

## Effect of macromolecular crowding on the rate of diffusion-limited enzymatic reaction

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**Abstract.** The cytoplasm of a living cell is crowded with several macromolecules of different shapes and sizes. Molecular diffusion in such a medium becomes anomalous due to the presence of macromolecules and diffusivity is expected to decrease with increase in macromolecular crowding. Moreover, many cellular processes are dependent on molecular diffusion in the cell cytosol. The enzymatic reaction rate has been shown to be affected by the presence of such macromolecules. A simple numerical model is proposed here based on percolation and diffusion in disordered systems to study the effect of macromolecular crowding on the enzymatic reaction rates. The model qualitatively explains some of the experimental observations.

**Keywords.** Enzyme kinetics; Monte Carlo; percolation; random walk; obstacle.

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### 1. Introduction

The aqueous phase of cell cytoplasm is crowded with macromolecules such as soluble proteins, nucleic acids and membranes [1]. The influences of such crowding on biochemical reactions inside physiological media are manifold [2]. Due to crowding, the average free energy  $\mu$  of a non-specific interaction between any molecule in the medium and a crowding molecule may change considerably which may influence the reaction activity  $\gamma = \exp(\mu/k_B T)$ , where  $k_B$  is the Boltzmann constant and  $T$  is the absolute temperature. Steric repulsion is the most fundamental of all interactions between macromolecules in solution at finite concentration and as an effect of such repulsion the macromolecules occupy a substantial volume fraction in the cell interior [3]. Significant volume fraction of macromolecules in the medium imposes a constraint on introducing any new macromolecule. As a consequence of crowding, macromolecular association reactions become increasingly favourable. Because of crowding, the molecular diffusion in the medium is expected to be anomalous [4]. The effect of macromolecular crowding on different kinetic steps of enzyme catalysis such as formation of enzyme–substrate complex and enzyme–product complex

were analysed through different equilibrium thermodynamic models [5]. A number of approaches have been proposed to study the effects of macromolecular crowding on the reaction kinetic rate laws such as the law of mass action [5], fractal-like kinetics [6], the power-law approximation [7], stochastic simulation [8] and lattice gas simulation [9]. In these analytic and numerical models, the influence of macromolecular crowding on both equilibrium thermodynamics and reaction rates were addressed and it was observed that the rate decays exponentially with time as expected in equilibrium kinetics. The influence of macromolecular crowding on the enzymatic reaction rates has been investigated experimentally using a variety of crowding agents [10]. These studies have also indicated a significant influence of crowding on the rate parameters of the enzymatic reaction. It was found that the effect of crowding on the enzymatic reaction may be different depending on whether the product formation in the enzyme reaction is limited by the diffusional encounter of substrate and enzyme or the formation of the transition state complex, an association of enzyme and substrate. Moreover, molecular diffusion is known to be the major determinant of many cellular processes and plays a key role in cell metabolism where the encounter of the free substrate with an active site of the enzyme is often the rate determining step. However, how the kinetics of an enzymatic reaction is dependent on the size and concentration of the crowding macromolecules is still not fully understood. The macromolecular crowding till date remains an underappreciated and neglected aspect of the intracellular environment [11]. It is hence essential to understand the experimental observations from microscopic origin.

In this paper, an approach based on non-equilibrium dynamics of enzymatic reactions in the diffusion-limited regime is considered. The aim is to understand qualitatively the influence of inert macromolecular crowding on the diffusion-limited enzymatic reactions governed by non-equilibrium thermodynamics. A simple numerical model in two dimensions (2d) based on molecular diffusion in disordered systems coupled with enzymatic reaction is proposed here. The disordered system is modelled by percolation clusters [12]. It is predicted that the rate of a diffusion-limited enzyme-catalysed reaction will experience a monotonic decrease with increase in the fractional volume occupancy of the crowding agent. The model explains qualitatively certain experimental observations.

