Bimolecular chemical reactions in crowded environments

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Abstract：Using two-dimensional Langevin dynamics simulations，we investigate the kinetics of the bimolecular chemical reactions in crowded environments. An important finding is that the dependence of the mean reaction time τ on the area fraction of crowders ϕ relies on the manner of varying ϕ. Specifically，When ϕ is increased by adding crowders into the circular domain，a monotonic increase in τ is observed. Moreover，the growth rate of τ becomes much faster once the percolation occurs in the system. As ϕ is tuned by varying the radius of the circular domain R，τ has a minimum as a function of ϕ，which is a result of two distinct dynamical regimes，i. e.，the crowding-dominated regime and the density-dominated regime. As the size of crowders becomes larger，the reaction process is found to be accelerated. Finally，we show that distributions of the reaction time obey the exponential ones，and the degree of crowding does not alter the distribution pattern.

Key words：bimolecular chemical reactions；reaction kinetics；crowding effect

拥挤环境下的双分子化学反应

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摘要：利用二维朗之万动力学模拟研究了拥挤环境下的双分子化学反应. 研究发现平均反应时间 τ 的分子拥挤剂面积分数 ϕ 依赖性取决于改变 ϕ 的方式. 当通过向体系中添加拥挤剂使 ϕ 改变，τ 随 ϕ 的增大单调增加. 而当体系中发生逾渗现象时，τ 的增长速率大大提高. 若通过调整体系尺寸大小来改变 ϕ，由于拥挤效应主导的动力学区域和拥挤剂浓度主导的动力学区域间的竞争，τ 随 ϕ 的增大出现极小值. 此外，发现随着拥挤剂尺寸的增大，双分子反应速率加快. 最后，模拟数据表明反应时间符合指数分布，且体系的拥挤程度不会改变这一分布模式.

关键词：双分子化学反应；反应动力学；拥挤效应

0   Introduction

The cytoplasm of living cells contains a variety of macromolecules, including nucleic acids, lipids, and cytoskeleton fibers, all of which can occupy as large as 40% of the volume[1-2]. Such a high concentration of macromolecules causes a very crowded environment inside cells. The crowding effect plays an important role in both thermodynamic and dynamic properties of biological processes in cells, such as protein folding and stability, aggregation, amyloid formation, and biochemical reactions[3-4].

Among these biological processes, the biochemical reactions are of significantly importance in maintaining the cellular functions, as they can prompt cellular responses to external stimulus. It has gained extensive attentions on the mechanism and the rate of biochemical reactions in living systems over the past decades[5]. *In virto* experiments are generally carried out in dilute aqueous solutions. However, biochemical reactions taking place in living cells have to suffer from a crowded environment. Theoretically, any reactions that increase the available volume of a system would be favored by the crowding effect[3]. Such reactions include the binding of a macromolecule to another one, the folding of the protein, and the folding of nucleic acid chains into more compact shapes. Bimolecular chemical reactions usually contain two steps, i. e., the formation of an encounter complex and the subsequent chemical transformation. In most cases, the former is the rate-controlled step, which implies that the reaction is a diffusion-limited process[6-7]. As a result, the effect of crowding on bimolecular chemical reactions is to decrease the reaction rate[7-9]. There have been lots of studies[7-21] focusing on how the crowding effect affects the bimolecular chemical reactions.

