Excluded volume effects in on- and off-lattice reaction–diffusion models

Lina Meinecke, Markus Eriksson

Abstract: Mathematical models are important tools to study the excluded volume effects on reaction–diffusion systems, which are known to play an important role inside living cells. Detailed microscopic simulations with off-lattice Brownian dynamics become computationally expensive in crowded environments. In this study, the authors therefore investigate to what extent on-lattice approximations, the so-called cellular automata models, can be used to simulate reactions and diffusion in the presence of crowding molecules. They show that the diffusion is most severely slowed down in the off-lattice model, since randomly distributed obstacles effectively exclude more volume than those ordered on an artificial grid. Crowded reaction rates can be both increased and decreased by the grid structure and it proves important to model the molecules with realistic sizes when excluded volume is taken into account. The grid artefacts increase with increasing crowder density and they conclude that the computationally more efficient on-lattice simulations are accurate approximations only for low crowder densities.

1 Introduction

Living cells are regulated by complicated signalling pathways that control which genes are expressed and how the cells behave. These reaction networks are spatially organised with important reaction complexes often bound to the cell membrane and with the DNA being confined inside the nucleus in eukaryotes. Moreover, the cytoplasm of cells is highly crowded, meaning that up to 40% of the volume is occupied by macromolecules, which are present only at very low concentrations. On membranes, crowding is even more severe, due to attaching actin filaments creating static barriers. Assuming only hard sphere repulsion between these crowding macromolecules, three excluded volume effects are the consequence: (i) it forces moving molecules (tracers) to diffuse around obstacles (crowders), slowing down the diffusion; (ii) it either decreases (diffusion limited case) or increases (reaction rate limited case or dimerisations) the reaction rates; and (iii) due to the impeded diffusion, it increases the spatial heterogeneity inside the cells, leading to spatial self-organisation. More complex interactions between the crowders and tracers such as transient binding or hydrodynamic effects have further effects on the reaction–diffusion behaviour inside cells. These are deterministic ordinary differential equations describing the concentrations of the reacting molecules, and they are applicable when the law of large numbers holds, meaning that the molecules are present at large copy numbers, and when they are well-mixed in a dilute system. Consequently, mathematical models are a crucial tool to understand reaction–diffusion processes in the intracellular environment.

One class of mathematical models are so-called particle based reaction–diffusion models (PBRD), where we resolve individual molecules by following their diffusive paths and by modelling reactions as random events. These are considered as the microscopic modelling level in reaction–diffusion simulations. One group of PBRD are Brownian dynamics (BD) simulations, where the diffusive motion is modelled as a continuous time continuous space stochastic process. When the particles are modelled as hard spheres these simulations inherently account for the excluded volume effects. However, the catch with these methods is, that they become computationally very expensive due to the high number of collision events when applied to dense environments. An approximation to BD simulations are lattice based approaches, such as cellular automata (CA), where we still follow individual trajectories, but the possible particle positions are restricted to an artificial grid. These methods are cheaper to simulate and in this paper we will investigate what influence the artificial lattice has on the excluded volume effects for the reaction and diffusion rates and the stochastic noise. We therefore simulate both models in a two-dimensional (2D) plane representing cellular membranes.

This microscopic modelling framework captures the internal and external noise, that often play an important role in computational systems biology. Reaction–rate equations (RREs) are a macroscopic approximation to this microscopic description. These are deterministic ordinary differential equations describing the concentrations of the reacting molecules, and they are applicable when the law of large numbers holds, meaning that the molecules are present at large copy numbers, and when they are well-mixed in a dilute system. They consequently do not capture the stochastic or space dependent effects inside cells, but provide suitable reference solutions in dilute media to compare the effect of macromolecular crowding to.

In the following section, we will present the on- and off-lattice microscopic tools in more detail. We will then perform simulations in environments with various crowder densities for static crowders and show that the diffusion is more severely slowed down when simulated in continuous than in discrete space. In Section 4, we will extend the simulations to reactions in a crowded environment, and find that they are non-linearly affected by the artificial lattice. We finally draw conclusions on the agreement between the BD and CA models when excluded volume effects are important in Section 5.

2 Particle based reaction–diffusion models

Particles in solution move due to their thermal energy, and the collisions with the smaller solvent molecules result in a random walk often modelled by Brownian motion. This causes them to diffuse from high to low concentrations. We will now present how to create sample trajectories of a reaction–diffusion system with on- and off-lattice simulations, where the particles diffuse with diffusion coefficient $D$. We demonstrate how to treat reaction events using the example of the association event

$$A + B \xrightarrow{k_A} C,$$

where $k_A$ is the intrinsic reaction rate.
2.1 Continuous space

BD simulations sample trajectories of Brownian motion in continuous space, or off-lattice. To simulate a trajectory, one can discretise time with a small enough fixed time step $\Delta t$. We then draw a normally distributed random number $\xi \sim \mathcal{N}(0, 1)$ to compute the new particle position as

$$x(t + \Delta t) = x(t) + 2 \gamma_0 \Delta t \xi,$$  \hspace{1cm} (2)

and equivalently for the other space coordinates, since Brownian motion is independent along the Cartesian axes. This algorithm is implemented among others in the freely available software package Smoldyn [12, 13]. Here, a time step dependent binding radius is chosen such that the simulated reaction rate equals $k_A$. Within this binding radius two reaction partners react with probability one and the method has been used for simulations in a crowded environment [14, 15, Ch. 4]. Similarly, in MCell [16] the position of a diffusing molecule after the discrete time step $\Delta t$ is sampled from the probability distribution of its position. Extensions to this basic implementation of reactions include the BD software ReaDDy [17], which allows for interaction potentials between the simulated particles and has been used for studies of the excluded volume effects in [18, 19] and SRSim [20], which allows for more complicated reactions such as rotational dependency by rule-based modelling. In coarse grained BD simulations, the particles move a fixed distance $\Delta x$ in each time step $\Delta t$, but in a random direction uniformly distributed in the disc or sphere around the particle. This approach has been used in [21, 22].

