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Feeling the tension: the bacterial mechanosensitive channel of large conductance as a model system and drug target

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The drug-resistance crisis has become dire and new antibiotic targets and strategies are required. Mechanosensitive channel of large conductance (MscL) is a conserved bacterial mechanosensitive channel that plays the role of 'osmotic-emergency-release-valve. It has the largest-gated pore known allowing osmoprotectants out, and other compounds into the cell. Inappropriate gating of the channel can lead to slow growth, decreased viability, and an increase in potency for many antibiotics. The 'membrane permeability' observed for some antibiotics, including streptomycin, is mediated by directly binding to and activating MscL. Novel compounds that are MscL agonists have also recently been isolated. Although the compounds are diverse, the binding sites of all characterized MscL-specific agonists are within the same general region of the MscL complex, leading to an *in silico* screening for compounds that bind this region. In sum, these studies demonstrate that MscL is a viable drug target that may lead to a new generation of antibiotics and adjuvants.

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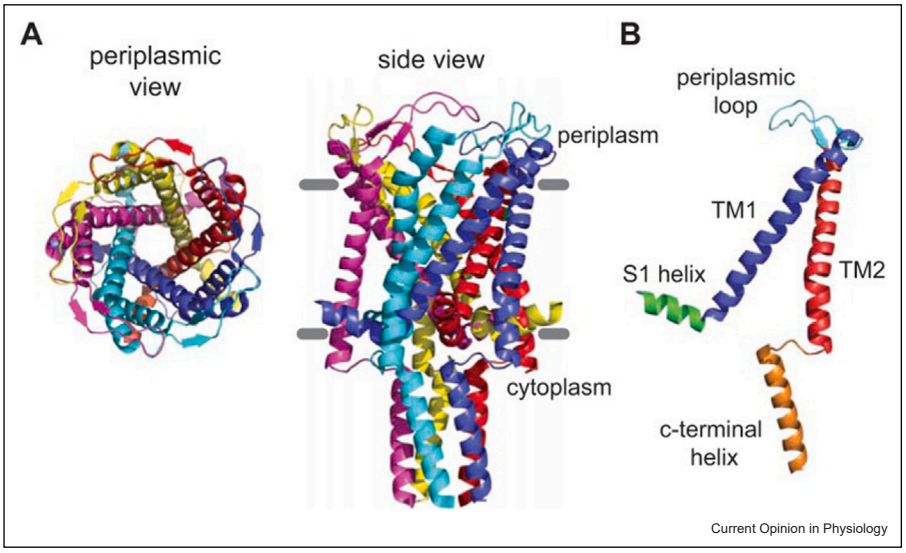
Introduction

Bacterial mechanosensitive channels were first identified by patch clamp of *Escherichia coli* native membranes [1]. We now know that there are two families of channels within bacteria, mechanosensitive channel of large conductance (MscL) and those genetically such as the *E. coli* small or smaller conductance (MscS); the latter family is large and diverse and often has several paralogs within any given species (*E. coli* has six [2]). The MscS family members appear to have variations on the theme, for example, MscK in *E. coli* is only active in high-potassium concentrations [3], and for many, their exact physiological conditions in which they function *in vivo* remain obscure [2]. In contrast, MscL is highly conserved [4] and although is found in the vast majority of bacteria, has only a single member per species [5].

The physiological function of bacterial mechanosensitive channels is that of biological emergency release valves [5]. In high osmolarity, compatible solutes are accumulated as osmoprotectants within the cytoplasm to keep cell turgor high, which is required for cell growth (for *E. coli*, about 2–4 atmospheres cytoplasmic pressure [6], but see Ref. [7]). Upon acute decreases in external osmolarity, the pressure within the cell climbs, leading to membrane tension that gates the channels and a rapid efflux of the accumulated solutes as well as other metabolites ([8–10] and reviews [5,11–17]). The MscS-styled channels within the cell open at lower membrane tensions [18], and MscL is the last-ditch-effort to release excess turgor. MscL forms the largest-gated pore known, estimated to be close to 30 Å [19,20], thus allowing release of larger, more valuable metabolites, making it a more desirable drug target; it is therefore the topic of this review.

