

A Mathematical Model for Voltage Gated Ion-channel Stationary Conductance

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Abstract

Opening of ion-channels has been popularly described by means of Markov processes with a small number of important channel states. The approximation of the number of important states is often ambiguous and overestimating this number creates unnecessary computational complexities. We propose a simple equation that will portray the essence of voltage regulated ion-channel gating in a functional cell. The equation is generated using concepts in elementary thermodynamics applied to protein Markov models and it matches well many published channel current-voltage characteristics. Further, parameters of the model are found to be identifiable and easily determined from experimental data.

Keywords:

Voltage gated ion-channels, Transition kinetics, Markov processes

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1. Introduction

The electrical activity of a living system is a function of the dynamic ionic transport across biological membranes. Ion channels are pore-forming protein ensembles that are responsible for the task of regulating or gating, ion flows. Gating arises as conformational changes in the proteins that comprise the channel. These conformational changes are driven by changes in the electric field or by molecules (ligands) that bind to them. For this reason, ion-channels are often classified into voltage-gated and ligand-gated categories.

Voltage-gated ion channels have charged domains that make their structure sensitive to variations in the external electric field. For a particular range of membrane potentials they adopt a conformation with a central hole, forming a channel for the free movement of ions. Such an 'open' state is further defined by certain 'selectivity filters' (often amino acids) that would render specificity for the protein (Beyl et al., 2007). At other membrane potentials, the flow of ionic current is blocked as a result of the 'closed' or 'inactive' conformations that the protein adopts. A channel protein can thus adopt various conformational states with varying degrees of conductance, and they can spontaneously switch between these states. The dynamics of such switches is central to any study that involves an ion channel.

Equation based kinetic models are useful to interpret the behaviour of a channel in a given situation. Starting with the model of Hodgkin and Huxley (Hodgkin and Huxley, 1952), several researchers have developed theoretical frameworks that partially explain observations made on channel activity. The Hodgkin-Huxley

formalism relies on an underlying model of average channel conductance and their equations describe the changes of ionic permeability with membrane potential. The model makes use of hypothetical gating particles to bring about the channel's function, by forcing their motion with respect to the electric field across the membrane. Although this model has been used in many instances, hypothetical gating particles do not appear to be consistent with underlying molecular mechanisms. The use of stochastic versions of the model was based on the understanding that ion-channels are essentially stochastic entities (Fox, 1997).

Further developments in the study of ion-channels made an attempt to give a mechanistic description to the gating phenomena. Such models consist of nonlinear ordinary differential equations (ODEs), including a current balance equation and the dynamics of conformational transitions incorporated as a 'gating variable' that corresponds to the state of the ion channels. Discrete-state Markov models have been used to describe the different states of the ion channel (Kienker, 1989) and have found good acceptance for providing a mechanistic description for the otherwise abstract Hodgkin-Huxley formalism based models.

Markov chain models are developed on the assumption that ion channels exist in a finite number of significant energy states, with time-homogeneous rates of transition between them. The model consists of a topology of allowed transitions between these states, together with the rates for these transitions. Fitting single-channel recording data with a Markovian kinetic scheme has been standard in neurophysiology for quite some time (Kienker, 1989; Milesco et al., 2005; Sansom et al., 1989). However, for a good agreement with experimental data, frequently

the number of closed states needed varies with the experimental protocol. Markov models benefit compared to the Hodgkin-Huxley formalism with the large degree of freedom in the model structure that brings it closer to experimental observations (Fink and Noble, 2009). Thermodynamic models are a two state Markovian description of channel flipping, the rate kinetics of which are described by concepts in thermodynamics (Destexhe and Huguenard, 2000; Ozer, 2004).

Fractal models (Liebovitch et al., 1987; Liebovitch, 1989) of ion channel gating provide a different description of the underlying mechanism compared to Markov models. Such models are characterised by equations having continuous rather than discrete states. The Diffusion models introduced by Millhauser (Millhauser et al., 1988), justify Fractal models at a microscopic level. Statistical analysis, however has often favored Markov models over Fractal models (Sansom et al., 1989; Nekouzadeh and Rudy, 2007).

A major hurdle in modelling ion-channel gating using a Markov-jump scheme is in the determination of an appropriate number of closed states. As the topology space expands with the number of states, generating appropriate kinetic schemes imposes ambiguity because it may be possible to come up with multiple schemes that are consistent with a given set of data. Moreover, numerical simulation of such models becomes time-consuming. This limits the model during parameter estimation and further in its use for multi-cellular simulations (Fink and Noble, 2009). An increasing complexity of the topology thus calls for model reduction. Kienker (1989) talks about the existence of equivalence in topologies of models that are identifiable within the same data set. This would imply that models with

a larger number of states are reducible. Keener (2009) illustrates the possibility of reducing the complexity to stable invariant manifolds. This approach would reduce the dimension of the system without suffering from large approximation errors. Furthermore, the time scale of the Markovian transitions are much faster than the main time scales involved in the aggregate cell voltage and ion behaviour (Aidley and Stanfield, 1996; Hille, 2001), which in turn offers options for model reduction.

Another difficulty in the acceptability of ion channel models is related to parameter identifiability. A model is said to have structurally unidentifiable parameters when multiple parameters are equally powerful in explaining observed data (Walter and Pronzato, 1997). Since channel models are primarily developed based on collected experimental information, a practical identifiability analysis based upon numerical parameter estimation makes sense. A large segment of ion-channel models in the literature lack parameter identifiability (Fink and Noble, 2009) and many others are only locally identifiable.

In this article, we seek a voltage-gated ion-channel conductance model with three main characteristics. To begin with, the model should have a clear mechanistic motivation based on the underlying thermodynamics. In addition, the model should be relatively simple to implement, with a small number of easily identifiable parameters. Finally, the model should match well experimental data from patch clamp and similar studies as reported in the literature.

2. Background

2.1. Stable conformations of ion channels

The probability of a protein molecule adapting a particular conformation in space is largely influenced by the electrical force field surrounding it. When subject to an electric field, charged groups within a protein will experience a force and may attain a new electrostatic equilibrium by incorporating angular changes in the dipoles associated with the peptide bond (Manna et al., 2007). If the thermodynamic kinetic energy is large enough to overcome the energy barrier, the protein takes up a new conformational state. Proteins can hence take up a large number of conformational states separated by small energy barriers. Despite the continuum of intermediate states, this dynamical system would typically have only a few stable equilibria (Keener, 2009) and hence a limited number of experimentally observable states.

2.2. Transition velocities

The rate at which the protein switches between such stable states is mainly determined by the driving forces that help in overcoming the thermodynamic energy barrier. However, there are some conformational changes that are not regulated by either electric field or ligands. They may be considered to have a constant energy barrier in the given environment, and hence may be thought to have a uniform rate. Classical thermodynamics identifies the rate of transition between two reaction states based on the free energy barrier between them, as

$$k = k_0 e^{-(\Delta G)/RT} \quad (1)$$

where, k is the rate of transition between the two states, k_0 is a constant, ΔG is the free energy barrier between the two states, R is the universal gas constant and T the absolute temperature.

