

COMMENTARY

Seeing spermine blocking of K⁺ ion movement through inward rectifier Kir2.2 channels

Sun-Joo Lee¹ and Colin G. Nichols¹ 

Inwardly rectifier potassium (Kir) channels are a major potassium channel sub-class whose function is regulated by ligand-dependent gating and highly voltage-dependent block by polyamines. With molecular dynamics simulations over previously unattainable timescales, Jogini et al. (*J. Gen. Physiol.* <https://doi.org/10.1085/jgp.202213085>) provide unprecedented visualization of K⁺ conduction through open Kir2.2 channels and of the molecular details of channel block by spermine.

Inwardly rectifying potassium (Kir) channels are a major potassium channel sub-class (Nichols and Lopatin, 1997), essential for maintenance of resting membrane potentials and local as well as whole body K⁺ homeostasis. Kir channels have a highly conserved architecture with a tetrameric assembly of identical or similar subunits, each of which comprises a selectivity filter (SF), transmembrane domain (TMD), and a large cytoplasmic domain (CTD), generating a classical K⁺-selective pore that extends from the SF through the inner cavity formed by the TMD and through the cytoplasmic pore generated by the CTD, which is gated at the bottom of the TMD. Two well-described mechanisms regulate the function of Kir channels; first, conformational changes driven by ligand binding that control gating, and second, the highly voltage-dependent binding of intracellular polycationic metabolites, i.e., polyamines, that block K⁺ movement through open channels at positive membrane potentials. Despite long recognition of these mechanisms, lack of high-resolution structures of open states and open structures in complex with polyamines have perpetuated gaps and controversies in understanding of the details.

In this issue, Jogini et al. (2022) have carried out molecular dynamics simulations, over hundreds of microseconds to milliseconds, time scales that are currently essentially unachievable by any other laboratories, to generate open Kir2.2 channels in silico, and to visualize K⁺ conduction through the open channels as well as blocking of K⁺ conduction by polyamines, that help to resolve some of the controversies regarding the molecular mechanisms of inward rectification as considered below.

Obtaining open and conducting Kir2.2 channels

Structural resolution of Kir channels in an unambiguously open conformation has been elusive until very recently. The first

high-resolution structure of chicken Kir2.2 in complex with the essential gating ligand PIP₂ unambiguously identified a PIP₂ binding site (Hansen et al., 2011) and revealed secondary structure rearrangements in the PIP₂-bound channel that tightly tethered the CTD to the TMD. However, the main helix bundle crossing (HBC) gate was still clearly too narrow to be open (Hansen et al., 2011). Introduction of a gain-of-function mutation that removed the additional requirement for a secondary anionic lipid binding generated the CTD-tethered structure even in the absence of PIP₂, although the HBC gate remained closed (Lee et al., 2016). Finally, following an approach initially employed to force opening of a bacterial Kir channel (Bavro et al., 2012), by including a charge mutation (G178D) at the HBC, we generated channels that were open even in the absence of PIP₂ and which crystallized in a conformation with slightly wider HBC, but still too narrow for hydrated ion permeation (Zangerl-Plessl et al., 2020). Only recently, a cryo-EM structure of Kir6.2 complexed with SUR1, including two Kir6.2 gain-of-function mutations that render the channel insensitive to ATP binding and force open the pore, resulted in the first clearly open conformation of a eukaryotic Kir channel (Zhao and MacKinnon, 2021).

Jogini et al. (2022) attempted two different computational strategies to generate an open conducting Kir2.2 channel. Their first attempt was to introduce the known positive regulators, PIP₂, and bulk anionic lipids, into the system and allow the protein to open following the normal activation process. Despite simulating all-atom behavior for 200 μs, much longer than previously attempted (Zangerl-Plessl et al., 2020), channels still failed to open, suggesting that the hydrophobic interactions maintaining closure of the HBC gate are very strong. By repeating the trick of introducing a charged residue at the bundle crossing (G178R in this case), opening of the HBC gate due to

¹Department of Cell Biology and Physiology, Center for Investigation of Membrane Excitability Diseases, Washington University in St. Louis, St. Louis, MO, USA.

Correspondence to Colin G. Nichols: cnichols@wustl.edu.

© 2022 Lee and Nichols. This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see <http://www.rupress.org/terms/>). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 4.0 International license, as described at <https://creativecommons.org/licenses/by-nc-sa/4.0/>).

strong electrostatic repulsion was observed to occur very quickly, within 5–10 ns.

