Computational Study of the Loss-of-Function Mutations in the Kv1.5 Channel Associated with Atrial Fibrillation

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ABSTRACT: Atrial fibrillation (AF) is a heart disease caused by defective ion channels in the atria, which affect the action potential (AP) duration and disturb normal heart rhythm. Rapid firing of APs in neighboring atrial cells is a common mechanism of AF, and therefore, therapeutic approaches have focused on extending the AP duration by inhibiting the K+ channels involved in repolarization. Of these, Kv1.5 that carries the I_Kur current is a promising target because it is expressed mainly in atria and not in ventricles. In genetic studies of AF patients, both loss-of-function and gain-of-function mutations in Kv1.5 have been identified, indicating that either decreased or increased I_Kur currents could trigger AF. Blocking of already downregulated Kv1.5 channels could cause AF to become chronic. Thus, a molecular-level understanding of how the loss-of-function mutations in Kv1.5 affect I_Kur would be useful for developing new therapeutics. Here, we perform molecular dynamics simulations to study the effect of three loss-of-function mutations in the pore domain of Kv1.5 on ion permeation. Comparison of the pore structures and ion free energies in the wild-type and mutant Kv1.5 channels indicates that conformational changes in the selectivity filter could hinder ion permeation in the mutant channels.

INTRODUCTION

Cardiac action potential (AP) is generated via rapid depolarization of the cells by Na+ ions entering through Na+ channels, which are then repolarized by K+ ions exiting through various K+ channels. Heart rhythm is determined by the AP duration, which depends on timely opening and closing of these ion channels. Dysfunction of ion channels due to environmental or genetic factors could change the AP duration, causing cardiac arrhythmias such as atrial fibrillation (AF) and ventricular fibrillation. Because prevalence of AF is increasing rapidly with the aging of the population, developing safe and effective treatments for AF is needed.1,2

A common mechanism of AF is complex re-entry between neighboring atrial cells, which causes rapid firing of APs.2,3 This can be prevented by inhibiting K+ channels, which will extend the repolarization period and hence the AP duration. However, the target K+ channel has to be specific to atria, otherwise the attempt to treat AF could cause arrhythmias in other parts of the heart. The hERG K+ channel is a well-known example of such a side effect—it carries the rapid delayed rectifier current I_Kr in all parts of the heart, so its blocking could cause ventricular arrhythmias.2,4 The voltage-gated potassium channel Kv1.5, which carries the ultrarapid delayed rectifier current I_Kur, is expressed mainly in atria.5-8 Thus, the Kv1.5 channel offers a promising target for the treatment of AF.9 Intriguingly, Kv1.5 expression is reduced and I_Kur is downregulated in chronic AF,10 indicating that there is no single target for curing AF, and a molecular-level diagnostics of AF is necessary for a proper treatment.

Population studies during the last decade have shown that AF runs in families,11,12 which has intensified the search for genetic factors involved in the onset of AF. In genetic studies of lone AF patients, several mutations were identified in the KCNA5 gene that encodes the Kv1.5 channel protein.13-16 Functional analysis showed that most of these mutations led to loss of function in Kv1.5, though some mutations were also found to exhibit gain of function.16 Treatment of AF patients—who have loss-of-function mutations in Kv1.5 and hence downregulated I_Kur—with Kv1.5 inhibitors would be counterproductive as this may lead to excessive prolongation of the atrial AP duration, which itself is a source of AF.17 Mechanistic insights into the functional role of the mutations involved in AF could improve the Kv1.5 pharmacology for the treatment of AF.18

Since the determination of the first crystal structure in 1998,19 potassium channels have been studied in numerous molecular dynamics (MD) simulations.20 Mechanism of ion permeation through recycling between the two and three K+ ion configurations in the selectivity filter (SF) has been established by free energy21,22 and brute-force MD simulations.23 Simulation methods have been very useful in
developing selective inhibitors of Kv channels involved in various diseases.24−26 MD simulations have also been used to probe the structural changes that occur in the SF during the C-type inactivation.27,28 An important question in this regard is the role of mutations in hindering ion permeation and whether they result in a permanent suppression of conductance or a C-type inactivation. So far, this issue has been explored mostly for mutations of the residues in the SF.29−32 In a very recent study, the link between a voltage sensor mutation and the C-type inactivation has been investigated.33 However, as far as we are aware, the effect of the pore domain mutations on the channel function has not been considered in simulation studies. In