MscL is a homopentamer (structure/function reviewed here: [17•]). To date, there are only two species with valid X-ray crystal structures [21–23] and none from cryo-EM. The *Mycobacterium tuberculosis* structure is shown in Fig. 1. At the N-terminus is an amphipathic α-helix, S1, that serves as a 'slide helix' stabilizing the first transmembrane domain (TM1) upon gating [17,24–26], S1 is connected via a glycine hinge to the pore-forming TM1 [27] constricting at the cytoplasm [21–23], the periplasmic loop serves as a spring element [17,28–31], and TM2 interacts with the lipids [32•–34] and ends in with a series of charged residues or 'knot in the rope' that guides TM2 movements [35]. Finally, the subunit ends in a helical bundle that does not separate upon channel gating [36,37]. Although there are currently no structures of the open state, evidence suggests that it

Figure 1



Structure of the *M. tuberculosis* MscL derived from X-ray crystallography. **(a)** MscL pentamer shown from a periplasmic view (left) and a side view (right) in which the approximate membrane location is indicated by gray lines. **(b)** A single isolated subunit is represented in which different MscL domains are indicated: N-terminal helix, called S1 helix (green), TM1 (blue), periplasmic loop (cyan), second transmembrane domain TM2 (red), and C-terminal helix (orange). Figure and legend originally published in Ref. [17], Copyright © 2020 American Society for Microbiology.

forms a huge pore size of about 30 Å [19,20], and TM1 and TM2 angles, thinning the membrane [19,38], and corkscrewing within it [19,39,40]. Of special interest here are interactions between the S1/TM1 region of one subunit with TM2 of another at the cytoplasmic interface: these change substantially upon gating [41•]. It is this interface that is the binding site of all known specific MscL agonists found to date.

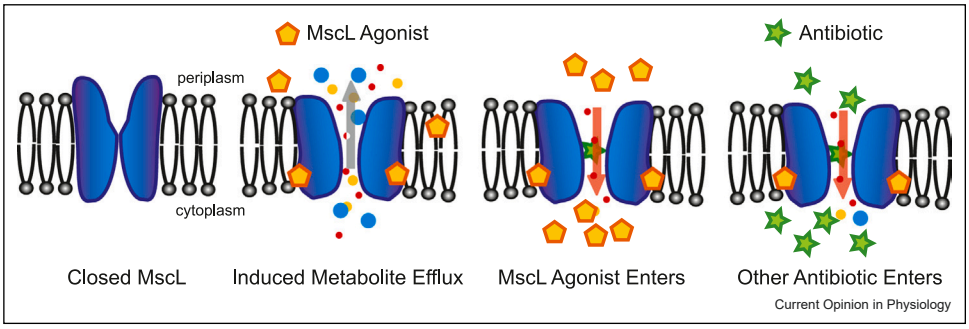
The arguments for mechanosensitive channel of large conductance as a drug target

From the first observation, it appeared that bacterial mechanosensitive channels sensed tension in the membrane [1]: this has been well substantiated [42,43]. While interactions with specific lipid headgroups have been proposed in some systems [44,45], they do not appear to be necessary in most species. It is the changes in the membrane tension profile that are important [42]. Microbial mechanosensitive channels were the first poster-children for what has been coined as the 'Force From Lipid' (FFL) hypothesis: upon membrane tension, it is the changing lipid interactions with the protein that serve as the energy for channel gating (see Ref. [46] for a recent historical perspective and review). Recently, the structural elements for ion passage through MscL have been identified by a series of equilibrium and steered molecular dynamics simulations [47•], and the FFL model has been supported by cryo-EM structures for MscS under membrane tension [48••].

Given the physiological function of MscL and the size of its open pore, it makes sense that it is tightly regulated. Amphipaths and other compounds change the tensions within the membrane-gated bacterial channels and were detrimental to the cell, but were also nonspecific [17,49]. An early forward-genetics study isolating gain-of-function mutants demonstrated that inappropriate MscL gating slowed cell growth and decreased viability [27]. Because the MscL pore is so large, it essentially permeabilizes the cell when it opens, allowing metabolisms out, and possibly drugs in. This led to the questions: could this be done pharmacologically? Could "specific" MscL agonists be found? And could they lead to novel antibiotics that would help to alleviate the antibiotic-resistance crisis?

