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A systematic investigation of the rate laws valid in intracellular environments

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Abstract

Recently there has been significant interest in deducing the form of the rate laws for chemical reactions occurring in the intracellular environment. This environment is typically characterized by low-dimensionality and a high macromolecular content; this leads to a spatial heterogeneity not typical of the well stirred in vitro environments. For this reason, the classical law of mass action has been presumed to be invalid for modeling intracellular reactions. Using lattice-gas automata models, it has recently been postulated [H. Berry, Monte Carlo simulations of enzyme reactions in two dimensions: Fractal kinetics and spatial segregation, Biophys. J. 83 (2002) 1891–1901; S. Schnell, T.E. Turner, Reaction kinetics in intracellular environments with macromolecular crowding: simulations and rate laws, Prog. Biophys. Mol. Biol. 85 (2004) 235–260] that the reaction kinetics is fractal-like. In this article we systematically investigate for the first time how the rate laws describing intracellular reactions vary as a function of: the geometry and size of the intracellular surface on which the reactions occur, the mobility of the macromolecules responsible for the crowding effects, the initial reactant concentrations and the probability of reaction between two reactant molecules. We also compare the rate laws valid in heterogeneous environments in which there is an underlying spatial lattice, for example crystalline alloys, with the rate laws valid in heterogeneous environments where there is no such natural lattice, for example in intracellular environments. Our simulations indicate that: (i) in intracellular environments both fractal kinetics and mass action can be valid, the major determinant being the probability of reaction, (ii) the geometry and size of the intracellular surface on which reactions are occurring does not significantly affect the rate law, (iii) there are considerable differences between the rate laws valid in heterogeneous non-living structures such as crystals and those valid in intracellular environments. Deviations from mass action are less pronounced in intracellular environments than in a crystalline material of similar heterogeneity.

Keywords: Macromolecular crowding; Law of mass action; Fractal-like kinetics; Lattice-gas automata; Brownian dynamics; Lattice effects

1. Introduction

There is growing evidence of the importance of reaction kinetics for the structural organization of the intracellular environment, which is far from the homogeneous, well mixed solution typical of in vitro experiments. A high degree of molecular crowding as well as the presence of indigenous obstacles in cellular media have important consequences in the physico-chemistry of the cell. The ramifications of this are only now becoming more generally understood. One of them is that it is not clear what are the rate laws governing reactions occurring in vivo [13]. The law of mass action and its stochastic and microscopic counterpart – the chemical master equation – are strictly applicable for homogeneous, three-dimensional environments and consequently of little or no use in modeling intracellular biochemical reactions [16]. In recent years, biochemists have been using various computational frameworks to extract rate laws or empirical reaction equations from direct numerical simulations. Among these approaches, simulations based on lattice-gas automata (LGA) are the most popular and widely used.

Lattice-gas automata simulations of simple reactions in low dimensional and fractal media (a particular class of heteroge-

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neous media) by Kopelman [10,11] have shown that in the asymptotic limit $t\to\infty$, the rate coefficient k is not a constant but rather has the time-dependent form

$$k(t) \sim t^{-(1-p)}, \tag{1}$$

where p is a non-dimensional index quantifying deviations from the classical law of mass action. The space in which the reaction occurs is assumed to be unbounded or very large. This type of kinetics is popularly referred to as fractal-like kinetics. For well-stirred reactions occurring in a homogeneous three-dimensional space, $p=1$. Thus, the law of mass action is valid. Typical values for fractal systems are near $p=2/3$ [11]. Recently it has been suggested that fractal-like kinetics is also a good description of the reaction kinetics of intracellular enzyme-catalyzed reactions [1,13], although to date an extensive analysis has not been performed.

In the following two subsections (Sections 1.1 and 1.2), we discuss in some detail what is currently known about the homogeneous and heterogeneous reaction kinetics. We also point out problems and open questions. This is followed by the aims of this paper (Section 1.3).

Note that both cases (homo- and heterogeneous reaction kinetics) are relevant to understanding the reaction kinetics in intracellular environments since it has been estimated that the cytoplasmic obstacle concentration is in the range $0.05 \le [O] \le 0.4$ [7,9]. Note also that even for homogeneous conditions the reactions occur in a different environment than that of in vitro since many reactions occur on 2D membranes or surfaces [13].

1.1. Homogeneous reaction kinetics

The homogeneous reaction kinetics has been studied for a number of simple reactions of the type: $A+A\to\emptyset$, $A+B\to B$ and $A+B\to C$ [14]. We will focus on the last reaction since this is the most generally applicable case. For the $A+B\to C$ pathway, there are two main variables: (i) the reaction probability P, i.e. the probability that when an A molecule meets a B molecule they react to product an inert C molecule, and (ii) the initial conditions A_0, B_0. A survey of the literature shows that by far the most well-studied case is $P=1$ and $A_0=B_0$, for which the index p, as defined in Eq. (1), takes the universal value $p=d/4$. Here d is the dimensionality of the space in which the reaction occurs. The most general result is that given by the theory of Bramson and Lebowitz [2], which shows that for initial conditions $[A_0]<[B_0]$ and if all species are mobile then $p=d/2$ for $d<2$ and $p=1$ for $d\ge 3$. In two dimensions, $p=d/2$ with logarithmic corrections. If the majority species is immobile then $p=d/(d+2)$ in all dimensions. These results are all valid in the asymptotic limit of very long times $t\to\infty$. Thus it is not clear if these results are of relevance to biochemical reactions occurring in a finite time: long-lived transients may dominate the experimentally observable kinetics; this means that the relevant reaction kinetics may be a function of both P and the initial conditions. It has been previously shown [14] that for one-dimensional (1D) simulations with $P\ll 1$ and $A_0=B_0$, the reaction kinetics exhibits a cross-over from classical kinetics ($p\backsimeq 1$) to non-classical fractal kinetics ($p=1/4$). The cross-over time is however so long that only the classical kinetics may be observed within the finite time scale of the simulation or the experiment. We expect that in higher dimensions (e.g. the 2D and 3D environments typical of intracellular conditions) such a cross-over time will be even larger than that found in 1D simulations although this has not been investigated to date. Thus it is possible that mass action kinetics is valid for some reactions occurring inside cells. If true, this would go against the current trend of thought that assumes that mass action is invalid inside cells. From present studies [14] it is unclear under what conditions this generally occurs, particularly if the initial conditions play a role in determining the type of kinetics.

