

Biological mechanism and identifiability of a class of stationary conductance model for Voltage-gated Ion channels

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The physiology of voltage gated ion channels is complex and insights into their gating mechanism is incomplete. Their function is best represented by Markov models with relatively large number of distinct states that are connected by thermodynamically feasible transitions. On the other hand, popular models such as the one of Hodgkin and Huxley have empirical assumptions that are generally unrealistic. Experimental protocols often dictate the number of states in proposed Markov models, thus creating disagreements between various observations on the same channel. Here we aim to propose a limit to the minimum number of states required to model ion channels by employing a paradigm to define stationary conductance in a class of ion-channels. A simple expression is generated using concepts in elementary thermodynamics applied to protein conformational transitions. Further, it matches well many published channel current-voltage characteristics and parameters of the model are found to be identifiable and easily determined from usual experimental protocols.

1. Introduction

The electrical activity of a living system is a dynamic function of the ionic transport across biological membranes. Ion channels are pore-forming protein ensembles that are responsible for the task of regulating 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 [6]. 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 steady distribution and 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 [15], 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.

Further developments in the study of ion-channels made an attempt to give a mechanistic description of 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 [20] and have the advantage of 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 [7, 20, 26, 32]. However, for good agreement with experimental data, frequently the number of closed states needed varies with the experimental protocol. A significant benefit of Markov models compared to the Hodgkin-Huxley formalism is that the large degree of freedom in the model structure allows it to fit more closely with experimental observations [12]. Thermodynamic models are a two state Markovian description of channel flipping, the rate kinetics of which are described by concepts in thermodynamics [10, 29].

Fractal models [22, 23] 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 et al. [27], justify Fractal models at a microscopic level. Statistical analysis, however has often favored Markov models over Fractal models [32, 28].

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 can give rise to 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. Thus the model has limitations from the point of view of parameter estimation and further in its use for multicellular simulations [12]. The increasing complexity of the topology

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generates a need for model reduction. Kienker [20] 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 [19] 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 [1, 14], 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 in noise-free perfect experiments [5, 36, 2]. Since it is well-known that a large segment of ion-channel models in the literature lack parameter identifiability in noise experiments [12], fundamental studies of structural identifiability are scarce due to the difficulty of solving the associated symbolic set of equations [8, 9].

It should be also noted that in usual experimental protocols optimised to improve the signal-to-noise ratio [4, 37], provide two sets of separated data: one used to characterise the steady-state, and the other with time constants, when dynamics are not sufficiently fast to be disregarded. With this in mind, Hodgkin-Huxley models employ empirical expressions for both steps that are not always realistic [37]. On the other hand, estimation in models based on Markov chains often make use of other types of experimental protocols, and do not exploit the large range of data available using the standard protocols.

In this article, we describe a voltage-gated ion-channel model of stationary conductance with three main characteristics. To begin with, the model has a clear mechanistic motivation based on the underlying thermodynamics. In addition, the model is relatively simple to implement, with a small number of easily identifiable parameters. Finally, we test the model using experimental data from patch clamp and similar studies reported in the literature. Our model can make use of classical experimental protocols and provide a mechanistic formulation for the calibration of steady-state characteristics in the Hodgkin-Huxley setting and, by identifying the form of the stationary distribution, constrain the estimation process in Markov processes, where the study of global structural identifiability is still an open problem. In addition, the simple model proposed here can be applied to study the gating of fast ion channels, such as fast persistent and fast activated Na^+ currents or transient activated K^+ current [18].

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 [24]. 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 [19] 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 (figure 1). 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 [26].

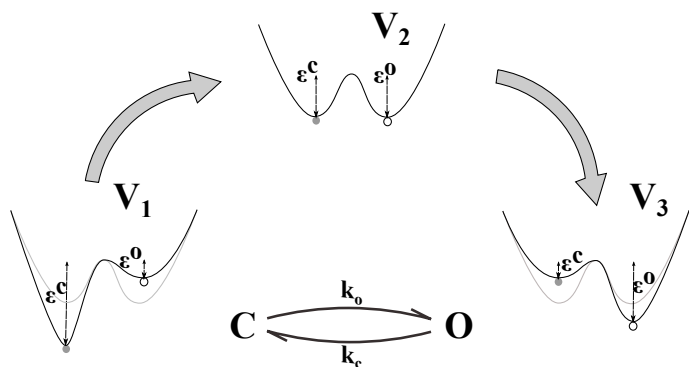


Figure 1: *Energy profile for the transitions between open and closed states in an ion channel, modulated by a change in membrane potential: a change in membrane potential brings in protein conformational changes and thereby free energies of protein conformation. The rates of channel closing and opening, k_c and k_o , are a function of the free energy barriers (ϵ^c and ϵ^o) defined by structural configurations of the respective states. The figure describes how the open probability of a channel is enhanced by a change in the ambient voltage from V_1 to V_3 by virtue of its conformational energy.*

