Control of Voltage-Dependent Biomolecules via Nonequilibrium Kinetic Focusing

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We show that voltage-sensitive macromolecules can, by the application of the right type of external voltage fluctuations, be focused with great probability into a desired kinetic substate. As an illustration we consider an eight-state model of the Shaker K^+ channel driven by dichotomous voltage fluctuations. This technique may provide a powerful new tool for the study of the kinetics of voltage sensitive ion channels.

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Ion channels are semipermeable macromolecular "pores" in the membranes of eukaryotic cells through which various ionic substances are selectively allowed to pass [1]. To a large degree, they determine the properties of excitable cells in nerve and muscle tissue. In voltage sensitive ion channels the coupling between membrane voltage and channel gating involves charges or electrical dipoles which act as sensors of the potential across the membrane. When the channel changes state these charges move, and produce capacitive currents called gating currents [2]. Information about the conformational states of channel molecules can be obtained by measuring the components of these gating currents, and indicates that voltage-dependent channels dwell in a number of states, most of them corresponding to closed conformations, and that the transition between these states is influenced by the membrane potential [3].

One of the principal goals of studying gating currents is to discover detailed information about the conformational substates of ion channels. It is hoped that this will lead to a knowledge of the corresponding molecular mechanisms, and eventually to a comprehensive understanding of the physiological role each channel plays. Here we propose a method we call *nonequilibrium kinetic focusing* in which a single kinetic substate of an ion channel can be selectively enhanced and its properties studied by the application of the right type of external voltage fluctuations.

As a particular example of the application of nonequilibrium kinetic focusing we will concentrate on one particular model system, the Shaker K^+ channel, which has been studied extensively in the last few years [4]. In a recent publication, Bezanilla, Perozo, and Stefani [5] (BPS) report on an eight-state linear kinetic model for this channel

$$C_0 \leftrightarrows C_1 \leftrightarrows C_{11} \leftrightarrows C_{12} \leftrightarrows C_2 \leftrightarrows C_3 \leftrightarrows C_4 \leftrightarrows 0\,,$$

which is able to reproduce many of the experimental observed gating currents. The "*C*" states all represent closed conformations of the channel, and the "*O*" state is the open conformation. The arrows indicate allowed kinetic transitions between substates.

One possible interpretation of this model is pictured in Fig. 1. The features in Fig. 1 are meant to represent various structural elements in the ion channel protein, and this is not the only conceivable arrangement [1]. The mobile voltage sensor can be in one of eight possible states, only one of which (the open state) allows for the passage of K^+ ions. The equilibrium position of the spring is determined by the membrane potential through the coupling to the mobile charged element. The mobile element moves in a stepping ratchet potential and is found the vast majority of the time near one of the potential energy minima, which represent the eight distinct kinetic substates observed experimentally [6]. We want to emphasize that the effect which we discuss here does not depend on the exact validity of this model. We introduce this model merely to set the stage for our ideas.

Occasionally the mobile element makes thermally activated jumps to neighboring states, and over time these jumps lead to an equilibrium occupation probability over the eight substates. The distribution of states of a system in thermal equilibrium at temperature T is very well understood. The probability of state i of a molecule with n

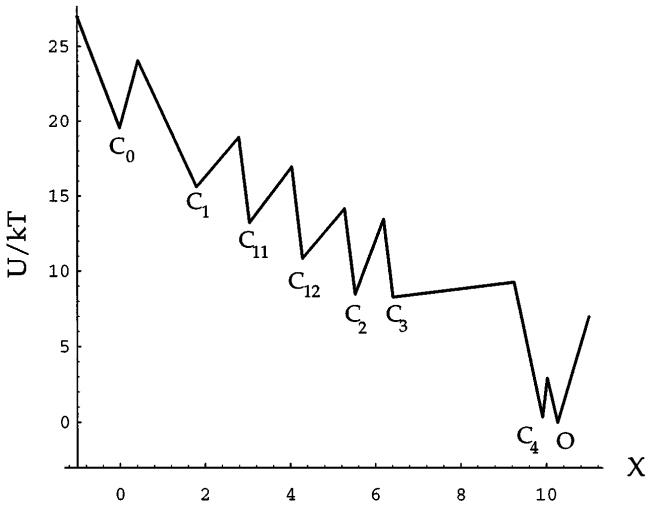

FIG. 1. Cartoon of one possible arrangement of the voltage sensor of the Shaker K^+ channel, shown here in the open state.

