On the mechanism of the GIRK2 channel gating by phosphatidylinositol bisphosphate (PIP2), sodium, and the Gβγ dimer

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ABSTRACT
G protein–gated inwardly rectifying K⁺ (GIRK) channels belong to the inward-rectifier K⁺ (Kir) family, are abundantly expressed in the heart, and require that phosphatidylinositol bisphosphate (PIP₂) is present so that intracellular channel-gating regulators such as Gβγ and Na⁺ ions can maintain the channel-open state. However, despite high-resolution structures and a large number of functional studies, we do not have a coherent picture of how Gβγ and Na⁺ ions control gating of GIRK2 channels. Here, we utilized computational modeling and all-atom microsecond-scale molecular dynamics simulations to determine which gates are controlled by Na⁺ and Gβγ and how each regulator uses the channel domain movements to control gate transitions. We found that Na⁺ ions control the cytosolic gate of the channel through an anti-clockwise rotation, whereas Gβγ stabilizes the transmembrane gate in the open state through a rocking movement of the cytosolic domain. Both effects altered the way by which the channel interacts with PIP₂ and thereby stabilizes the open states of the respective gates. These studies of GIRK channel dynamics present for the first time a comprehensive structural model that is consistent with a great body of literature on GIRK channel function.

The abbreviations used are: GIRK2-Apo, G protein- gated inwardly rectifying K⁺ channel; GIRK2-PIP₂ or GIRK2, GIRK2 in complex with phosphatidylinositol bis-phosphate; GIRK2-PIP₂-Na⁺ (GIRK2-Na⁺), GIRK2-PIP₂ in complex with intracellular sodium; GIRK2-PIP₂-Gβγ (GIRK2-Gβγ), GIRK2-PIP₂ in complex with the Gβγ dimer; GIRK2-PIP₂-Gβγ-Na⁺ (GIRK2-Gβγ-Na⁺), GIRK2-PIP₂ in complex with the Gβγ dimer and intracellular sodium; SF, selectivity filter; HBC, helix bundle crossing; CTD, cytosolic domain; TM, transmembrane; Ca, α-carbon; EF, electric field; Po, open probability; PCA, principal component analysis.

G protein-gated inwardly rectifying K⁺ (GIRK) channels include four mammalian members (GIRK1-4 or Kir3.1-Kir3.4) and belong to the potassium inward rectifier K⁺ (Kir) channel family (1,2). They are expressed abundantly in the heart (GIRK1, GIRK4) and in the brain (all members), among other tissues, where they function both as homotetramers and/or heterotetramers with each other. As their name implies, physiologically they are directly gated by G proteins, specifically the Gβγ dimer of GTP-binding (G) proteins (Gβγ) (3-5), ones that associate with pertussis-toxin sensitive Gα subunits. GIRK channels are dependent on phosphoinositides, especially PIP₂, which via direct interactions stabilize their gates in the open state (6-8). Another physiological regulator of GIRK activity is intracellular Na⁺ that is in part coordinated by a specific Asp residue found in the GIRK2 and GIRK4 channel members (9-12). Both Na⁺ and Gβγ have been shown to work by increasing the affinity of the channel to PIP₂ and to synergize via this mechanism in activating the channel, while PIP₂ itself is unable to gate efficiently the channel on its own, unlike other Kir channel family members (2). GIRK activation inhibits excitability, slowing the rate of pacemaker and atrial cell firing in the heart, while inhibiting transmitter release by pre-synaptic neurons or opposing excitation of post-synaptic neurons. GIRK inhibition through hydrolysis or dephosphorylation of PIP₂ causes channel desensitization that lasts as long as it takes to re-synthesize PIP₂ (13, 14). GIRK channels are thought to be good therapeutic targets for multiple conditions that call for a decrease in excitability or arrhythmogenesis, such as epilepsy in the brain and atrial fibrillation in the heart (15-17).
Structural studies, using crystallography or computational modeling, have produced three-dimensional (3-D) models of GIRK channels in complex with each and all of these physiological regulators, PIP_2, Gβγ, and Na^+ (4, 5, 8, 18). Atomic resolution crystal structures of GIRK channels have been limited to a truncated GIRK2 channel construct that reproduces functional characteristics of the full-length channel (5, 8, 19). The GIRK2 structures have provided direct evidence for the multiple gate hypothesis of these channels. These structures point to three constrictions along the permeation pathway: the Selectivity Filter (SF), where K^+ ions are selected over other ions; the Helix Bundle Crossing (HBC) gate, also referred in the literature as the inner helix gate, located near the inner leaflet of the plasma membrane bilayer; and a cytosolic gate that is unique to Kir channels, referred to as the G-loop gate (Fig. S1). The HBC gate is comprised by the side chains of F192, while the G-loop gate is comprised by two methionine pairs at the top and bottom of the G loop (Fig. S2B).

The GIRK2 structures in complex with the various regulators, obtained mainly from the MacKinnon lab, have provided a wealth of structural information. Yet no satisfying structural gating model has resulted that is consistent with the physiological regulation of the channel. None of the structures with physiological regulators have captured the channel gates in the open state. Even the channel bound to all its gating molecules, PIP_2, Na^+, and Gβγ, which in electrophysiological experiments has been found to activate the channel the most (20), did not capture the channel gates in the open state. The structure of a poorly functional point mutant, GIRK2 (R201A), has come the closest to capturing the gates open, where two of the subunits bound to PIP_2 depicted open conformations but the remaining two that were not PIP_2 bound had their gates closed (5). It was hypothesized that the crystal packing prevented PIP_2 from binding to all four subunits leaving two of the four gates in the closed state. Based on this structure, models were created in an effort to understand what structural changes could be predicted, if all subunits would reach the open state, as expected. The binding of Gβγ was predicted to cause an anti-clockwise movement of ~4° of both the cytoplasmic and transmembrane (TM) domains that put the channel in a “pre-open” state. The R201A mutant channel showed an additional ~4° twisting movement to cause full gate opening (5, 21). Table S1 and Fig. S3 show all the crystal structures determined under different conditions with both minimal and α-carbon (Cα) distances between the gates of opposite-facing subunits. This heroic work has answered many questions, but at the same time has left many other questions unanswered. It has also generated puzzling conclusions, perhaps the most troubling of which is the question: does the poorly functional R201A mutant of the GIRK2 channel represent a valid model of a constitutively active channel that mimics the natural open channel state? Critical mechanistic questions remain, such as which gates are controlled by the two different activators (Na^+ versus Gβγ or both) and how the movements caused by these regulators tie into the regulation of the gates by PIP_2. Given the nature of these dynamic questions, we pursued them using computational modeling and molecular dynamics (MD) simulations based on the pre-open crystal structure of GIRK2.

