

From Biochemical Reaction Networks to 3D Dynamics in the Cell: the ZigCell3D Modeling, Simulation and Visualisation Framework

Pablo de Heras Ciechomski*
ScienceVisuals Sarl
Lausanne, Switzerland

Michael Klann†
Robin Mange‡
ETH Zurich, Switzerland

Heinz Koeppl§
ETH Zurich, Switzerland
IBM Research Zurich

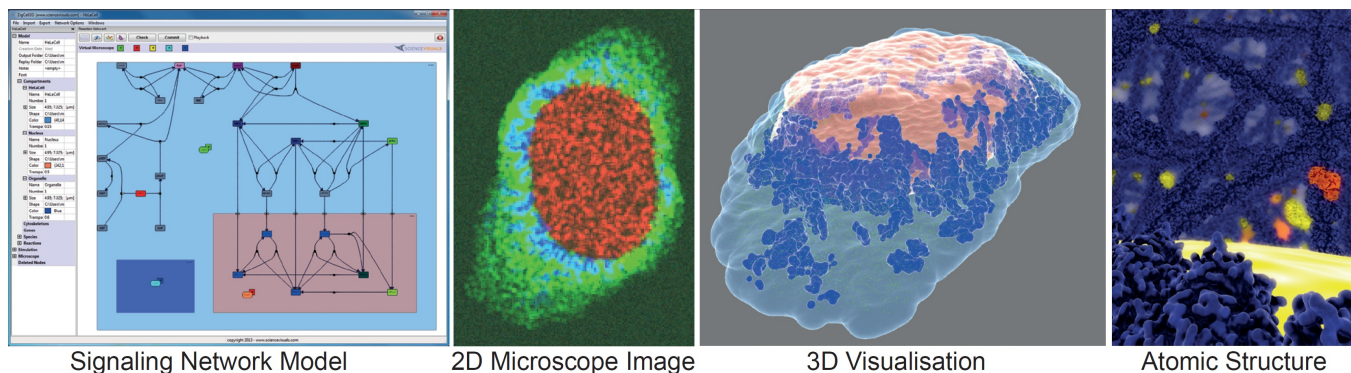


Figure 1: Signaling model in a HeLa cell with resulting virtual microscope image and detailed visualisation showing the cell, nucleus, an organelle, and molecules. On the nanoscopic level even the atomic structure of signaling molecules and cytoskeleton can be shown.

ABSTRACT

Systems-oriented research accelerates our understanding of biological processes and helps in identifying novel drug candidates. However, development of good models and our intuition is hampered by the biological complexity. To be able to see how candidate models evolve in front of the user in an interactive virtual 3D cell at various zoom levels, therefore is a crucial aspect and a challenging problem.

The motivation for creating the ZigCell3D software, is thus a holistic view ranging from being able to change model parameters, see how they affect 3D versions of the cell at molecular levels, while at the same time being able to verify the simulated model against a real experimental fluorescence microscopy image.

ZigCell3D is a virtual 3D whiteboard approach to chemical reaction modelling. It aims to provide a realtime interactive environment, where complex biophysics research is turned into a creative and game-like 3D environment. The complete system entails modelling, simulation and visualisation as part of a unified framework.

The core visualisation is based on a multi-core parallel C/C++ ray tracing engine, that builds a complete 3D iso-surface model of the cell, its organelles and molecules down to the atomic level using PDB files. The simulator itself is based on coarse-grained Brownian motion of the individual molecules, which is visualised in detail in a tightly coupled manner. Using a virtual fluorescence microscope the virtual simulation environment can be benchmarked against real life experimental data.

*e-mail: pablo@sciencevisuals.com

†e-mail: mklann@control.ee.ethz.ch

‡e-mail: rmange@control.ee.ethz.ch

§e-mail: koeppl@ethz.ch

Index Terms: I.3.7 [Computer Graphics]: Three-Dimensional Graphics and Realism—Raytracing; I.4.10 [Image Processing and Computer Vision]: Image Representation—Volumetric; I.6.5 [Simulation and Modeling]: Model Development—Modeling Methodologies; J.3 [Life and Medical Sciences]: Biology and Genetics—;

1 INTRODUCTION

Systems biology aims at a quantitative model-based description of biological processes for instance metabolism or signaling [24]. Cellular signal transduction is required to control the cellular state with respect to environmental conditions or stimuli, which can be observed in the macroscopic phenotype of a cell. The presence, state and spatial distribution of signaling proteins determines the cellular state, therefore we developed an integrated framework to model, simulate and visualise the molecular interactions and activation dynamics in a cell on the level of single molecules.

Several examples from cell polarization for chemotaxis [18] over long-range signaling [19] to local patterns and clustering [29] show the importance of localisation and spatial effects in signaling. Simultaneously stochastic effects can be significant due to low particle numbers [32]. Particle-based Brownian dynamics and also simulations based on the Reaction Diffusion Master Equation (RDME, with less spatial resolution but better performance) include both effects and facilitate their integration over the cellular domain such that spatio-temporal dynamics of signaling can be analysed [21]. The present work describes how the models for these simulations can be developed and the 3D results can be visualised.

Biological processes are very complex such that a large variety of modeling strategies has been developed to describe them. Systems biology graphical notation (SBGN) and markup language (SBML) [14, 27] aim at formalisation and standardisation of these models, such that different models and modules can be connected and exchanged by different modelers. While the formalism should be as concise as possible, it still has to be flexible enough in order to describe all biological processes and not to limit the scientific

creativity of the researchers. Likewise the formalism should be intuitive to learn especially in the interdisciplinary field of systems biology, where mathematicians, computer scientists and wet lab biologists work together. Ideally the modeling framework provides the foundation for the different disciplines to be able to collaborate efficiently. In addition, the formalism should enforce the biophysical principles. Biophysical or thermodynamic constraints can limit the number of possible models and thus help to find the most likely model for a certain signaling pathway.

