brief communication

# Percolation model of ionic channel dynamics

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ABSTRACT The nonexponential closed-time distributions observed for ionic channels have been explained recently by quasi-one-dimensional models of structural diffusion (Millhauser, G. L., E. E. Salpeter, and R. E. Oswald. 1988. *Proc. Natl. Acad. Sci. USA.* 85:1503–1507; Condat, C. A., and J. Jäckle. 1989. *Biophys. J.* 55:915–925; Levitt, D. G. 1989. *Biophys. J.* 55:489–498). We generalize this treatment by allowing for more complex trajectories using percolation theory. We assume that the gating transition depends on marginally connected conformational states leading to the observed spread in time scales.

## INTRODUCTION

The closed-time densities of unitary currents in ion channels, as revealed by single channel recording, are usually heterogenous. Whether the gating kinetics is best represented by a sum of exponentials, related to a few kinetic states (McManus et al., 1988, 1989; Horn and Korn, 1989) or by a (fractal) continuum of states (Liebovitch and Sullivan, 1987; Liebovitch, 1989) is still controversial. Simplified models of protein dynamics such as defect diffusion may help to understand how local interactions between the many atoms in a protein ultimately lead to global changes in structure and function (Läuger, 1988). Recently three one-dimensional random walk models of structural fluctuations in channel proteins have been proposed (Millhauser et al., 1988; Condat and Jäckle, 1989; Levitt, 1989). The gating transition is assumed to occur when the effective conformational variable assumes a particular value or values within a narrow range. One of the essential predictions of the one-dimensional approach is the algebraic long-time decay of the closed-time density $f(t) \sim t^{-1.5}$. The NG 108-15 channel (McGee et al., 1988) indeed shows this behavior over four decades in time. However, to obtain four decades, one has to assume a linear chain of about 100 states, $N \sim (t/t_0)^{0.5}$. It is therefore desirable to construct models which account for more complex trajectories. In this communication we show that the dynamic behavior of the channel can be explained alternatively as the signature of a percolating network.

Proteins are molecular networks of hydrogen bonds. Almost all C=O and NH groups of the polypeptide backbone are hydrogen bonded, typically 90% (Baker and Hubbard, 1984). A conformational change implies a shift in the network structure depending on the flexibility of the bonds involved. Hydrogen exchange experiments demonstrate a large spread in the stability of hydrogen bonds (Englander and Kallenbach, 1984). A large-scale conformational change connecting two given conformational states i and j may proceed through a series of local rearrangements of bonds. A rigid bond encountered along the path forces the system to turn back. An alternative path has to be selected. The trajectory of a protein in conformational space may thus resemble the walk of an ant in a labyrinth. Such connectivity problems can be handled by percolation theory: If a network of conducting wires is diluted by randomly removing the conducting elements, one observes that the overall conductivity vanishes at a critical concentration of intact wires (Zallen, 1983; Stauffer, 1985). A percolation cluster of connected sites near its threshold is shown in Fig. 1.

## MODEL

In the following we consider structural transitions $i \rightarrow j$ in conformational space controlled by hydrogen bonds in analogy to the local unfolding model of hydrogen exchange. If the bond fluctuations are fast compared to the structural change, one can write the structural transition rates $W_{ij}$ in the following form (Nakanishi et al., 1974):

$$W_{ij} = \frac{K_{ij}}{1 + K_{ij}} W^{\circ}_{ij}. \quad (1)$$

$K_{ij}$ is the dissociation constant of the H bond controlling the transition $i \rightarrow j$ and $W^{\circ}_{ij}$ denotes the transition rate in the case of an open bond. In hydrogen exchange experiments one generally observes a fast and a slowly exchang-


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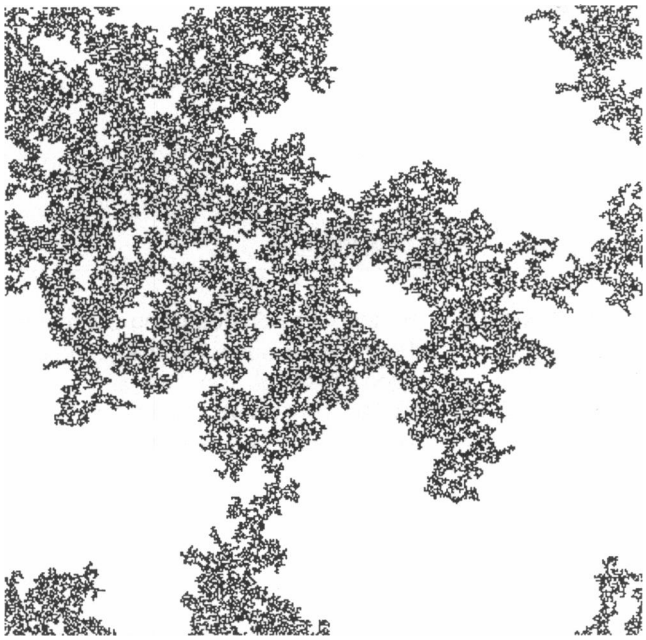

