Supporting Information

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SI Text

I. Simplification of the Coupled Equilibrium Constant at Very Low Voltage. We consider the four different macrostates which contribute to P_{11}. Taking the ratios of the Boltzmann weights of the macrostates (1,1,1), (1,1,0), and (1,0,1) to $z(1,0,0)$, we get:

$$\frac{z(1,1,0)}{z(1,0,0)} = K_2 \qquad \textbf{[S1.1]}$$

$$\frac{z(1,0,1)}{z(1,0,0)} = K_3 \qquad \textbf{[S1.2]}$$

$$\frac{z(1,1,1)}{z(1,0,0)} = K_2 K_3 \theta_{23} \qquad \textbf{[S1.3]}$$

Both K_2 and K_3 are small at low voltages (Eq. **1**) and, therefore, the ratios defined in the above equations will become negligible. In other words, the magnitude of the $z(1,0,0)$ term will be much larger than $z(1,0,1)$, $z(1,1,0)$, and $z(1,1,1)$.

The coupled equilibrium constant for 1st particle would be expressed as:

$$\varepsilon_1 = \frac{(K_1 K_2 K_3 \theta_{12} \theta_{23} \theta_{13} + K_1 K_2 \theta_{12} + K_1 + K_1 [K_3 \theta_{13}])}{(1 + K_3 + K_2 + K_2 K_3 \theta_{23})} \qquad \textbf{[S2]}$$

Each of the apparent intrinsic equilibrium constants is an exponential function of voltage. Thus $\ln \varepsilon_1$ would be a highly nonlinear function of voltage. However, using the simplification described in Eqs. **S1.1**, **S1.2**, and **S1.3**, the expression for ε_1 at very low voltages would be greatly simplified (Eqs. **9.1** and **9.2**).

II. Definition of Particle. For the purposes of our discussion, we use the word "particle" to signify a specific structural unit of a protein. A protein is considered to be an ensemble of interlinked particles. When a site-specific probe is used to monitor the dynamics of a specific structural unit, the remaining structural units are considered to be a subspace of the entire ensemble. χ^{diff} for this specific structural unit reports its net interaction with the entire subspace. For example, in case of the voltage-gated sodium channels, when the activation of a specific voltage-sensor is monitored (say using a fluorescent probe), the remaining structural units (i.e., all the other voltage-sensors and the pore domain) constitute the subspace. In a recent study on BK channels, fluorescent probes were used to monitor the activation of S2 and S4 segments of the voltage-sensing domain separately (1). When monitoring S2, the rest of the protein forms the subspace. Thus the definition of particle largely depends on the choice of the site-specific probe and the knowledge about the conformational transition that the probe is reporting.

III. Solution for an $\mathcal{N}$-Particle Ensemble. We consider an $\mathcal{N}$-particle system, in which each of the particles undergoes a voltage-dependent transition from its resting to activated microstate, and each particle interacts with every other the particle of the system through four state-dependent interactions as described for the three particle system. For any particle 'i' in this system, we define the particle matrix, $\bar{S}_i$, which is a 1×2 vector, with the intrinsic chemical factors as its elements,

$$\bar{S}_i = [\,\omega_{i0} \quad \omega_{i1}\,] \qquad \textbf{[S3]}$$

where the intrinsic chemical factor of 'i' in a specific conformation 's' is:

$$\omega_{is} = e^{-G_{is}\beta} \qquad \textbf{[S4]}$$

The system can exist in 2^n possible macrostates and therefore, we can write a 1×2^n vector where each element represents the intrinsic chemical factor of a macrostate of this system. This system matrix, $\bar{\Lambda}$, can be defined as:

$$\bar{\Lambda} = \otimes_{i=1}^{n} \bar{S}_i \qquad \textbf{[S5]}$$

where $\otimes$ indicates the Kronecker[*] product of all the particle matrices. The elements of the system matrix $\bar{\Lambda}$ do not include the contributions of the interaction energies between the particles. To incorporate the coupling interaction terms, we define another 1×2^n coupling vector, $\bar{C}$, where each element indicates the stabilization conferred to an individual macrostate of the system by the pairwise interactions.