While a reaction network as in Figure 1 shows the interactions that are possible in the system, it tells nothing about the actual state. Visualising the complete process becomes challenging because the dynamics cover several orders of magnitudes: single molecules with sizes of a few nanometers interact when they collide, but the cell has a size of several micrometers, contains thousands of molecules, and signaling covers many interactions until the cellular system has changed or adjusted its state [11, 12]. Therefore a scalable visualisation method is needed that can cover several orders of magnitude and link the nano with the macro scale.

One of the fundamental aspects of visualising the result of simulations is to inspect, debug and refine models. Therefore the simulation outcome has to be compared to reference data. Here we show how the 3D simulation results can be compared to microscope images, which are a common means of measuring cellular signaling dynamics. In addition, the schematic visualisation of ZigCell3D [36] depicts how the molecules move and interact in the cell. Thus our system shows how the macroscopic dynamics emerge from the molecular level.

A joint modeling, simulation and visualisation framework becomes an intuition builder as the immediate connection between the simulation and the visualised result is presented. Interacting, selecting and moving parts of the simulated 3D particle cloud as made possible by the application, then also highlights the underlying rules that created the component or molecule. What is normally a guessing game for the modeler, becomes a 3D colouring book annotated with mathematical expressions. We therefore can help to bridge the divide between the hard-core quantitative sciences and math-free wet-lab biology.

ZigCell3D consequently focuses on the mesoscopic particle level. This level connects the actions resulting from the molecular state, which can be analysed with molecular dynamics simulations, to the macroscopic state of the cell, which is normally described by ordinary or partially differential equations (ODE/PDE) [21]. This transition is the key step from the genotype to the phenotype of organisms and therefore justifies that the performance of the detailed simulation is lower than those of ODE models. Here we will present applications that highlight the applicability and usability of our system.

2 RELATED WORK AND APPLICATIONS

Only a few mature integrated modeling, simulation and visualisation platforms exist for systems biology applications. CellDesigner [14] and TinkerCell [4] so far only cover non-spatial models and simulations, while VCell includes space [28]. VCell originally only used PDE simulations, but can now also use the particle based simulator Smoldyn [1] as simulation engine, to include spatial and stochastic effects. Smoldyn itself is a C/C++ Smoluchowski/Brownian dynamics simulation package developed by S. Andrews. It outputs the resulting 3D particle positions using OpenGL but lacks the integrated aspect where users can enter the model in a GUI. More simulation methods are reviewed for instance in [21]. The need to have interactive modeling tools also lead to development of RuleBender (for rule-based models), in contrast to text-file or command line control of simulations [33]. RuleBender can also present and compare the simulation results, while it uses existing BioNetGen simulation technology [9].

When it comes to the visualisation, there are quite a few notable related projects such as the standalone BioInspire platform [7], which also serves as underlying architecture of ZigCell3D. It allows massive 3D models to be loaded and has support for simulations. There is no specialised support for molecular dynamics in BioInspire as it is a more generic platform, made available to create different types of scenarios. VMD [34] runs on the same principle to visualise results of external performant molecular dynamics simulations. In addition it can export files for rendering in external ray tracers.

The visualisation systems closest to the proposed one are those with an integrated interactive and high quality rendering such as FvNano [5] which uses a GPU accelerated ray caster to visualise the scene, as well as [10] and [35].

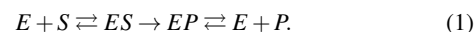
3 ZIGCELL3D OVERVIEW

ZigCell3D shows the spatiotemporal dynamics of a signaling model that is built/selected in the modeling window and simulated using a particle-based method. Since it is important to understand what shall be visualised, we will explain modeling and simulation first.

3.1 GUI and Modeling

The cellular biochemistry comprises a limited set of interaction/reaction types, such that formalisation for graphical representation of the reaction network requires only a few symbols. SBGN aims at providing a standardised graphical notation for biochemical reaction networks. However, it is still challenging to enforce the usage of the exact symbolic representation in the daily science, especially the symbol menu of the SBGN design tool CellDesigner [14] might scare untrained users away. Here we present a method to cover the formalisation of SBGN with a more intuitively to use design tool.

In this context it is worth noting, that mathematical models of signaling often rely on Michaelis-Menten or Hill-kinetics to simplify complex processes. Such higher order or nonlinear kinetics just provide a black-box model of the actual biochemical process. In reality for instance a substrate S to product P conversion, which is catalysed by an enzyme E : $S + (E) \rightarrow P + (E)$, includes the formation of an intermediary enzyme substrate complex



A detailed particle-based simulation requires to break higher order kinetics into the elementary first and second order association, transformation and dissociation steps. ZigCell3D likewise enforces this biophysical principle by only supporting elementary reactions (exceptions are given in the special section).

3.1.1 Reaction Smart Box to Simplify SBGN

ZigCell3D only needs one reaction smart box element plus reaction arrows. Depending on the connections of the box, the respective SBGN symbol is chosen by the GUI automatically. Input (output) arrows can be connected to three connectors on the left (right) side of the box. In addition, triggers, modifiers or inhibitors can be connected to the top or bottom. Figure 2 gives an overview of the symbols. Note that a reaction can not have more than 2 educts/inputs, the formation of trimers for instance has to be described by first forming dimers that then bind another monomer to complete the trimer molecule.

The reaction box can be rotated if required by the reaction network layout. The reaction network can be printed without showing the reaction box in order to obtain the SBGN look of the reaction network. Furthermore a grid can be enabled with snap to grid functions to align all symbols.