More generally, the free energy barrier may be dependent on the electric field, that is, the membrane potential V . In this case we have

$$k(V) = k_0 e^{-\Delta G(V)/RT} \quad (2)$$

Here $\Delta G(V)$ is the free energy barrier between the two states defined by the voltage (Milescu et al., 2005).

In the case of voltage based transition, the activation energy can be expressed in a general form by using a Taylor series expansion (Destexhe and Huguenard, 2000; Ozer, 2004), as follows:

$$\Delta G(V) = a + bV + cV^2 + \dots$$

and the rate of transition $k(V)$ may be written as,

$$k(V) = k_0 e^{-(a+bV+cV^2+\dots)/RT} \quad (3)$$

Here a corresponds to the free energy independent of the electric field, the linear

term, bV corresponds to interactions between electric field and isolated charges and rigid dipoles on the protein. The higher order terms correspond to the influence of polarization and deformation within the protein structure as well as mechanical constraints. These effects are usually negligible as the trans-membrane voltage variations are generally small (Destexhe and Huguenard, 2000).

In what follows, we are mainly interested in models where the free energy is linear in the membrane potential. The effect of temperature variations may be neglected for the system under consideration, as long as the physiological environment remains unaffected. In this case, the following lemma, helps us derive a simple expression for voltage regulated conformational transitions.

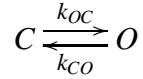
Lemma 1. *For the case $\Delta G(V)$ is affine in V , then for any transition between two stable states of the protein with a transition rate $k(V)$; at uniform ambient temperatures, $k(V)$, $\frac{k_i(V)}{k_j(V)}$ and $\prod \left(\frac{k_i(V)}{k_j(V)} \right)$ may be expressed as $e^{[(V-V_h)s]}$*

Proof: see Appendix A

2.3. Markov Models for Conformation transitions

A Markov process is a stochastic series of events with event probability depending only on the state of the system at the time immediately preceding the event. The series of molecular changes associated with the opening of an ion channel has been popularly demonstrated with Markov models with discrete states (Kienker, 1989; Sansom et al., 1989; Keener, 2009). Consider a simple form of transition between an open and closed state, let O and C represent the probability that the molecule is in the corresponding open and closed state at the given time.

Since the equation governing changes in probabilities for a single molecule has a form similar to the rate equation for a large number of molecules, the transition may be represented by a kinetic scheme, as follows:



where, k_{ij} represents the rate of transition from state j to state i . The above kinetic scheme has a transition intensity matrix \mathcal{K} ,

$$\begin{bmatrix} -k_{CO} & k_{OC} \\ k_{CO} & -k_{OC} \end{bmatrix}$$

and a corresponding steady state probability for the channel to be in an open state as

$$\mathcal{O} = \frac{1}{1 + \frac{k_{OC}}{k_{CO}}}$$

and using Lemma (1) we obtain,

$$\mathcal{O} = \frac{1}{1 + e^{[(V-V_h)s]}} \quad (4)$$

The expression is the modified Boltzmann's expression used in modeling ion-channel gating (Beyl et al., 2007; Huang et al., 2009; Xu and Lipscombe, 2001). This sigmoidal function of voltage is symmetric about the half-activation voltage, V_h . However, experimental data frequently show one or both of: (i) activation and inactivation behaviour (ii) asymmetric behaviour, and hence it is hard to fit

data with a single Boltzmann function. This implies that use of only two states to define the system with linear energy barriers, does not ably model experimental observations.

Three possible ways to resolve this are (i) model the system as having more than two macro-states (stable conformational states), (ii) model the system as having transition rates that change with the dwell times, leading to a fractal model; or, (iii) use non-linear terms in the energy expression. The second option is not considered as the system is being described as a time-homogenous Markov process. Again, the final option is also not taken into account as we have already justified the physical assumption to neglect higher order terms so as to develop a simple model to describe the dynamics. Also, as we shall see in section (6) the use of higher order non-linear energy models does not appear to give simpler models, nor does it give more accurate fits to experimental data.

Models developed on the basis of Markovian dynamics often have more than three states for a good agreement with experimental observations (Kienker, 1989; Milescu et al., 2005). In such models the rate constant is often defined with an exponential or a Boltzmann function in voltage, both obtained from a linear approximation of equation (3).

The above two-state model may be extended into a network of macrostates for further analysis. Voltage-gated ion channels are characterised by a central pore formed from the union of four similar subunits or domains (Aidley and Stanfield, 1996). Each of these domains contains six trans-membrane alpha helical structures (S1 to S6), the segment S4 often acting as the voltage sensor. Such a three

dimensional entity often provide options for parallel transitions among the various sub-states. In any case, transitions to the open-state are achievable.

3. A Multiple Conformation Extension of the ‘Modified Boltzmann Function’

3.1. The Master Equation and Transition Rates

Here we consider the transition network of the ion-channel as a system with a set of \mathcal{N} stable states, marked $i = 0, 1, \dots, n$; with $n = \mathcal{N} - 1$ closed or inactive states and 0 being the open state. In sections that follow, we would consider models with only a single open state, as it is logical with respect to the molecular structure of ion channels and reflects fine details in experiments (Liebovitch, 1989). If S_i denotes the probability for the protein to be in state i at any time t , the system obeys the *master equation*,

$$\dot{S} = \mathcal{K}S \tag{5}$$

where, $S = (\mathcal{O}, S_1, S_2, \dots, S_n)$ and $\mathcal{K} \in \mathbb{R}^{\mathcal{N} \times \mathcal{N}}$ is a transition matrix with $k_{ij} \geq 0$ giving the rate of transit from state j to state i . The diagonal elements satisfy, $k_{ii} = -\sum_{i:i \neq j}^{\mathcal{N}} k_{ij}$, to conserve the probabilities. Again, $k_{ij} \neq 0$ if and only if there is a transition from state j to state i . To obtain a unique solution, we impose the normalization, $\mathbb{1}_{\mathcal{N}}^T S = 1$, $\mathbb{1}_{\mathcal{N}}$ being the unit column vector of size \mathcal{N} . The stationary probability distribution of the ion channel state S may be derived as

$$\begin{bmatrix} \mathbb{1}_{\mathcal{N}}^T \\ \mathcal{K}_{2:\mathcal{N},\bullet} \end{bmatrix} S = \begin{bmatrix} 1 \\ \mathbb{O}_n \end{bmatrix},$$

where $\mathcal{K}_{2:\mathcal{N},\bullet}$ denotes rows 2 to \mathcal{N} of the transition matrix \mathcal{K} and \mathbb{O}_n is a zero vector of size n . Then by Cramer's rule,

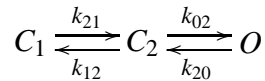
$$O = \frac{\begin{vmatrix} 1 & \mathbb{1}_n^T \\ \mathbb{O}_n & \mathcal{K}_{2:\mathcal{N},2:\mathcal{N}} \end{vmatrix}}{\begin{vmatrix} \mathbb{1}_{\mathcal{N}}^T \\ \mathcal{K}_{2:\mathcal{N},\bullet} \end{vmatrix}} = \frac{1}{1 + \frac{\sum_{j=2}^{\mathcal{N}} (-1)^{j+1} |\mathcal{K}_{2:\mathcal{N},\bullet-j}|}{|\mathcal{K}_{2:\mathcal{N},2:\mathcal{N}}|}} \quad (6)$$

where, $\mathcal{K}_{2:\mathcal{N},\bullet-j} \in \mathbb{R}^{n \times n}$ denotes a sub-matrix of \mathcal{K} obtained by deleting the first row and the j -th column. Note therefore that in general, equation (6) will be quite complex, with the denominator including a ratio of sums of products of elements of \mathcal{K} .

