

## Viewpoint

## Speeding up in a crowd

Huan-Xiang Zhou

Department of Physics and Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306, USA

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*New simulations show that a high concentration of molecules can significantly increase the rate of reaction between two molecules, but does the setup reflect realities of biochemical reactions inside cells?*Subject Areas: **Statistical Mechanics, Biological Physics****A Viewpoint on:****Diffusion-Limited Reactions in Crowded Environments**

N. Dorsaz, C. De Michele, F. Piazza, P. De Los Rios and G. Foffi

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There is not a lot of “alone time” inside a cell. Rather, cellular biochemical reactions take place in a high concentration of reactant molecules and bystander macromolecules [1]. Interest in understanding reaction dynamics in the presence of such “crowded conditions” is now motivating theorists to extend the equations that have worked so well at describing reactions at low concentrations to a new regime [2, 3]. In a paper appearing in *Physical Review Letters*, Nicolas Dorsaz at the University of Fribourg and colleagues at EPFL, both in Switzerland, and at Università di Roma La Sapienza, Italy, describe one approach to doing this that makes a step forward in predicting how reaction rates are affected by a high concentration of reactants [4].

When two reactant particles come together to form a product, there are two steps which can limit the rate at which the reaction occurs [3]. The first is the formation of an intermediate product, known as the “transient” or “encounter” complex, which consists of configurations in which the reactant particles have relative separations and orientations close to those in the final product. The second potential rate-limiting step occurs when the reactant molecules, starting from the transient complex, undergo configurational rearrangements to form the final product.

Reactant particles approach the first step through translation and rotation, motions that, in solution, are generally diffusive. For this reason, biomolecular reactions where the formation of the transient complex limits the rate of reaction are said to be diffusion-limited.

A theoretical framework for modeling diffusion-limited bimolecular reactions was established by the Polish scientist Marian Smoluchowski [5] nearly a century ago. When a single reactant particle, labeled A, is surrounded, at time  $t = 0$ , by an equilibrium distribution of reactant particles, labeled B [Fig. 1(a)], the sur-

vival probability  $S(t)$  of particle A at time  $t$  satisfies

$$\frac{dS(t)}{dt} = -c_b k(t) S(t). \quad (1)$$

Here,  $c_b$  is the bulk concentration of the B reactant, and  $k(t)$  is a time-dependent rate coefficient, which is determined by the so-called distribution function,  $p(\mathbf{r}, t)$ , of an A-B reactant pair. For reactions between spherical particles, only relative motion between the particles matters and the distribution function satisfies the Smoluchowski equation:

$$\frac{\partial p(\mathbf{r}, t)}{\partial t} = \nabla \cdot D_0 e^{-\beta V(r)} \nabla e^{\beta V(r)} p(\mathbf{r}, t), \quad (2)$$

where  $\beta$  denotes the inverse thermal energy,  $D_0$  is the translational diffusion constant of a B reactant particle relative to the A reactant particle, and  $V(\mathbf{r})$  is their interaction energy [Fig. 1(b)]. Without the two factors containing  $V(\mathbf{r})$ , Eq. (2) is just the usual diffusion equation. The two extra factors in the Smoluchowski equation account for the fact that the interparticle relative diffusion is under the influence of their interaction energy. A diffusion-limited reaction between A and B is modeled by an absorbing boundary condition—that is, one where the distribution function is zero—when the two reactant molecules are in contact. The rate coefficient  $k(t)$  is given by the total flux of the pair-relative diffusion across the contact surface.