**Results**

**Na^+ or Gβγ couple to distinct gates but when present simultaneously show synergism in gating the channel**

Five modeled systems ranging from 160,000 to 400,000 atoms were simulated in the absence and presence of an electric field (EF) for a total of 1.0 to 1.5 μs respectively (Table S2). The largest system with all the components is illustrated in Fig. S2A. K^+ ion permeation is taken as the criterion to designate a channel conformational state under specific conditions as “closed” or
“open”. In the GIRK2-Apo system, no ion permeation was seen regardless of whether an EF was applied or not. In contrast, in all other systems of the GIRK2 channel, such as with PIP2 (GIRK2-PIP2 or GIRK2), with PIP2+Na+ (GIRK2-Na+), with PIP2+Gβγ (GIRK2-Gβγ), and with PIP2+Gβγ+Na+ (GIRK2-Gβγ-Na+), K+ ions permeated, albeit inefficiently, even in the presence of EF across the membrane (0.06 V/nm or Vm=-200 mV). However, in the GIRK2-Gβγ-Na+ system, an almost 10-fold greater number of ions permeated under the transmembrane EF (Table 1). The relative permeation seen in these simulations agrees with electrophysiological results (20). Moreover, in the absence of an external electric field, the MD simulations also showed outwardly directed flow of K+ (intracellular to extracellular) (data not shown).

To better understand the gating mechanism, we set the production stage in the presence of an EF, since gating proved more efficient under these conditions (Table 1). All systems equilibrated by 150 ns of simulation as seen in the RMSD plots (Fig. S4). The average distance required to occlude permeation was 5.69 Å and was derived from the GIRK2-Na+ system, which has been shown experimentally to be the least conductive (20). When both the HBC and G-loop gates exceeded the minimal distance of 5.69 Å, the channel was considered to be in the open state. As designated by vertical lines in Fig. 1, this representation of activity would correspond to the open probability (Po) of the channel in the nanosecond time scale, even though not every time the channel was permeable an ion did in fact permeate. From this simplified analysis, it became apparent that Na+ or Gβγ subunits alone could open GIRK2 and could do so in a synergistic manner when applied together (Fig. 1).

In fact, the synergism between the Gβγ subunits and Na+ can be seen in records of single channel activity (cell-attached recordings) of the highly related GIRK4 channel. The gating behavior of certain GIRK4 mutants that mimic the Na+ (i.e. I229L), Gβγ (i.e. S176P), or Na+ + Gβγ (i.e. I229L + S176P) conditions (Fig. 2) (22, 23) bears remarkable pattern similarity to the simulated records of Po, even though the time scales separating these records are at least 6 orders of magnitude apart.

**Na+ ions predominantly stabilize the G-loop gate in the open state.** The GIRK2-PIP2 system revealed that although the HBC gate distances were distributed around the minimal distance for permeation, the G-loop gate distances became limiting (Fig. 3), explaining the inefficient open probability seen in Figure 1. The open probability of each of the two gates individually (HBC and G loop) is shown in figure 4. Once Na+ was included in the GIRK2-PIP2 system, there was a clear right shift of the G-loop gate minimum distributions to the open state (Fig. 3). This effect on the G-loop gate was in sharp contrast to the HBC gate, whose minimal distance distributions were not affected by Na+, remaining centered around the non-permissive 5.69 Å distance (Fig. 3). The G-loop gate Po increased by more than 7-fold, whereas the HBC gate Po, if anything, was somewhat decreased (Table 2 and Fig. 4). As a result, the HBC became the limiting gate to ion flow in the presence of intracellular Na+ ions.

**Gβγ dimer acts to predominantly stabilize the HBC gate in the open state.** A clear right shift of the HBC gate minimum distributions to the open direction was seen when Gβγ was included in the GIRK2-PIP2 system (Fig. 3). The effect of Gβγ on the G-loop gate also showed a right shift (compared to GIRK2-PIP2) but there was a significant component of distance distributions that Gβγ was not able to affect (Figs. 3, 4, and Table 2). Opening of the G-loop gate may become a rate limiting step to the conductive state when channels get activated by Gβγ. Since this limitation was less than that imposed by the HBC gate in the GIRK2-Na+ system, a higher overall Po of the channel was achieved (Fig. 1 and Table
This observation is consistent with experimental results suggesting that in comparison, gating by Gβγ is greater than that by intracellular Na⁺ (20).

**Na⁺ and Gβγ subunits work together to activate the channel the most.** When both Gβγ and intracellular Na⁺ ions were included in the simulations, the distance distributions of both gates were predominantly in the open state (Fig. 3) resulting in the greatest Po (Fig. 4 and Table 2). In the presence of an EF this condition produced the most efficient permeation of K⁺ ions (Table 1).

**Rocking, tilting, and rotation movements of GIRK2 domains lead to channel activation**

As discussed earlier, the only GIRK2 crystal structure depicting both the HBC and G-loop gates in the open state has been the GIRK2(R201A)+PIP₂ (3SYQ) structure (8). Electrophysiological recordings of the mutant have revealed very small ionic currents (8, 24), casting doubt as to whether this mutant provides a meaningful model of the channel open structure. Principal component analysis (PCA) was performed on our simulated trajectories to discern channel domain movements linked to gating. This analysis suggests that the HBC and G-loop gates move in opposite directions as the channel gates (Movie S1). This kind of movement is in contrast to the proposal based on the 3SYQ “open” structure (R201A-PIP₂), in which the two gates move in the same direction. In fact, when we performed PCA on the crystal structures excluding the 3SYQ structure, similar results to our simulations were obtained, supporting a movement of the two gates in opposite directions (Movie S2). When PCA was repeated including the 3SYQ, the two gates were shown to move in the same direction. In contrast to the proposal based on the 3SYQ “open” structure (R201A-PIP₂), in which the two gates move in the same direction. In fact, when we performed PCA on the crystal structures excluding the 3SYQ structure, similar results to our simulations were obtained, supporting a movement of the two gates in opposite directions (Movie S1). This kind of movement is in contrast to the proposal based on the 3SYQ “open” structure (R201A-PIP₂), in which the two gates move in the same direction. In fact, when we performed PCA on the crystal structures excluding the 3SYQ structure, similar results to our simulations were obtained, supporting a movement of the two gates in opposite directions (Movie S2). When PCA was repeated including the 3SYQ, the two gates were shown to move in the same direction as previously suggested (5). These results as a whole have suggested the GIRK2(R201A)+PIP₂ mutant involves perhaps a distinct reaction path and mechanism leading to the conformational state depicted by the crystal structure and may not represent a good model of the channel open state achieved by natural gating molecules.