The degree of crowding in a system can be mediated by two distinct manners. In *in virto* experiments, this is generally realized by adding crowders to the system with a constant volume[9]. In this case, the density of reactant particles and the degree of crowding are independent. On the contrary, the number of crowders is relatively constant in *in vivo* experiments, and the degree of crowding could be meditated by contracting or expanding of cells through osmotic shifts[9]. Consequently, a higher degree of crowding also brings about a higher density of reactant particles, which makes the reaction kinetics difficult to be determined. It is the original motivation for us to investigate the kinetics of bimolecular chemical reactions under crowded environments. By changing the degree of crowding in two distinct manners, we expect to achieve a deeper understanding about the effect of crowding on the kinetics of bimolecular chemical reactions. For example, as the degree of crowding is increased by adding crowders into the system, a monotonic increase in the mean reaction time τ is observed. Interestingly, the occurrence of percolation in the system with the area fraction of crowders ϕ exceeding 0.50 leads to a crossover behavior of τ. Furthermore, another important factor that may influence the kinetics of bimolecular chemical reactions is the size of crowders. Therefore, we use 2D Langevin dynamics simulations to investigate the effects of crowding, the manner of varying the degree of crowding, and the size of crowders on the kinetics of bimolecular chemical reactions in the present work.

The frame of the paper is as follows. In the next section, our model and the simulation technique is briefly described. Detailed results and corresponding discussions are presented in section 2. Finally, we give a summary in section 3.

1   Model and methods

The two-dimensional model geometry we considered in this work is sketched in Fig. 1, where a reactant particle of a size σ searches for another one (also called target) located at the center of a circular domain. In our model, N_c

crowders of a uniform size σ_c ranging from 1σ to 3σ diffuse inside the circular domain with a radius R, which results in a crowded environment. A reflecting boundary is applied for the reactant particle and crowders. The target is treated as an absorbing domain of a radius 2.5σ from the center.
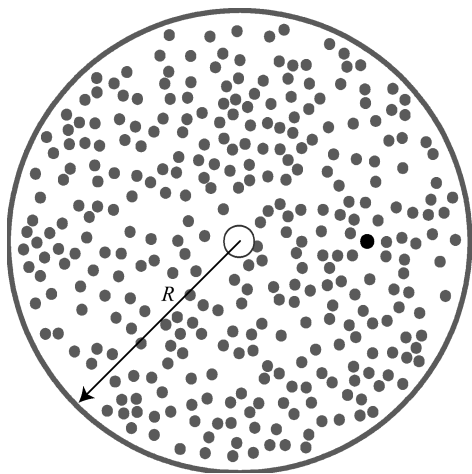

Fig. 1   **Schematic illustration of a reactant particle （in black）searching for another one located at the center of a circular domain of a radius R in a crowded environment to complete the bimolecular chemical reaction. The crowders are shown in grey**

In the simulations, the interactions between the reactant particle and crowders are modeled by the repulsive Lennard-Jones （LJ） potential

$$U_{LJ}(r) = \begin{cases} 4\varepsilon\left[\left(\dfrac{\sigma_{LJ}}{r}\right)^{12} - \left(\dfrac{\sigma_{LJ}}{r}\right)^{6}\right] + \varepsilon, & r \leqslant 2^{1/6}\sigma_{LJ} \\ 0, & r > 2^{1/6}\sigma_{LJ} \end{cases}$$

$$(1)$$

Here ε is the well depth of the LJ potential, and $\sigma_{LJ} = (\sigma + \sigma_c)/2$ is the LJ radius. The repulsive interactions between the crowders are also depicted by the above LJ potential with a LJ radius $\sigma_{LJ} = \sigma_c$ at this time. The LJ parameter $\varepsilon = 1$, the size of the reactant particle σ, and the mass of the reactant particle m fixed the energy, length and mass scales of the system, respectively. The time scale is then given by $t_{LJ} = (m\sigma^2/\varepsilon)^{1/2}$. The thermal energy of the system is set as $k_B T = 1.2\varepsilon$.