Another algorithm for simulations in dilute media is Green's functions reaction dynamics (GFRD) [23], where single particles or particle pairs are surrounded by protective domains. An asynchronous time step is chosen in an event driven algorithm to be the time when the first particle leaves its protective domain and in this way one avoids simulating all the relatively uninteresting jumps between collision events. An exact implementation is the so called first-passage kinetic Monte Carlo algorithm [24–26], used in the software packages ECell [27] and eGFRD [28]. The GFRD algorithms, however, become computationally very expensive [29], when applied to non-dilute systems and we will perform BD simulations with basic reactions with the software Smoldyn to investigate the excluded volume effects in continuous space.

Volume exclusion effects are inherently simulated with these algorithms [30], when the particles are represented as hard spheres rather than point particles and BD simulations have been performed to study crowding effects in [15, 31]. The review article [11], summarises which of the available software packages allow for this feature.

2.2 Discrete space

To gain further efficiency when simulating PBBD we will now restrict the particles’ trajectories to an artificial lattice. The so-called cellular automata (CA) or lattice gas automata models [32] are widely used to investigate excluded volume effects on both the diffusion rates [33–37] and the reaction rates [2, 6, 38, 39]. First, the domain is discretised into a grid or lattice. Each lattice site can then contain at most one molecule and at each time step. The molecules are picked in random order to jump to a neighbouring site. If the sampled target site is occupied, either a reaction happens with probability $p_A$ or the move is rejected, which models the excluded volume effects. Since the mean square displacement (MSD) of a diffusing molecule in $d$ dimensions is

$$\langle x^2(t) \rangle = 2d \gamma_0 t,$$  \hspace{1cm} (3)

We choose the time step

$$\Delta t = \frac{h^2}{2d \gamma_0},$$  \hspace{1cm} (4)

for a lattice spacing $h$. The algorithm for the simple reaction (1) until final time $T$ then reads as shown in Algorithm 1 (see Fig. 1). This algorithm has been used for Cartesian and hexagonal lattices and in [38] it is shown that resulting reaction dynamics simulated with the algorithm are sensitive to the choice of grid in a crowded environment and that the artificial grid in general overestimates the excluded volume effects on the reaction rates as compared with BD simulations. In [40] binding and unbinding of ligands to a single target site is simulated on a Cartesian grid and the derived reaction constants agree with BD simulations. The algorithm has been extended in [33] to model different molecule shapes, which consist of combinations of Cartesian lattices, and the

---

**Algorithm 1**

1. Place initial numbers of $A$, $B$ and $C$ molecules randomly on the grid.
2. while $t < T$ do
3.   Choose molecules in random order.
4.     for each molecule do
5.       Randomly choose a nearest neighbor site as target.
6.         if target site is empty then
7.           Move molecule.
8.           else
9.             if molecule is $A(B)$ and target is occupied by $B(A)$ then
10.                Generate a random number $\xi$.
11.                   if $\xi < p_A$ then
12.                      Replace $A$ and $B$ with a $C$ molecule at target site.
13.                   else
14.                      Reject the jump.
15.                end if
16.            else
17.                   Reject the jump.
18.                end if
19.          end if
20.     end for
21. end while

---

**Fig. 1** Algorithm 1: cellular automata
To test if this time step is sufficiently small, we performed the guarantee that the off-lattice simulations are time step independent. Algorithm 1 (see Fig. 1), referred to as CA, and use the open simulations, we choose a finer resolution with participate in the reaction processes.

In all the experiments, we choose \( h = 1 \) and the 2D domain \( 50 \times 50 \) to perform the on-lattice simulations according to Algorithm 1 (see Fig. 1), referred to as CA, and use the open source software Smoldyn for the off-lattice simulations, referred to as BD. We investigate three different lattices (Cartesian, Cartesian with diagonal jumps and hexagonal, see Fig. 1 in supplementary material) in the CA simulations. For all simulations, we choose the molecule radius \( r = 0.5 \). Assuming that each lattice sight can at most hold one molecule, this leads to a lattice size of \( h = 1 \) for both the Cartesian and hexagonal lattices. The time and space resolutions of the on-lattice simulations are defined by \( h \) and by \( (4) \), which leads to the time step \( \Delta t_{CA} = 0.25 \) for the CA models on a classical Cartesian grid and the hexagonal grid where all jump lengths are equal. In the Cartesian case, where longer diagonal jumps are possible we choose the time step as in \( [41] \), resulting in fewer jumps with \( \Delta t_{CA, \text{diag}} = 0.2947 \) and the probability that a jump occurs along a diagonal \( p_{\text{diag}} = 0.1788 \). For the BD simulations, we choose a finer resolution with \( \Delta t_{BD} = 0.001 \), to guarantee that the off-lattice simulations are time step independent. To test if this time step is sufficiently small, we performed the same simulations with a smaller time step of \( \Delta t_{BD} = 0.0001 \) and observed no difference in the diffusive behaviour.

diffusivity of moving molecules among static crowders is computed for a mix of shapes.

In the following sections, we will perform both, BD and CA simulations in crowded environments to examine in more detail if CA is a good approximation of BD for simulating both reaction and diffusion dynamics in this setting. The crowders are here represented as static and inert particles, which do not actively participate in the reaction processes.