Figure 1. (A) Sequence alignment of hKv1.5 with rKv1.2, which is used to build the homology model of hKv1.5 (see Methods). Mismatched residues in the alignment are less than 10% (indicated with green). Genetic mutations in AF patients are indicated with purple in the third row and with red in WT Kv1.5. (B) Homology model of hKv1.5. Only two cross-monomers are shown for clarity. The SF is represented in licorice, and the K+ ions in the filter and cavity are shown in green. The residues D469, P488, and T527 involved in genetic mutations are explicitly indicated. The kink angle in the S6 segment (θ ≈ 155°) is defined between the two lines shown, where the first line is through the residues 491−511 and the second line is between the residues 512−525.

Figure 2. Time series of the ion positions in the SF and cavity of WT Kv1.5 (D) show that two K+ ions initially occupy the S1 and S3 sites in the SF and a K+ ion is in the cavity, which is the S1−S3 state (A). Within 2 ns, the ion pair at S1−S3 move to S2−S4 and the cavity ion goes out. After ~60 ns, a K+ ion moves from bulk to S0, forming the S0−S2−S4 state (B). In the mutant Kv1.5 channels (E−G), the two K+ ions remain at S1−S3, whereas the cavity ion moves to S4 within 10 ns, forming an inactivation state with three K+ ions at S1−S3−S4 (C).
particular, several mutations in the pore domain of Kv1.5 result in loss of function, and it will be interesting to study their effect on ion permeation in MD simulations.

Here, we present the results of a computational study, where we investigate the effects of three loss-of-function mutations in the pore domain of human Kv1.5, namely, D469E, P488S, and T527M. All-atom MD simulations are performed in both wild-type (WT) and mutant Kv1.5 channels to explore the effect of the mutations on the channel structure and function. The changes in the structures are evaluated using both distance and angle measurements among the pore residues. The energetic consequences of the mutations are studied by comparing the potential of mean force (PMF) of K+ ions in WT and mutant Kv1.5 channels. Our results indicate that all three mutations lead to conformational changes in the SF, which would result in the suppression of the conductance relative to WT Kv1.5.

RESULTS AND DISCUSSION

Loss-of-Function Mutations in the Pore Domain of hKv1.5. A homology model for the pore domain of the hKv1.5 channel is constructed from the crystal structure of the rKv1.2 channel using the sequence alignment in Figure 1A. The positions of the three loss-of-function mutations in the pore domain of Kv1.5 (D469E, P488S, and T527M) are indicated with red and purple in the alignment diagram (Figure 1A). The physical locations of these residues in the Kv1.5 model are shown in Figure 1B. The residue T527 is at the cytoplasmic end of S6, D469 is in the pore helix, and P488 is in the filter-S6 linker. Although the locations of the three mutations are far from the SF, they all result in substantial loss of function. To investigate the effect of these mutations on ion permeation, we have constructed models for the three mutant Kv1.5 channels, Kv1.5[D469E], Kv1.5[P488S], and Kv1.5[T527M], and performed MD simulations on them together with WT Kv1.5 for comparison. In the following, all the quantities used in comparisons of structures are calculated from the 100 ns of production data for each channel.

Effect of the Loss-of-Function Mutations on the Ions in the SF. We first discuss permeation of K+ ions in WT Kv1.5, which has some differences from other potassium channels because of the presence of four arginine residues at the pore mouth (see Figure S1 in the Supporting Information). In a typical waiting state in Kv channels, two K+ ions occupy the S1 and S3 sites in the SF, another K+ ion is in the cavity, and three water molecules occupy the S0, S2, and S4 sites (Figure 2A). We will name the states according to the K+ occupancy of the sites, so this state will be called S1−S3. A conducting state is obtained when the K+ ions in the SF move from S1−S3 to S0−S2 and the K+ ion in the cavity enters S4 (S0−S2−S4 state). The time series of the ion positions from MD simulations of the WT Kv1.5 channel are shown in Figure 2D. The two K+ ions that are initially placed at S1−S3 are seen to move quickly to S2−S4 within 2 ns, whereas the cavity ion exits to the cytoplasm. The ions at S2−S4 make brief excursions to S1−S3, but they mostly remain at S2−S4. Thus, unlike other Kv channels, two K+ ions preferentially occupy the S2−S4 sites in Kv1.5 rather than S1−S3. This is presumably caused by the arginine charges near the S0 site that push the K+ ions down the filter. After ~60 ns, the conducting S0−S2−S4 state is formed with the entry of a K+ ion from bulk to S0 (Figure 2B,D). The S0−S2−S4 state appears to be more stable than the S1−S3 or S2−S4 states where the S0 site is not occupied by a K+ ion. Because the rate of C-type inactivation depends on the occupancy of S0, the S0−S2−S4 state may have an effect on the occurrence of the C-type inactivation in WT Kv1.5.