To denote every macrostate of this $\mathcal{N}$-particle system we use an $\mathcal{N}$-tuple variable $\nu_k(x_1^k, x_2^k, x_3^k, \ldots, x_{n-1}^k, x_n^k)$. The components of ν_k are x_i^k, $(i = 1,2,\ldots,n)$, which indicate the microstate of the ith particle in the kth macrostate of the system $(k = 1,2,\ldots,2^n)$. x_i^k is 1 or 0 $(\forall i,k)$ and indicates whether the ith particle is in the activated or resting conformation. The kth element of $\bar{\Lambda}$ corresponds to the macrostate ν_k and can be expressed as:

$$\Lambda_k = \prod_{i=1}^{n} \omega_{i x_i^k} \qquad \textbf{[S6]}$$

The net coupling factor for the $\mathcal{N}$-particle system in the kth macrostate will be:

$$C_k = \prod_{i=1}^{n} \prod_{j=1, j \neq i}^{i} \theta_{i_0 j_0}^{(1-x_i^k)(1-x_j^k)} \theta_{i_1 j_1}^{x_i^k x_j^k} \theta_{i_0 j_1}^{(1-x_i^k) x_j^k} \theta_{i_1 j_0}^{x_i^k (1-x_j^k)} \qquad \textbf{[S7]}$$

Λ_k and C_k are the kth elements in the system matrix, $\bar{\Lambda}$ and the coupling matrix, $\bar{C}$ respectively. Thus, the Boltzmann weight of the kth macrostate, ν_k, becomes:

$$z(\nu_k) = \Lambda_k C_k \qquad \textbf{[S8]}$$

The Boltzmann weights of all the macrostates of the system can be collectively represented in the form of a 1×2^n matrix, $\bar{\zeta}$, whose kth element is $z(\nu_k)$:

$$\bar{\zeta} = [z(\nu_1) z(\nu_2) z(\nu_3) \ldots\ldots z(\nu_{2^n-1}) z(\nu_{2^n})] = [\bar{\Lambda} \circ \bar{C}] \qquad \textbf{[S9]}$$

where $[\circ]$ indicates the Hadamard[†] product of the two matrices.

The canonical partition function for the entire system is:

$$\hat{Z} = \sum_k z(\nu_k) = \bar{\Lambda}.\bar{C}^T \qquad \textbf{[S10]}$$

The probability of the ith particle being in the activated conformation as:

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[*] Consider matrices $\bar{A} = (a_{ij})$ of order $m \times n$ and $\bar{B} = (b_{kl})$ of order $p \times q$. The Kronecker product of the two matrices is given by $\bar{A} \otimes \bar{B} = (a_{ij}\bar{B})_{ij}$ where $a_{ij}\bar{B}$ is of the order of $p \times q$ and $\bar{A} \otimes \bar{B}$ is of the order of $mp \times nq$ (2).

[†] Consider matrices $\bar{A} = (a_{ij})$ and $\bar{B} = (b_{ij})$ of order $p \times q$. The Hadamard product of the two matrices is given by $[\bar{A} \circ \bar{B}] = (a_{ij} b_{ij})_{ij}$ where $a_{ij} b_{ij}$ is a scalar and $[\bar{A} \circ \bar{B}]$ is of the order of $p \times q$ (2).

$$P_{i1} = \frac{\sum_k x_i^k z(\nu_k)}{\hat{Z}} \qquad \textbf{[S11]}$$

And the probability of the ith particle being in the resting conformation can be expressed as:

$$P_{i0} = \frac{\sum_k (1 - x_i^k) z(\nu_k)}{\hat{Z}} \qquad \textbf{[S12]}$$

At extreme potentials these probability terms would also be dominated by a single macrostate of the system—at low voltages the macrostate where i is activated and all others are resting would dominate P_{i1}, and the macrostate where all particles are resting would dominate P_{i0}. At high voltages P_{i1} would be dominated by the macrostate where all particles are activated, while P_{i0} would be dominated by the macrostate where only i is resting and all the others are activated. With these approximations the χ parameters for i in an $\mathcal{N}$- particle system can be easily derived in the explicit or normalized forms.

