A multiscale model linking ion-channel molecular dynamics and electrostatics to the cardiac action potential

Jonathan R. Silva 1, Hua Pan 1, Dick Wu 1, Ali Nekouzadeh 1, Keith F. Decker 1, Jianmin Cui 2, Nathan A. Baker 2, David Septa 2, and Yoram Rudy 3, 4, 2

1Department of Biomedical Engineering and Cardiac Bioelectricity and Arrhythmia Center, 2Department of Biochemistry, and 3Department of Cell Biology and Physiology, Washington University, St. Louis, MO 63130

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Ion-channel function is determined by its gating movement. Yet, molecular dynamics and electrophysiological simulations were never combined to link molecular structure to function. We performed multiscale molecular dynamics and continuum electrostatics calculations to simulate a cardiac K+ channel (IKs) gating and its alteration by mutations that cause arrhythmias and sudden death. An all-atom model of the IKs α-subunit KCNQ1, based on the recent Kv1.2 structure, is used to calculate electrostatic energies during gating. Simulations are compared with experiments where varying degrees of positive charge—added via point mutation—progressively reduce current. Whole-cell simulations show that mutations cause action potential and ECG QT interval prolongation, consistent with clinical phenotypes. This framework allows integration of multiscale observations to study the molecular basis of excitation and its alteration by disease.

Cardiac arrhythmias | Long QT syndrome

The current carried by ion channels during the action potential (AP) in excitable tissues is determined by dynamic changes of the ion channel conformation during gating. Recently determined structural crystals of ion channel proteins (e.g., ref. 1) provide a structural basis for computer simulations of the ion-channel protein molecular dynamics (MD) during conformational changes. In parallel, recently acquired experimental data on ion-channel electrophysiological function have provided the basis for detailed Markov models of channel electrical function during gating (2, 5). These models can be used to link cellular consequences of genetic mutations in cardiac ion channels that alter channel kinetics to cardiac arrhythmias [see ref. 4 for review]. Although consistent in reproducing the kinetics of channel opening and closing, the molecular conformations represented by the states of these Markov models are not identified.

In this article, we develop a multiscale computational approach that combines molecular dynamics simulations and Poisson-Boltzmann continuum electrostatic calculations with electrophysiological modeling for establishing structure-function relationships between ion channel movement during gating, its function as a carrier of transmembrane ionic current, and its role in the whole-cell AP. Detailed understanding of these relationships is necessary for the development of effective genetic/molecular therapies and specific drug treatment of disease states that involve abnormal electrical function of excitable tissues. We present an example of a cardiac ion channel, the slow delayed rectifier IKs, which participates in AP repolarization and its rate dependence (4). We consider mutations to its alpha subunit, KCNQ1, that lead to reduction of IKs current and prolongation of the AP. The clinical phenotype associated with such mutations is the Long-QT syndrome type 1, LQT1; it presents as QT interval prolongation on the ECG and predisposes patients to cardiac arrhythmia and sudden death.

Results and Discussion

Several charged residues that participate in Kv1.2 (Shaker homolog) gating are conserved in KCNQ1 (Fig. 1A). We focus on the voltage sensing region of each of the 4 channel subunits, which contains transmembrane segments S1-S4 (Fig. 1B). Negatively charged residues on S2 and S3 can interact with positive charges on S4, forming energetically favorable salt bridges between oppositely charged residues during channel activation. The S4 residues in KCNQ1 are highly conserved and, as in most K+ channels, enable channel opening and closing in response to transmembrane potential, Vm. The molecular KCNQ1 model is constructed by aligning the KCNQ1 sequence with Kv1.2 (Fig. 1A) using its open state as a template. The model is then refined with MD (SI Appendix, Section 1, Figs. S1 and S2). The resulting KCNQ1 configuration is shown in Fig. 1B. Close examination (Fig. 1C) shows that water penetrates and interacts with charged S4 residues. In Shaker, the ability of water to solvate part of the voltage sensor has been implicated in its ability to transport protons when R2 and R3 are mutated to histidine (5). Additionally, a single point mutation to R1, such as R1C, enables ionic current (omega current) to pass through the voltage sensing region (6).

To simulate S4 motion during channel gating, we translated it 12 Å [experiments suggest 7–13 Å (7, 8)] from the permissive state (or up state) down toward the intracellular space and rotated it 90° counterclockwise (observed from extracellular space) about its axis (Fig. 1D) (SI Appendix, Section 2). The probability that S4 resides at a given position is primarily determined by the electrostatic energy, which is computed with the Poisson-Boltzmann Equation (PBE) to generate a 2-dimensional energy landscape as a function of S4 movement, Fig. 1F (9–11). For the energy contributed by Vm (calculated below) to determine channel opening and closing, we needed to scale the solvation and coulombic energy contributions. This scaling had the effect of reducing barriers between minima corresponding to stable channel states [Details can be found in SI Appendix, Section 4]. We label the minima (right to left) as a deep closed state, an intermediate closed state and a permissive state of the voltage sensors. These labels are associated with specific protein conformations, of which representatives are shown in Fig. 1F. Channels can open only when all voltage sensors are in the permissive state. The presence of multiple closed states (minima)...
is consistent with the sigmoidicity of KCNQ1 and $I_K$, which facilitates participation in AP rate adaptation (3).