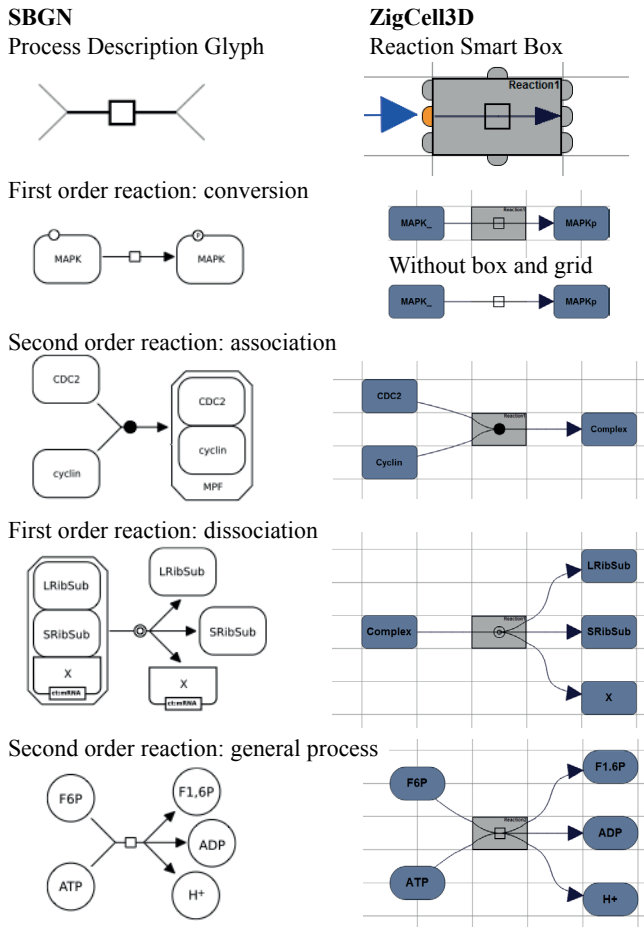


Figure 2: Elementary reactions: comparison of symbols in SBGN and ZigCell3D. The smart reaction box changes the reaction symbol according to the connections, which can be added to 8 connection points.

3.1.2 Special Reaction Models

Enzymatic reactions are supported in ZigCell3D by using the algorithm presented by Falk et al. [10] to mimic Michaelis-Menten reaction kinetics in the simulation. However we recommend to split it into its elementary reactions as in (1) in order to have a more accurate simulation. Since the enzyme counts as an educt (basically it appears both on the right and left hand side of the reaction), an enzymatic catalysis box can only have one regular input (Figure 3). **Inhibitions** of a reaction, however, are not supported because the elementary process is undefined. Instead the user has to implement the exact inhibition or chose one of the templates, e.g. those in Figure 3.

3.1.3 Transporters and Association with Cellular Structures

The reaction box can also be placed in a compartment boundary. Then it obviously becomes a (reversible) transport reaction (see Figure 4). The input of the transport reaction is defined as the molecule species outside of the compartment, while the output is inside.

Likewise the reaction box can be used to define binding interactions between mobile molecules and fixed cellular structures. Binding reactions do not change the molecular species but only affect the mobility state: instead of diffusing through the compartment the molecule stops moving and stays bound to the structure. If bound to

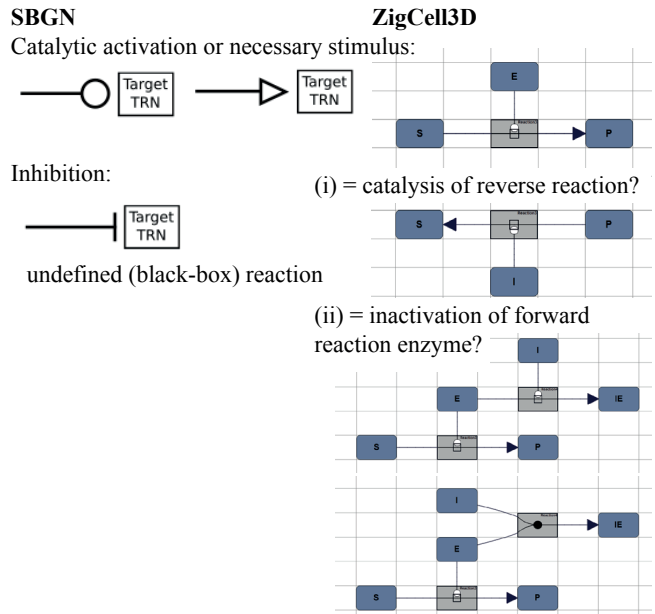


Figure 3: Non elementary reactions: while catalysis is well defined, inhibition could mean several possible reactions. In any case it has to be broken into the elementary reactions. Inactivation of the forward reaction enzyme can occur either in an enzymatic reaction or by complexation.

a cytoskeleton filament, it can also move along the structure (mimicking motor protein transport in 1D) as suggested in [11]. If bound to a compartment surface, it can diffuse on the respective side of the surface in 2D, only receptors reach through the surface (cf. Figure 4).

Note that the binding rate constants for structural association reactions depend on the compartment/structure. While non-spatial models often use transport or binding rate constants with units s^{-1} , in the ZigCell3D spatial simulation molecules will bind to the structure if they are closer than a critical distance. Therefore the binding rate of the simulation will be proportional to the surface of the object, and the GUI has to scale the rate constants accordingly (cf. Table 1).

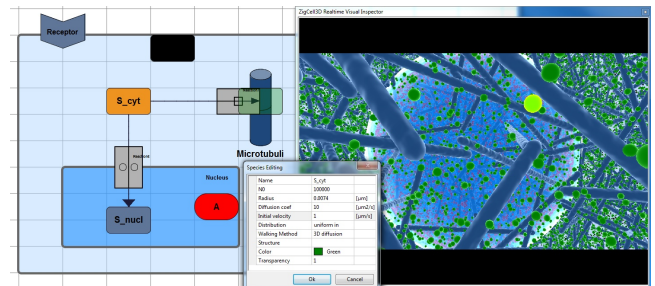


Figure 4: (Left window) Interactions with structures of the reaction volume, e.g. transport through surfaces or association to cytoskeleton filaments. Note that the structure acts like a modifier in the reaction. Molecules can snap to the surface/boundary of a compartment and become surface bound, moving in 2D along the surface. (Right window) Selecting a molecule in the 3D visualisation also highlights its species in the network. Double-clicking an object in either window opens the properties dialog for the species (pop-up window).