We next discuss permeation of K+ ions in the three mutant Kv1.5 channels. The time series of the ion positions are shown in Figure 2E−G for the D469E, P488S, and T527M mutations, respectively. In all three cases, the K+ ions at S1−S3 remain there throughout the simulations, whereas the K+ ion in the cavity moves into S4 after a brief transient period lasting less than 10 ns, forming the S1−S3−S4 state (Figure 2C). In WT Kv1.5, the transition of the K+ ion at S3 to S4 is almost immediate (Figure 2D). The fact that this does not happen in the mutant Kv1.5 channels suggests that the mutations have caused a bottleneck at the S3−S4 interface, hindering the S3−S4 transition of the ion. Instead, the S4 site is occupied by the cavity ion in less than 10 ns, and the three K+ ions at S1−S3−S4 form a stable state in the rest of the simulations (Figures 2E−G, and S2 in the Supporting Information). Formation of a stable S1−S3−S4 state is rather unexpected because of the strong Coulomb repulsion between the neighboring K+ ions, so we check its feasibility from PMF calculations.

Energetics of S4 Occupancy from PMF Calculations. The S1−S3−S4 state was shown to be unstable in Kv1.2 from PMF calculations, and a similar result is expected in other Kv1 channels. To see if such a state is feasible in the mutant Kv1.5 channels, we construct the PMF for the K+ ion moving from the cavity to S4 in the S1−S3 state of Kv1.5[P488S] (Figure 3A). For comparison, the same PMF calculation is also performed in WT Kv1.5. Moving the K+ ion from the cavity to S4 costs +2.5 kcal/mol in WT Kv1.5, which indicates that the S1−S3−S4 state is unstable, consistent with other Kv1 channels. In contrast, the same free energy difference is −2 kcal/mol in Kv1.5[P488S], showing that a stable S1−S3−S4 state is indeed energetically feasible in the mutant Kv1.5 channel. We note that the S4 well in the S1−S3−S4 state of

8884 DOI: 10.1021/acsomega.8b01094
ACS Omega 2018, 3, 8882−8890
mutant channels compared to the carbonyl oxygens and water between the two K+ ions at the S3→S4 transition in the S1 channels. PMFs for moving the K+ ion from S3 to S4 and sites (Figure S3 in the Supporting Information).

The average K′–O distances obtained from the last 30 ns of MD simulations for each channel are listed (in Å). A water molecule from the cavity is in the coordination shell, but because of frequent exchanges with cavity waters, only a range can be given.

Table 1. Free Energy Differences (in kcal/mol) That Affect the S3 → S4 Transition

| free energy (kcal/mol) | WT Kv1.5 | Kv1.5[P488S] | Kv1.5[P488S] |
|------------------------|----------|--------------|--------------|
| W(S4)−W(S3)            | −0.5 ± 0.2 | 2.4 ± 0.3     | 0.2 ± 0.3     |
| U0(S3 → S4)            | 1.9 ± 0.2  | 3.3 ± 0.3     | 3.3 ± 0.3     |

“Free energies are determined from the PMFs in Figure 3B. W(S4)−W(S3) gives the free energy difference between the S4 and S3 sites, and U0(S3 → S4) measures the energy barrier faced by the K+ ion at S3 to move to S4.

| K′−O (A) | 2.78 ± 0.19 | 3.83 ± 0.38 | 3.75 ± 0.48 |
| K′−O (B) | 2.91 ± 0.41 | 3.84 ± 0.49 | 3.74 ± 0.51 |
| K′−O (C) | 2.85 ± 0.23 | 3.83 ± 0.41 | 3.75 ± 0.49 |
| K′−O (D) | 2.94 ± 0.33 | 3.84 ± 0.51 | 3.74 ± 0.43 |
| K′−O (H2O) | 2.82 ± 0.36 | 2.64 ± 3.17 | 2.64 ± 3.17 |

Kv1.5[P488S] extends further out from its usual position at z = −6.5 to about −8 Å. In normal occupancy states, the S3–S4 distance is about 3.5 Å (see Figure 3B). However, because of the strong Coulomb repulsion between the K′ ions, this could not be sustained in the S1−S3−S4 state. The average distance between the two K′ ions at the S3−S4 sites is about 5 Å, which is 1.5 Å longer than the usual separation between the S3−S4 sites (Figure S3 in the Supporting Information).