**Movements controlling HBC gating.** Once the channel was gated from a pre-open to the open state, rocking movements of the CTD and tilting of the TM2 were observed (Movie S1). In order to quantitatively depict these movements two angles were defined: a) a dihedral angle (TM2-CTD) formed by the Cα atoms of S196 with I244, I281 on one hand and with Q287 on the other hand, and b) a TM2 tilt angle formed by the TM2 terminal and the vertical axis of the TM domain (Fig. 5). The larger the TM2-CTD dihedral angles the more the CTD rocks outwardly, away from the vertical axis of the TM domain. Comparison of the closed and open structures reveal an outward CTD rocking movement during opening, an outward TM2 helix tilting and local twists in the Gβγ binding βL-βM and βD2-βE1 loops (Fig. 5). 3-D plots were then employed to compare the relationship between the dihedral angle, the tilt angle, and the HBC Cα distance. Fig. 6A shows clearly that as the TM2-CTD dihedral angles and the TM2 tilt angles increase (move outwards), so do the HBC Cα distances. Generally speaking, increases in both the TM2-CTD dihedral angles and the TM2 tilt angles translate into opening the HBC gate.

It has been proposed that a four-degree anticlockwise rotation (of the CTD relative to the TMD and as viewed from the membrane) will allow transition of the closed (3SYA or GIRK2-PIP₂+Na⁺) to the preopen (4KFM or GIRK2-PIP₂+Gβγ+Na⁺) conformation (5). We used the geometry center of the TMD and CTD excluding the N- and C-termini, as mentioned in the methods, to calculate this angle and estimated it to be around 3.68° (Fig. 7A), consistent with the previously proposed angle of 4° (5). Yet, no correlation was found between rotation angles and HBC Cα distances in the conductive systems (GIRK2-Na⁺, GIRK2-Gβγ, GIRK2-Gβγ-Na⁺)
that showed progressive opening of the HBC gate (Fig. 7B). In other words, we were unable to correlate the HBC gate opening to an anti-clockwise rotation activation mechanism alone.

**Binding of two Gβγ subunits (unlike a single Na⁺ ion) cause the cytosolic domain to rock.** Na⁺ ions bind to the CD-loop of GIRK2 (and GIRK4) (10, 12) and stabilize its interactions with the G-loop gate (18). In contrast, Gβγ binds GIRK channels at the inter-subunit interface between the βD2-βE1 loop of one subunit and the βL-βM loop of its adjacent subunit (4, 5). Since the common edge between the two planes that defined the TM-CTD dihedral angle is at the βD2-βE1 loop level, we aimed to assess relative contributions of Gβγ and Na⁺ to the local movements of both loops and further to the rocking motion described above.

Changes in the local conformations of the βD2-βE1 and βL-βM loops that comprise the Gβγ binding site were examined first. Two critical residues to the binding and activation by Gβγ, F254 in the βD2-βE1 loop and L344 in the βL-βM loop (4, 5, 25-27), are close to one another in the absence of Gβγ and farther apart as Gβγ occupies the cleft between the two loops. Fig. S6 shows that consistent with the 4KFM crystal structure both Gβγ-present systems (GIRK2-Gβγ/GIRK2-Gβγ-Na⁺) show a larger F254-L344 Cα distance compared to the systems in the absence of Gβγ (GIRK2/GIRK2-Na⁺). Addition of Gβγ that interacts with both βD2-βE1 and βL-βM loops causes clear changes in the local conformations (Figs 8, S5C, and D). The reason for the local twists was assessed by the psi-phi (ψ-φ) angles as a function of the gating molecules. Significant differences in the ψ-φ distribution of the systems in the presence of Gβγ from those in its absence take place in both loops (Fig. S7). Q248 was reported to form contacts with N75, S98, and W99 on the Gβγ subunit and mutations at S98 and W99 diminish Gβ activation of GIRK (28, 29). Our simulation results are consistent with these experimental findings. In fact, with the exception of S250 that did not display differences in the ψ-φ distribution of the systems in the presence of Gβγ, all other residues have been reported to interact with Gβγ (5). Similarly, the ψ-φ distributions are consistent with the experimental work, showing that Gβγ rather than Na⁺ could induce strong interactions with both loops except residue S250. As a result, both the βD2-βE1 and the βL-βM loops seem to act like a crank when Gβγ subunits are present. The stronger (GIRK2-Gβγ-Na⁺ and GIRK2-Gβγ > GIRK2-Na⁺) the twist rotations are, the more the CTD will rock.

For each GIRK2 subunit, only one Na⁺ binds to the βC-βD₁ loop while two Gβγ subunits bind to the βD2-βE1 and βL-βM loops respectively (Fig. S24). Due to its distant binding location, Na⁺ seems unable to induce strong rotations of both loops. Collectively, two Gβγ subunits help one CTD rock more on both sides compared to the binding of a single Na⁺ ion. These differences between the extent of rocking movements caused by Gβγ versus Na⁺ are consistent with the greater channel activation seen by the Gβγ subunits versus Na⁺ (20).

** Movements controlling the G-loop gate.** We also examined the relationship of the TM2-CTD dihedral angle, the anti-clockwise rotation angle between TM and CTD, and the G-loop gate minimal distances in a 3-D plot. In Fig. 6B, as the rotation angles increase and the TM2-CTD dihedral angles decrease (as shown by the arrow), the G-loop gate distances increase. In other words, an anti-clockwise rotation and/or an inwardly CTD rocking movement enlarge the G-loop gate.

**PIP₂ interactions as a result of gating by Na⁺ and/or Gβγ**

PIP₂ has been shown to be a necessary co-factor for activating GIRK channels (6, 7). In contrast to its ability to gate other Kir channels, PIP₂ is unable to gate efficiently GIRK channels on its own but requires the presence of Na⁺/Gβγ (12). Furthermore, Na⁺ and Gβγ together display synergism in activating GIRK channels (20). Thus, we proceeded to examine the interactions
between residues comprising the GIRK2 binding site for PIP2 and phosphates 4' (P₄) and 5' (P₅) atoms on its inositol ring.

