

A method for determination of energetical structure of conformational states of voltage dependent ionic channels

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Abstract. The mechanism of state transitions has been applied as a base to investigate energetical structure of conformational states of ionic channels. The kinetics of transitions between conformational states, governing the dynamics of the action potential, was represented in the form of a matrix equation. Transition coefficients have been expanded as a function of the external potential to provide a set of parameters. Resulting theoretical equations have been incorporated into the Hodgkin-Huxley model of the action potential. Model parameters, related to energy levels and barriers between conformational states, can be evaluated by fitting a theoretical curve to the experimental one.

Key words: ionic channels, spike, membrane, squid axon

INTRODUCTION

Hodgkin and Huxley (1952 a-d) have shown that the course of the action potential (spike) results predominantly from the interaction of two ionic currents - which, in turn, is determined by the voltage dependent membrane permeability for these particular ions. In the squid axon studied by Hodgkin and Huxley the dominant pair of ions consisted of sodium and potassium. It seems that this composition prevails throughout the nervous system although calcium ions can possibly replace sodium ions in their role (Traub and Miles 1991).

The Hodgkin and Huxley model of the spike assumed the existence of specific ionic channels. The nature of these channels was not known at the time of the model's development. Only the last few years have brought conclusive prove that the channel's function is being realized by specialized proteins. Noda et al. (1984) revealed the primary structure of the voltage dependent sodium channel and the Jan group did the same with the potassium channel (Pazian et al. 1987, Tempel et al. 1987). Moreover, both natural (Hartshorne et al. 1985) and artificially induced channels (Noda et al. 1986a,b; Timpe et al. 1988) have been reconstructed in lipid membranes and proved to retain their function.

Results referenced above have given strong support to the conviction accepted long before that protein conformation changes constitute the basis of channel functioning (Armstrong and Matteson 1984, Stevens 1987). In the present work, an attempt is made to extract the information characterizing the channel protein on the base of this assumption.

BACKGROUND, FUNDAMENTALS, CONVENTIONS

The Hodgkin-Huxley model outline

The Hodgkin-Huxley model consist of the following set of equations:

$$C \frac{dV}{dt} = g_k n^4 (V - V_k) + g_{Na} m^3 h (V - V_{Na}) + g_L (V - V_L)$$

$$\frac{dn}{dt} = \alpha_n (1 - n) - \beta_n n$$

$$\frac{dm}{dt} = \alpha_m (1 - m) - \beta_m m$$

$$\frac{dh}{dt} = \alpha_h (1 - h) - \beta_h h$$

The first equation describes the current balance. Sodium and potassium currents are expanded in two following formulas based on variables n for potassium and m, h for sodium current. They can be interpreted as channel population in the open (n) and inactivated (h) state. A power index has been imposed on these variables to fit experimental data in a better way; it can be interpreted as reaction order by analogy with chemical reactions. The index value of four is the highest that could be accepted for hand-made calculations before the computer era.

In turn, the time evolution of variables m , h and n depend on voltage dependent functions α , β . These functions have been measured experimentally.

Modifications

The model used for the present work differs from the original Hodgkin-Huxley model in two ways. First, the constant field equation, nonlinear voltage dependence of ionic currents has been incorporated (after Hodgkin and Katz, 1949).

The second modification appears substantial for this work since it enables inferences of channel characteristics. The general rule of state transitions has been applied to conformation changes of each channel involved in spike generation; hence, the fraction of channels assuming any state can be monitored. Detailed description of the equations introduced will be given later on.

Notation convention for channel populations

The evolution of channel populations assuming different conformational states is governed by a dif-

ferential equation based on transition coefficients. If only two states are involved, the population of channels assuming one of them is given by an equation adopted by Hodgkin and Huxley:

$$\frac{dp}{dt} = -\alpha p + \beta(1-p)$$

where p is a channel population assuming particular state, α is a transition coefficient of leaving this state and β - transition coefficient of entering this state from the other. If many states are allowed for the system, the number of appropriate equations increases and their size grows. We will introduce a notation convention to keep formula form short.