In the case of voltage based transition, the activation energy can be expressed in a general form by using a Taylor series expansion [10, 29], 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 and bV corresponds to interactions between the 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 [10].

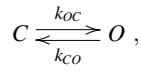
In what follows, we are mainly interested in models where the free energy barrier is linear in the membrane potential. The effect of temperature variations may be neglected for the system under consideration, as long as the physiological environment remains unaffected. In this

case, the following trivial lemma, helps us derive a simple expression for voltage regulated conformational transitions.

Lemma 1. Consider a set $\{\Delta G_1(V), \dots, \Delta G_M(V)\}$ of activation energies, where each $\Delta G_i(V)$ is affine in V . Denote the corresponding transition rates by $\{k_1, \dots, k_M\}$. Then any expression of the form $\prod_{p=1}^P \left(\frac{k_{ip}(V)}{k_{jp}(V)} \right)$ may be expressed as $e^{[(V-V_h)s]}$

2.3. Markov Models for Conformation transitions

The series of molecular changes associated with the opening of an ion channel is often described using Markov models with discrete states [20, 32, 19]. To begin, consider the simple case of a transition between an open and closed state; let O and C represent the probabilities that the molecule is in the corresponding open and closed state at a 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 the steady state probability for the channel to be in an open state leads

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

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 [6, 17, 39]. 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 the two-state Markov chain 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.

We focus on the first option here for the following reasons. The second option (ii) would give rise to time-inhomogeneous Markov chains, which are typically very difficult to analyse. Moreover, the identification task becomes less tractable in this case. As mentioned previously, the last option (iii) includes higher order terms that are usually neglected. Also, as we shall see later in section (6) the use of higher order non-linear energy models does not appear to result in simpler models, nor does it give more accurate fits to experimental data.

In fact, usually models developed on the basis of Markovian dynamics have more than three states [option (i)] for a good agreement with experimental observations [20, 26]. 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).

We will now analyse how best we can extend the two-state model by option (i) to have a network with limited and identifiable macro-states.

3. A Multiple Conformation Extension of the 'Modified Boltzmann Function' for stationary conductance

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 the following, we consider models with a single open state, as this reflects the molecular structure of many ion channels as detailed in experiments and is not a generalization [23, 33] If S_i denotes the probability for the protein to be in state i at any time t , the system obeys the master equation

$$\dot{S} = \mathcal{K}S, \quad (5)$$

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, $k_{ii} = -\sum_{i \neq j} k_{ij}$, to ensure the system evolves on the probability simplex. The entry $k_{ij} \neq 0$ if and only if there is a transition from state j to state i . The stationary probability distribution S satisfies $\mathcal{K}S = 0$, $\mathbb{1}_{\mathcal{N}}^T S = 1$, where $\mathbb{1}_{\mathcal{N}}$ is the column vector of size \mathcal{N} with all entries equal to one.

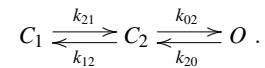
As is standard, we associated a directed graph with the Markov process described by (5) consisting of the nodes $\{0, 1, \dots, n\}$ with an edge from state j to state i ($i \neq j$) if and only if $k_{ij} \neq 0$.

In the following subsection, we derive explicit formulae for the form of the stationary vector in some simple cases; emphasising that in these cases the open state probability \mathcal{O} takes a particularly simple form. We also describe a general condition on the structure of the graph associated with the Markov process that is sufficient for this simple form to hold.

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



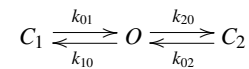
Here O represents an open state and C_1 and C_2 represent closed or inactive conformations. The steady-state probability for the channel to be in the open state can be calculated as

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

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

$$\mathcal{O} = \frac{1}{1 + e^{[(V-V_{h_1})s_1]} + e^{[(V-V_{h_2})s_2]}}. \quad (6)$$

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 (6).

