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MULTI-RESOLUTION SPATIAL SIMULATION FOR MOLECULAR CROWDING

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ABSTRACT

Spatial phenomena attract increasingly interest in computational biology. Molecular crowding, i.e. a dense population of macromolecules, is known to have a significant impact on the kinetics of molecules. However, an in-detail inspection of cell behavior in time and space is extremely costly. To balance between cost and accuracy, multi-resolution approaches offer one solution. Particularly, a combination of individual and lattice-population based algorithms promise an adequate treatment of phenomena like macromolecular crowding. In realizing such an approach, central questions are how to specify and synchronize the interaction between population and individual spatial level, and to decide what is best treated at a specific level, respectively. Based on an algorithm which combines the Next Subvolume Method and a simple, individual-based spatial approach, we will present possible answers to these questions, and will discuss first experimental results.

1 INTRODUCTION

With advanced biological methods, e.g. high content screening or high resolution microscopy, an increasing amount of information is generated that is incorporated into more and more complex models. Recent findings emphasize the central role space plays in inter- and intracellular dynamics, for example that molecular crowding, i.e. a dense population of macromolecules, alters diffusion, hydration, and other properties of individual molecules. Thereby, the processes are quite intricate, e.g. depending on the concrete setting molecular crowding can promote the association of chaperones and thus decrease aggregation of denatured proteins, but in the absence of chaperones and when the protein folding process is too slow it might increase aggregation (Kinjo and Takada 2003). In intracellular environments macromolecules often occupy more than 1/3 of the total volume. They represent together with their hydratic shell a large excluded volume, which affects the physico-chemical kinetics of various intracellular processes (Zimmermann and Minton 1993, Hall and Minton 2003).

The simulation of this type of phenomena is rather costly. To reduce the effort required in simulation, different approaches are already exploited in computational biology. One is to use parallel simulation engines (Jeschke, Ewald, Park, Fujimoto, and Uhrmacher 2008), another is to trade accuracy for efficiency. The latter can be done by a more coarse grained execution (Gillespie 2001), or by a combination of different simulation algorithms, e.g. a numerical integration algorithm and a stochastic discrete event approach (Takahashi, Kaizu, Hu, and Tomita 2004). In both cases temporal resolution forms the basis. In (Gillespie 2001) the simulation proceeds in larger time steps approximating the number of reaction events that happened in between. In (Cao, Gillespie, and Petzold 2005), reactions that happen faster are executed by a numerical integration algorithm whereas the slower reactions in which fewer species are involved are calculated by a Gillespie variant in a discrete event mode. The principal idea of this multi-resolution simulation can be adopted for the spatial variant. However, in this case time and space has to be taken into account. In simulating macromolecular crowding it makes sense to simulate the larger molecules individually and the smaller ones with less detail, i.e. at population level. This combination of population, i.e. concentration-based, and individual-based approaches leads us to multi-level modeling and simulation. In (Uhrmacher, Ewald, John, Maus, Jeschke, and Biermann 2007) we introduced a specific formalism which relates individual and population level. The downward causation reflects the impact that the population level has on the individuals, e.g. the high density will promote phenomena like channeling between the molecules and the rates of macromolecular reactions will be affected (Hall and Minton 2003), the upward causation describes how the activities of the individuals affect the dynamics at population level, e.g. due to moving macromolecules the space for other molecules is diminished and in this tightly packed environment some reactions are more likely

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to happen (Zimmermann and Minton 1993). Thus, one of the central challenges is to connect both levels, however, first one has to decide what algorithm to use for each of the two levels.

The paper is structured as follows: section 2 gives an overview about algorithms for different levels of abstraction. Next, in section 3 the multi-algorithm, multi-resolution simulation approach is introduced that includes a description of the coordinator element and the realization of the interactions between the distinct simulation levels. As the approach is used for simulating systems under crowding conditions, section 4 gives a short introduction to this phenomena. The experimental setup and simulation results are presented in sections 5 and 6. The paper concludes with an overview of projects related or similar to the presented approach in section 7 and a summary (section 8).