IV. General Expression for the χ Values. At extreme voltages:

$$\ln \varepsilon = qF\beta V + \chi \qquad \textbf{[S13]}$$

$qF\beta$ is the slope of the $\ln \varepsilon$ vs V plot at extreme voltages. Thus Eq. **S13**, may be rewritten as:

$$\ln \varepsilon = \left(\frac{\partial \ln \varepsilon}{\partial V}\right) V + \chi \qquad \textbf{[S14]}$$

Eq. **S14** can be converted into an exact differential as:

$$-\frac{\chi}{V^2} = -\frac{\ln \varepsilon}{V^2} + \frac{1}{V}\frac{\partial \ln \varepsilon}{\partial V} = \frac{\partial}{\partial V}\left(\frac{\ln \varepsilon}{V}\right) \qquad \textbf{[S15]}$$

Thus χ may be expressed as:

$$\chi = -V^2 \frac{\partial}{\partial V}\left(\frac{\ln \varepsilon}{V}\right) \qquad \textbf{[S16]}$$

χ_+ and χ_- values are the limiting values of the above expression, at very high and very low voltages respectively.

For a two-state process, the sum of measured probability of activation (P_A) and measured probability of resting (P_R) of a structural unit will always be unity. Thus the coupled equilibrium constant can be written as:

$$\varepsilon = \frac{P_A}{1 - P_A} \qquad \textbf{[S17]}$$

Using Eqs. **S16** and **S17** we can easily arrive at Eq. **18**.

1. Pantazis A, Gudzenko V, Savalli N, Sigg D, & Olcese R (2010) Operation of the voltage sensor of a human voltage- and Ca2+-activated K+ channel. *Proc Natl Acad Sci USA* 107(9):4459–4464.
2. Horn RA & Johnson CR (1994) *Topics in Matrix Analysis* (Cambridge Univ Press, Cambridge).

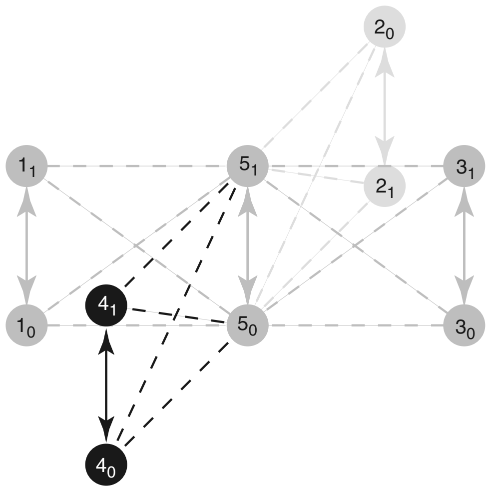

Fig. S1. Hypothetical model of a voltage-dependent sodium channel. The model represents a voltage-dependent sodium channel. Particles 1 through 4 represent the four nonidentical voltage-sensing domains of the ion channel, each capable of existing in two conformations: resting (designated as i_0) and activated (designated as i_1) ($i = 1, 2, 3$, or 4). The central pore domain, designated as particle 5, can also exist in two conformations: open (5_1) or closed (5_0). The vertical double-arrowed solid lines represent the intrinsic activation constants for the conformational change of the particles. The diagonal and horizontal lines represent the microstate dependent pairwise coupling factors between the particles: The diagonal lines indicate the cross-interactions between particles that are in unlike states, whereas the horizontal lines represent the like interactions between particles in the same microstate. Each of the voltage-sensing domains is directly coupled to the pore, but none of the voltage sensors interact with each other directly.

