Realistic simulation of the activation of voltage-gated ion channels

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Understanding the detailed mechanism of the activation of voltage-gated ion channels has been a problem of great current interest. Reliable molecular simulations of voltage effects present a major challenge because meaningful converging microscopic simulations are not yet available and macroscopic treatments involve major uncertainties regarding the dielectric constant used and other key features. The current work has overcome some of the above challenges by using our recently developed coarse-grained (CG) model in simulating the activation of the Kv1.2 channel. The CG model has allowed us to explore problems that cannot be addressed at present by fully microscopic simulations, while providing insights on some features that are not usually considered in continuum models, including the distribution of the electrolytes between the membrane and the electrodes during the activation process and thus the nature of the gating current. Furthermore, the clear connection to microscopic descriptions combined with the power of CG modeling offers a powerful tool for exploring the energy balance between the protein conformational energy and the interaction with the external potential in voltage-activated channels. Our simulations have reproduced the observed experimental trend of the gating charge and, most significantly, the correct trend in the free energies, where the closed channel is more stable at negative potential and the open channel is more stable at positive potential. Moreover, we provide a unique view of the activation landscape and the time dependence of the activation process.

membrane potential | stability | free energy

The elucidation of the structure of voltage-activated ion channels and biophysical studies (e.g., refs. 1–5) have provided key information about the relationship between the membrane voltage and the gating process. However, despite these great advances, we still do not have a clear picture of the corresponding structure–function correlation. Furthermore, although there has been significant progress in the computational modeling of the energetics of ion channels (e.g., refs. 6–10), the understanding of the voltage activation process is rather limited. Not only have the exact structural changes not been fully determined, but the energetics of the conformational transition and the coupling to the external voltage are far from being understood.

To clarify the current challenges, it is useful to follow the general concept depicted in the schematic diagram of Fig. 1*A*. As shown in the figure, in the resting state (with a negative potential), the channel is in its closed state, where the free energy of moving to the open state is positive. The application of a positive potential stabilizes the open state, leading to a conformational transition to this state, where the channel allows ion transport. The overall process can be captured once we have the relevant free energy landscape, which should follow the trend of Fig. 1*B*. This landscape depicts a path from a region of closed channel at negative potential to a region of open channel at positive potential. The fundamental challenge is to capture the energetics of the conformational transition and to reproduce the effect of the external potential that would overcompensate the conformational energy and move the system to the open state. Further challenge is associated with capturing the microscopic nature of the gating charge and the barrier for the conformational transition, as well as the fluctuations during the gating process.

One of the major difficulties in simulating voltage-activated ion channels is the treatment of the membrane potential and its effect on the protein configurations and the electrolyte distribution. These issues have been explored by interesting Poisson–Boltzmann (PB) studies (11, 12), but the results reflect a macroscopic perspective and problematic dielectric constants (for a review, see ref. 13). In principle, one may try to use molecular dynamics simulations with all-atom explicit models (14), but at present we believe that such an approach is not likely to provide converging free energies (due to both the challenge of obtaining stable solvation free energies in protein interiors and the difficulties in capturing the response of the ionic atmosphere). Probably the most effective current strategy should involve the use of coarse-grained (CG) models with proper electrostatics.

In view of the above considerations, we have recently developed a unique strategy based on our existing CG model, while adding the crucial effect of the ions in solution and the membrane potential. This model enables us to simulate membrane proteins in the presence of external potential and electrolyte solutions. In the present work, we focus on modeling the function of the Kv1.2 voltage-activated channel. The simulations provide interesting insights about the energy balance, gating charges, and gating fluctuations in voltage-activated channels.

Key Features of the Modeling Approach

Our CG treatment (which is described in *Methods* and *SI Text*) is aimed at modeling the protein/membrane system and its interaction with the external potential. The modeling of the protein/membrane system has been described in detail elsewhere (15). Here we outline some of key points about the description of the electrolyte solution and the membrane potential (where we basically capture the physics of systems which are described formally in the *SI Text*).

Our simulation system for studies of membrane potential is described in Fig. 2. The system includes a simulation box that explicitly includes the membrane containing the protein (region I), an optional region with explicit ions (region II, which is not considered in the present study), and a grid representing the electrolyte solution (region III). We also add a "bulk region" far away from both the membrane and electrode surfaces, as a specialized way for spanning the space between the membranes to the electrodes, without using an enormous grid.