Having confirmed the feasibility of the S1−S3−S4 state in the mutant Kv1.5 channels, we next consider the energetics of ion movement from S3 to S4 in the S1−S3 state to see if there are any factors hindering the S3 → S4 transition in these channels. PMFs for moving the K′ ion from S3 to S4 and cavity are shown in Figure 3B. The critical quantities in the PMFs that affect the S3 → S4 transition are listed in Table 1. In the WT Kv1.5 PMF, the S4 site has 0.5 kcal/mol lower free energy than S3 and the barrier between them is less than 2 kcal/mol. The favorable free energy and low energy barrier are consistent with the fast S3 → S4 transition and prevalence of the S4 occupation observed in the MD simulations (Figure 2D). In the PMFs of the mutant channels, the S4 site has a higher free energy than S3, and the energy barriers for the S3 → S4 transition are 1–2 kcal/mol higher compared to that in WT Kv1.5. Thus, the PMF calculations explain why the S3 → S4 transition is hindered in the mutant channels. Further, from the relative depths of the S3 and S4 minima, we can infer that the S1−S3−S4 state would be most stable in Kv1.5[P488S], followed by Kv1.5[D469E] and Kv1.5[T527M]. Because stability of a state adversely affects ion permeation, we expect the current blocking strength to follow the same order.

### Structural Changes at the S4 Site Due to Mutations

The PMF results focus our attention on the T480 residues in the SF. The carbonyl oxygens of T480 are at the S3−S4 interface and, together with the side-chain oxygens of T480, they form the S4 site. Inspection of the coordination shell of the K′ ion at S4 confirms the shift of the ion position toward the cavity in the mutant channels (Table 2). Overall, the hydroxyl oxygens of T480 and a water molecule appear to provide a tighter coordination shell for the K′ ion in the mutant channels compared to the carbonyl oxygens and water in WT. The tightest coordination shell occurs in Kv1.5-[P488S], which has the most stable state for the ions in the SF.

A more intriguing observation from Table 2 is that the K′−O distances are almost the same in all four monomers in the mutant channels, which does not happen in WT Kv1.5. To pursue the symmetry issue further, we examine the cross-monomer distances between the Ca, Oα, and OOH atoms of T480 (Table 3). All three distances differ by 0.2−0.3 Å between the monomers A−C and B−D in WT Kv1.5, but they become equal in the mutant channels. That this is not the result of averaging can be seen from the time series of the O−O distances with 0.3 ns block data averaging (Figure S5 in the Supporting Information), which show that the symmetry between the A−C and B−D distances in the mutant channels is retained at much smaller time scales. Mutations also cause a small tightening at the S4 site; the B−D distances become ~0.2 Å shorter in the mutant channels compared to WT. The symmetric and tighter conformation of the T480 residues are expected to contribute to the increase in the S3 → S4 barrier and the S3 well depth in the mutant channels relative to WT (Table 1, Figure 3B).
We have examined the other residues in the SF (V481, G482, and Y483) for the cross-monomer symmetry between A–C and B–D and found that the symmetry is maintained in the mutant channels but does not happen in WT Kv1.5. We do not observe any decrease in the carbonyl oxygen distances for these residues in the mutant channels, so tightening in the SF occurs only at the S4 site. To see whether the symmetric conformation propagates down the channel, we consider the kink angles in S6, which are involved in channel gating (Figure 1B). The distribution of the kink angles for monomers A–D in WT and mutant Kv1.5 channels is shown in Figure 4. In WT Kv1.5, the kink angles peak at 155° in monomers A and D, whereas B and C have higher and lower peak values, respectively. In all three mutant channels, the kink angles exhibit nearly identical distributions in all four monomers, peaking at ~155°. Because there is no direct link between the SF and the kink region in S6, this is likely to be enforced by the presence of two K⁺ ions at S3–S4. Indeed, after replacing the K⁺ ion at S4 with a water molecule, the kink angles are

