A Microscopic Model of Enzyme Kinetics

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ABSTRACT Many in vivo enzymatic processes, such as those of the tissue factor pathway of blood coagulation, occur in environments with facilitated substrate delivery or enzymes bound to cellular or lipid surfaces, which are quite different from the ideal fluid environment for which the Michaelis-Menten equation was derived. To describe the kinetics of such reactions, we propose a microscopic model that focuses on the kinetics of a single-enzyme molecule. This model provides the foundation for macroscopic models of the system kinetics of reactions occurring in both ideal and nonideal environments. For ideal reaction systems, the corresponding macroscopic models thus derived are consistent with the Michaelis-Menten equation. It is shown that the apparent K_m is in fact a function of the mechanism of substrate delivery and should be interpreted as the substrate level at which the enzyme vacancy time equals the residence time of ES-complexes; it is suggested that our microscopic model parameters characterize more accurately an enzyme and its catalytic efficiency than does the classical K_m. This model can also be incorporated into computer simulations of more complex reactions as an alternative to explicit analytical formulation of a macroscopic model.

INTRODUCTION

Enzymes are frequently characterized by the kinetics of reactions in which specific substrates are converted to products. The simplest single enzyme, E, single substrate, S, reaction is illustrated in Model I, where k_1 is a second-order rate constant and k_2 and k_{cat} are first-order rate constants for the dissociation of enzyme-substrate complexes, ES, and the formation of product, P:

$$E + S \underset{k_1}{\overset{k_2}{\leftrightarrows}} ES \xrightarrow{k_{cat}} E + P$$

Model I

The kinetics of this reaction are generally described by ordinary differential equations indicating the rate of change of the constituents. Explicit full time course solution of these equations is not possible; however, approximate singular power series solutions can be established that are valid for either short or long time intervals, and these can then be asymptotically matched (Tichonov, 1952; Murray, 1977). The more common approach is to invoke a steady-state assumption that results in the classical Michaelis-Menten equation for the initial velocity of product formation, v_0, in terms of the initial substrate and enzyme concentration, $[S_0]$ and $[E_0]$ (Michaelis and Menten, 1913; Briggs and Haldane, 1925):

$$v_0 = \frac{V_{max}[S_0]}{K_m + [S_0]}, \quad (1)$$

where the Michaelis constant $K_m = (k_2 + k_{cat})/k_1$ and $V_{max} = k_{cat}[E_0]$. The kinetics of the enzyme reaction presumably is characterized by the parameters K_m and k_{cat}, which are assumed to be constant, and their ratio k_{cat}/K_m is referred to as the catalytic efficiency of the enzyme.

The Michaelis-Menten equation adequately describes the initial velocity of ideal reactions involving purified substrate and enzyme in solution; however, it may not be applicable to more complex systems and in vivo reactions. In particular, when the delivery of substrate is not via simple diffusion or the enzyme is sequestered or bound to a surface the first-order processes leading to the Michaelis-Menten equation are not valid. An example is provided by the reactions involved in the tissue factor pathway of blood coagulation. The primary reaction of this physiological process is the activation of factor X by a tissue factor-factor VIIa enzyme complex (TF:VIIa) that is irreversibly bound to a lipid or cellular surface. This reaction in vivo clearly does not occur in the ideal environment assumed for Eq. 1 because the substrate delivery is mediated by the fluid flow of the blood. To model the kinetics of such reactions, we have developed a sequential microscopic model, describing the velocity of catalysis for a single enzyme. This model then provides the basis for macroscopic models reflecting various reaction environments, e.g., systems with convection, surface interactions, or the presence of other molecular species. The proposed microscopic model focuses on the sequence of events that occur at a single enzyme between successive product formations. Specific model parameters associated with these events characterize the enzyme and its kinetic specificity for a particular substrate and also reflect the kinetic dependency on the mechanism of substrate delivery. The associated macroscopic models describing the

Received for publication 2 February 1995 and in final form 11 May 1995.
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0006-3495/95/08/356/06 $2.00

kinetics of complex systems can be quite complicated, depending on the mechanism of substrate transport, and may prove difficult to solve and analyze mathematically. An alternative approach is to incorporate the proposed microscopic model into a stochastic macroscopic computer model that simulates the reaction (Gentry et al., 1995).