Although a crystal structure of the closed state of Shaker is not available, experiments suggest spatial constraints that localize R1 on S4 between E1 on S2 and near another residue close to the top of S1 (12). A second study, examining site-specific fluorescence, indicates a rotation of 180° (8) and translation of 6–8 Å with an additional degree of freedom for S4 that tilts its axis as it moves intracellularly. Applying 90° rotation from the open to the closed state in our KCNQ1 simulations implies that R2 moves near E1 whereas R1 has only long distance interactions with it. Data suggesting otherwise in KCNQ1 have not been published. However, $Cd^{2+}$ bridge formation (12), fluorescence mapping, and site specific mutations (8), could examine this proposed model as done in Shaker.

The movement of positive charges on S4 (R1-R6) across the membrane during channel opening and closing, causes gating to depend on $V_m$. The energy landscape in the presence of $V_m$ (Fig. 2A) is computed with a modified PBE (often referred to as the PB-V) (13) (SI Appendix, Section 5). The presence of water that penetrates into the voltage sensor focuses the electric field, reducing the distance that S4 positive charges need to travel to cause gating, as shown experimentally (5, 6, 14) and computationally (15). The energy because of charges moving through the electric field ($V_m = 100 \text{ mV}$) is shown in Fig. 2B (Left); it shows permissive state stabilization of $\approx 10 \text{ kJ/mol}$ per 100 mV relative to the deep closed state. Since residues in S4 are translated, we plot (Fig. 2B, Right) their individual gating charge contribution (calculated as in ref. 13) at opposite ends of the translated region. The dominant role of R1-R4 in conferring voltage dependence is observed experimentally in Shaker (16); however, the total gating charge in our model is less ($\approx 6$ charges move across the field vs. $\approx 13$ in Shaker), which is to be expected because the R3 position in KCNQ1 is filled with a glutamine. Additional gating charge may also be generated by movement of residues that we postulate to participate in gating (17).

Adding the contribution of $V_m$ to the energy landscape shown in Fig. 1F, allows us to visualize its effect on the minima that define the 3 stable states of the model. In the presence of a negative $V_m = -80 \text{ mV}$, stabilization of the closed states occurs (Fig. 2C). Applying a positive potential ($+60 \text{ mV}$), causes the stable region to shift toward the permissive state. The scheme in Fig. 2D utilizes the energy landscapes of the 4 voltage-sensing

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**Fig. 1.** KCNQ1 model and energy landscape. (A) Kv1.2/KCNQ1 alignment. Symbols: * identical, : conserved, and . semiconserved substitutions. Red residues are charged. (B) Top-down (extracellular) view of the all atom system. Each of the 4 channel subunits (consisting of S1–S6 segments) is color coded. Pore regions (S5–S6) of one subunit interact with the adjacent subunit voltage-sensing region (S1–S4). (C) Voltage-sensing region with lipid of the 4 channel subunits (consisting of S1–S6 segments) (gray) and water (light blue) solvent molecules. (D) S4 translation and rotation. β-carbon of R4 labeled with red beads shows motion. Arrows indicate stable configurations (labeled): S1, Yellow; S2, Red; S3, White; S4, Green; S5, Blue. (E) Implicit membrane. Isocountours at dielectric constant $\varepsilon = 78$ (blue) and $\varepsilon = 2$ (transparent) show the transition region representing the lipid esters. Water (blue) can penetrate the protein as in C and is represented by $\varepsilon = 78$. (F) Energy landscape at $V_m = 0 \text{ mV}$ and associated conformations. Increasing translation corresponds to movement toward the intracellular space; increasing rotation is counterclockwise as viewed from extracellular space. Left of dotted white line is permissive state. Positively/negatively charged residues are labeled with red/blue letters. Energy landscape minima (deep blue) correspond to stable configurations; right to left: deep closed state, intermediate closed state, permissive state. In the deep closed state conformation, interactions occur between R2 and E1 and between R4 and E2. Intermediate closed state shows interactions between R4 and D1 and E2. In the permissive state, interactions are observed between R6 and E2 and between R4 and E1. Views of conformations were selected to show the interactions between residues. For movement of S4 during gating, see Movie S1.
domains and their voltage dependence to compute the probability of their residency in the permissive state using a Monte Carlo simulation. For these simulations, each voltage sensor is allowed to perform a random walk on its own energy landscape, and if all 4 reach the permissive state the channel can transition to the open states (Fig. 2E). This current is then calculated as the sum of many (1,000) single channel currents (Fig. 2F). This current is then compared with experimentally recorded currents. As shown in Fig. 3, simulated current traces at varying pulse potentials are similar to experimental traces with some slowing of activation rate at lower potentials, indicating that the simulated activation barrier is higher than that in the oocyte-expressed KCNQ1 channels in the experiment.

Further validation of the model and its predictive capability is obtained by introducing (simulating) mutations at the molecular level. We examined mutations at E160 (E1) on S2 that is the site of E160K, a naturally occurring LQT1 mutation. We tested 3 mutations: Q (polar side chain), A (neutral) and K (positively charged). These mutations progressively reduce the negative charge carried by glutamic acid (E) and correspondingly slow activation, caused by a higher energy barrier for reaching the permissive state. Experiments also show a significant reduction in total E160A current because of reduced channel expression and/or conductance, which is accounted for in the model. For E160K an electrostatic repulsion is present between R4 and the substituted K at E1. Experimentally, these channels do not generate membrane current, so macroscopic conductance is set to 0.