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,

$$O \xrightleftharpoons[k_{01}]{k_{10}} C_1 \xrightleftharpoons[k_{12}]{k_{21}} C_2 \cdots C_i \cdots C_{n-1} \xrightleftharpoons[k_{n-1,n}]{k_{n,n-1}} C_n$$

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

$$C_1 \xrightleftharpoons[k_{12}]{k_{21}} C_2 \xrightleftharpoons[k_{23}]{k_{32}} \cdots C_{p-1} \xrightleftharpoons[k_{p-1,0}]{k_{0,p-1}} O \xrightleftharpoons[k_{0,p+1}]{k_{p+1,0}} \cdots C_{n-1} \xrightleftharpoons[k_{n-1,n}]{k_{n,n-1}} C_n$$

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 1. 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

More generally, consider a transition network in which a unique simple path exists from every stable state to the open state; so in the directed graph associated with the matrix \mathcal{K} , there is a unique path from every $j \neq 0$ to the node 0. We also assume that the matrix \mathcal{K} is irreducible [16].

The celebrated Markov Chain Tree Theorem [21] allows us to characterise the form of the steady state probability of the open state in this case. This result is usually stated for column stochastic matrices or discrete Markov chains; however, it is trivial to see that an exact analogue also holds for continuous chains with matrices of the form \mathcal{K} . We state a restricted version of this result below but first introduce some notation.

For the directed graph G associated with \mathcal{K} , a rooted spanning tree T_i at $i \in \{0, \dots, n\}$ consists of the vertices $\{0, 1, \dots, n\}$ and has the following properties: (i) T_i is acyclic; (ii) for every $j \neq i$, there exists exactly

one outgoing edge from j ; (iii) there exist no edge outgoing from i . We denote by $w(T_i)$ the weight of T_i , which is given by the product of the entries of \mathcal{K} corresponding to the edges in T_i . For $0 \leq i \leq n$, let \mathcal{T}_i denote the set of all directed spanning trees rooted at i , and define $w_i = \sum_{T_i} w(T_i)$. Note that each w_i will be a sum of terms of the form

$$k_{i_1 j_1} k_{i_2 j_2} \cdots k_{i_n j_n} \quad (7)$$

The following result is now a simple re-wording of the Markov Chain Tree Theorem as presented in [21] and elsewhere.

Theorem 1. Assume the matrix \mathcal{K} is irreducible. The unique stationary probability vector associated with \mathcal{K} , π is given by

$$\pi_i = \frac{w_i}{\sum_j w_j}$$

where w_i is defined as above for $0 \leq i \leq n$.

If there is a unique path from every node $j \neq 0$ back to the node 0, then it follows immediately that there is exactly one directed spanning tree T_0 rooted at 0 (which represents the open state). It then follows that the steady state probability of the channel being in the open state is of the form

$$\mathcal{O} = \frac{w(T_0)}{\sum_j w_j} \quad (8)$$

As there is only a single term of the form (7) in the numerator, it follows readily by combining (8) with Lemma 1 that \mathcal{O} can will take the form

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

A few examples of network topologies for which this form is guaranteed by this analysis 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 [23], 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 be minimized. The distance may be measured as square of the L_2 norm:

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

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

<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>Star topology, $O = \frac{1}{1 + \sum_{i=1}^3 e^{(V-V_{hi})S_i}}$</p> </div> <div style="text-align: center;"> <p>Extended star topology, $O = \frac{1}{1 + \sum_{i=1}^9 e^{(V-V_{hi})S_i}}$</p> </div> <div style="text-align: center;"> <p>Tree topology, $O = \frac{1}{1 + \sum_{i=1}^6 e^{(V-V_{hi})S_i}}$</p> </div> </div>	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>Star topology, $O = \frac{1}{1 + \sum_{i=1}^2 e^{(V-V_{hi})S_i}}$</p> </div> <div style="text-align: center;"> <p>Ring topology with a unique simple path to the open state $O = \frac{1}{1 + \sum_{i=1}^3 e^{(V-V_{hi})S_i}}$</p> </div> </div>	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>Ring topology with a unique simple path to the open state $O = \frac{1}{1 + \sum_{i=1}^3 e^{(V-V_{hi})S_i}}$</p> </div> <div style="text-align: center;"> <p>Mesh topology with a unique simple path to the open state $O = \frac{1}{1 + \sum_{i=1}^5 e^{(V-V_{hi})S_i}}$</p> </div> </div>
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Table 1: Examples of ion-channel topologies that satisfy the criteria of a unique simple path to the open state