3.1.4 Compartments, Molecules, Properties

Molecule species of the reaction model are represented by a glyph, e.g. a rectangle (see Figure 4). The properties of each species include the initial number and initial spatial distribution, radius r and diffusion coefficient D , as well as the colour for visualisation. For a super-detailed visualisation also the corresponding PDB-file with the atomic structure of the molecule can be specified.

Compartments, for instance the cell, nucleus, or other sub-cellular organelles, can be organised on the canvas to contain the molecules and sub-compartments that reside inside of them. Besides geometric shapes like spheres, ellipsoids, cylindrical or cubic volumes also arbitrary shapes are supported in ZigCell3D, such that for instance a HeLa Cell model from CellOrganizer [31] can be used as reaction compartment (cf. Figure 1). For sub-compartments with several instances like vesicles or for obstacles like cytoskeleton filaments the spatial distribution in the parent compartment can be selected as well. Semi-transparent compartments enable to see through the outmost compartments in the visualisation.

3.1.5 Model Consistency Check before Simulation

Whenever a model shall be executed, ZigCell3D checks whether all reactions obey the biophysical limits. Of special importance is the diffusion limit of reactions: no pair of molecules i and j can react without being in contact. Coming into contact is limited by the speed of the molecules, hence diffusion D , and the collision cross-section, hence the molecular radii r , which are both defined in a spatial model. For 3D reactions the limit is given by the collision process, namely the rate constant $k_D(i, j) = 4\pi(r_i + r_j)(D_i + D_j)$. All macroscopic reaction rate constants of the model must be smaller than the respective $k_D(i, j)$. Furthermore all reactants (inputs and outputs) of a reaction must be in the same reaction compartment (except for transporters). And of course for nested geometries (molecules in the nucleus that is inside of a cell, which is inside of a test tube compartment) each sub-element has to be smaller than its parent such that it fits inside.

3.1.6 Interactive Simulation and Visualisation Control

Once a simulation is started, it returns updated particle positions for visualisation at a user specified frame-rate. The current number of molecules of selected species as well as further properties can also be displayed in an online plot window. For publications or post-processing, however, the numbers can also be exported in several formats.

Colours of molecules and compartments can be changed during the simulation and are immediately changed also in the 3D visualisation window as well as the plot window. Selecting an object in the visualisation window also highlights its counterpart in the abstract reaction network, and its properties can be updated (see Figure 4). In addition, the user can specify perturbations to reactions (change rate constant at a specified time or immediately) or molecule species (add N molecules or remove all molecules) to excite the system such that its dynamics can be probed. Fluorescence tags can be added to each species (even while the simulation is running), in order to visualise their instantiations in the simulation using the virtual microscope (cf. Section 3.4).

3.2 Simulation

The particle-based simulation of the biochemical reaction-diffusion process in 3D space is described in detail in [22]. The key features are a discrete time continuous space random walk of the molecules and the implementation of reactions based on the lambda-rho model [8]. In the physiological range of signaling, the parameters of molecule radii are $r = 2 - 10nm$, diffusion coefficients are $D = 0.1 - 10\mu m^2/s$ and rate constants $k < 1 \times 10^9 M^{-1} s^{-1}$. Then the simulator step length has to be in the range of $\Delta t = 10^{-3} - 10^{-8}s$ in order to have reaction probabilities smaller than 0.2 [22]. The

spatial step $\Delta x = \sqrt{2D\Delta t}$ is then in the range of several nm. Thus the step length determines both the spatial/temporal resolution and the computational costs [21]. In order to improve the performance also a two-regime method [13] is available, which switches to a Monte Carlo simulation of the reaction-diffusion master equation (RDME) for those regions of the cell that are not in the field of view of the visualisation [20].

The ZigCell3D simulation (dll/dylib) is implemented in C/C++ and parallelised using pthreads. It can be replaced in principle by any other simulation with the same interfaces defined by BioInspire. Especially the simulation has to be able to return/provide updated particle positions and numbers every Δt_{CPUs} in order to enable a smooth visualisation and recognise modified parameters from the GUI.

3.3 3D Cell Visualisation

The visualisation component in ZigCell3D software is running the BioInspire parallel ray tracing core which is a pure CPU implementation on top of C/C++. The ray tracer is responsible for populating a 3D world with a one-to-one correspondence to the input simulation components such as molecules, cell cytoskeleton and the cell membrane. All surfaces in the ray tracer are mathematical descriptions of the underlying geometry, using iso surfaces [30] and a force tension model for all components, even down to atomic level for the molecular surfaces using PDB files. The reasons behind using iso surfaces are many but most importantly: reduction of memory usage by only storing a position, radial extension or force for each emitter in the molecules for example; for the cytoskeleton it allows the merging of several actin filaments, connected one after the other, using the elastic surface property of the iso surface; it gives a more biological and life-like visualisation as the surfaces have no hard edges such as is usual in meshed geometry; there is no need for levels of detail as the surface is sampled per ray and not per surface discrete element as is done in rasterisation; the ability to reflect the simulation properties immediately without having to rebuild the geometry as the ray automatically builds the surface on a per pixel basis; the possibility of making distance queries in the vicinity of the molecule with a high precision and many more important advantages.