The open state structures were stable and hence allowed rigorous testing of different parameters that affect K⁺ conduction through the open pore. If channels were complexed with PIP₂, half of the simulated channels stayed open for at least 120 μ s after mutating G178R back to the wild-type glycine. With these stable open structures, they were able to test the roles of residues near the PIP₂ binding site on the gating transition, and the roles of charged cytoplasmic pore-lining residues on K⁺ conductance, as well as spermine binding. These issues had been probed in previous simulation studies on Kir2 and Kir3 channels (Bernsteiner et al., 2019; Chen et al., 2020; Zangerl-Plessl et al., 2020), with essentially similar findings, but the far more extensive simulations available in the current study provide considerably greater confidence in the conclusions.

Resolving spermine interaction with the channel pore

Strong inward rectification, a unique feature of Kir channels, results from channel block by polyamines, primarily spermine and spermidine (Nichols and Lopatin, 1997). Extensive studies of the structural requirements for block, both of the channel and of the polyamine analogs (Kurata et al., 2004; Kurata et al., 2010), show that they enter the pore as charged molecules, but there have been unsettled controversies regarding how this blocking actually takes place, particularly regarding the exact location(s) of polyamine binding (Nichols and Lee, 2018). Two major mechanistic models have been considered (see Fig. 1). The first, which we refer to as “long pore plugging”, proposes that spermine blocks by entry deep in the pore, into the SF, and that the strong voltage-dependence arises from entry of the charged spermine into the membrane field (Lopatin et al., 1995). The second, which we refer to as “extended pore filing”, proposes that spermine sits at the bundle crossing interacting with the acidic residues both in the TMD and the CTD (Guo et al., 2003), and that voltage-dependence of block arises from displacement of a column of K⁺ ions lined along the pore axis between the cytoplasmic domain and the SF. Two critical factors, K⁺ ion distribution within the pore and regions of single filing, need to be considered to find support for either of these mechanisms. Thus far, attempts to obtain crystal structures of Kir channels in the presence of spermine have failed to resolve bound spermine molecules within the pore, and MD simulations of open Kir2.2 channels can therefore be critically beneficial, illustrating K⁺ and spermine movements and interactions within the channel.

Single filing

The notion of single filing of K⁺ ions is particularly important for the extended pore filing model since it assumes that K⁺ ions along the pore cannot bypass one another and get knocked on as a spermine molecule moves up to its final position within the pore. MD simulations consistently indicate that single filing is not taking place below the narrow region defined by the G-loop at the top of the cytoplasmic pore (see Fig. 1); even with a strong electrical field imposed across the channel, K⁺ ions move freely into and out of the cytoplasmic pore, with neither single filing nor any coupled movement between the ions (Bernsteiner et al.,

2019; Zangerl-Plessl et al., 2020). On the other hand, movements of K⁺ ions above the G-loop are strongly coupled to one another. After K⁺ ions neutralize the local negative charges between the G-loop and the SF, entrance of a new K⁺ ion into the region results in the coupled movement of K⁺ ions all the way to the top of the SF, leading to exit of one K⁺ ion to the extracellular space (Zangerl-Plessl et al., 2020) even though the K⁺ ions above the G-loop do not physically align along the pore axis. Indeed, in the most recent simulations, 5–6 K⁺ ions within the region above the G-loop are directly expelled by spermine, consistent with K⁺ ions above the G-loop being the primary charge movements responsible for the steep voltage dependence of spermine blocking.

K⁺ ion density along the pore

The question then arises just how many K⁺ ions reside above the G-loop in a normal open and conducting channel. While there has long been a notion that one K⁺ ion sits in the inner cavity, based on the assignment of centrally located electron density to K⁺ in the original K channel crystal structure (Doyle et al., 1998), it seems reasonable that the number of K⁺ ions in the inner cavity will be dependent on the charged states of the rectification controller (RC) residues (i.e., the four D173 of Kir2.2). In our simulations, with all four negatively charged, the mean K⁺ ion number in the inner cavity was 3.7 (Zangerl-Plessl et al., 2020) and in the present study, interestingly with two negatively charged rectifying controllers (and two as neutralized glutamic acids), an average of two K⁺ ions resided in the inner cavity. Experimentally, divalent cation density has been observed in the cKir2.2 inner cavity (Tao et al., 2009), supporting the idea that at least two positive charges can reside in the inner cavity in the closed state, and the number could increase as the gate opens with higher water levels (Bernsteiner et al., 2019) and hence higher bulk polarizability.