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:

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

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 [11], 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:

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

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

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 stationary distributions of voltage gated ion-channels can be adequately modeled as dwelling predominantly among three different macro-states 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

Most papers in the literature follows the voltage step protocol where steady-state voltage-current characteristics are obtained independently of the ion channel dynamics. Several were selected for utilizable data on single channel studies. We collect data from both, stationary conductance obtained from peak currents (with less signal-to-noise ratio [3]) or from raw steady-state measurements. 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 [11] implemented in MATLAB was used for making the fits. The resulting data fits are presented in figures 2,3,4 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.

6. Comparison with non-linear thermodynamic models

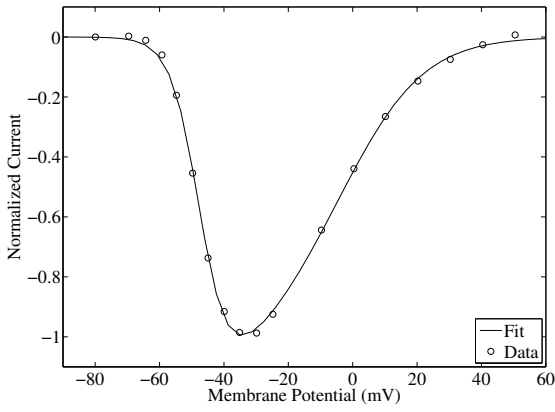
The estimation of the open-state probability of voltage-gated ion channels to an equation of form as (9) would rather be equivalent to a steady-state approximation of the Hodgkin-Huxley formalism. However, as we seek to look beyond the Hodgkin-Huxley formalism for a good mechanistic description, the 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 [10] show very poor performance in even small extrapolations from the given data (Figure 5). 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 [29, 30] 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 [30] as,

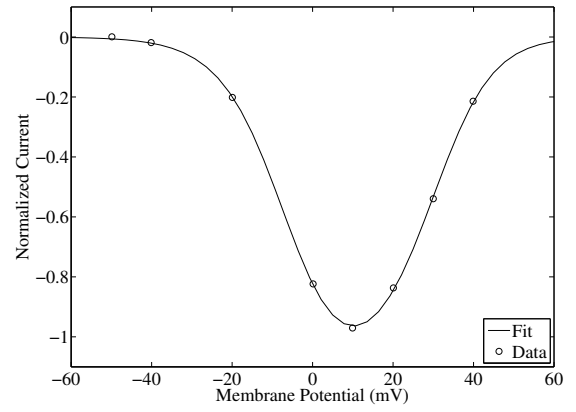
$$\mathcal{O} = \frac{1}{\sum_{i=1}^n \alpha_{0,i} e^{[(V - V_{\alpha,i})s_{\alpha,i}]^2} + 1 + \frac{\sum_{i=1}^n \beta_{0,i} e^{[(V - V_{\beta,i})s_{\beta,i}]^2}}{\sum_{i=1}^n \beta_{0,i} e^{[(V - V_{\beta,i})s_{\beta,i}]^2}}} \quad (14)$$

where, n was defined as the number of distinct transitions defined by different energy barriers. It should be noted that in work [30] was also able to get acceptable fits with two transitions. The problem with this model however seems to be in the existence of unidentifiable parameters, as is the case with majority of the existing Markov Models [12]. Further, if no manipulation is carried out to collect group of parameters that are no identifiable only with stationary data, the model asks for fitting a minimum of twelve parameters (provided two macro-transitions ($n = 2$) gives acceptable fits).