## **2. The model**

In brief, the enzyme kinetic reaction in the cell cytoplasm can be described as substrate molecules diffusing through crowding macromolecules and binding to the active site of the freely floating enzymes. Subsequently, a product is formed if the reaction is energetically favourable and this product diffuses through the same crowd of macromolecules. The classical Michaelis–Menten equilibrium enzyme kinetic reaction is given as [13]



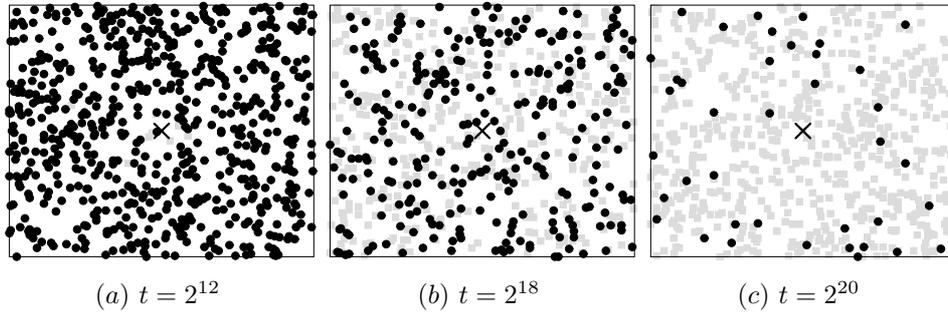
where E represents enzyme, S represents substrate, P represents product and ES is the intermediate enzyme–substrate complex.

### *Rate of diffusion-limited enzymatic reaction*

In the present model, the reaction is limited by diffusion only and the formation of the transition state complex ES is not taken into account. The conversion of substrate to product is also assumed to be instantaneous. Note that diffusion has the slowest time scale in this problem. Hence, the above enzymatic reaction reduces to an irreversible one as



The final equilibrium state corresponds to conversion of all substrates to products. A Monte Carlo (MC) algorithm has been developed to study diffusion-limited enzymatic reaction as in eq. (2) in the presence of inert macromolecules. The algorithm is developed on the 2d square lattice of size  $L \times L$ . For simplicity, the motion of the macromolecules is ignored and these act as immobile and inert obstacles. The inert obstacles do not interact either among themselves or with the substrate or product. The obstacles (O), enzyme (E), substrate (S) and product (P) are all represented as point particles in this model. It is also assumed that there exists only one immobile enzyme in the whole system. The enzyme is placed at the centre of the lattice. After placing the enzyme, the obstacles and the substrates are distributed randomly over the lattice sites with their specified concentrations  $C_O$  and  $C_S$  respectively. A random number  $r$  is called from a uniform distribution of random numbers between 0 and 1 corresponding to each lattice site. If  $r \leq C_S$ , the site is occupied with a substrate and if  $C_S < r \leq a_f$  the site is occupied with an obstacle where  $a_f = C_S + C_O$  is the area fraction. The excluded volume condition is maintained, i.e., at any instant of time one lattice site cannot be occupied by more than one molecule of the same or different species. The substrate molecules diffuse through the space not occupied by the obstacles which will be referred as empty space later. As soon as an S reaches E, a product P is produced with unit probability. The diffusion of substrate or product in the system is modelled by simple random walk in the presence of obstacles or disorder. At each MC time step, all the random walkers (all S and P) make an attempt to move to one of their nearest neighbours. The destination site, a site out of the four neighbours, of a random walker is chosen calling a random number  $r$  uniformly distributed between 0 and 1. With respect to the present site, the destination site is going to be on the left if  $0 < r \leq 1/4$ , it is at the top if  $1/4 < r \leq 1/2$ , it is on the right if  $1/2 < r \leq 3/4$ , and it is at the bottom if  $3/4 < r \leq 1$ . The destination site could be either empty or occupied by S, P, O or E. Depending on the status of the destination site, there are then four possibilities: (a) if the destination site is empty, the present S or P moves to the destination site, (b) if the destination site is occupied by an S or P, S or P remains on the same site, (c) if the destination site is occupied by an O, P or S also remains on the same site, and (d) if the destination site is occupied by the enzyme E, P remains on the same site but S is converted to P with unit probability. If all the molecules of S and P are checked for an attempt of motion, time  $t$  (the MC time step) is increased to  $t + 1$ . To ensure percolation of the substrate molecules, the maximum area fraction  $a_f = C_S + C_O$  is taken as 0.4, far below the percolation threshold,  $\sim 0.59$  on the square lattice [12]. Note that, the present non-equilibrium diffusion-limited enzymatic reaction model is substantially different from that of lattice gas model incorporating equilibrium reaction rates proposed by Schnell and Turner [9] which leads to an unusual equilibrium constant equal to zero in the crowded environment [14].