At the base of the BioInspire ray tracer is a bounding volume hierarchy (BVH [25]) that is created using a modified median cut algorithm. By first radix sorting the elements in the tree by the three dimensions x , y and z jointly in a parallel fashion, the CPU has to jump around less in memory thus reducing the memory latency cost. This is done by creating nodes that keep the three floating point values inside and the three next pointers (as 32 bit integer indices) for the list nodes. The parallel sort itself is done using 16 bit key values, giving rise to 65536 long tables (one for each thread) that are counted and spread on the second step of the radix algorithm. Thus sorting is reduced to a two part radix that is parallelised and memory access conservative.

Once the sorting is done the top down building is started such that elements that are too large are kept at a higher node so that they don't enlarge the following child nodes of the BVH. At each node the largest axis is approximately chosen dependent on the pre-sorted order of the elements (min and max center points) to avoid having to update the min and max values on each node splitting, who also depend on the radius and not necessarily exclusively on the center points. The building itself at the moment is only parallelised after the second step of the algorithm, once four sub-trees are present.

This BVH consists of entities or instances of underlying iso surface elements, that are themselves either direct surface emitters, sub trees of surface emitters or algorithmic generators of surfaces or other instances.

The root solver that is making up the base of the intersection

algorithm is based on polynomial formulations of the primitives, that are solved with a Bezier clipping approach [35], as opposed to the recently very popular re-surfaced ray marching approach [15], notably in the 3D demo graphics scene. Using a partial analytic solver, removes many trouble spots such as floating point accuracy. It is also easier to allow transparency as the solver does not stop merely when finding the first intersection.

The main reason for not using the GPU to accelerate the ray tracer is the amount of extra development necessary, the great diversity of drivers and bugs in graphics hardware, the limited memory support and the constraint on having to equip each computer with a specialized high performance graphics card. Yet another reason is that performance is easier to predict on a multi-core CPU implementation, especially for random access memory algorithms such as ray tracing.

3.4 Virtual Microscope Visualisation and Reaction Fluxes

The virtual fluorescence microscope rasterizes the simulated domain and bins the fluorescence intensity of all labeled objects into voxels like a confocal fluorescence microscope. In the present examples a Gaussian point spread function is used to determine the distance dependent contribution of each fluorescent object to each voxel. Rasterisation is done on the molecular positions of a given time-point for all voxels simultaneously, while real microscopes need time for scanning through the volume and obtain the last voxel only with significant delay.

The GUI enables the selection of a certain 2D image from the 3D intensity map (z-stack), a certain range from the z-stack or the whole 3D intensity pattern in the BioInspire window (cf. Figure 5). Different colour/label channels can be (de-)selected as well.

Labeling of reactions enables visualisation of spatiotemporal dynamics of reaction-fluxes. Each time a reaction is executed, a fluorophore is created at the position of the event. The lifetime of the reaction label fluorophore determines how long a past reaction is visible. Figure 5a and b compares constant versus exponentially distributed reaction label lifetimes, showing only marginal differences. The undirected diffusion of the source molecules of the reaction in any case leads to an unstructured cloud of reaction labels.

A virtual fluorescence microscope was already suggested by Falk et al. [11], but without binning the intensity to a rather low-resolution microscope image. The present virtual microscope model thus is closer to reality. It could also produce training datasets for image deconvolution methods, e.g. [16]: the virtual microscope can produce arbitrary noise levels, such that the influence of noise on image segmentation as well as segmentation for 3D objects from 2D image layers can be tested.

4 APPLICATIONS

The following applications highlight the importance of space and stochasticity in biochemical reaction networks, as well as the contribution of visualisation for the appreciation of the results.

4.1 Spatial Gene Expression and Protein Interaction

First we exemplify the principle steps of signaling, namely the transcription and translation step from genes via mRNA to proteins. Figure 5 shows how mRNA distribute around a gene and the resulting proteins distribute around the mRNA [6]. Figure 5 furthermore presents different visualisation methods. The detailed particle visualisation actually hides the global information about protein distributions in space, which can be better seen in the blurry microscope images. Especially we can also use fluorescence labels for reactions in ZigCell3D (see Section 3.4). Thus not only molecules but also reaction-fluxes can be visualised in the image.

The limited number of genes (actually 1) and mRNA (a few) gives rise to bursting kinetics and strong noise in protein numbers.

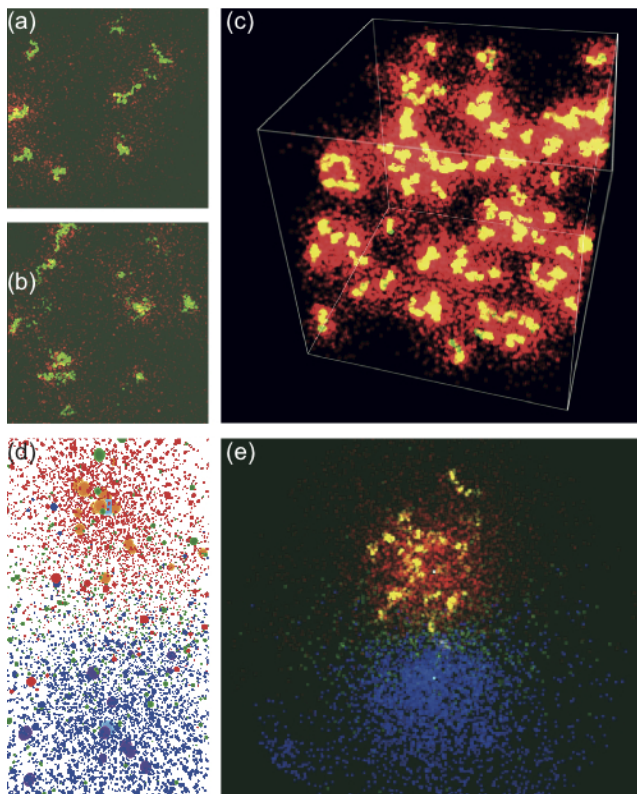


Figure 5: Virtual fluorescence microscope images: (a) and (b) show proteins in red and the events where they are created in green/yellow. (a) fixed lifetime of the reaction label, (b) exponentially distributed lifetime of the reaction label. (c) 3D representation of the microscope data. (d) and (e) show interacting proteins from 2 genes (at the cyan spot) that are exactly 1 micrometer apart in a spatial gene expression model. While the 3D particle based visualisation of (d) lets the user focus on single molecules, the virtual microscope image highlights the spatial distribution, it directly shows the local density.