1.2. Heterogeneous reaction kinetics

The best example of heterogeneous kinetics in the literature is the reaction kinetics of excitons in molecular macro-clusters (inside crystalline isotopic alloys) that were prepared as fractals [11]. In particular, Kopelman et al. studied the exciton-fusion reaction $T+T\to S$, where two triplet (T) excitations fuse and form a singlet (S) excitation. In perfect crystals, the kinetics is observed to be classical whereas in isotopically mixed crystals, the experimentally determined kinetics appears to be described by fractal-like kinetics. These results are reproduced by simulations of random walkers on 2D (square lattice) percolation clusters; the range of occupation probabilities ranges from critical to unity. Please note that the effective topology of the laboratory experiments is that of a square lattice [12] implying that the LGA lattice in such a case is not a convenient artificial construct but rather mimics reality. Square grid LGA simulations (with periodic boundary conditions) have recently been employed by Berry [1] and Schnell and Turner [13] to study the reaction kinetics of enzyme-catalyzed reactions in intracellular environments with macromolecular crowding. The authors conclude that the kinetics is fractal-like, at least for moderate to long times. There are three main problems with these studies:

i. Contrary to crystalline alloys (as in the experiments of Kopelman), intracellular environments do not possess a regular square topology, i.e. the square lattice of LGA simulations of intracellular reaction kinetics is an artificial construct.
ii. It is not possible to make any definite conclusions on the type of kinetics from the limited temporal data provided by the LGA simulations. The kinetics is deduced from log–log plots of $k(t)$ versus time. In these plots, the apparent power–law behavior of $k(t)$ is only clearly evident over about half a decade of time series data. Indeed in a number of cases, it can be shown that the kinetics appears to be better described by a law of the form $k(t)\propto\log(t)$.

iii. The reactions occur on a toroidal surface of fixed radius, i.e. periodic boundary conditions and a space of fixed size. It is not clear if these constraints influence the kinetics. In fact, biochemical reactions can occur on membranes or surfaces of different sizes. The geometry of these surfaces may be that of a torus (periodic boundary conditions), a flat plane (fixed boundary conditions), or some other form.

1.3. Aims of the present work

In this paper we address four questions concerning the rate laws governing the reaction kinetics in the intracellular environment, which are raised from the review made in the last two subsections.

i. What rate law governs the intracellular reaction kinetics over the biologically realistic reaction time? Is it possible that both mass action and fractal-like kinetics are valid in intracellular environments, depending on various factors?
ii. Are the rate laws in heterogeneous ordered structures, for example crystalline isotopic alloys, different than those in intracellular environments, where there is also a significant heterogeneity but no natural spatial lattice?
iii. Are the reaction kinetics dependent on the geometry and the size of the intracellular surfaces on which the reactions occur?
iv. How do the kinetics vary with the reaction probability and initial conditions?

In this study we have chosen the elementary reaction $A+B\rightarrow C$ in a two-dimensional (2D) environment, where C is an inert molecule, as a model intracellular reaction. This pathway can, for example represent the irreversible binding of a ligand and a receptor. In previous studies [1,13] the reaction model has been the single enzyme, single substrate Michaelis–Menten mechanism. We have selected a more elementary reaction because it has a smaller number of variables and thus its kinetics is easier to characterize. Our chosen environment is 2D since many intracellular reactions occur on 2D membranes or surfaces [13]. We study the reaction occurring under two different conditions: (i) homogeneous space, i.e. obstacle-free conditions (Section 3.1), and (ii) heterogeneous space, that is, a space in which a certain number of obstacles are randomly and uniformly distributed throughout (Section 3.2). The obstacles model the effects of the macromolecular crowding agents present in the cytoplasm.

In order to answer the above questions, we have carried out a set of simulations (see Section 2 for more details) to compute the non-dimensional parameter p (as defined in Eq. (1)) during the course of the reaction. There are three possible types of kinetics which can be deduced from these simulations: (i) $p=constant=1$ implies mass action, i.e. classical kinetics; (ii) $p=constant<1$ implies non-classical fractal-like kinetics (iii) $p\neq constant$ implies non-classical kinetics not compatible with fractal kinetics. We conclude our paper with a brief discussion (Section 4).

2. Methods

2.1. Determination of the fractal exponent p

The equation of motion for the chemical concentration of species A molecules, $[A(t)]$, is given by

$$\mathrm{d}[A(t)]/\mathrm{d}t = -k(t)[A(t)][B(t)]. \tag{2}$$

The initial chemical concentrations A_0 and B_0 are generally not equal. We assume that $A_0<B_0$ (A is the minority or limiting species). The two reactant species are consumed in equal amounts, $[A(t)]=A_0-x$ and $[B(t)]=B_0-x$, and thus Eq. (2) simplifies to:

$$\mathrm{d}x/\mathrm{d}t = -k(t)(A_0-x)(B_0-x). \tag{3}$$

This differential equation can be easily solved by standard methods [4] to give the solution:

$$[A(t)] = \frac{A_0(B_0-A_0)}{B_0\exp(y)-A_0}, \tag{4}$$

where

$$y = (B_0-A_0)\int_0^t k(s)\mathrm{d}s. \tag{5}$$

Assuming that the kinetics in intracellular environments follows the form proposed by Kopelman, i.e. $k(t)\sim t^{-(1-p)}$, $t>0$, and using Eqs. (4) and (5), it can be shown that

$$p\log t = \text{constant} + \log\left[-\log\left\{\frac{B_0[A(t)]}{[A_0(B_0-A_0+[A(t)])]}\right\}\right]. \tag{6}$$

Thus, if fractal kinetics is the resultant intracellular reaction kinetics, plots of the second term on the R.H.S. versus $\log t$ will be linear with gradient p. Graphs of p versus time were derived from the slope of the latter plot.

2.2. Computational models

First we shall describe lattice-gas models (LGA) [5]. Initially molecules of two different kinds A and B are uniformly distributed on a two-dimensional lattice with unit spacing and periodic or fixed boundary conditions. A third kind of molecule, an obstacle molecule, is also similarly distributed. Obstacle molecules can be static or mobile. We will describe in detail the algorithm for static obstacles; the one for mobile obstacles is the same, with the exception that the "subject" molecules in this case include obstacle molecules. At each time step, a "subject" molecule (a molecule of type A, B or C) is randomly chosen and moved or reacted upon according to the following rules:

1. Randomly choose the nearest neighbor destination site.
2. If the destination site is empty, move to it.
3. Otherwise, if the "subject" molecule is A or B and the molecule occupying the destination site (the "target" molecule) is respectively B or A, generate a random number between 0 and 1. If this number is lower than the reaction

probability P, replace the "target" molecule with C, and remove the "subject" molecule.