![Figure 4. Distribution of the kink angles for four monomers: (A) WT Kv1.5, (B) Kv1.5[D469E], (C) Kv1.5[P488S], and (D) Kv1.5[T527M]. In each figure, the distributions in four monomers are indicated by A (blue), B (red), C (black), and D (orange).](image)

Structural Changes in Kv1.5[D469E]. In WT Kv1.5, the D469E residues are at the far end of the pore helix and interact electrostatically with K494 in the S6 helix of the next monomer (Figure 5A and Table 4). The D469E mutation improves this interaction between the monomers A–B and C–D, but it is broken between B–C and D–A, where E469 makes links with S465/S466 in the same monomer (Figure 5B and Table 4). The broken interdomain links between B–C and D–A are replaced with even stronger R487–D485 interactions (Figure 5B and Table 4). Thus, the overall effect of the D469E mutation is to stabilize the top of the pore helix. The bottom of the pore helix is stabilized when the S3 and S4 sites are occupied by K⁺ ions in the S1–S3–S4 state. To quantify the effect of the D469E mutation on the pore helices better, we compare their root-mean-square deviations (rmsds) in WT Kv1.5 with those in the mutant channels (Figure 6). In WT Kv1.5, the pore helices in monomers A and C are stabilized by the K494–E469 interaction but not in monomers B and D (Table 4). As a result, the pore helices in monomers B and D fluctuate much more compared to those in monomers A and C (Figure 6A). In Kv1.5[D469E], all four pore helices are stabilized similarly and adapt a symmetric configuration. Most importantly, they exhibit similar fluctuations that are suppressed compared to WT Kv1.5 (Figure 6B). Intriguingly, the same behavior of the pore helices is also observed in the Kv1.5[P488S] and Kv1.5[T527M] channels (Figure 6C,D).

Structural Changes in Kv1.5[P488S]. In WT Kv1.5, P488 is in the P loop that connects the SF to the S6 helix. The distinctive cyclic structure of proline restricts the bulky side chains of Trp to move in the outer pore direction (Figure 5A). The P488S mutation creates space for the reorientation of the Trp side chains (Figure 5C). In addition, S488 makes a hydrogen bond with the carbonyl oxygen of G493 in the S6 helix in all four monomers and tightens the P loop with the S6 helix compared to the WT Kv1.5. The reorientation of the W–W domains in the P loop and the S488–G493 hydrogen bond improve the K494–E469 interaction over the WT (Figure 5C), which become uniformly tighter (Table 4). Compared to the Kv1.5[D469E] results, the average K494–D/E469 distances are both uniform and much shorter, which points to a more symmetric configuration of the pore helices. The pore helices in Kv1.5[P488S] indeed have similar rmsd values and fluctuate much less compared to those in Kv1.5[D469E] (Figure 6C). Noting that symmetric conformations with suppressed fluctuations hinder ion permeation, this result correlates well with the PMFs in Figure 3B.

Structural Changes in Kv1.5[T527M]. In both WT and mutant Kv1.5 channels, Y523 makes a hydrogen bond with E433 in the S5 helix and has a hydrophobic interaction with V514 in the S6 helix of the next monomer (Figure 5D). In Kv1.5, the TS27 residue is exposed to the cytoplasm and is not involved in any interaction. In contrast, M527 in Kv1.5[T527M] makes a π–π interaction with Y523 in the same monomer (Figure 5D). This extra interaction helps to stabilize the V514–Y523–E433 link compared to WT. To demonstrate

DOI: 10.1021/acsomega.8b01094
ACS Omega 2018, 3, 8682−8690
this effect, we compare the V514(Cγ)–Y523(Cε) distances (Table 4). As in the previous two cases, the distances indicate that this region of the pore adapts a symmetric conformation after the T527M mutation. The V514 residue is in the PVPV motif, which controls gating via the kink angle (Figures 1B and 5D). We have already shown that the kink angle exhibits a symmetric distribution for all monomers in Kv1.5[T527M] (Figure 4), which is clearly caused by the T527M mutation. A small stress on the PVPV motif is known to modulate the pore domain in Kv channels.36,37 In the case of the T527M mutation, it leads to a symmetric conformation of the pore helices with suppressed fluctuations (Figure 6D).