2 ALGORITHMS FOR DIFFERENT ABSTRACTION LEVELS

Simulation algorithms exploit numerous abstractions to simulate the dynamics of cellular systems: from the actual physical processes, described by quantum mechanics etc., over the abstraction to entire atoms (*molecular dynamics*), toward approaches that consider molecules, compartments, or entire cells (Vaidehi and Goddard III 2001). Simulation algorithms for a sub-molecular scale rely on natural laws that are of continuous nature (Takahashi, Arjunan, and Tomita 2005). At the level of molecules, approaches abstract from the natural laws by assuming that the molecules move randomly (i.e., Brownian Motion). A coarse classification of the algorithms can be achieved by grouping them into three classes - microscopic, mesoscopic, and macroscopic (Takahashi, Arjunan, and Tomita 2005, Tolle and Le Novere 2006).

Microscopic algorithms operate with single particle detail, tracing the position in space of every element. The position of the entities is updated individually by calculating the forces acting on the particles (e.g. quantum mechanical or Newtonian) and applying the results to determine the new velocity and position (molecular dynamics). By adding stochastic fluctuations to the forces, random movements of an individual, caused by permanent collisions with smaller particles of the solvent, can be simulated (*Brownian motion*). While molecular and Brownian dynamics provide high accuracy, it is impossible to simulate larger models with many particles as the effort required for position update and collision detection is significant.

When the amount of particles in a volume changes discretely and stochastically and individual features of species elements do not need to be accounted for, algorithms that operate on a mesoscopic abstraction level can be used for simulation. The evolution of the simulated system can be seen as a Markovian process. The governing equation is the master equation that describes the time evolution of a probability density function $p(S;t)$ representing the probability that the system is in state S at a time t. In (Gillespie 1977), Gillespie presented the stochastic simulation algorithm (SSA) that generates trajectories of an underlying master equation. Based on the probability that two sphere-shaped particles collide and undergo a reaction in the next infinitesimal time interval, he introduced the *reaction propensity* as the product of a stochastic constant and the number of possible reaction pairs. This propensity is used to sample the next event time from an exponential distribution and to determine the corresponding reaction. As this algorithm calculates every single reaction, improvements have been presented to speed up computation by aggregating events (Gillespie 2001, Cao, Gillespie, and Petzold 2006) or introducing efficient data structures and reusing random numbers (Gibson and Bruck 2000). One key assumption of SSA is that the distribution of the species inside the volume is homogeneous. To simulate systems that do not adhere to this assumption, other approaches that allow to consider compartments and the diffusion of species are necessary, e.g. (Kholodenko 2006). A common way of introducing diffusion on mesoscopic level is partitioning space into sub-volumes and extending the master equation with a diffusion term, resulting in the reaction-diffusion master equation (RDME) (Gardiner 1996). The solution of the RDME is intractable for all but very simple systems, leading to the development of the Next Subvolume Method (Elf and Ehrenberg 2004), an algorithm that generates trajectories of an underlying RDME, similarly to SSA sampling a chemical master equation.

At a macroscopic abstraction level the law of mass conservation is used to derive ordinary differential equations (ODE) that describe the change of concentrations of species. This continuous, deterministic approach is applicable when the number of species elements is high enough to ignore stochastic effects. While the equations for simple models can be solved analytically, more complex models must be simulated with numerical solvers. It is possible to include space by using partial differential equations (PDE) instead of ODEs. For example, partial differential equations have been used to represent signaling processes in membranes assuming a homogeneous distribution of receptors (Haugh 2002). However, stochastic effects cannot be taken into account easily and, as a homogeneous distribution is assumed, they are not suitable for more realistic representations of membrane micro-domains (Mayawala, Vlachos, and Edwards 2005). Small numbers of actors, different geometries of components, and tracing of individual components pose additional problems. Furthermore, population-based approaches suffer from state space explosion (Tolle and Le Novere 2006). This motivates the development of new spatial individual-based approaches, like e.g. *SpacePi* (John, Ewald, and Uhrmacher 2008), which abstracts from detailed quantum

Algorithm 1 Short version of the Next Subvolume Method pseudo code description.