In addition to the observation that inappropriate gating of the channel was detrimental to bacteria, there are other reasons to believe that MscL is a viable drug target. There are indications that bacterial mechanosensitive channels play a role in pathogen virulence, host colonization, and transitioning between the host and environment and back (reviewed in Ref. [50••]). MscL is unique to microbes, not found in mammals, is highly conserved, and found in most bacterial species, including pathogens [5]. MscL channels are expressed in all phases of bacterial growth, even upregulated in stationary phase [51], and do not require cellular metabolism or any cellular energy source; consequently, stationary-phase cultures, biofilms, quiescent cells, and

Figure 2



Three mechanisms by which a MscL agonist could have antibacterial activity. To the left, the MscL channel is normally tightly regulated and thus closed, unless there is gating tension within the membrane. The second panel shows that a MscL agonist can lead to undesirable fluxes of osmolytes and metabolites from the cell. The third panel shows that the MscL agonist can enter the cell through MscL where it can potentially have a second mode of action. The fourth and final panel shows that other antibiotics that target cytoplasmic proteins can enter the cell through MscL, thus, the agonist can serve as an adjuvant that increases potency and specificity of other antibiotics.

nodules should be susceptible. One study identified MscL as one of the top 20 potential drug targets [52].

We believe the most effective way to target MscL would be by agonists that increase channel gating, rather than antagonists or blockers that may decrease pathogen transmission. As shown in Fig. 2, there are three potential modes by which an agonist may serve an antimicrobial function: (1) allow efflux of valuable metabolites; (2) allow the passage of the agonist into the cytoplasm where it may have other targets, that is, dual-targeting compounds; (3) allow passage of other drugs/antibiotics that have cytoplasmic targets, thus increasing their specificity and potency and serving as an adjuvant. As shown in the following sections, each of these have been observed.

A high-throughput screen

To find MscL agonists, we performed a high-throughput screen (HTS) and ultimately assayed for compounds that decreased *E. coli* growth in a MscL-dependent manner [53]. Many compounds that also decreased growth in a MscS-dependent manner were discarded because of nonspecificity that indicated the compound may simply change membrane tension.

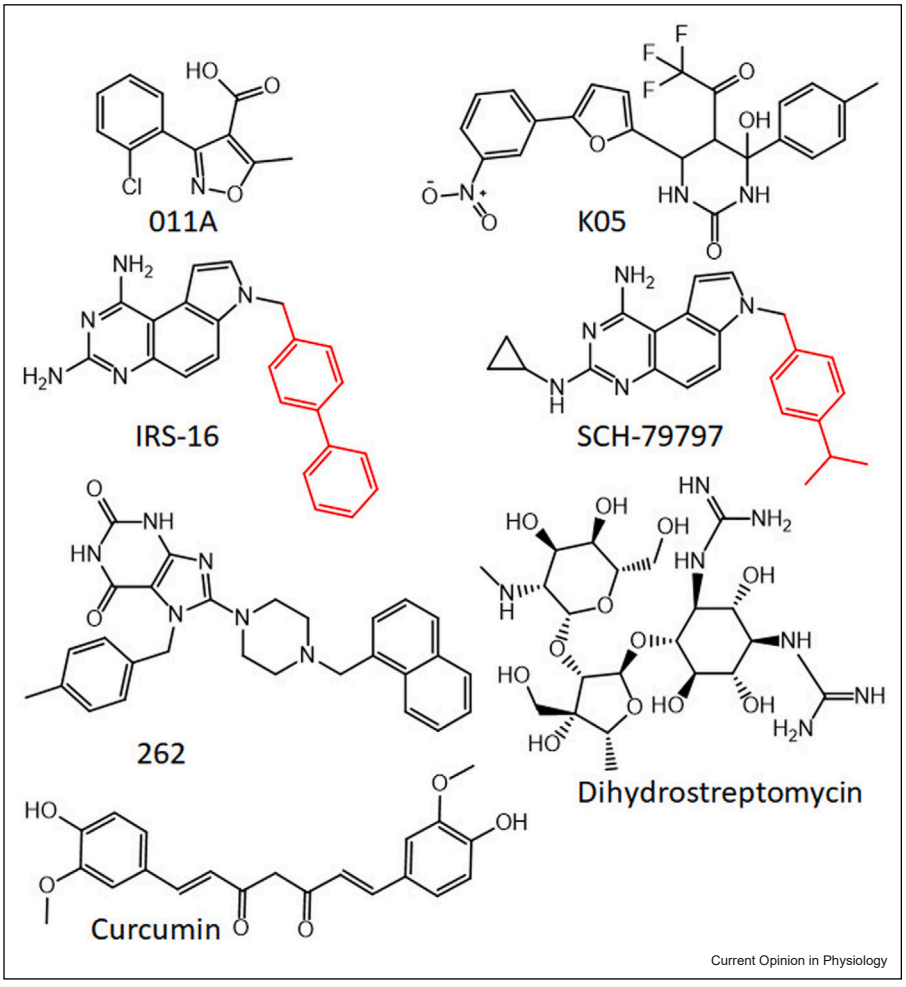
Known antibiotics are mechanosensitive channel of large conductance agonists?