3 Diffusion simulations

In all the experiments, we choose \( \phi = 1 \) and the 2D domain \( 50 \times 50 \) to perform the on-lattice simulations according to Algorithm 1 (see Fig. 1), referred to as CA, and use the open source software Smoldyn for the off-lattice simulations, referred to as BD. We investigate three different lattices (Cartesian, Cartesian with diagonal jumps and hexagonal, see Fig. 1 in supplementary material) in the CA simulations. For all simulations, we choose the molecule radius \( r = 0.5 \). Assuming that each lattice sight can at most hold one molecule, this leads to a lattice size of \( h = 1 \) for both the Cartesian and hexagonal lattices. The time and space resolutions of the on-lattice simulations are defined by \( h \) and by \( (4) \), which leads to the time step \( \Delta t_{CA} = 0.25 \) for the CA models on a classical Cartesian grid and the hexagonal grid where all jump lengths are equal. In the Cartesian case, where longer diagonal jumps are possible we choose the time step as in \( [41] \), resulting in fewer jumps with \( \Delta t_{CA, \text{diag}} = 0.2947 \) and the probability that a jump occurs along a diagonal \( p_{\text{diag}} = 0.1788 \). For the BD simulations, we choose a finer resolution with \( \Delta t_{BD} = 0.001 \), to guarantee that the off-lattice simulations are time step independent. To test if this time step is sufficiently small, we performed the same simulations with a smaller time step of \( \Delta t_{BD} = 0.0001 \) and observed no difference in the diffusive behaviour.

To investigate the diffusive motion over long times without encountering boundary effects we implement periodic boundary conditions on all boundaries.

The MSD of a diffusing molecule surrounded by static crowders is plotted in Fig. 2 for an increasing fraction of occupied volume \( \phi \). In CA we simulate \( 10^4 \) trajectories each in a different crowder distribution and in BD we simulate 100 different crowder distributions with each 1000 trajectories. Note that the more accurate BD simulations with the small time step \( \Delta t_{BD} \) run ca. 58 times slower than the CA simulations for \( \phi = 0.4 \).

If no crowders are placed in the system (\( \phi = 0 \)), all models agree with the theoretical MSD in dilute medium \( (3) \) (see Fig. 2 in supplementary material). However, for an increasing \( \phi \) the on- and off-lattice models increasingly differ and the speed of diffusion decreases, which is expected. The unexpected finding is that the diffusion is slowed down more severely with BD than with CA simulations. This appears counter-intuitive at first, since the artificial grid decreases the available directions of movement and the off-lattice model with infinitely many degrees of freedom (dof) is expected to simulate more mobile particles. However, the lattice also orders the particles, so that they effectively exclude less space, as shown in Figs. 3a and b. This principle can be understood intuitively when considering a parking lot with predefined parking spots, imagine finding a spot or leaving the lot if all cars were parked randomly instead. The restricted number of degrees of freedom also makes it more probable to choose the possible direction out of a finite number of lattice directions as compared with the probability to sample a jump in the small angular direction \( \varphi \), as shown in Fig. 3c. The increased flexibility on the hexagonal lattice leads to even faster diffusion than on a Cartesian, but allowing for diagonal jumps does not increase diffusion any further, since most of the jumps (82%) are sampled along the Cartesian axes. Hence, the Cartesian grid with only 4 dof has the closest agreement with the off-lattice simulations with infinitely many dof, contrary to the findings in \( [38] \) for fractal-like reaction

Fig. 2 MSD for a diffusing molecule, where \( \phi \) is the fraction of occupied volume of static crowders. First row: \((\langle x^2(t) \rangle - t)\). The reference line is the MSD in dilute medium \( (3) \). Second row: \((\langle x^2(t) \rangle - t)\). Increasing \( \phi \) here leads to increasingly slower diffusion and increases the difference between the models, where excluded volume effects are strongest in the off-lattice simulations

(a) \( \phi = 0.2 \), (b) \( \phi = 0.4 \), (c) \( \phi = 0.2 \), (d) \( \phi = 0.4 \)
kinetics. For very high and unbiological crowder densities \((\phi = 0.6)\), particles in BD and CA simulations on a Cartesian mesh effectively no longer move and only the on-lattice models with more dof allow the particles to find a free passageway (plots not shown).

For normal diffusion, in a dilute medium in integer dimension \(d\) the MSD grows linearly with time \((3)\). In the second row in Fig. 2, we further observe that the MSD is non-linear for all times \([34, 42, 43]\). The percolation thresholds for lattices are \(\phi = 40.73\%\) (Cartesian) and \(\phi = 50.30\%\) (hexagonal) \([44]\). For the off-lattice case with partially overlapping disks for the excluded volume as illustrated in Fig. 3b, it is more difficult to find exact values, but if the free space would consist of fully overlapping spheres 67.63\% \([45]\) would have to be freely available which would leave us with an occupancy fraction of 32.37\% for the effective excluded volume. In the second row in Fig. 2, we see that the simulations reveal sustained anomalous diffusion for the cases when the percolation threshold has been exceeded.

To quantify the excluded volume effects on the effective diffusivity, we evaluate the diffusion constant \(\gamma (\phi)\) after the transient phase by taking the last value of the plot \(\langle x^2(t) \rangle / (4t)\) for the cases converging to normal slower diffusion, as shown in Fig. 5. As expected, the effective diffusion constant \(\gamma (\phi)\) decreases with increasing crowder density and we can clearly observe the lattice artefact of underestimating the excluded volume effect on the diffusivity. It also appears that \(\gamma (\phi)\) depends linearly on \(\phi\) for all models, and the decrease in diffusivity for BD agrees with the findings in \([46]\) for equally sized spheres.

We now simulate 100 trajectories for one specific crowder distribution and plot the mean together with the 95\% confidence interval in Fig. 6, to investigate the excluded volume effects on the variance of the diffusive motion. As \(\phi\) increases, less and less space becomes available for the particles’ diffusion and hence the lattice artefact of underestimating the excluded volume effect on the variance becomes more pronounced. Another interpretation is that the higher number of particles in the system leads to a more deterministic behaviour. Similarly, the BD simulations resulting in slower diffusion have a smaller trajectory to trajectory variance, but we do not observe a grid effect for the variance otherwise.