The motions of the reactant particle and the crowders are governed by the Langevin equation

$$m_i\ddot{r}_i = -\nabla U_i - \xi_i v_i + F_i^R \qquad (2)$$

where m_i, ξ_i, and v_i is the mass, frictional coefficient, and velocity of the ith particle, respectively. $-\nabla U_i$, $\xi_i v_i$, and F_i^R is the conservative, frictional and random forces subjected by the corresponding particle, respectively. Wherein, F_i^R satisfies the fluctuation-dissipation theorem[22]. The dimensionless parameters for the reactant particle are chosen to be $m = 1$ and $\xi = 0.7$. Thus, the mass and frictional coefficient of crowders are determined subsequently as $m_c = (\sigma_c/\sigma)^2$, $\xi_c = 0.7\sigma/\sigma_c$. The Langevin equation is integrated in time by the method proposed by Ermak and Buckholz[23].

The initial positions of N_c crowders are generated randomly in the circular domain. Then, N_c crowders are under thermal motions described by the Langevin thermostat to obtain the equilibrium state of the system. Meanwhile, the reactant particle is fixed with a distance 10σ from the center of the circular domain. By checking the mean radical density profile of crowders, we could judge whether the system is in equilibrium. Once achieved, the reactant particle is released and the target search process begins. The reaction time τ_i is given by the first passage time of the reactant particle reaching the target. Typically, the mean reaction time τ is obtained by averaging 1 000 search events to reduce statistical errors, i. e.,

$$\tau = \sum_{i=1}^{1\,000} \frac{\tau_i}{1\,000}.$$

2   Results and discussions

2.1   Theory

To shed light on the kinetics of bimolecular chemical reactions in crowded environments, we assume that the reactant particle diffuses in dynamic channels formed by the crowders in the circular domain. Certainly, there exists fluctuations in the diameters of these channels, and even some randomly distributed short bottlenecks[24-26].

For a circular domain of a radius R containing N_c crowders of a diameter σ_c, the area fraction of crowders ϕ can be written as

$$\phi = \frac{N_c \pi \left(\frac{\sigma_c}{2}\right)^2}{\pi R^2} \quad (3)$$

The average distance between the center of neighboring crowders is $d = R \cdot (\pi/N_c)^{1/2}$. Thus, the average distance between the surface of neighboring crowders, denoted by D, is

$$D = d - \sigma_c = R \cdot \left(\frac{\pi}{N_c}\right)^{\frac{1}{2}} - \sigma_c \quad (4)$$

By substituting Eq. (3) into Eq. (4), we get

$$D = \sigma_c \left(\frac{1}{2} \sqrt{\pi/\phi} - 1\right) \quad (5)$$

D can be regarded as the average width of the channels formed by crowders from a statistical perspective. Obviously, D increases as σ_c becomes larger at a fixed ϕ, and decreases with increasing ϕ at a fixed σ_c. By defining L as the average total length of the channels, we have

$$LD = \pi R^2 (1 - \phi) \quad (6)$$

Thus, L can be expressed as

$$L = \frac{\pi R^2}{\sigma_c} \left[\frac{1 - \phi}{\frac{1}{2} \sqrt{\pi/\phi} - 1}\right] \quad (7)$$

In contrast to D, L gets larger with increasing ϕ, and decreases as σ_c is smaller, as shown in Fig. 2.
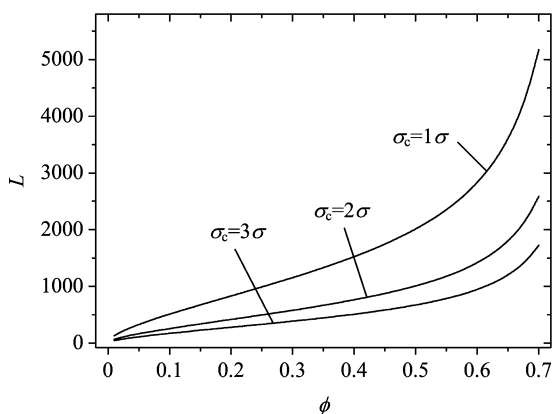

Fig. 2 The average total length of the channels L given in Eq. (7) is plotted as a function the area fraction of crowders ϕ for three different diameters of crowders σ_c. Here the radius of the circular domain is $R = 18$

2.2 The ϕ-dependent mean reaction time

2.2.1 Varying ϕ by adding crowders

In *in virto* experiments, the degree of crowding is generally adjusted by adding crowders to the system with a constant volume[9]. In the following, we study the bimolecular chemical reaction kinetics in a crowded environment where the area fraction of crowders ϕ is varied in this way.