If the lifetime of all molecules is limited, protein distributions are localised around the gene [6]. Signaling molecules can only interact with each other if they live long enough to meet in space or if they are created close enough, i.e. if their spatial distributions are overlapping [3]. Figure 5d and e also highlights the interactive region between two signaling molecule species, where green molecules are created by fusion of red and blue molecules, which are created by two different genes in the presented example.

4.2 FCS in in-vivo like environments

The application of a virtual fluorescence microscope is not limited to visualisation but can also be used to probe local physical properties of the medium. In Figure 6 we show the autocorrelation curve obtained from a simulated Fluorescence Correlation Spectroscopy (FCS) experiment. This example shows how the mobility of molecules – which eventually also determines collisions between molecules and thus reaction rates – depends on the microscopic structure of the medium. In the presented example we compare the influence (i) of a dense actin cytoskeleton as reported by Medalia et al. [26] and visualised in Figure 6a/b and (ii) a ER-like structure as reported by Hiroi et al. [17], visualised in Figure 6c/d to a reference simulation without any obstacles. Compared to the reference simulation without obstacles with $\tau_D = 0.16ms$ and $\langle N \rangle = 2.05$ the crowded environment (i) leads to a reduced diffusion and corresponding larger $\tau_D = 0.24ms$, while $\langle N \rangle = 2.00$ stays similar in

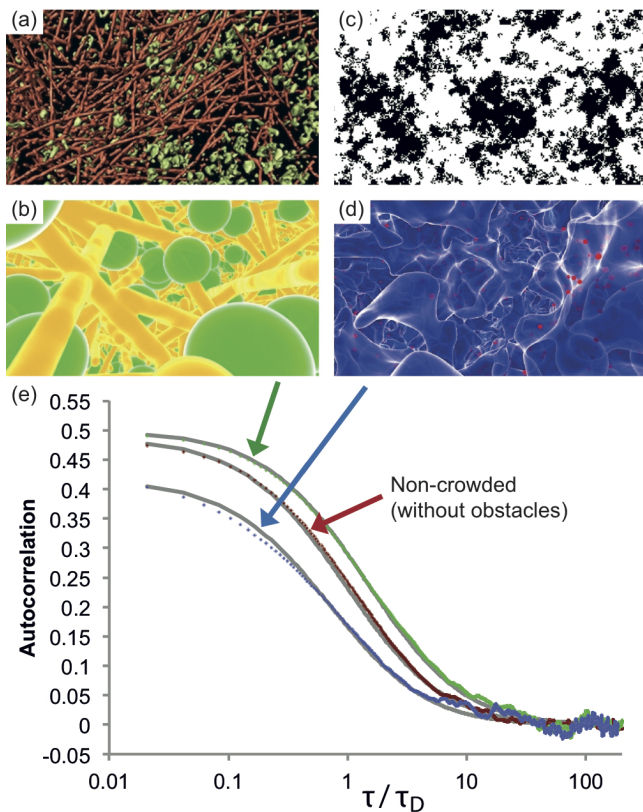


Figure 6: (a) Intracellular environment as reported by Medalia et al. [26]. (b) ZigCell3D model of (a). (c) Intracellular environment as reported by Hiroi et al. [17]. (d) ZigCell3D model of (c) with small test molecules in red. (e) Simulated Fluorescence Correlation Spectroscopy (FCS) in empty space, the crowded environment of (a), and in the structured environment of (c). τ is scaled relative to the reference τ_D of the empty space simulation.

(see Figure 6e). The structured environment (ii) however leads to shorter residence times $\tau_D = 0.13ms$ and the relatively large obstacles redistribute the test molecules into the focal volume in the presented example such that $\langle N \rangle = 2.40$. Possibly, crowding leads to anomalous diffusion [2], a fact that is represented here in the deviation between the fitted FCS especially for the structured environment (Figure 6c, d), which can be further investigated using more simulations.

Thus ZigCell3D helps to connect spatial properties with dynamics, in the presented example with the diffusion coefficient of molecules. The advantage of the combined simulation and visualisation framework is that the spatial structures of the environment can be readily seen by the user.

4.3 Receptor clustering and its effect on signaling

Clusters, i.e. non-uniform spatial distributions of receptors are a prominent example where spatial modeling becomes important. Here we combine the density and feedback dependent clustering model of Jilkine et al. [18] with a proposed signaling mechanism from Mugler et al. [29]. The two step activation process with very unstable intermediary state of the signaling molecule proceeds more likely if the activator density is (locally) high. Therefore the clustered receptors activate the signal stronger, and eventually more active signaling molecules enter the nucleus (see Figure 7). It is worth noting, that although the number of molecules in the nucleus is small, their concentration therein is bigger than in the cytoplasm.