Note that the algorithm does not allow more than one molecule to occupy any one lattice position, thus enforcing a hard-sphere molecular repulsion. The above sequence is repeated $n_{\text{total}}(t)$ times, where $n_{\text{total}}(t)$ is the current number of distinct molecules on the lattice (excluding obstacles) at time t. After one such sequence the time is incremented by one. We perform the simulations with two different lattice topologies: a square grid with coordination number 4 and a triangular grid with coordination number 6. The size of the lattice with the triangular grid has been scaled so that the obstacle concentration is the same for both types of grid.

We also construct an off-lattice Brownian Dynamics (BD) model. Each molecule's motion (for molecules of types A, B and C) is defined by an equation of motion of the following type

$$\dot{\mathbf{x}}_i(t) = \xi_i(t), \tag{7}$$

where i is a subscript identifying each molecule. These equations, called Langevin equations [6,8], are derived from Newton's second law in the limit of high friction. $\mathbf{x}_i(t)$ is defined to be the position of the center of mass of molecule i. The stochastic variable ξ_i is white noise defined by $\langle \xi_i^m(t)\rangle = 0$ and $\langle \xi_i^m(t)\xi_j^n(t')\rangle = 2D\delta_{i,j}\delta_{m,n}(t-t')$, where D is the diffusion coefficient of the molecules and the superscripts m,n refer to the spatial components of the noise vector. The angular brackets denote the statistical average. Molecules are modeled as circles of diameter d_0.

At each time step, a "subject" molecule i (of type A, B or C) with coordinates (x_i, y_i), is randomly chosen and moved or reacted upon according to the following rules:

1. Using the Langevin equation (Eq. (7)), the new position of the molecule's center of mass (x', y') after time δt is calculated.
2. If there exists no molecule (of any kind) occupying these destination coordinates then move the subject molecule to this new position. Formally this corresponds to the condition:

$$(x_j - x')^2 + (y_j - y')^2 \geq d_0^2, \forall j \text{ such that } j \neq i \tag{8}$$

3. Otherwise, if the "subject" molecule is A or B and the molecule occupying the destination coordinates ("target" molecule) is respectively B or A and the distance between the center of mass of the two molecules is less than d_0, generate a random number between 0 and 1. If this number is smaller than $P\delta t$, replace the "target" molecule with C, and remove the "subject" molecule.

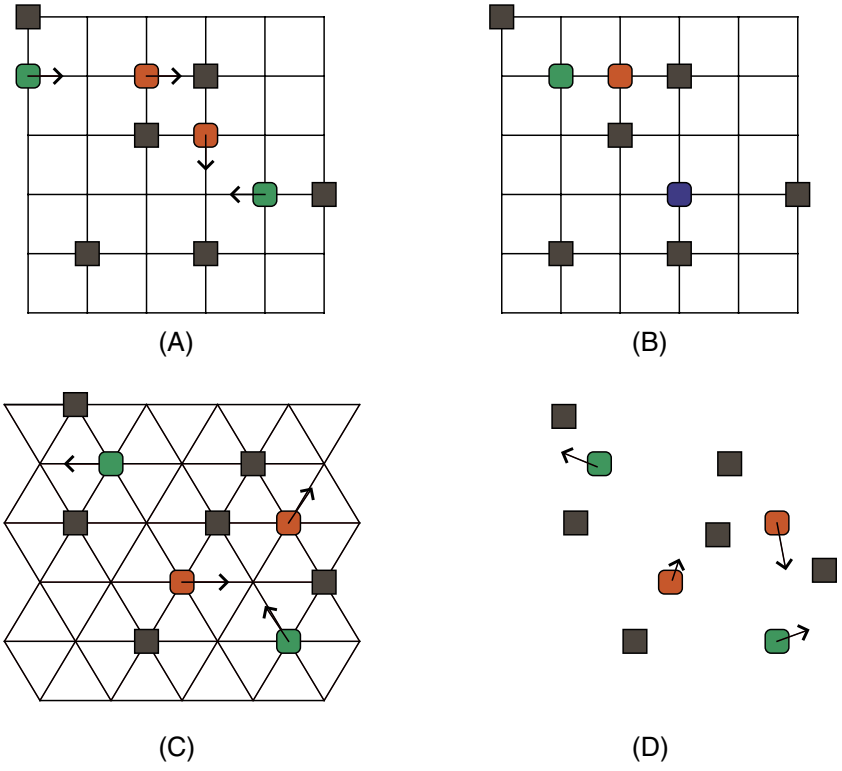

Fig. 1. Schematic illustrating the three simulation methods employed in this study: (A) and (B) show LGA based on a square grid, (C) shows LGA based on a triangular grid, (D) shows off-lattice Brownian dynamics. The large black squares represent the static obstacles (i.e. the intracellular macromolecules), the red circles represent molecules of species A, the green molecules represent molecules of species B and the blue molecules represent molecules of species C. The arrows represent the velocity of the molecules (the arrow's length is a measure of the magnitude of the velocity). For the square and triangular lattices, the velocity can be only in a small number of discrete directions and is always of the same magnitude whereas off-lattice the velocity can be in any direction and have any magnitude. We illustrate the dynamics of the molecules for the reaction $A+B \rightarrow C$ in (A) and (B). The molecules are initially randomly distributed on the square lattice with a random distribution of velocities (A); after a time step, molecules move to neighboring lattice points in which there are no obstacles and react with compatible molecules (B).

The algorithm enforces a volume-exclusion condition, as was done for LGA. The above sequence is repeated $n_{\text{total}}(t)$ times, where $n_{\text{total}}(t)$ is the current number of distinct molecules (obstacles are not counted, if they are static) at time t. After one such sequence the time is incremented by δt. Note that δt must be chosen small enough so that the increment in position coordinates (as calculated by the Langevin equation) is significantly less than the molecular diameter d; otherwise molecules could "jump" over other molecules, leading to an unrealistic and erroneous situation. The data from BD simulations is strictly speaking only valid in the limit $\delta t \to 0$. All BD simulations were done with $\delta t = 1$ and $\delta t = 0.1$: data obtained from simulations with these time steps agree, thus implying that our results are independent of the time step.

All three models (LGA with square lattice, LGA with triangular lattice and off-lattice BD) are schematically illustrated in Fig. 1.

3. Results

3.1. Reaction kinetics in 2D homogeneous environments

In this part of the paper we study the reaction kinetics of the reaction $A+B \to C$ in a two-dimensional homogeneous (i.e. obstacle-free) environment. Molecules of species B are stationary unless otherwise stated. Here we undertake the task of measuring p over the relevant reaction time, i.e. the time until at least 99% of the limiting (or minority) species, A, has reacted with the majority species B. This guarantees that the rate laws deduced from our simulations are biologically relevant, unlike the common practice of deducing the rate laws in the biologically unrealistic limit $t \to \infty$. Since the time required for a reaction to finish (i.e. the relevant reaction time defined above) depends on the particular values of the initial concentrations and the reaction probability, then it follows that the time axes in our graphs of p versus time will vary depending on the various factors involved. An added realism of our simulations is that they are in 2D; this is more realistic than the conventional 1D simulations, since it is well known that many intracellular reactions occur on 2D membranes or surfaces [13].