Let us organize a distribution of channels' population into a vector, and transition probabilities in matrix form

$$P = \begin{bmatrix} p_1 \\ p_2 \\ \dots \\ p_k \end{bmatrix}, \quad A = \begin{bmatrix} \alpha_{11} & \alpha_{12} & \dots & \alpha_{1k} \\ \alpha_{21} & \alpha_{22} & \dots & \alpha_{2k} \\ \cdot & \cdot & \cdot & \cdot \\ \alpha_{k1} & \alpha_{k2} & \dots & \alpha_{kk} \end{bmatrix}$$

for k states. Vector elements P_i denote populations (fractions) of channel molecules in i -th state. Off-diagonal elements α_{ji} are probabilities of transition from i -th to j -th state.

The sum of populations P_i must be equal to 1 any time since the number of channels is constant. This restriction can be incorporated into the coefficient matrix by assuming diagonal elements to be the negative of probability of leaving corresponding state:

$$\alpha_{ii} = -\sum_{j \neq i} \alpha_{ji}$$

With the above conventions, the differential equation governing transitions assumes the form

$$\frac{dP}{dt} = AP$$

The solution of this equation is given by

$$P(t) = P_0 e^{At}$$

where P_0 is starting population distribution.

Time-scale induced approximations

If the number of states is large, coefficients α_{ij} may be distributed over a wide range of magnitude. On the other hand, investigated processes are usually restricted to some period of time. Consequently, there always exists a time scale appropriate for the process under consideration. It makes some transitions "out of the scope" if they run too quickly or too slowly.

Particularly, it is easy to show that if for some states there is

$$|\alpha_{lm}| \gg |\alpha_{ij}| \text{ for } l, m \in K' \wedge i, j \notin K'$$

where K' is a subset of indexes, then these states remain near equilibrium throughout the evolution of the system (maybe except for a short period after the start of the evolution). As far as one is interested in processes long in comparison with the largest reciprocal of α_{lm} , these states may be replaced by single state in main system and additional matrix describing internal dynamics of this state treated as another (dependent) system:

$$\begin{bmatrix} \alpha_{11} & \alpha_{12} & \dots & \alpha_{1k} \\ \alpha_{21} & \alpha_{22} & \dots & \alpha_{2k} \\ \cdot & \cdot & \cdot & \cdot \\ \alpha_{l1} & \alpha_{l2} & \dots & \alpha_{lk} \\ \cdot & \cdot & \cdot & \cdot \\ \alpha_{k1} & \alpha_{k2} & \dots & \alpha_{kk} \end{bmatrix} \sim \begin{bmatrix} \alpha_{11} & \alpha_{12} & \dots & \tilde{\alpha}_{1l} \\ \alpha_{21} & \alpha_{22} & \dots & \tilde{\alpha}_{2l} \\ \cdot & \cdot & \cdot & \cdot \\ \tilde{\alpha}_{l1} & \tilde{\alpha}_{l2} & \dots & \tilde{\alpha}_{ll} \end{bmatrix} \wedge \begin{bmatrix} \alpha_{ll} & \dots & \alpha_{lk} \\ \cdot & \cdot & \cdot \\ \alpha_{k1} & \dots & \alpha_{kk} \end{bmatrix}$$

where strongly connected states were numbered with largest indices starting from 1 ($K' = \{i:l_i_k\}$). New coefficient values can be easily derived from old ones:

$$\begin{aligned} \tilde{\alpha}_{il} &= \sum_{j=1}^k \alpha_{ij} p_j & \text{for } i < l \\ \tilde{\alpha}_{ll} &= \sum_{i=1}^k \alpha_{ii} p_i & \text{for } i \geq l \\ \tilde{\alpha}_{lj} &= \sum_{i=1}^k \alpha_{ij} & \text{for } j < l \end{aligned}$$

where p_i are equilibrium fractions of united states.

In some cases, a state or a group of states can be separated from the rest of the system in the sense that time constants of transitions between any state belonging to this group and any other state are much greater than the time of the process under consideration. If it is the case, the whole population of this state(s) is practically constant during the process and can be disregarded except in the situation when the slow influx from the group is the only factor determining population in some "sensitive" state.

Transition coefficients

Transition coefficients c_{ij} can be written in the form of the widely used approximation:

$$\alpha_{ij} = \delta_{ij} \epsilon_{ij}$$

where δ is constant geometrical factor and E is Boltzmann factor:

$$\epsilon_{ij} = \begin{cases} e^{-\frac{B_{ij}}{kT}} & \text{for } B_{ij} > 0 \\ 1 & \text{for } B_{ij} \leq 0 \end{cases}$$

The maximum value of 1 has been introduced to preclude the possibility of attaining negative values of potential barrier B . Such possibility must be excluded as it is physically not allowed (a negative barrier height would mean that the two states merged).

