

COMMENTARY

Opening leads to closing: Allosteric crosstalk between the activation and inactivation gates in KcsA

 Lucie Delemotte 

Voltage-gated potassium (Kv) channels control a number of different physiological processes, including the firing rate in axons. Such K⁺ channels display a reduction of conductance after exposure to a prolonged activating stimulus. This process, referred to as inactivation, causes repolarization of the cell membrane after the depolarizing phase of an action potential. The transient openings that result from it also allow neurons to readily fire a new action potential. Two types of inactivation mechanisms have been described in Kv channels (Hoshi et al., 1990). Fast inactivation, also called N-type inactivation, results from a mechanism that has been ascribed to pore blocking by a N-terminal peptide. Slow inactivation, or C-type inactivation, is revealed upon suppression of fast inactivation and is thought to be due to a conformational change occurring within the pore of the channel. While the structural basis of C-type inactivation appears to have been established, how it is dynamically coupled to channel activation remains to be understood in detail. In the *Journal of General Physiology*, a new study (see Li et al. in this issue) proposes an intriguing mechanism for the allosteric control of C-type inactivation by the activation gate in the bacterial K⁺ channel KcsA.

KcsA has long been used as a prototypical model to study C-type inactivation (Yellen, 2002). KcsA activates at low intracellular pH and subsequently inactivates within seconds. As is the case for Kv channels, its inactivation is promoted by low extracellular K⁺ concentrations, and can be modulated by mutation of pore residues (Yellen, 2002). Compared with Kv channels, however, KcsA has the advantage of structural simplicity: although the transmembrane (TM) portion of Kv channels is a tetrameric assembly of six TM segments, KcsA is made of the tetrameric assembly of only two TM segments. Nevertheless, the KcsA pore closely resembles that of Kv channels: the main activation gate is found at the crossing between TM segments on the intracellular side of the channel and the C-type inactivation site is located toward the extracellular side at the level of the selectivity filter (SF). Thus, KcsA is believed to be a good model for the structural and dynamical underpinnings of C-type inactivation without the

complications associated with other protein domains, which may influence C-type inactivation in a nontrivial manner.

Since the advent of high-resolution x-ray crystallography of membrane proteins in the mid-1980s, multiple structures of KcsA have been resolved in different conditions, which appear to have revealed the basis of channel gating. A “conductive” SF configuration is promoted by high K⁺ concentrations, a closed intracellular gate, and mutations that inhibit C-type inactivation. On the other hand, low K⁺ concentrations, an open intracellular gate, and mutations that favor C-type inactivation, promote an SF configuration that is “pinched” or “inactivated” (Fig. 1; Zhou et al., 2001; Cuello et al., 2010b). Thus, it is understood that the loss of conduction during C-type inactivation is caused by pinching of the selectivity filter at the level of G77, which occurs upon rotation of the peptide bond between V76 and G77. This conformational change is coupled to the loss of occupancy of the central binding site in the SF by K⁺ ions, and the filling of cavities located behind the SF with three tightly bound water molecules, coined “inactivating water molecules” (Ostmeyer et al., 2013; Weingarth et al., 2014). This structural view of the inactivation process is supported by a range of experiments and computer simulations (Cuello et al., 2017; Li et al., 2017; Tilegenova et al., 2017; van der Crujjsen et al., 2017; Xu et al., 2017). Although not universally accepted (Devaraneni et al., 2013; Liu et al., 2015), that pinching of the SF is the rate-limiting conformational change associated with C-type inactivation appears to be the most satisfactory theory currently available (Li et al., 2017).

In this new contribution, Li et al. (2018) build upon this theory and investigate how the intracellular gate controls the structure and dynamics of the SF, i.e., whether the SF is conductive or inactive (Li et al., 2018). To do so, the authors elegantly used atomistic, explicit solvent, molecular dynamics (MD) simulations, as well as electrophysiological measurements.

MD simulations have become a mainstream approach in molecular physiology, because the technique is capable of revealing the dynamics of all components in a molecular system at atom-

 Department of Applied Physics, Science for Life Laboratory, KTH Royal Institute of Technology, Stockholm, Sweden.