Initialization

1. Distribute the initial particles over N sub-volumes
2. Calculate the sum s_i of diffusion rates d_i and reaction rates r_i for all sub-volumes

$$s_i = r_i + d_i, 0 \leq i \leq N$$

3. Sample time of next event for all sub-volumes

$$\tau_i = -ln(u)/s_i,$$

with u being a sample from the uniform distribution $U(0,1)$ and enqueue sub-volumes according to their event times

Main loop

1. Take top sub-volume from event queue
2. Determine type of event w.r.t. to diffusion and reaction rates
   - Reaction: determine specific reaction, update the propensities of the current sub-volume and sample new event time (see (Gillespie 1977) for details)
   - Diffusion: determine species type and diffusion target, update propensities and sample new event times for both sub-volumes

mechanics and allows to attribute properties and individual motions to individuals. However, also at a coarse-grained level individual-based approaches require significant calculation efforts. Therefore, we introduce in the following an approach that combines individual- and population-based spatial simulation.

3 MULTI-ALGORITHM MULTI-RESOLUTION SIMULATION

The proposed multi-algorithm, multi-resolution simulator will focus on the combination of discrete-event with individual-based algorithms. Central to our approach is a coordinator responsible for the synchronization, but first let us introduce the two algorithms that we use in our case study. The algorithm for the lattice (population) level is based on the Next Subvolume Method (NSM, see Algorithm 1) (Elf and Ehrenberg 2004).

The NSM separates between two types of events: reaction events and diffusion events. What event type takes place next is determined using a two step algorithm. First the sub-volume with the minimum next event time is calculated. This calculation is based on the sum of diffusion and reaction rates. During the second step the actual type of event is identified. Here the individual values for reaction and diffusion rates are taken into account. In case of a reaction event the state of the system is updated similar to Gillespie's SSA. If a diffusion takes place the target sub-volume is randomly selected from all neighbors of the source sub-volume and the number of elements for the diffusing species is adjusted for both cells. The NSM algorithm continues by determining the sub-volume with the least next event time.

The algorithm used for the individual level is currently a rather simplistic one assuming random movements. Spherical macro objects move through space with position updates taking place in fixed time intervals. The model description for this abstraction level provides a movement function and a value representing the update interval for the individuals. The simulator uses this information to perform collision detection, update the position of the object, and to calculate the new movement vector and the next event time. As this implementation of the individual simulator is very basic, more complex algorithms are currently under development, e.g. a simulator for models described in the *SpacePi* formalism (John, Ewald, and Uhrmacher 2008).

Both levels of abstraction can influence each other in numerous ways.

Individual level → lattice level Macromolecules occupy a certain amount of volume. With their movement through space, the volume that is available for the small molecules and their interactions changes. Thus, the density of the particles in a sub-volume is increased or decreased, which affect the reaction activity at population level because the free volume is taken into account when calculating reaction rates. As the available volume of a lattice cell decreases, particles that are currently inside the cell get distributed among its neighbors. This differs from normal diffusion as particles get "pushed" out of the cell by the object. Furthermore, the location of the macromolecules will have an impact into which adjacent cell a particle is more likely to diffuse. Also reactions between macromolecules and particles can take place, thereby, those particles are no longer available within the sub-volume for other reactions. In addition, it is possible that, due to being bound to the moving macromolecule, they will be able to move faster into certain regions in the cell than only based on diffusion if, for example, the macro object takes part in an active transport.

Lattice level → individual level The population level can also influence the behavior of the macromolecules by, for example, providing reaction and binding partners. To determine the number of possible reaction partners is not trivial as a macromolecule might cover more than one sub-volume. It is also possible that, given different sub-volumes, the movement characteristics (speed and direction) of an individual might be influenced by the concentrations in these sub-volumes.

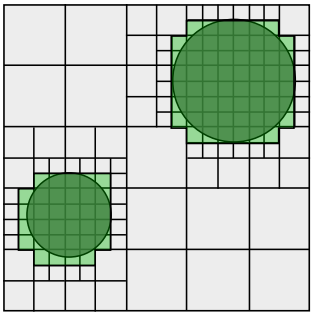

Figure 1: Subdivision of lattices in two-dimensional space with $d = 2$ to approximate two macro objects. The sum of all highlighted sub-compartments represent the area occupied by the appropriate objects.

3.1 The Coordinator Component

The coordinator represents the connecting part between the different algorithms operating on distinct levels of abstraction and controls the simulation process. It keeps track of the next event times for the involved algorithms and schedules the simulation part with the least event time to process next. The coordinator also manages the interactions between the algorithms and updates the appropriate models dynamically to reflect changes made at another abstraction level.