The barrier height between two states is not symmetrical and depends on relative energy levels as well as mean barrier height. Hence, it is possible to break up this value into symmetrical and antisymmetrical components. Let us define:

$$E_{ij} = -E_{ji} = E_i - E_j$$

Thus

$$B_{ij} = b_{ij} + \frac{1}{2} E_{ij}$$

$$B_{ji} = b_{ij} - \frac{1}{2} E_{ij}$$

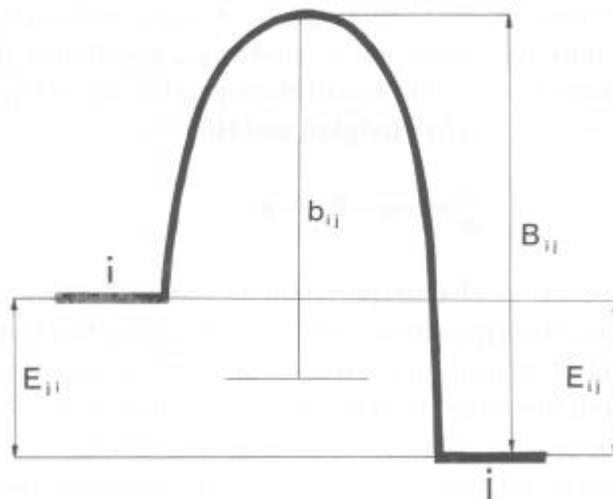


Fig. 1. Parameters of the energetical barrier: E_i and E_j - differences of energy levels of two states; b_{ij} - mean barrier height; B_{ji} - barrier height for transition from j -th state to the i -th. See text for details.

Figure 1 depicts relations between B_{ij} and B_{ji} . Both components B_{ij} and B_{ji} change with external electric field, causing the barrier height to evolve. Taylor expansion of the above formula near null electric potential gives

$$B_{ij}(V) = b_{ij} + \frac{1}{2} E_{ij} + \frac{1}{2} \frac{dE_{ij}}{dV} \Delta V + \frac{db_{ij}}{dV} \Delta V + \dots$$

Assuming that the curvature of barrier dependence on electric field is small enough in the range of spike potentials to preserve only the dominant component, we obtain the simplest linear approximation of transition coefficients in the form of

$$\alpha_{ij} = d_{i,j} \cdot e^{-\frac{\frac{1}{2} E_{ij} + w_{ij} V}{kT}}$$

where symmetrical voltage-independent elements have been merged and the first derivative expression has been reduced to single factor:

$$d_{i,j} = \delta_{ij} e^{-\frac{b_{ij}}{kT}}$$

$$w_{ij} = \frac{1}{2} \frac{dE_{ij}}{dV} + \frac{db_{ij}}{dV}$$

The above approximation provides three parameters for each pair of states. These parameters can be expanded to four provided that values of \sim for at least two temperatures are known.

The model

1) Conventions

External resistance has been assumed to be zero. Consequently, external potential is always zero.

Inward current is positive.

2) Glossary

The meanings of symbols used in equations are as follows:

- E - inside potential (negative in the rest)
- V - membrane voltage (equal -E)
- C - membrane capacity
- I_{Na} - sodium current produced by sodium voltage-dependent channels
- I_K - potassium current produced by potassium voltage-dependent channels
- I_L - leakage current
- p_{Na} - population of open sodium channels
- p_K - population of open potassium channels
- \tilde{p}_{Na} - sodium permeability
- \tilde{p}_k - potassium permeability
- Na_o - outside concentration Na ions
- Na_i - inside concentration Na ions
- K_o - outside concentration of K
- K_i - inside concentration of K
- G_L - leakage conductance
- σ - surface density of membrane channels
- ξ - specific conductance of the open channel
- ρ - permeability per channel
- α_{ij} - transition coefficient from the state j to i (for $i=j$ probability density of leaving i-th state)
- δ_{ij} - geometrical factor of transition coefficient
- B_{ij} - energy barrier height between j and i states as seen from state j
- b_{ij} - basal energy barrier between states j and i
- E_{ij}^0 - basal i-th and j-th states energy difference (at $V=0$)
- w_{ij} - voltage dependence factor of energy barrier