To demonstrate the consistency of the proposed microscopic model, we establish the associated macroscopic model corresponding to an ideal reaction and identify the microscopic parameters with those of Eq. 1. This provides an alternative representation of the Michaelis-Menten parameters. In particular, this approach demonstrates that the apparent K_m, $K_{m\text{-app}}$, which is the estimated substrate concentration at which half the apparent maximum velocity is attained, is *not* a characteristic of the enzyme per se because it also depends critically on the mode of substrate delivery and the environment of the enzyme. Our microscopic model parameters reflect more specifically an enzyme's true kinetic properties and affinity for a substrate and, thus, provide a better characterization of the reaction than does the Michaelis-Menten parameters.

A MICROSCOPIC KINETIC MODEL OF ENZYMATIC ACTIVATION

A model of product formation at a single enzyme can be formulated by considering the sequence of events that lead to product formation. Between successive productive ES-complexes leading to product formation, P, there may be several unproductive complexes that dissociate and release unmodified substrate. The time interval between successive product formations is thereby partitioned into subintervals in which the enzyme is alternately vacant and occupied. We consider first the simplest situation in which no other molecules compete with the substrate for the enzyme's active site. This scenario is illustrated in Fig. 1, where the filled segments represent periods in which the enzyme is complexed with substrate, blank segments represent the time the enzyme is not occupied, and the arrows indicate dissociation of substrate or product formation. The enzyme vacancy time between successive complex formations, t_v, will vary as will the residence time of a complex, t_r. The total time between two successive products, t_p, is given by

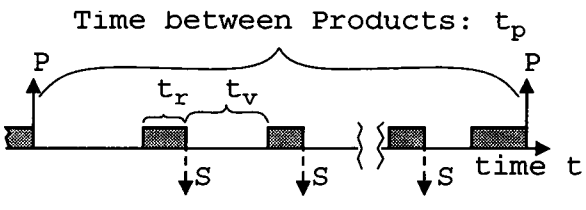

FIGURE 1 Time sequence of events between successive product formations at an enzyme site; shaded regions represent enzyme-complexes, and arrows indicate dissociation of a complex-releasing substrate, S, or product, P.

$$t_p = \sum_1^n (t_v + t_r)$$

when there are n complexes formed between successive product formations. These parameters are stochastic variables, not constants, and are assumed to be independent. However, because of the proximity of a dissociated substrate to the enzyme, for dilute substrate concentrations the second vacancy time may be less than the initial vacancy time after product release. Over a long time interval, these variables can be represent by their expected values, which are denoted by capital letters as

T_v = average enzyme vacancy time;

T_r = average ES complex residence time;

N= average number of substrate bindings between product formations;

T_p= average time between product formations.

Thus, the mean time between successive bindings is

$$T_p = N\,(T_v + T_r).$$

The reaction velocity per enzyme (molecules/s), on average, is therefore

$$v_e = \frac{1}{N(T_v + T_r)} = \frac{P_a}{T_v + T_r}, \qquad (2)$$

where $P_a = 1/N$ denotes the probability that an ES complex is productive.

The physiochemical reaction milieu influences vacancy time through the delivery rate of substrate to the enzyme, whereas the residency time primarily reflects the innate chemical specificity of the enzyme for substrate, e.g., the Gibbs free energy of a complex and the enzyme's catalytic efficiency, although it has been suggested that these also can be affected by environmental factors like the fluid viscosity (Somogyi et al., 1978).

The maximum velocity per enzyme, $v_{max} = P_a/T_r$, occurs when the enzyme is never vacant, $T_v = 0$. A second observation derived from Eq. 2 provides a more insightful interpretation of the substrate concentration traditionally referred to as the $K_{m\text{-app}}$.

In a system without competitive inhibition, the ***apparent*** K_m *is the substrate concentration for which the mean vacancy time equals the mean residence time for an enzyme-substrate complex*:

$$K_{m\text{-app}} = [S] \quad \textit{at which} \quad T_v = T_r.$$

This relationship emphasizes the fact that the $K_{m\text{-app}}$ is as much an indicator of the reaction system's substrate deliv-

ery process as it is an indicator of the enzyme's affinity for the substrate and catalytic mechanisms.