Surprisingly, four known antibiotics, including the aminoglycosides spectinomycin and dihydrostreptomycin (DHS), were MscL-specific 'hits' at the concentration used in the HTS. Upon scrutiny, it became clear that MscL expression increased the potency of the drugs [53]. Further study of DHS solved two 50-year-old mysteries [54••]. First, how does this bulky and highly charged compound cross the membrane? Some mechanisms were previously proposed, but this work showed that MscL is a primary pathway into the cytoplasm. Second, in the early 1960s, it was shown that upon streptomycin treatment, an efflux of potassium occurred before any decrease in viability [55]. We showed that this early efflux, as well as that of glutamate, another accumulated osmoprotectant, was MscL-dependent. In sum, the data demonstrated that DHS directly and specifically binds to and activates MscL. The binding site is deep within the pore, near the cytoplasmic interface where S1/TM1 of one subunit slides along the TM2 of another. After DHS causes effluxes of potassium and glutamate via the open MscL, the drug then uses the open pore to pass into the cytoplasm [54]. Consistent with these findings, there is also recent evidence that MscL is gated upon cell freezing, which can induce aminoglycoside uptake and potentiation [56]. MscL is likely a pathway to the cytoplasm for other antibiotics [53].

Novel compounds from the high-throughput screen

Thus far, two compounds identified from the HTS have been well characterized: 011A [57,58] and K05 [59]. In both instances, *E. coli* cell growth and viability decrease upon treatment, they increase MscL activity as determined by patch clamp, they work on multiple species, they can act as adjuvants increasing the potency of antibiotics, and they bind to the same general location, close to where S1/TM1 slides along the TM2 of another subunit upon channel opening — the hydrophobic nature of the compounds appears to allow them to pass through the membrane for the initial access to this site [57]. However, there are differences between the two agonists in structure and *in vivo* and computational studies where even a single binding in the homopentamer triggered movements consistent with initial gate opening [57,59]. These data were the first to indicate

Figure 3



2D structures of some known agonists and antibiotics. Most agonists shown in this figure allosterically bind to the S1/TM1 region to facilitate MscL channel open. Note that the leftmost portion of SCH-79797 and IRS-16 is associated with disrupting the folate pathway, while the rightmost 'cumene' of the former and the dibenzyl structure in IRS-16 (both highlighted in red) are thought to be associated with MscL activation and thus membrane permeabilization. Direct binding and potential binding sites for curcumin have yet to be determined.