Spermine binding sites

The present simulations show spermine residing at different locations along the pore, depending on the applied voltage. At computationally low voltages ($V < 185$ mV), spermines are interacting with acidic residues in the CTD, resulting in reduction of K⁺ entry to the deeper pore and hence an incomplete block of channel currents. At intermediate voltages ($185 \text{ mV} < V < 215$ mV), spermines bind to the region between the RC and the SF, completely blocking passage of K⁺ ions, and hence causing persistent block of conductance. In general, these observations provide support for what we have termed the “cavity trapping” model of rectification (Nichols and Lee, 2018), in which entry of spermine into the inner cavity and SF forces K⁺ ion movement through the SF and hence across the membrane electric field, combining elements of the original long pore plugging model (Lopatin et al., 1995), with the extended pore filing model (Guo et al., 2003). At the highest voltages ($V > 250$ mV), spermine completely occupies the SF and then punches through to the outside, restoring K⁺ conductance at these extreme conditions. Importantly, while no significant punch-through phenomenon has been experimentally detected in Kir2 channels over the tractable voltage range (approximately ± 120 mV), it may be

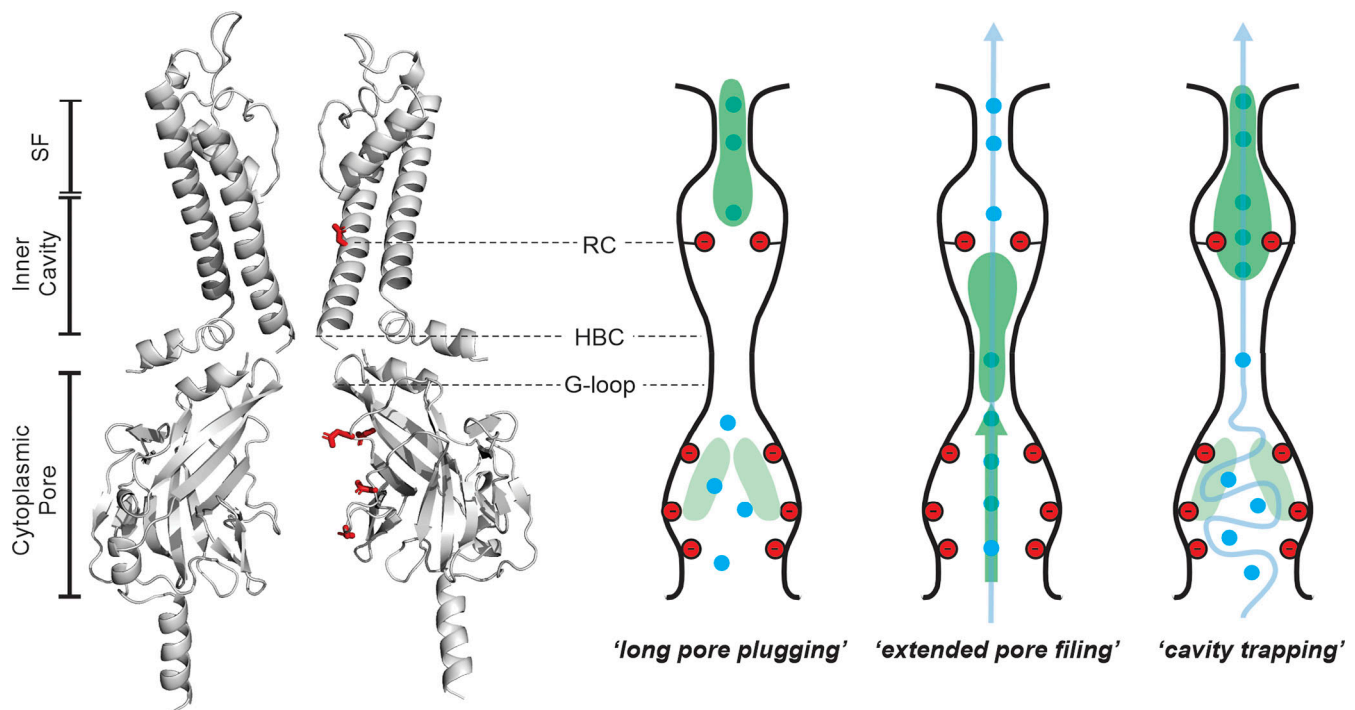


Figure 1. **Models for inward rectification mechanisms.** Left: Two opposing subunits of Kir2.2 (PDB accession no. 5KUM) show the key structural features of the channel pore, including the SF, transmembrane inner cavity, and cytoplasmic pore regions, as well as residues that control polyamine-induced rectification (red). Right: Cartoon illustrations of the distinct proposed models for inward rectification. In the long pore plugging model, polyamines are assumed to cause very weak voltage-dependent block by interaction with negatively charged residues in the cytoplasmic pore (pale site), but cause steep voltage-dependent rectification by moving deep into the pore, beyond the rectification controller and into the selectivity filter (dark site), thereby moving substantial polyamine charge across the electric field and displacing K^+ ions from the SF in the process. In the extended pore filing model, rectification is assumed to arise from movement of the polyamine into the cytoplasmic pore, and through the HBC towards the rectification controller (dark site), obligatorily “pushing” a single-file “chain” of K^+ ions (blue) through the SF in the process. The cavity trapping model represents a hybrid model that assumes weak block arising from movement of the polyamine into the cytoplasmic pore (pale site), but with the major charge movement associated with steep rectification arising from displacement of K^+ ions from the inner cavity through the SF, due to obligatory single file movement of positive charges above the G-loop, plus movement of polyamine charge itself into the electric field, as in the long pore plugging model. Redrawn from Nichols and Lee, 2018.