3.1.1 Cell update at lattice level

With the introduction of moving individual objects the spatial configuration at the lattice level changes with each update at the individual level. The volume of a lattice cell can increase or decrease depending on the movement of the macro objects. To dynamically change the available volume of a cell a subdivision algorithm based on an octree partitioning is introduced to determine the fraction of the total cell volume that is occupied by the individual object (see Algorithm 2). During this algorithm the original cell is recursively subdivided into smaller sub-compartments and for each an overlapping test with the macro object is performed. If the object intersects the sub-compartment, the subdivision steps are repeated until a predefined subdivision depth is reached. The sum of the smallest sub-compartments that intersect the macro object is the volume occupied by this object in the specific cell. Figure 1 shows a subdivision example for the two-dimensional case. The higher the subdivision depth is chosen, the finer the macro object is approximated at the lattice level but this also increases the effort for the subdivision process. In the currently implemented NSM algorithm for the lattice level the volume of a cell is used to calculate both the diffusion and reaction rates whereas the rate is inversely proportional to the volume. Furthermore,

Algorithm 2 subdivide(c, d, n); recursive calculation of the number of occupied sub-compartments for a lattice cell

```
Require: n = 0
  if macro object intersects compartment then
    subdivide c into sub-compartments sc
    if d > 1 then
      subdivide(sc, d-1, n)
    else
      n++
    end if
  end if
```

the free area between two neighboring cells and the fraction of the cell that is occupied by an individual are taken into account when processing a diffusion event. In the basic NSM the probability for selecting a specific neighbor cell is $1/N$ with N being the total number of neighbors. Now, collisions with the macro object must be taken into account. Therefore, the process for determining the diffusion target consists of two phases: First, a test if a collision occurs is performed (see section 3.1.2 for details), if not, a target cell is selected with respect to the fraction of the border between the source and each neighbor that is occupied by a macro object.

As mentioned before, an additional effect of a macro object entering a cell is a displacement of particles inside the cell and the lattice level algorithm has to account for this. Therefore, the NSM algorithm has been extended to handle this by distributing particles from cells that are completely occupied by the macro object among the cells at its "rim", i.e. among the cells partly occupied by the object.

Not only macro objects exclude space, but also elements at lattice level. To simulate crowding effects at this level, a parameter that represents the amount of volume an element occupies has been introduced. Thus, the volume of a cell is not only reduced by a macro object but also by the elements inside the cell. For now, this parameter is only considered during the diffusion step of the NSM algorithm. A particle can only diffuse into a neighboring cell if there is enough available space. Inside a cell the volume of an element is assumed to be negligible compared to the volume of the cell, so this parameter does not influence the calculation of reaction and diffusion rates. In (Gillespie, Lampoudi, and Petzold 2007) it is shown for the one-dimensional case how the size of reactants can influence stochastic kinetics. It is also pointed out that the generalization to two or three dimensions is rather complicated (if not impossible) with the presented approach. The inclusion of crowding effects inside a lattice cell is therefore left for future work.

3.1.2 Inter Reactions

Additionally to intra reactions at lattice level and individual level, reactions might also take place between elements of

different levels. For example, a small cofactor can bind to an apoenzyme and assist during transformation of the enzyme to its active state (holoenzyme). The lattice level simulator should support this inter reactions by providing information about species concentration in cells that are partly occupied by a specific macro object. The coordinator can then determine whether and when a next interaction might take place between this macro object and an element of a species that is simulated at lattice level. Let

$$s_{X,sv} = n|X|D/l_{sv}^2$$

be the diffusion rate for a species X with diffusion constant D in sub-volume sv that can diffuse into n directions (Elf and Ehrenberg 2004). For cells that are neither completely nor partly occupied by a macro object, n is the number of neighbors that can be selected as diffusion targets. Let us now consider the case when the available cell volume is decreased by a macro object and a fraction of the cell is not available for particles. Here, the macro object "cuts off" parts of the cell. Let $A_{sv,M}$ be the surface area of the macro object M inside cell sv and V_{sv} the available volume of sv. The required values for $A_{sv,M}$ and V_{sv} can be obtained during the subdivision process mentioned above. The rate for particles that diffuse into the occupied volume, i.e. that "collide" with the object, can be calculated as

