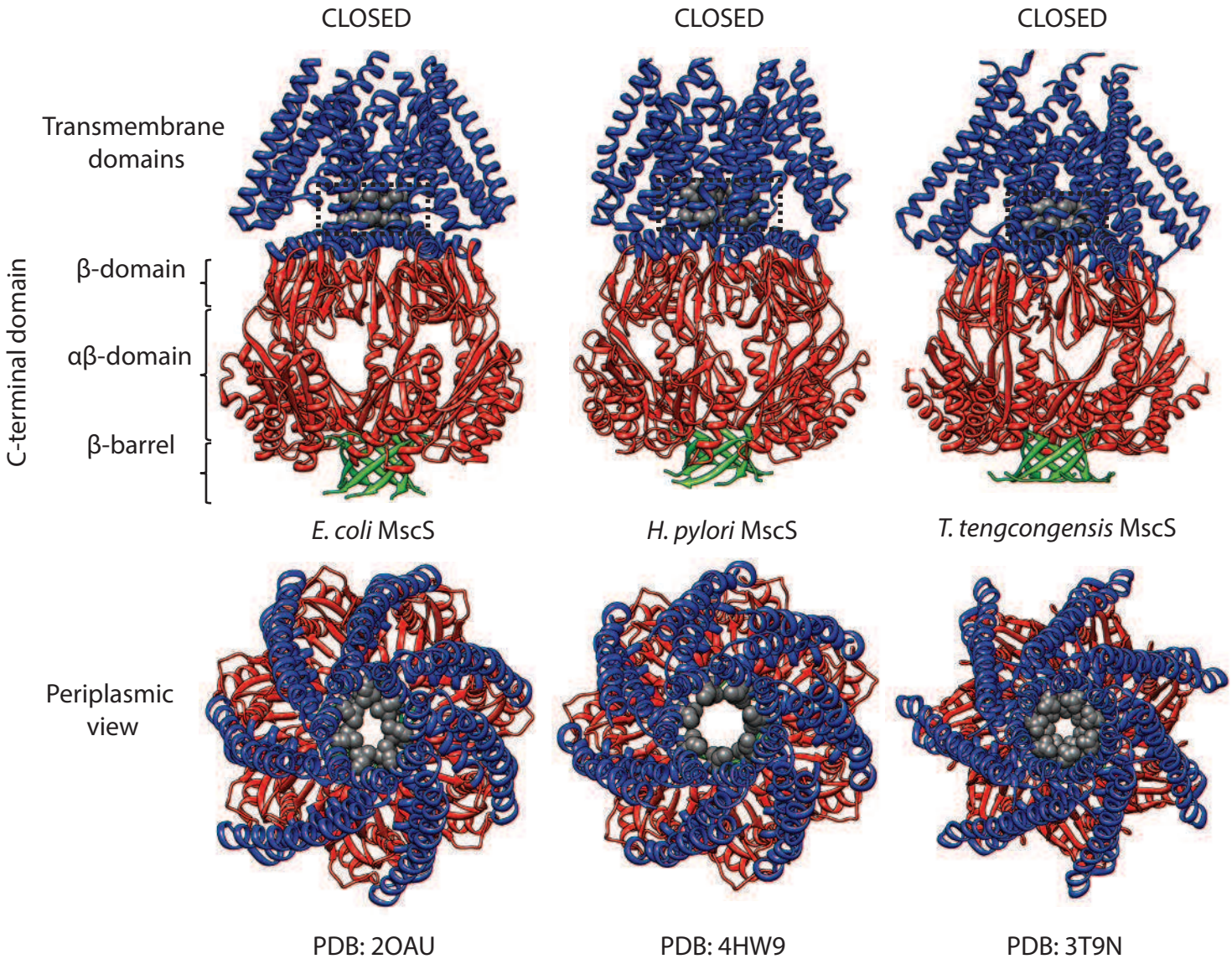
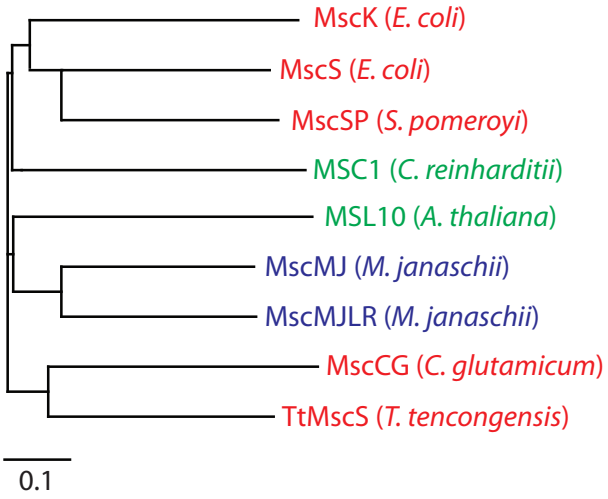