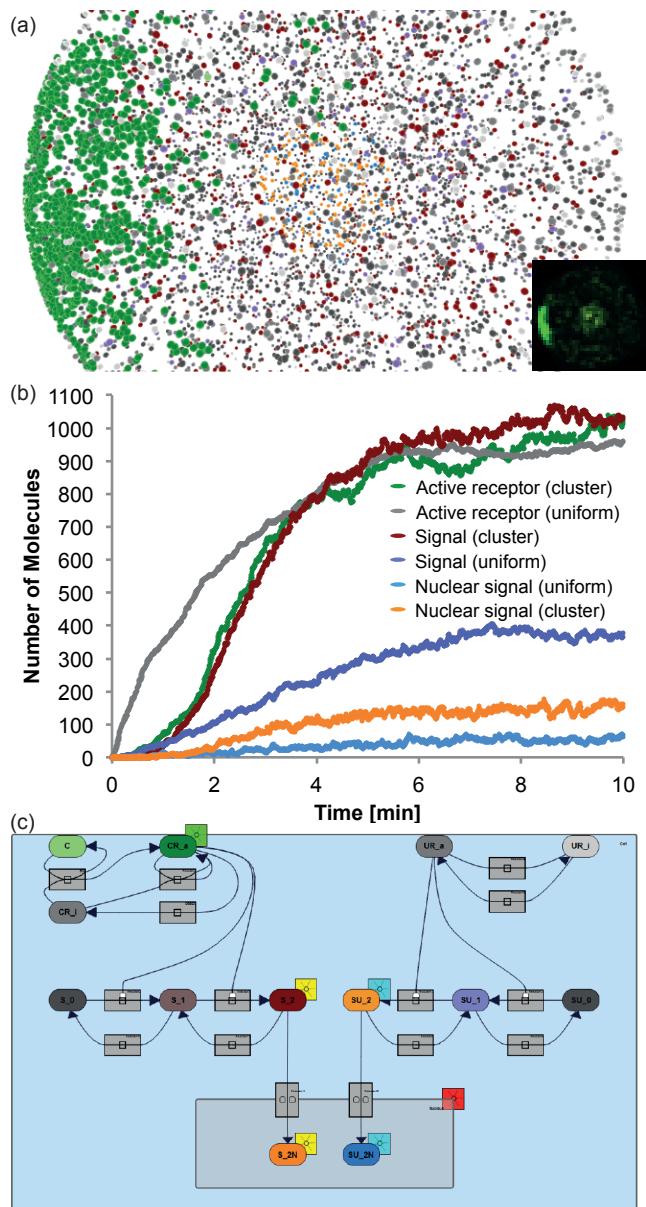


Figure 7: Clustering of receptors and signaling towards the nucleus. (a) 3D view, the insert shows a virtual microscope image with the accumulation of receptors in a cluster in green and accumulation of signaling molecules in the nucleus in yellow. (b) Dynamics of the process. (c) Reaction network for clustered and uniformly distributed receptors. Note the fluorescence labels on the active receptors (green) and signaling molecules (yellow/cyan).

Table 1: Parameters of the clustering model. * (Conversion of the transport rate constants based on the surface to volume ratio of the nuclear surface.)

Species	Location	N_0	Reaction; Parameters
C	PM (2D)	1	$C+CR_i \rightarrow C+CR_a; k = 1.0 \times 10^5 M^{-1}s^{-1}$
CR_i	Cyt. (3D)	2000	$CR_a+CR_i \rightarrow 2 CR_a; k = 1.0 \times 10^5 M^{-1}s^{-1}$
CR_a	PM (2D)	0	$CR_a \rightarrow CR_i; k = 5.0 \times 10^{-2} s^{-1}$
UR_i	PM (2D)	2000	$UR_i \rightarrow UR_a; k = 4.0 \times 10^{-3} s^{-1}$
UR_a	PM (2D)	0	$UR_a \rightarrow UR_i; k = 3.7 \times 10^{-3} s^{-1}$
S_0	Cyt. (3D)	2000	$R_a+S_0 \rightarrow R_a+S_1; k_{cat}=10s^{-1}; k_M=10^{-4} M$
S_1	Cyt. (3D)	0	$R_a+S_1 \rightarrow R_a+S_2; k_{cat}=10s^{-1}; k_M=3 \times 10^{-5} M$
S_2	Cyt. (3D)	0	$S_{1/2} \rightarrow S_{0/1}; k_{1 \rightarrow 0} = 50s^{-1}; k_{2 \rightarrow 1} = 0.01s^{-1}$
$S_{2,N}$	Ncl. (3D)	0	$S_2 \leftrightarrow S_{2,N}; k_{in} = 0.05 \mu m s^{-1}; k_{out} = 0.01 \mu m s^{-1}$
			* $S_2 \leftrightarrow S_{2,N}; k_{in} = 0.0135s^{-1}; k_{out} = 0.1s^{-1}$

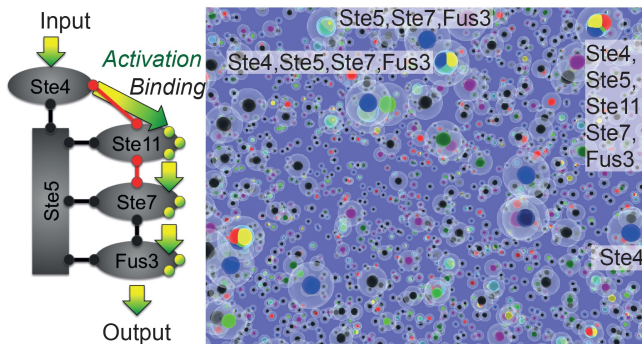


Figure 8: Rule-based MAPK signaling simulation example, where the signaling molecules can co-localise along the scaffold Ste5 in yeast. The image shows a snapshot of different complexes that formed in the simulation [23]. Each complex is enclosed in a bigger sphere to highlight the content of different complexes.

Here the nucleus has 1/3 of the diameter of the cell, i.e. about 1/27 of the volume only! The example also shows, how two modules or components of signaling can be easily combined in ZigCell3D in order to test their joint properties. The network also shows, that two different signaling models can be tested in the same cell and thus in the same simulation in order to compare them.