$$s_{X,sv} = A_{sv,M}^2|X|D/V_{sv}^2.$$

This can be done for the set C_M consisting of all cells partly occupied by a specific object M. With this rates the next interaction time for object M with a species X can be calculated as the minimum of the next event times sampled from an exponential distribution for each sub-volume in C_M, that is

$$t_{M,X} = \min_{sv \in C_M} -\frac{\ln r}{s_{X,sv}},$$

with r being a random number from the uniform distribution $U(0,1)$. Note that this gives only the time of the next collision between an object and an element of species X. The individual simulator is responsible for deciding if a reaction actually takes place. Furthermore, a recalculation of the next interaction time is necessary when a sub-volume $sv \in C_M$ is selected as a diffusion target for an element of species X during a simulation step at lattice level.

As an individual can occupy a number of cells it is possible to include orientation dependent reactions (see e.g. (Schmitz and Schurr 1972) how orientation of reactants might affect reaction kinetics) at the individual level by mapping a specific binding site of a macromolecule to a lattice cell.

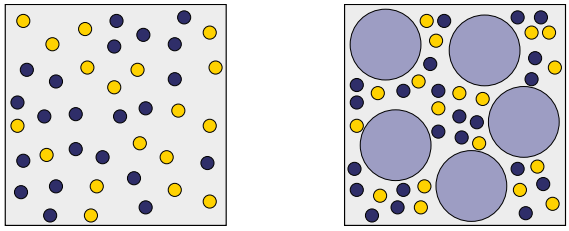

Figure 2: **Macromolecular crowding. Left**: Particles are distributed homogeneously inside a volume. **Right**: Macro objects occupy volume that is not available for particles. Thus, the effective volume decreases.

4 MACROMOLECULAR CROWDING

Whereas interactions of macromolecular species within in-vitro experiments that are carried out in dilute solutions are not hampered by other macromolecules (e.g. actin filaments or ribosomes), in living cells macromolecules can occupy up to 30% - 40% of the available volume. This effect is called *macromolecular crowding* and can influence diffusion coefficients for species and effective rates for reactions taking place among species (Chebotareva, Kurganov, and Livanova 2004).

For diffusion coefficients, with increasing concentration of crowding molecules the movement of particles is perturbed and a reduction of the coefficients by a factor of ten is estimated (Ellis 2001). In contrast, reaction constants increase as the particles are less randomly distributed compared to a solution without other macromolecules, resulting in a higher effective concentration and an increased probability for a reactive collision. However, it must be differentiated between reactions whose participating species diffuse fast enough that the rate limiting component is the reaction process itself and reactions including slowly diffusing species (diffusion-limited reactions). In the latter case the effective reaction constant can decrease under crowding conditions (Ellis 2001). As also mentioned by Ellis, the crowding effect on reaction activity only applies to entities of a specific size, whereas the change of the diffusion constant is present for all particles. For example, while an ion is too small for macromolecular crowding to exert effects on its activity the movement is still affected by the presence of other molecules that occupy space.

To simulate the effect of crowding on test solutes within experiments, background molecules in high concentrations are introduced that preferentially can only interact with the test particle via steric repulsion without undergoing a reaction, i.e. the interaction is limited to non-reactive collisions.

A good example for the effect of crowding conditions is the competition between protein folding and aggregation with molecular chaperones as studied in (Kinjo and Takada

2003). Chaperone proteins assist in the folding and unfolding process of other proteins by preventing the aggregation of these structures to non-functional units. Kinjo and Takeda show that crowding enhances the folding process of slow-folding proteins and inhibit their aggregation with the help of chaperones.

5 EXPERIMENTS

Experiments for analyzing the effect of molecular crowding on reaction propensities were performed on a 40x40x1 grid with lattice side length $l = 1\mu m$. Gillespie (Gillespie 1977) defined the propensity a_R of a reaction R as $a_R = c_R N_R$ with c_R being the stochastic rate constant and N_R the number of distinct molecular reactant combinations. The probability that reaction R takes place during the infinitesimal time interval Δt is $a_R \Delta t$. The stochastic rate constant c_R can be decomposed into a constant r_R that depends on the physical properties of the reactants as well as the temperature and the volume V of the cell (Versari and Busi 2007), so that the propensity can be rewritten as $a_R = V^{-1} r_R N_R$. Hence, the propensity of a reaction is inversely proportional to the available volume. For the experiments, the sum of all reaction propensities was used to measure the reaction activity of a lattice cell.