As plotted in Fig. 3, the mean reaction time τ grows monotonously with the increasing ϕ. At a fixed $\sigma_c = 1$, an increase in ϕ leads to a smaller D, indicating the reactant particle diffuses in narrower channels. Meanwhile, a larger L means the reactant particle needs to diffuse a longer distance to react with another one. Obviously, these two factors severely impede the reaction process.
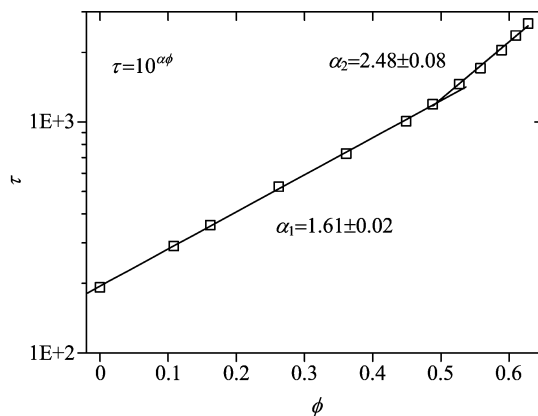

Fig. 3 Semi-logarithmic plot of the mean reaction time τ as function of the area fraction of crowders ϕ. The black and red solid lines are the linear fittings to the data point. Here, $\sigma_c = 1$ and $R = 18$

Interestingly, we find that the data points could be well fitted by the exponential functions $\tau = 10^{\alpha_1 \phi}$ for $\phi < \phi_c$ with $\alpha_1 = 1.61 \pm 0.02$, and $\tau = 10^{\alpha_2 \phi}$ for $\phi > \phi_c$ with $\alpha_2 = 2.48 \pm 0.08$. Here, $\phi_c \approx 0.50$ is the critical area fraction of crowders which divides the two different increasing regimes. This crossover behavior of τ with increasing ϕ can be understood by the percolation theory. It has been suggested that a bond to bond percolation occurs at the percolation threshold $p_c = 0.50$[27]. At $\phi < \phi_c$, the liquid is the continuous phase with crowders randomly distributing in it. This indicates that the channels formed by crowders are connected. However, at $\phi > \phi_c$, the crowders becomes the

continuous phase so that the channels are divided into small pieces and get discontinuous. When a reactant particle diffuses in such short and discontinuous channels and is confronted with a blocking, it either goes back or just waits a long time for the opening of the blocking to escape. Consequently, τ increases much faster once the percolation occurs in the system. As expected, the observed critical area fraction of crowders ϕ_c equals to the percolation threshold $p_c = 0.50$. The percolation theory provides a right physical insight into the crossover behavior of τ with increasing ϕ.

2.2.2 Varying ϕ by contracting or expanding the circular domain

In striking contrast to *in virto* experiments, the number of crowders is relatively constant in *in vivo* experiments, and the density of crowders could be meditated by contracting or expanding of cells through osmotic shifts[9]. In this situation, the density of reactant particles and the degree of crowding are coupled. The degree of crowding aggravates with the contraction of cells. However, it also suggests that the distance of a reactant particle diffusing to react with another one reduces. In other words, the effective density of reactant particles increases. The interplay between these two competitive factors makes the reaction kinetics complicated.