**Figure 1.** The system morphology on a  $256 \times 256$  square lattice is shown at three different times (a)  $t = 2^{12}$ , (b)  $t = 2^{18}$  and (c)  $t = 2^{20}$  for substrate concentration  $C_s = 0.01$  and area fraction  $a_f = C_s + C_o = 0.1$  ( $C_o = 0.09$ ). The black dots represent the substrates and the gray boxes represent the products. For clarity, obstacles are not shown. The enzyme is represented by a cross at the center of the lattice. Products are formed due to the enzymatic reaction and in the long time limit almost all the substrates are converted into products.

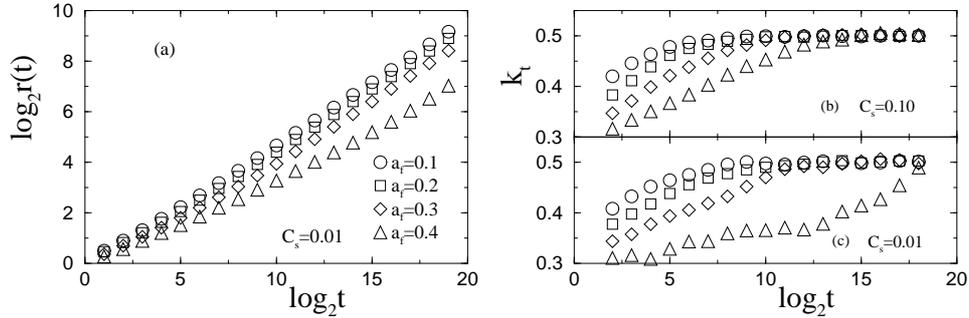
Cyclic boundary condition has been applied in the motion of S and P. The simulation has been performed up to  $10^6$  MC time steps on a  $256 \times 256$  square lattice. The data are averaged over 100 ensembles. The time evolution of the system morphology for  $a_f = 0.1$  with  $C_s = 0.01$  is shown in figure 1 at three different times. The black dots represent the substrates and the gray boxes represent the products. For clarity, obstacles are not shown. It can be seen that the initial black dots are converted to gray boxes at the end. That means, the substrate molecules are diffusing, reacting with the enzyme, and are getting converted into products. In time, almost all the substrate molecules are converted to products and the product molecules also diffuse and spread all over the space uniformly. Lin and coworkers [15] simulated some elementary kinetic reactions like  $A + B \rightarrow 0$  with no obstacles under reflective boundary condition and observed Zeldovich cross-over (segregation of A and B) [16]. Such segregation is not observed with periodic boundary condition in the present simulation. Effect of impenetrable boundary on diffusion-limited reaction like  $A + A \rightarrow 0$  leads to different behaviour depending on different boundary conditions [17].

### 3. Results and discussion

Classical diffusion of a tracer particle in disordered systems has already been studied extensively and the results are well-understood [18]. Generally the diffusion is modelled by random walk and the disordered system is modelled by spanning percolation clusters [12]. For studying diffusion, a quantity of interest is the root mean square (rms) distance  $r(t)$  covered by the diffusing particle in time  $t$ . The rms distance  $r(t)$  in 2d is given by

$$r^2(t) = 4D \times t^{2k}, \quad (3)$$

Rate of diffusion-limited enzymatic reaction



**Figure 2.** (a) Plot of diffusion length  $r(t)$  against time  $t$  in double logarithmic scale for different area fractions  $a_f$  keeping substrate concentration  $C_S$  at 0.01. (b) and (c) Plot of the local exponent  $k_t$  vs. time  $t$  for  $C_S = 0.1$  and  $C_S = 0.01$  respectively. The same symbol set of (a) has been used in (b) and (c) for different area fractions  $a_f = C_S + C_O$ .