3.2. Examples with similar form of solution

3.2.1. Solution for a special case: A three state linear system with reversible transitions

A three state transition diagram for channel opening is given below



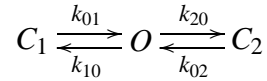
Here O represents an open state and C_1 and C_2 represent closed or inactive conformations. The steady-state probability for the channel to be in the open state may be deduced as

$$\mathcal{O} = \frac{1}{\left(1 + \frac{k_{12}}{k_{21}} + \frac{k_{12} k_{20}}{k_{21} k_{02}}\right)}$$

By using lemma 1, there exist $V_{h_1}, s_1, V_{h_2}, s_2$ such that

$$\mathcal{O} = \frac{1}{1 + e^{[(V-V_{h_1})s_1]} + e^{[(V-V_{h_2})s_2]}} \quad (7)$$

In the case of a slightly different topology,



the probability of the channel to be in an open conformation would be

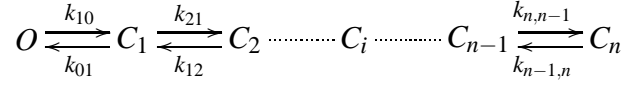
$$\mathcal{O} = \frac{1}{\left(1 + \frac{k_{10}}{k_{01}} + \frac{k_{20}}{k_{02}}\right)}$$

and once again, using lemma 1, the stationary probability of being open can be represented in the form (7).

3.2.2. Linear Networks

This scheme can be generalized for n macrostates depending on the position of the open state in the entire topology. A topology involving the open state on

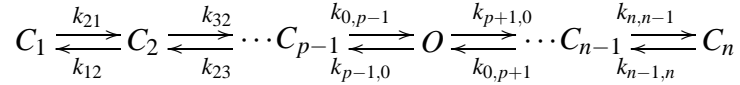
the network extremum,



would have an open state probability,

$$\mathcal{O} = \frac{1}{\left(1 + \sum_{j=1}^n \prod_{i=1}^j \frac{k_{i,i-1}}{k_{i-1,i}} \right)}$$

and a topology,



would yield,

$$\mathcal{O} = \frac{1}{\left(1 + \sum_{j=1}^{p-1} \prod_{i=1}^j \frac{k_{i,i+1}}{k_{i+1,i}} + \sum_{j=p}^n \prod_{i=1}^j \frac{k_{i+1,i}}{k_{i,i+1}} \right)}$$

In either case, with respect to the argument in lemma (1), the open state probability can be reduced and in general, a system with N macro-states related in order of their conformational transitions would have an open state probability,

$$\mathcal{O} = \frac{1}{1 + \sum_{i=1}^n e^{(V-V_{h,i})s_i}}$$

where, $n = \mathcal{N} - 1$, is the number of transitions in the linear network.

Remark: For the networks considered so far, rendering one of the transitions irreversible, would make the network absorbing in nature. In other words, the system would reach and never leave a fixed conformation. Since such a possibility is not physically reasonable for ion-channels under consideration, this case is not considered further.

3.2.3. Other networks

Case: For a transition network in which a unique simple path exists from every stable state to the open state, we have $|\mathcal{K}_{2:\mathcal{N}, 2:\mathcal{N}}| = \prod_{p=1}^n k_{i_p, j_p}$ for some indices $i_p, j_p \in \{1 \dots, n\}$ (See Theorem 1 in Appendix B). For such cases,

$$\mathcal{O} = \frac{1}{1 + \frac{\sum (-1)^{q+1} \prod_{p=1}^n \mathcal{K}_{i_p, j_{p,q}}}{\prod_{p=1}^n \mathcal{K}_{i_p, j_p}}} \quad (8)$$

for some indices $j_{p,q} \in \{0, 1 \dots, n\}$.

A stationary probability distribution in a form given by equation (8) is observed in network topologies that can be distinguished into hierarchical or tree, star and extended-star categories. In the case of ring and mesh networks the existence of the unique simple path to the open state from every stable state becomes

a necessary condition. The open state stationary probability is obtained as

$$\mathcal{O} = \frac{1}{1 + \sum_{i=1}^N e^{(V-V_{h,i})s_i}} \quad (9)$$

where $N \geq n$; for all cases considered. A few examples are illustrated in table (1).

4. Numerical analysis and low order approximation

We have observed so far that open-state probabilities of ion-channels may be expressed in a similar form to the modified Boltzmann equation, but with sum of exponentials replacing the single exponential term as in equation (9). The value of N is generally not less than the number of transition macro-states. The ambiguity in the value of N (which is at large dependent on the network structure) together with computational and identifiability issues for large N motivate us to consider models with small N . Of course, when considering such approximations, we potentially open up inaccuracies in the model (Liebovitch, 1989), and it is therefore important to check that any such reduction still accurately captures the observable behaviour of the system.

A good way of approximating the model would be to search for the minimum number of exponential terms that yields a good fit for experimentally observed ion channel current-voltage characteristic data. Let us denote the M -vector of ionic currents obtained from patch clamp experiments on a certain channel by $\mathbf{I}^m \in \mathbb{R}^M$. Let $\mathbf{V}^m \in \mathbb{R}^M$ be the corresponding voltage vector. In order to fit these data to equation (9), the distance between the experimental data and the model needs to

	<p>Star topology,</p> $O = \frac{1}{1 + \sum_{i=1}^3 e^{(V-V_{h,i})s_i}}$		<p>Extended star topology,</p> $O = \frac{1}{1 + \sum_{i=1}^9 e^{(V-V_{h,i})s_i}}$		<p>Tree topology,</p> $O = \frac{1}{1 + \sum_{i=1}^6 e^{(V-V_{h,i})s_i}}$
	<p>Ring topologies with a unique simple path to the open state</p>		<p>Mesh topologies with a unique simple path to the open state</p>		

Table 1: Examples of ion-channel topologies that satisfy the criteria of a unique simple path to the open state

be minimized. The distance may be measured as square of the L_2 norm:

$$J(\mathbf{I}^m, \mathbf{I}(\mathbf{V}^m)) = \|\mathbf{I}^m - \mathbf{I}(\mathbf{V}^m)\|_2^2 = \sum_{j=1}^m (I_j^m - I(V_j^m))^2$$

where $\mathbf{I}(\mathbf{V}^m) \in \mathbb{R}^M$ is the vector of ionic current predicted by the model given membrane potentials in the vector \mathbf{V}^m . I_j^m and V_j^m are respectively the j^{th} position in vectors \mathbf{I}^m and \mathbf{V}^m . Let g_{max} be the maximal conductance of the channel and V^* , the equilibrium potential of the ion transported by the channel. Mathematically, this optimization problem can be formulated as following:

$$\min_{\{V_{h,i}\}_{i=1}^N, \{s_i\}_{i=1}^N, g_{max}} J(\mathbf{I}^m, \mathbf{I}(\mathbf{V}^m)) \quad (10)$$

subject to the conductance expression:

$$I(V_j) = \frac{g_{max}(V_j - V^*)}{1 + \sum_{i=1}^N e^{(V - V_{h,i})s_i}} \quad \forall j = 1, \dots, m \quad (11)$$

and with bounds that are physiologically justified.