We investigate how the reaction kinetics varies as a function of P (Figs. 2 and 3), the initial conditions (Figs. 2 and 3) and the mobility of the majority species (Fig. 2A). We also study the relationship between the reaction kinetics and the geometry of space (i.e. torus or a flat plane) in which the reaction occurs (Fig. 4A) and the size of this space (Fig. 4A). Finally we investigate whether there exists a dependence on the lattice of the LGA simulations (Fig. 4B).

Note that the LGA simulations with square and triangular grids represent kinetics occurring in regular structures such as crystalline materials whereas the off-lattice simulations represent kinetics in intracellular environments where there is no underlying spatial lattice. An alternative way of looking at our simulations is that we are only simulating reaction kinetics in intracellular environments and that the grid, if present, is just an artificial construct of the simulation method.

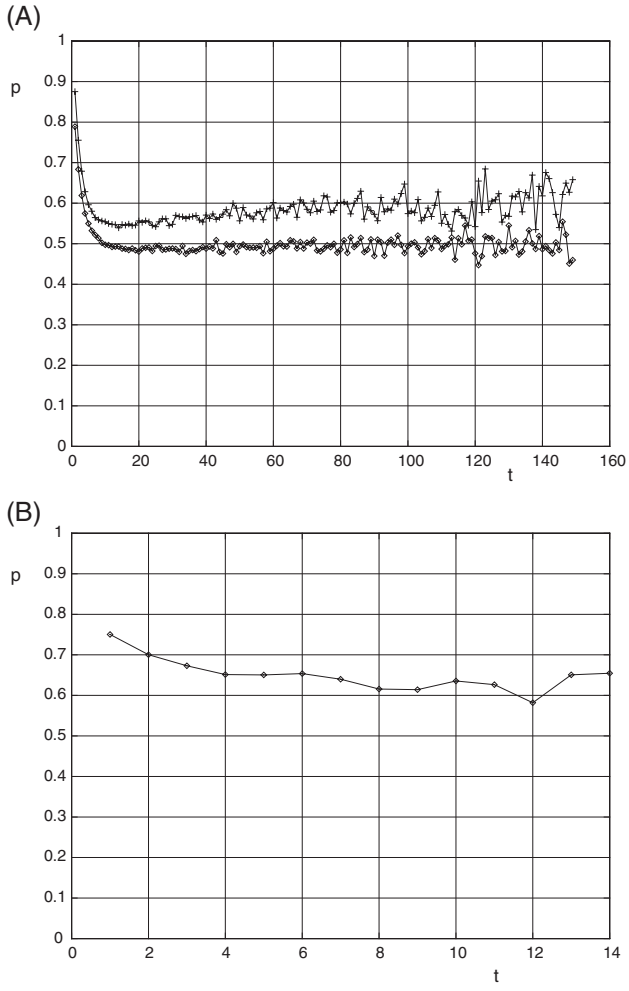

Fig. 2. Reaction kinetics as a function of the initial conditions in a homogeneous environment for large reaction probability. In this figure we plot the index p versus time for reactions in a homogeneous 2D plane with periodic boundary conditions. Here, $P=1$ and the initial conditions are (A) $A_0=0.4$, $B_0=0.5$ and (B) $A_0=0.1$, $B_0=0.5$. Molecules of species B are stationary except for the cross data points in (A), in which case the B molecules have the same diffusivity as the A molecules. We took 10 samples for (A) and 100 samples for (B). The data was generated using a 1000×1000 square lattice LGA.

We find that the reaction kinetics is well-approximated by fractal-like kinetics (since $p \backsimeq constant$) during the course of the reaction. The kinetics is non-classical, i.e. $p \backsimeq 1/2$ (this value agrees with the theoretical value of $p=d/4$ mentioned in Section 1.1) in the limit of large P and $A_0 \backsimeq B_0$, whereas classical kinetics ($p \backsimeq 1$) is recovered in the limit of small P and $A_0 \ll B_0$. We can explain these observations as follows: (i) For small P, molecules of species A tend to explore large regions of space before reacting, in the process "mixing" the solution thus increasing the homogeneity of the environment. (ii) For $A_0 \ll B_0$ molecules of species A find molecules of the abundant species B very easily, a phenomenon qualitatively similar to that found in well-stirred conditions. As illustrated in Fig. 3A, there is a small change in the value of p (20%) if the simulations are repeated with diffusing molecules of species B rather than stationary ones. All the following results are qualitatively robust to the mobility of B.

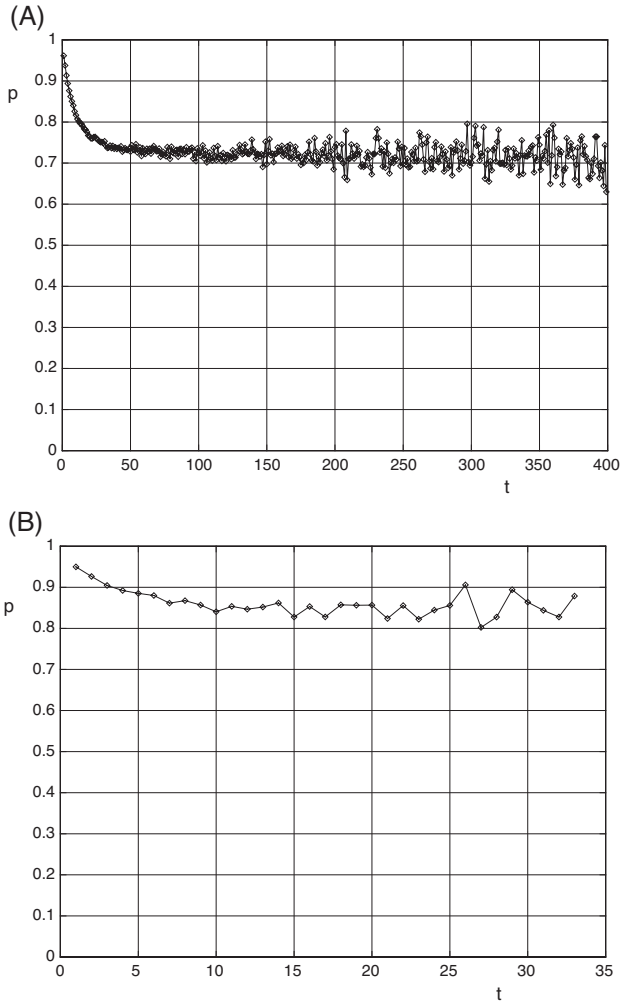

Fig. 3. Reaction kinetics as a function of the initial conditions in a homogeneous environment for small reaction probability. In this figure we plot the index p versus time for reactions in a homogeneous 2D plane with periodic boundary conditions. Here, $P=0.1$ and the initial conditions are (A) $A_0=0.4$, $B_0=0.5$ and (B) $A_0=0.1$, $B_0=0.5$. Molecules of species B are stationary. We took 10 samples for (A) and 100 samples for (B). The data was generated using a 1000×1000 square lattice LGA.