CLOSED
CLOSED
CLOSED
Transmembrane domains
C-terminal domain
β-domain
αβ-domain
β-barrel
E. coli MscS
H. pylori MscS
T. tengcongensis MscS
Periplasmic view
PDB: 2OAU
PDB: 4HW9
PDB: 3T9N

FIGURE 2



	Selectivity P_{Cl}/P_K	***Inactivation***
MscK (*E. coli*)	~1	No
MscS (*E. coli*)	1.2 - 3	Yes
MscSP (*S. pomeroyi*)	1.4	No
MSC1 (*C. reinharditii*)	7	Yes
MSL10 (*A. thaliana*)	5.9*	No
MscMJ (*M. janaschii*)	0.17	No
MscMJLR (*M. janaschii*)	0.20	No
MscCG (*C. glutamicum*)	0.3	No
TtMscS (*T. tencongensis*)	~9	No

* This value is P_{Cl}/P_{Na} rather than P_{Cl}/P_K

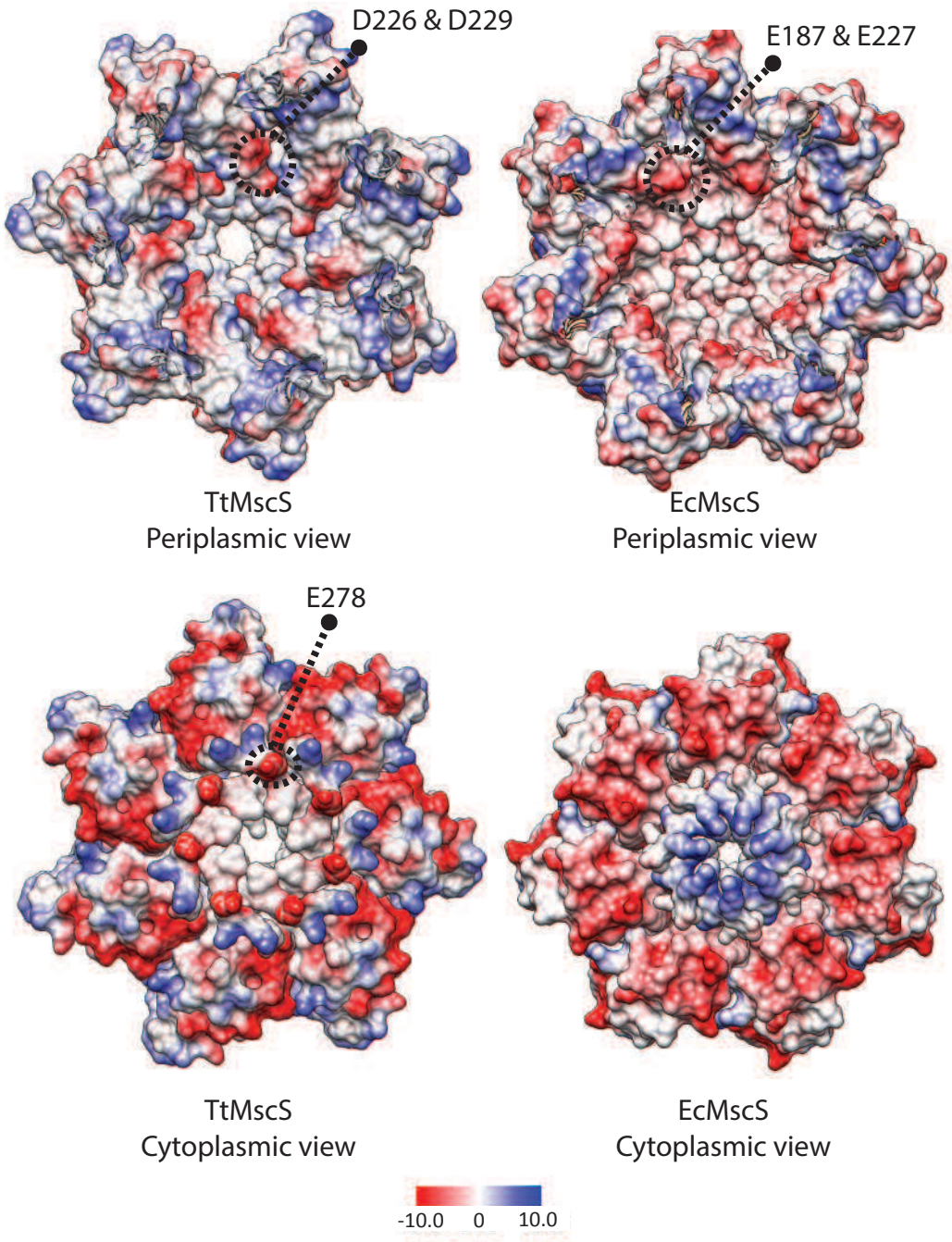
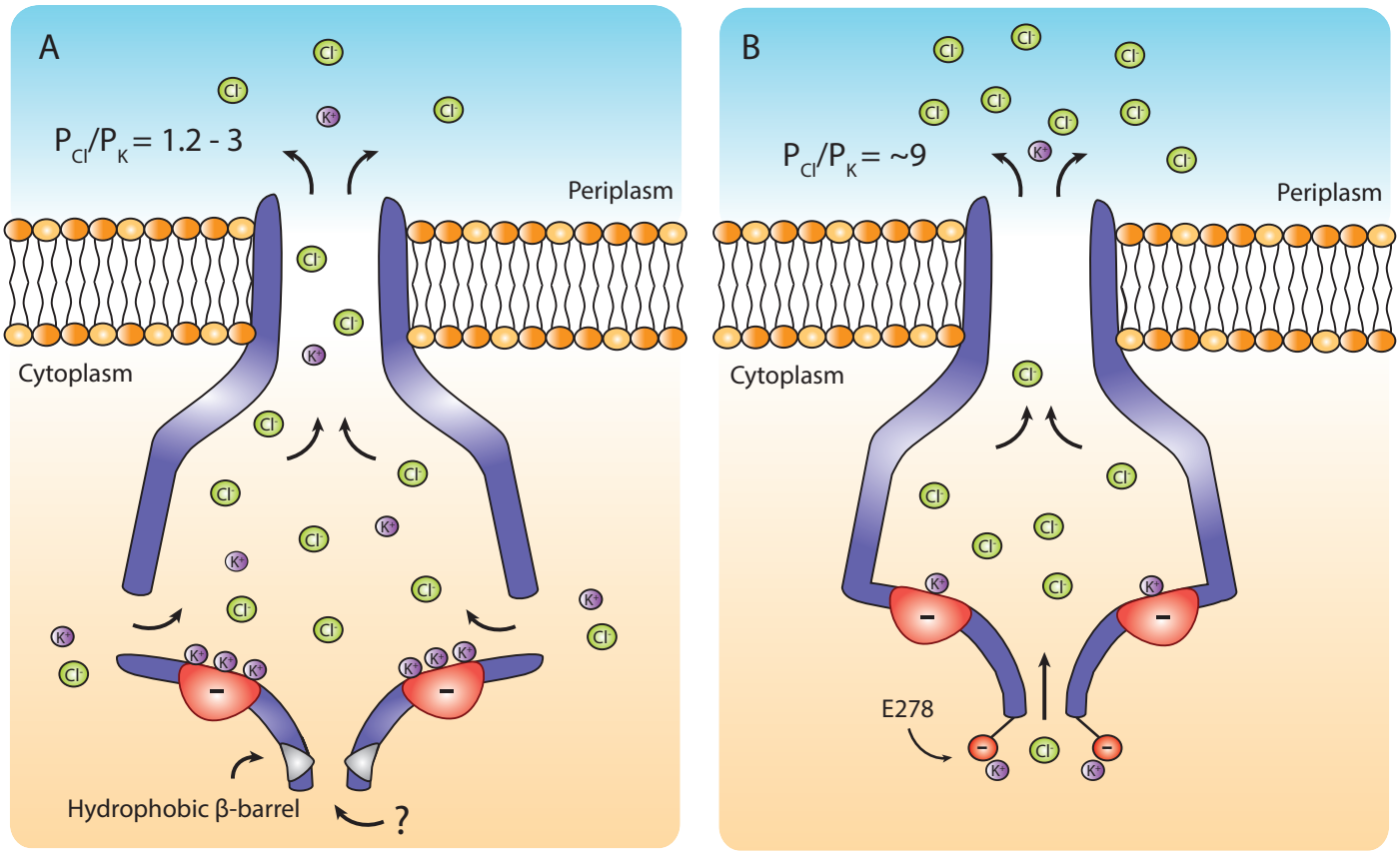

