Lattice Model of Diffusion-Limited Bimolecular Chemical Reactions in Confined Environments

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We study the effect of confinement on diffusion-limited bimolecular reactions within a lattice model where a small number of reactants diffuse among a much larger number of inert particles. When the number of inert particles is held constant, the rate of the reaction is slow for small reaction volumes due to limited mobility from crowding and for large reaction volumes due to the reduced concentration of the reactants. The reaction rate proceeds fastest at an intermediate confinement corresponding to a volume fraction near 50%. We generalize the model to off-lattice systems with hydrodynamic coupling and predict that the optimal reaction rate for monodisperse colloidal systems occurs when the volume fraction is approximately 19%. Finally, we discuss the implications of our model for bimolecular reactions inside cells and the dynamics of confined polymers.

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It is a somewhat surprising fact that the total concentration of protein within a cell rivals that within a protein crystal [1]. This highly crowded environment plays an important role in dynamical processes, such as chemical reactions, and thermodynamic properties, such as chemical equilibria, observed *in vivo* [2]. While the volume fraction of macromolecules within the cell may exceed 30%, at any given time there may be just a few copies of a given protein corresponding to a concentration of a few nanomolars for a cell of volume 1 μm^3 [3,4]. This is in stark contrast to *in vitro* experiments, where the reactants are present at a relatively high concentration with a negligible level of crowding molecules. Therefore, caution is required when interpreting *in vivo* biochemical experiments as it is not always obvious what effect the presence of crowding molecules will have. For example, the rate of a reaction may be increased if the crowding favors a compact transition state, decreased if the reaction is diffusion-limited, or unaffected if the reactants are small compared to the crowding species [5].

In this Letter, we study the effect of crowding on reaction rates in a finite system with fixed numbers of crowding and reacting particles. This is in contrast to a typical *in vitro* experiment, where the level of crowding is adjusted by adding inert particles to the system. However, for *in vivo* experiments the number of particles in the cell is often fixed, and their density can be adjusted by osmotic shifts of the cell volume. In this situation the concentration of reactants and the degree of crowding are not independent as is implicitly assumed in previous work [1–5]. Instead, they change concomitantly with the volume of the system, and this, we find, leads to a nonmonotonic dependence of the reaction rate on the volume. This nonmonotonicity is the result of two distinct dynamical regimes. When the system volume is very large, the effect of the crowding particles is negligible. Therefore, the rate of reaction is dictated by the time required for the reactants to diffuse the mean separation between reactive particles which increases with the system size. We will call such systems ''concentration-limited.'' At the opposite extreme is the case where the system volume is very close to the sum of the total volume taken up by the reactant and crowding particles. In this case the separation between reactants may be quite small, but the reaction proceeds slowly because the high density of crowding particles severely impedes the diffusion of the reactants. Such systems are ''crowding-limited.'' Between these two limits, there is an optimal volume at which the reaction proceeds the fastest. We note that the existence of a maximum rate as a function of confinement is predicated on the assumption that the particle number is kept fixed while the system volume is changed. Varying the system volume while keeping the concentration fixed (or vice versa) will result in a monotonic change in the reaction time.

The competition between the concentration-limited and crowding-limited regimes may be understood from a simple argument. Take the bimolecular reaction $A + B \rightarrow A$, where the surface of the A particles, taken to be a sphere, absorbs B particles. The flux of particles at the A surface (i.e., the reaction rate) is $4\pi acD$, where a is the radius of the A particle, and the concentration of B particles far from the sphere, c, is inversely proportional to the volume of the system $c \propto R^{-3}$[6]. The effect of crowding particles that do not react with the absorbing boundary may be included through a rescaling of the diffusion constant D provided the distance the reactant particles must travel is large compared to the mean spacing between crowding particles. For the case of a lattice-based system, a mean-field diffusion constant (which is a good approximation when the crowding particles move much faster than the reactants) is achieved by multiplying the ''bare'' diffusion constant D_0 by the success probability for an attempted hop to a neighboring site. This results in a diffusion constant $D \approx D_0 p$, where $p = (1 - c)$ is the probability that a neighboring site is unoccupied and c is the number density of particles on the lattice. Therefore, the flux at the absorbing surface

scales as $a(1 - Nb^3R^{-3})/R^3$, where N is the total number of particles in the system and b is the lattice constant. This expression is nonmonotonic in R, and it has a maximum when the system size is such that the particle density is $1/2$.