The Smoluchowski theory reduces a many-body problem to essentially a one-body problem [Fig. 1(a) and (b)]. It is strictly valid only if the A reactant particle is immobile, the B reactant particles do not interact with each other, and motions of the B particles are diffusive over all timescales. However, when the concentration of the B reactant increases, the interactions between them can't be ignored [Fig. 1(c)]. As a result, there have been

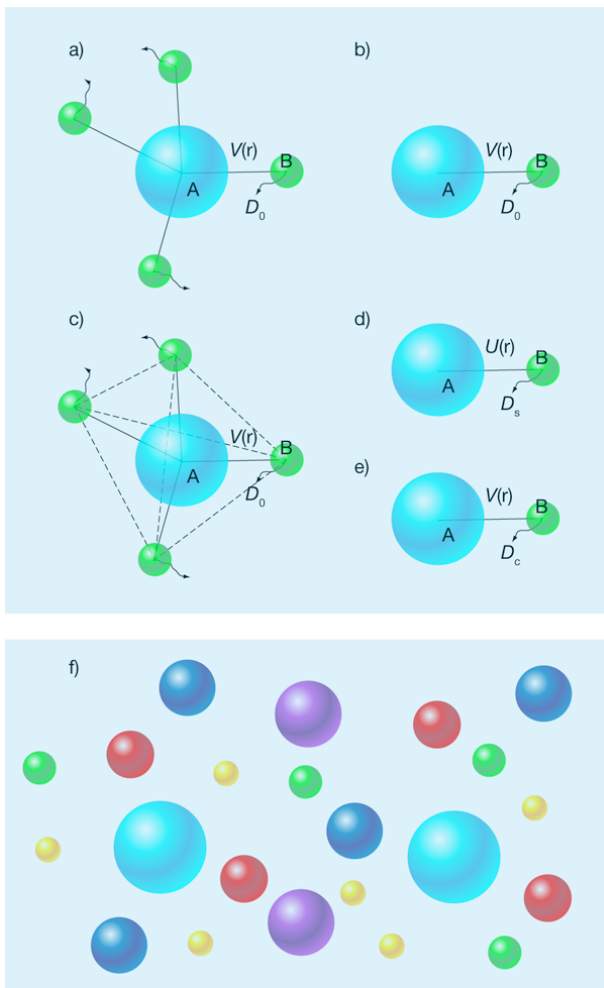


FIG. 1: Models of biochemical reactions make different assumptions about how molecules interact. At low concentrations (a) each B type reactant interacts with an A reactant independently of other B particles. The “many-body” problem in (a) can be reduced to a single pair representation (b). A more realistic assumption is that the B particles interact with each other (dashed lines), as in (c). This can be accounted for by assuming that either (d) the interaction in the reduced pair representation is modified or (e) the diffusion constant depends on the local concentration. (f) The true distribution of macromolecules and reactants inside cells involves many types of molecules. (Credit: Alan Stonebraker)

various attempts to extend or modify the Smoluchowski theory to account for these interactions.

One approach [6, 7] is based on the assumption that the pair distribution function is still governed by the Smoluchowski equation, but the A-B pair interaction potential  $V(\mathbf{r})$  is replaced by  $U(\mathbf{r})$ , the potential of mean force for the pair [Fig. 1(d)]. The potential of mean force accounts for the effect of the other B particles (and other bystander solute particles if present) on the interaction of the A-B pair. Similarly, the effects of the other B particles on the diffusion constant are accounted for by replacing  $D_0$  with the self-diffusion constant  $D_s$ . Self-

diffusion of a B particle is slowed down by the presence of other hard particles; hence  $D_s < D_0$  [8].

This extended Smoluchowski approach was tested against simulation results of a system of hard-sphere particles where the volume fraction of the particles (reactants and bystanders) was 41%, which is a high concentration [9]. In the simulations, one particle was labeled as A, various numbers of other particles labeled as B, and the remaining particles were bystanders. Up to moderate concentrations of the B reactant (less than 10% volume fraction), predictions of the survival probability by the extended Smoluchowski approach agreed well with the simulation results. At higher B fractions, however, the predictions underestimated the decay rate of the survival probability.