s3) Equations

The basic equation of current balance has a form:

$$C \frac{dV}{dt} = I_{Na} + I_K + I_L$$

Currents have been evaluated with constant field equation (Hodgkin and Katz 1949), except linear formula adopted for leakage:

$$I_{Na} = \tilde{p}_{Na} V \frac{[Na]_o - [Na]_i \exp\left(-\frac{VF}{RT}\right)}{1 - \exp\left(-\frac{VF}{RT}\right)}$$

$$I_K = \tilde{p}_K V \frac{[K]_o - [K]_i \exp\left(-\frac{VF}{RT}\right)}{1 - \exp\left(-\frac{VF}{RT}\right)}$$

$$I_L = G_L V$$

Ionic permeabilities for sodium and potassium ions are connected with the population of channels assuming the open state with the formula

$$\tilde{p}^* = \sigma \rho p_o$$

where open state population P_o is the element of the population vector ascribed to that state. The whole population vector follows differential equation discussed earlier:

$$\frac{dP}{dt} = AP$$

The value of permeability per channel can only be found on the ground of experimental measurements. The only measurable value characterizing channel permeability is channel conductance. To find an appropriate permeability value, let us notice that if ion concentrations at both sides of the membrane are equal, the constant field equation can be simplified to the form (for sodium ions):

$$[Na]_o = [Na]_i \Rightarrow$$

$$I_{Na} = P^* \frac{VF^2}{RT} \frac{[Na] - [Na] \exp\left(-\frac{VF}{RT}\right)}{1 - \exp\left(-\frac{VF}{RT}\right)} = P_{Na} \frac{[Na] F^2}{RT} V$$

In consequence, the permeability can be evaluated according to conductance g and ion concentration during experiment $[Na]$:

$$\rho_{Na} = \frac{RT}{[Na] F^2} g_{Na}$$

With this quantity the constant field equation assumes the shape adopted above.

The value of 25 pS in 0.5 M NaCl has been used in calculations for sodium channel, as cited by Montal (1990). No corresponding data have been found concerning the potassium channel. As well, no report has been found on measurements performed in different concentrations, which could validate the assumption of concentration - permeability relation linearity.

DETERMINATION OF ENERGETICAL STRUCTURE OF CHANNEL CONFORMATIONS

There exist a number of model parameters, such as channel density, maximal permeability, membrane capacity etc. Values of these parameters are measurable experimentally and the majority of them can be found in the literature. We will refer to these parameters as hidden ones.

In addition, with the linear approximation adopted, dynamics of conformational transitions between each pair of states of each ionic channel depends on three parameters.

Given the values of hidden parameters, any set of model parameters' values implies the unique course of action potential generated by the model. The main idea of this paper is that a channel molecule characteristics can be found by fitting the model action potential to the experimental data. With model parameter values achieved by fitting, it is possible to calculate physically interpretable energy parameters, in the present study referred to as the energetical structure of conformational states.

A number of states of real channels remains still unknown. At least one open state exist, but there can be more (Armstrong and Matteson 1984). Estimations of a number of closed states range from two (Montal et al. 1987) to five (Armstrong and

Matteson 1984). Two inactivated states must exist according to Montal et al. (1987).

For preliminary calculations presented in this paper one open, two inactivated and three closed states have been adopted. A linear order of states has been assumed for simplicity, implying five energetical barriers between states. With this arrangement, fifteen parameters for each channel, i.e. total of thirty parameters must be evaluated.

The set of direct parameters is minimal in the sense that no more parameters are necessary to simulate the spike course at particular temperature. Additionally, barrier heights can be evaluated if at least two measurements of action potential in various temperatures are accessible.

Details of calculations

Experimental records of Hodgkin and Huxley (1952d, Fig. 12) have been digitized and adapted with a set of programs.

First, deformations due to oscilloscope screen convexity and photographic distortions have been removed. In that purpose, geometrical relationship between oscilloscope image and its photography was mathematically described. The oscilloscope screen was assumed to be spherical, since that was the most preferred shape before flat screens came into use (results confirmed this assumption). Subsequently, images of two reference lines (originally straight) were fitted to their photographic counterparts. Resultant parameters were used to restore courses of remaining lines.

Resultant parameters seem to be reasonable, supporting correctness of the procedure. The camera was placed probably 5-6 inches from the screen at the position most suitable for particular image. Replacements of the camera enabled to minimize unrecoverable distortions due to screen deviation from sphere, present at periphery. At the central part of every image, the agreement was satisfactory.