The explicit grid model reflects a compromise between the fully microscopic models of the electrolyte (in an implicit solvent) to a macroscopic model (see *SI Text* for details). The specific treatment of the electrode potential is outlined in the *SI Text*.

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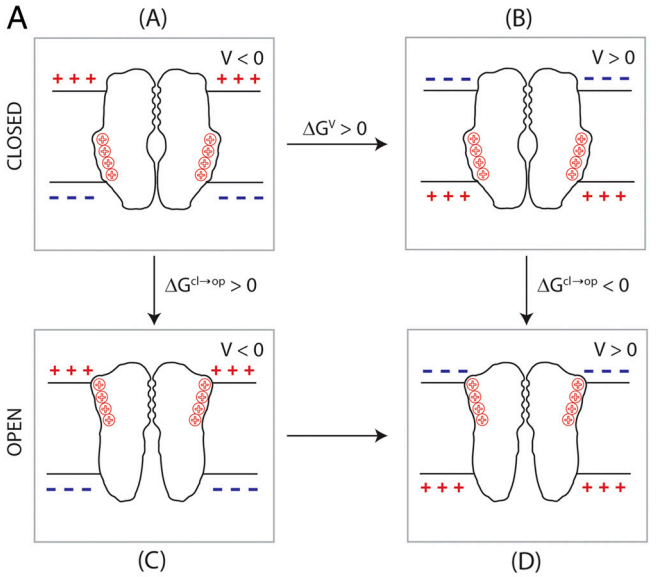

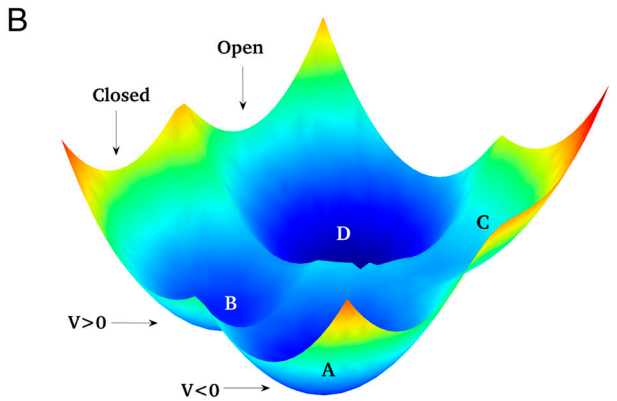

Fig. 1. Illustrating the free energy balance in a voltage-activated ion channels. (*A*) The figure considers schematically the activation process, where the protein moves from the closed to open state due to the allosteric effect of the external potential (V). When the external potential is negative ($V < 0$), the closed conformation is more stable and the channel is blocked. Application of a positive potential ($V > 0$) stabilizes the open conformation and the system moves to its ion conduction state. (*B*) A tentative landscape for activation of the Kv1.2 system in the two-dimensional space defined by the protein closed to open conformational coordinate and the external potential. Note that the potential coordinate is a "coordinate" of an external constraint and as such the motion from negative to open potential does not have to be downhill (because it does not include the external power source). Note also that the surface was generated with empirical valence bond-type parabolas and from the data of Table 1. Thus the increase in energy, upon moving from the minima toward the boundary of the map, does not reflect the real surface.