As expected, the E160A energy landscape closely resembles E160Q, because the residues only differ by side-chain polarity. However, E160A activation kinetics are modestly slower than E160Q (as in experiment). This difference is caused by the absence of a minimum near the intermediate closed state in the E160A landscape that is present for E160Q (Fig. 3A, arrow). Experiments also show a significant reduction in total E160A current because of reduced channel expression and/or conductance, which is accounted for in the model. For E160K an electrostatic repulsion is present between R4 and the substituted K at E1. Experimentally, these channels do not generate membrane current, so macroscopic conductance is set to 0.

Native IKs is carried by a heteromeric channel that consists of KCNQ1 and a modulatory subunit KCNE1 that slows activation (18). We account for the slowing of S4 motion by KCNE1
through a decrease in the diffusion constant between points on the energy landscape. With this change, we reproduce the voltage dependent activation of $I_{\text{Ks}}$ and that of the mutant channels, in the presence of KCNE1 (Fig. 3C). This result is consistent with experiments showing proximity of KCNE1 and S4, supporting the idea that KCNE1 directly affects S4 motion.

To simulate the whole-cell phenotype, we insert the channel into a recently updated Hund-Rudy canine ventricular myocyte model (19). Whole-cell WT and mutant currents at different rates are shown in Fig. 4. In the absence of current (most severe LQT1 mutation E160K), the AP is significantly prolonged. As rate increases, the AP shortens, a process termed adaptation. The slower activation of the mutant currents E160Q and E160A also results in a prolonged AP and a severe phenotype. Note that AP prolongation increases at slow heart rate (Fig. 4C), a hallmark of LQT Syndrome. The KCNE1 mutation L51H reduces the amount of expressed KCNQ1 by 30% (20). In our simulations (Fig. 4, orange) this mutation causes moderate AP prolongation, as seen clinically for LQT5 patients (21).

The duration of the ECG QTc interval (QT corrected for rate) in patients reflects the time between ventricular depolarization and repolarization, and nominally has a duration of 348 to 467 ms (22). In patients with LQT1, QTc is prolonged significantly to an average 493 ms. QTc in patients with LQT5 is much less prolonged at 457 ms (21). To evaluate the potential ECG changes of the simulated mutations, we calculate pseudo ECGs (23) (Fig. 4D), showing QT prolongation similar to the clinical phenotypes in the absence of β-adrenergic stimulation.

Dramatic alteration of channel kinetics caused by charge switching and neutralization mutations in Shaker and KCNQ1 suggest that the electrostatic energy is a primary component of the channel free energy, which determines opening and closing rates. However, several additional factors may also contribute, including: intramolecular interactions, solvent rearrangement, entropic terms, nonpolar energies, and the force required to move the channel gate. A simulation estimating such contributions is provided (Fig. S3). In addition to S4, the surrounding S2 and S3 helices may also move, and S4 may have more degrees of freedom in addition to translation and rotation (24).

Experimental clarification of the S4 pathway will enable more complete MD simulations to provide a better picture of the channel molecular dynamics.

**Fig. 3.** Mutation effects. (A) Energy landscapes for wild-type KCNQ1 and mutants E160Q, E160A, and E160K at $V_m = -100 \text{ mV}$ in the presence of KCNE1. (B) Comparison of macroscopic current between experiment (black) and simulation (cyan) for homomeric KCNQ1 mutants. (C) $I_{\text{Ks}}$ (KCNQ1 + KCNE1) is accurately simulated by slowing S4 movement. Mutations cause slowing of activation, in addition to KCNE1 effect.

**Fig. 4.** (A) AP and ECG. $V_m$ and $I_{\text{Ks}}$ currents are color coded. All currents, with the exception of $S_{\text{L1H}}$, are in the presence of KCNQ1. (A) Slow cycle length (CL = 1000 ms). (B) Fast rate (CL = 300 ms). (C) Adaptation curves, which plot AP duration (APD) dependence on CL (time between beats). (D) Simulated pseudoECGs. LQT1 mutation E160K shows significant QT interval prolongation, compared with mild prolongation by LQT5 mutation L51H, consistent with clinical phenotypes (21) (E160A closely overlaps E160K).
Materials and Methods
A detailed description of methods is provided in the SI Appendix. Briefly, the homology model is built with Modeller (25) using the Kv1.2 open-state crystal structure as a template (26). This model is refined using molecular dynamics in the NAMD simulation package (27). Visualization of these molecular models is accomplished with VMD (28). Poisson Boltzmann calculations are computed with APBS (11), which is modified to compute the PB-V equation to include V_m effects (13). These modifications also enable computation of gating charges as in ref. 13. The adjoint form of the Smoluchowski equation (29) is used to calculate transition rates between points on the energy landscape (9, 30), which are incorporated into macroscopic Markov models of the current.

Macroscopic current models are incorporated into the Hund-Rudy canine ventricular action potential model (HRd) (31). PseudoECG traces are calculated from a strand as in ref. 23.

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Supporting Information

1 Homology Model

Homology models were constructed with Modeller v9.1(1), which minimizes an objective function that accounts for dihedral angles, bond lengths and torsions, electrostatic interactions in addition to the conformation indicated by the Kv1.2 template. One hundred unique models were generated and eight, with the most favorable objective value, were chosen for further evaluation. Procheck can be used to compute a G-factor, which reflects the correspondence of the Ramachandran plot (Figure S1), torsion angles and covalent geometry to known protein structures. The model chosen for molecular dynamics studies had an overall G-factor of -0.3, which is within the range (> -0.5) of known high-resolution protein structures (Figure S2).