FIGURE 1 Computer generated two-dimensional percolation cluster of connected sites on a square lattice ($Z = 4$) at the threshold $x_c = 0.59$.

ing component (Nakanishi et al., 1974). We may thus approximate the distribution of dissociation constants by two sets of mobile ($K_{ij} \approx K_o$) and rigid ($K_{ij} \approx 0$) hydrogen bonds. The probability density of rates $p(W)$ is then given by:

$$p(W) = x\,\delta(W - W_o) + (1 - x)\,\delta(W - W_1). \qquad (2)$$

$0 < x < 1$ is the concentration of mobile bonds associated with the rate $W_o$, and $W_1 \ll W_o$ denotes the transition rate constrained by a rigid bond.

We first assume $W_1 = 0$. We further split the set of conformational states {i} into two macrosets $\{C_i\}$, $\{O_i\}$ corresponding to the experimentally observed closed and open channel states. These assumptions lead to a typical percolation problem. A global transition $\{C_i\} \rightarrow \{O_i\}$ involves a sequence of allowed local transitions $i \rightarrow j$. Percolation implies the existence of a pathway linking the two macrosets. The evolution of the system in conformational space can be described by a master equation

$$\frac{dn_i}{dt} = -\sum_j W_{ij} n_i + \sum_j W_{ij}\, n_j. \qquad (3)$$

$n_i$ denotes the occupation probability of state i. The dimension of the conformational space $D$ and the coordination number $Z$ of the transition $i \rightarrow j$ is arbitrary. The case $D = 1$, $Z = 2$, $W_{ij} = W_o$ leads to one-dimensional diffusion (Condat–Jäckle model, 1989; Millhauser et al., 1988; Levitt, 1989). Eq. 3 can be solved analytically using the effective medium approximation (Movaghar and Schirmacher, 1981). Within this approximation the global behavior of such a system is well described. The dynamics of the channel is thus explained as a network property and not by the construction of an active site.

In particular one derives the survival fraction of the system $\phi(t)$. This denotes the probability that the protein, initially in the closed set, still occupies this set after a time $t$. The Laplace transform of $\phi(t)$, $\phi(p) = \int_o^\infty e^{-pt}\phi(t)dt$, can be written in the form:

$$\phi(p) = \frac{1}{p + m(p)}. \qquad (4)$$

The relaxation kernel $m(p)$ is given by the self-consistent equation (Schirmacher, 1987, 1988):

$$m(p) = Z a_p \left\langle \frac{1}{\frac{1}{m(p) + p} + \frac{1}{W_{ij}}} \right\rangle. \qquad (4a)$$

$a_p$ is a density renormalization constant ($a_p = 0.368$ for $D = 3$) which accounts for double counting and $\langle \ldots \rangle$ denotes the average over the distribution of rates $p(W)$. For the model of Eq. 2, Eq. 4a can be written in the following form:

$$m(p) = Z a_p x \frac{1}{\frac{1}{m(p) + p} + \frac{1}{W_o}}, \qquad (4b)$$

which gives

$$m(p) = \frac{\epsilon W_o - p}{2} + \left[\left(\frac{p + \epsilon W_o}{2}\right)^2 + W_o p\right]^{0.5}. \qquad (5)$$

This type of kernel was first derived by Götze et al. (1981) using a self-consistent mode coupling theory. $\epsilon$ is the separation parameter denoting the distance from the percolation threshold. In particular: $\epsilon = x/x_o - 1$. $x$ is the fraction of flexible bonds and $x_o = 1/Z\,a_p$ denotes the critical concentration of flexible bonds. Inversion of the Laplace transform leads to the following $\phi(t)$, shown in Fig. 2:

$$\phi(t) \approx \begin{cases} e^{-W_o t} & t \lesssim 1/W_o \\ e^{-(\epsilon^2/4)W_o t}\{(\pi W_o t)^{-0.5} - \epsilon e^{(\epsilon^2/4)W_o t} erfc[\epsilon(W_o t)^{0.5}]\} & t > 1/W_o \end{cases} \qquad (6)$$