4.3.1 Performance

The clustering model containing 8000 molecules ($D \leq 0.1 \mu\text{m}^2/\text{s}$) was integrated with $\Delta t = 1.67\text{ms}$. The simulation proceeded with ≈ 2000 steps per second, i.e. about 2 s per second of CPU time on a standard desktop machine. Visualisation reached interactive frame rates for this model, enabling a smooth exploration of the 3D particle cloud.

4.4 Visual Inspection of a Spatial Rule-Based Model

ZigCell3D can load position data of previous/other simulations to replay them. This ‘sideloading’ feature is used here to visualise particle positions from a spatial rule-based simulation of Mitogen activated protein kinase (MAPK) signaling in yeast from [23] (see Figure 8). Thus also spatiotemporal dynamics of other particle-based simulations can be analysed with ZigCell3D, which is especially useful if the source simulation package does not include visualisation, but outputs particle positions and numbers.

5 USER FEEDBACK

Steven Andrews acknowledged that a GUI to enter the model and an interactive visualisation is still missing in Smoldyn [1], especially in order to promote it as a tool for wet-lab biologists and was excited about the ZigCell3D user interface and the 3D visualisation. We discussed the use case where simulations are executed in realistic cellular geometries, e.g. from CellOrganizer [31] with Robert Murphy. While he enjoyed to see interactive navigation through the cell models, he questioned the informative value of simulations in such models, given the higher computation time required and as long as only one instance of a cell is simulated. We agree in this issue, because it touches the question how abstract a model has to be or can be in order to be a good model.

We used ZigCell3D with several students, mostly with biology background. They liked the fact that abstract models (‘a box for a cell?’) become realistic geometries in the simulation/visualisation, which helps the conception of modeling. Since they are familiar with microscope images, they could directly link their experience and expectation to the outcome of ZigCell3D, and appreciated that they could dive into the simulated scene with the schematic 3D visualisation. Since ZigCell3D does not require Nvidia/Cuda GPUs

or other specialised hardware, it could be tested by everybody interested. For some domain experts, e.g. in non-spatial stochastic modeling, ZigCell3D acted as a reminder that there are more aspects, especially space and crowding, that have to be considered in biology. Thus we conclude that ZigCell3D can bridge the divide between the different disciplines working together in systems biology.

6 DISCUSSION

The applications have shown various aspects where visualisation is crucial to understand the interplay of spatial and dynamic components of the system, highlighting the importance of space for the dynamics of biochemical reactions. The gene expression example and Figure 5 highlighted the localization of molecules around their source. The distribution is better seen in a less detailed virtual microscope image, while a detailed visualisation focusing on individual molecules misses the context. The distribution and interactivity of molecules is driven by diffusion, which is hampered by all the obstacles in the cell. Figure 6 showed different 3D obstacle configurations and the resulting diffusion properties such that users can better understand the influence of the in vivo environment. Interactive rotation and navigation through the environment furthermore allows exploration of the spatial properties.

In contrast, Figure 8 focuses on complex formation, showing various states of the possible configurations. In that case, focusing on single complexes is necessary to see their configuration. Still, different complexes can have specific sub-cellular locations. Figure 7 shows how clustering of receptors along the membrane can lead to signal activation, while uniformly distributed receptors only lead to significantly lower signal transmission into the nucleus. The clustering example also shows the modularity of signaling models. In ZigCell3D modules developed by different groups can be combined into a larger model. Eventually complete signaling pathways could be simulated.

However we admit, that ZigCell3D aims at small modules rather than complete signaling pathways due to its detailed simulation method. Large-scale signaling networks require immense CPU times, and if possible should therefore be simulated using less detailed and less costly methods. Still ZigCell3D can for example compute effective rate constants for ODE models in order to include spatial effects implicitly in the non-spatial model.

In the current version, the system does not support rule-based modeling, which significantly simplifies modeling of components that have many states. Still, simulations of such models can be loaded and visualised as exemplified in Section 4.4. In general, modeling in ZigCell3D is less flexible because more biophysical constraints have to be considered. This might look like a limitation in the first place, especially when creating nonlinear phenomena for signaling transfer functions. But after all it just reflects the fact that also in the cell complex dynamics have to be created bottom-up in several steps by interactions that only together create the desired behaviour. Understanding how the non-linear response of cells emerges from simple mass-action reaction schemes is one of the key challenges to understand why in-vivo signaling networks are wired the way they are.

7 CONCLUSIONS AND OUTLOOK

We have shown a system that enables understanding of biophysical reaction models on various levels. Especially it enables linking of the particle level (random motion and stochastic reactions) with deterministic reaction networks, including the spatial component of the system from the nano-scale to the scale and structure of a cell. Users are able to navigate through the 2D reaction graph or the 3D particle cloud and can interactively select molecules, highlighting them simultaneously in all different windows. By this development we envision a stronger connect between models and results,

but also between 2D microscopical images and further batches of experimental data. Future work aims at improving the usability of perturbations in the system, i.e. virtual experiments, which provide direct feedback to the users action. Every perturbation requires to run a different simulation for comparison, which could be executed in parallel on several nodes of a cluster. Future improvements are expected by further decoupling of simulation and visualisation into different processes to improve speed by a better distribution of the workload. The challenge lies in maintaining the connection for user feedback from visualised data back to the simulation.

ACKNOWLEDGEMENTS

The authors wish to thank Martin Falk for sharing his ideas and experience about GUI and visualization with us. Furthermore we want to thank Jonas Ries for the introduction to FCS as well as Steve Andrews, Robert Murphy and Maike Roth for valuable discussions and feedback.

This work was supported in part by a grant from the Swiss Commission for Technology and Innovation (CTI), Project 12532.1 PFLS-LS. Heinz Koepl acknowledges funding from Swiss National Science Foundation, grant no. PP00P2_128503.

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