Results and Discussion

Validations and Examinations of the Model. The main features of our approach have been validated recently by reproducing the trends in Debye–Huckel and Gouy–Chapman models, as well as the trend in the more challenging problem of two electrodes and electrolyte solution (15). We have also simulated the expected capacitance of a neutral membrane in an electrode–electrolyte system and demonstrated that the ions in the solution provide almost a complete screening of the external potential, which starts to increase only when it reaches the membrane boundaries. This behavior is similar to what is expected from macroscopic considerations. Our calculated charge distribution for the membrane system (see *SI Text*) appears to be different than that obtained in conventional PB studies (16). That is, the PB results are normalized with n_{bulk} rather than by a treatment that guarantees electroneutrality of a finite system. More significantly (for the present study), the PB charge distribution does not conserve the electroneutrality in each side of the of the membrane (see ref. 16 for a representative study). This PB treatment creates a problem in describing the quasi-equilibrium in the short time before the ions pass through the channel, where, for example, for a system with an electroneutral protein that was equilibrated before the application of the potential, we should have an electroneutral solution on each side of the membrane (zero net charge on each side) shortly after the potential is turned on. Thus, the formulation used in calculations of the capacitance of the membrane using PB treatments (where the capacitance charges are evaluated by integrating the charge density on the left side of the membrane from $-\infty$ to the membrane surface, ref. 16) is formally problematic because the integrated charge is zero in the complete treatment of the system. Apparently the PB treatment does not look at the ions in the bulk or near the electrodes as explicit ions that should be combined with the ions near the membrane when considering the specific charge balance on each side of the membrane. Thus the electrolyte ions that move to the electrodes are considered in the same way that one would consider the electrons in the electric wires (namely, these ions are not considered explicitly). We believe that looking explicitly at the electrolyte ions that balance the charge migration toward the membrane can provide an interesting time-dependent insight.

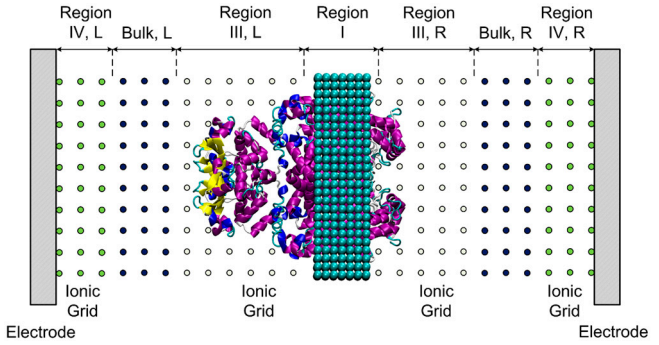

Fig. 2. A schematic description of the treatment of the simulation system. The regions considered are (*i*) the protein membrane system (region I); (*ii*) the region of the solution and the ions that is treated with explicit ions and Langevin dynamics simulation (region II), which is not shown because this region is optional and is not being used in the present study; (*iii*) the region with explicit grid points (region III); (*iv*) the bulk region. The region between the bulk and the electrodes (region IV). The indices L and R stand for the left and right sides, respectively.

Simulating the Potential and Electrolyte Charges in the Kv1.2/Membrane System. As stated in the introduction, our challenge is to model the energy balance in voltage activation channels. Thus we took as a test system the Kv1.2 voltage-activated channel and started our study by evaluating the potential in the open and closed models of the protein/membrane system. The calculations started with the structural models built by Rosetta (with structural information on the open state; ref. 3) and used by Pathak et al. (17) for both the open and closed structures In each case, we started by determining the ionization state of the protein ionizable groups using the CG model and our constant pH simulation approach (see refs. 15 and 18).

We started the calculations with initial Monte Carlo (MC) procedure for determination of the initial ionization states of the protein. We then introduced the electrolytes and the external potential and allowed the complete system to equilibrate in terms of the electrolyte distribution and the ionization states (which was done by performing the MC procedure at equal intervals of the electrolyte equilibration steps). The calculated potential profile and electrolyte charge distribution are depicted in Fig. 3 and the *SI Text*, respectively, for the closed and open channel. The change in potential occurs mainly in the membrane protein

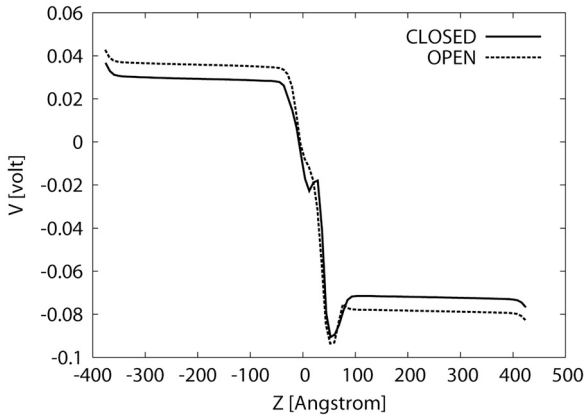

Fig. 3. The potential in the Kv1.2 system in the open and closed states.

region, establishing the fact that our electrolyte model provides basically infinite dielectric screening in the bulk region. The fact that we obtained the screening (without assuming it) is encouraging because we are using a model with discrete features, where it is not guaranteed that we will get the physically correct trend.