So far, we have shown that all three mutations lead to symmetric conformations of the S6 and pore helices with suppressed fluctuations (Figures 4 and 6). These changes are likely to cause the compression of the T480 residues in the filter (Table 3) via the interaction of V505 on S6 with T480. The side chains of these residues are in contact, so any stress on V505 will be directly transmitted to T480 (Figure S9 in the Supporting Information).

Discussion. In order to compare the predicted suppression of conductance in mutant Kv1.5 channels relative to WT Kv1.5 with experiments, single-channel conductance measurements are needed for all channels. Unfortunately, the loss-of-function experiments were performed using whole-cell patch-clamp recordings.14,16 Further, the D469E and P488S mutants displayed reduced surface expressions in HEK293 cells,16 which made it impossible to determine how much of the loss of function could be attributed to the impaired conduction of ions. Although there is evidence for the suppression of conductance in the T527M mutant,14 this is a relatively weak effect (30%). To test our predicted order of current blocking as P488S > D469E > T527M, measurement of the single-channel conductance of all the mutant channels is highly desirable. Experimental verification of the blocking order will provide evidence for the novel inactivation mechanism proposed here.

Table 4. Average Values of the K494(N)−D/E469(O), R487(N)−D485(O), and V514(Cγ)−Y523(Cε) Distances (in Å) in WT and Mutant Kv1.5 Channels

|         | K494(N)−D/E469(O) | R487(N)−D485(O) | V514(Cγ)−Y523(Cε) |
|---------|------------------|-----------------|-------------------|
|         | WT D469E P488S   | WT D469E        | WT T527M          |
| A−B     | 5.0 ± 0.5        | 4.7 ± 0.3       | 4.5 ± 0.5         |
| B−C     | 4.2 ± 0.4        | 6.5 ± 0.5       | 4.5 ± 0.4         |
| C−D     | 5.4 ± 0.6        | 4.6 ± 0.5       | 4.5 ± 0.5         |
| D−A     | 4.3 ± 0.2        | 7.5 ± 0.3       | 4.4 ± 0.4         |

CONCLUSIONS

We have performed a computational study of the three loss-of-function mutations in the Kv1.5 channel that are identified in...
AF patients. Despite their different locations on the pore, all three mutations lead to very similar conformational changes in the channel, namely, the S6 and pore helices in all four monomers assume symmetric conformations and their rmsd fluctuations are suppressed compared to WT Kv1.5. This results in a slight compression of the S4 site in the SF, which can hold three K+ ions in the cavity to construct the waiting state for ion conduction.23

In Figure 6, the conformations of the four monomers appear to be the signature of this blocking mechanism. It will be interesting to study the loss-of-function mutations in other Kv channels to see if a similar blocking mechanism occurs in those cases.

The insights gathered from this study on the effect of the loss-of-function mutations in Kv1.5 on ion permeation could be useful in the development of effective therapies for AF patients who have these genetic mutations. One possible strategy is to break the symmetric conformation of the four monomers in the mutant Kv.5 channels by binding a small drug to one of the monomers in the cavity. Conversely, one can help to induce this blocking mechanism in WT Kv1.5 channels in order to regulate the I_{Kur} current. This could be achieved using a drug that modulates all S6 monomers at the PVPV site or binds in between the S5—S6 helices near the pore region to suppress the fluctuations of S6 and pore helices. This could provide an alternative therapy for AF patients who have WT Kv1.5. Overall, the mechanism proposed here has the potential to open up new areas for drug discovery not only for Kv1.5 but also for other Kv1 channels.

### METHODS

**Homology Models.** The crystal structure of the open state of rKv1.2 (PDB ID: 2R9R) is used to construct the homology models for hKv1.5 and its mutant forms, Kv1.5[D469E], Kv1.5[P488S], and Kv1.5[T527M]. The sequence alignment between the pore domains of rKv1.2 and hKv1.5 (residues 308–421 in rKv1.2) reveals over 90% of sequence identity (Figure 1). Point mutations are sufficient to create the homology models for WT and mutant Kv1.5 channels because of the 1-1 amino acid correspondence, that is, there are no missing residues in the sequence alignment. VMD (version 1.9.2) mutator plugin40 is used for point mutations to create the homology models. We used VMD to create homology models for the Kv1.1—Kv1.3 channels in past studies and obtained good results.24 For further justification, we also generated homology models for the sequence alignment in Figure 1 using Swiss-Model.41 Top scored homologs have been found to have very similar structures as homology models created by VMD (rmsd between the models generated using VMD and Swiss-Model is about 0.5 Å). A fourfold symmetry is maintained in the WT and the three mutated channels.