Another approach, which is what Dorsaz *et al.* follow, is based on the “local concentration,”  $c(\mathbf{r}, t)$ , of the B particles around the A particle [9]. The local concentration also satisfies Eq. (2), but with  $D_0$  replaced by the so-called collective diffusion constant,  $D_c$  [9, 10] [Fig. 1(e)]. The latter depends on the local concentration and is given by [10]

$$D_c(c) = M(c) \frac{d\Pi(c)}{dc}, \quad (3)$$

where  $M(c)$  is the local mobility, and  $\Pi(c)$  is the local osmotic pressure. If hydrodynamic interactions between the B particles are absent, then  $M(c) = \beta D_0$ . When  $c \rightarrow 0$ , the effects of interactions between the B particles are negligible, and consequently  $\beta\Pi(c) \rightarrow c$ . In this limit,  $D_c(c) \rightarrow D_0$ . When  $c > 0$ ,  $D_c(c) > D_0$  for hard particles. That is, the collective diffusion constant of the B reactant is greater than the diffusion constant of a single B particle in the dilute limit. This is in contrast to the self-diffusion constant  $D_s$ , which, as noted above, is smaller than  $D_0$ . The collective diffusion flux across the A-B contact surface is taken to be  $c_b k(t)$ . The steady-state rate constant  $k_{ss}$  predicted from the linearized Smoluchowski equation for  $c(\mathbf{r}, t)$  agrees well with simulation results up to moderate B concentrations (less than 20% for hard particles) [9].

In their paper, Dorsaz *et al.* push this theoretical approach and simulations to higher B concentrations. For the case where there is no interaction between the particles [ $V(\mathbf{r}) = 0$ ], they found  $k_{ss}$  by solving the steady-state Smoluchowski equation for  $c(\mathbf{r}, t)$  without approximation and tested the solution against simulations. The system they simulated consisted of an immobilized A particle with an absorbing contact surface, surrounded by diffusing B particles. All the particles were hard spheres. The theory agreed well with the simulations, provided the A particle was much larger than the B particles. Relative to the dilute limit, Dorsaz *et al.* found an eightfold increase in  $k_{ss}$  at high reactant concentrations. However, when the A particle was smaller than the B particles, the theory significantly overestimated  $k_{ss}$  at high concentrations.

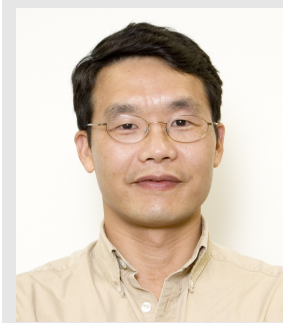
Dorsaz *et al.*'s study brings attention to an important

question: What is the appropriate governing equation for modeling bimolecular reactions at high reactant concentrations? Up to moderate concentrations, both of the approaches outlined above are supported by simulations. However, both exhibit some failings at high reactant concentrations. The approach based on collective diffusion seems to work at high reactant concentrations at least when the A particle is much bigger than the B particles. Dorsaz *et al.* attempted to remedy the problem with the theory when the A particle was smaller than the B particles by introducing an effective interaction potential (along with additional improvisations). However, this remedy is *ad hoc* and apparently double counts the effects of the other B particles on the pair potential, since these effects are supposed to have already been captured by the collective diffusion constant. It is also unclear how the governing equation for the local B concentration should be modified when other bystander particles are present. For example, Dorsaz *et al.* found a significant decrease in  $k_{ss}$  when just a tiny fraction of the B particles were replaced by bystanders.

Since understanding reactions in cells is a motivating factor in these kinds of studies, it is important to point out a crucial difference between the condition simulated by Dorsaz *et al.* and that inside cells. While the total concentration of intracellular macromolecules is very high, the concentration of each individual species is actually quite low [Fig. 1(f)]. Ultimately, theories and simulations will have to be tailored to this condition to be of physiological relevance.

## About the Author

### Huan-Xiang Zhou



Huan-Xiang Zhou received his Ph.D. from Drexel University in 1988. He did postdoctoral work at the National Institutes of Health. After faculty appointments at HKUST and Drexel, he moved to Florida State University in 2002. His group does theoretical, computational, and experimental research on protein-protein binding kinetics, crowding and confinement effects of cellular environments, and dynamics-function correlations of ion channels.

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