Evaluating the Gating Charges. The gating charge is a crucial parameter that represents the shift of the relative free energy difference between the closed and open configurations, due to the change in an external potential. This parameter, which was initially postulated by Hodgkin and Huxley (19), provides a qualitative explanation of the coupling of the external potential to the channel activation. The evaluation of the gating charge is usually done in an indirect way, using reasonable but not necessarily microscopic assumptions. One may simply determine the relative population of open and closed channels as a function of the applied potential and determine the Boltzmann probability for the voltage-induced structural change (1). This treatment is equivalent to the assumption that the energy needed to move the gating charge, Q_{gate}, in the applied electric field is equal to the work of moving the protein charges between the two configurations under the membrane electric field. This assumption can be formulated as (also see *SI Text*)

$$Q_{\text{gate}}\Delta V = \Delta G^{\text{cl}\to\text{op}}, \qquad [1]$$

where $\Delta G^{\text{cl}\to\text{op}}$ is the contribution of the membrane potential to the work of moving from the closed to open configuration, and ΔV is the change in the electrostatic potential between the initial and final position of the protein effective charge (for a case with many protein charges, see *SI Text*). Under the assumption of linear membrane potential, it is simple to calculate Q_{gate} if the structures of the open and closed states are known, and a useful related insight has been obtained from macroscopic studies (17, 20). An attempt to evaluate Q_{gate} with the philosophy of Eq. **1** using microscopic simulations has also been reported (14) (see *SI Text* for related discussion). However, there are some problems with the current strategies, including the fact that the potential is not really linear or uniform in the protein membrane system (in contrast to the implicit assumption of one of the versions of the treatment of ref. 14). At any rate, we must consider the fact that the real observables are $\Delta G^{\text{cl}\to\text{op}}(V)$ and the actual measured gating current (obtained as discussed in, e.g., ref. 21). In other words, despite the remarkable conceptual importance of the gating charge obtained from Eq. **1**, modeling approaches should arguably focus on reproducing $\Delta G^{\text{cl}\to\text{op}}(V)$ and the experimentally measured integrated gating current. Thus we focused on the actual measured quantity, namely, the gating current, rather than on its interpretation. Our explicit calculations of the gating charge was done by first applying the potential in the closed structure, letting the electrolytes equilibrate, and then fixing the number of positive and negative ions on both sides of the membrane. Next we allowed the protein to move from the closed to the open structure, while allowing the electrolytes to equilibrate with the crucial constraint that they cannot pass through the channel.

In considering the gating current or its integral over time, we note that the movement of the positively charged protein residues to the Z direction leads to movement of negative solution ions toward the membrane protein system (this polarization is clearly captured by the PB treatments). However, the measured current is actually due to the movement of the compensating positively charged ions toward the electrode. Obviously, this current has the same magnitude (with opposite sign) as the negative current that moved toward the membrane, but the physics is better described by considering the actual gating current. Thus we evaluate the gating charges by (see *SI Text*)

$$Q_{\text{gate}}(V) \approx \int_{-\infty}^{Z'} (\Delta\Delta q_{\text{grid}}(Z,V)/\Delta Z)dZ, \qquad [2]$$

where $\Delta\Delta q_{\text{grid}}(Z,V)$ is the difference between the accumulative sum of $\Delta q_{\text{grid}}(Z,V)$ of the open and closed channels, and Z' is the point to the left of Z_1, where the electrolyte charge distribution near the membrane changes sign. At this point, the integrated charge reaches a plateau and then starts to decrease. Note that the integral evaluates the charges generated by the current after it equilibrates on the left side of the membrane, but before it actually penetrates the membrane.