where  $\mathcal{D}$  is the diffusivity of the system. The exponent  $k$  has a value  $1/2$  for diffusion on a regular lattice in the  $t \rightarrow \infty$  limit. On the percolation cluster, diffusion is found to be anomalous and the value of  $k$  becomes less than  $1/2$  [18]. The enzyme kinetic reaction inside a cell cytoplasm involves (i) diffusion of a large number of substrate molecules through the random structure of inert macromolecules, (ii) reaction with the enzyme to have products, and (iii) finally diffusion of products from the enzyme through the same macromolecular crowding. The diffusion process involved here is then a collective motion of a large number of particles in the presence of disorder which is a complicated process compared to diffusion of a single tracer particle in a disordered medium. Self-diffusion is expected to play a non-trivial role along with the diffusion of S or P in the presence of disorder in these systems. In order to check whether the enzyme kinetic reaction considered here is diffusion-limited or not, one needs to analyse the diffusive behaviour of either the substrates or the products. To calculate the average diffusion length of the product particles, the coordinates  $\{x_i(t), y_i(t)\}$  of each product  $i$  is recorded with time  $t$ . Time is measured starting from the birth of a product. The rms distance  $r(t)$  travelled in time  $t$  is then calculated as

$$r^2(t) = \frac{1}{N_P(t)} \sum_{i=1}^{N_P(t)} [\{x_0 - x_i(t)\}^2 + \{y_0 - y_i(t)\}^2], \quad (4)$$

where  $(x_0, y_0)$  is the coordinate of the enzyme at the centre of the lattice and  $N_P(t)$  is the number of products of age  $t$ . The data are then sample averaged over 100 ensembles.

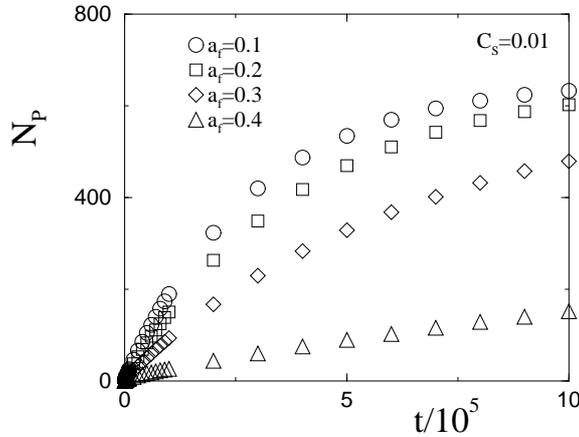
In figure 2a,  $r(t)$  is plotted against time  $t$  in double logarithmic scale for different area fractions  $a_f = C_S + C_O$  keeping the substrate concentration constant at  $C_S = 0.01$ . It can be seen that the magnitude of the diffusion length decreases with increasing  $a_f$ . The collective motion of the particles is then affected more and more by the presence of increasing inert macromolecules in the system. However, in order to check the diffusive behaviour of the particles one needs to estimate the exponent

$k$  defined in eq. (3). The local slope  $k_t = d \log_2 r(t) / d \log_2 t$  of the curve  $\log_2 r(t)$  vs.  $\log_2 t$  is determined by employing central difference method. In figures 2b and 2c,  $k_t$  is plotted against time  $t$  for two different substrate concentrations  $C_S = 0.10$  (b) and  $C_S = 0.01$  (c) for the same set of area fractions  $a_f$  as in figure 2a. The value of  $k_t$  saturates to  $1/2$  starting from a smaller value as  $t$  tends to a large value. Thus, a cross-over from sub-diffusive to diffusive behaviour has occurred for all area fractions in the long time limit. In the case of low substrate concentration  $C_S = 0.01$  and high area fraction  $a_f = 0.4$ ,  $k_t$  shows certain anomalous behaviour. Note that, at this parameter regime the macromolecular concentration is  $0.39$  which is just below  $1 - p_c \approx 0.41$  since the percolation threshold for a  $2d$  square lattice  $p_c \approx 0.59$ . The empty sites provide the connectivity for the substrate molecules all over the lattice. However,  $p_c$  is defined on an infinitely large system. For a smaller system, even at the concentration of  $0.39$  the connectivity of empty sites may be lost for some of the ensembles considered. Consequently, the product may be trapped in a localized region around the enzyme and this may be the reason behind the anomalous behaviour observed in this parameter regime.