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states is approximately

$$P_i \approx \exp(-E_i/kT) \Big/ \sum_{j=1}^{n} \exp(-E_j/kT)\,. \qquad (1)$$

We derived the energy function picture in Fig. 2 by fitting the kinetic data in [5]. The information provided in [5] is not sufficient to fill in a very detailed model of the conformational dynamics [7]. However, by making use of some mild auxiliary assumptions we were able to combine this information into a continuous model which is consistent with the observation of BPS, and which contains the basic details.

The gating states of the channel are described by a single dimensionless reaction coordinate $X = zx/\lambda$, where z is the effective charge valence and x is the coordinate of the mobile unit. Thus, large distances between the stable states represent large gating currents. The dimensionless energy function $U(X) = E(x)/kT$ has a complicated form with several minima, each of which corresponds to one of the states of the ion channel.

In BPS the kinetic features of particular regions of substates of Shaker K^+ channels are deduced by measuring the gating current during relaxation after applying a 120 ms pulse from a hyperpolarized holding potential of −90 mV. The time of the pulse is long enough for an equilibrium state to form, and by adjusting its voltage a certain limited degree of control over the system is achieved. An external voltage V across the membrane changes the energies in Eq. (1) in a linear way, $E_i \rightarrow E_i + VX_i$, where X_i are the relative positions of the states. Figure 3 shows the equilibrium probabilities of the eight states of the Shaker K^+ channel as the external voltage is varied. As observed experimentally in BPS there are major charge motions around −44 and −63 mV. To observe the relaxation kinetics in the region of a particular substate one should enhance that substate as much as possible by pulsing to the voltage at which the probability is a maximum. For instance, the voltage which produces the largest probability for the state C_3 is around −45 mV. This situation represents a fundamental constraint which limits the experimentalist's ability to study a particular substate and its kinetics.

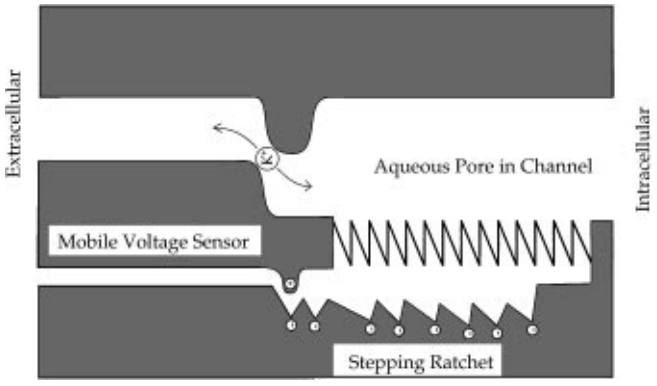

FIG. 2. Conformational energy function for the Shaker K^+ channel (at 0 mV membrane potential).

Let us then consider an alternative to this situation where an external time dependent voltage $V_0 + V(t)$ [where $\langle V(t)\rangle = 0$] is applied across the membrane. The gating of the channel is modeled as the motion of a mobile subunit with charge valence z and position x in an internal potential $u(x)$ subject to thermal fluctuations,

$$\gamma\dot{x}(t) = -u'(x) + z[V_0 + V(t)]/\lambda + \sqrt{2\gamma kT}\,\xi(t)\,,$$

where γ is the friction coefficient (inverse mobility) of the mobile unit, and we have assumed that its motion is overdamped. The length scale λ is the membrane thickness, and $\xi(t)$ is white noise with $\langle\xi(t)\rangle = 0$ and $\langle\xi(t)\xi(0)\rangle = \delta(t)$. After converting to dimensionless units $X = zx/\lambda$, $\tau = (z^2kT/\gamma\lambda^2)t$, $v_0 = V_0/kT$, and $v(t) = V(\tau)/kT$, and $U(X) = u(x)/kT$, which is in fact the definition of the energy function in Fig. 2, we have

$$\dot{X}(\tau) = -U'(X) + v_0 + v(\tau) + \sqrt{2}\,\zeta(\tau)\,, \qquad (2)$$

where $\langle\zeta(\tau)\rangle = 0$ and $\langle\zeta(\tau)\zeta(0)\rangle = \delta(t)$.