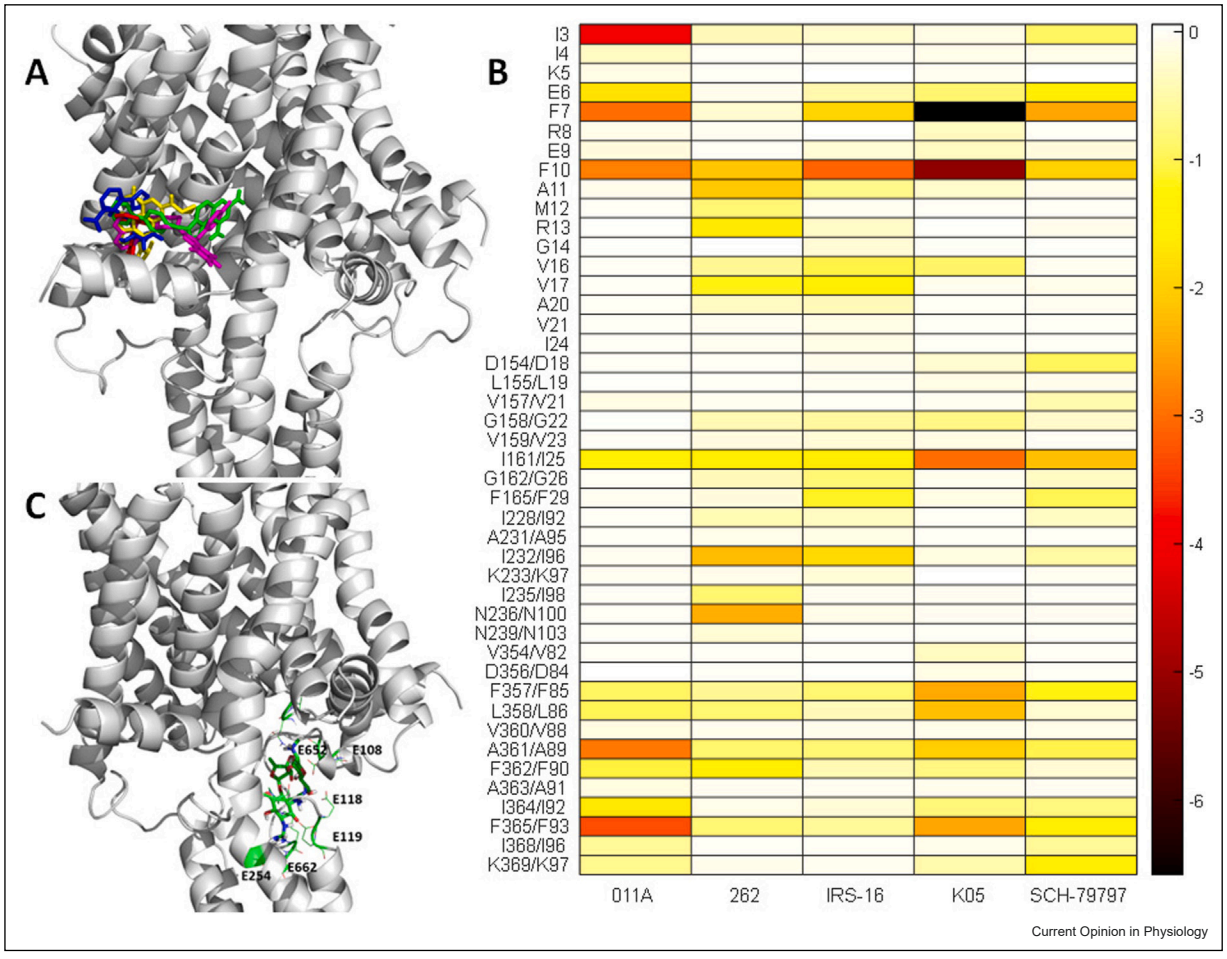
that there may be multiple families of compounds that bind the same general location but have slightly different agonist properties.

Dual-targeting compounds

One of the ways to avoid drug resistance is to combine drugs with different modes of action, or better yet, combine two modes of action within a single compound. We have been on the lookout for these 'dual-targeting' compounds where one of the modes of action involves the specific 'permeabilization' of membranes by some undefined mechanism. This has led to two discoveries where MscL activation is one of the modes of action, leading to membrane permeabilization: one is curcumin, the other is SCH-79797 and related compounds.

Curcumin is a flavonoid polyphenol isolated from the rhizome of turmeric (*Curcuma longa*) that has been shown to have antibacterial properties [60]. It has three physiological effects in bacteria: an apoptosis-like response involving RecA [61], inhibiting septation [62], and membrane permeabilization [63]. It also works synergistically with other antibiotics [64–66]. We found that both MscL and RecA are required for curcumin-dependent decreases in growth and viability, but that even though little if any decrease in growth is observed in RecA-minus/MscL-plus cells, curcumin treatment still led to potassium and glutamate fluxes as well as increases in potency of other antibiotics, consistent with curcumin opening the MscL channel [67•]. Curcumin's membrane permeabilization, inhibition of septation, and ability to work in synergy with other antibiotics, are all dependent upon MscL expression [67•]. The pKa

Figure 4



The agonists (011A, K05, IRS-16, SCH-79797, and 262) bind to MscL at the same S1/TM1 region (Panel A), but demonstrate different binding modes (Panel B). Panel A shows the docking poses that have the best binding-free energies in the subsequent molecular dynamics simulations and free-energy analysis. The agonists are shown as red, blue, green, yellow, and magenta sticks, respectively. The docking scores are – 7.1, – 7.2, – 8.4, – 8.8, and – 11.3 kcal/mol for the agonists, respectively. Panel B is the heatmap of the binding profiles with the ligand-residue-binding free energies being smaller than – 0.1 kcal/mol. Panel C, on the other hand, illustrates the cytoplasmic binding site of DHS. The antibiotics stably resided the binding site during a 100-nanosecond MD simulation. Most of the surrounding residues are glutamate residues.

values of the three acidic functional groups were determined to be 7.8, 8.5, and 9.0, respectively; at neural pH, about 14% of curcumin is ionized, making it a possible membrane thinner [68]. A binding site has not yet been identified, so it is possible that it could work by membrane thinning [69]; more research is needed in this area.