Every particular spike course has been separated and the membrane potential reconstructed by thin-

ning the original lines. All potential values have been evaluated in relation to reference lines. The resultant tabularized function has been utilized in the minimalization (fitting) procedure with references to intermediate points realized by linear interpolation.

A computer program was written for the PC microcomputer to realize the minimalization procedure. Preliminary calculations were held on a PC 386 with a coprocessor, under DOS system. Turbo Pascal compiler was used.

Spike simulations were conducted with modified Runge-Kutta method. The modification was oscillation-sensitivity capable of time step shortening at the moment the first derivative appeared instable.

The most time-consuming procedure - matrix exponent - has been calculated with a trick based on

the assumption that diagonal elements are much greater than others (validity of this assumption has been controlled). Hence, the multiplication could be done in a straightforward manner. Moreover, the Taylor series of exponent had been cut at the second derivative in majority of runs since such accuracy appeared sufficient.

Many fitting procedures have been used utilizing a variety of minimizing functions, basing on square distance sum. This multiplicity reflects the great variety of minimalization strategies.

For present calculations, the linear structure of transitions has been forced (e.g., only between "adjacent" states). The reason for this restriction was the low precision of experimental data used along with the need of simplicity of preliminary calculations.

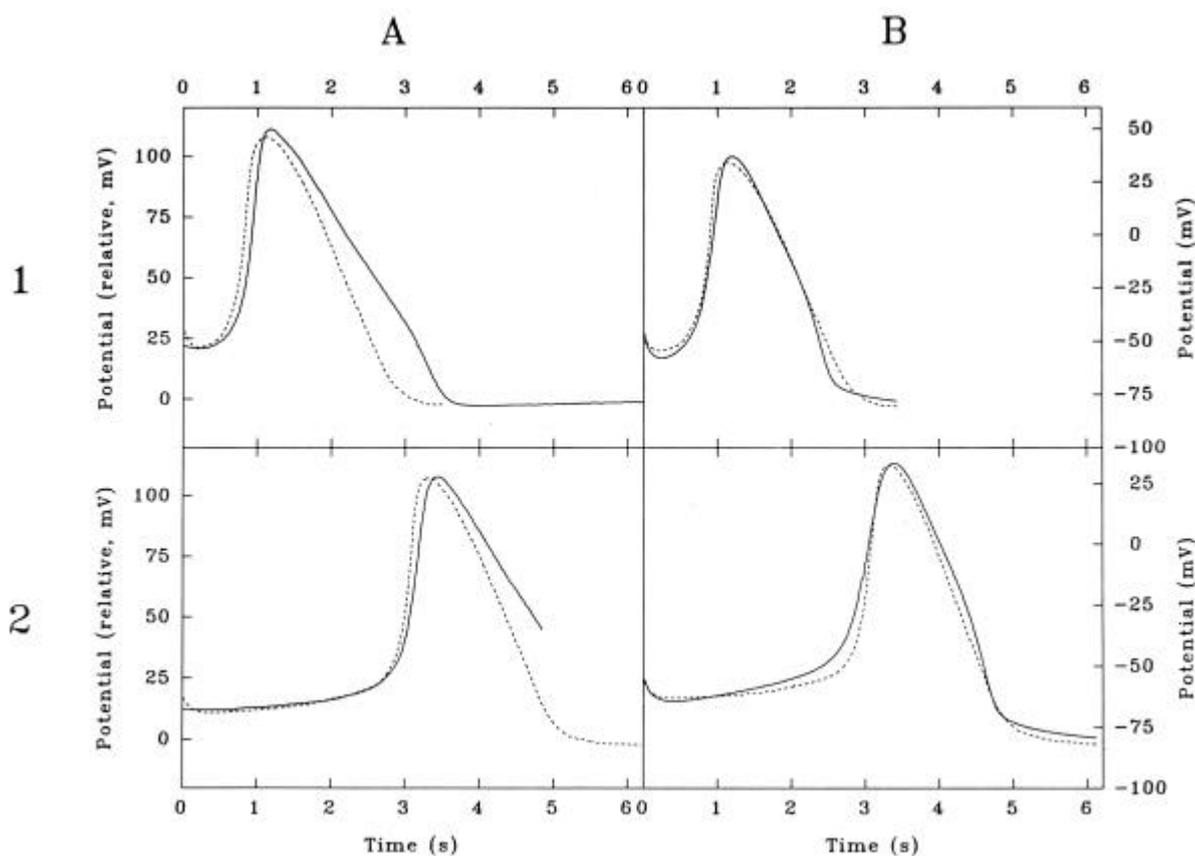


Fig. 2. Comparison of model and experimental data spike courses. Column A, the simulation of Hodgkin-Huxley (1952d), results as presented at Fig. 12; column B, present model. Row 1, initial depolarisation 20mV (simulated by Hodgkin and Huxley for 15mV); row 2, initial depolarisation 12 mV (simulated by Hodgkin and Huxley for 7mV). Dashed lines, experiment; continuous lines, simulations. Original curves of Hodgkin and Huxley have been numerically displaced and overlapped. Only relative potential scale is accessible for these data.

The fit for squid axon

An illustration of a sample result during the fitting procedure is shown at Fig. 2. Experimental data were those of Hodgkin and Huxley for squid axon.

It can be seen that some details of the action potential course - especially the initial inflexion - are mimicked better by the present model than by the original Hodgkin-Huxley model, although the fit is not satisfactory at the presented stage of minimization.

The final fit along with evaluated parameters will be published later.

DISCUSSION

The Hodgkin-Huxley model proved to be a very powerful tool for membrane potential studies. It has been used to study single spike course (Hodgkin and Huxley 1952d, Frankenhauser and Huxley 1964, Kernell and Sjöholm 1972), long-term neural activity properties (accommodation) by Frankenhauser and Vallbo (1965) and repetitive firing by Kernell and Sjöholm (1973). Qualitatively satisfactory results of these studies manifest the universality of the model thus encouraging further development.