Pull on the TM2 helix gets the HBC gate opened. Gβγ relative to Na⁺ stabilizes key interactions of the positively charged K194 residue with the negatively charged phosphates P₅ and P₄ of PIP2 (Fig. 9A, B). Meanwhile, K200 in the GIRK2-Gβγ-Na⁺ system loses its interaction with PIP2 (especially with the P₄ phosphate), stabilizing the salt bridge interactions of the K199 with both phosphates of PIP2 (Fig. 9A, B). The CTD rocking and its anticlockwise rotation are likely to be driving the stronger binding of K199 to the P₅/P₄ atoms and the concomitant unbinding of K200. This interaction rearrangement may underlie the pull on the TM2 helix to cause its tilting movement (Fig. 5) and as a consequence causing the HBC gate to open. Unlike the induction by Gβγ, Na⁺ in the GIRK2-Na⁺ system does not exert much influence on K194, K199 or K200 and it is thus unable to influence the HBC gate (Fig. 9B).

The presence of PIP2 alone with the channel in our study resulted in ion permeation, albeit low, indicating that GIRK2-PIP2 could be active (Table 1). Although electrophysiological experiments have shown that PIP2 on its own is not efficient to stabilize the channel gate in the open conformation (6), low basal activity can be seen (53). The low permeation in the GIRK2-PIP2 system simulations could also be due to the pre-open conformation as the initial structure for MD, which has overcome part of the energy barrier required for the CTD rocking movement. When we took an HBC closed conformation as our initial model, for example the last snapshot of the GIRK2-Apo system, PIP2 alone could not activate the channel even when the simulation time scale was extended to 1 µs. In other words, PIP2 alone could not overcome the CTD rocking energy barrier in this time scale. Furthermore, the HBC Po of GIRK2-PIP2 (44.86%) seems larger than that of GIRK2-Na⁺ (35.14%) as shown in Fig. 4A and Table 2. However, in an additional 0.5 µs simulation, the corresponding Po of GIRK2-PIP2 and GIRK2-Na⁺ showed an approximate 7% decrease and 34% increase respectively (data not shown), which suggests that PIP2 alone is insufficient to stabilize the HBC gate in the open state.

G-loop gate is stabilized by the interactions with the CD loop/βI strand. The G-loop gate of GIRK2 consists of seven residues (MVEATGM). Other than the terminal methionines with longer side chains, it is difficult for other residues to induce strong short range VDW interactions. Instead, the long-range electrostatic interactions elicited by the only charged G loop residue E315 become critical for its movement. The G-loop gate has been found in a GIRK1 chimera to be stabilized in the open state by the equivalent Glu residue through a hydrogen bond of E304 with H222 of the CD loop (18). A similar hydrogen bond also exists in GIRK2. For example, in the GIRK2-Na⁺ system, the hydrogen bond between E315(N) and H233(O) is observed with significant occupancy 10.40% (Table 3). This bond is lost in GIRK2-Gβγ/GIRK2-Gβγ-Na⁺ systems due to longer distances (Fig. 10). The other G-loop gate stabilization in the closed state of the GIRK1 chimera was proposed to be the inter-subunit E304-R313 salt bridge (18). However, in GIRK2 this interaction seems to favor G-loop gate opening. E315 (G loop)-R324 (adjacent βI strand) was formed in the open state of the GIRK2-Na⁺/GIRK2-Gβγ-Na⁺ systems due to longer distances (Fig. 10). During channel activation by Na⁺, this salt bridge causes E315 to be pulled outward (Fig. 11A), which facilitates the opening movement of G-loop gate via an anti-clockwise rotation (Fig. 6).
GIRK2-IP2 salt bridge interactions in the G-loop gate stabilize the closed G-loop gate structure (18). This interaction switches to R66 (R77 in GIRK2)-IP2 to stabilize the G-loop gate open structure (18). Similarly, in the GIRK2-Na+ system open G-loop gate simulated structure, the R77-IP2 salt bridge was established. The carbonyl oxygen atom of residue R77 was positioned to interact with R230, which is two positions away from the key D228 residue that coordinates Na+, and D81 in a R230-R77-D81 triad interaction pattern (Fig. 11 A). In contrast to the GIRK2-Na+ system, in the GIRK2-Gbγ/GIRK2-Gbγ-Na+ systems the R77-IP2 interactions are almost lost, which instead introduces a larger repulsion between R230-R77 and consequently limits R230 to interact only with residue D81 (Fig. 11 B). Considering the CTD anti-clockwise rotation, the outwardly pointing orientation of R230 (GIRK2-Na+) is preferred to the inwardly pointing orientation (GIRK2-Gbγ/GIRK2-Gbγ-Na+) and is more likely to stabilize the rotated conformation (Figs 11 and 12). As the rotation angles decrease (GIRK2-Na+ > GIRK2-Gbγ > GIRK2-Gbγ-Na+), the E315-H233 and E315-R324 stabilization is lost step by step. It is thus inferred that the IP2-mediated R230-R77/D81 interactions probably affect the stabilization of the G-loop gate in view of the CTD anti-clockwise rotation.

As a whole, the G loop is likely to be gated as follows: the larger K64-IP2 distance fluctuations facilitate the formation of an inter-subunit H68(N-terminus) - V351(LM loop) hydrogen bond (Fig. 11 and Table 3). Based on the inter-subunit R77-IP2 interactions, R230 in the CD loop interacts with D81 (GIRK2-Gbγ/GIRK2-Gbγ-Na+) or D81+R77 (GIRK2-Na+). Different binding patterns determine whether the G-loop gate could be stabilized by the adjacent βI strand or the βI+CD loop in view of CTD anti-clockwise rotation.

Discussion

In the past decade, great advances have been made in obtaining structural snapshots of a truncated GIRK2 channel that is in contact with its physiological intracellular regulators, IP2, Na+, and Gbγ. Yet, no coherent structural dynamic model has emerged that is consistent with over three decades of functional data on the gating of GIRK channels. Here, we used all-atom microsecond-scale MD simulations to discern the large movements leading to key molecular interactions that underlie channel gating by Na+ and Gbγ. In the conceptual model that has emerged, Na+ binding to the CD loop causes it to interact intra-molecularly with the G-loop gate (E315-H233) and stabilize it in the open state, with the aid of an inter-molecular interaction of the G loop with the adjacent βI strand (E315-R324). Gbγ binds to the βD2-βE1 and βL-βM cleft and causes a large change in the TM2-CTD dihedral angle, which serves as a crank to initiate a rocking movement of the CTD and as a consequence the tilting of TM2 helix and the opening of the HBC gate.