Since the parameter regime here is limited by diffusion, the enzyme kinetic reaction is then expected to be diffusion-limited. Due to the enzyme kinetic reaction (given in eq. (2)) the substrates are converted to products in time with unit probability on their encounter. In order to characterize the enzyme kinetic reaction, the number of products  $N_P$  are counted as function of time  $t$ , the MC time step, for different substrate concentrations  $C_S$  and area fractions  $a_f = C_S + C_O$ . In figure 3, the product numbers  $N_P$  is plotted against time  $t$  for different area fractions  $a_f$  with  $C_S = 0.01$ . Initially,  $N_P$  increases linearly, then slows down and finally saturates in the long time limit. For low area fraction, it can be seen that the reaction is almost complete, i.e., most of the substrates given initially,  $N_S(0) = C_S \times L^2 \approx 655$ , are converted to products exponentially as in classical equilibrium Michaelis–Menten kinetics though in the present model a non-equilibrium kinetics is considered. However, note that there is a considerable decrease in the product yield with increase in area fraction and their profiles are found not to follow an exponential increase. It has already been predicted by numerical simulations that classical Michaelis–Menten kinetics may not apply to enzymatic reactions in crowded media [19]. In a 1d model of reaction diffusion with disorder, Doussal and Monthus [20] also found large time decay in the species density via real space renormalization group calculations. The macromolecular crowding then could have a considerable and non-trivial effect on the enzymatic reaction rate.

Initial rate of enzymatic reactions determines most of the molecular processes and thus is an important quantity to estimate. Since non-equilibrium enzymatic reaction is considered here, the reaction rate  $R$  is defined as the ratio of the number of products  $N_P$  to time  $t$  for 10% conversion of the substrates.  $R$  is then sample averaged. A similar analysis has also been performed for  $N_P$  vs.  $t$  plots corresponding to  $C_S = 0.1$  for different area fractions  $a_f$ . In figure 4a, the normalized reaction rate  $R_n = R/C_S$  is plotted against obstacle concentration  $C_O$  for two different substrate concentrations  $C_S = 0.01$  (circles) and  $C_S = 0.1$  (squares). Note that, area fraction  $a_f = C_S + C_O$  is not a good parameter to study the reaction rate since  $a_f$  will remain finite for finite  $C_S$  even at  $C_O = 0$ . In the inset,  $R_n$  is also plotted against  $C_O$  in semi-logarithmic scale. There are few things to notice. First, the reaction rate is

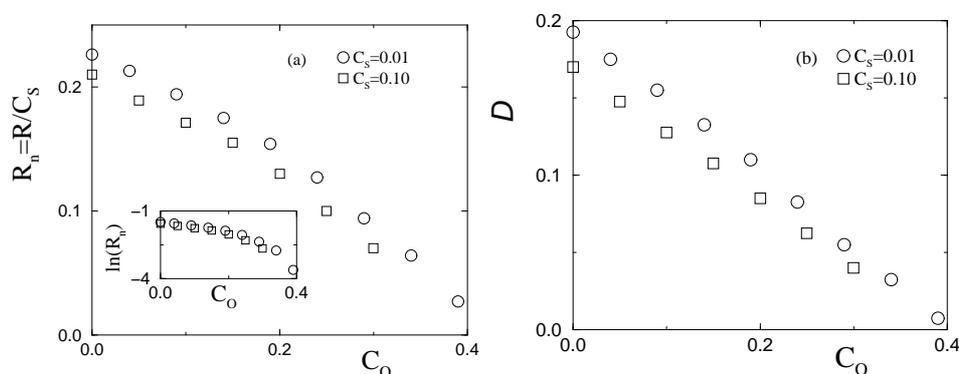
Rate of diffusion-limited enzymatic reaction