The computed charge distributions ($\Delta q_{\text{grid}}(Z,V)$) and the gating charge (Q_{gate}) obtained from $\Delta\Delta q_{\text{grid}}(Z,V)$ are shown in the *SI Text* and Fig. 4, respectively. The value of Q_{gate} appears to depend on the dielectric constant used for the interaction between the electrolyte grid points and the protein charges. Here we have found that using an effective dielectric constant of 40 and 80 gives gating charge (Q_{gate}) of 10 e and 5 e, respectively, whereas the observed value is 12–14 e (21, 22). A more careful comparison to the experimentally determined value of Q_{gate} should involve simulation of the integrated current at different potentials and consideration of the effect of the landscape on the conformational transition.

The Energetics of the Voltage Activation Process. At present, it is extremely challenging to explore the conformational landscape by explicit simulations. Alternatively, we expect to obtain semiquantitative information by our CG model (e.g., see our recent work in ref. 23). Here we have focused on the energetics at the end points (namely, closed and open structures) at different values of the

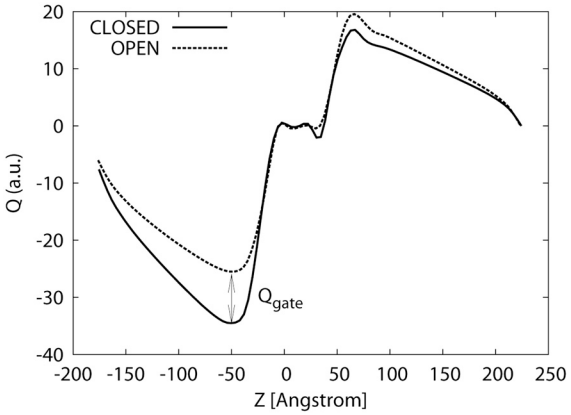

Fig. 4. The gating charge for the Kv1.2 system. The figure presents the integrated charge following Eq. **2**. The difference in the maximum accumulated charge between two states is taken as the gating charge (see text), which is approximately 10 in this figure.

external potential. We have also examined the energetics of an intermediate structure, which was done by pushing the system from the closed to open structure, using targeted molecular dynamics, locating intermediate structures, and then evaluating the CG free energy at the corresponding region. The calculated results are summarized in Table 1. The most important finding that has emerged from the calculations is that we succeed in reproducing the effect of moving the channel from its closed to open configuration upon change of the external potential from a negative to positive value. The overall change in energy is about 30 kcal/mol (+14 and −16 kcal/mol for −200 and +200 mV, respectively), reflecting both the conformational energy and the effect of the external potential. Most significantly, it appears that our model gives stable results and that the trend obtained in Table 1 is retained even when we change the parameters in the model in a reasonable range. For example, changing the grid-protein dielectric from 80 to 40 results in overall change of energy (upon change of potential from −200 to +200 mV) of about 40 instead of 30 kcal/mol. It is also important to note that fully microscopic calculations are expected to produce (at present) errors of more than 100 kcal/mol (see discussion of the related simulations of F1-ATPase, ref. 23). Omitting the hydrophobic contributions lead to an even smaller change in trend than the one obtained with change of dielectric.

The challenge of evaluating the barrier for the conformational transition has been addressed here in a preliminary way, considering only the intermediate configuration discussed above. As seen from Table 1, the barrier at the intermediate structure increases when the membrane potential becomes positive. This trend appears to be stable (with regard to the dielectric used) and associated with contributions from many residues rather than just a few. The height of the free energy barrier, however, is significantly larger than the experimental estimate of about 5 kcal/mol at $V = 0$ (24). The calculated barrier can be reduced by several factors, including conformational pathway where the gating Arg residues move closer to the protein and even by a path where the four subunits of the tetramer move sequentially, rather than moving together. The actual height of the barrier and the exact nature of landscape should be evaluated by more detailed mapping and this can be done by our renormalization approach (18).

The general trend of the calculations was used to generate the tentative surface of Fig. 1*B* by constructing an empirical valence bond-type conformational surface (e.g., ref. 25) with the calculated $\Delta G^{\mathrm{cl}\to\mathrm{op}}$ at different voltage values and with the experimental estimate for the activation barrier (5 kcal/mol at $V = 0$), modulated by a dependence on the potential which follows the calculated trend (which is, however, drastically scaled down). This drastic modification of the barrier reflects the need for more careful mapping at the intermediate region, including the need to allow the system to find the least energy path with the applied potential.