This optimization problem brings forth two significant difficulties: (i) the cost function is not convex and (ii) the number of dominant conformations that the protein adopts is usually unknown and hence the order of the exponentials, N is unknown. To guarantee that the global optimum is achieved, an initial guess should be carefully selected. It is possible to have a calculated guess for the V_h values from the current-voltage characteristics, positioned centrally over the range

of values at which the curve shows steady activation or inactivation. The steepness of the tangent drawn at this estimated voltage may be used as an initial estimate of the slope value. Alternatively, the recently developed global optimization Scatter Search based methodology, SSm GO (Egea et al., 2009), can be used. This algorithm combines a population-based metaheuristic method with a local optimization.

Regarding the number of exponentials, the aim is to curtail the number of conformational transitions so that it leads to minimum parameters to fit the data. For this purpose an algorithm was implemented which calculates the global optimum for several order of exponentials until the confidence in the fit is accomplished. The algorithm starts by $N = 1$ and progressively increases until the best fit between the model and the data has a relative error less than ε times the original data. The measure considered is again the square of the L_2 norm and mathematically, this criterion may be formulated as:

$$J(\mathbf{I}^m, \mathbf{I}(\mathbf{V}^m)) < \|\varepsilon \mathbf{I}^m\|_2^2$$

For all the cases we have considered, a good fit is obtained by setting $\varepsilon = 0.10$.

Interestingly, for all these cases we have examined, a maximum of two exponentials, i.e. $N = 2$ was required. The data could be accurately fitted by using a two dimensional state space ($N = 1$) for simple symmetric data-plots, and for all other cases, a three dimensional state space ($N = 2$) could accurately explain the data. This would imply that to a large extent voltage gated ion-channels can

be adequately modeled as dwelling predominantly among three different macrostates even if they are able to change between several conformations. The time that the protein dwells in some of these states may be considerably smaller than that of the three dominant conformations. The three macrostates may incorporate some of the effects of the minor conformations rather than completely negating their influences; as reflected from the data-fits.

With the above argument we propose the following equation for the open state probability corresponding to a three state system,

$$\mathcal{O} = \frac{1}{1 + e^{[(V-V_{h_1})s_1]} + e^{[(V-V_{h_2})s_2]}} \quad (12)$$

as a good approximation to represent the stationary probability of voltage gated ion-channels to remain open. The channel current may hence be calculated with the following equation:

$$I_i = \frac{g_{max,i}(V - V_i^*)}{1 + e^{\{V-V_{h,1}(i)\}s_1(i)} + e^{\{V-V_{h,2}(i)\}s_2(i)}} \quad (13)$$

where, I_i is the channel current; V_i^* , the reversal potential of ion i ; V , the membrane potential; $g_{max,i}$, the maximal conductance of the channel for the ion i and $V_{h,1}(i), s_1(i), V_{h,2}(i), s_2(i)$ being the parameters for the activation function as defined by equation (12).

5. Fitting of experimental data to the model

Several papers in the literature involve experimental observations on the relationship between membrane potential and ion-channel current. Several were selected for utilizable data on single channel studies. Data were extracted from the published curves using the Engauge Digitizer 4.1. The digitized data sets were fit by the equation (13). The global scatter search algorithm, SSm GO (Egea et al., 2009; Banga, 2009) implemented in MATLAB was used for making the fits. The resulting data fits are presented in figures 1,2,3 and summarized in table 2. In all cases, with $N \leq 2$, we are able to represent the observed data well using the model structure proposed.

Table 2: Fitted parameters of Channel current - membrane potential data of a few ion-channels using equation 12

Figure	Reference	Channel type	V_{h_1} (mV)	s_1 (mV)	V_{h_2} (mV)	s_2 (mV)	g_{max}
1(a)	Talavera and Nilius (2006)	$Ca_v3.1$	-47.2228	-0.22613	0.617753	0.07519	0.0069 (mV^{-1})
1(b)	Beyl et al. (2007)	$Ca_v1.2$	-5.36225	-0.12598	31.7746	0.13336	0.0098 (mV^{-1})
1(c)	Xu and Lipscombe (2001)	$Ca_v1.2$	-2.89138	-0.13021	38.9031	0.13943	0.002 ($\mu A/mV$)
1(d)	Xu and Lipscombe (2001)	$Ca_v1.3$	-22.4361	-0.11182	26.4652	0.08824	0.0022 ($\mu A/mV$)
2(a)	Stühmer et al. (1987)	$Na_v1.2$	-34.1391	-0.14523	28.3072	0.10388	8.8843 (pA/mV)
2(b)	Ruben et al. (1997)	$Na_v1.2a$	-25.6539	-0.14232	38.2236	0.088449	0.0138 (mV^{-1})
3(a)	Zou et al. (2003)	$K_v10.2$	-64.5494	-0.17538	-29.4952	-0.02195	0.1009 ($\mu A/mV$)
3(b)	Zou et al. (2003)	$K_v11.3$	-41.6897	-0.11727	-6.79872	0.053344	0.0630 ($\mu A/mV$)
3(c)	Zou et al. (2003)	$K_v2.1$	40	-0.021769	3.51185	-0.13161	0.0476 ($\mu A/mV$)

6. Comparison with non-linear thermodynamic models

The estimation of the open-state probability of voltage-gated ion channels to an equation of form as (9) would rather be equivalent to a steady-state approximation of the Hodgkin-Huxley formalism. For example, the dimensionless variable

n from equation (6) of Hodgkin and Huxley (1952) may be approximated to equation (9) with $N = 15$. However, as we seek to look beyond the Hodgkin-Huxley formalism for a good mechanistic description, the given model need to be compared with efforts made to represent the system on a realistic perspective. Thermodynamic formalism comes closest to this effort. Although the fundamentals are in place, such models (Destexhe and Huguenard, 2000) show very poor performance in even small extrapolations from the given data (Figure 4). We would also argue that the biophysical basis of the multiple conformation model is much clearer than that of a Taylor's series dual conformation model.

Ozer (2004, 2007) modified the non-linear model to a functional form by lumping the different transitions in the protein to a single event. The model which uses a sum of Gaussian distribution, seems to give the best fits for experimental data. The steady-state open probability of ion-channels written with respect to this model, may be arrived using equation (8) of Ozer (2007) as,

$$\mathcal{O} = \frac{1}{1 + \frac{\sum_{i=1}^n \alpha_{0,i} e^{[(V-V_{\alpha,i})s_{\alpha,i}]^2}}{\sum_{i=1}^n \beta_{0,i} e^{[(V-V_{\beta,i})s_{\beta,i}]^2}}} \quad (14)$$

where, n was defined as the number of distinct transitions defined by different energy barriers. It should be noted that Ozer (2007) was also able to get acceptable fits with two transitions. The problem with this model however seems to be in the existence of practically unidentifiable parameters, as is the case with majority of

the existing Markov Models (Fink and Noble, 2009). Further, the model asks for fitting a minimum of twelve parameters (provided two macro-transitions ($n = 2$) gives acceptable fits).