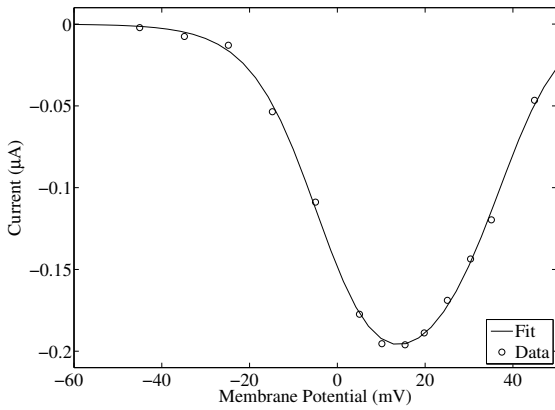
A mathematical model for a biological phenomenon is assured to have uniquely estimated parameters if the model structure ensures identifiability [36]. Conventionally, ion-channel data has limited interpretations by producing indistinguishable models [20]. In-silico representation of ion-channel dynamics is yet to come up with a 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. The model that we have presented in this article, is found



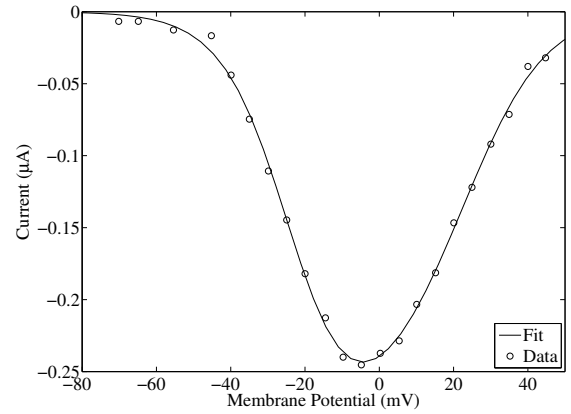
(a) Normalized current-voltage relationship of the T-type calcium channel from the digitized data of Talavera and Nilius (2006) [35]



(b) Normalized current-voltage relationship of the wild-type calcium channel [6]

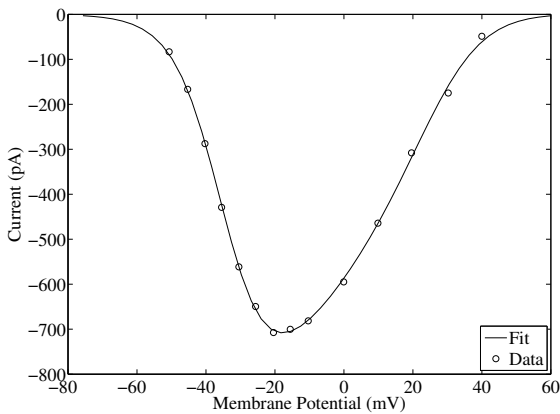


(c) Averaged, peak current-voltage plots of $Ca_v1.2\alpha$ L-type calcium channels [39]

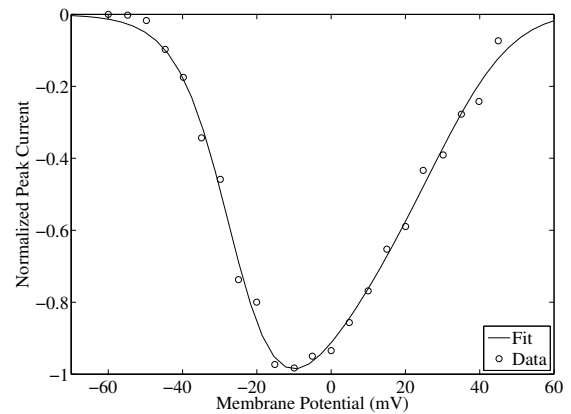


(d) Averaged, peak current-voltage plots of $Ca_v1.3\alpha$ L-type calcium channels [39]

Figure 2: 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.

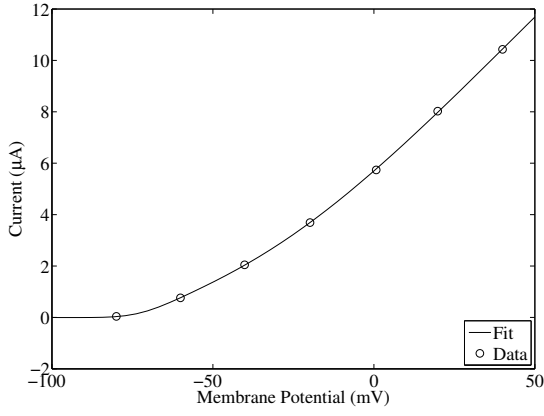


(a) Peak inward current vs. voltage relation for the current records of sodium channel [34] fitted with equation (12)