**Figure 3.** Plot of the number of products  $N_P$  vs. time  $t$  for different area fractions  $a_f = C_S + C_O$  keeping substrate concentration constant at  $C_S = 0.01$ .

decreasing with the increase in obstacle concentration  $C_O$  in a nonlinear fashion. Second, the reaction rates are different for a particular  $C_O$  even after normalizing by the substrate concentration  $C_S$ . Third, there is a monotonic decrease of  $\ln(R_n)$  for small  $C_O$  and deviates from linear decrease for large  $C_O$ . The decrease in reaction rate with increasing crowding concentration is expected and also observed in experiments [10,21]. However, the dependence of the rate on the crowding concentration is different from the prediction made by Minton [5] in the transition state as well as diffusion-limited enzymatic reaction in which a hump in the  $\ln(R_n)$  vs.  $C_O$  curve is expected for an intermediate  $C_O$ . Fourth, the normalized reaction rate is approaching zero as  $C_O$  approaches  $1 - p_c \approx 0.41$ . Beyond  $C_O = 0.41$ , the obstacles could block the spanning clusters of the empty sites. Consequently the enzymatic reaction will be localized and the reaction rate is expected to go to zero.

The above observations can qualitatively be understood in terms of diffusion and percolation phenomena. As  $C_O$  increases, diffusivity is expected to decrease because of the crowding due to obstacles. The influence of macromolecular crowding on the diffusion of solutes has been investigated in recent experiments utilizing different crowding agents and a reduced solute diffusion coefficient was observed with increasing size and concentration of crowding macromolecules [22]. An estimate of diffusivity  $\mathcal{D} = (dr^2(t)/dt)/4$  (as given in eq. (3)) has been made utilizing the data of diffusion length  $r(t)$  for different sets of substrates ( $C_S$ ) and obstacles ( $C_O$ ) concentrations. In figure 4b,  $\mathcal{D}$  is plotted against  $C_O$  for  $C_S = 0.01$  (circles) and  $C_S = 0.10$  (squares). Like reaction rate, diffusivity  $\mathcal{D}$  is also studied as a function of obstacle concentration  $C_O$  instead of  $a_f$ . It can be seen that  $\mathcal{D}$  also decreases with  $C_O$  in a nonlinear fashion. First of all, it is interesting to note that the whole dependence of  $R_n$  on  $C_O$  is in accordance with the behaviour of  $\mathcal{D}$  with  $C_O$ . The enzymatic reaction rate in this parameter regime is therefore mostly governed by diffusion and can be considered a purely diffusion-limited enzymatic reaction. It is important now to consider the low  $C_O$  values, especially the case of  $C_O = 0$ . For low  $C_O$  values,  $\mathcal{D}$  is slightly less for  $C_S = 0.1$  than that of  $C_S = 0.01$  for the



**Figure 4.** (a) Plot of normalized reaction rate  $R_n = R/C_S$  against  $C_O$  for two different  $C_S$  values 0.01 (circles) and 0.1 (squares).  $\ln(R_n)$  is plotted against  $C_O$  for the same  $C_S$  values in the inset. The same symbol set for different  $C_S$  values is used. (b) Plot of diffusivity  $\mathcal{D}$  against  $C_O$  for  $C_S = 0.01$  and  $C_S = 0.1$ . The same symbol set of (a) is used.