Although there are an unlimited number of types of nonequilibrium fluctuations which can be applied, for the purpose of illustration we will confine ourselves here to what is known as dicotomous noise, or telegraph noise. This is noise which has two states $V_0 + V_\pm$. Transitions between states occur at random moments with probabilities $W_\pm$ out of the plus and minus states, respectively. For this reason this noise is known as Markovian colored noise. This noise is very suitable as a first choice because it is easy to apply experimentally [8] and because together with the sawtooth form of the effective potential picture in Fig. 3, it allows for a particularly simple analysis. We have already considered this noise in another context [9]. It is also important to note that while we use this particular noise as an illustration, the basic principles behind our results will also apply to a host of other types of noise. While more detailed work needs to be done to determine if other

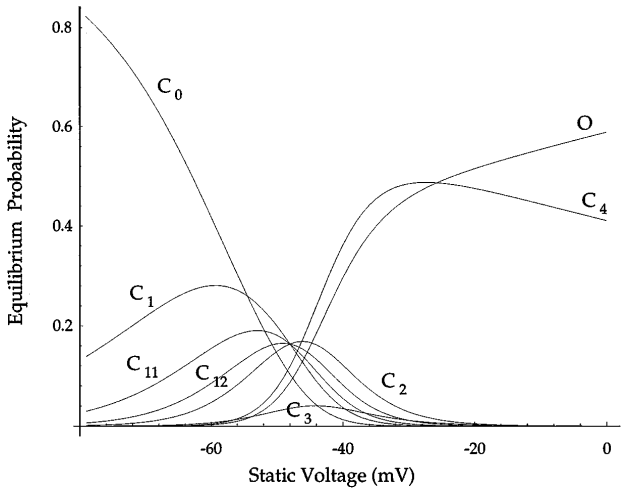

FIG. 3. Equilibrium probabilities for the eight states of the Shaker K^+ channel.

types of easily applied voltage fluctuations (e.g., nonwhite Gaussian noise [10]) will work better in this or other situations, the basic principles should apply regardless of the specific type of nonequilibrium voltage fluctuations applied across the membrane.

We start by putting the external voltage fluctuation in dimensionless form $v_\pm = V_\pm/kT$ where we chose

$$v_+ = \sqrt{\frac{D}{\tau_v}\left(\frac{1+\epsilon}{1-\epsilon}\right)}, \qquad v_- = -\sqrt{\frac{D}{\tau_v}\left(\frac{1-\epsilon}{1+\epsilon}\right)}. \tag{3}$$

The transition probabilities w_+ from the plus to the minus state and w_- from the minus to the plus state are

$$w_+ = (1+\epsilon)/2\tau_v\,, \qquad w_- = (1-\epsilon)/2\tau_v\,. \tag{4}$$

This noise has mean zero $\langle v(t)\rangle = 0$ and correlation function $\langle \eta(t)\eta(0)\rangle = (D/\tau_v)\exp(-\tau/\tau_v)$. The dicotomous noise thus has three characteristic dimensionless parameters, the "amplitude" D, the correlation time τ_v, and an asymmetry parameter ϵ. This noise goes over to white noise with strength D as $\tau_v \to 0$ and $\epsilon \to 0$.