A mathematical model for a biological phenomenon is assured to have uniquely estimated parameters if the model structure ensures identifiability (Walter and Pronzato, 1997). Conventionally, ion-channel data has limited interpretations by producing indistinguishable models (Kienker, 1989). In silico representation of ion-channel dynamics is yet to come up with an epic model for the reason that the model structure needs to be tweaked each time to incorporate experimental observation. This indeed is a question of parameter identifiability. Ion-channel experiments being quantitative in nature rather ask for locally identifiable parameters in its model structure. The model that we have presented in this article, is found to have *structurally output locally identifiable (s.o.l.i)* parameters, according to the following lemma.

Lemma 2. *The model for ion channel open state probability described by means of a Markov chain with three macro-states [equation (12)] is s.o.l.i by the Taylor approximation of fourth order.*

Proof: see Appendix C

7. Conclusions

In this article, we have defined the physical transitions of channel proteins (which helps them to function as a gate), by means of free energy changes associ-

ated with changes in its environmental electric field, from a thermodynamic perspective. The paper brings forth a simple kinetic expression (equation 12) which approximates the open probability of the channel for a given membrane potential. The approximation which assumes the existence of a unique simple path of transition, from every stable state to the open state, is logical with respect to conformational dynamics. The model thus postulates the behaviour of the system to be defined by a scheme of three (hypothetical) states (one in the open conformation and two closed).

Our model has the limitation that it has been developed for steady state and may not represent ion-channel dynamics in its entirety. Nonetheless, the model is identifiable and requires smaller number of parameters compared to existing models. This would in turn reduce the computational cost associated with regular Markov Models, without compromising much from the mechanistic viewpoint.

Appendix A. Proof of Lemma 1

Proof. If ‘ k_1 ’ and ‘ k_2 ’ are the rates of two separate transitions appearing in a transition network, their corresponding ratio could be written as,

$$\frac{k_1(V)}{k_2(V)} = \frac{k_{1,0}}{k_{2,0}} e^{-((a_2-a_1)+(b_2-b_1)V+\dots)/RT} \quad (\text{A.1})$$

This expression takes the form of equation (3). In conjunction with the reasoning provided in section (2.2), we may approximate equations (3) and (A.1) by

neglecting higher order terms as:

$$k(V), \frac{k_1(V)}{k_2(V)} = e^{[(V-V_h)s]} \quad (\text{A.2})$$

where s would represent the slope at a voltage of V_h . Product of rate ratios may also be approximated as follows:

$$\begin{aligned} \prod \left[\frac{k_i(V)}{k_j(V)} \right] &= \left[e^{\{(V-\hat{V}_{h,a})\hat{s}_a\}} e^{\{(V-\hat{V}_{h,b})\hat{s}_b\}} \dots \right] \\ &= e^{[(V-\hat{V}_{h,a})\hat{s}_a + (V-\hat{V}_{h,b})\hat{s}_b + \dots]} \\ &= e^{[(V-V_h)s]} \end{aligned}$$

□

Appendix B. Case when a unique simple path exists from every stable state to the open state

In Section (3.1), it was claimed the open channel probability takes the special form (8) for any network in which a unique simple path exists from every stable state to the open state. Note that the claim follows immediately provided we can prove that $|\mathcal{K}_{2:\mathcal{N},2:\mathcal{N}}| = \prod_{p=1}^n k_{i_p j_p}$ for some indices $i_p, j_p \in \{1, \dots, n\}$. We shall prove this fact here.

In the next theorem, we adopt the following notational convention. For a matrix $Q \in \mathbb{R}^{n \times n}$ with $q_{ij} \geq 0$ for $i \neq j$, we define a node 0, distinct from the entries of Q such that $q_{0j} = -\sum_{i=1}^n q_{ij}$ for $1 \leq j \leq n$. The graph $D_0(Q)$ consists of the

vertices $\{0, 1, \dots, n\}$ and has a directed edge from vertex j to vertex i if $q_{ij} \neq 0$. It is important to note that $\mathcal{K}_{2:n,2:n}$ satisfies the hypotheses of the theorem.

Theorem 1. *Let $Q \in \mathbb{R}^{n \times n}$ satisfy the following properties:*

- (i) $q_{ij} \geq 0$ for all $i \neq j$;
- (ii) *there exists a unique simple path in $D_0(Q)$ from j to 0 for every vertex j , $1 \leq j \leq n$.*

Then the determinant of Q takes the form

$$|Q| = \prod_{p=1}^n k_{i_p j_p} \text{ for some indices } i_p, j_p \in \{0, \dots, n\}.$$

Proof. We proceed by induction on the size n of the matrix Q . The first non-trivial case is $n = 2$. For $n = 2$, it follows that

$$Q = \begin{pmatrix} -(q_{01} + q_{21}) & q_{12} \\ q_{21} & -(q_{02} + q_{12}) \end{pmatrix}.$$

A simple calculation shows that

$$|Q| = q_{01}q_{02} + q_{01}q_{12} + q_{02}q_{21}.$$

Each of the three terms in the above expression describes a different pair of paths from the vertices 1, 2 back to the vertex 0. As there exists a unique path from each vertex back to 0, exactly one of these terms is non-zero. This proves the result for

the case $n = 2$. We next assume that the result holds for matrices Q of size p with $2 \leq p \leq n$. We shall show that, under this induction hypothesis, the result also holds for matrices of size $n + 1$

Let $Q \in \mathbb{R}^{(n+1) \times (n+1)}$ satisfy (i) and (ii) and let π_j denote the unique simple path in $D_0(Q)$ from j to 0 for $1 \leq j \leq n + 1$. It follows from (ii) that there must exist at least one index $i \in \{1, \dots, n + 1\}$ such that $q_{0i} \neq 0$. Consider the set $I \subseteq \{1, \dots, n + 1\}$ given by

$$I := \{j : 1 \leq j \leq n + 1, \text{ the edge } (i, 0) \in \pi_j\}.$$

The set I consists of those vertices j for which the final edge on the simple path from j to 0 in $D_0(Q)$ is the edge $(i, 0)$. As $i \in I$, it follows that I is non-empty. There are two distinct cases to consider.

First, suppose that $I \neq \{1, \dots, n + 1\}$, so that we can write

$$\{1, \dots, n + 1\} = I \cup I'$$

where $I' = \{1, \dots, n + 1\} \setminus I$, is also non-empty. If $i \in I$ and $j \in I'$, then it follows from (ii) that both $q_{ij} = 0$ and $q_{ji} = 0$. Otherwise there would exist two simple paths from i to 0 in the former case, or from j to 0 in the latter case. Without loss of generality (by permuting the vertices if necessary), we can assume that $I = \{1, 2, \dots, \phi\}$ for some integer ϕ with $1 \leq \phi < \mathcal{N}$, $I' = \{\phi + 1, \dots, n + 1\}$. Then,

our discussion shows that

$$Q = \begin{pmatrix} Q_1 & 0 \\ 0 & Q_2 \end{pmatrix}$$

where $Q_1 \in \mathbb{R}^{\phi \times \phi}$, $Q_2 \in \mathbb{R}^{(n+1-\phi) \times (n+1-\phi)}$. From the block diagonal form of Q , it follows immediately that both Q_1 and Q_2 satisfy conditions (i) and (ii) of the theorem. From the induction hypothesis the determinants of both Q_1 and Q_2 are simple products of the elements q_{ij} , $0 \leq i, j \leq n+1$. However, $|Q| = |Q_1||Q_2|$ and hence the determinant of Q also has the desired form.