The method described above converts the classical Hodgkin and Huxley problem: the extraction of channel parameters on the basis of the measured potential course is proposed, instead of membrane potential simulation based on measured parameters. The most prominent feature of this approach is simplification of experimental procedures along with widening the class of neurones suitable for measurements. Another gain arises from the link between functional characteristics of the membrane and physically interpretable channel parameters introduced by the modified model.

Applications

The method for investigation of energetical structure of conformational states can supply important information helpful in studies of protein second- and third order structure. The suitability of voltage dependent channels for that purpose lies in

their ability to produce easily observable electrical phenomena - conformational transitions of no other protein can be monitored so directly. Moreover, the sensitivity of the neurone activity for some agents suggests that even minor modifications of transition coefficients produced by these agents can be detected on the ground of the course of action potential.

The second possible application of the method is differentiation of various genetic and functional lines of channels at the level of the single intact cell. This task can not be accomplished on the basis of simple action potential comparison since it can be modified by many factors, for example environment composition. Biochemical methods, in turn, require a comparatively large amount of material and introduce the danger of protein modification during processing.

The last application we want stress here is the possibility of modeling the basic unit of neural networks as close as possible to reality. All existing models are based on arbitrary assumptions, essentially distorting model neurone characteristics. Taking precise action potential dynamics into account may disclose important features of the network.

Limitations and restrictions

The validity of the presented method depends on features of the particular neurone under consideration. First, there must be just one channel protein for each ion involved. Otherwise, the resultant conformation structure does not reflect the actual one (unless the proportion of the two channels is known and had been introduced into the model).

The relevance of parameters obtained with the method described depends on the consistency of the assumed system of states structure with actual channels' scheme. It is possible (Miller 1988, Schwarz et al. 1988) that there can exist many subtypes of ionic channel even in the same cell. In that case, if parameters of different subtypes are significantly different, fitting oriented to a single-type channel can be successful only by chance. Conversely, if the two subtypes are similar, they can not be resolved by fitting the model.

If just one type of each channel is present, there still exists a problem of choosing the appropriate number of conformational states. It can be reasonably assumed that an insufficient number of states should produce poor fit; too many states introduce a danger of false minima. Consequently, the method can resolve only the minimal set of states having the greatest impact on conformation dynamics.

Two kinds of false minima are possible. There can exist a real local minima in parameter space and apparent minima produced dynamically by minimalization procedure. The latter kind of false minima has been encountered. They could be easily recognized and avoided by adequate minimalization strategy. Real local minima can not be excluded; however, their existence is dubious, at least in the investigated case.

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