A probabilistic treatment of Eqs. (2)–(4) leads to the set of coupled equations [11]

$$\frac{\partial \rho_\pm(X,\tau)}{\partial \tau} = \frac{\partial}{\partial X}\left[\tilde{U}'(X) - v_\pm + \frac{\partial}{\partial x}\right]\rho_\pm(X,\tau) \mp w_+\rho_+(X,\tau) \pm w_-\rho_-(X,\tau)\,,$$

where $\rho_\pm(X,\tau)$ are the conditional probability densities for the ion to be at X when the noise is in the plus or minus state $v(\tau) = v_\pm$. The total probability density is just $P(X,\tau) = \rho_+(X,\tau) + \rho_-(X,\tau)$. For simplicity we have introduced $\tilde{U}(X) = U(X) - v_0 X$.

After introducing the auxiliary variable $Q = w_+\rho_+(X,\tau) - w_-\rho_-(X,\tau)$, and rewriting the above set of equations in terms of $P(X,\tau)$ and $Q(X,\tau)$ we obtain

$$\frac{\partial P(X,\tau)}{\partial \tau} = \frac{\partial}{\partial X}\left[\tilde{U}' + \frac{\partial}{\partial X}\right]P - \frac{A}{\delta}\frac{\partial}{\partial X}Q\,,$$

$$\frac{\partial Q(X,\tau)}{\partial \tau} = \frac{\partial}{\partial X}\left[\tilde{U}' - \frac{\theta}{\delta} + \frac{\partial}{\partial X}\right]Q - \frac{Q}{\tau_v} - \frac{1}{\delta A}\frac{\partial P}{\partial X}\,,$$

where $\delta = \sqrt{\tau_v/D}$, $\theta = 2\epsilon/\sqrt{1-\epsilon^2}$, and $A = 2\tau_v/\sqrt{1-\epsilon^2}$.

We wish to find the stationary probability distribution $P_0(X)$, $Q_0(X)$ of the above equations, where $\partial P_0/\partial\tau = 0$ and $\partial Q_0/\partial\tau = 0$. If we further note that $\partial_t P(X,\tau) = -\partial_x J(X,\tau)$ where J is the probability current, and that the stationary current must vanish in this closed system, we can obtain the stationary state equations

$$0 = \tilde{U}' P_0 + \frac{\partial P_0}{\partial X} - \frac{A}{\delta}Q_0\,, \tag{5}$$

$$0 = \frac{\partial}{\partial X}\left[\tilde{U}' - \frac{\theta}{\delta} + \frac{\partial}{\partial X}\right]Q_0 - \frac{1}{\tau_v}Q_0 - \frac{1}{\delta A}\frac{\partial P_0}{\partial X}\,. \tag{6}$$

We will consider only the case where the amplitude of the driving is large with respect to the thermal fluctuations, and where $T \to 0$, and $\sqrt{D/\tau} > \sup|U'|$. Substituting Eq. (5) into Eq. (6) and integrating and solving for the stationary distribution for the potential in Fig. 2, we obtain the result we are after:

$$P_0(X) \approx Ne^{-\Phi(X)}, \qquad \Phi(X) = \int^X \frac{U'(y) - v_0}{W(y)}\,dy\,,$$

$$W(y) = 1 + D[1 - \delta^2[\tilde{U}'(y)]^2 + \delta\theta\tilde{U}'(y)]. \tag{7}$$

The stationary probabilities of the substates are then given approximately by

$$P_i \approx \exp[-\Phi(X_i)/kT] \Big/ \sum_j \exp[-\Phi(X_j)/kT]. \tag{8}$$

This expression can be evaluated on the computer for the potential shown in Fig. 2. More detailed theoretical and numerical studies with a wider range of applicability will appear elsewhere.