The experimental model included three species A, B, and C at lattice level. For each species a diffusion coefficient $D_{A,B,C} = 3.5 \times 10^{-13}\, m^2 s^{-1}$ was defined and a single element occupies a volume $V_{A,B,C} = 0.0045\,\mu m^3$. As an initial distribution, each cell contained 10 elements of species A and B, respectively, resulting in 32000 elements in total. Two simple reactions (forward and backward reaction)

$$R_f : A + B \xrightarrow{r_f} C$$
$$R_b : C \xrightarrow{r_b} A + B$$

were defined with $r_f = 1.0\,\mu m^3 s^-1$ and $r_b = 10.0\,\mu m^3 s^-1$. The crowding condition was modeled using a specific amount of globular crowding agents simulated at individual level. The number of individually simulated entities depends on a crowding parameter ξ, which is defined as the ratio of occupied volume V_o to the total model volume V_t, hence $\xi = V_o / V_t$. Each entity has a diffusion constant of $D = 7.3 \times 10^{-14}\, m^2 s^{-1}$, a radius $0.5\,\mu m$ and occupies a volume of approx. $0.5\,\mu m^3$. A setup for a single experiment consisted of a value for the crowding parameter ξ and 50 simulation runs.

For analyzing the effect of crowding on diffusion-dependent reactions, a 10x10x1 grid with lattice side length $l = 1\mu m$ was constructed. Here the focus was on measuring the time it takes until the first reaction event takes place under different crowding conditions. For this, species A ($D_A = 3.3 \times 10^{-13}\, m^2 s^{-1}, V_A = 0.005\,\mu m^3$) and

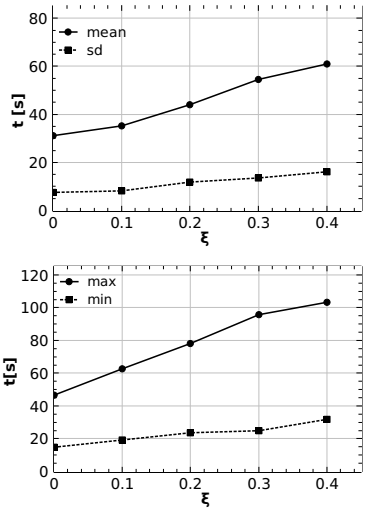

Figure 4: Mean and standard deviation (top) and minimum and maximum values (bottom) of the minimum reaction times for different values of the crowding parameter ξ.

B ($D_B = 0.0 m^2 s^{-1}, V_B = 0.0\,\mu m^3$) can react according to reaction R_f, but no backward reaction was defined. At the start of the simulation 200 elements of species A are located in cell $(0,0,0)$ and only one element of species B in cell $(9,9,0)$. The experiment was conducted for different values of the crowding parameter ξ, with 100 simulation runs per parameter value.

For all experiments, the subdivision depth was set to $d = 3$, which means that a cell was partitioned into at most 512 sub-compartments.

6 RESULTS

Figure 3 shows color map plots for the reaction propensities for all cells of the 40x40x1 model. Overall, crowding can increase reaction propensities by a factor of about two or three. It can be seen that a decrease in the available volume can have a significant local effect with the maximum propensity for single cells being about five times higher than the maximum in the dilute case. Figures 3c and 3f show outputs from an experiment with fewer but larger macro objects ($r = 2.0$). Note that in this case an amount of object volume is not considered in the simulation as the model has only a z-dimension of one. This was compensated by increasing the number of macro objects. The maximum propensity for the experiment with the dilute solution is $264\,s^{-1}$, whereas for the crowded environment this value is nearly six times higher ($1201\,s^{-1}$).