Correspondence to Lucie Delemotte: lucie.delemotte@scilifelab.se.

© 2018 Delemotte 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/>).

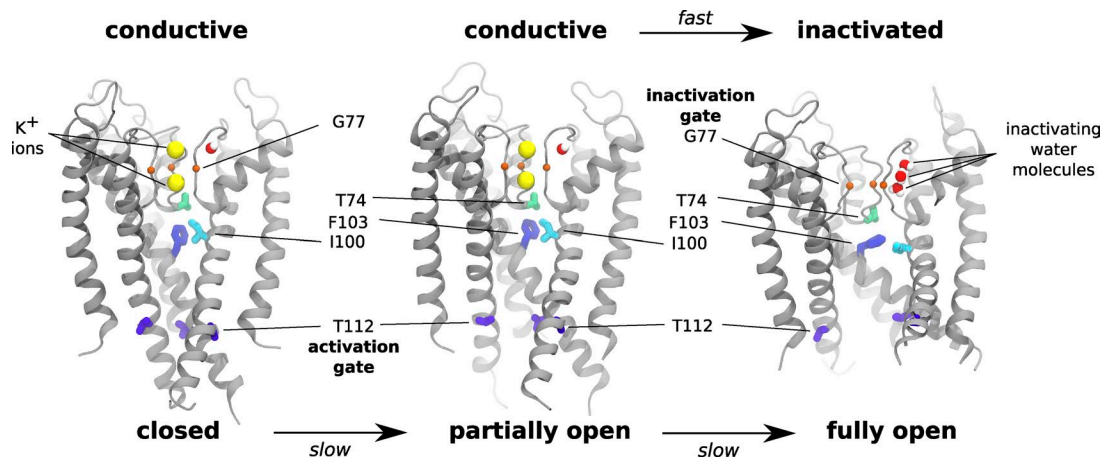


Figure 1. **Representative configurations and key molecular determinants of the closed/conductive, partially open/conductive, and fully open/conductive states of KcsA.** Three of four subunits of the channel are displayed as gray ribbons, SF-bound K⁺ ions are yellow spheres, and inactivating water molecules in one of the subunits are red and white spheres. Key residues are shown as sticks: T74, green; G77, orange; I100, cyan; F103, blue; T112, purple. Opening of the activation gate is shown to be slow while inactivation of the SF is fast.

ic-level resolution. Like any other computational modeling approach, however, MD simulations are very sensitive to the input data and the parameters of the model. Crucial aspects are the quality of the initial protein model (and thus of the protein structure used to build said model) and the accuracy of the physical model used to describe interparticle interactions (the so-called “force field”). Ion conduction and selectivity in K⁺ channels, in particular, are extremely sensitive to variations in these elements (Köpfer et al., 2014; Heer et al., 2017; Kopec et al., 2018). The SF conformational change studied by Li et al. (2018) is in many ways related to the question of ion conduction, and a fine balance of the interactions between the protein, ions and water is likely required to control the process. Li et al. (2018) addressed potential concerns with the modeling protocol through a careful, systematic study design: they conducted extensive MD simulations and evaluated the stability of different models of the various states of the channel (open, semi-open and closed gate/conductive, inactivated SF), and they complemented these analyses with free energy calculations and simulations under electric potentials to promote ion conduction. As controls, they compared configurations obtained during simulations to newly available crystal structures; conducted simulations of noninactivating mutants; and contrasted the resulting hypothesis with new electrophysiology experiments.

To date, no structure of wild-type KcsA with an open intracellular gate and a conductive SF has been resolved. Therefore, to examine this state, Li et al. (2018) constructed two independent models: the first used the coordinates of a KcsA crystal structure with a fully open gate, in which the inactivated SF is rebuilt using the coordinates of a conductive SF (extracted from a structure with a closed gate); the second model was the experimental crystal structure of a fully-open noninactivating mutant (E71A), with all mutations reverted back to the WT sequence. Multiple replicates of microsecond-time scale MD simulations consistently showed that these models are stable over hundreds of nanoseconds, but ultimately relax spontaneously and rapidly toward an inactivated-like SF conformation. Comparison of the resulting

configuration of the inactivated SF with that observed in a structure of an open-inactivated state (Cuello et al., 2017) revealed an impressive agreement, particularly in regard to the position of inactivating water molecules behind the SF. The simulations explained how K⁺ ions within the SF are destabilized, by revealing a beautiful tetrameric inactivated SF symmetry in which the innermost inactivating water molecule is shown to coordinate the carbonyl of V76 and the NH group of G77 in the adjacent subunit, thus displacing the V76 carbonyl away from its K⁺-binding position. The symmetric assembly also provides a rationale for the cooperative nature of the transition of the four chains of the SF to the inactivated state.