The model was further refined by simulating its motion in an all-atom lipid and water solvent using the NAMD simulation package(3). This enables the channel to settle into a stable conformation, taking into account physical intramolecular forces and solvent effects that affect the channel structure. For our simulations we used a recent version of the CHARMM force field(4)—CHARMM 22 for proteins(5) and CHARMM 27 for lipids(6). The initial step in simulating KCNQ1 in an all-atom environment is to insert the channel into a pre-equilibrated phosphatidylcholine (POPC) membrane, which is the main membrane constituent in a cardiac myocyte(7). This was done by aligning the protein with the membrane top and bottom as suggested experimentally, the R1 α-carbon at 13.7 Å from the membrane center(8, 9). Then lipid or water groups that overlapped or were within 0.8 Å of the protein were removed and water was inserted 10 Å above and below the protein. Cl- ions were added to compensate for an excess of positively charged residues and make the system electroneutral. The system contained 248 lipid groups and 23,939 water molecules (120,577 total atoms).

2 Motion of S4

In Shaker K+ channels, experimental evidence indicates that channel opening involves translation and rotation of S4 that replaces the interaction between the outermost glutamic acid (E1) on S2 and the second arginine on S4 (R2) with interaction between E1 and the fourth arginine on S4 (R4)(10). Several experiments also suggest that in addition to rotation, S4 translates between 7 and 13 Å during channel opening(11-14). We translated S4 12 Å from the open state toward the intracellular space with an increment of 0.25 Å. The direction of translation was along the helix axis, calculated from the positions of the alpha carbons of residues 234 to 237 using vector algebra(15).

At each translation, S4 was also rotated ~2π/5 with an increment of π/100 around this axis to sample its motion (Figure 1D, in manuscript). A model at each position was created by creating a harmonic restraint for each of the new S4 α-carbon (Cα) positions in Modeller, while the Cα in S1, S2, S5 (from the neighboring subunit) were fixed.

For these studies, we isolated the voltage sensing region of the channel, S1, S2, S3 and S4 from a single subunit and S5 from the neighboring subunit. Long extra- and intracellular loops were not expected to contribute significantly to the electrostatic energy (due to the high dielectric constant of these spaces) and were removed. Since the S4-S5 linker was not included in the simulations, the intracellular end of S4 was constrained to
stay within reach of its position in the crystal structure (15 Å, a value near to the distance that S4 was translated, 12 Å).

After each model is created, PDB type files are converted to PQR format by using PDB2PQR(16, 17) in preparation for energy calculations. At this point, the protonation state of H5 is checked to verify that its pKa is less than 7.4 (body pH). This ensures that the free charge carried by this residue remains intact. pKa determination is accomplished by using PropKa, which uses an empirical algorithm based on many known protein structures(18) and is included in PDB2PQR.

3 Insertion of the Protein into an Implicit Membrane

The automated insertion of the channel into an implicit membrane requires several considerations including:

1.) the ability of water and free ions to penetrate clefts in the protein
2.) the irregular shape of the protein
3.) the transition from a low dielectric core to a higher dielectric constant in the regions where there are ester and phospholipid groups near the surfaces of the membrane.

The varying dielectric (ε= 78 for water, 7 for the transition region, 2 for the membrane core) is incorporated by creating 3 sets of dielectric and free charge accessibility maps, one for each region, in APBS. Maps in APBS are based on a cubic spline surface of the protein(19) (APBS srfm option spl2), with the protein interior set to ε=2, and solvent accessibility to 0. After the maps are created, a top and bottom surface of the protein and membrane are defined by stepping from the top of the volume of interest to the first point where free charges are excluded (κ=0). If there is no protein midway through the volume the top is assigned the defined membrane value (for dimensions see Figure 1E).

These three maps are then combined such that: if a point resides on either side of the membrane the point is assigned the value of the water map, if a point is inside the transition region and beneath the membrane surface it is assigned the transition map value, and if a point is inside the membrane core and beneath the membrane surface it is assigned the core map value. To account for the penetration of water into the voltage sensing region a fourth map is created. Points indicated by this map are assigned the value of the high dielectric (ε=78) map. The isocontours showing the boundaries between each region are shown in Figure 1E.

For the membrane potential computations, described below in section 5, it is necessary to add charge to one side of the membrane. To accomplish this, we used the definition of the membrane top and added charge to any point above this surface.
4 Protein Energy Calculations

The electrostatic energy at a given conformation can be found by integrating the potential $\phi(\vec{r})$, found by the Linearized Poisson-Boltzmann equation (LPBE):

$$-
abla \cdot [\varepsilon(\vec{r})\nabla \phi(\vec{r})] + \kappa^2(\vec{r})\phi(\vec{r}) = -\frac{e_\epsilon}{k_B T} 4\pi \rho(\vec{r})$$

where $\phi = \frac{e\Phi}{k_B T}$ is the reduced electrostatic potential and

$\Phi$ is the electrostatic potential (statvolt);

$\varepsilon$ is the inhomogeneous dielectric constant (statcoul$^2$ erg$^{-1}$ cm$^{-1}$);

$\rho$ is the density of charge within the protein (statcoul cm$^{-3}$);

$e_\epsilon$ is the electron charge (statcoul);

$k_B$ is the Boltzmann constant (erg K$^{-1}$);

$T$ is the temperature (K).

and $\kappa^2 = \varepsilon_\epsilon \kappa^2$, with

$$\kappa^2 = \frac{4\pi}{k_B T} \sum_a q_a^2 \bar{\rho}_a / \varepsilon_w$$

$\kappa^{-1}$ is the Debye-Hückel screening parameter (cm),

$q_a$ is the charge (statcoul) and $\bar{\rho}_a$ (cm$^{-3}$) is the bulk ion density of each ion type $\alpha$

and $\varepsilon_\epsilon$ is the dielectric constant of water (statcoul$^2$ erg$^{-1}$ cm$^{-1}$).