The second case to consider is where $I = \{1, \dots, n+1\}$. Again, without loss of generality we can assume that the index $i = 1$. In this case, for every vertex $j \in \{1, 2, \dots, n+1\}$, the final edge on the path from vertex j to 0 in $D_0(Q)$ is the edge $(1, 0)$. As we are assuming that there is a unique simple path from each j to 0, it follows that $q_{0j} = 0$ for $2 \leq j \leq n+1$. Thus Q can be written in the form:

$$Q = A + B$$

where all column sums of A are zero and every entry of B is zero apart from $b_{11} = -q_{01}$. As the determinant is a multi-linear function of the columns of a matrix the form of Q implies that

$$\begin{aligned} |Q| &= |A - q_{01}\hat{Q}| \\ &= -q_{01}|\hat{Q}|. \end{aligned} \tag{B.1}$$

where $\hat{Q} \in \mathbb{R}^{n \times n}$ is the submatrix of Q formed by removing the first row and column. Write \hat{q}_{ij} for the (i, j) entry of \hat{Q} . We note the following facts,

- (a) $\hat{q}_{ij} \geq 0$ for $i \neq j$.
- (b) The column sums in Q are given by $q_{0j} = 0$ for $2 \leq j \leq n+1$. It follows immediately that for \hat{Q} , $\hat{q}_{0j} = -\sum_{i=1}^n \hat{q}_{ij} = q_{1,j+1}$ for $1 \leq j \leq n$.
- (c) For each vertex $j \in \{2, \dots, n+1\}$ in $D_0(Q)$, the unique simple path in $D_0(Q)$ from j to 0 has $(1, 0)$ as its final edge. It follows that there is a unique simple path in $D_0(Q)$ from j to 1 for $j \in \{2, \dots, n+1\}$.

It follows from the above points (and from point (c) in particular) that in the graph $D_0(\hat{Q})$ associated with \hat{Q} , there is a unique simple path from every vertex $j \in \{1, \dots, n\}$ to the vertex 0. (In fact, in $D_0(\hat{Q})$, the vertex 0 corresponds to the vertex 1 in the original graph $D_0(Q)$.)

The above discussion shows that \hat{Q} satisfies the conditions (i) and (ii) in the statement of the theorem and hence by the induction hypothesis, it follows that the determinant $|\hat{Q}|$ is a simple product of terms q_{ij} , $1 \leq i, j \leq n+1$. It now follows immediately from (B.1) that the determinant $|Q|$ also has the desired form. This completes the proof. □

Appendix C. Model Identifiability, Proof of Lemma 2

In order to study the parameter identifiability of model [given by equation (12)], let us consider the following general mathematical structure:

$$M(\theta) : y(\theta) = f(\theta, x)$$

where $\theta \in \mathbb{R}^{n_\theta}$ is the set of parameters to be estimated and $y(\theta) \in \mathbb{R}$ and $x \in \mathbb{R}$ denote the output and input of the system, respectively. In addition, let us denote the search space of the parameter by $\Theta \subseteq \mathbb{R}^{n_\theta}$.

Definition 1. *The model $M(\theta)$ is said to be **structurally output globally identifiable** (s.o.g.i), if for any $\tilde{\theta} \in \Theta$, except for the points of a subset of measure zero, and for all $x \in \mathbb{R}$:*

$$y(\theta, x) \equiv y(\tilde{\theta}, x) \implies \theta \equiv \tilde{\theta}$$

*If the same conditions is fulfilled only in the neighborhood of θ then the parameters will be **structurally output locally identifiable** (s.o.l.i.)*

This definition is quite general and is often difficult to be put into practice. One of the usual approach in such instance is the so-called "Taylor approach" (Walter and Pronzato, 1997) where both outputs $y(\theta, x)$ and $y(\hat{\theta}, x)$ are approximated by a Taylor expansion around a point $x^* \in \mathbb{R}$. If we denote by $\mathcal{U}_{x^*}^n$ the ball around x^* , where the Taylor approximation of order n holds; the general identifiability definition is therefore set as:

Definition 2. *The model $M(\theta)$ is said to be s.o.g.i in $x \in \mathcal{U}_{x^*}^n$ if the system of*

equations:

$$\left[\frac{\partial^i y(\theta, x)}{\partial x^i} \right]_{x=x_0} = \left[\frac{\partial^i y(\tilde{\theta}, x)}{\partial x^i} \right]_{x=x_0} \quad \forall i = 1, \dots, n \quad (\text{C.1})$$

has an unique solution. In the same way, the model will be s.o.l.i in $x \in \mathcal{U}_{x^*}^n$ if a finite number of solution is obtained.

Proof. It has to be proved that, the ion channel open state probability represented by the equation,

$$G(\theta) = \frac{1}{1 + e^{(V-V_{h_1})s_1} + e^{(V-V_{h_2})s_2}} \quad (\text{C.2})$$

is s.o.l.i for any $V \in \mathcal{U}_{V^*}^n$, by using the Taylor approximation of fourth order. It may be noted that studying the identifiability property for $G(\theta)$ with parameters

$$\theta = [V_{h,1}, s_1, V_{h,2}, s_2] \in \mathbb{R}^4 \quad \tilde{\theta} = [\tilde{V}_{h,1}, \tilde{s}_1, \tilde{V}_{h,2}, \tilde{s}_1] \in \mathbb{R}^4$$

is equivalent to studying this property for the model

$$M(\theta) = e^{(aV+b)} + e^{(cV+d)}, \quad \theta = [a, b, c, d]$$

where

$$a = s_1, \quad b = -V_{h_1}s_1, \quad c = s_2, \quad d = -V_{h_2}s_2$$

$$\tilde{a} = \tilde{s}_1, \quad \tilde{b} = -\tilde{V}_{h_1}\tilde{s}_1, \quad \tilde{c} = \tilde{s}_2, \quad \tilde{d} = -\tilde{V}_{h_2}\tilde{s}_2$$

Therefore, the system of equations built by using (C.1) leads to:

$$e^{aV_0+b} + e^{cV_0+d} = e^{\tilde{a}V_0+\tilde{b}} + e^{\tilde{c}V_0+\tilde{d}} \quad (\text{C.3a})$$

$$ae^{aV_0+b} + ce^{cV_0+d} = \tilde{a}e^{\tilde{a}V_0+\tilde{b}} + \tilde{c}e^{\tilde{c}V_0+\tilde{d}} \quad (\text{C.3b})$$

$$a^2e^{aV_0+b} + c^2e^{cV_0+d} = \tilde{a}^2e^{\tilde{a}V_0+\tilde{b}} + \tilde{c}^2e^{\tilde{c}V_0+\tilde{d}} \quad (\text{C.3c})$$

$$a^3e^{aV_0+b} + c^3e^{cV_0+d} = \tilde{a}^3e^{\tilde{a}V_0+\tilde{b}} + \tilde{c}^3e^{\tilde{c}V_0+\tilde{d}} \quad (\text{C.3d})$$

with the following two solutions obtained with Mathematica software (Wolfram Research, 2008):

$$a = \tilde{c}, \quad b = \tilde{d}, \quad c = \tilde{a}, \quad d = \tilde{b}$$

$$a = \tilde{a}, \quad b = \tilde{b}, \quad c = \tilde{c}, \quad d = \tilde{d}$$

Therefore, the system is s.o.l.i in the neighbourhood of V^* where the Taylor approximation holds. \square

Corollary 1. *If the model (C.2) is completed with the following condition:*

$$s_1 > s_2$$

it is trivial to see that the system (C.3) now has only one solution and the model is s.o.g.i. for any $V \in \mathcal{U}_{V^}^n$.*

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References

References

Aidley, D. J., Stanfield, P. R., 1996. Ion channels: molecules in action. Cambridge University Press.