**Simulation Details.** We use CHARMM-GUI to create the simulation system. Each channel protein is embedded in a pre-equilibrated POPC lipid bilayer and solvated with explicit water molecules in a simulation box with dimensions 80 × 80 × 81 Å³. Ions are added to maintain a physiological concentration of 150 mM KCl in the system. The whole system is neutralized and contain about 50 000 atoms. To study ion conduction in WT and mutated channels, we place two K⁺ ions at the S1 and S3 sites in the SF in all cases. Rest of the sites in the SF are filled with water molecules.27,42 We also place a K⁺ ion in the cavity to construct the waiting state for ion conduction.23

Restraints on channel proteins are released gradually over 10 ns. First, the side chains are released by reducing the harmonic force constant as 30, 10, 5, 1, and 0.1 kcal/mol/Å, whereas the backbone is restrained with a 30 kcal/mol/Å force. Each step is performed for 1 ns. Next, the backbone atoms are released following a similar protocol. After the equilibration, each system is simulated for 100 ns without any restraints for production run. Trajectory data are saved at every 5 ps for the analysis.
MD simulations are performed using the NAMD code (version 2.9) with the CHARMM36 force field. Simulations are performed in the NPT ensemble with the temperature and pressure maintained at 300 K and 1 atm, respectively, via Langevin coupling with a damping coefficient of 5 ps$^{-1}$. All bonds to hydrogen atoms are maintained using the SHAKE algorithm. Periodic boundary conditions are employed with the particle-mesh Ewald algorithm to compute the long-range electrostatic interactions. Lennd-Jones potential is switched off within 10–12 Å using a force-switching function. A nonbonded pair list cutoff of 14 Å is used. A time step of 2 fs is maintained throughout the simulations. The average interatomic distances and kink angles presented in the figures and tables are obtained from the trajectory data using the VMD software.

### PMF Calculations

PMF calculations are performed to investigate the stability of the S1–S3–S4 and S1–S3 combinations of K$^+$ ions in the SF and to calculate the free energy profile along the SF and cavity. The PMFs are constructed between a binding site in the SF and cavity. The umbrella windows are generated at 0.5 Å steps by pulling the K$^+$ ion in the cavity to the SF. At each window, the z-coordinate of the K$^+$ ion is sampled using a harmonic potential with a force constant of 10 kcal/mol/Å$^2$. The raw position densities obtained with the application of the harmonic potential are unbiased at each window, and the corresponding PMFs are combined using the weighted histogram analysis method to find the total PMFs of K$^+$ ions in WT and mutated channels. Convergence of PMFs is studied using 2 ns blocks sliding over the whole data in 0.5 ns steps (Figure S4 in the Supporting Information). The final PMFs are calculated from the production data that exclude the equilibration data.

### ASSOCIATED CONTENT

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b01094.

Arg 487 residues in the mouth of the Kv1.5 channel, snapshots of the formation of the S1–S3–S4 state in Kv1.5[P488S] channel, time series of the distance between the S3 and S4 ions in the S1–S3–S4 state of the mutated channels, convergence of the PMFs for WT and mutated channels, time series of the cross-monomer distances between the carbonyl oxygens of T480 residues, distribution of the kink angles in Kv1.5-[T527M] after replacing the K$^+$ ion at S4 with a water molecule, snapshots of the last frames from the MD simulations of the Kv1.5[V481I] and Kv1.5[L499V] channels showing the ion occupancy of the filler, distribution of the kink angles for four monomers in Kv1.5[V481I] and Kv1.5[L499V], and interaction of the V505 side chains on S6 with the T480 side chains (PDF)

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### ACKNOWLEDGMENTS

We thank Shigehiko Hayashi for helpful discussion at the start of this work and we would also like to thank Shigehiko Hayashi and Irene Yarovskyy for computational support. Computations were performed using the supercomputers at Kyoto University, National Computational Infrastructure (Canberra), and Victorian Life Sciences Computation Initiative (Melbourne).

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**Notes**

The authors declare no competing financial interest.
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