The position of the channel in the membrane determines which residues come in contact with free ions in the intra and extracellular solutions. The screening of the protein charges by these ions reduces the distance at which charged residues interact and is accounted for by $\kappa^2$ in the above equation. We assume $\kappa$ to be 0 inside the membrane as in reference(20), which implies that free ions do not reside within the membrane. The new formulation of $\kappa^2$ then becomes:

$$\kappa^2 = \frac{4\pi}{k_B T} f(\vec{r}) \sum_a q_a^2 \bar{\rho}_a / \varepsilon_w,$$

where $f(\vec{r})$ is 0 within the protein and membrane and 1 elsewhere.

To simulate the natural environment of the protein, a multilayer implicit membrane was constructed that is accounted for in the LPBE by $\varepsilon(\vec{r})$. The core of the membrane, which consists of the hydrophobic lipid tails, spans a region of 19 Å and is assigned a dielectric constant $\varepsilon_m$=2. Near the outer edge of the membrane, an increase in the dielectric constant is associated with the ester groups in the lipid headgroups. This transition region spans 3 Å on either side of the membrane and has a dielectric constant, $\varepsilon_\text{w}=7$. The phosphate groups that line the outer surface of the membrane correspond to a region with a very high dielectric constant, and this region is lumped with the water
region on each side of the membrane with $\varepsilon_w = 78(21)$. The water seen penetrating the pore in the all-atom simulation is mapped accordingly and assigned a value of $\varepsilon_w$ (**Figure 1E**, in manuscript).

The LPBE can be solved by using a multigrid method, implemented in the Adaptive Poisson Boltzmann Solver, APBS(22). Two levels of refinement are used—a coarse pass followed by a focusing step that includes only the volume of interest. For the coarse grid, the size of the box is 180x180x180 Å$^3$ which is then focused to 65x60x75 Å$^3$. Both steps utilize a mesh with 97 gridpoints in each of the x and y directions and 129 points in z. The maximum grid spacing is 0.67 Å.

The electrostatic energy of the protein inserted in this membrane is

$$W^p(q) = \frac{1}{2} \int_V \Phi^p (\vec{r}, q) \rho (\vec{r}, q) d^3r$$

where $q$ is the position of S4 and W is the energy in kJ/mol.

Discretizing the box in which the protein resides into a grid can result in charged atoms being split between more than one gridpoint. Upon solving, energy from the self-interaction of the split charges contributes to the energy landscape. To overcome this difficulty, maps were computed for the heterogeneous dielectric (where the solvent dielectric differs from the protein) and for a homogeneous dielectric ($\varepsilon=2$). The homogeneous energy was then subtracted from the heterogeneous energy to create a solvent response map. To obtain the final energy, the coulombic energy was added back in analytically ($\varepsilon=2$) by using Coloumb, a program that is included in the APBS package. Since the analytic solution does not split the charges, but instead considers them as point charges, it can be used as an appropriate substitute for the subtracted charges (which had contained self-interactions).

The energy of a channel in a given region of space can be reduced according to:

$$W^c = -k_B T \log \int \int \exp \left( -W(\vec{r}, \theta) / k_B T \right) d\vec{r} d\theta$$

where $W^c$ is the effective energy for the volume, $\vec{r}$ is translation and $\theta$ is rotation.

This equation returns a value close to that of the minimum energy in the region, which corresponds to the most likely state of the protein. To create our energy landscape, we used a region containing 12 conformations that spanned a translation of 1 Å and 1/25 π radians.

While electrostatic energy provides an approximation to the free energy of the channel, the sudden changes in magnitude with small perturbations in translation and rotation prevent us from using it directly to study channel kinetics. The primary cause is a high energy barrier that must be overcome to move charged residues from the aqueous extra and intracellular environments to the transmembrane region. To compensate for these effects barriers were corrected by a factor of 0.125 to enable the energy conferred by $V_m$ to open and close the channel as required (**Figure S3**).
5 \( V_m \) Energy Calculations

The contribution to the energy landscape from the transmembrane potential can be found with a modified Poisson-Boltzmann equation(20):

\[
-\nabla \cdot [\varepsilon(r)\nabla \phi_m + \phi_m] + \kappa^2 \phi_m = \frac{e^2 V_m}{k_B T} \Theta(r),
\]

where \( V_m \) is the transmembrane potential (statvolt) with respect to the extracellular space,

\[
\phi_m = \frac{e\Phi_m}{k_B T}
\]

is the reduced electrostatic potential due to \( V_m \),

\( \Phi_m \) is the electrostatic potential,

and \( \Theta(r) \) is a Heaviside step function

whose value is 1 for \( r \) within the intracellular space and 0 elsewhere.

The modified LPBE can be solved with APBS, with some slight modification to enable introduction of appropriate boundary conditions (23) (see Appendix for changes and derivation of boundary potentials). As can be seen in manuscript Figure 2A, the ability of water to penetrate into the voltage sensing region causes a nonlinear potential variation across the membrane.