4 Reaction–diffusion simulations

The excluded volume also has a thermodynamic effect on the reaction rates. For a high diffusion constant, the system can be

---

**Fig. 3** (a) Diffusion in an environment with static crowders. Before the diffusing molecule encounters the first obstacles it moves with the dilute diffusion speed 0 (green / dark grey). When it starts colliding the diffusion slowed down (orange / light grey), until an average slower diffusion is observed on long time scales (red / dark grey). (b) The anomalous behavior of the MSD (solid line) and the constant behavior of diffusion in dilute medium (dashed line) (see online version for colour.)

**Fig. 4** (a) Diffusion in an environment with static crowders. Before the diffusing molecule encounters the first obstacles it moves with the dilute diffusion speed 0 (green / dark grey). When it starts colliding the diffusion slowed down (orange / light grey), until an average slower diffusion is observed on long time scales (red / dark grey). (b) The anomalous behavior of the MSD (solid line) and the constant behavior of diffusion in dilute medium (dashed line) (see online version for colour.)

**Fig. 5** Effective long time diffusivity \(\gamma (\phi)\) obtained from particle based on and off-lattice simulations. Due to the random distribution of crowders in BD, the diffusivity is the slowest in the off-lattice simulations and increases with an ordering grid and more possible jump directions.
considered well-mixed, and the rate of reaction rate limited reactions is increased since the obstacles decrease the reaction volume. Moreover, dimers effectively exclude less space than two monomers, so that dimerisation is also favoured by crowding effects [47]. Diffusion-limited reactions on the other hand are impeded due to the slowed down diffusion and the increased time for the reaction partners to meet. There exist many models for reactions in the crowded cell environment, such as fractional dynamics [2, 38, 39, 48], a power law approximation of the RREs [49], and fractional [3] and multifractional Brownian motion [15], or scaled particle theory to compute the reaction rates using statistical physics [21, 47, 50, 51]. To examine the excluded volume effects on the reaction rates and in particular if CA can capture the same effects as the more accurate BD simulations, we compare association, dissociation and reversible reactions

\[ A + B \rightarrow C, \quad C \rightarrow A + B, \quad A + B \rightleftharpoons C, \quad (5) \]

when simulated with BD and with CA. These basic reactions can be combined to simulate more complex reactions such as the Michaelis–Menten enzyme dynamics, see, e.g. [40]. In these complex reaction systems, CA simulations are often performed to investigate the excluded volume effects [2, 6, 39]. The aim of this section is to examine to which extent the computationally more efficient CA are applicable to this aim. All CA simulations are here performed on a Cartesian grid, since it shows the best agreement with off-lattice simulations in the pure diffusive case. We model the complex C in two ways: (i) C has the same size as A and B; (ii) C has double the size of A and B, meaning it is a \(2 \times 1\) molecule in CA and a sphere with radius \(r_c = \sqrt{2}r_A\) in BD, as shown in Fig. 7.

The latter model appears to be more realistic when we simulate a system where the molecules occupy volume, since no volume is lost in a binding reaction and the resulting complex occupies the same volume as the two reacting molecules together. In Model I on the other hand, only half of the volume fraction is occupied after an A and a B molecules reacted with each other. This means that the system is loosing mass, which makes it more unrealistic. The restriction that molecules have to be composed of Cartesian voxels in CA, however, forces us to choose a rather unrealistic representation of C in that case. To account for the larger size of C we adjust its diffusion constant \(\gamma_C = \frac{\gamma_A}{2}\), and we extend the CA model to simulate particles of different sizes by adding an extra time step \(\Delta t_C\) for the jumps of C.

In this section, \(a(t)\) denotes the number concentration of \(A\) molecules

\[ a(t) = \frac{A(t)}{V}. \quad (6) \]

where \(A(t)\) is the number of \(A\) molecules in the system with volume \(V\) at time \(t\). We focus on diffusion-limited reactions by choosing the reaction probability to be one and compute 10^3 sample trajectories in 10^3 different crowder distributions and plot their mean values in all experiments. The crowders are represented as an additional molecular species, which is static and inert and hence does not actively affect the reactions.

4.1 Association events

We first consider the bimolecular binding reaction

\[ A + B \xrightarrow{k_+} C. \quad (7) \]

When volume exclusion is taken into account the reaction radius in Smoldyn is not well calibrated in relation to the macroscopic reaction constant [52]. To investigate the effect of excluded volume on the reaction dynamics when simulated on- and off-lattices, we

Fig. 6 MSD and the 95% confidence interval simulated for 100 trajectories in one crowder distribution. The variance decreases with increasing crowder density, as there is less space available for the molecules to diffuse in
(a) BD, (b) Cartesian, (c) Hexagonal, (d) Diagonal

Fig. 7 We model the complex C in two ways. Model I: C has the same size as A and B. Model II: C has double the size of A and B.
first calibrate Smoldyn such that the two models agree in the absence of crowders. To this aim, we first experimentally find the concentration a(t) of A molecules for the association \( A + B \rightarrow C \) in a dilute environment with volume occupancy \( \phi \), simulated with CA (solid lines) and BD (dashed lines). Left column: a(t). Right column: \( a(t) - a_d(t) \). For an increasing crowder density the reactions are slowed down compared to the dilute (9) and the difference between the on- and off-lattice models increases (Model I). In Fig. 8, we plot the concentration \( a(t) \) and the difference to the dilute solution \( a_d(t) \) for each \( \phi \).