Banga, J. R., October 2009. Ssm go toolbox. <http://www.iim.csic.es/~gingproc/ssmGO.html>.

Beyl, S., Timin, E. N., Hohaus, A., Stary, A., Kudrnac, M., Guy, R. H., Hering, S., 2007. Probing the Architecture of an L-type Calcium Channel with a Charged Phenylalkylamine. *J. Biol. Chem.* 282 (6), 3864–3870.

Destexhe, A., Huguenard, J., 2000. Nonlinear thermodynamic models of voltage-dependent currents. *Journal of Computational Neuroscience* 9, 259–270(12).

Egea, J., Balsa-Canto, E., García, M., Banga, J., 2009. Dynamic optimization of nonlinear processes with an enhanced scatter search method. *Ind. Eng. Chem. Res* 48 (9), 4388–4401.

Fink, M., Noble, D., 2009. Markov models for ion channels: versatility versus identifiability and speed. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* 367 (1896), 2161–2179.

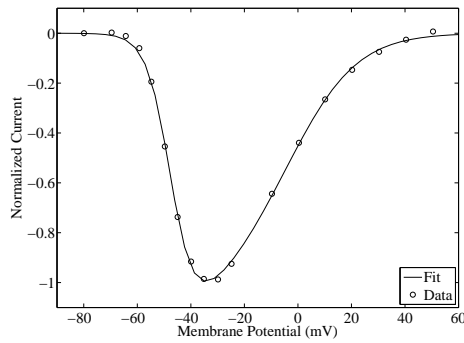
Fox, R., 1997. Stochastic versions of the hodgkin-huxley equations. *Biophysical Journal* 72 (5), 2068 – 2074.

Hille, B., 2001. *Ion Channels of Excitable Membranes*. Sinauer Associates, Inc.

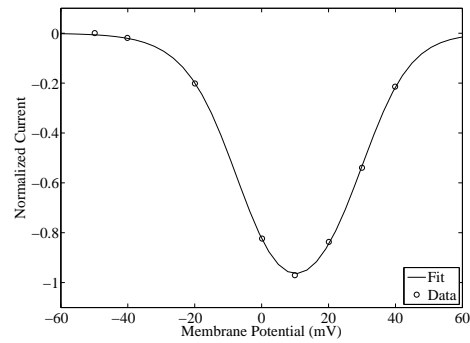
- Hodgkin, A. L., Huxley, A. F., August 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J Physiol* 117 (4), 500–544.
- Huang, S., Cai, Q., Liu, W., Wang, X., Wang, T., 2009. Whole-cell recordings of voltage-gated calcium, potassium and sodium currents in acutely isolated hippocampal pyramidal neurons. *Journal of Nanjing Medical University* 23 (2), 122 – 126.
- Keener, J., 2009. Invariant manifold reductions for markovian ion channel dynamics. *Journal of Mathematical Biology* 58, 447–457.
- Kienker, P., 1989. Equivalence of Aggregated Markov Models of Ion-Channel Gating. *Proceedings of the Royal Society of London. B. Biological Sciences* 236 (1284), 269–309.
- Liebovitch, L., 1989. Testing fractal and markov models of ion channel kinetics. *Biophysical Journal* 55 (2), 373 – 377.
- Liebovitch, L. S., Fischbarg, J., Koniarek, J. P., Todorova, I., Wang., M., Jan 1987. Fractal model of ion-channel kinetics. *Biochim Biophys Acta.* 896 (2), 173–180.
- Manna, S., Banerjee, J., Ghosh, S., 2007. Breathing of voltage dependent anion channel as revealed by the fractal property of its gating. *Physica A: Statistical Mechanics and its Applications* 386 (1), 573 – 580.

- Milescu, L., Akk, G., Sachs, F., APR 2005. Maximum likelihood estimation of ion channel kinetics from macroscopic currents. *Biophysical Journal* 88 (4), 2494–2515.
- Millhauser, G. L., Salpeter, E. E., Oswald, R. E., 1988. Diffusion models of ion-channel gating and the origin of power-law distributions from single-channel recording. *Proceedings of the National Academy of Sciences of the United States of America* 85 (5), 1503–1507.
- Nekouzadeh, A., Rudy, Y., 2007. Statistical properties of ion channel records. part ii: Estimation from the macroscopic current. *Mathematical Biosciences* 210 (1), 315 – 334.
- Ozer, M., 2004. An improved non-linear thermodynamic model of voltage-dependent ionic currents. *Neuroreport* 15 (12), 1953–7.
- Ozer, M., 2007. A comparative analysis of linear, nonlinear and improved nonlinear thermodynamic models of voltage-dependent ion channel kinetics. *Physica A: Statistical Mechanics and its Applications* 379 (2), 579 – 586.
- Ruben, P. C., Fleig, A., Featherstone, D., Starkus, J. G., Rayner, M. D., 1997. Effects of clamp rise-time on rat brain iia sodium channels in xenopus oocytes. *Journal of Neuroscience Methods* 73 (2), 113 – 122.
- Sansom, M., Ball, F., Kerry, C., McGee, R., Ramsey, R., Usherwood, P., 1989. Markov, fractal, diffusion, and related models of ion channel gating. a compar-

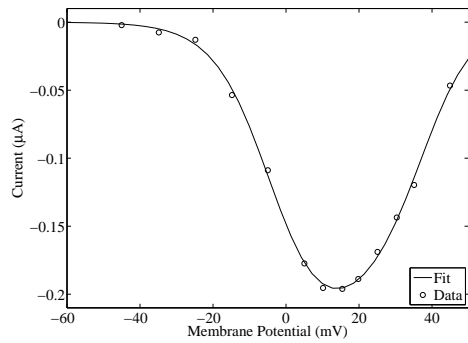
- ison with experimental data from two ion channels. *Biophysical Journal* 56 (6), 1229 – 1243.
- Stühmer, W., Methfessel, C., Sakmann, B., Noda, M., Numa, S., 1987. Patch clamp characterization of sodium channels expressed from rat brain cdna. *European Biophysics Journal* 14 (3), 131–138.
- Talavera, K., Nilius, B., 2006. Biophysics and structure-function relationship of t-type Ca^{2+} channels. *Cell Calcium* 40 (2), 97 – 114, t-type calcium channels: from old physiology to novel functions.
- Walter, E., Pronzato, L., 1997. *Identification of Parametric Models from Experimental Data*. Springer.
- Wolfram Research, I., 2008. *Mathematica*, version 7.0 Edition. Wolfram Research, Inc.
- Xu, W., Lipscombe, D., 2001. Neuronal $CaV1.3\alpha1$ L-Type Channels Activate at Relatively Hyperpolarized Membrane Potentials and Are Incompletely Inhibited by Dihydropyridines. *J. Neurosci.* 21 (16), 5944–5951.
- Zou, A., Lin, Z., Humble, M., Creech, C. D., Wagoner, P. K., Krafte, D., Jegla, T. J., Wickenden, A. D., 2003. Distribution and functional properties of human KCNH8 (Elk1) potassium channels. *Am J Physiol Cell Physiol* 285 (6), C1356–1366.