Simulating the Gating Fluctuations. One of the most challenging issues in modeling ion channels is the ability to provide a microscopic description of the time dependence of the gating charge.

Table 1. The free energy at different regions of the conformation/voltage landscape

Voltage, volt	Closed	Intermediate	Open
−0.2	**−203**	−201	−189
−0.1	**−201**	−165	−193
0.0	**−201**	−130	−200
+0.1	−201	−6	**−208**
+0.2	−202	−65	**−218**

The effect of the change in potential in each structure was adjusted, subtracting the capacity work in the open structure from the energy of all the other structures.

Here one can use the relevant experiments in constructing effective models (e.g., ref. 24). However, such models cannot be related directly to the underlying structures and also are not likely to be unique. Although our study has not yet produced the complete landscape, it is instructive to demonstrate here the potential of the use of a calculated landscape in simulating the time-dependence relationship between the gating current and the applied voltage. Such a demonstration is provided here by running Langevin dynamics simulations based on the effective landscape of Fig. 1 (at $V = 0$), while using the effective friction determined by our renormalization approach in a similar system (25). The calculated time dependence of the effective conformational coordinate can then be translated to current by assuming that the electrolytes respond rapidly to the protein charge displacement (see *SI Text*). The simulated result presented in Fig. 5 provides a first glimpse of the origin of the fluctuations of the gating current. The phenomenological pioneering studies of ref. 24 have addressed the same issue, but have not tried to use structure-based simulations.

The above analysis can be further advanced in a more realistic direction, which can range from establishing a clear correlation between the reaction coordinate and the combined effective position of the protein charges, to simulating the time dependence of the fluctuations of the CG model. Further insight can be obtained by using our full renormalization approach (18) to simulate the time dependence of the system under fast changing potential pulse.

Concluding Remarks

This work used a recently developed CG model for studies of the effect of external potentials on membrane proteins and explored the utility of such a model in simulating the effect of membrane potential on ion channels. The model used involves our early CG model of the protein membrane system and a different model of the electrolyte solution and the external electrodes. The electrolyte model allows one to navigate between the more microscopic MC modeling to faster mean field models and helps in providing some light on various elements that are needed in order to understand the nature of the external potential.

Applying the CG model to study the energetics of the voltage-activated Kv1.2 channel provided several advances. These include the evaluation of the balance between the protein conformational energy and the applied potential, while obtaining the correct trend in this balance without the need of any specifically adjusted parameter. The CG simulations also provided a clearer nonstandard description of the gating current and gating charge, allowing one to look directly at the change in the electrolyte charges rather than the interaction between the linearized exter-

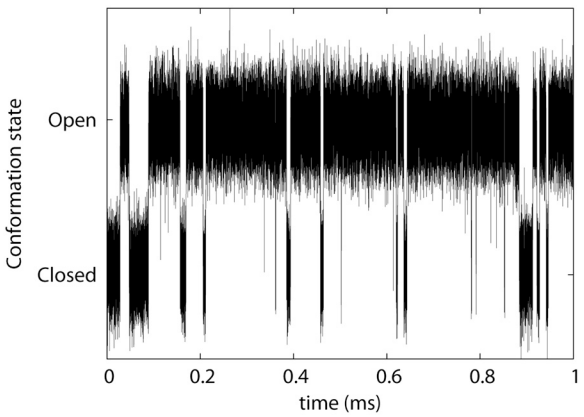

Fig. 5. Simulating the gating fluctuations. The figure describes the results of the Langevin dynamics simulations on the tentative surface of Fig. 1*B* for $V = 0$. The simulations were done with a friction of 150 ps^{-1}.

nal potential and the protein charges. Finally, our study has introduced a landscape-based simulation of the gating fluctuations and basically the gating current.

At this point, it may be useful to point out that, although it is interesting to understand the nature of the gating charge and the corresponding contribution from different residues, it is arguably more important to analyze the energy contribution of each residue (e.g., *SI Text*). That is, to understand the interplay between the external potential and the protein/membrane conformational energy, we have to see what are the free energy contributions of the different residues. Now, although the main effects are electrostatic, they reflect the interactions between the residue charges and their surroundings (plus the external electrode potential), rather than just the interaction between the charges and the linearized potential.