Additional microsecond-time scale MD simulations revealed that the conductive SF is only stable in the presence of closed or semi-open intracellular gates (Fig. 1). What, then, are the relative free energies of the conductive and inactivated SF states, and how are they reshaped by the state of the intracellular gate? Obtaining an answer to these questions using regular MD simulations would require hundreds of independent runs. Li et al. (2018) thus astutely resorted to enhanced-sampling MD simulations—a type of advanced MD simulation scheme that has already allowed major insights into conformational dynamics of membrane proteins (reviewed in Harpole and Delemotte, 2018). Li et al. (2018) specifically used replica-exchange Umbrella Sampling (RE-US) simulations to encourage sampling of regions of the conformation landscape of high free energy. Crucially, these calculations revealed that a partially open intracellular gate favors a single free-energy minimum corresponding to a conductive SF state. In contrast, a fully open intracellular gate promoted two separate free energy minima, both corresponding to pinched, inactivated states of the SF, with two different K⁺ occupation patterns. Complementary 100-ns simulations under a 300-mV depolarizing potential further depicted a consistent picture: in the presence of a partially open gate, the SF indeed conducted K⁺ ions, whereas a fully open gate led the SF to rapidly become nonconductive.

Can the allosteric control of the SF state by the intracellular gate be rationalized by analyzing the MD simulations? Li et al.

(2018) proved that this can be done by monitoring the probability distribution of the states of residues T74, I100, and F103, which are located below the SF and were previously implicated in cross-talk between the SF and gate (Pan et al., 2011). As the gate proceeds from partially to fully open, F103 intercalates between T74 and I100 in a process that is coupled to a change in the rotameric populations of the sidechains of the two bulky residues I100 and F103, which favors the inactivated state of the SF (Fig. 1). Furthermore, long-timescale simulations of two mutants that have been previously shown not to inactivate (I100A and F103A; Pan et al., 2011) barely show signs of SF pinching, thus verifying the important allosteric role played by these residues and proving further that the modeling protocol used by Li et al. (2018) reproduces the expected channel behavior.

The paper concludes with experimental validation of the proposed mechanism. Currents elicited by low pH applied to a C-terminal-less KcsA channel inactivate faster than those recorded with the full-length channel. Because the truncated KcsA has a propensity to be more open than the full-length KcsA (Cuello et al., 2010a), these experiments are compatible with the conclusion drawn from the simulations: the more open the gate, the more the SF tends to inactivate.

Intriguingly, the measured inactivation timescale for the truncated KcsA is ~ 1.7 s, whereas the simulations of Li et al. (2018) suggest that relaxation of the SF to an inactive state occurs on the 100-ns timescale. To reconcile these seemingly divergent observations, the authors propose that the rate-limiting conformational change underlying C-type inactivation is the full opening of the intracellular gate from a semi-open state. This view is contrary to the widely accepted idea that the inactivating conformational change of the SF is the slowest molecular process. To support this hypothesis, Li et al. (2018) show that several replicas of semi-open gate simulations with different protonation states remain stable over the microsecond timescale, without ever reaching the fully open-state configuration. Full opening thus seems to occur on timescales beyond the current capabilities of these MD simulations. To fully validate or refute this hypothesis, longer simulations will be needed, possibly using enhanced MD simulation schemes, as well as spectroscopy studies that will allow a dynamical insight into the relationship between the gate and the SF.