Similarly lattice size effects and the geometry of space (Fig. 4A) do not tend to have an appreciable effect on the reaction kinetics. Such effects would be expected to be appreciable only when the condition $\sqrt{Dt}\sim L$ is fulfilled, where D is the diffusion coefficient of the molecules and L is the size of space. Unless L is very small (or D very large or a combination of the two), these effects are generally only significant in the limit $t\to\infty$, and thus are not of much experimental relevance. This effect is further exacerbated by the fact that diffusion coefficients of macromolecules in the cytoplasm are usually 5–20 times smaller than their values in saline solution [17]. The finiteness of the environment implies that there is a long wavelength cutoff in the available diffusion modes, which translates into an increase in the value of p as the system size becomes smaller. Note that this increase is small: a 97% decrease in the linear dimension of the 2D space causes a 6% increase in the value of p. We find that the results are fairly robust to changes in the type of lattice (square or triangular) and even to the 'removal' of the lattice (off-lattice Brownian Dynamics (BD)] (Fig. 4B). There is a small noticeable increase of p with increasing rotational symmetry of the space in which the reaction occurs ($p_{\text{off-lattice}}>p_{\text{triangle}}>p_{\text{square}}$). This is because any grid will restrict the movement of molecules to some extent. Thus the system is slightly less stirred for simulations on a square grid than on a triangular grid; similarly it is less stirred on a triangular grid than when there is no grid at all. However, as we previously observed, these artifacts do not change the results qualitatively and only cause very minor changes to the quantitative results.

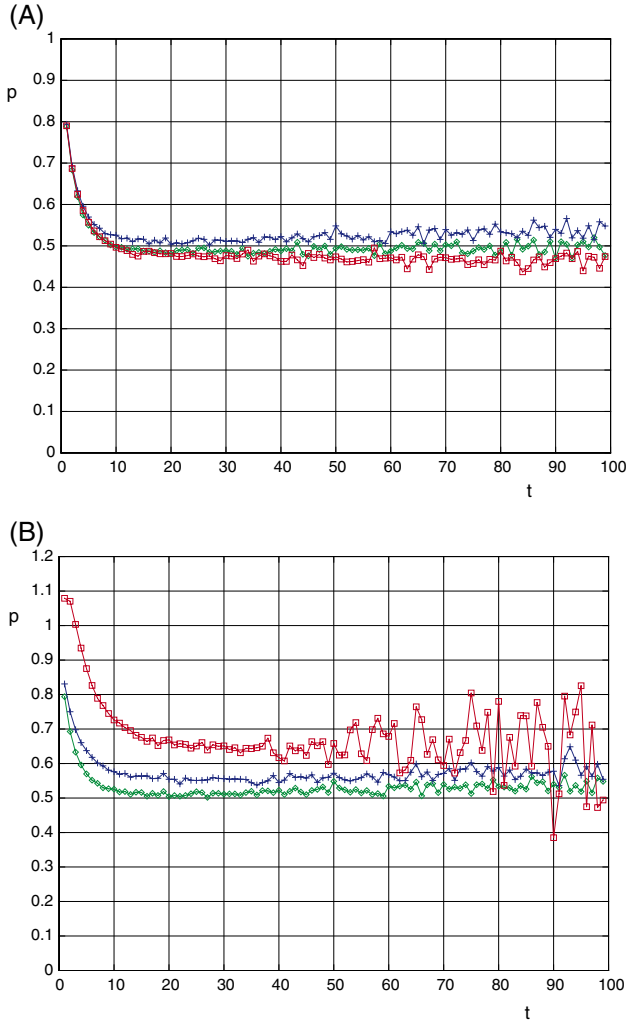

Fig. 4. Reaction kinetics as a function of the lattice size, topology and boundary conditions in a homogeneous environment. (A) Plots of the index p versus time for reactions in a homogeneous 2D plane. Here, $P=1.0$ and the initial conditions are $A_0=0.4$, $B_0=0.5$ on a square lattice. We evaluate lattice size (ls) and boundary condition (bc) effects on the kinetics: (green) ls=1000×1000, bc=periodic; (blue) ls=30×30, bc=periodic; (red) ls=30×30, bc=fixed. (B) Plots of the index p versus time for reactions in a homogeneous 2D plane. Here, $P=1.0$ and the initial conditions are $A_0=0.4$, $B_0=0.5$ in a 30×30 periodic box. We evaluate lattice topology effects on the kinetics: (green) square lattice LGA; (blue) triangular lattice LGA; (red) off-lattice BD. We took 10^4 samples to generate the data, with the exception of the data with ls=1000×1000 (10 samples) and the data for the off-lattice BD (20 000 samples).

3.2. Reaction kinetics in 2D heterogeneous environments

In this subsection we study the reaction kinetics of the reaction $A+B\rightarrow C$ in a two-dimensional heterogeneous (obstacle-ridden) environment. Molecules of species A, B and C are mobile while the obstacles molecules are static, unless otherwise stated. There are three main variables in this case: (i) the reaction probability P (ii) the initial conditions A_0, B_0 and (iii) the obstacle concentration $[O]$. Our choice of obstacle concentration values ($[O]=0.1,0.2,0.3,0.4$) spans the experimentally estimated range: $0.05\leq[O]\leq 0.4$ [7,9] of macromolecular crowding agents in the cytoplasm.

We compare the heterogeneous and homogeneous kinetics. We find that the dependence of the behavior on P and the initial conditions is qualitatively the same in both environments. That is, the reaction kinetics can be either classical or non-classical. We investigate how the reaction kinetics depends on (i) the type of spatial lattice (square or triangular) used in LGA simulations (Fig. 5), (ii) the obstacle mobility (Fig. 6), (iii) the geometry of the space (i.e. torus or a flat plane) in which the reaction occurs (Fig. 7), (iv) the size of this space (Fig. 7) and (v) the presence or absence of a natural spatial lattice (LGA or off-lattice BD) (Fig. 8).