By integrating the product of the potential with the protein charges, the contribution of \( V_m \) to the total energy can be found:

\[
W^m(q) = \int \Phi^m(\vec{r}, q) \rho(\vec{r}, q) d^3 r.
\]

6 Computing the Currents

The transition rates between points on the energy landscape can be found with the Smoluchowski equation(24, 25):

\[
\frac{\partial p(x,t)}{\partial t} = \frac{\partial}{\partial x} D \left( \frac{\partial}{\partial x} p(x,t) - \beta F(x) p(x,t) \right)
\]

\( p(x,t) \) is the probability of finding a particle at a position \( x \) (Angstrom) at time \( t \) (ms).

\( D (\text{Å}^2 \text{ms}^{-1}) \) is the diffusion constant, \( F(x) \) is the force on the particle (kJ \cdot \text{mol}^{-1} \cdot \text{Å}^{-1}),

and \( \beta \) is \( 1/(k_B T) \) (kJ \cdot \text{mol}^{-1})^{-1}.

The Smolochowski equation has two terms: 1) a diffusive term that causes particles to disperse from a region in which they are concentrated, and 2) an advective term that accounts for the force on the particles introduced by the energy landscape. If \( F = -\nabla W \), which means that the force on the particle depends on the gradient of the potential (a conservative field), the adjoint Smoluchowski equation can be used and solved with finite differences(26):
\[
\frac{\partial p(x,t)}{\partial t} = \frac{\partial}{\partial x} D(x) e^{-pW(x)} p \frac{\partial}{\partial x} \left( e^{pW(x)} p \right).
\]

Noise variance analysis and studies in the presence of rubidium indicate that the channel can transition into a state-independent flickery blocked state, which would be reflected on the single channel recordings(27-29). In the model, channels can transition from any state to the blocked state, and the transition rates back and forth from the blocked state are independent of the state of the channel (Figure 2C, in manuscript).

Single channel activity can be simulated by using the scheme in manuscript figure Figure 2C and employing a Monte-Carlo simulation. A single channel has four voltage-sensing subunits, and each subunit is initialized to a position on the energy landscape. For a given timestep, \(dt\), the channel can make a transition from its current point to an adjacent point only if a random number (between 0 and 1, found with the Mersenne-Twister algorithm(30)) is less than the product of \(dt\) and the transition rate (found by the Smoluchowski equation). Then, if all 4 subunits are in the activated region of the energy landscape, the full channel can make a cooperative transition into the open state \(O_1\).

Once the channel has made this transition, the subunits are locked in the activated region of the energy landscape. From the first open state the channel can then transition to open states that are further from the closed state (\(O_2-O_5\)). The presence of these states is inferred from a delay before the onset of inactivation and an increase in the time constant of deactivation that is proportional to the length of a depolarizing pulse(31, 32). The inactivated state is therefore only accessible when the channel reaches the final open state (\(O_5\)).

7 Pseudo ECG

A recently published model of the canine epicardial myocyte (33) was used for action potential simulations. A one dimensional fiber of 160 model cells connected by gap junctions was used to represent the planar wavefront that travels from endocardium to epicardium during normal ventricular excitation. Experimentally observed transmural differences in APD in control (34) were reproduced by incorporating regions of endocardial, M and epicardial cells with heterogeneity in \(I_{Ks}\) (35), \(I_{NaL}\) (36) and \(I_{to1}\) (37). Distribution of cell types and ion channel densities are shown in the table below.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Endocardial</th>
<th>M</th>
<th>Epicardial</th>
</tr>
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<tr>
<td>Cell Numbers</td>
<td>1-20</td>
<td>21-80</td>
<td>81-160</td>
</tr>
<tr>
<td>(g_{Ks}) (mS/(\mu)F)</td>
<td>1*epi</td>
<td>0.5*epi</td>
<td>1*epi</td>
</tr>
<tr>
<td>(g_{NaL}) (mS/(\mu)F)</td>
<td>0.01495</td>
<td>0.0221</td>
<td>0.0130</td>
</tr>
<tr>
<td>(g_{to1}) (mS/(\mu)F)</td>
<td>0.3375</td>
<td>2.49</td>
<td>2.6182</td>
</tr>
</tbody>
</table>

To allow computationally tractable single cell and strand simulations, ion concentrations were initialized using steady state data from simulations using a previously published model of \(I_{Ks}\) (33). Single cell and strand results are reported for the fourth paced beat following concentration initialization. Single cell action potentials and adaptation are shown for a single epicardial cell simulation. Pseudo-ECG results are shown for the fourth beat at CL = 1 s.
The pseudo ECG is calculated as in reference (38) by

\[ \Phi_e(x', y', z') = \frac{a^2 \sigma_i}{4 \sigma_e} \int -\nabla V_m \cdot \nabla \frac{1}{r} dx \]

\[ r = \left[ (x - x')^2 + (y - y')^2 + (z - z')^2 \right]^{1/2} \]

where \( \nabla V_m \) is the spatial gradient of \( V_m \), \( a \) is the radius of the fiber, \( \sigma_i \) is the intracellular conductivity, \( \sigma_e \) is the extracellular conductivity, and \( r \) is the distance from a source point \((x, y, z)\) to a field point \((x', y', z')\).

\( \Phi_e \) is computed at 2.0 cm away from the epicardium along the fiber axis.

8 Oocyte Experiments

Whole-cell currents were recorded by two-electrode voltage clamp using a Dagan CA-1B amplifier (Dagan, Minneapolis, MN). Records were low-pass filtered at 100 Hz or 1 kHz with the amplifier's built-in Bessel filter and digitized at 500 Hz or 5 kHz, respectively. The electrodes were filled with 3 M KCl solution. Oocytes were bathed in ND96 or high K+ solution as indicated in Fig 3. The ND96 solution contained (mM) 96 NaCl, 4 KCl, 1.8 MgCl2, 0.1 CaCl2, 5 Hepes; pH 7.6. To measure voltage-dependent activation, currents were elicited by testing voltage pulses from -40 to 20 mV in 10 mV increments. Holding potential was −80 mV. Leak current was subtracted digitally.