For very short times, the reaction speed is increased in Fig. 8, because initially close reaction partners are kept in the vicinity of each other by the surrounding obstacles. However, generally the diffusion limited reactions simulated here are slower for higher crowder densities. Introducing the artificial lattice, leading to a faster diffusion than in the BD simulations, has a non-linear effect on the reaction rates. Since early times, it slows down the reactions, since close reaction partners have a higher chance of escaping each other. Then, a cross-over between the CA and BD curves occurs where the increased diffusivity of the on-lattice simulations leads to a faster encounter of initially distant reaction partners and the reaction rate is higher in the CA simulations. In Fig. 9, we plot the concentration \( a(t) \) simulated with both BD and CA in a highly crowded environment to explain the different phases. We only observe Phase III when the starting concentrations of \( A \) and \( B \) are 0.2 and \( \phi = 0.4 \), leading to the unbiological overall occupancy of 60\%. In this case, the diffusivity in CA is as obstructed as in BD (see Fig. 2f), but the grid makes it more difficult for molecules to pass each other, such that the moving C molecules can permanently block \( A \) and \( B \) molecules from reacting, whereas in the off-lattice case there are more chances to pass moving obstacles.

The discrepancy between on- and off-lattice simulations increases for higher crowder densities and higher initial concentrations. We call the latter effect self-crowding, meaning we can observe excluded volume effects even without adding explicit obstacles, since the \( A \), \( B \) and \( C \) molecules themselves act as obstacles to one another. Since \( C \) is not actively participating in the reaction, we only simulate Model I, but the self-crowding effect of \( C \) would be increased with Model II.

### 4.2 Dissociation events

In this section, we examine the dissociation reaction

\[ C \xrightarrow{k_d} A + B \]

(Model I). In Fig. 8, we plot the concentration \( a(t) \) and the difference to the dilute solution \( a_d(t) \) for each \( \phi \).

For very short times, the reaction speed is increased in Fig. 8, because initially close reaction partners are kept in the vicinity of each other by the surrounding obstacles. However, generally the diffusion limited reactions simulated here are slower for higher crowder densities. Introducing the artificial lattice, leading to a faster diffusion than in the BD simulations, has a non-linear effect on the reaction rates. Since early times, it slows down the reactions, since close reaction partners have a higher chance of escaping each other. Then, a cross-over between the CA and BD curves occurs where the increased diffusivity of the on-lattice simulations leads to a faster encounter of initially distant reaction partners and the reaction rate is higher in the CA simulations. In Fig. 9, we plot the concentration \( a(t) \) simulated with both BD and CA in a highly crowded environment to explain the different phases. We only observe Phase III when the starting concentrations of \( A \) and \( B \) are 0.2 and \( \phi = 0.4 \), leading to the unbiological overall occupancy of 60\%. In this case, the diffusivity in CA is as obstructed as in BD (see Fig. 2f), but the grid makes it more difficult for molecules to pass each other, such that the moving C molecules can permanently block \( A \) and \( B \) molecules from reacting, whereas in the off-lattice case there are more chances to pass moving obstacles.

The discrepancy between on- and off-lattice simulations increases for higher crowder densities and higher initial concentrations. We call the latter effect self-crowding, meaning we can observe excluded volume effects even without adding explicit obstacles, since the \( A \), \( B \) and \( C \) molecules themselves act as obstacles to one another. Since \( C \) is not actively participating in the reaction, we only simulate Model I, but the self-crowding effect of \( C \) would be increased with Model II.

### Table 1

| Parameter | Mean binding time |
|-----------|-------------------|
| BD        | \( k_{BD} = 20.3 \) | \( \tau_A = 105.23 \pm 1.06 \) |
| CA        | \( p_A = 1 \)      | \( \tau_A = 103.81 \pm 1.20 \) |
| RRE       | \( k_A = 3.8270 \) | \( \tau_A = 104.52 \) |
for the two different models for the size of \( C \). We first choose the dissociation probability in CA to be 
\[ p_D = 0.1 \]
and then compute the mean time until a dissociation event happens with expected number of necessary events until a dissociation occurs times the time step between the events, and obtain
\[ \tau_D = \Delta t \sum_{j=1}^{\infty} p_D (1 - p_D)^{j-1} = 2.5, \]  
(11)
which we use in the BD simulations. In a dilute medium, the mean concentration of \( A \) then follows the macroscopic RREs, which lead to the dilute concentration
\[ a_d(t) = a_{d0} + c_{d0} \left( 1 - e^{-k_D t} \right), \]  
(13)
when \( C \) follows Model I extra space is needed to place one of the reaction products \( A \) or \( B \) and the dissociation might be rejected if another molecule occupies the position sampled for the extra particle. This effect, however, is not possible to achieve with the software Smoldyn, as it always places the new particle in the system and decides in the next time step if a possible rebinding or a diffusive jump happen. Hence, we only examine CA simulations for different fractions of occupied volume \( \phi \) and compared them to the analytic solution in the dilute medium in Fig. 10.

Again, we observe self-crowding effects, as the simulations in the dilute medium for \( \phi = 0 \) do not follow the dilute concentration (13). The \( A \) and \( B \) molecules here act as moving obstacles that block the one-dimensional passageways forming in the CA geometry and hence separate \( A \) and \( B \) permanently from each other, while the moving \( C \) allows \( A \) and \( B \) to collide more easily in the off-lattice simulations.

For the different models for the size of \( C \). We first choose the dissociation probability in CA to be \( p_D = 0.1 \) and then compute the mean time until a dissociation event happens with expected number of necessary events until a dissociation occurs times the time step between the events, and obtain
\[ \tau_D = \Delta t \sum_{j=1}^{\infty} p_D (1 - p_D)^{j-1} = 2.5, \]  
(11)
with \( \Delta t = \Delta t^{CA} = 0.25 \). The macroscopic reaction rate for the instantaneous dissociation event is the inverse of its expected time, so that
\[ k_D = \frac{1}{\tau_D} = 0.4, \]  
(12)
when we use in the BD simulations. In a dilute medium, the mean concentration of \( A \) then follows the macroscopic RREs, which lead to the dilute concentration
\[ a_d(t) = a_{d0} + c_{d0} \left( 1 - e^{-k_D t} \right). \]  
(13)
4.3 Reversible reactions

We now combine our results to examine the reversible binding reaction

\[
A + B \underset{k_d}{\overset{k_i}{\rightleftharpoons}} C,
\]

where we choose the reaction constants in the same way as in the previous sections. To guarantee the correct rebinding probability in the Smoldyn simulations, we manually set it to 0.25 to agree with the rebinding probability in CA, where a newly produced particle jumps with probability 0.25 into the adjacent reaction partner and they react with \( p_{ij} = 1 \). The RREs for (14) are non-linear and hence not analytically solvable, but we can obtain a numerical solution with MATLAB's ode45 function.