We find that LGA simulations of reactions in heterogeneous environments are sensitive to the type of spatial lattice. This is contrary to the results for homogeneous environments. We show that LGA simulations with a square grid magnify the effect of macromolecular crowding in comparison to simulations using a triangular grid (Fig. 5). The simulations on the two grids differ qualitatively as regards the rate law. Square grid simulations give non-classical and non-fractal kinetics (i.e. $p<1$ and $p\neq$ constant). Triangular grid simulations give non-classical and approximately fractal kinetics ($p<1$ and $p\simeq$ constant) for low to moderate obstacle concentrations ($0<[O]<0.3$). For a high obstacle concentration ($[O]=0.4$) we see non-classical and non-fractal kinetics ($p<1$ and $p\neq$ constant). If we plot log $k(t)$ versus log time, as is the convention, deviations from fractal-like kinetics are less apparent. This is particularly true when the temporal change

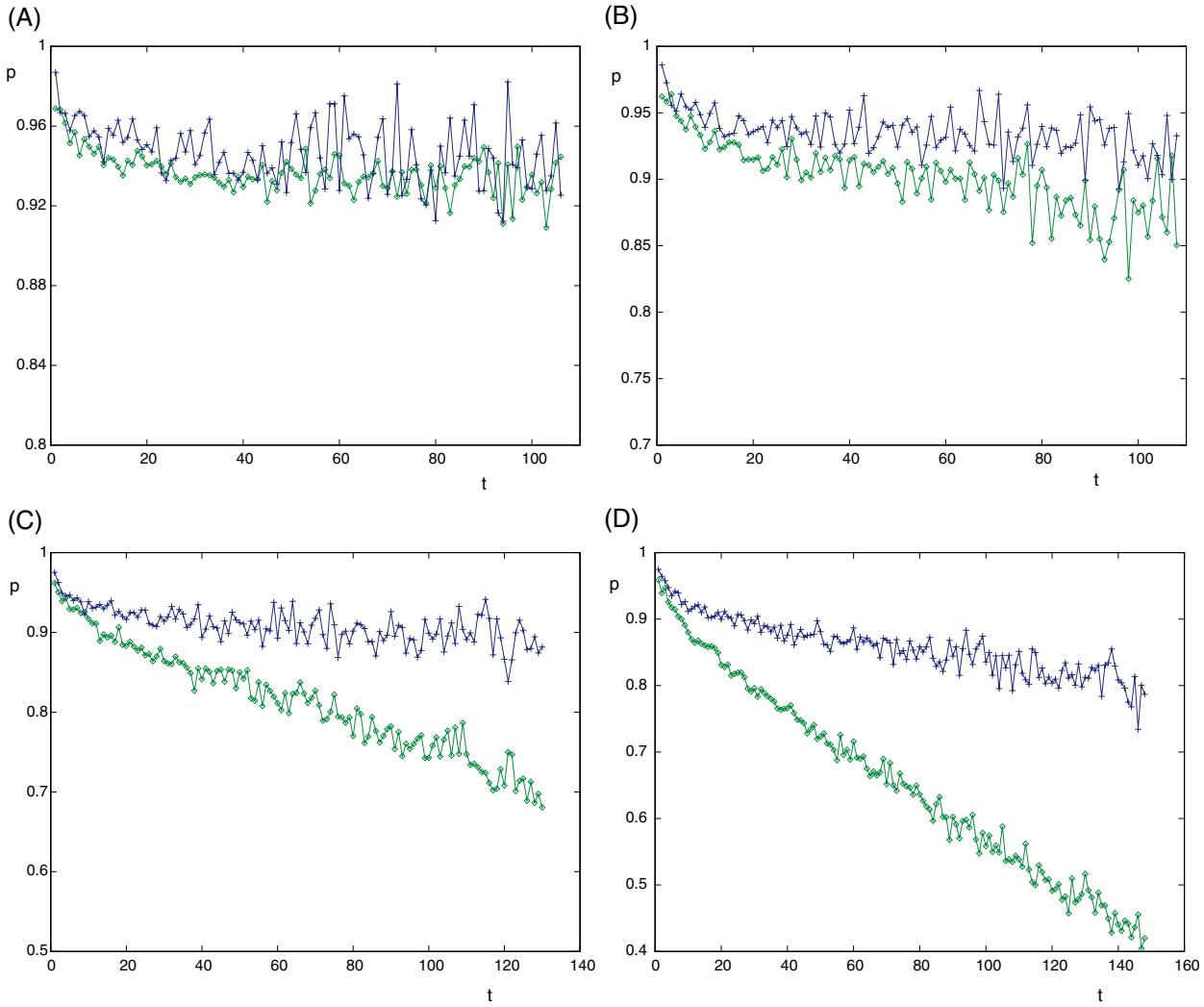

Fig. 5. Effects of the lattice topology on the reaction kinetics in a heterogeneous environment. We plot the index p versus time for reactions in a heterogeneous 2D plane with periodic boundary conditions. Here, $P=0.2$ and the initial conditions are $A_0=0.01$, $B_0=0.1$. The plots are generated by averaging over 500 samples of data obtained from a 500×500 square lattice LGA (green data points) and a 500×500 triangular lattice LGA (blue data points). Molecules of all species (A, B and C) are mobile. The static obstacle concentration is (A) $[O]=0.1$, (B) $[O]=0.2$, (C) $[O]=0.3$, (D) $[O]=0.4$.

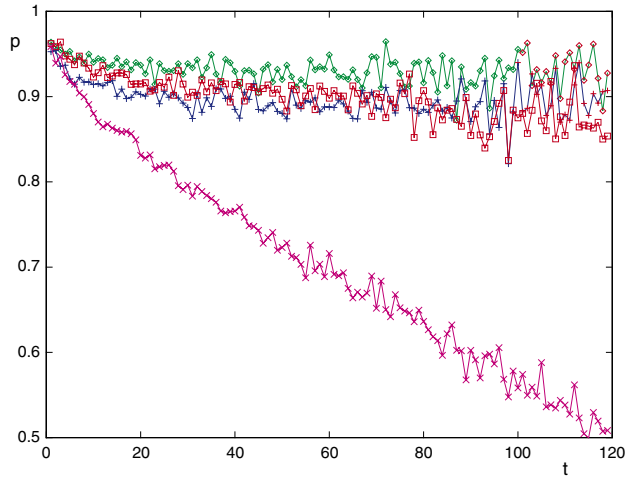

Fig. 6. Variation of the reaction kinetics in a heterogeneous environment with obstacle mobility. We plot the index p versus time for reactions in a heterogeneous 2D plane with periodic boundary conditions. Here, $P=0.2$ and the initial conditions are $A_0=0.01$, $B_0=0.1$. The plots are generated by averaging over 500 samples of data obtained from a 500×500 square lattice LGA. Molecules of species A, B and C are mobile. In two of the four cases shown, the obstacles are static with obstacle concentrations $[O]=0.2$ (red) and $[O]=0.4$ (pink). In the other two remaining cases, the obstacles are mobile with obstacle concentrations $[O]=0.2$ (green) and $[O]=0.4$ (blue).