In order to go beyond this simple argument, we explore the transition from concentration-limited reactions to crowding-limited reactions using a model with two reactant particles that react instantaneously upon contact confined to a spherical volume in the presence of inert crowding particles. The reaction rate is then the inverse mean first passage time for the particles to find each other. If we make the further simplification of holding one of the reactants stationary, the mean first passage time is given by

$$\bar{\tau} = \frac{1}{V}\int_V \tau(\vec{x}) d^d\vec{x}, \tag{1}$$

where the integral is over the d-dimensional volume of the system. Assuming that the motion of the mobile reactant is diffusive, $\tau(\vec{x})$, the average time for it to reach the stationary particle starting from position $\vec{x}$, satisfies the equation [7]

$$D\nabla^2\tau(\vec{x}) = -1. \tag{2}$$

D is the effective diffusion constant in the presence of crowders and is a function of the crowder concentration. Equation (2) is subject to a reflecting boundary condition at the system periphery and an absorbing boundary condition at the surface of the stationary reactant.

If we place the fixed reactant at the center of the spherical domain, as shown in Fig. 1, then Eq. (2) is exactly solvable with the result (in two and three dimensions, respectively)

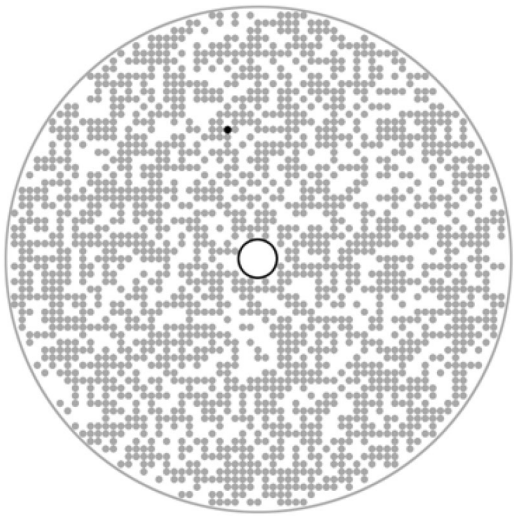

FIG. 1. Snapshot from the simulation described in the text. The stationary target is indicated by the black circle in the center, and the mobile reactant is the black dot halfway between the target and the top of the outer circle. Also shown are the 2000 inert crowding particles (gray).

$$\tau_{2d}(r) = \frac{1}{D}\left[\frac{R^2}{2}\ln\left(\frac{r}{a}\right) - \frac{r^2}{4} + \frac{a^2}{4}\right], \tag{3}$$

$$\tau_{3d}(r) = \frac{1}{D}\left(-\frac{r^2}{6} - \frac{R^3}{3r} + \frac{a^2}{6} + \frac{R^3}{3a}\right). \tag{4}$$

Here r is the radial position of the starting point, a is the radius of the stationary target, and R is the radius of the confining domain. Equations (3) and (4) may be used with Eq. (1) to yield expressions for the inverse ''reaction rate''

$$\bar{\tau}_{2d} = \frac{1}{2D(R^2 - a^2)}\left[R^4\ln\left(\frac{R}{a}\right) - \frac{3R^4}{4} + R^2a^2 - \frac{a^4}{4}\right], \tag{5}$$

$$\bar{\tau}_{3d} = \frac{1}{D(R^3 - a^3)}\left(\frac{R^6}{3a} - \frac{3R^5}{5} + \frac{R^3a^2}{3} - \frac{a^5}{15}\right). \tag{6}$$

In the spirit of the mean-field argument introduced earlier, we employ a lattice-gas model and utilize an excellent approximation for the concentration-dependent diffusion constant derived by van Beijeren and Kutner [8]. For the case when the reactant and crowding particles have equal mobilities and move on a two-dimensional square lattice, this diffusion constant takes the form

$$D(c) = \frac{\Gamma b^2}{8}[\sqrt{4(1 - c) + c^2(\pi - 1)^2} - c(\pi - 1)], \tag{7}$$

where b is the lattice spacing, Γ is the attempt rate for particle moves, and c is the ratio between the total number of mobile particles to the number of accessible sites $c \simeq b^2(N + 1)/[\pi(R^2 - a^2)]$. Combining Eqs. (5) and (7), we find an expression for the reaction time which is plotted in Fig. 2. We emphasize that the key assumption made here is that the only effect of the crowding particles on the motion of the reactive particle is to change its diffusion constant, and the motion remains diffusive up to time scales comparable to the reaction time.

To test the validity of this assumption, we have performed Monte Carlo simulations of the lattice model. The simulations consist of one reactant and N inert particles confined to a two-dimensional circular domain of radius R with a circular target of radius a at the center, as shown in Fig. 1. Each simulation begins with a random configuration of the reactant and crowding particles and ends when the reactant reaches the center target at which point the first passage time is recorded. The average reaction time is determined from at least 1500 such runs.