To clarify the reaction kinetics in this case, we resort to numerical simulations where the number of crowders is a constant at a fixed σ_c, and the area fraction of crowders ϕ is changed by varying the radius of the circular domain R. As shown in Fig. 4, a nonmonotonic dependence of the mean reaction time τ on ϕ is observed. When the circular domain is very small, the crowding effect is the dominated factor determining the reaction kinetics. The reaction kinetics in such a system is crowding-dominated. With increasing R, the degree of crowding decreases rapidly so that the reaction rate is accelerated. However, with a further increase in R, the reactant particle needs to diffuse a longer distance to react with another one.

Namely, the reaction kinetics in such a system is density-dominated. The extreme situation is that the bimolecular chemical reaction between two reactant particles proceeds in a vast space. Although the degree of crowding is negligible at this time, the reaction rate is extremely slow. Therefore, the competition between the two distinct dynamical regimes leads to the nonmonotonic dependence of τ on ϕ.
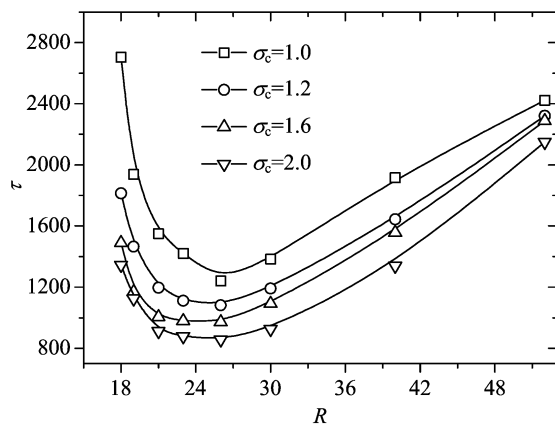

Fig. 4　The mean reaction time τ as a function of the radius of the circular domain R at different sizes of crowders σ_c. Here, the number of crowders at $\sigma_c = 1.0$ is fixed at $N_c(\sigma_c = 1.0) = 770$. At the same R, ϕ is a constant for different σ_c, i.e., $N_c(\sigma_c) = N_c(\sigma_c = 1.0)/\sigma_c^2$

Furthermore, the optimal reaction rate appears at $\phi \approx 28.5\%$, and is independent of the sizes of crowders σ_c we have studied. However, if the reactant particles immerse in a sea of crowders with smaller sizes, a depletion attraction would be introduced between the reactant particles[28]. In this case, the optimal reaction rate may occur at a higher ϕ since the depletion attraction is very likely to benefit the bimolecular chemical reaction.

2.3　The σ_c -dependent mean reaction time

In the following, we study the effect of the size of crowders σ_c on the reaction kinetics. Fig. 5 shows that the mean reaction time τ decreases monotonously as the crowders become larger. Eq. (5) suggests that as σ_c increases, the average width of the channels D gets larger. Meanwhile, Eq. (7) indicates that the average total length of the

channels L is shorter. Thus, the diffusion of the reactant particle becomes faster. Here ϕ is changed by varying the number of crowders N_c. In fact, a similar behavior of τ with increasing σ_c can be observed when ϕ is tuned by varying the radius of the circular domain R, as implied by Fig. 4.
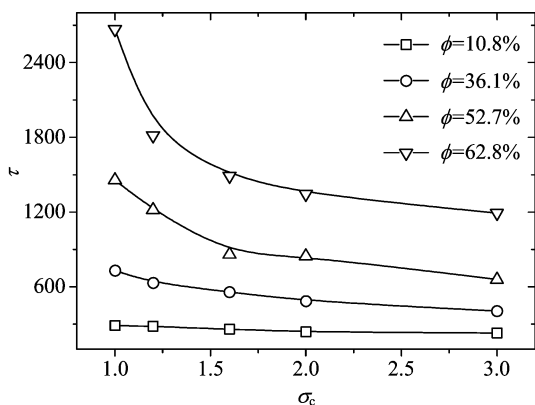

Fig. 5 The mean reaction time τ as a function of the size of crowders σ_c at different ϕ which is changed by varying N_c. Here the radius of the circular domain is $R = 18$