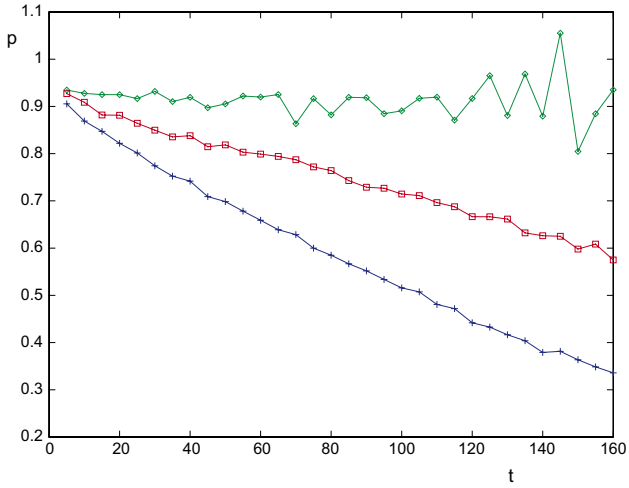

Fig. 8. Comparison of the reaction kinetics in a heterogeneous environment using lattice and off-lattice methods. We plot the index p versus time for reactions in a heterogeneous 2D plane with periodic boundary conditions. Here, $P=0.2$, the initial conditions are $A_0=0.01$, $B_0=0.11$ and the static obstacle concentration is $[O]=0.44$. The data is generated using (i) a 30×30 square lattice LGA (blue), (ii) a 30×30 triangular lattice (red), (iii) an off-lattice BD in a 30×30 box (green). 10^5 samples were used for cases (i) and (ii), and 10^4 samples for case (iii). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

in p is small, as for example for $[O]=0.2$. The method we employ to extract p enables us to determine more easily and precisely any deviations from fractal kinetics; these deviations cannot be easily appreciated on conventional log–log plots.

If we take the point of view that the lattice is a natural one, then our results imply that the kinetics inside heterogeneous crystalline materials depends strongly on the lattice symmetry. If we take the alternative point of view, namely that we are simply simulating reactions occurring in intracellular environments and that there is no real lattice, then we conclude that using LGA simulations based on a square grid over-estimates macromolecular crowding effects. In this regard, simulations with a triangular grid are more realistic than with a square grid. The natural free packing of discs is hexagonal, since this is the densest regular arrangement possible. In our simulations, molecules are represented by discs. The triangular grid allows this dense packing, whereas the square grid cannot. On the square grid the molecules can only move or interact in four directions which is significantly less than for the triangular grid. This difference becomes more pronounced with the introduction of obstacles. As we increase the obstacle concentration, the square grid becomes congested much more rapidly than the triangular grid. This explains the over-estimation of crowding effects. The differences between the two types of LGA are more noticeable when there are a significant number of obstacles in the environment, and particularly if these obstacles are relatively static compared to the reactant molecules. These two conditions are not uncommon in intracellular environments.

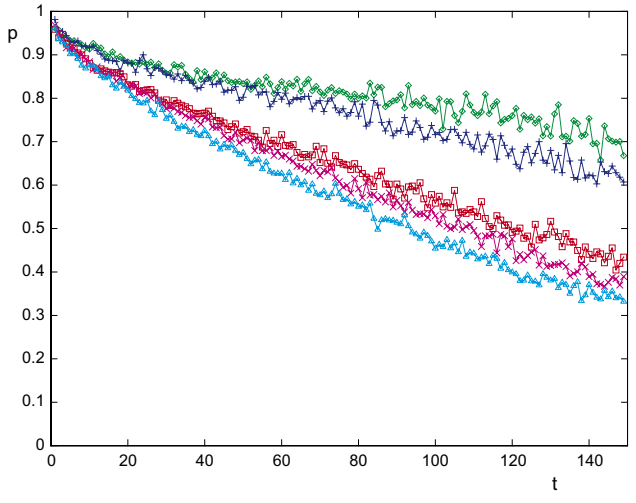

Fig. 7. Effects of the boundary conditions and lattice size on the reaction kinetics in a heterogeneous environment. We plot the index p versus time for reactions in a heterogeneous 2D plane. Here $P=0.2$, the initial conditions are $A_0=0.01$, $B_0=0.11$ and the static obstacle concentration is $[O]=0.44$. The data is generated using (i) a triangular lattice LGA on a 500×500 grid (green, 500 samples) and a 30×30 grid (blue, 10^5 samples), (ii) a square lattice LGA on a 500×500 grid (red, 500 samples) and a 30×30 grid (pink, 10^5 samples), (iii) a square lattice LGA on a 30×30 grid with fixed boundary conditions (cyan, 10^5 samples). Boundary conditions are periodic for all cases except case (iii).

We now consider the situation where the obstacles are free rather than fixed. In the cytoplasm, obstacle molecules are most probably macromolecules, which are relatively immobile compared to the smaller reactant molecules. Therefore simulating fixed obstacles is a good first approximation. We investigate the effect of obstacle mobility on the kinetics. To this end, we now simulate the case where the obstacle molecules have the same diffusivity as the reactant molecules. The result is behavior closer to classical (larger values of p)

than for static obstacles (Fig. 6). In particular, for the square grid, we see fractal-like kinetics whereas for static obstacles we found non-fractal (but still non-classical) kinetic behavior. Differences between the results for static and non-static obstacles increase with obstacle concentration. The movement of the obstacles allows greater movement for all molecules. This type of dynamics is closer to that modeled by the classical rate law, that of a well-mixed, homogeneous environment. Of course, the kinetics of our system is still heterogeneous and therefore non-classical.

We now briefly consider the effect of lattice size and geometry on the kinetics, reverting to fixed obstacles (Fig. 7). We find that these factors play a minor role in determining the reaction kinetics, as for the homogeneous environment. In fact, these effects are even less pronounced than in the homogeneous case, since the effective diffusivity in an obstacle-ridden space is significantly smaller than that in free space.