References


29. Sesti F & Goldstein SA (1998) Single-channel characteristics of wild-type IKs channels and channels formed with two minK mutants that cause long QT syndrome. *J Gen Physiol* 112(6):651-663.


Appendix I

Transition Rates

KCNQ1 Rates

\[ I_{KCNQ1} = \overline{G_{KCNQ1}} \cdot O_{KCNQ1} \cdot (V_m - E_{Ks}) \]

Where maximum conductance, \( \overline{G_{KCNQ1}} \), is:

\[ \overline{G_{KCNQ1}} = 2.4 \cdot 10^{-3} \cdot \left( 1 + \frac{0.6}{1 + \left( \frac{3.8 \cdot 10^{-5}}{[Ca^{2+}]_i} \right)^{1.4}} \right) \]

Accounts for dependence ← of conductance on intracellular calcium concentration \([Ca^{2+}]_i\).

and open probability, O:

\[ O_{KCNQ1} = O_1 + O_2 + O_3 + O_4 + O_5 \]

\[ E_{Ks} = \frac{R \cdot T}{F} \cdot \log \left[ \frac{[K^+]_o + P_{Na/K} \cdot [Na^+]_i}{[K^+]_i + P_{Na/K} \cdot [Na^+]_i} \right] \]

All rates in ms\(^{-1}\)

\[ \theta = 3.19 \cdot 10^{-1} \]

\[ \eta = 3.09 \cdot 10^{-2} \cdot \exp(-2.08 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T}) \]

\[ \psi = 2.54 \cdot 10^{-2} \cdot \exp(6.46 \cdot 10^{-2} \cdot \frac{V_m \cdot F}{R \cdot T}) \]

\[ \omega = 1.78 \cdot 10^{-2} \cdot \exp(-5.32 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T}) \]

\[ \lambda = 2.32 \cdot 10^{-2} \cdot \exp(1.21 \cdot 10^{-1} \cdot \frac{V_m \cdot F}{R \cdot T}) \]

\[ \mu = 6.19 \cdot 10^{-2} \cdot \exp(-9.46 \cdot 10^{-2} \cdot \frac{V_m \cdot F}{R \cdot T}) \]

\[ x_\psi = 8.74 \cdot 10^{-1} \]

\[ x_\omega = 3.29 \cdot 10^{-1} \]

\[ \psi_y = \psi \cdot x_\psi^{-1} \]

\[ \omega_y = \omega \cdot x_\omega^{-1} \]

\[ D = 27.0 \text{ Å}^2 \text{ms}^{-1} \text{ for translation and rotation and radius of S4 is 5 Å} \]
Flickery Block

$\rho = 0.72$
$\sigma = 2.0$

Mutation Effects

$x_\phi = 8.23 \cdot 10^{-1}$

For E160A,
$G_{KCNQ1}$ is divided by 5.

For E160Q,
$G_{KCNQ1}$ is equal to $G_{Ks}$

For E160K,
$G_{KCNQ1}$ is 0.

KCNE1 Effects

$x_\phi = 8.23 \cdot 10^{-1}$

$\eta = 8.24 \cdot 10^{-2} \cdot \exp(-5.20 \cdot 10^{-1} \cdot \frac{V_m}{R \cdot T})$

$G_{KCNQ1} = 0.017 \cdot \frac{0.6}{1 + (3.8 \cdot 10^{-5})^{1.4}} \left(1 + \frac{0.6}{1 + \left(\frac{3.8 \cdot 10^{-5}}{[Ca^{2+}]_i}\right)^{1.4}}\right)$

$D = 0.75 \text{ Å}^2 \text{ms}^{-1}$
1.1

Appendix II

Derivation of the Boundary Potentials

For each region of the membrane the potential obeys (20),

\[ \phi''_i(z) = \kappa^2 \phi_i(z) \]
\[ \phi''_2(z) = \phi''_3(z) = \phi''_4(z) = 0 \]
\[ \phi''_5(z) = \kappa^2 (\phi_5(z) - V_m) \]

Region 1 is the extracellular space, 2 the transition between extracellular region and the core, 3 the core region, 4 the transition between the core and intracellular region, and 5 the intracellular region.

We assume: 1) that the potentials far away from the membrane on the extra and intracellular sides (at \( z = +\infty, -\infty \)) are 0 and \( V_m \), respectively, and 2) that the potential at the interface between the solution and the membrane is continuous.