Vacancy time analysis

Enzyme vacancy time depends on the rate of substrate delivery to the enzyme, R_c, and the probability that a substrate-enzyme interaction results in complex formation, P_{on}. Usually, $P_{on} < 1$ and, therefore, the average vacancy time will exceed the average collision time, T_c, because $T_v = T_c/P_{on}$. These time parameters are functions of the general reaction conditions, such as temperature, convection, etc. and of the *effective* substrate concentration or density (which will be considered in detail in the Discussion). Loosely, this is the concentration/density of substrate in a region surrounding the enzyme that may be characterized by the fact that changes in substrate concentration/density outside of this region will not alter appreciably the observed velocity. The *effective* concentration is usually directly dependent on the bulk substrate concentration; however, this may not be the case for an *effective* density due to surface saturation. Writing $T_c([S])$ to emphasize this functional dependence, Eq. 2 can be expressed as

$$v_e = \frac{P_a}{T_r + T_c([S])/P_{on}} \quad \text{or} \quad v_e = \frac{P_a \cdot P_{on}}{T_r \cdot P_{on} + T_c([S])}. \tag{3}$$

MACROSCOPIC SYSTEM MODELS BASED ON THE MICROSCOPIC MODEL

Macroscopic velocity models are formed by establishing the functional form of $T_c([S])$ for specific reaction environments and by multiplying Eq. 3 by the enzyme concentration (or density) to give the overall system velocity. As we show next, when the reaction occurs under ideal conditions this leads to a system velocity consistent with the Michaelis-Menten equation. However, this will not be the case when substrate delivery is mediated or the enzyme's movement or accessibility is modified.

A macroscopic model for simple diffusion controlled substrate delivery: an alternative view of the classical Michaelis-Menten kinetics

Consider a simple ideal reaction enzyme-substrate diffusion-controlled reaction in a fluid with no convection. Assuming independent diffusion, the probability of a single substrate colliding with a given enzyme in any time interval is proportional to the interval length. The proportionality constant, λ, is a function of the reaction volume and the diffusion coefficients. The distribution of times between successive collisions is then exponential, with mean time to a collision $1/\lambda$. For an ensemble of independently diffusing substrate molecules the rate of collision with the enzyme is $\Gamma = \lambda \cdot [S] \cdot N_A$, where N_A is Avogadro's number. The mean time between collisions of any substrate with the enzyme is then

$$1/\Gamma = 1/(R_c [S]),$$

where $R_c = \lambda \times N_A$ is the collisions/s/mole. Thus, $T_c([S]) = \{R_c \cdot [S]\}^{-1}$ and substituting into Eq. 3 gives the microscopic enzyme velocity equation:

$$v_e = \frac{P_a}{T_r + \{R_c \cdot [S] \cdot P_{on}\}^{-1}}. \tag{4}$$

The average rate at which ES complexes dissociate or form product, k_{off}, provides an upper bound for v_e. Setting $k_{off} = T_r^{-1}$, Eq. 4 can be written as

$$v_e = \frac{P_a \cdot k_{off} \cdot [S]}{[S] + k_{off}\{R_c \cdot P_{on}\}^{-1}}. \tag{5}$$

The corresponding macroscopic initial velocity model for an ideal system with enzyme concentration $[E_0]$, assuming that each enzyme generates product independently, and substrate concentration $[S] \approx [S_0]$, is

$$v_0 = v[E_0] = \frac{P_a \cdot k_{off} \cdot [E_0][S_0]}{[S_0] + k_{off}\{R_c \cdot P_{on}\}^{-1}}. \tag{6}$$

Comparing the structural form of Eq. 6 to the classical Michaelis-Menten Eq. 1, the parameters associated with Model I can be identified with the microscopic model parameters. Because the microscopic off rate is the sum of the first-order off rates of Model I, for an ideal system

$$\text{Michaelis Constant:} \quad K_m = \frac{k_{off}}{R_c \cdot P_{on}} = \frac{k_2 + k_{cat}}{R_c \cdot P_{on}} \tag{7}$$

and

$$\text{Maximum Initial Velocity:} \quad V_{max} = P_a \cdot k_{off} \cdot [E_0]. \tag{8}$$

The unidirectional rate constants of Model I can be expressed conversely in terms of the microscopic model parameter as

$$\begin{aligned} k_1 &= R_c \cdot P_{on} \\ k_2 &= (1 - P_a)k_{off} \\ k_{cat} &= P_a \cdot k_{off}. \end{aligned} \tag{9}$$

Thus, the "catalytic efficiency" ratio k_{cat}/K_m is actually the product of the efficiency of the enzyme when complexed with a substrate, P_a, the ease with which the enzyme binds substrate, P_{on}, and the fundamental substrate delivery rate, R_c, which in turn reflects the substrate diffusion rate and concentration.