The dual-action SCH-79797 compound [70] is effective against antibiotic-resistant strains and it is very difficult for bacteria to develop resistance to it. It inhibits the dihydrofolate reductase pathway, and 'increases membrane permeability' causing depolarization [71]. A derivative, Irresistin-16 (IRS-16), has increased potency, less toxicity, and was effective against *Neisseria gonorrhoeae* in a mouse vaginal infection model. We found that SCH-79797 and IRS-16 permeabilize the membrane by directly activating MscL [72]. In addition, we showed that the component of the SCH-97979 compound responsible for permeabilization, cumene, was also MscL-dependent [72]. These findings strongly suggest that addition of a chemical moiety that serves as a MscL agonist onto an antibiotic could significantly increase the potency and efficacy of the drug. The binding pocket for SCH-79797 is in the same location as other agonists [72].

Other possibilities?

We would be delinquent if we did not point out that there are other possibilities, described in the literature, where compounds could work by directly binding to and/or specifically activating MscL. For example, *Bacillus subtilis* strain 168 produces the lantibiotic

sublancin 168, and a previous study demonstrated that MscL expression is required for its antibacterial activity *in vivo* [73]. However, no MscL channel activation or direct interactions have thus far been shown.

Antibacterial agents that affect 'membrane permeabilization' by undefined mechanisms are also candidates. Given the findings with aminoglycosides, curcumin, and SCH-79797 described above, MscL activation by antibiotics may be much more common than previously anticipated.

Finally, one study suggests decreases in extracellular ions or osmolytes increase potency of aminoglycosides in a MscL- and MscS-dependent manner: MscS may even directly bind to the antibiotics [74]. Because the MscS family is more diverse and less conserved than MscL, the discovery of MscS-specific agonists could serve to target specific bacterial species, thus leading to narrow-spectrum antibiotics and adjuvants.

Commonalities and an *in silico* screen

There are now several MscL agonists identified that facilitate channel opening. As illustrated in Fig. 3, these compounds are structurally diverse. In all instances characterized, the binding sites are at the S1/TM1 and TM2 cytoplasmic interface region and, as determined by static molecular docking, have some affinity to the closed channel even though they appear to destabilize the closed state. However, using an endpoint free-energy method called MM-GBSA [75], it is clear that it adopts different ligand-residue-interaction profiles and binding modes (Fig. 4). The most common hotspot residues are hydrophobics/aromatics, and many interactions have been confirmed *in vivo* by multiple mutagenesis experiments [54,57,59,72,76].

An *in silico* screening approach was used to discover potential MscL agonists that bind to the cononical site. First, a virtual docking screen was conducted using a homology of the *E. coli* MscL model [54] and a subset of ZINC drug-like database chosen by 'fingerprint-based similarity' [77]. From the hits, we found a new family of compounds, exemplified by 262, which are MscL agonists: molecular dynamics simulations, free-energy analysis, and mutagenesis verify that the compound stably binds the canonical site with a decent binding affinity [76••]. These findings yield promise that additional compounds with higher potency and efficacy will be identified using this approach.

Conclusions

In sum, MscL is a very promising drug target that can be applied to develop novel antibiotics and adjuvants at this critical time of the antibiotic-resistance crisis. Recent studies demonstrate that MscL agonists slow growth and viability and increase the potency of antibiotics, and there is promise that MscL-agonist potency and efficacy can be improved. There are several challenges in MscL drug discovery. It is extremely difficult to rationally design agonists without knowing details of the underlying molecular mechanisms to achieve the final active state, and valid structures are limited: no crystal or cryo-EM structure of *E. coli* MscL has been reported, and none exist for the open state for any species, presumably because of its small size and dynamics. Thus, there is an urgent need to understand the gating mechanisms of MscL gating using variable experimental and molecular simulation approaches. For example, a computational protocol to simulate the passage of antibiotics through MscL, which is accelerated by an external electric field applied to the antibiotics, has been developed [57,58]. This 'virtual passage' approach can quantitatively measure a potential agonist's ability to induce conformational changes that enhance this passage. There are also accelerated sampling techniques such as microbubble cavitation [78] and Gaussian-accelerated molecular dynamics simulations [79] that have yet to be applied to study MscL gating. With further study using an array of approaches, high-quality open-channel structures can be modeled and serve as receptors for virtual screening and *de novo* design.

Conflict of interest statement

None.

Data Availability

No data were used for the research described in the article.

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