We first investigate Model I for \( C \) by simulating the reversible reaction only with CA for different \( a_0 = b_0 \) and \( c_0 = 0 \). In the first row of Fig. 11, we observe that increasing crowding stabilises the complex \( C \) and decreases the amount of \( A \) and \( B \) molecules in the system at steady state as compared with the dilute case, in agreement with the findings in [40, 53, 54]. There are two reasons for this: (i) a particle already occupies the lattice for the newly created molecule and the dissociation event is rejected; and (ii) the rebinding time for \( A \) and \( B \) is decreased, since they escape from each other more rarely. The decreased time for \( A \) and \( B \) to meet in a crowded environment does here not affect the steady-state concentrations. The self-crowding effect is of the same order as the crowding effect, indicating that the \( A \) and \( B \) molecules themselves heavily stabilise the complex \( C \).

To investigate the grid effect on the reversible reaction, we will now perform the same experiments for Model II, the second row in Fig. 11. As compared to the CA simulations for Model I we see that the steady-state levels are slightly elevated, which is expected since the complex \( C \) already occupies the space needed to dissociate into \( A \) and \( B \). The steady-state level, however, still lies beneath the analytically predicted one for dilute media, indicating that the predominant effect is the decreased rebinding time of \( A \) and \( B \) in a crowded environment. The on-lattice simulations here underestimate the excluded volume effect as it is easier for \( A \) and \( B \) to diffuse away from each other. In the case of the reversible reaction, investigated here, the grid artefact on the reaction rates is linear with no cross-over between CA and BD, and increases with increasing \( \phi \). The computational time between the two models differs considerably, for the case with \( a_0 = b_0 = 0.2 \) the CA simulations were ca. 42 faster than the more accurate BD simulations.

Last, we investigate the variance in the reversible reaction system and how it depends on the level of crowding. We performed the same experiments as for Fig. 11 second row, but with only 100 trajectories in one crowder distribution, but there was no visible difference in the variance of the overall concentration of \( A \). In Fig. 12, we depict snapshots of the distributions of \( A, B \) and \( C \) molecules together with the obstacles when the system has reached steady state. Here, we observe that an increased crowder density leads to spatially more inhomogeneous distributions of \( A \) and \( B \), since close reactants are stabilised in the complex \( C \).

5 Conclusions

To understand complex gene regulatory networks in the crowded cell it is essential to perform realistic and computationally efficient reaction–diffusion simulations capturing the excluded volume effects, due to the high concentration of crowding macromolecules. In this paper, we perform rigorous reaction–diffusion simulations in discrete and continuous space to compare how well the computationally efficient on-lattice models approximate the more accurate off-lattice models, when applied to crowded environments. Due to the computational complexity we hereby restrict our study to static crowders, to diffusion-limited reactions with reaction probability one, and to the 2D case, providing insight into reaction-diffusion processes on biological membranes, where many important bio-chemical processes are known to take place [3, 34, 35].
where all molecules are assumed to be the size of one lattice site. Hence, the CA model does not always succeed in reproducing the reactions. For a reversible reaction, we find that the on-lattice grey) and C (light grey) and the static obstacles (black) in the steady state of the reversible reaction (4.10) and initial concentrations a₀ = b₀ = 0.2 and c₀ = 0. With increased crowded density the spatial heterogeneity increases (see online version for colour).

with off-lattice simulations, which was not expected as they are more flexible in the number of directions a molecule can move. A possible explanation to this phenomenon is, that the artificial grid orders the particles such that they effectively excluded less space. The consequences of the lattice on the outcome of diffusion limited association reactions are twofold: for short times the bimolecular reaction rates are decreased by the artificial grid, but for long times they are increased due to the faster hitting times between the reactants. For a reversible reaction, we find that the on-lattice simulations underestimate the crowding effects compared with the more detailed BD model. In all experiments, the excluded volume effects increase for higher initial concentrations of the reactants, indicating that they themselves act as crowders, an effect we call self-crowding. When modelling dissociation or reversible reactions we illustrated that it is important to model the molecules with their actual size, a feature usually not considered in the CA models, where all molecules are assumed to be the size of one lattice site. Hence, the CA model does not always succeed in reproducing the results obtained from BD simulations, but on the other hand, we have given examples of the considerable decrease in its computational time as compared to the costly off-lattice simulations.

We observe that the overall variance of the concentration of molecules is independent of the presence of crowding molecules, but the spatial variation in a reversible reactive system is increased, since close reactants are more likely to be bound in a complex and distant reactants are more hindered to meet each other.

To summarise, the main aim of this paper is to investigate the accuracy of on-lattice approximations to BD simulations, since they are popular for investigating excluded volume effects. We find that the artificial lattice in the computationally more efficient CA model has significant artefacts, especially on the diffusive behaviour and the steady-state concentration of a reversible reaction. Hence it can be regarded as an alternative to the more accurate off-lattice simulations only for low crowder densities.

6 Acknowledgments

This work was supported by the Swedish Research Council grant 621-2001-3148. The authors thank Per Lötstedt for his support and Andreas Hellander for helpful comments on this manuscript.