These movements alter the interactions of GIRK2 with IP2 that serve to stabilize the gates in the appropriate state causing channel gating to take place. In the case of the G-loop gate the pivotal role of the CD loop interactions with the G loop is regulated on one hand by D228 and H233 that are utilized for Na+ ion coordination (8, 30) and on the other hand by R230 that is influenced by a slide helix IP2-interacting residue, R77. Based on the inter-subunit R77-IP2 interaction, R230 in the CD loop interacts with D81 (GIRK2-Gbγ/GIRK2-Gbγ-Na+) or D81+R77 (GIRK2-Na+), which determines whether the G-loop gate can be stabilized by the adjacent βI strand or βI+CD loop (Fig. 11). On the other hand, the R77-IP2 interaction seems to act as the connection of the G-loop gate to the HBC. The negatively charged head of IP2 is located between the positively charged R77 and K194, experiencing competitive interactions (Fig. 12). Once the R77-IP2 interaction takes place, the
K194-PIP$_2$ interaction is impaired (GIRK2-Na$^+$). Otherwise, the stronger K194-PIP$_2$ interaction in the GIRK2-G$\beta$γ/GIRK2-G$\beta$γ-Na$^+$ systems pulls the bottom of TM2 to open the HBC gate (Figs. 5, 9, and 12). The key amino acid residues predicted by our simulations to be involved in the stabilization of channel-PIP$_2$ interactions, such as K194, R230, D81, E315, and R324, are highly conserved among Kir channels (Fig. S8) further underscoring their important role proposed by our study. The importance of these residues proposed by our computational studies ought to be validated experimentally in future studies.

Our observations from both the HBC and G-loop gates do account for the movement of the two gates in opposite directions, as indicated by the PCA. In the presence of only Na$^+$ ions, the HBC of the GIRK2-Na$^+$ system is barely affected due to the limited CTD rocking movement; the G-loop gate is enlarged by an anti-clockwise rotation with the aid of the E315-H233 and E315-R324 interactions. On the other hand, in the presence of only G$\beta$γ, the HBC in the GIRK2-G$\beta$γ system is widened by the induced larger CTD rocking; in contrast, the G-loop gate is somewhat destabilized by the loss of the E315-H233 hydrogen bond. Lastly, in the presence of Na$^+$ and G$\beta$γ, the HBC in the GIRK2-G$\beta$γ-Na$^+$ system assumes the largest size but both the E315-H233 and E315-R324 stabilization interactions of the G-loop gate are lost. In other words, a larger outward CTD rocking movement enlarges the HBC but destabilizes the G-loop gate. In fact, none of the published GIRK2 wild-type crystal structures existed with both gates closed, based on the minimum distance (5.69 Å) required for permeation.

The synergism between Na$^+$ and G$\beta$γ in gating GIRK channels (20) was also observed in our simulations. The Po of the HBC gate is the highest in the GIRK2-G$\beta$γ-Na$^+$ system, while that of the G-loop gate is similar in the GIRK2-Na$^+$ and GIRK2-G$\beta$γ systems (Table 2). Thus, the synergism is likely to correlate with the control of the HBC gate. D228 acts as a key residue in the coordination of Na$^+$. Na$^+$ in the GIRK2-G$\beta$γ-Na$^+$ system causes a decrease in the distance of D228-R201 compared to the GIRK2-G$\beta$γ system (Figs 12C and D). A rotation by 7.74 ° of the C-linker is induced, which disrupts the K200-PIP$_2$ interaction that was present in the GIRK2-G$\beta$γ system. As a result, the K194-PIP$_2$ interaction is enhanced and a larger and more stabilized HBC gate results in the GIRK2-G$\beta$γ-Na$^+$ system. Using both experimental and MD simulation approaches, K200 was previously identified as a key player in GIRK2 channel gating by G$\beta$γ or ethanol (54). Disruption of the K200-PIP$_2$ interaction through neutralization mutations caused channel activation and enhancement of channel-PIP$_2$ interactions, a result consistent with the conclusions from the present simulation studies.

A recent computational study examined conduction through the GIRK2 channel pore but also gating in the absence on the gating molecules G$\beta$γ or Na$^+$ (55). By comparing these two systems the authors found that PIP$_2$ enlarged the HBC gate, while the G-loop gate was found to be narrower than the HBC gate. Yet, the PIP$_2$-bound GIRK2 channels were conductive, albeit inefficiently. These results are consistent with the results of our study. Moreover, since our study focused on gating, including the effects of the gating particles G$\beta$γ and Na$^+$, it elucidated the relative efficiencies of gating under different conditions (i.e. Na$^+$, G$\beta$γ, or Na$^{+}+G\beta\gamma$). Our results are in complete agreement with experimental findings. Bersteiner and colleagues quoted the pivotal 1998 Huang et al., study as providing evidence that PIP$_2$ alone could activate GIRK1/4 channels (55, 7). This is in fact not the case, as Na$^+$ was also present in the solutions used by Huang et al., to result in efficient GIRK channel gating. Thus, the experimental literature agrees that Na$^+$ or G$\beta$γ alone or together enhance the ability of PIP$_2$ to gate GIRK channels to different levels. In fact, a single point mutant has been shown to strengthen...
channel-PIP$_2$ interactions sufficiently, such that PIP$_2$ could now gate alone (without Na$^+$ or G$\beta\gamma$) (22) mimicking gating by Na$^+$ (see Fig. 2).

An interesting residue in the PIP$_2$ binding pocket is W91 that also showed a dependence of its distance from K194 based on the level of opening of the HBC gate. Fig. S9 shows that W91 was 9.0 Å away from K194 in GIRK2-G$\beta\gamma$ but reached closer to K194 in GIRK2-G$\beta\gamma$-Na$^+$, approximately within 7-8 Å. Even though this is still not close enough for W91 and K194 to engage in cation-pi interactions, these subtle conformational differences in the Na$^+$-G$\beta\gamma$ system over the G$\beta\gamma$ demonstrate the sensitivity of dynamic experiments (MD simulations) over static ones (X-ray crystal structures).

In our simulations, the GIRK2 system showed a pre-open to HBC-closed process, which suggested that the HBC gate closing comes ahead of the G-loop gate. Consistent with our previous results (18), it can be concluded that the open/close movements of the HBC and G-loop gates are sequential but not simultaneous. From close to open, the G-loop gate opening precedes that of the HBC gate (18, GIRK1 chimera), while from open to close, the HBC closing precedes the G-loop gate (present study, GIRK2).

Differences between GIRK2 and other GIRK homomeric (e.g. GIRK4) or heteromeric channels (e.g. GIRK1/2) remain to be explored in order to offer a molecular understanding of how intracellular channel modulators may differentially regulate the activity of GIRK channels.