Figures 4 and 5 show results for the experiments analyzing the effect of molecular crowding on diffusion-dependent reactions at lattice level. Figure 4 shows an approximately

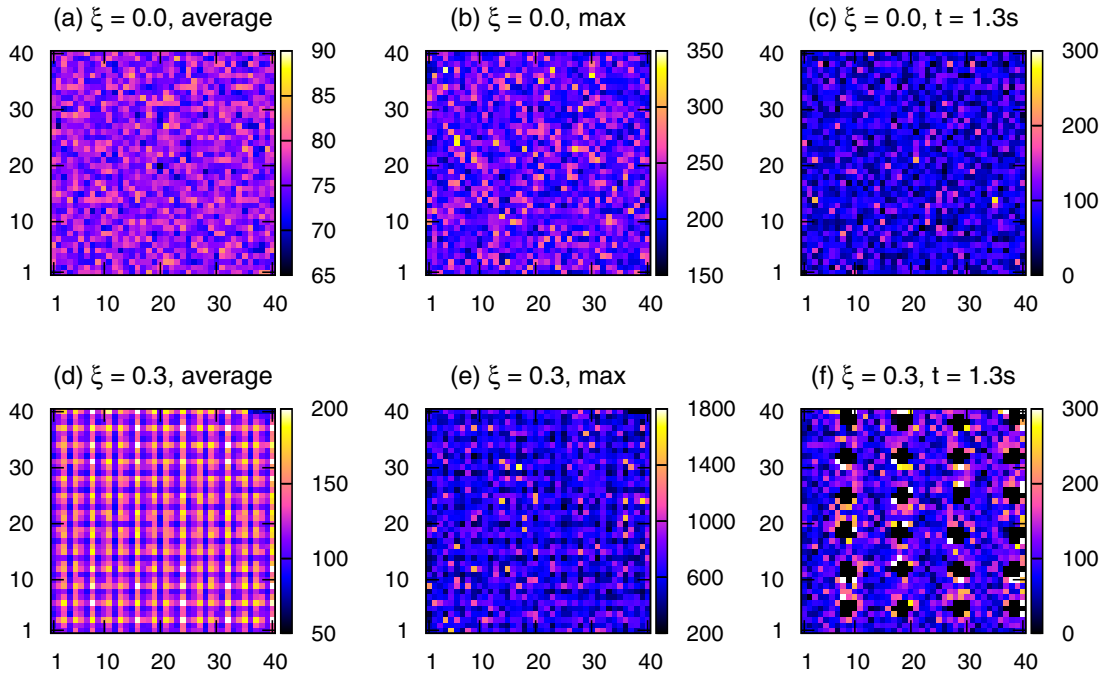

Figure 3: Color map plots for the average (a,d) and maximum (b,e) propensity for each cell of the 40x40x1 model, plotted for $\xi = 0.0$ (dilute) and $\xi = 0.3$ (crowded). The plots were created from 50 independent simulation runs. Plots c and f show a sample output from an experiment with macro objects having a radius of $r = 2.0$. Note that the z-axis scale differs between plots.

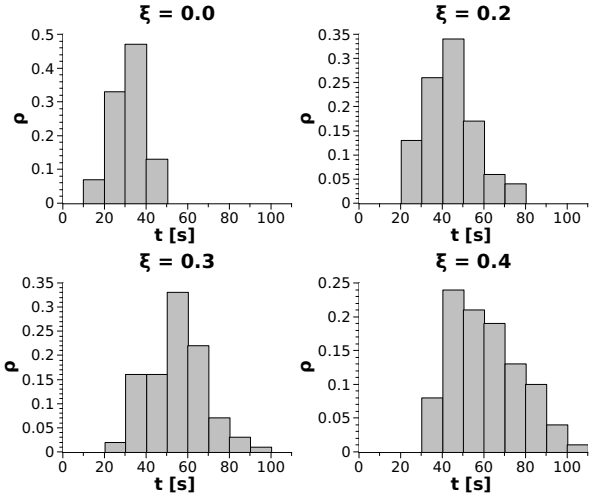

Figure 5: Distribution of minimum reaction times for different values of ξ. The height of a bar for an interval $[t_{i-1}, t_i)$ gives the ratio ρ of the number of samples whose minimum reaction times lie in this interval to the total samples count.

twofold increase of the average time it takes until the first reaction occurs. The maximum time for the crowded case with $\xi = 0.4$ can be about threefold higher than the average time in the dilute case ($\xi = 0$). The movement of elements is hampered by collisions with a macro object and by the inability to diffuse into cells without sufficient available volume, thus it takes longer for an element to reach a specific target cell. The difference between the predicted tenfold decrease in the diffusion rate and the measured value might arise from the simplification that crowding effects are not considered inside a single cell.