A microscopic model with a competitive inhibitor

A similar microscopic model can be developed for a reaction system that includes a species of molecules, B, that also

bind to the enzyme and thereby inhibit the reaction. In this case, some of the nonproductive complexes illustrated in Fig. 1 may represent EB-complexes. We therefore introduce a complete compliment of basic model parameters, with subscripts S or B as appropriate. For example, the mean residence times $T_{r.S}$ and $T_{r.B}$ refer to ES and EB complexes, respectively. The average time between product formation is then

$$T_P = N_S \cdot T_{r.S} + N_B \cdot T_{r.B} + N \cdot T_v,$$

where N_S and N_B denote the average number of ES and EB complexes between successive product formations and $N = N_S + N_B$. The mean vacancy time T_v depends on the *effective* concentrations and mode of delivery of both S and B molecules and on their respective binding probabilities. Clearly, if B represents the product of the reaction, its *effective* concentration will greatly exceed its bulk concentration because upon formation a product is ipso facto very close to an enzyme that has just become vacant.

A macroscopic velocity model for an ideal system with competition

For an ideal diffusion system with independently diffusing substrate and competing molecule B, the rate of collision of either species with an enzyme is the sum of the rates λ_S and λ_B associated with the individual species. Thus, in a system with bulk concentrations $[S]$ and $[B]$, the time to a first collision with the enzyme of either molecule is a function of their independent collision rates, $R_{c.B}$ and $R_{c.S}$:

$$T_c = \{R_{c.S}[S] + R_{c.B}[B]\}^{-1}.$$

However, the mean vacancy time is not simply T_c^{-1} because it depends on both binding probabilities, $P_{on.S}$ and $P_{on.B}$. The general macroscopic model will be complicated if these are different, because then the model must include all possible sequences of S-E and B-E collisions that do not result in complex formations, followed by the formation of EB or unproductive ES complexes, with their probabilities of occurrence, repeated until a productive ES complex forms. To avoid this complexity, assume that $P_{on.S} = P_{on.B} = 1$. Because the independent collision rates are

$$R_{c.S} = \lambda_S N_A \quad \text{and} \quad R_{c.B} = \lambda_B N_A,$$

the resulting enzyme velocity equation is

$$v_e = \frac{1}{T_{r.S}N_S + T_{r.B}N_B + N \cdot \{[R_{c.S}[S] + R_{c.B}[B]]\}^{-1}},$$

where

$$N_S = \frac{1}{P_a} \qquad N_B = \frac{\lambda_B[B]}{\lambda_S[S]} \cdot N_S \quad \text{and} \quad N = N_S + N_B.$$

This enzyme velocity equation can be expressed as

$$v_e = \frac{P_a k_{off.S}[S]}{[S] + \frac{k_{off.S}}{R_{c.S}} + \frac{k_{off.S}}{k_{off.B}} \frac{R_{c.B}}{R_{c.S}}[B]},$$

which reduces to Eq. 5 when $[B] = 0$. Introducing apparent Michaelis constants, $K_{M.S}$ and $K_{M.B}$, as in Eq. 7, as the ratio of the respective off rates and rates of collision with the enzyme, the macroscopic model for an ideal reaction with competitor has the Michaelis-Menten form

$$v = \frac{k_{cat} \cdot [S] \cdot [E_0]}{[S] + K_{M.S}\left(1 + \frac{[B]}{K_{M.B}}\right)}.$$

Reactions with "facilitated" substrate delivery

In blood coagulation and many other physiological processes, substrate delivery to immobilized enzymes is by the blood. These in vivo reactions may involve very dilute substrate concentrations and enzymes, such as the tissue factor molecule, tightly bound to cells or lipids (Nemerson, 1988). Blood flow can facilitate substantially the "delivery" of substrate to a static enzyme, thereby increasing the *effective* substrate concentration. Restricting the enzyme to a surface can increase the substrate-enzyme collision rate through reduction in the dimensionality of the diffusive path and increase the *effective* substrate concentration when the substrate also binds to the surface. Surface binding constricts the enzyme's rotational diffusion, which can increase P_{on}, and can enhance its efficiency P_a by inducing conformational changes to its active site. Reactions involved in the tissue factor pathway of coagulation have been investigated in an experimental flow system analogous to a blood vessel (Gemmell et al., 1990). A macroscopic model for such systems requires a description of the substrate transport in a flow system.