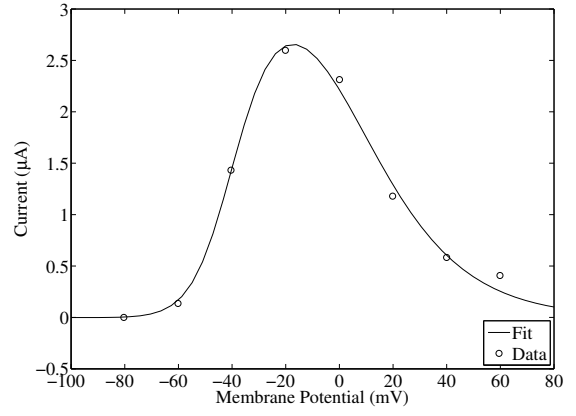


(b) Normalized current-voltage relationship of sodium channel from the macropatch current [31] fitted with equation (12)

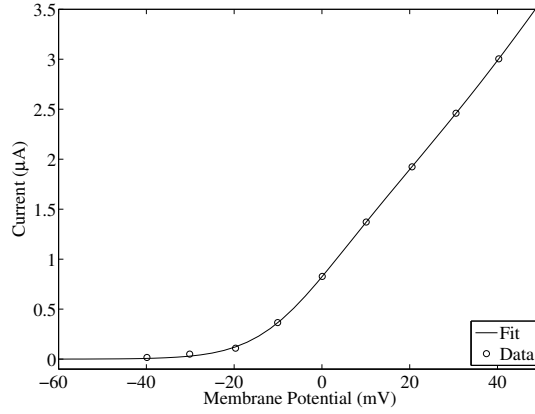
Figure 3: Current-Voltage relationship of Voltage-gated Na^+ channels fitted with equation 12.



(a) KCNH5

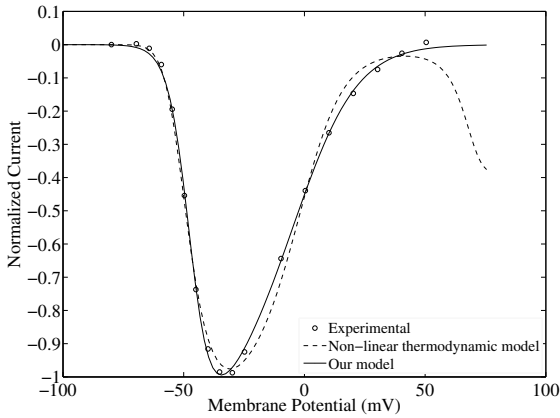


(b) KCNH7

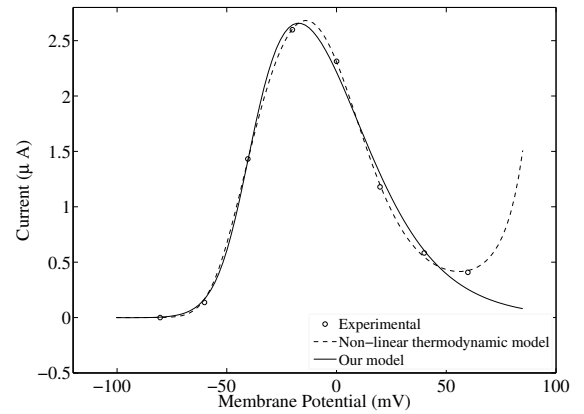


(c) KCNB1

Figure 4: Current-voltage relations for KCNH5, KCNH7 and KCNB1 class of potassium channels expressed in oocytes [40] fitted with equation 12.



(a) Calcium channel[35]



(b) Potassium channel[40]

Figure 5: Current-voltage relationship of ion-channels obtained from the studies [35, 40] fitted with a third order non-linear model and modified model presented in this article according to equation (12)

to have structurally output locally identifiable (s.o.l.i) parameters (see A for formal definition), 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 A

7. Conclusion

In this article, we bring forth an important criterion in modelling ion channel activity using Markov jump schemes. We propose that the behaviour of the system may be developed by an analytical design that is simple and adaptable; and towards this, a minimum of three Markov