same  $C_O$ . This slight decrease in  $\mathcal{D}$  is due to diffusion through the self-crowding at higher  $C_S$ . On the other hand, the reaction rate at zero obstacle concentration is expected to be proportional to  $C_S$  and  $\mathcal{D}$  and  $R$  can be obtained as  $R \approx C_S \times \mathcal{D}$ . It can be seen that the normalized reaction rate  $R_n$  obtained here is very close to the corresponding values of  $\mathcal{D}$  at  $C_O = 0$  for both the  $C_S$  values. At  $C_O = 0$ , the self-diffusion of the substrate molecules eventually determines the reaction rates and might be responsible for a slight decrease in  $R_n$  for  $C_S = 0.1$  with respect to  $C_S = 0.01$  as seen in figure 4a. The effect of  $C_S$  in the absence of obstacles has been verified numerically for several higher values of  $C_S$  and a considerable effect of self-crowding has been observed on the reaction rate as well as on diffusivity. Note that,  $R_n$  values are slightly greater than  $\mathcal{D}$  for almost all values of  $C_O$  as it can be seen by comparing figures 4a and b. This might have happened firstly due to the fact that the initial yield occurs only from the locally available substrate molecules. The diffusion length of these substrate molecules are very less in comparison to the expected diffusion length. Secondly, one should note that the initial reaction rate for a given  $C_O$  has to be calculated keeping the substrate concentration  $C_S$  fixed. However, in the present model the substrate concentration is decreasing with time as the substrates are being converted into products. The effect will be predominant for low  $C_S$  and small system size. Consequently, the rate determination will be erroneous in the  $t \rightarrow 0$  limit due to low yield. Hence, extreme care has to be taken in determining the initial reaction rate. The enzymatic reaction considered here is completely diffusion-limited and the results obtained are explained in terms of diffusion in disordered systems. It is therefore intriguing to note that such a simple model of enzymatic reaction, based only on diffusion and percolation phenomena, is able to explain qualitatively the experimental observations [10,21] as well as results obtained in complicated models [5–9]. Hence, diffusion is observed to be playing a crucial role in determining the enzymatic reaction rates.

It should be emphasized here that enzymatic reactions occur in three-dimensional space in living systems whereas the simulation is performed in two dimensions here. The simulation results obtained here agree qualitatively with the experimental observations and it is expected that the features of the model will be retained in higher dimensions also. The main difficulty in 3d simulation is in parallel updating of a large number of substrate and product molecules during time evolution through a large number of MC time steps. Time required for the full conversion of substrate to product increases exponentially with the number of molecules ( $N_S = C_S \times L^d$ ) which strongly depends on the dimensionality of space for a fixed substrate concentration. However, for quantitative comparison of the results obtained in simulation with that of experiments, the model must be extended to three dimensions.

The biochemical events in the densely crowded mitochondrial matrix, the site for TCA cycle and fatty acid oxidation pathway are largely governed by large macromolecules of various sizes. It is thus important to investigate the influence of crowding as exerted by macromolecules of different sizes. A decrease in reaction rate has been observed in experiments for varying obstacle sizes keeping the obstacle concentration constant [21]. It seems that the complex interaction between obstacles and the substrate is size dependent and might be governing the enzymatic reaction rate. It is expected that the diffusion of substrates across large macromolecules might be slow due to the complex interactions with the obstacles. In the present model of enzymatic reaction, this complex interaction between obstacle and substrate may be incorporated by introducing a residence time for the substrate molecules at each encounter with the obstacle. A slowing down in the reaction rate with increasing residence time has been observed in the simulation in accordance with the experimental results [21]. The details will be reported elsewhere.

#### **4. Summary**

The effect of macromolecular crowding on the enzymatic reaction rates has been modelled by an MC algorithm based on diffusion and percolation phenomena. The substrates, products, obstacles and enzyme all are represented by point particles. A single immobile enzyme is considered and placed at the centre of the lattice. The obstacles and the substrates are distributed randomly with their specific concentrations following a uniform distribution of random numbers between 0 and 1. The obstacles remain immobile throughout the simulation. It is found that the reaction is solely diffusion limited under these conditions. The diffusion of substrates and products are modeled by a collective random walk. The products form gradually and subsequently almost all the substrates are converted into products after a long time. The initial reaction rate has been estimated for different substrate and obstacle concentrations. The normalized reaction rate has a nonlinear dependence on the obstacle concentration and found slightly dependent on the substrate concentration. The dependence of reaction rate on the substrate as well as obstacle concentrations is then qualitatively understood with the help of diffusion and percolation theory. The results qualitatively explains the experimental observations.

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