Analytical descriptions of flow reactors involve coupling the convective-diffusive mass transport of substrate with its depletion via activation by the enzymes. The simplest model, mathematically, considers an idealized laminar flow system with a totally reactive tube surface, so that the reaction rate varies as the rate of mass transfer of substrate to the tube wall. This rate varies with the distance x from the tube inlet and is given by a convolution integral

$$N(x) = -\int_0^x K_L(x - \zeta) \frac{dS_w(\zeta)}{d\zeta} d\zeta, \qquad (10)$$

where $S_w(\zeta)$ is the effective substrate concentration near the wall at $x = \zeta$ and K_L is the transfer rate to the wall in a diffusion-controlled environment, which is equivalent to the delivery rate λ in the ideal static model (Kobayashi and Laidler, 1974). For a tube of radius R and length L, the

transport rate of a substrate with diffusion coefficient D and a mean flow velocity v is

$$K_L = \frac{1}{\Gamma(1/3)}\left[\frac{12D^2v}{RLx}\right]^{1/3}.$$

The assumption that any substrate reaching the surface is instantly activated corresponds to setting $P_{on} = 1$ and $T_r = 0$. The corresponding macroscopic model is derived by setting $T_c = 1/K_L(x)$ in Eq. 3 where x is the distal coordinate of the enzyme. To establish a system velocity, one must first establish the *effective* substrate concentration, S_w, by solving an integral-differential equation formed by equating $N(x)$, given by Eq. 10, to the rate of change in S_w, which is provided by the enzyme velocity equation. For the case of Michaelis-Menten kinetics, the integral equation can be transformed into an alternative form and solved by series methods (Rusu, 1995). Simple analytical solutions are not possible, and as an alternative approach we have simulated such systems (Gentry and Ye, 1995). With simulations, one does not have to assume totally reactive surfaces and, in fact, can demonstrate that the reaction kinetics of a flow system depends on both the density and distribution of the enzymes. The point to be gleaned from this analysis is that in a flow-reaction system the *effective* substrate concentration experienced by enzymes is facilitated by convective-diffusive substrate transport and varies with the enzyme's location because of substrate depletion in the boundary layer.

DISCUSSION

The classical Michaelis-Menten equation describes remarkably well the velocities of ideal enzyme reactions in diffusion-controlled environments, particularly for in vitro experiments with purified proteins. It is commonly recognized, however, that it does not describe adequately nonideal reactions in nonstatic environments or when enzyme-substrate interactions are not diffusion-controlled. To establish a more robust model of enzyme kinetics, we have introduced a sequential microscopic model that is mechanistic and reflective of the actual events that effect reaction kinetics. An important feature of this model is that it isolates the substrate delivery components from the catalytic characteristics of the enzyme. As a working model, it uses a set of parameters that can be studied independently by various experimental techniques to characterize an enzyme's kinetic properties.

To model reaction systems with various substrate delivery mechanisms or constraints on enzyme accessibility, associated macroscopic models can be established on the basis of our microscopic model. For an ideal diffusion-controlled system, the corresponding macroscopic model is consistent with the Michaelis-Menten equation. In this case, the macroscopic and Microscopic Model parameters can be identified, as in Eqs. 7–9, with the standard unidirectional rate constants and the classical Michaelis-Menten parameters. For other reaction systems, such as those with facilitated or restricted substrate delivery, the associated system velocity equation can only be established by a detailed analysis of the enzyme-substrate collision rate. For simpler systems, the appropriate model parameters can be identified, but for more complicated environments such theoretical analysis is intractable. In these situations, an alternative strategy is to study the reaction system via computer models, based on our microscopic model, in which the substrate transport can be simulated. Such simulations can provide extremely detailed information about a reaction and provide insight into molecular level interactions that cannot be observed experimentally. Although such simulations do not establish new mechanisms, they can be used to compare competing theories about a reaction (see, for example, our companion paper).