7 References

[1] Luby-Phelps, K.: ‘Cytoskeleton and physical properties of cytoplasm: volume, viscosity, diffusion, intracellular surface area’, Int. Rev. Cytol., 1999, 192, pp. 189–221
[2] Schnell, S., Turner, T.E.: ‘Reaction kinetics in intracellular environments with macromolecular crowding: simulations and rate laws’, Prog. Biophys. Mol. Biol., 2004, 85, (2–3), pp. 235–260
[3] Krapf, D.: ‘Chapter five: mechanisms underlying anomalous diffusion in the plasma membrane’, Car. Top. Membr., 2015, 75, pp. 167–205
[4] Jin, S.; Verkman, A.S.: ‘Single particle tracking of complex diffusion in membranes: simulation and detection of barrier, raft, and interaction phenomena’, J. Phys. Chem. B, 2007, 111, (14), pp. 3625–3632
[5] Medalia, O., Weber, I., Franjukis, A.S., et al.: ‘Macroarchitectural architecture in eukaryotic cells visualized by cryoelectron tomography’, Science, 2002, 298, (5596), pp. 1209–1213
[6] Berry, H.: ‘Monte Carlo simulations of enzyme reactions in two dimensions: fractal kinetics and spatial segregation’, Biophys. J., 2002, 83, (4), pp. 1891–1901
[7] Hansen, M.M.K., Meijer, L.H.H., Spruigt, E., et al.: ‘Macromolecular crowding creates heterogeneous environment of gene expression in picoliptre droplets’, Nat. Nanotechnol., 2015, 11, (October), pp. 1–8
[8] Ando, T., Skolnick, J.: ‘Crowding and hydrodynamic interactions likely dominate diffusion dynamics’, Proc. Natl. Acad. Sci., 2010, 107, (43), pp. 18457–18462
[9] Penington, C.J., Hughes, B.D., Landman, K.A.: ‘Building macroscale models from micromolecular simulations for nonlinear diffusion and multispecies phenomena’, Phys. Rev. E, 2011, 84, (4), p. 041120
[10] Saxton, M.J.: ‘A biological interpretation of transient anomalous subdiffusion’, J. qualitative model’, Biophys. J., 2007, 92, (6), pp. 1178–1181
[11] Schönöberg, J., Ulrich, A., Noé, F.: ‘Simulation tools for particle-based reaction-diffusion dynamics in continuous space’, BMC Biophys., 2014, 7, (11), p. 11
[12] Andrews, S., Bray, D.: ‘Stochastic simulation of chemical reactions with spatial resolution and single molecule detail’, Phys. Biol., 2004, 1, (3–4), pp. 157–151
[13] Andrews, S.S., Addy, N.J., Brent, R., et al.: ‘Detailed simulations of cell biology with Smoldy21’, PLoS Comput. Biol., 2010, 6, (3), p. e1000705
[14] Gilbert, D., Heiner, M., Takahashi, K., et al.: ‘Multiscale spatial computational systems biology (Dagstuhl Seminar 14841)’, Dagstuhl Rep., 2015, 4, (11), pp. 138–226
[15] Marquez-Lago, T.T., Leier, A., Burrage, K.: ‘Anomalous diffusion and multicompartimentation: simulating molecular crowding and spatial obstacles in systems biology’, IET Syst. Biol., 2012, 6, (4), p. 134
[16] Stiles, J.R., Van Helden, D., Bartol, T.M., et al.: ‘Miniature endplate current rise times less than 100 microseconds from improved dual recordings can be modelled with passive acetylcholine diffusion from a synaptic vesicle’, Proc. Natl. Acad. Sci. USA, 1996, 93, (12), pp. 5747–5752
[17] Schönöberg, J., Noé, F.: ‘RealDy – a software for particle-based reaction-diffusion dynamics in crowded cellular environments’, PLoS One, 2013, 8, (9), p. e74261
[18] Klann, M.T., Lapin, A., Reuss, M.: ‘Agent-based simulation of reactions in the crowded and structured intracellular environment: influence of mobility and location of the reactants’, BMC Syst. Biol., 2011, 5, (31), p. 71
[19] Schönöberg, J., Heck, M., Hofmann, K.P., et al.: ‘Explicit spatiotemporal simulation of receptor-G protein coupling in rod cell disk membranes’, Biophys. J., 2014, 106, (1), pp. 1042–1053
[20] Gruenert, G., Ibrahim, B., Lenser, T., et al.: ‘Rule-based spatial modeling with diffusing, geometrically constrained molecules’, BMC Bioinform., 2010, 11, p. 307
[21] Ridgway, D., Broderrick, G., Lopez-Campisustros, A., et al.: ‘Coarse grained molecular simulation of diffusion and reaction kinetics in a crowded virtual cytoplasm’, Biophys. J., 2008, 94, (10), pp. 3748–3759
[22] Xie, Z.R., Chen, J., Wu, Y.: ‘A coarse-grained model for the simulations of biomolecular interactions in cellular environments’, J. Chem. Phys., 2014, 140, (5), p. 054112
[23] van Zon, J.S., ten Wolde, P.R.: ‘Green’s-function reaction dynamics: a particle-based approach for simulating biochemical networks in time and space’, J. Chem. Phys., 2005, 123, (23), p. 234910
[24] Donè, A., Bulatov, V.V., Oppenheim, T., et al.: ‘First-passage kinetic Monte Carlo algorithm for complex diffusion-reaction systems’, J. Comput. Phys., 2010, 229, (9), pp. 3214–3236
[25] Oppenheim, T., Bulatov, V.V., Donè, A., et al.: ‘First-passage kinetic Monte Carlo method’, Phys. Rev. E, 2009, 80, (6), pp. 1–14
[26] Takahashi, K., Tanase-Nicola, S., ten Wolde, P.R.: ‘Statio-temporal correlations can drastically change the response of a MAPK pathway’, Proc. Natl. Acad. Sci. USA, 2010, 107, (6), pp. 2473–2478
[27] Tomita, M., Hashimoto, K., Takahashi, K., et al.: ‘E-CELL: software environment for whole-cell simulation’, Bioinformatics, 1999, 15, (1), pp. 72–84
[28] Van Zon, J.S., Rein Ten Wolde, P.: ‘Simulating biochemical networks at the particle level and in time and space: Green’s function reaction dynamics’, Phys. Rev. Lett., 2005, 94, (12), pp. 1–4
[29] Lee, H., LeDuc, P.R., Schwartz, R.: ‘Stochastic off-lattice modeling of molecular self-assembly in crowded environments by Green’s function reaction dynamics’, Phys. Rev. E, 2008, 78, (3), p. 031911
[30] Takahashi, K., Vel Arjunan, S.N., Tomita, M.: ‘Space in systems biology of signaling pathways – towards intracellular macromolecular crowding in silico’, FEBS Lett., 2005, 579, (8), pp. 1783–1788