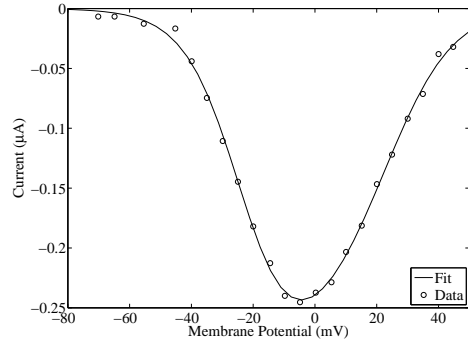
(a) Normalized current-voltage relationship of the T-type calcium channel from the digitized data of Talavera and Nilius (2006)



(b) Normalized current-voltage relationship of the wild-type calcium channel from the digitized data of Beyl et al. (2007)

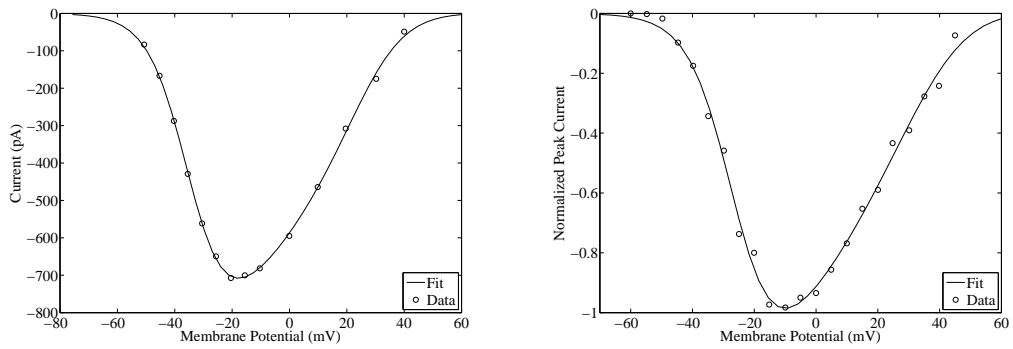


(c) Averaged, peak current-voltage plots of $Ca_v1.2\alpha$ L-type calcium channels from the digitized data of Xu and Lipscombe (2001)



(d) Averaged, peak current-voltage plots of $Ca_v1.3\alpha$ L-type calcium channels from the digitized data of Xu and Lipscombe (2001)

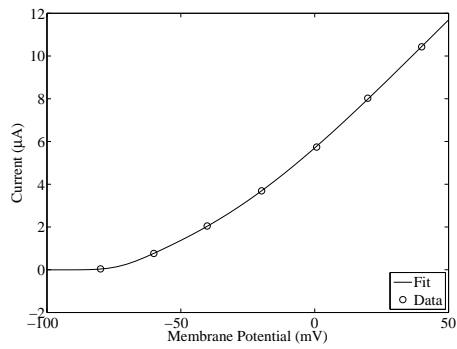
Figure 1: Current-Voltage relationship of Voltage-gated Ca^{2+} channels fitted with equation (12). Voltage-gated Ca^{2+} channels (VGCC) can be classified into high-voltage-activated (HVA) and low-voltage-activated (LVA) channels, which implies that LVA channels activate at 20 to 30 mV more negative potentials than HVA channels. T-type calcium channels are LVA and show fast macroscopic inactivation whereas L-Type calcium channels are HVAs.



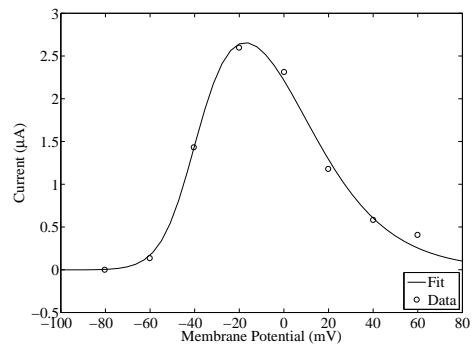
(a) Peak inward current vs. voltage relation for the current records of sodium channel from the digitized data of Stühmer et al. (1987) fitted with equation (12)

(b) Normalized current-voltage relationship of sodium channel from the macropatch current records of Ruben et al. (1997), fitted with equation (12)

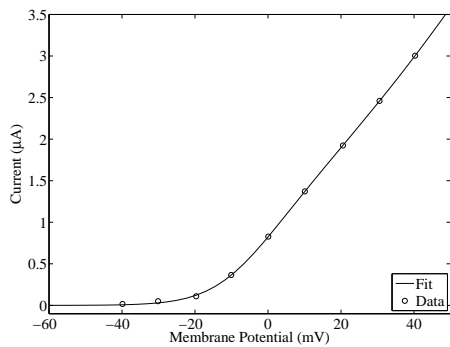
Figure 2: Current-Voltage relationship of Voltage-gated Na^+ channels fitted with equation 12.



(a)

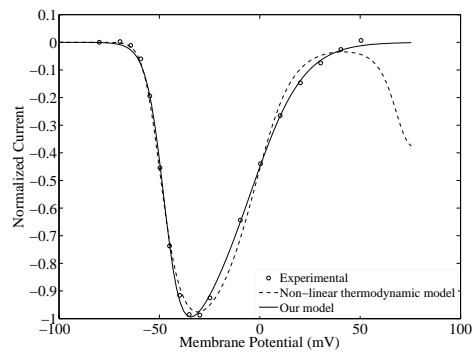


(b)

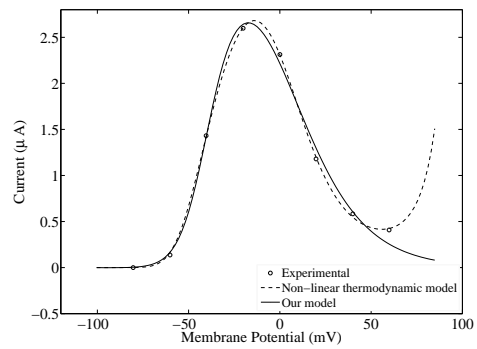


(c)

Figure 3: Current-voltage relations for KCNH5 (3(a)), KCNH7 (3(b)) and KCNB1 (3(c)) class of potassium channels expressed in oocytes, from the study by Zou et al. (2003) fitted with equation 12.



(a) Calcium channel



(b) Potassium channel

Figure 4: Current-voltage relationship of ion-channels obtained from the studies of (a) Talavera and Nilius (2006) and (b) Zou et al. (2003) fitted with a third order non-linear model and modified model presented in this article according to equation (12)