One of the most important questions regarding the present model is its stability in terms of the crucial ionization states and the overall energetics. We will only mention here that we believe that the current model provides one of the most reliable, comprehensive, and stable treatments of the electrostatic energies in protein/membrane systems, and we consider this important issue as well as the ionization states of the protein residues in the *SI Text*.

The CG model may appear to some to represent an oversimplification that overlooks the many substates of the real surface and thus the origin of the fluctuating current. In fact, our renormalization approach generates an effective friction that should represent the fluctuations between the different substates (see ref. 18). Furthermore, the effective CG free energy does include the entropic contributions to the overall free energy. Thus we expect that more careful landscape mapping will be able to address questions about the role of entropic effects (e.g., ref. 24).

Another important issue is the control of ion selectivity, which is most probably due to change in ion–ion interaction in multiion conductance (6). Here it would be important to explore the effect of the fluctuations in the substates of the open state.

The present work also provides important insight on the overall function of voltage-activated channels. That is, as described in Fig. 6, the opening of the channel can be viewed as moving a positive charge from a region near the gate to a region farther away, in response to the applied potential. In fact, viewing the voltage activation as electrostatic allosteric process of interplay between the interaction between the applied potential and the protein positive groups, which are in turned interacting (indirectly) with the transferred charge, seems to be a useful generalization. That is, in the closed state, the positive protein charges are closer to the site where the conducted ions have a higher barrier, where the actual barrier for the ion transfer is due to the local nonpolar environment in the closed gate (see ref. 10). When the positive charges move away, following activation, the indirect interaction with the transferred ion is reduced. This change in interaction involves the opening of the nonpolar region, which is, however, due to the change of its interaction with the positive charges. In other words, the force that moves the nonpolar region to its location is the interaction between the gate region and the gating charges (see a related case in the function of hemoglobin in ref. 26).

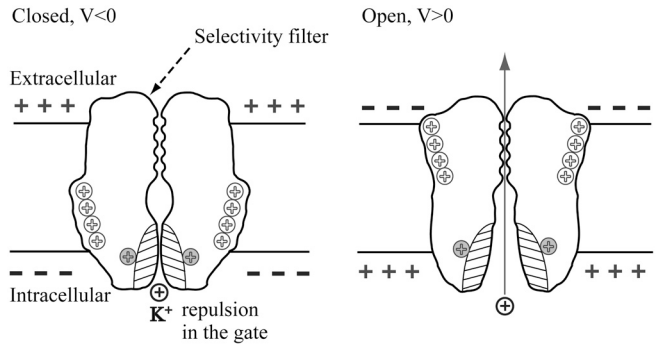

Fig. 6. A conceptual figure of the activation of the Kv1.2 and related channels. The figure considers the allosteric effect of the potential, where the change of position of the positive gating charges reduces the repulsion between theses charges and the positive elements of the gating region, allowing the attached nonpolar region to open and to reduce the desolvation penalty of a transferred ion.

Regardless of the general qualitative insight, the key point of the present study is in highlighting the ability to use initial structural models and to explore their consistency with the functional constraints about the energy balance and the remarkable sets of experimental constraints. In particular, a more refined CG structure-based energy landscape should be very useful in exploring the molecular meaning of the current fluctuations with different applied voltage pulses and their relationship to the biological function of voltage-activated channels. Furthermore, the model should provide an effective way for extracting detailed molecular information from different mutational studies.

Methods

Our general strategy involves a refinement of our recent CG model and the extension of this model to the incorporation of external potential in the simulation of protein/membrane systems. The protein system is treated by a CG model that describes the main chains by an explicit model that represents the side chains as a simplified united atom model, whereas the membrane is described by a grid of nonpolar groups. This CG model provides a more advanced treatment of electrostatic effects than most current CG models (for more details, see ref. 18 and the *SI Text*).