A consistent observation that is emerging from recent work on the inactivation mechanism of KcsA (Li et al., 2018) as well as recent spectroscopy studies (Cuello et al., 2017; Tilegenova et al., 2017; Xu et al., 2017) is that there is a tight allosteric coupling between the activation and inactivation gates. The details of the molecular mechanism at play are also becoming steadily clearer with the involvement of a cluster of residues below the SF. This progress underlies the potential for discovery in MD simulations because of their ability to provide detailed atomic-resolution insights without introducing any perturbation to the system. The complexity of the questions that can be investigated going forward can thus be increased: what is the effect of the environment and the role played by lipids, specifically, in the regulation of C-type inactivation (Kim et al., 2016; van der Crujisen et al., 2017; Xu et al., 2017)? How does this mechanism translate to more complex channels that are regulated by other stimuli and by the presence of

regulatory subunits? In Kv channels in particular, what is the role of the voltage sensor domains (VSDs) and the apparatus that links the VSDs and the pore in inactivation (Conti et al., 2016; Kalstrup and Blunck, 2018)? Let's venture to exploit the synergies between structural, functional, spectroscopy, and simulation studies to shed further light on these complex questions in the near future.

Acknowledgments

Marina A. Kasimova is greatly acknowledged for insightful discussions and comments on a manuscript draft.

José D. Faraldo-Gómez served as editor.

References

- Conti, L., J. Renhorn, A. Gabrielsson, F. Turesson, S.I. Liin, E. Lindahl, and F. Elinder. 2016. Reciprocal voltage sensor-to-pore coupling leads to potassium channel C-type inactivation. *Sci. Rep.* 6:27562. <https://doi.org/10.1038/srep27562>
- Cuello, L.G., V. Jogini, D.M. Cortes, A.C. Pan, D.G. Gagnon, O. Dalmas, J.F. Cordeiro-Morales, S. Chakrapani, B. Roux, and E. Perozo. 2010a. Structural basis for the coupling between activation and inactivation gates in K⁽⁺⁾ channels. *Nature*. 466:272–275. <https://doi.org/10.1038/nature09136>
- Cuello, L.G., V. Jogini, D.M. Cortes, and E. Perozo. 2010b. Structural mechanism of C-type inactivation in K⁽⁺⁾ channels. *Nature*. 466:203–208. <https://doi.org/10.1038/nature09153>
- Cuello, L.G., D.M. Cortes, and E. Perozo. 2017. The gating cycle of a K⁺ channel at atomic resolution. *eLife*. 6:e28032. <https://doi.org/10.7554/eLife.28032>
- Devaraneni, P.K., A.G. Komarov, C.A. Costantino, J.J. Devereaux, K. Matulef, and F.I. Valiyaveetil. 2013. Semisynthetic K⁺ channels show that the constricted conformation of the selectivity filter is not the C-type inactivated state. *Proc. Natl. Acad. Sci. USA*. 110:15698–15703. <https://doi.org/10.1073/pnas.1308699110>
- Harpole, T.J., and L. Delemotte. 2018. Conformational landscapes of membrane proteins delineated by enhanced sampling molecular dynamics simulations. *BBA-Biomembranes*. 1860:909–926. <https://doi.org/10.1016/j.bbmem.2017.10.033>
- Heer, F.T., D.J. Posson, W. Wojtas-Niziuński, C.M. Nimigeon, and S. Bernèche. 2017. Mechanism of activation at the selectivity filter of the KcsA K⁺ channel. *eLife*. 6:e25844. <https://doi.org/10.7554/eLife.25844>
- Hoshi, T., W.N. Zagotta, and R.W. Aldrich. 1990. Biophysical and molecular mechanisms of Shaker potassium channel inactivation. *Science*. 250:533–538. <https://doi.org/10.1126/science.2122519>
- Kalstrup, T., and R. Blunck. 2018. S4-S5 linker movement during activation and inactivation in voltage-gated K⁺ channels. *Proc. Natl. Acad. Sci. USA*. 115:E6751–E6759. <https://doi.org/10.1073/pnas.1719105115>
- Kim, D.M., I. Dikiy, V. Upadhyay, D.J. Posson, D. Eliezer, and C.M. Nimigeon. 2016. Conformational heterogeneity in closed and open states of the KcsA potassium channel in lipid bicelles. *J. Gen. Physiol.* 148:119–132. <https://doi.org/10.1085/jgp.201611602>
- Kopec, W., D.A. Köpfer, O.N. Vickery, A.S. Bondarenko, T.L.C. Jansen, B.L. de Groot, and U. Zachariae. 2018. Direct knock-on of desolvated ions governs strict ion selectivity in K⁺ channels. *Nat. Chem.* 10:813–820. <https://doi.org/10.1038/s41557-018-0105-9>
- Köpfer, D.A., C. Song, T. Gruene, G.M. Sheldrick, U. Zachariae, and B.L. de Groot. 2014. Ion permeation in K⁺ channels occurs by direct Coulomb knock-on. *Science*. 346:352–355. <https://doi.org/10.1126/science.1254840>
- Li, J., J. Ostmeyer, E. Boulanger, H. Rui, E. Perozo, and B. Roux. 2017. Chemical substitutions in the selectivity filter of potassium channels do not rule out constricted-like conformations for C-type inactivation. *Proc. Natl. Acad. Sci. USA*. 114:11145–11150. <https://doi.org/10.1073/pnas.1706983114>
- Li, J., J. Ostmeyer, L.G. Cuello, E. Perozo, and B. Roux. 2018. Rapid constriction of the selectivity filter underlies C-type inactivation in the KcsA potassium channel. *J. Gen. Physiol.* [jgp.201812082](https://doi.org/10.1085/jgp.201812082). <https://doi.org/10.1085/jgp.201812082>
- Liu, S., P.J. Focke, K. Matulef, X. Bian, P. Moënné-Loccoz, F.I. Valiyaveetil, and S.W. Lockless. 2015. Ion-binding properties of a K⁺ channel selectivity