Let us consider a limiting case that the crowders is so large that there is only one crowder in the circular domain. In this case, the reactant particle does not need to search the area occupied by the large crowder of a poor mobility. Therefore, if σ_c further increases and is larger than the biggest size of crowders $\sigma_c = 3$ we have studied in this work, τ is expected to continue to decrease. Note that the mobility of a crowder not only depends on its size and the degree of crowding, but also the temperature and the viscosity of the system. When the size and ϕ is fixed, the mobility of crowders could be enhanced by increasing the temperature or decreasing the viscosity of the system. Obviously, these two changes are beneficial to the diffusion of reactant particles such that the bimolecular reaction should be accelerated.

2.4 Distributions of the reaction time

The distributions of the reaction time τ_i can reveal the statistical characteristics of the reaction process. As shown in Fig. 6(a), a negative
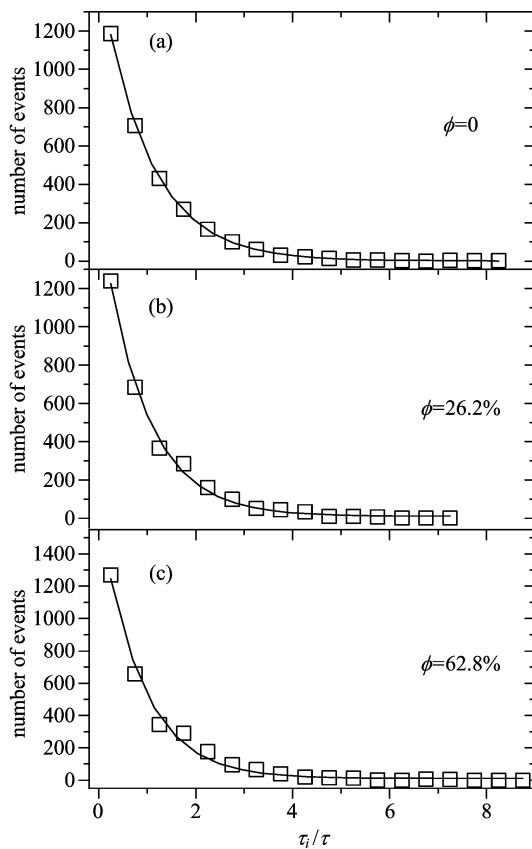

Fig. 6 Distributions of the reaction time τ_i normalized by their mean value τ at three different area fraction of crowders ϕ. Here the size of crowders is $\sigma_c = 1.0$, and the radius of the circular domain is $R = 18$. The solid lines are the single exponential decay fittings to the data

exponential distribution of τ_i/τ at $\phi = 0$ is observed. Figs. 6(b)~(c) suggest that the degree of crowding does not alter the distribution pattern of τ_i/τ even if ϕ exceeds the percolation threshold $p_c = 0.50$.

The negative exponential distribution of τ_i/τ indicates that the bimolecular chemical reaction is a memoryless and Poissonian process. Assuming that the two reactant particles are connected, the reaction would be the cyclization reaction between two polymer monomers, i.e., the loop formation. It has been suggested recently that the loop formation is also a Poissonian process[29].

3 Conclusion

To summarize, we have investigated the kinetics of the bimolecular chemical reactions in

crowded environments by using 2D Langevin dynamics simulations. We show that the dependence of the mean reaction time τ on the area fraction of crowders ϕ relies on the manner of varying ϕ. When ϕ is increased by adding crowders into the circular domain, a monotonic increase in τ is observed. Moreover, the growth rate of τ becomes much faster once the percolation occurs in the system. As ϕ is tuned by varying the radius of the circular domain R, τ has a minimum as a function of ϕ, which is a result of two distinct dynamical regimes. With an increase in the size of crowders σ_c, the reaction process is found to be accelerated. Finally, we show that distributions of the reaction time obey the exponential ones, and the degree of crowding does not alter the distribution pattern.

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