In conclusion, the GIRK2 channel is activated by the intracellular regulators PIP$_2$, Na$^+$ ions, and/or G$\beta\gamma$ subunits via rocking and anti-clockwise rotation movements of the CTD. Due to the lack of direct or allosteric interactions in either the $\beta$D$_2$-$\beta$E$_1$ or the $\beta$L-$\beta$M loops, the Na$^+$ ion action is limited to the control of the G-loop gate through the same subunit E315-H233 and adjacent subunit E315-R324 hydrogen bonds thus inducing an anti-clockwise rotation. In contrast, binding of the G$\beta\gamma$ subunits in the cleft created by the $\beta$D$_2$-$\beta$E$_1$ and the $\beta$L-$\beta$M loops of adjacent subunits twists both the loops in a crank-like manner to rock the CTD and pull the HBC gate to open with the aid of K194-PIP$_2$ salt bridge interactions. The interactions of R77(Slide helix)-PIP$_2$ seems to be what connects the G-loop to the HBC gate. Na$^+$ enlarges the G-loop gate and drives formation of the R77-PIP$_2$ interaction that in turn destabilizes the K194-PIP$_2$ interaction and the conductive state of HBC. The synergism of Na$^+$+G$\beta\gamma$ is likely to be attributed to a 7.74° rotation of the C-linker that as a result disrupts the K200-PIP$_2$ and enhances the K194-PIP$_2$ attraction.

**Experimental Procedures**

**Setting up GIRK2 model systems for MD simulations**

The crystal structure of GIKR2 channel (PDB ID: 4KFM), which is considered to be in the pre-open state, was used to build the initial models by adding different endogenous regulators, such as PIP$_2$, Na$^+$, and G$\beta\gamma$. To characterize the role of each regulator alone or in combination, five simulation systems in total were constructed, which are termed: GIRK2, GIRK2-PIP$_2$, GIRK2-PIP$_2$-Na$^+$ (GIRK2-Na$^+$), GIRK2-PIP$_2$-G$\beta\gamma$ (GIRK2-G$\beta\gamma$), and GIRK2-PIP$_2$-G$\beta\gamma$-Na$^+$ (GIRK2-G$\beta\gamma$-Na$^+$), respectively (Table S2). All the model systems were built by taking the following steps:

1. All the missing side chains of residues, I55, R73, E127, F141, K165, K301, and E303 in GIRK2; R42 and R214 in the G$\beta$ subunit; E58 and E63-F67 in the G$\gamma$ subunit (PDB ID: 4KFM) were added by Discovery Studio 2017 software.

2. All the hydrogen atoms were added using the H++ website server (http://biophysics.cs.vt.edu/) (31, 32). The protonated states of the titratable residues were determined by pKa calculations at neutral physiological condition (pH = 7.0).
The PIP₂ molecule was built based on the DiC₁PIP₂ in the crystal structure (PDB ID: 4KFM) by adding alkyl tails using Maestro (Schrödinger, LLC). The geometry of PIP₂ structure was optimized by ab initio quantum chemistry at the Hartree-Fock/6-31G* level, followed by restrained electrostatic potential (RESP) charge calculations using the GAUSSIAN 09 program (33). The antechamber module of AmberTools17 was employed to generate the required force field parameters for the PIP₂ based on the derived RESP charges and the GAFF2 force field (34-36).

The GIRK2 channel and complexes were immersed in explicit lipid bilayer of POPC, POPE, POPS, and cholesterol with molecular ratio of 25:5:5:1 (37) and a water box (116 × 116 × 150 Å³ for GIRK2, GIRK2-PIP₂, and GIRK2-Na⁺, and 162 × 162 × 170 Å³ for GIRK2-Gβγ and GIRK2-Gβγ-Na⁺, a box edge of at least 14 Å from the protein periphery in each dimension using periodic boundary conditions) by using CHARMM-GUI Membrane Builder webserver (http://www.charmm-gui.org/?doc=input/membrane) (38-41). 150 mM KCl was added into the system with the K⁺ ions and water molecules from crystal structure retained.

The tleap module of Ambertools17 was used to neutralize the complexes by adding additional K⁺ or Cl⁻ ions prior to generation of the topology and coordinates files. The FF14SB, LIPID17, and GAFF2 force fields were chosen for protein, mixed lipid membrane, and PIP₂ respectively. The simulation systems obtained contain a range from 160,000 to 400,000 atoms (Table S2).

All-atom microsecond-scale MD simulations
A two-stage energy minimization protocol, steepest descent algorithm (10,000 steps) and conjugate gradient (10,000 steps) was performed for each model system. The systems were heated from 0 to 300 K using Langevin thermostat algorithm with a 0.5-femtoseconds (fs) timestep to avert internal disturbance. In the heating stage, the protein and lipid bilayer were initially fixed to remove any potential steric clashes from K⁺ or Cl⁻ ions, and water molecules; followed by gradually reduced position restraints on the protein and membrane (10 to 0.1 kcal/mol·Å² in 10 steps of total 20 nanoseconds). 0.5-microsecond (μs) MD simulations were conducted using the constant-temperature, constant-pressure ensemble (NPT) without electric field, followed by the same time scale using the constant-temperature, constant-volume ensemble (NVT). To accelerate the permeation events in the limited time scale of simulations, an external voltage 0.06V/nm (18, 42) was employed, under which the secondary structure of all 4 subunits of GIRK2 was well maintained. However, higher electric fields resulted in structural instability. The PMEMD.CUDA program in AMBER16 was used to conduct the simulations. Long range electrostatics will be calculated using the particle mesh Ewald (PME) method with a 10 Å cutoff. A 4-fs timestep by employing hydrogen mass repartition algorithm for system solutes (43) was used to accelerate the MD simulations. SHAKE algorithm was applied on the solvent molecules.

Analysis of MD Simulation results
All the analysis was done on the trajectories with external electric field, unless otherwise mentioned. Geometry analysis (distances and dihedral angles), principal component analysis (PCA), and generalized Born surface area (MM-GBSA) binding free energy calculations were implemented by Amber16 and Ambertools17 (44). The channel gating mechanism was studied by PCA based on the concatenated trajectories of GIRK2 (pre-open to closed) and GIRK2-Gβγ-Na⁺ (pre-open to open). For comparison, the PCA was also performed on available GIRK2 crystal structures (PDB ID: 3SYA, 3SYC, 3SYO, 3SYP, and 4KFM) with and without the “open” conformation R201A with PIP₂ (PDB ID: 3SYQ).
In order to evaluate the TM-CTD rotation angle, larger-displacement residues in the N- and C- termini were excluded. To make the angle more reasonable, the relative tilting of TM domain to CTD occurred during simulations was removed by our program in Python 2.7.5, implemented in MDAnalysis 0.18 (45, 46). The delta TM-CTD rotation angle was obtained by taking the same reference, a closed conformation (PDB ID: 3SYA) (5). The HOLE program v2.2 was used for analysis of the dimensions of the pore in GIRK2 channel structures (47, 48). The sequence conservation analysis was performed using the ConSurf server (http://consurf.tau.ac.il/) (49-51).