Figure	Reference	Channel type	V_{h_1} (mV)	s_1 (mV)	V_{h_2} (mV)	s_2 (mV)	g_{max}
2(a)	Talavera and Nilius (2006) [35]	$Ca_v3.1$	-47.2228	-0.22613	0.617753	0.07519	0.0069 (mV^{-1})
2(b)	Beyl et al. (2007) [6]	$Ca_v1.2$	-5.36225	-0.12598	31.7746	0.13336	0.0098 (mV^{-1})
2(c)	Xu and Lipscombe (2001) [39]	$Ca_v1.2$	-2.89138	-0.13021	38.9031	0.13943	0.002 ($\mu A/mV$)
2(d)	Xu and Lipscombe (2001) [39]	$Ca_v1.3$	-22.4361	-0.11182	26.4652	0.08824	0.0022 ($\mu A/mV$)
3(a)	StÅijhmer et al. (1987) [34]	$Na_v1.2$	-34.1391	-0.14523	28.3072	0.10388	8.8843 (pA/mV)
3(b)	Ruben et al. (1997) [31]	$Na_v1.2a$	-25.6539	-0.14232	38.2236	0.088449	0.0138 (mV^{-1})
4(a)	Zou et al. (2003) [40]	$K_v10.2$	-64.5494	-0.17538	-29.4952	-0.02195	0.1009 ($\mu A/mV$)
4(b)	Zou et al. (2003) [40]	$K_v11.3$	-41.6897	-0.11727	-6.79872	0.053344	0.0630 ($\mu A/mV$)
4(c)	Zou et al. (2003) [40]	$K_v2.1$	40	-0.021769	3.51185	-0.13161	0.0476 ($\mu A/mV$)

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

states are necessary to model the dynamics.

To illustrate the criterion, we describe the physical transitions of a particular class of voltage gated ion channel proteins by means of free energy changes associated with perturbations in its environmental electric field from a thermodynamic perspective. For this class of ion-channels we assume the existence of a unique simple path of transition, from every stable state to the open state. The resulting model is a simple kinetic expression (equation 12) which approximates the stationary open probability of the channel for a given membrane potential and is a generalized form of the single exponent modified Boltzmann's function.

The model proposed in this article, is identifiable and requires small 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. Its main limitation is that it has been developed for steady-state data and may not represent ion-channel dynamics in its entirety but only fast ion channels. Nonetheless, when dynamics are relevant, the proposed scheme may be extended and the resulting model calibrated by using voltage-current data from classical experimental protocols.

Acknowledgment

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A. Model Identifiability, Proof of Lemma 2

Simplicity and identifiability of ion channel model would be of advantage especially, when such models are employed to develop larger frameworks describing metabolic activity of a cellular system, such a pacemaking neurons [13] or cardiac cells [25]. 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" [36] where both outputs $y(\theta, x)$ and $y(\tilde{\theta}, x)$ 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:

$$\left[\frac{\partial^i y(\theta, x)}{\partial x^i} \right]_{x=x_0} = \left[\frac{\partial^i y(\tilde{\theta}, x)}{\partial x^i} \right]_{x=x_0} \quad \forall i = 1, \dots, n \quad (15)$$

has a 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,

$$G(\theta) = \frac{1}{1 + e^{(V-V_{h_1})s_1} + e^{(V-V_{h_2})s_2}} \quad (16)$$

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 (15) leads to:

$$e^{aV_0+b} + e^{cV_0+d} = e^{\tilde{a}V_0+\tilde{b}} + e^{\tilde{c}V_0+\tilde{d}} \quad (17a)$$

$$ae^{aV_0+b} + ce^{cV_0+d} = \tilde{a}e^{\tilde{a}V_0+\tilde{b}} + \tilde{c}e^{\tilde{c}V_0+\tilde{d}} \quad (17b)$$

$$a^2 e^{aV_0+b} + c^2 e^{cV_0+d} = \tilde{a}^2 e^{\tilde{a}V_0+\tilde{b}} + \tilde{c}^2 e^{\tilde{c}V_0+\tilde{d}} \quad (17c)$$

$$a^3 e^{aV_0+b} + c^3 e^{cV_0+d} = \tilde{a}^3 e^{\tilde{a}V_0+\tilde{b}} + \tilde{c}^3 e^{\tilde{c}V_0+\tilde{d}} \quad (17d)$$

with the following two solutions obtained with Mathematica software [38]:

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

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

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

Corollary 1. If the model (16) is completed with the following condition:

$$s_1 > s_2$$

it is trivial to see that the system (17) now has only one solution and the model is s.o.g.i. for any $V \in \mathcal{U}_V^n$.