The simulation results for 2000 crowding particles show a minimum in the reaction time very near the minimum of $36.9b$ predicted by Eq. (5) (corresponding to 47% of the sites being occupied). The reaction times diverge sharply when the confining radius becomes less than 29 lattice sites or the concentration exceeds ~75%. Similar agreement is found for 4000 (Fig. 2, teal line) and 8000 (not shown) crowding particles. At large confining radii, the reaction

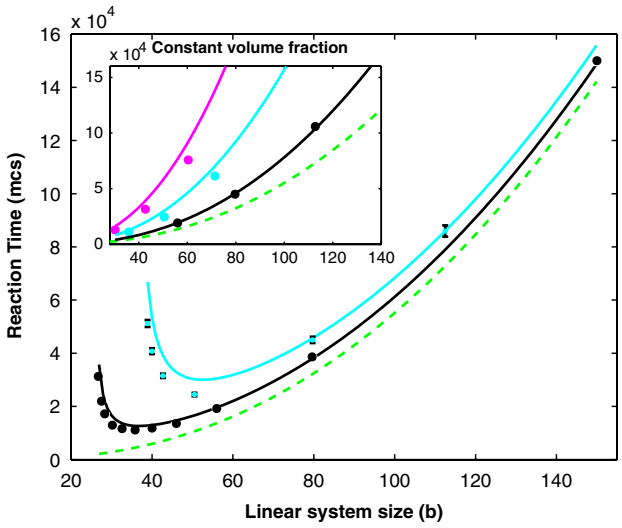

FIG. 2 (color online). Comparison of the reaction time predicted by Eq. (5) (solid lines) to the simulation data (dots) for 2000 (black) and 4000 (teal) crowding particles. (Inset) Reaction times at constant densities of 0.2 (black), 0.5 (teal), and 0.7 (purple) for 2000, 4000, and 8000 crowding particles showing the expected monotonic dependence, as well as the inaccuracy of Eq. (7) at higher densities. In both plots the green dashes indicate the reaction time predicted in the absence of crowding particles.

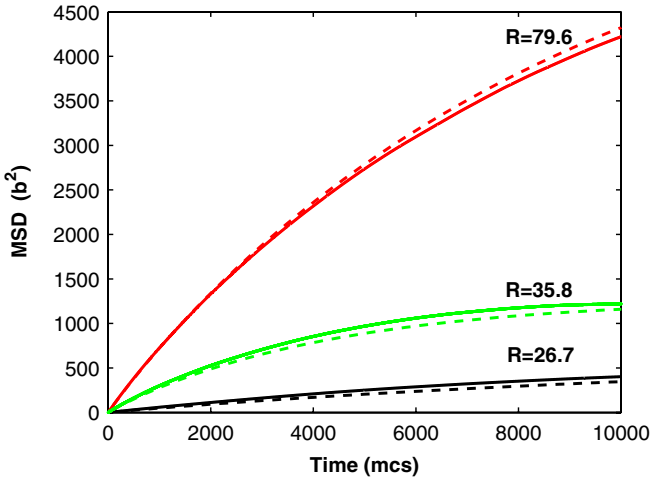

FIG. 3 (color online). Comparison of the MSD of the mobile reactant (solid lines) to MSD calculated in Ref. [8] (dashed lines) for systems of size $R = 79.6$, 35.8, and 26.7, corresponding to concentrations of $c = 0.1$, 0.5, and 0.9, respectively ($N = 2000$). The theoretical curves are the solution of Fick's second law in a circular geometry.

times increase as R^2 and approach the reaction rate in the absence of crowding particles (green line). As shown in Fig. 2, the difference between the crowded and uncrowded reaction times is nonzero at all system sizes. This can be explained by noting that, while the probability that a given site is occupied scales as R^{-d}, the required number of steps scales like R^d. The result is that the number of time steps where the particle is unable to move due to crowding remains nearly constant as the system size is increased.

The reaction times in Fig. 2 deviate from the theoretical curve at confining radii below $50b$ with a maximum error of 35% at the highest concentration simulated. This discrepancy is due to a nondiffusive correction to the mean squared displacement (MSD) in the lattice-gas model [9], which allows the reactant to more efficiently sample the space compared to purely diffusive motion. This point is illustrated in Fig. 3, where the MSD observed in our simulations is compared to purely diffusive behavior with D given by Eq. (7). For small system sizes and long times, where the nondiffusive behavior is expected to be significant, the simulation MSD systematically exceeds the theoretical value.