Fig. 12 The spatial distributions of A (blue / dark grey), B (red / dark grey) and C (light grey) and the static obstacles (black) in the steady state of the reversible reaction (4.10) and initial concentrations a₀ = b₀ = 0.2 and c₀ = 0. With increased crowded density the spatial heterogeneity increases (see online version for colour).
[31] McGuffee, S.R., Elcock, A.H.: ‘Diffusion, crowding & protein stability in a dynamic molecular model of the bacterial cytoplasm’, PLoS Comput. Biol., 2010, 6 (3), p. e1000094

[32] Boon, J.P., David, D., Kapral, R., et al.: ‘Lattice gas automata for reactive systems’, Phys. Rep., 1996, 273, (2), pp. 55–147

[33] Ellery, A.J., Baker, R.E., Simpson, M.J.: ‘Calculating the Fickian diffusivity for a lattice-based random walk with agents and obstacles of different shapes and sizes’, Phys. Biol., 2015, 12, (6), p. 066010

[34] Saxton, M.J.: ‘Lateral diffusion in an archipelago. The effect of mobile obstacles’, Biophys. J., 1987, 52, (6), pp. 989–997

[35] Saxton, M.J.: ‘Lateral diffusion in a mixture of mobile and immobile particles. A Monte Carlo study’, Biophys. J., 1990, 58, (5), pp. 1303–1306

[36] Saxton, M.J.: ‘Anomalous diffusion due to obstacles: a Monte Carlo study’, Biophys. J., 1994, 66, (2 Pt 1), pp. 394–401

[37] Saxton, M.J.: ‘Lateral diffusion in an archipelago. Single-particle diffusion’, Biophys. J., 1993, 64, (6), pp. 1766–1780

[38] Grima, R., Schnell, S.: ‘A systematic investigation of the rate laws valid in intracellular environments’, Biophys. Chem., 2006, 124, (1), pp. 1–10

[39] Mourão, M., Kreitman, D., Schnell, S.: ‘Unraveling the impact of obstacles in diffusion and kinetics of an enzyme catalysed reaction’, Phys. Chem. Chem. Phys., 2014, 16, (10), pp. 4492–4503

[40] Gomez, D., Klumpp, S.: ‘Biochemical reactions in crowded environments: revisiting the effects of volume exclusion with simulations’, Front. Phys., 2015, 3, (June), pp. 1–14

[41] Meinecke, L., Löfstedt, P.: ‘Stochastic diffusion processes on Cartesian meshes’, J. Comput. Appl. Math., 2016, 294, pp. 1–11

[42] Ben-Avraham, D., Havlin, S.: ‘Diffusion and reactions in fractals and disordered systems’ (Cambridge University Press, 2000)

[43] Weigel, A.V., Ragi, S., Reid, M.L., et al.: ‘Obstructed diffusion propagator analysis for single-particle tracking’, Phys. Rev. E, 2012, 85, (4), pp. 1–8

[44] Newman, M.E.J., Ziff, R.M.: ‘Efficient Monte Carlo algorithm and high-precision results for percolation’, Phys. Rev. Lett., 2000, 85, (19), pp. 4104–4107

[45] Quintanilla, J., Torquato, S., Ziff, R.M.: ‘Efficient measurement of the percolation threshold for fully penetrable discs’, J. Phys. A, Math. Gen., 2000, 33, (42), pp. L399–L407

[46] Meinecke, L.: ‘Multiscale modeling of diffusion in a crowded environment’. Preprint arXiv:1603.05605, 2016, pp. 1–27

[47] Hall, D., Minton, A.P.: ‘Macromolecular crowding: qualitative and semi-quantitative successes, quantitative challenges’, Biochim. Biophys. Acta – Proteins Proteomics, 2003, 1649, (2), pp. 127–139

[48] Pitulice, L., Vilaseca, E., Pastor, I., et al.: ‘Monte Carlo simulations of enzymatic reactions in crowded media. Effect of the enzyme-obstacle relative size’, Math. Biosci., 2014, 251, (1), pp. 72–82

[49] Savageau, M.A.: ‘Biochemical systems analysis: a study of function and design in molecular biology’ (Addison-Wesley, 1976)

[50] Grassberger, B., Minton, A.P., DeLisi, C., et al.: ‘Interaction between proteins localized in membranes’, Proc. Natl. Acad. Sci. USA, 1986, 83, (17), pp. 6258–6262

[51] Grima, R.: ‘Intrinsic biochemical noise in crowded intracellular conditions’, J. Chem. Phys., 2010, 132, (18), p. 185102

[52] Andrews, S.: ‘User Manual for Smoldyn’

[53] Ellis, R.J.: ‘Macromolecular crowding: obvious but underappreciated’, Trends Biochem. Sci., 2001, 26, (10), pp. 597–604

[54] Zimmermann, S.B., Minton, A.P.: ‘Macromolecular crowding: biochemical, biophysical, and physiological consequences’, Annu. Rev. Biophys. Biomol. Struct., 1993, 22, pp. 27–65