A crucial issue in modeling the effect of external potentials on membrane proteins is the proper modeling of the effect of the solvent molecules and the bulk ions. The solvent is modeled implicitly but the ions in the solutions are considered more explicitly. That is, in trying to introduce the electrolytes, it is important to retain the option of multistage modeling by describing a key part of the system in a fully discrete model. For example, we can treat the immediate region near the protein by a primitive model with the explicit ions moving by Langevin dynamics treatment (6). Here, however, we have focused on the next layer of surrounding, where the electrolytes are described in simpler way, which is done by adopting a compromise between full MC and grid-type approaches. That is, we would like to start conceptually by placing ions on grid points (with an assumed separation) and then use a MC model of the type we and others applied in determining the ionization states in proteins. However, for practical purposes, some additional simplifications were introduced, which can also be removed (the details of the model are described in the *SI Text*). At any rate, we ended up with a semimacroscopic strategy applied in our previous electrostatic modeling (27), which is similar to the approach introduced originally by Klein and Pack (28) but it retains a more microscopic view. In this approach, we generate a grid whose spacing is taken here as Δ with a volume element ($\tau = \Delta^3$) and place at the center of the ith grid point a residual charge (q_i^g) determined by

$$q_i^g = q_i^+ + q_i^-, \qquad [3]$$

where

$$q_i^{\pm} = \frac{\alpha^{\pm}(N_{\text{box}}^{\pm} + N_{\text{bulk}}^{\pm})e^{\mp\beta\phi_i}}{\left(\sum_{i\in\text{box}} e^{\mp\beta\phi_i} + N_{\text{bulk}}^{\text{grid}} e^{\mp\beta\phi_{\text{bulk}}}\right)}, \qquad [4]$$

where q_i^+ and q_i^- are, respectively, the positive and negative fractional charges that are assigned to the ith grid point, $\alpha^{\pm}$ is the ion charge of the electrolyte ions in atomic units (namely, ± 1 for the 1:1 electrolyte used in our calculations), $N_{\text{box}}^{\pm}$ is the total number of cations/anions in the simulation box, $Q_{\text{box}}^{\pm}$ is the total charge of cations/anions in the simulation system given by $Q_{\text{box}}^{\pm} = \alpha^{\pm} N_{\text{box}}^{\pm}$, φ_i is the electrostatic potential (times a unit charge) at the ith grid point, $\beta = (k_B T)^{-1}$. $N_{\text{bulk}}^{\text{grid}}$ is the number of grid points within the bulk system, and ϕ_{bulk} is a constant potential on the bulk grid points (see *SI Text* for more details). We can express ϕ_i as

$$\phi_i = 332\sum_j \frac{q_j^P}{\varepsilon_{\text{eff}}^{gp} r_{ij}} + 332\sum_{k\neq i} \frac{q_k^g}{\varepsilon^{\text{wat}} r_{ik}} + V_i^{\text{ext}}, \qquad [5]$$

where V_i^{ext} represents the external potential on the ith grid point, which will be described below.

Here q_j^p is the charge of the jth protein residue (these charges are evaluated by MC procedure described above) and q_k^g is the point charge at the kth grid point (representing the excess net charge of the kth volume element). Eq. **5** should also include the term $-RT\ln(C_i/C_0)$ because of the concentration dependence, in case of membrane potential with different concentration of electrolytes in the two sides of the membrane. The treatment of the boundary conditions is discussed in the *SI Text*.

The final set of the grid charges (q^g) are obtained iteratively (see *SI Text*), and the effect of the ionic strength is evaluated as outlined in the *SI Text*.

In order to model the effect of the external potential, one can consider formally the membrane/protein/water system as a capacitor. In this case, it is possible to use the well-known macroscopic capacitor model (e.g., ref. 29), where the external potential induces surface charges (σ_f) whose value will be defined below, and creates the corresponding displacement vector $\mathbf{D}^0$. In this case, we have

$$\mathbf{D}^0 = 4\pi\boldsymbol{\sigma}_f. \qquad [6]$$

Our first task is to determine the membrane potential and the electrolytes charges, so we can evaluate the free energy of the protein charges in the presence of this potential, which is done by expressing the external potential as

$$V_{\text{ext}}^i = \int_{Z_0}^{Z_i} (D_z^0/\bar{\varepsilon}(Z))dZ, \qquad [7]$$

where Z_0 is the Z coordinate at the left electrode (in the current work, we define the left side as the side with the smaller value for the Z coordinates and the right side with the larger Z value). An alternative strategy is to replace the treatment of Eq. **6** by simply having a finite grid of point charges on the electrode. Both treatments are described in the *SI Text*.

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