The mean-field treatment we have employed in the two-dimensional lattice model can be extended to the more physically relevant three-dimensional, off-lattice system with a few modifications. We recall that hard sphere systems undergo a glass transition at a volume fraction $\phi_c \sim 0.58$, which is less than the close-packing density [10,11]. Although the system is not completely frozen, the relevant time scales diverge sharply. Therefore, we restrict our analysis to densities below ϕ_c. In the fluid regime, for $\phi < \phi_c$, the self-diffusion constant is modified not only by the short range excluded volume interactions but also by long range hydrodynamic coupling mediated by the solvent [12]. In this case the diffusion constant is approximately given by $D = D_0(1 - \phi/\phi_c)^2$ [12]. If we use this formula in Eq. (6) and assume $R \gg a$, we find that the reaction time for monodisperse Brownian spheres is of the form $\bar{\tau}_{3d} \propto \phi^{-1}(1 - \phi/\phi_c)^{-2}$ and has a minimum for volume fraction $\phi = \phi_c/3 \approx 0.19$.

Experimentally, a crowded reaction with adjustable volume could be realized using microfluidic techniques. In this case the tracer and crowding particles would be confined to a microdroplet reaction vessel whose volume could be controlled through osmotic gradients across a semipermeable barrier [13]. This system mimics *in vivo* experiments that show increased tracer particle diffusion in osmotically swollen cells [14,15].

In most systems of interest, the target reactant would be mobile rather than stationary. One expects that the ratio of the reaction time for the stationary target to the reaction time for two mobile reactants in a fixed box size should be independent of the number of crowding particles present, as the crowding particles merely rescale the diffusion time. Our simulations support this intuition, and we find that the ratio is ~ 1.6 and ~ 2.0 in two and three dimensions, respectively, with corrections on the order of a/R.

In the context of these theoretical results, it is natural to ask to what extent do cells optimize biochemical reaction rates by adjusting their size? At first glance it would seem unlikely that cell volume would be a useful parameter for the cell to use to regulate reaction rates due to the large number of reactions that occur simultaneously involving

reagents that differ in size by many orders of magnitude. Furthermore, differential protein expression rates during the cell cycle could modify the overall protein concentration and thereby alter the cytoplasmic diffusion rate [16]. However, it is well known that the cell is more complicated than a ''bag of enzymes,'' and therefore the potential exists for the cell to establish subcellular compartments in such a way that the various reaction volumes are individually tunable. For example, the digestion of a pathogen by a macrophage occurs within a vesicle created by endocytosis. The volume of this vesicle is, in principle, adjustable by the amount of membrane used during vesicle creation. Similarly, other membrane-bound organelles such as Golgi, endoplasmic reticulum (ER), mitochondria, and the nucleus could be individually adjusted to optimize reactions occurring within. This is consistent with the finding that diffusion rates in the mitochondria and ER can differ substantially from the cytoplasm [17,18].

The case of the cell nucleus deserves special consideration. Here the primary reactions of importance involve the manipulation of the genome which is encoded by DNA. In analogy to our two-reactant model, many of the genome management functions the cell performs require two specific portions of the DNA to find each other. These internal cyclization reactions may occur between monomers separated by polymer spacers ranging from less than a persistence length, up to lengths on the order of the chromosome size [19,20]. If we identify the polymer segments that flank and bridge the reacting monomers as crowding particles, then we can immediately generalize the previous argument for the rate of crowded reactions to the internal cyclization of a confined polymer. Specifically, the rate of internal cyclization will have a nonmonotonic dependence on the size of the box containing the polymer. This nonmonotonicity has been previously observed in computer simulations [21].

The onset of crowding-limited dynamics at small system sizes leads to nonmonotonic behavior in the relaxation of other structural properties of the polymer such as its end-to-end vector. This nonmonotonicity is quantitatively different from that of crowded reactions due to the subdiffusive motion of the monomers imposed by the connectivity of the polymer. However, it can be easily explained by noting that the initial effect of reducing the volume accessible to the polymer to smaller than its unconfined size is to reduce the conformational phase space that the polymer can sample. This allows the polymer to sample space faster resulting in shorter relaxation times. However, like the free particle case, as the monomer density approaches the close-packing limit the polymer becomes jammed and the relaxation times increase.

We have shown that a simple, analytically tractable model is able to quantitatively predict reaction times occurring within crowded environments. This model can be generalized to off-lattice systems and systems with explicit solvent with slight modification. The nonmonotonicity in the reaction times shown here has broad implications for reactions within cells as well as the dynamics of confined polymers.

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