All the calculations were conducted on servers equipped with NVIDIA Tesla K80, K40, and GeForce GTX-1080 graphical cards. The executables were built under the Community Enterprise Operating System (CentOS) version 7 and NVIDIA Compute Unified Device Architecture (CUDA) toolkit version 8.0.

**Ion channel expression.** Various ion channel cDNAs were generated by introducing point mutations on the background of the channel GIRK4(S143T) (56) using the Quickchange site-directed mutagenesis kit (Stratagene, LaJolla, CA). cRNAs were in turn generated by in vitro transcription using the “Message Machine” kit (Ambion, Austin, TX). The cRNA concentrations were estimated by comparing the fluorescence intensity of diluted cRNA with cRNA Marker (GIBCO, Gaithersburg, MD) and in parallel through electrophoresis on formaldehyde gels. Ion channel proteins were expressed in *Xenopus* oocytes by injecting the desired amount of cRNA into the oocytes. The desirable expression level for single channel recording was achieved by incubating the oocytes at 18 C° for 1 to 2 days after injecting 0.01 to 0.1 ng cRNA per oocyte. Oocytes were isolated and microinjected as previously described (57). The IACUC approved protocol for use of *Xenopus laevis* frogs to isolate oocytes at Northeastern University was last approved on December 2018 by the protocol #:17-0102R-A1.

**Single channel recording and analysis.** The single-channel activity was recorded using an Axopatch 200A amplifier (Axon Instruments). The pipette solution contained (in mM) KCl 96, MgCl2 1, and HEPES 10 (pH 7.40). The bath solution contained (in mM): KCl 96, EGTA 5, and HEPES 10 (pH 7.40). 100 µM gadolinium was routinely included in the pipette solution to suppress native stretch channel activity in the oocyte membrane. Chemicals were purchased from Sigma (St. Louis, MO). Single-channel currents were filtered at 1–2 kHz with a six-pole low-pass Bessel filter, sampled at 5–10 kHz and stored directly into the computer’s hard disk through the DIGIDATA 1200 interface (Axon Instruments). Single-channel analysis was carried out with pClamp8 (Axon Instruments). The open probability of a single-channel in a recording was calculated by dividing the sum of the durations that the channel dwelling at the open state by the total length of recording. The length of the recordings used for computing the channel open probability ranged from 100.0 to 1804.8 seconds (573.8 ± 499.9 Seconds, n=18).

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**Conflict of Interest**

The authors declare that they have no conflicts of interest with the contents of this article.
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Figure and Tables

**Table 1** The number of K⁺ for each system that got through the GIRK2 channel and the channel state without or with electric field (EF, V/nm) in the last snapshot of MD simulations.

| Systems                  | EF = 0          | EF = 0.06         |
|--------------------------|-----------------|-------------------|
|                          | No.  | State | No.  | State       |
| GIRK2-Apo                | 0    | Closed| 0    | Closed      |
| GIRK2-PIP₂ (GIRK2)       | 2    | PO*   | 3    | PO          |
| GIRK2-Na⁺                | 2    | PO    | 3    | PO          |
| GIRK2-Gβγ                | 2    | PO    | 4    | PO          |
| GIRK2-Gβγ-Na⁺            | 1    | Open  | 36   | Open        |

*PO: partially open

**Table 2** The open probability (%) of the constrictions HBC, G-loop gate, and both for the last 350 ns that is in the equilibrium stage of MD in each simulation system.

| Systems                  | HBC   | G-loop gate | Both |
|--------------------------|-------|-------------|------|
| GIRK2-Apo                | 1.43  | 54.00       | 0.29 |
| GIRK2-PIP₂ (GIRK2)       | 44.86 | 11.71       | 4.86 |
| GIRK2-Na⁺                | 35.14 | 87.71       | 30.57|
| GIRK2-Gβγ                | 80.57 | 81.14       | 65.43|
| GIRK2-Gβγ-Na⁺            | 86.57 | 82.86       | 71.14|

**Table 3** The key hydrogen bonds (number / total occupancy over simulation time) of cytosolic domain proposed to facilitate the G loop gating.

| HB                        | GIRK2-Na⁺ | GIRK2-Gβγ | GIRK2-Gβγ-Na⁺ |
|----------------------------|-----------|-----------|---------------|
| E315 (G loop) – H233 (CD loop) | 2 / 10.40% | 3 / 0.60% | 0 / 0         |
| E315 (G loop) – R324 (βI strand) | 1 / 41.00% | 1 / 44.20% | 1 / 1.60%    |
| H68 (N-terminus) – V351 (LM loop) | 1 / 32.80% | 1 / 34.20% | 1 / 39.60%   |
**Figure 1.** Overall GIRK2 channel burst characteristics (each vertical line per ns) over simulation time. MD simulations were run on five systems: GIRK2-Apo, GIRK2-PIP$_2$ (GIRK2), GIRK2-Na$^+$, GIRK2-G$\beta\gamma$, and GIRK2-G$\beta\gamma$-Na$^+$. Channels with their HBC and G-loop gates larger than a minimum distance (5.69 Å) that is required for permeation were considered to be open. This minimum distance estimate was derived from the least conductive system GIRK2-Na$^+$ compared to the other two systems studied that have been shown experimentally to display measurable currents (i.e. GIRK2-G$\beta\gamma$ and GIRK2-G$\beta\gamma$-Na$^+$).

**Figure 2.** Unitary activity of GIRK4$^*$ mutants (using as control the highly active GIRK4-S143T). GIRK4$^*$ mutants mimic gating by Na$^+$ (I229L), G$\beta\gamma$ (S176P) and G$\beta\gamma$/Na$^+$ (S176P, I229L) in A, a compressed and B, more expanded time scales. Open probability of S176P (+/- 0.019; N=6), I229L (+/- 0.014; N=5), and S176P/I229L (+/- 0.093; N=7). These data obtained from mutants representing the endogenous gating molecules (PIP$_2$, Na$^+$, G$\beta\gamma$, Na$^+$/G$\beta\gamma$) are consistent with prior reports (53).

**Figure 3.** Minimal distances for HBC and G-loop gate opening. Histograms of distributions of minimum distances of the HBC gate (left) and the G-loop gate (right) based on the simulation (per ns) of the MD trajectory. The same five systems as in Fig. 1 were analyzed, namely GIRK2-Apo, GIRK2-PIP$_2$ (GIRK2), GIRK2-Na$^+$, GIRK2-G$\beta\gamma$, and GIRK2-G$\beta\gamma$-Na$^+$. The red-dashed line indicates the cutoff distance under which no K$^+$ permeation took place.