Figure 5 shows a shift in the distribution of average minimum reaction times towards higher intervals. As the crowding parameter ξ is increased, the number of samples with larger reaction times increases as well. In the dilute case, most of the samples have reaction times between 20 and 40 seconds. In contrast, with 40% of the available volume occupied, the distribution of the minimum reaction times shows clearly an increase of samples with times larger than 50 seconds, with some samples exceeding 90 seconds.

These results support the assumption that the rates of diffusion-controlled reactions are affected by macromolecular crowding and that they can decrease at least by a factor

of one-half and, in some cases, down to one-third of the value obtained for dilute solutions.

7 RELATED WORK

In (Takahashi, Kaizu, Hu, and Tomita 2004) a discrete event meta-algorithm for simulating models that span multiple scales is introduced, though the focus is on multi-timescale simulation. Different algorithms can be combined in a modular mode, each simulating the dynamics of a sub-set of state variables. When for two algorithms the intersection of the state variable sets is not empty, then these algorithms must notify one another whenever they change the value of a shared variable. Three classes of algorithms are supported: discrete event, discrete time, and differential. It is shown with the heatshock model that a combination of stochastic, discrete event and numerical integration modules can run faster than single algorithm simulations (both ODE and discrete event Gillespie).

In (Versari and Busi 2007) an approach is presented that applies Gillespie's algorithm to dynamical compartments. For this, the average volume of an element for each species is calculated and the volume of a compartment is increased or decreased according to this value when an element enters or leaves it. Therefore, in contrast to Gillespie's algorithm particles are no longer treated as zero dimensional points without volume but each element occupies an amount of space. This average volume depends on the physical properties of the species.

Smoldyn (Andrews and Bray 2004) is a simulation system that is based on the Smoluchowski equation and operates on a microscopic, single molecule level. In (Lipkow, Andrews, and Bray 2005) *Smoldyn* was used to simulate, among other things, the effect of molecular crowding on signal transduction in Escherichia coli chemotaxis. As *Smoldyn* only supports dimensionless particles, obstacles in the cytoplasm of the simulated cell that reduces the available space were introduced. One result was a steeper concentration gradient of the signaling species inside the cell because crowding increased its local concentration at one end of the cytoplasm and, due to the hampered diffusion movement, reduced it at the other end.

8 SUMMARY & CONCLUSION

As experimental results show molecular crowding influences significantly intra-cellular dynamics. Therefore, suitable modeling and simulation methods are highly needed. The presented approach supports different spatial levels by combining simulation algorithms operating on individuals and populations within a lattice. A coordinator manages the interaction between the simulators which includes an octree-based subdivision algorithm for approximating an individual at the lattice level, the distribution of particles when an individual occupies a cell, and the scheduling of inter-level reactions. The coordinator was tested with an adapted version of the Next Subvolume Method and a simple individual-based simulation algorithm. Experiments for analyzing the effects of crowding showed that the dynamics of a system are influenced by the amount of volume occupied by the species. Compared to a dilute solution, rates for reactions that are not limited by the diffusion of the reactants can increase two- and threefold due to the reduced amount of available volume, whereas rates for diffusion-dependent reactions can decrease to one-third of the value obtained from experiments with a dilute solution as a result of the restricted movement of the elements.

In the presented approach molecular crowding was induced by the macro elements simulated at individual level and took effect at the population level, as the reactions in and diffusions between lattice cells were influenced. Current work is aimed at replacing the simple individual-based approach by a *SpacePi* simulator, so that reactions that take place at individual-level (between the macromolecules) as well as interdependencies between individual- and population-based level can be more easily expressed. The crowding phenomena was also induced by taking the size of species at population level into account which influences the diffusion. This is one step towards simulating crowding at population level within the cells of the lattice. A more advanced approach for molecular crowding at population level based on SSA has been introduced in (Gillespie, Lampoudi, and Petzold 2007), however, being currently restricted to the one-dimensional case. In comparison to other approaches, we see the virtue of the presented one in combining two levels of abstractions, and thus in offering a link between highly accurate individual (with a lattice size $l \to 0$) and approximative, but often faster, population-based simulations ($l \to \infty$).

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