present in related Kir4 channels (Kucheryavykh et al., 2007), and is the most likely explanation for relief of rectification at positive voltages that is seen in CNG channels (Lu and Ding, 1999), and conceivably might happen in Kir2 channels if very high voltages could be applied experimentally.

Further study

The present study provides a beautifully detailed view of just how K^+ ions permeate Kir2.2 channels, consistent with and greatly extending previous simulations. Most significantly perhaps, it provides detailed visualization of just how polyamines block the channel current, with spermine binding deeply in the inner cavity up to the SF and the steep voltage-dependence of block resulting from the forced exit of K^+ ions occupying the space above the G-loop. Yet these fascinating observations still leave questions—and raise new questions—particularly regarding the details of rectification. One question is how does relatively strong rectification emerge in Kir channels that lack any negatively charged inner cavity residues, such as Kir3.2 (Girk2) channels (Yi et al., 2001) and Kir2.1[D172N] mutant channels with the RC residues neutralized (Wible et al., 1994)? Also diamine molecules all carry two charges, yet exhibit different levels of rectification with different lengths of the carbon chains (Pearson

and Nichols, 1998; Xie et al., 2003). Is it possible that, in addition to the electrical coupling, steric occlusion may be involved in forcing K^+ ions from the constricted space of the inner cavity? With the necessary computational firepower in hand, the D.E. Shaw group are well-placed to provide some answers.

Acknowledgments

Crina M. Nimigean served as editor.

This work is supported by National Institutes of Health R35 grant HL140024 to C.G. Nichols and R03 grant TR003670 to S.J. Lee.

The authors declare no competing financial interests.

References

- Bavro, V.N., R. De Zorzi, M.R. Schmidt, J.R. Muniz, L. Zubcevic, M.S. Sansom, C. Vénien-Bryan, and S.J. Tucker. 2012. Structure of a KirBac potassium channel with an open bundle crossing indicates a mechanism of channel gating. *Nat. Struct. Mol. Biol.* 19:158–163. <https://doi.org/10.1038/nsmb.2208>
- Bernsteiner, H., E.M. Zangerl-Plessl, X. Chen, and A. Stary-Weinzinger. 2019. Conduction through a narrow inward-rectifier K^+ channel pore. *J. Gen. Physiol.* 151:1231–1246. <https://doi.org/10.1085/jgp.201912359>