**Figure 4.** GIRK2 channel burst characteristics for individual gates (each vertical line per ns) over simulation time. MD simulations were run on five systems: GIRK2-Apo, GIRK2-PIP$_2$ (GIRK2), GIRK2-Na$^+$, GIRK2-G$\beta\gamma$, and GIRK2-G$\beta\gamma$-Na$^+$. A, Channels with their HBC and B, G-loop gates larger than a minimum distance (5.69 Å) that is required for permeation were considered to be open. The minimum distance estimate was derived as in Fig. 1 from the least conductive system GIRK2-Na$^+$ compared to the other two systems studied that have been shown experimentally to display measurable currents (i.e. GIRK2-G$\beta\gamma$ and GIRK2-G$\beta\gamma$-Na$^+$).

**Figure 5.** GIRK2 activation mechanism schemes: the closed- and open-structures are colored yellow and green respectively with both HBC and G-loop gates labeled. Cytosolic domain (CTD) rocking, TM2 helix tilting, and the local twists of the $\beta$L-$\beta$M and $\beta$D$_2$-$\beta$E$_1$ loops are indicated by the black/red arrows. A, view of two opposing subunits with the front and back subunits removed for clarity. B, 90° counter-clockwise rotation showing the two loops ($\beta$L-$\beta$M and $\beta$D$_2$-$\beta$E$_1$) that form the cleft where one G$\beta\gamma$ subunit binds each channel subunit individually.

**Figure 6.** Parameters describing HBC and G-loop gate opening. A, 3-D plots of TM2 helix (V188-S196) tilt angles, TM2-CTD dihedral angles (C$_\alpha$ atoms of S196, I244, I281, and Q287), and average HBC C$_\alpha$ distances; B, 3-D plots of TM2-CTD dihedral angles, relative rotation angles of TM2 and CTD, and average G-loop gate minimal distances. The arrow indicates the direction of increasing rotation angles and decreasing TM2-CTD dihedral angles that result in increasing G-loop minimal gate distances, best exemplified by the GIRK2-Na$^+$ system.
Figure 7. Movements during the opening of the HBC gate. A, The calculated anti-clockwise rotation angle of CTD of the X-ray crystal structures 3SYA (closed) and 4KFM (pre-open). The estimated 3.68° is consistent with the previously proposed angle of 4° (5); B, The average HBC Ca distances versus the relative anti-clockwise rotation angles of TMD and CTD domains by snapshot (per ns) of MD simulations shown for four systems: GIRK2-Apo, and PIP2 containing: GIRK2-Na+, GIRK2-Gβγ, and GIRK2-Gβγ-Na+. The system containing all gating molecules best exemplifies the most open HBC gate.

Figure 8. Changes in local conformations of the Gβγ binding loops. The dihedral angles (°) of βL-βM (left) and βD2-βE1 (right) loop from trajectories with the largest distributions in GIRK2-Na+ labeled by dashed lines for comparison: the dihedral angle was defined by the Ca atoms of Y349, D346, E345, and E350 for βL-βM loop, and Q248, S250, E251, and G252 for βD2-βE1 loop.

Figure 9. Changes in specific residue-PIP2 interactions leading to HBC gating. A, Distances between the N atom of the proposed key Lys residues (panel C) and the P4 (left) or P5 (right) atoms of PIP2 taking the crystal structure (PDB ID: 4KFM) as a reference in red; B, PIP2-Lys pairwise Gibbs free energy change with a standard deviation bar; C-F, binding patterns between the key Lys that are involved in regulating HBC by the PIP2 identified in the crystal structure (PDB ID: 4KFM), GIRK2-Na+, GIRK2-Gβγ, and GIRK2-Gβγ-Na+. The major contributors to binding are highlighted in cyan.

Figure 10. Changes in specific residue-PIP2 interactions leading to G-loop gating. The average non-bonded interactions with an error bar and the distances between the major residues involved in G loop gating over simulation time. The crystal structure (PDB ID: 4KFM) is taken as a distance reference in red. SC/MC in atomic labels represent side chain/main chain.

Figure 11. Stabilization of the G-loop gate in the open state. Network of the non-bonded interactions among G loop, CD loop, Slide helix, LM loop, N-terminus, and βI strand for A, GIRK2-Na+ and B, GIRK2-Gβγ-Na+. Two adjacent subunits (yellow and green) are shown with the front PIP2 removed for clarity.

Figure 12. Cartoon of the PIP2 mediated gating mechanism of the G-loop gate. A, GIRK2-Na+; B, GIRK2-Gβγ-Na+ and the synergism effect caused by the C-linker rotation: C, GIRK2-Gβγ; D, GIRK2-Gβγ-Na+. The facing subunits are colored in green (A-D) with an adjacent in orange (A and B). One PIP2 only is shown in each panel for clarity. Sodium ion is shown in a purple ball. The positive and negative groups are designated blue and red respectively in Fischer projection style. Once the R230-R77-D81 triad interaction pattern (A) other than the R230-D81 (B) is induced, the opening movement of G-loop gate is facilitated by a larger anti-clockwise rotation with the aid of E315-H233 and E315-R324 hydrogen bond interactions. The K200-PIP2 interactions (C) is disrupted by a 7.74° rotation of the C-linker which is induced by the shorter D228-R201 distance (D).
Fig. 1

Simulation time (ns)
Fig. 6

A. HBC Cα distances (Å)
- GIRK2-Apo
- GIRK2-Na⁺
- GIRK2-Gβγ
- GIRK2-Gβγ-Na⁺

B. G-loop gate min distances (Å)
- GIRK2-Apo
- GIRK2-Na⁺
- GIRK2-Gβγ
- GIRK2-Gβγ-Na⁺
Fig. 9

(A) N(K)-P$_{i}$(PIP$_{2}$) distance vs time

(B) ΔG (kcal/mol)

(C) GIRK2-Na$^{+}$

(D) GIRK2-G$\beta$$\gamma$

(E) GIRK2-G$\beta$$\gamma$-Na$^{+}$

F) GIRK2-Na$^{+}$

Simulation time (ns)

Distance (Å)

K194

K199

K200

K194

K199

K200

0

100

200

300

400

500

6.0

5.5

5.0

4.1

3.3

9.4

ΔG (kcal/mol)

K194

K199

K200

0

-5

-10

-15

-20

-25

-30
Fig. 11
Fig. 12

A: GIRK2-Na+

B: GIRK2-Gβγ-Na+

C: GIRK2-Gβγ

D: GIRK2-Gβγ-Na+

CTD anti-clockwise rotation

5.47°

3.44°

C-linker rotation

7.74°

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