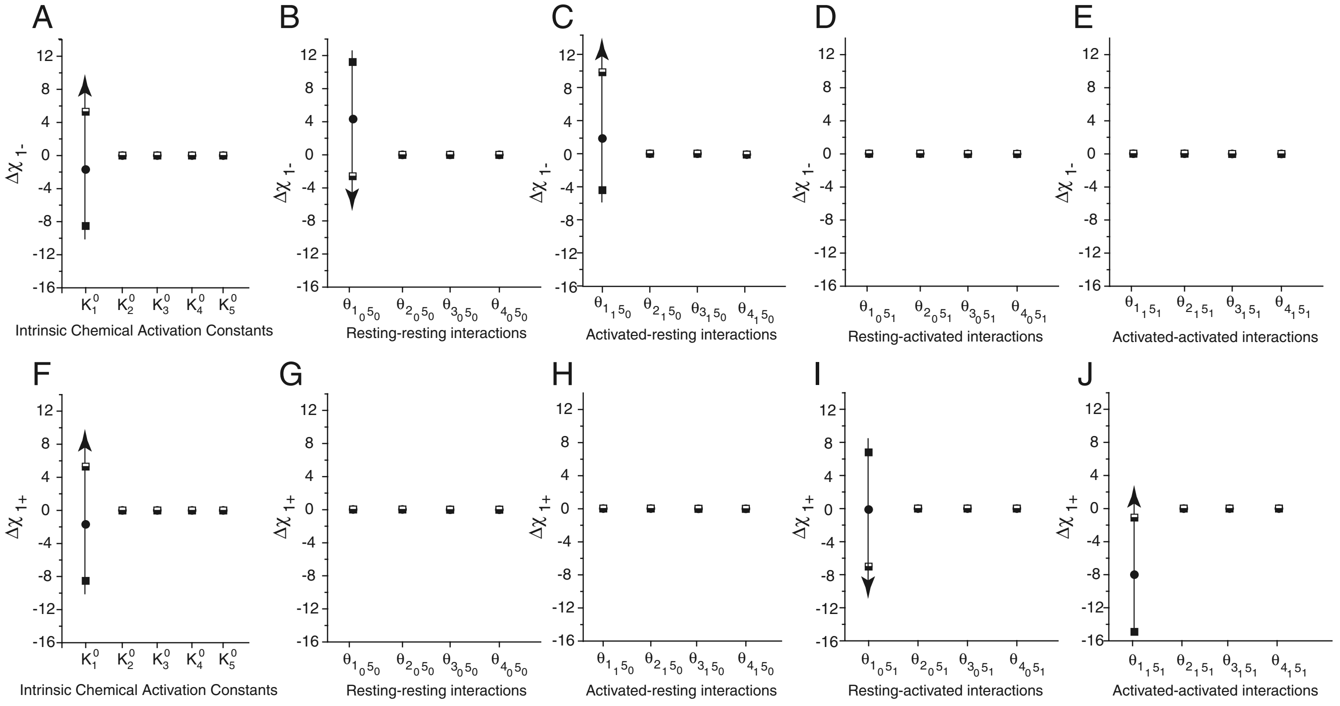

Fig. S2. Change in the χ values ($\Delta\chi = \chi^{\text{Mutant}} - \chi^{\text{WT}}$) of a particle in response to perturbation of different model parameters. Simulations were performed using the model of sodium channel as described in Fig. S1. Each of the model parameters was perturbed one at a time (while retaining the control values for the rest of the parameters) and the changes in the values of χ_{1-} (*A–E*) and χ_{1+} (*F–J*), relative to their control (initial) values are shown. Resting-resting and activated-activated state interactions imply the "like" state interactions between two particles which are in the same microstate. The "resting-activated" interaction implies the interaction between resting particle 1 and another particle that is activated. The "activated-resting" interaction implies that between an activated particle 1 and another particle that is resting. The arrows point towards increasing value of the thermodynamic parameter.

Table S1. The parameters and their values used for the numerical solution of the model sodium channel

Parameter	Value
$\hat{K}_1^0$	5
q_1	1.5
$\hat{K}_2^0$	10
q_2	1.5
$\hat{K}_3^0$	20
q_3	2
$\hat{K}_4^0$	1
q_4	1
$\hat{K}_5^0$	0.001
q_5	0.5
$\theta_{1_0}\theta_{5_0}$	75
$\theta_{1_1}\theta_{5_0}$	0.05
$\theta_{1_0}\theta_{5_1}$	1
$\theta_{1_1}\theta_{5_1}$	30
$\theta_{2_0}\theta_{5_0}$	50
$\theta_{2_1}\theta_{5_0}$	0.05
$\theta_{2_0}\theta_{5_1}$	0.01
$\theta_{2_1}\theta_{5_1}$	50
$\theta_{3_0}\theta_{5_0}$	100
$\theta_{3_1}\theta_{5_0}$	5
$\theta_{3_0}\theta_{5_1}$	0.5
$\theta_{3_1}\theta_{5_1}$	20
$\theta_{4_0}\theta_{5_0}$	75
$\theta_{4_1}\theta_{5_0}$	10
$\theta_{4_0}\theta_{5_1}$	1
$\theta_{4_1}\theta_{5_1}$	15

These parameters were used to calculate three extreme values of $\ln\varepsilon$ to check the parity between the numerical simulations and the expected (calculated) values of $\ln\varepsilon$ (and thus the consistency of the simulation code). The comparison table is provided as Table S2 (XLS).