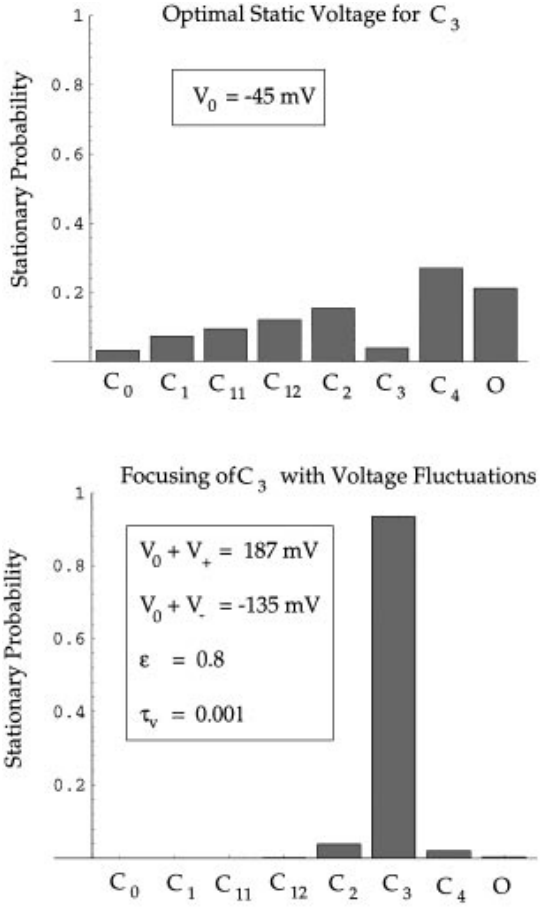

FIG. 4. C_3 Focusing results for a model of the Shaker K^+ channel.

Figure 4 shows the focusing effect we are after. We have chosen to illustrate the enhancement of the C_3 substate because it is the state with the smallest probability under optimal equilibrium conditions, and because the effects are particularly dramatic. Qualitatively similar results can be obtained for any state. The correct noise parameters are found by trial and error by evaluating Eqs. (7) and (8) [12]. The probabilities of any state can be significantly enhanced from the optimal equilibrium values. For the case pictured in Fig. 4, the optimal probability under static conditions for C_3 is about 0.04. After nonequilibrium kinetic focusing the probability of this state has been enhanced to 0.96, some 25 times the optimal static value. More importantly, under optimal static conditions most of the other states are present in even greater proportions, and the state C_3 is smothered by signals from the other states. After focusing with nonequilibrium fluctuations the state C_3 is by far the predominant state and under these circumstances it is no exaggeration to talk about focusing the channels into a specific state.

Since nonequilibrium stationary states do not have the same restrictions as equilibrium ones, they can be manipulated and controlled to a much greater extent. The effect which makes this control possible is nonequilibrium bias created when the voltage fluctuations interact cooperatively with the underlying dynamics of channel gating. Any voltage sensitive transition (and hence any voltage sensitive gating mechanism) can be biased by nonequilibrium voltage fluctuations. The basic principle remains to support transitions into a particular state of interest, and to suppress transitions out of that state. This principle is equally valid for multiple branchings of the state tree, and for any physical interpretation of the states, as long as some of the transitions into and out of the states are voltage dependent. For the same reason these methods should be equally applicable in totally different contexts, such as the control of chemical reactions.

A certain amount of generic intuition about this behavior can be gleaned from earlier work [13] and from more recent work on fluctuation-induced transport [9,10,14,15]. For the dicotomous noise used here there are three principal factors to consider. The first is amplitude of the fluctuations. If the amplitude is too small, there is little or no effect while if it is too large, the response of the voltage sensors will be so large as to smooth out the probability distribution. Second, if there is some spatial asymmetry about a particular substate, the addition of time correlated voltage fluctuation will cause an enhancement of the activation rates out of the state in one direction over another. Last there is temporal asymmetry (in the present case parametrized by ϵ) which is an asymmetry in the voltage noise itself due to a nonvanishing of the higher order odd correlation functions of the fluctuations. Temporal asymmetry can bias the transport in either direction, and can compete with the effects due to a spatial asymmetry [9,15]. For nonwhite Gaussian noise (not discussed here), it is the shape of the power spectrum of the noise that controls the behavior [10]. Generally speaking it can be shown that "narrow" states are made more stable relative to "wide" states by Gaussian fluctuations whose power spectrum has positive curvature at zero frequency, and that the opposite is true when the power spectrum has negative curvature at zero frequency.

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