- Chen, X., M. Bründl, T. Friesacher, and A. Stry-Weinzinger. 2020. Computational insights into voltage dependence of polyamine block in a strong inwardly rectifying K⁺ channel. *Front. Pharmacol.* 11:721. <https://doi.org/10.3389/fphar.2020.00721>
- Doyle, D.A., J. Morais Cabral, R.A. Pfuetschner, A. Kuo, J.M. Gulbis, S.L. Cohen, B.T. Chait, and R. MacKinnon. 1998. The structure of the potassium channel: Molecular basis of K⁺ conduction and selectivity. *Science*. 280: 69–77. <https://doi.org/10.1126/science.280.5360.69>
- Guo, D., Y. Ramu, A.M. Klem, and Z. Lu. 2003. Mechanism of rectification in inward-rectifier K⁺ channels. *J. Gen. Physiol.* 121:261–275. <https://doi.org/10.1085/jgp.200208771>
- Hansen, S.B., X. Tao, and R. MacKinnon. 2011. Structural basis of PIP₂ activation of the classical inward rectifier K⁺ channel Kir2.2. *Nature*. 477: 495–498. <https://doi.org/10.1038/nature10370>
- Jogini, V., M.O. Jensen, and D.E. Shaw. 2022. Gating and modulation of an inward-rectifier potassium channel. *J. Gen. Physiol.* 155. <https://doi.org/10.1085/jgp.202213085>
- Kucheryavykh, Y.V., W.L. Pearson, H.T. Kurata, M.J. Eaton, S.N. Skatchkov, and C.G. Nichols. 2007. Polyamine permeation and rectification of Kir4.1 channels. *Channels*. 1:172–178. <https://doi.org/10.4161/chan.4389>
- Kurata, H.T., L.R. Phillips, T. Rose, G. Loussouarn, S. Herlitz, H. Fritzen-schaft, D. Enkvetchakul, C.G. Nichols, and T. Baukrowitz. 2004. Molecular basis of inward rectification: Polyamine interaction sites located by combined channel and ligand mutagenesis. *J. Gen. Physiol.* 124: 541–554. <https://doi.org/10.1085/jgp.200409159>
- Kurata, H.T., E.A. Zhu, and C.G. Nichols. 2010. Locale and chemistry of spermine binding in the archetypal inward rectifier Kir2.1. *J. Gen. Physiol.* 135:495–508. <https://doi.org/10.1085/jgp.200910253>
- Lee, S.J., F. Ren, E.M. Zangerl-Plessl, S. Heyman, A. Stry-Weinzinger, P. Yuan, and C.G. Nichols. 2016. Structural basis of control of inward rectifier Kir2 channel gating by bulk anionic phospholipids. *J. Gen. Physiol.* 148:227–237. <https://doi.org/10.1085/jgp.201611616>
- Lopatin, A.N., E.N. Makhina, and C.G. Nichols. 1995. The mechanism of inward rectification of potassium channels: “Long-pore plugging” by cytoplasmic polyamines. *J. Gen. Physiol.* 106:923–955. <https://doi.org/10.1085/jgp.106.5.923>
- Lu, Z., and L. Ding. 1999. Blockade of a retinal cGMP-gated channel by polyamines. *J. Gen. Physiol.* 113:35–43. <https://doi.org/10.1085/jgp.113.1.35>
- Nichols, C.G., and S.J. Lee. 2018. Polyamines and potassium channels: A 25-year romance. *J. Biol. Chem.* 293:18779–18788. <https://doi.org/10.1074/jbc.TM118.003344>
- Nichols, C.G., and A.N. Lopatin. 1997. Inward rectifier potassium channels. *Annu. Rev. Physiol.* 59:171–191. <https://doi.org/10.1146/annurev.physiol.59.1.171>
- Pearson, W.L., and C.G. Nichols. 1998. Block of the Kir2.1 channel pore by alkylamine analogues of endogenous polyamines. *J. Gen. Physiol.* 112: 351–363. <https://doi.org/10.1085/jgp.112.3.351>
- Tao, X., J.L. Avalos, J. Chen, and R. MacKinnon. 2009. Crystal structure of the eukaryotic strong inward-rectifier K⁺ channel Kir2.2 at 3.1 Å resolution. *Science*. 326:1668–1674. <https://doi.org/10.1126/science.1180310>
- Wible, B.A., M. Taglialatela, E. Ficker, and A.M. Brown. 1994. Gating of inwardly rectifying K⁺ channels localized to a single negatively charged residue. *Nature*. 371:246–249. <https://doi.org/10.1038/371246a0>
- Xie, L.H., S.A. John, and J.N. Weiss. 2003. Inward rectification by polyamines in mouse Kir2.1 channels: Synergy between blocking components. *J. Physiol.* 550:67–82. <https://doi.org/10.1113/jphysiol.2003.043117>
- Yi, B.A., Y.F. Lin, Y.N. Jan, and L.Y. Jan. 2001. Yeast screen for constitutively active mutant G protein-activated potassium channels. *Neuron*. 29: 657–667. [https://doi.org/10.1016/S0896-6273\(01\)00241-0](https://doi.org/10.1016/S0896-6273(01)00241-0)
- Zangerl-Plessl, E.M., S.J. Lee, G. Maksaev, H. Bernsteiner, F. Ren, P. Yuan, A. Stry-Weinzinger, and C.G. Nichols. 2020. Atomistic basis of opening and conduction in mammalian inward rectifier potassium (Kir2.2) channels. *J. Gen. Physiol.* 152:e201912422. <https://doi.org/10.1085/jgp.201912422>
- Zhao, C., and R. MacKinnon. 2021. Molecular structure of an open human K_{ATP} channel. *Proc. Natl. Acad. Sci. USA*. 118:e2112267118. <https://doi.org/10.1073/pnas.2112267118>