Next we compare the LGA results with off-lattice Brownian Dynamics (BD). The two simulation techniques differ in approach, which means we can only make an approximate comparison. LGA model reactions by simulating point particles moving and reacting on a lattice. The 2D off-lattice method models reactions by simulating the movement and reaction of discs in free space. In an off-lattice simulation the diameter of the particle corresponds to the lattice spacing in an LGA. In our LGA the diffusion coefficient is $D=\delta x^2/2\delta t$ where $\delta x=1$, the lattice spacing and $\delta t=1$ is the time step. We see that the two methods roughly agree for small obstacle concentrations and progressively diverge as this increases (Figs. 4B and 8). As the obstacle concentration increases, compartmentalization occurs. In the off-lattice simulation molecular movement of course remains smooth. However in the LGA, as the compartment size decreases, the movement of molecules becomes sensitive to the lattice topology. If we are using the LGA to model intracellular kinetics then this is a problem since it means that data artifacts increase proportionately with the obstacle concentration. One solution is implementing a simulation in which molecules are modeled as circles moving on a fine mesh. The spacing of this grid has to be much smaller than the molecular diameter. This is different from LGA in which the molecular diameter is always the same as the lattice spacing. This approach is not used because it offsets the inherent simplicity of LGA, and it is usually assumed that grid effects are negligible. In general the differences between LGA with triangular grid and off-lattice simulations are small, except when the obstacle concentration is high. The off-lattice simulation always gives fractal-like kinetics independent of the extent of spatial heterogeneity. This shows that the effect of macromolecular crowding on the value of p is not as significant as LGA-based studies have previously suggested. An alternative viewpoint is that reaction kinetics inside heterogeneous crystalline structures deviates more from mass action than the reaction kinetics in intracellular environments of comparable heterogeneity.

As we remarked in Introduction, p is a non-dimensional index quantifying deviations from the classical law of mass action. Clearly it would be desirable to be able to have a mathematical expression from which we could deduce the value of p for any given system of interest. Unfortunately this is not possible in general since as we have shown, the value of p depends on too many factors (the type of reaction, the mobility of the species, the dimensionality, geometry and size of the space, the mobility of the obstacles, and the obstacle concentration) to enable such a precise quantification. Indeed the problem of exactly solving the kinetics of the $A+B\rightarrow C$ in a one-dimensional homogeneous space (which is far simpler than what we have considered in this article) has only been solved recently [3], even though the problem was first posed more than 20 years ago [15]. It must be emphasized however that in this article we have shown for the first time what is the proper simulation approach to determine the valid intracellular kinetics; thus given a particular system of interest, one can use this approach to computationally determine p.

Given that one can determine p from the simulations, one might ask what are the practical implications of such an endeavor. The index p measures deviation of the kinetics from those predicted by the law of mass action. The closer p is to unity, the closer are the kinetics to mass action. One might ponder for what values of p are the complications arising from crowding biologically significant. For example say $p=0.9$; is this value close enough to unity to warrant the use of the classical law and ignore crowding? To answer such a question we briefly quantify the error in choosing to ignore crowding effects (i.e. assuming $p=1$ even though $p<1$).

Consider the situation where an intracellular reaction $A+B\rightarrow C$, with initial conditions $A_0 \ll B_0$, is characterized by a value p. Then based on Eq. (4), the decay of the minority species is given by:

$$[A(t)] \approx A_0 \exp\left[-\frac{(B_0 - A_0)t^p}{p}\right]. \tag{9}$$

A measure of the total reaction time is given by the time when the concentration of the limiting species A has decayed to A_0/e which is given by:

$$t^* \approx \left(\frac{p}{B_0 - A_0}\right)^{1/p}. \tag{10}$$

If one ignored crowding effects then the total reaction time would be given by Eq. (10) but with $p=1$. Then the error in ignoring crowding is easily shown to be given by the approximate formula:

$$E \approx B_0^{1-1/p} p^{1/p} - 1. \tag{11}$$

Thus given this equation, one can get a rough estimate for the error introduced by assuming that the classical law holds in cases where it actually might not. It is easy to generalize the above expression for all initial conditions though the form of the error expression becomes significantly more complex.

4. Discussion

We have presented a thorough computational analysis of an elementary reaction in an environment modeling features of the

cytoplasm. We have shown that it is possible for the reaction kinetics in intracellular environments to be either classical (following the law of mass action) or non-classical (following Kopelman's fractal-like kinetics). Both situations seem likely to occur. The type of reaction kinetics is not determined by the heterogeneity of the space but rather by the value of the reaction probability P and by the initial conditions (A_0 relative to B_0). In agreement with previous results, the kinetics is always fractal in the asymptotic limit $t\to\infty$. Here, the asymptotic value of the fractal index is $p\to 1/2$ independent of P or initial conditions. We have shown that classical or fractal-like kinetics governs reactions during the transient, until 99% of the minority species is exhausted. In particular, we find that classical kinetics is recovered in the limit of small P and for $A_0 \ll B_0$. Therefore, we have shown that the many asymptotic results in the literature of diffusion-limited chemical reactions, though correct, may not be of experimental relevance.

We find that the results are virtually independent of the geometry and size of the 2D space in which the reaction occurs, for both homogeneous and heterogeneous environments. This means reactions occurring in physically dissimilar parts of the cell (for example a small toroidal vesicle or a comparatively flat portion of the plasma membrane) will essentially exhibit the same type of kinetics. Note that we have only studied volume-exclusion effects stemming from macromolecular crowding but have neglected more complicated effects such as ionic or covalent interactions of the molecules with the surface and with each other. Including such effects may possibly introduce a dependence of the kinetics on the surface geometry.

LGA have long been the preferred method for simulating chemical reactions due to their simplicity and ease of implementation. The discrete spatial lattice on which the point molecules hop approximates well the conditions in some types of heterogeneous kinetics, e.g. the exciton fusion experiments in isotopically-mixed crystals, but have no counterpart when modeling the intracellular environment. Thus it is possible that the data obtained from LGA simulations of intracellular reactions might have artifacts induced by the grid. To check for such a dependency we compared data produced by LGA with both square and triangular lattices with off-lattice BD. We find that there is no significant difference between the three methods for homogeneous conditions. However, as the obstacle concentration increases, the differences increase proportionately, showing both qualitative and quantitative differences between all three simulations. In general we conclude that (i) inside cells (off-lattice BD) the reaction kinetics is very well-described by fractal-like kinetics, (ii) LGA simulations, particularly those based on a square grid, over-estimate the effect of intracellular crowding and consequently predict the wrong type of kinetic laws.

An alternative point of view is that in comparing LGA with different lattices and the off-lattice BD, we are actually comparing the kinetics in heterogeneous structures with a natural spatial lattice such as inside crystals and the kinetics inside cells, where there is no natural spatial lattice. From this perspective, our results show that deviations from mass action are much more pronounced inside heterogeneous crystalline structures than in intracellular environments of comparable heterogeneity.

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