D226 & D229
E187 & E227
TtMscS
Periplasmic view
EcMscS
Periplasmic view
E278
TtMscS
Cytoplasmic view
EcMscS
Cytoplasmic view
-10.0
0
10.0

FIGURE 4



EcMscS

TtMscS

FIGURE 5

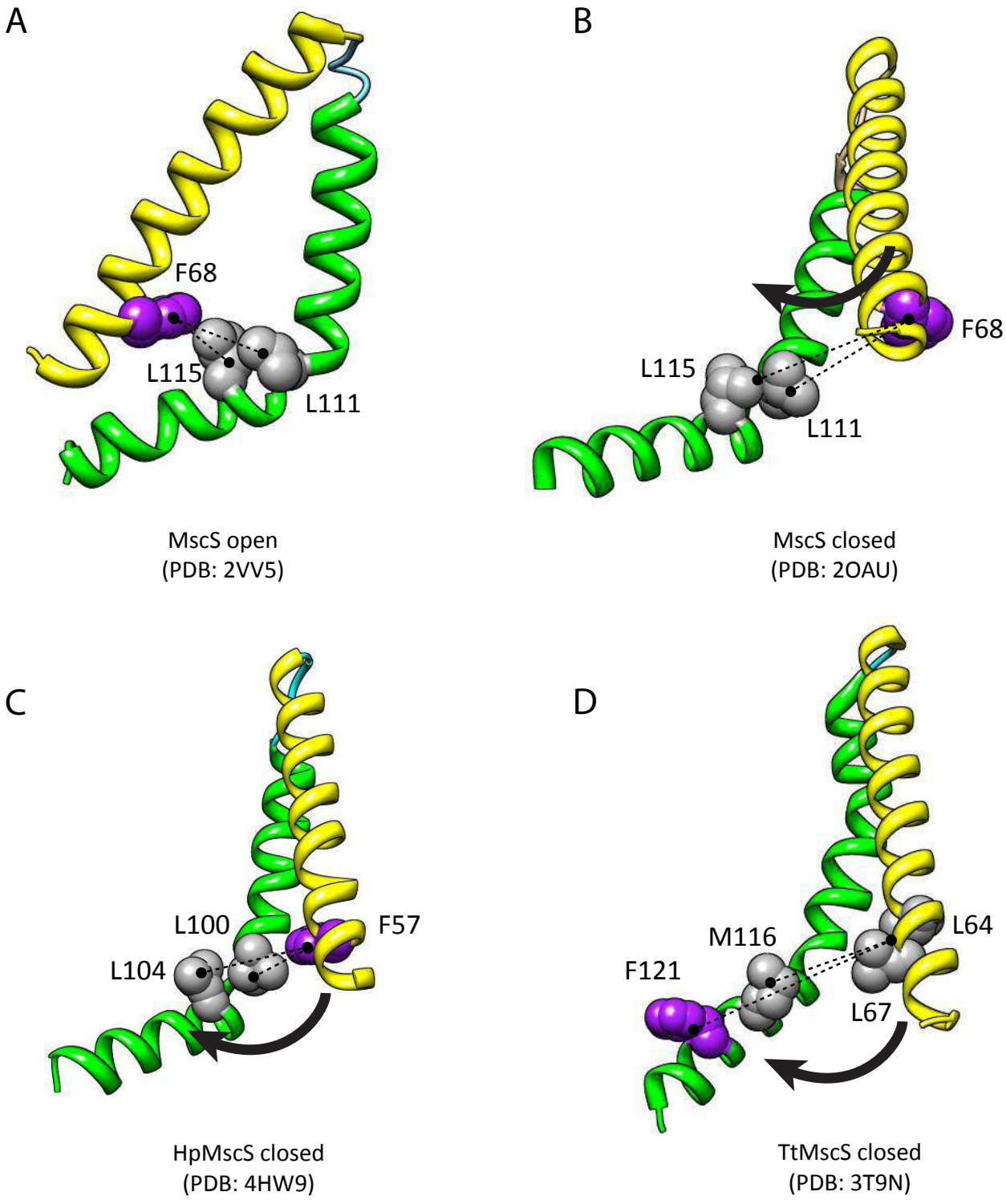

A
F68
L115
L111
MscS open
(PDB: 2VV5)
B
F68
L115
L111
MscS closed
(PDB: 2OAU)
C
L100
F57
L104
HpMscS closed
(PDB: 4HW9)
D
M116
L64
F121
L67
TtMscS closed
(PDB: 3T9N)

Figure 6