\[ \phi_1(-\infty) = 0 \]
\[ \phi_3(+\infty) = V_m \]
\[ \phi_1(0) = \phi_2(0) \]
\[ \phi_3(L_{\text{int}}) = \phi_4(L_{\text{int}}) \]
\[ \phi_3(L_m - L_{\text{int}}) = \phi_4(L_m - L_{\text{int}}) \]
\[ \phi_5(L_m) = \phi_5(L_m) \]

\[ \varepsilon_{\text{w}} \phi_1'(0) = \varepsilon_{\text{w}} \phi_2'(0) \]
\[ \varepsilon_{\text{w}} \phi_2'(L_{\text{int}}) = \varepsilon_{\text{w}} \phi_3'(L_{\text{int}}) \]
\[ \varepsilon_{\text{w}} \phi_3'(L_m - L_{\text{int}}) = \varepsilon_{\text{w}} \phi_4'(L_m - L_{\text{int}}) \]
\[ \varepsilon_{\text{w}} \phi_4'(L_m) = \varepsilon_{\text{w}} \phi_5'(L_m) \]
\[ \phi_1 = C_1 \exp(\kappa z) \Rightarrow \phi'_1 = \kappa C_1 \exp(\kappa z) \]
\[ \phi_2 = C_2 + C_3 z \Rightarrow \phi'_2 = C_3 \]
\[ \phi_3 = C_4 + C_5 z \Rightarrow \phi'_3 = C_5 \]
\[ \phi_4 = C_6 + C_7 z \Rightarrow \phi'_4 = C_7 \]
\[ \phi_5 = C_8 \exp(-\kappa z) - V_m \Rightarrow \phi'_5 = -\kappa C_8 \exp(-\kappa z) \]

Then the following system of equations can be set up to find the constants \( C_1 \) to \( C_8 \).

\[
\begin{bmatrix}
1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & L_{\text{int}} & -1 & -L_{\text{int}} & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & L_m - L_{\text{int}} & -1 & L_{\text{int}} - L_m & 0 \\
0 & 0 & 0 & 0 & 1 & L_m & -\exp(-\kappa L_m) & 0 \\
0 & 0 & \varepsilon_i & 0 & -\varepsilon_m & 0 & 0 & 0 \\
\varepsilon_u \kappa & 0 & -\varepsilon_i & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & \varepsilon_m & 0 & -\varepsilon_i & 0 \\
0 & 0 & 0 & 0 & 0 & \varepsilon_i & \varepsilon_u \kappa \exp(-\kappa L_m) & C_8
\end{bmatrix}
= 
\begin{bmatrix}
C_1 \\
C_2 \\
C_3 \\
C_4 \\
C_5 \\
C_6 \\
C_7 \\
C_8
\end{bmatrix}
= 
\begin{bmatrix}
0 \\
0 \\
0 \\
V_m \\
0 \\
0 \\
0 \\
0
\end{bmatrix}
\]

After solving, \( \phi(z) \) to \( \phi_5(z) \) are-

\[
A = \frac{V_m \varepsilon_i \varepsilon_m}{2 \varepsilon_i \varepsilon_m - 2 \varepsilon_i \varepsilon_u \kappa L_{\text{int}} + 2 \varepsilon_u \varepsilon_i \kappa L_{\text{int}} + \varepsilon_i \varepsilon_u \kappa L_m}
\]
\[ \phi_1(z) = A \exp(\kappa z) \]
\[ \phi_2(z) = A \left( 1 + \frac{\varepsilon_u \kappa z}{\varepsilon_i} \right) \]
\[ \phi_3(z) = A \left( 1 + \frac{\varepsilon_u \kappa L_{\text{int}}}{\varepsilon_i} - \frac{\varepsilon_u \kappa L_m}{\varepsilon_m} \right) + \frac{A \varepsilon_u \kappa z}{\varepsilon_i} \]
\[ \phi_4(z) = \frac{A \left( \varepsilon_m \varepsilon_u \kappa (2 L_{\text{int}} - L_m) + \varepsilon_i \left( \varepsilon_m + \varepsilon_u \kappa (-2 L_{\text{int}} + L_m) \right) \right)}{\varepsilon_i \varepsilon_m} + \frac{A \varepsilon_u \kappa z}{\varepsilon_i} \]
\[ \phi_5(z) = -A \exp(\kappa (L_m - z)) + V_m. \]

Which reduces to the previously published equations(20) when \( L_{\text{int}} \) is set to 0.
Fig. S1. Ramachandran plot. Red regions are highly favored in terms of steric interactions, yellow regions are allowed, white regions are unfavored. Triangles are glycine residues (unrestricted by \( \phi \) and \( \psi \) because of lack of a side chain), all other residues are indicated by squares. Residues in disallowed regions, by the steric criteria, are in red.
Fig. S2. Procheck assessment. Comparison of Ramachandran plot and G-factor to known structures. For the Ramachandran plot, as the crystal becomes better resolved more residues tend to be found in the favored regions (90% in the highest resolution structures), which is what we observe. The G-factor is a similar comparison, but includes many more criteria such as the angles of the side chains for each residue, bond lengths and planarity (2).
Estimation of error in energy calculations. (A) Membrane potential $V_m = 100 \, \text{mV}$ causes stabilization of the open state and introduces a net difference of $-10 \, \text{kJ/mol}$ between closed and open configurations. (B) Energy cycle. Each energy landscape represents the energy difference between two different environments as indicated. Difference is from conditions (indicated in boxes) at arrow head to those at arrow tail. For example, the topmost energy landscape on the right represents "Charges + Homogeneous Environment" minus "No Charges + Homogeneous Environment". The dielectric constant for the homogeneous environment is $\varepsilon = 78$. Large increases within the energy landscape are associated with the introduction of the protein environment and further by the presence of the membrane environment. Scaling of the energy landscape was performed to account for the effect of individual water molecules and other environmental contributions not explicitly computed in the simulations. With this correction, the energy barriers are in the range of $V_m$ as required.

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Movie S1. Movie shows S4 movement through the energy landscape from open to closed. At stable conformation (open, intermediate, closed, deep closed) the protein is rotated showing 3D structure.

Movie S1 (AVI)

Other Supporting Information Files

SI Appendix (PDF)