The model, depending on $\epsilon \lesssim 0$, describes a continuous dynamical transition (Götze et al., 1981). Below the threshold ($\epsilon < 0$) one obtains a nonergodic term in $\phi(t)$: $\phi(t \rightarrow \infty) = |\epsilon| \neq 0$, implying that the two macrosets are dynamically disconnected. Above and below the threshold, $\phi(t)$ has a region of algebraic decay with exponent 0.5. Because the closed-time density is given by the time

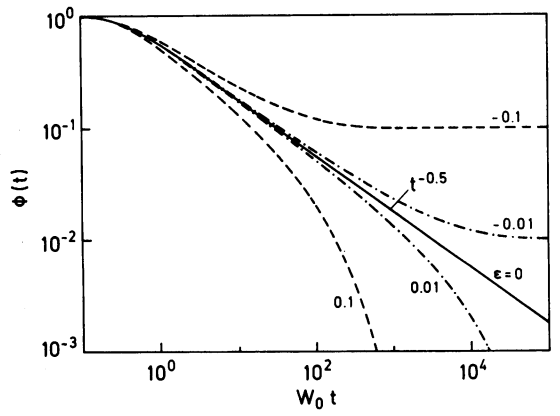


FIGURE 2 Survival fraction in the closed state $\phi(t)$ at different values of $\epsilon = \pm 0.1, \pm 0.01, 0$ according to Eq. 6.

derivative of $-\phi(t)$, one obtains the result of the one-dimensional model: $f(t) \sim t^{-1.5}$. However, the power law is now explained as the signature of a dynamical transition. Its validity range $(1/W_o, 1/\epsilon^2 W_o)$ depends on the distance from the percolation threshold. The observed four decades imply $|\epsilon| < 10^{-2}$. The protein is thus close to the percolation limit.

## DISCUSSION

We have shown that a percolation model of structural transitions reproduces the quasi–one-dimensional power law observed for the NG 108-15 channel. Physically this results from the highly ramified structure of the percolation cluster containing dead ends and bottle necks. The power law is a critical phenomenon, and thus a special case. Below and above the threshold a fit by a few exponentials will account for the data. A small distance from the percolation limit may guarantee restricted flexibility consistent with stability and function. Each main chain residue has the potential to form approximately three hydrogen bonds (C=O [two], NH [one], Baker and Hubbard, 1984). Associating each transition i → j with one H bond, we obtain $Z = 3$. For $D = 3$ this implies $x_c = 0.91$ close to the value $x_c = 1$ for one-dimensional motion. $Z = 4$ (including the side chains) would give $x_c = 0.68$. Thus, a substantial fraction of H bonds has to be flexible to give $\epsilon > 0$. The relevance of H bonds can be determined by investigating the voltage and pH dependence of $K_{ij}$. Further a decrease in temperature should drastically reduce the number of flexible bonds. The distribution $p(W)$ could be measured by hydrogen exchange experiments.

We have also considered the case of a finite $W_1 \ll W_o$. This results in a final decay of $\phi(t)$ with rate $W_1$ even for $\epsilon < 0$ and may account for data such as displayed in Fig. 2 of McManus et al. (1988).

We would like to comment on the notions "non-Markovian" and "fractal" which appear in the controversial discussion mentioned in the introduction. In our model we start from a set of microscopic Markovian rate equations. The averaging procedure reduces the number of states to essentially two, open and closed. This reduction leads to a non-Markovian description. Eq. 4 transformed into the time domain reads:

$$\frac{d}{dt}\phi(t) = -\int_o^t m(t-t')\,\phi(t')dt'. \qquad (7)$$

$m(t)$ is the Laplace transform of $m(p)$. Eq. 7 is non-Markovian because it samples all transition rates of the trajectory in the past $t'$ leading to the final transition at time $t$. Here, the microscopic trajectory is a fractal object (percolation cluster) which implies a nonexponential time dependence of $\phi(t)$. The time-dependent rate $k(t) = -d/dt \ln \phi(t)$ is not a simple power law, $k(t) \propto t^{-x}$ (Eq. 6). In the regime where $\phi(t) = t^{-0.5}$ one has of course $k(t) = t^{-1}$.

Our concept of nonuniform, clustered protein trajectories, leading to a hierarchy of processes is supported by molecular dynamics (Levitt, 1983) and Monte Carlo simulations (Go and Nogutchi, 1989) and by experiments performed on small proteins (Ansari et al., 1985; Careri et al., 1986; Iben et al., 1989). The protein dynamics approach explains nonexponential relaxation not as a singular feature of channel proteins but as a natural consequence of local heterogeneity in protein structures. Finally, one has to show how the formal concept of nonuniform protein trajectories applies to three-dimensional changes of the channel structure, such as sliding of helices (Catterall, 1988).

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