- filter in different conformations. *Proc. Natl. Acad. Sci. USA*. 112:15096–15100. <https://doi.org/10.1073/pnas.1510526112>
- Ostmeyer, J., S. Chakrapani, A.C. Pan, E. Perozo, and B. Roux. 2013. Recovery from slow inactivation in K⁺ channels is controlled by water molecules. *Nature*. 501:121–124. <https://doi.org/10.1038/nature12395>
- Pan, A.C., L.G. Cuello, E. Perozo, and B. Roux. 2011. Thermodynamic coupling between activation and inactivation gating in potassium channels revealed by free energy molecular dynamics simulations. *J. Gen. Physiol.* 138:571–580. <https://doi.org/10.1085/jgp.201110670>
- Tilegenova, C., D.M. Cortes, and L.G. Cuello. 2017. Hysteresis of KcsA potassium channel's activation- deactivation gating is caused by structural changes at the channel's selectivity filter. *Proc. Natl. Acad. Sci. USA*. 114:3234–3239. <https://doi.org/10.1073/pnas.1618101114>
- van der Cruysen, E.A.W., A.V. Prokofyev, O. Pongs, and M. Baldus. 2017. Probing Conformational Changes during the Gating Cycle of a Potassium Channel in Lipid Bilayers. *Biophys. J.* 112:99–108. <https://doi.org/10.1016/j.bpj.2016.12.001>
- Weingarh, M., E.A.W. van der Cruysen, J. Ostmeyer, S. Lievestro, B. Roux, and M. Baldus. 2014. Quantitative analysis of the water occupancy around the selectivity filter of a K⁺ channel in different gating modes. *J. Am. Chem. Soc.* 136:2000–2007. <https://doi.org/10.1021/ja411450y>
- Xu, Y., M.P. Bhate, and A.E. McDermott. 2017. Transmembrane allosteric energetics characterization for strong coupling between proton and potassium ion binding in the KcsA channel. *Proc. Natl. Acad. Sci. USA*. 114:8788–8793. <https://doi.org/10.1073/pnas.1701330114>
- Yellen, G. 2002. The voltage-gated potassium channels and their relatives. *Nature*. 419:35–42. <https://doi.org/10.1038/nature00978>
- Zhou, Y., J.H. Morais-Cabral, A. Kaufman, and R. MacKinnon. 2001. Chemistry of ion coordination and hydration revealed by a K⁺ channel-Fab complex at 2.0 Å resolution. *Nature*. 414:43–48. <https://doi.org/10.1038/35102009>