Effective substrate concentrations

The rate of substrate-enzyme collisions is a key parameter of our microscopic model that depends on the substrate delivery mechanism and on the *effective* substrate concentration, i.e., the apparent substrate concentration that an enzyme actually experiences. In nonideal systems, different enzymes may experience different *effective* concentrations. An important facet of this problem for surface-mediated reactions is that if a priori binding of substrate to the surface is a requirement for forming an ES-complex, it is the surface density of the substrate, rather than its fluid concentration, that is critical. Various approaches to this have been considered. One approach has been to define a "local" concentration by identifying a local reaction volume and extrapolating from velocity data what apparent concentration of substrate would be required in this volume to achieve the observed velocity with predetermined kinetic parameters. This approach has been taken to introduce shells around lipid vesicles in which the substrate concentration and the K_m and k_{cat} parameters are presumably different from those for the bulk reaction volume (Nesheim et al., 1984). Another approach is to define a "recognition volume" about an enzyme that is small enough that an ES-complex will form whenever a substrate enters this volume (Welch et al., 1983). We suggest that both of these are inappropriate, because the first does not recognize the linkage between the mechanism of substrate movement and the true catalytic rate of the enzyme, whereas the second is too restrictive. A more functional approach is to define a "region of influence" as the volume/surface area of the reaction system from which a substrate molecule can reach the enzyme in a fixed influence time, T_i. The choice of T_i is arbitrary but, because $T_v = T_r$ when $[S] = K_{m.app}$ (by definition), a reasonable choice is to set $T_i = m \times T_r$ for $m \gg 1$. This essentially would eliminate consideration of substrate that on average could not effect the reaction velocity. The *effective* concentration/density is then that of this region of influence.

When extrapolating the enzyme velocity v_e to form a macroscopic system velocity, it is often assumed that the enzymes in the system function independently of each other. Although in ideal fluid systems it is often assumed that the enzyme concentration is quite low, this need not be the case for coagulation reactions in which surface-bound enzymes may cluster (Andree, et. al., 1995). When the enzyme density is great enough, individual enzyme's region of capture will intersect resulting in enzyme-competition for the substrate. This will be most pronounced when $[S]$ is low. Generally, at less than saturating substrate concentrations, the reaction velocity associated with a cluster of enzymes will not be the product of a single-enzyme velocity and the number of enzymes in the cluster.

It has long been recognized that the rate of substrate delivery may be increased for surface-bound enzymes because of the reduction in diffusion dimensionality for substrates that bind to the surface and then diffuse to the enzyme (Noyes, 1961; Berg and von Hippel, 1985; Adam and Delbrück, 1968; Berg, 1985). However, on a surface the maximum velocity will depend on both the enzyme and substrate surface density. Because of the saturability of surface-binding sites, a situation that simply does not arise in fluid environments, no matter how high the fluid concentration $[S]$ is raised, the surface density cannot exceed a specific upper bound. If ES complexes can only be formed from surface-bound substrate, then enzymes may never experience the high substrate collision frequency required for the velocity to approach V_{max}. This is particularly the case for clustered enzymes because enzymes interior to the cluster clearly cannot be as accessible as those on the perimeter. Surface saturation resulting from increasing $[S]$ can actually decrease the *effective* substrate density as surface saturation reduces surface diffusion and, thus, the region of influence.

In conclusion, we suggest that the proposed microscopic model of enzyme kinetics offers flexibility in describing the catalytic properties of an enzyme-substrate reaction and allows the separation of the truly kinetic properties of the molecular reaction from the mechanistic aspects of the substrate delivery system. As such, the microscopic model is more functional and applicable to a wider class of enzyme reactions systems than the classical Michaelis-Menten model. The parameters of the microscopic model correlate directly with physical factors that significantly effect reaction kinetics. This model is robust, in the sense that when the basic kinetic parameters, other than R_c, are established for one reaction system, they will apply to the same reaction in other systems with different substrate transport mechanisms. This may allow more accurate a priori prediction of system kinetics for nonstandard systems, both through theoretical analysis and using computer simulations.

This work supported in part by grant HL 29019 from National Institutes of Health and grant OPG0009100 from the Natural Science and Engineering Research Council, Canada.

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