

Discrete Event Multi-level Models for Systems Biology

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Abstract. Diverse modeling and simulation methods are being applied in the area of Systems Biology. Most models in Systems Biology can easily be located within the space that is spanned by three dimensions of modeling: continuous and discrete; quantitative and qualitative; stochastic and deterministic. These dimensions are not entirely independent nor are they exclusive. Many modeling approaches are hybrid as they combine continuous and discrete, quantitative and qualitative, stochastic and deterministic aspects. Another important aspect for the distinction of modeling approaches is at which level a model describes a system: is it at the “macro” level, at the “micro” level, or at multiple levels of organization. Although multi-level models can be located anywhere in the space spanned by the three dimensions of modeling and simulation, clustering tendencies can be observed whose implications are discussed and illustrated by moving from a continuous, deterministic quantitative macro model to a stochastic discrete-event semi-quantitative multi-level model.

1 Introduction

The goal of Systems Biology is to analyze the behavior and interrelationships between entities of entire functional biological systems [1, 2]. As the systems under study do not support an easy experimental access and analysis, models play an important role in gaining an insight into the systems’ behavior and structure. Models can be evaluated differently. For instance, properties of the system can be derived by using methods like model checking [3]. Simulation is a different approach as it means an experiment based on the model. Thereby, it completes the *in-vivo* or *in-vitro* experiments of Systems Biology by *in-silico* experiments [1, 2]. Diverse modeling and simulation methods are being applied in the area of Systems Biology. Efforts like the “Systems Biology Workbench” are aimed at integrating different data analysis, visualization, modeling, and simulation tools [4]. In this context SBML (Systems Biology Markup Language) is being developed to support the exchange of models between different simulation systems

[5]. Similar to CELLML [6], SBML focuses on continuous systems modeling and simulation. In continuous systems models, the system is described by a set of state variables, whose time-dependent changes are usually specified by a set of differential equations [7, 8, 9, 10].

Aside from the continuous modeling approaches the discrete approaches also have increasingly gained momentum: the behavior of the system is modeled by states changing at arbitrary points on a still continuous time scale [11, 12]. State transitions are triggered by external and internal events, which is in fundamental contrast to the continuous state changes of a differential equation system. With the discrete approaches models have emerged that integrate qualitative and stochastic aspects: the values of some variables and/or of the modeling parameters are qualitatively scaled or taken from probability distributions [13]. Qualitative continuous systems modeling approaches exist as well even though those are more rare [14, 15]. The same can be observed with respect to stochastic continuous approaches, e.g. [16].

Most models in Systems Biology can easily be located within the space that is spanned by the three dimensions of modeling: continuous and discrete [17]; quantitative and qualitative; stochastic and deterministic, although this categorization is neither exclusive in each dimension, nor are the dimensions entirely independent.

In the following we will add a comparatively less explored dimension to distinguish modeling approaches in Systems Biology: the question at which level a model describes a system: is it at the “macro” level, at the “micro” level, or at multiple levels of organization.

The more mature a field becomes, the more hybrid approaches gain ground. In Systems Biology, the number of models steadily increases that are no longer purely quantitative or qualitative, or purely continuous or discrete. Thus, we expect the same to happen with respect to the organizational level: one level of explanation will hardly suffice. The more so, as the goal of Systems Biology is to describe the dynamics of cellular systems in their entirety [1]. In this context, not only interdependencies at one organizational level but between different ones become of interest, as “the whole is to some degree constrained by the parts (upward causation), but at the same time the parts are to some degree constrained by the whole (downward causation).” [18]. The importance of these interdependencies has been emphasized for systems in general [19] and biological systems in particular [20, 21], and also recently for Systems Biology with increasing urgency [22, 23, 24, 25] motivating the development of concrete models, e.g. [26, 27, 28, 29].

This paper is organized as follows: in chapter two the modeling approaches in the area of Systems Biology are categorized into the three modeling dimensions, which were described above (quantitative-qualitative, continuous-discrete and stochastic-deterministic); chapter three introduces the idea of the different organization levels for the distinction of the modeling approaches (micro, macro and multi-level models) and interrelates the categorization approach to the modeling dimensions studied in chapter two with a focus on multi-level models; after

theoretical considerations about the different modeling dimensions and their interdependencies, chapter four illustrates the explorations based on a biological application example; chapter five gives a more in-detail discussion of the composition and interaction of multi-level models; it is followed by a general discussion, summary and outlook.

2 Structuring the Space of Model Approaches

A formal model is described in a formal language to be interpretable by a computer system. Each model forms an abstraction of a system to support some concrete objective. Thus, we follow the definition of Minsky [30] that “A Model (M) for a system (S) and an experiment (E) is anything to which E can be applied in order to answer questions about S.”. As Cellier [31] points out, this definition does not describe “models for systems” per se, a model is always related to the tuple system and experiment. A model of a system might therefore be valid for one experiment and invalid for another. One consequence of this definition is that it is very unlikely to derive a model, which is valid for all possible experiments, unless it is an identical copy of the system and thus no longer a model. Modeling is a process of abstraction. It involves simplification, aggregation, and omission of details. Although processes of omission and simplification become particularly obvious if the model is described in a formal language, these processes play also a role in *in-vitro* and *in-vivo* experiments. Whereas it seems natural to conclude that the physical medium of *in-vitro* or *in-vivo* experiments restrain the experiments and thus the question that can be answered, one is often not aware about that and what constraints are implied by the respective modeling approach. However, the diversity of modeling approaches applied in Systems Biology illustrates and suggests that, depending on the biological system, the available data and knowledge about the system, and the objective of the simulation study, modeling approaches are chosen deliberately on demand and thus address the diverse needs of modeling and simulation in Systems Biology - if we do not assume that the diversity is caused merely by the diverse backgrounds which the modelers come from. So the question is to be asked what do certain approaches offer in modeling biological systems when compared to others. By introducing and discussing the dimension of organizational levels we will try to partially answer this question for the case of discrete-event, multi-level modeling approaches.

First we will use the dimensions of continuous and discrete, quantitative and qualitative, and stochastic and deterministic modeling to structure the space of modeling approaches applied in Systems Biology.

2.1 Continuous, Discrete, and Hybrid System Models

Distinguishing between continuous and discrete systems modeling and simulation has a comparatively long tradition. Although different modeling formalisms do exist, e.g. systems dynamics, bond graphs, or block diagrams, the continuous realm of modeling and simulation is unified by differential equations for model

representation and numerical integration algorithms for execution [31]. Thereby time-dependent variables are assigned to different measuring or non-measurable quantities of the system. The continuous state changes are modeled by a sum of rates describing the increase and decrease of the quantities amounts. Frequently kinetic rate equations, like the Michaelis-Menten or some mass action kinetics, are used for that purpose. Such modeling approaches are perfectly suited for the reproduction of measured time-dependent trajectories and also easily allow the fitting of the model parameters. Continuous systems models are the dominant type of model used in Systems Biology [32]. A series of simulation tools for continuous systems modeling and simulation in general and Systems Biology applications in particular support a comfortable developing of these types of models, e.g. Gepasi [33], ProMoT/Divi [34], Jarnac [35], DBsolve [36], and Cellerator [37]. Continuous models reflect nicely what is measured in cellular biology. Small samples of cell cultures are analyzed by extracting the DNA, enzymes, or metabolites, and by quantifying the concentration of the respective species over time.

Often a cell's activity is perceived as being discrete rather than continuous motivating the design of discrete systems models. In contrast to continuous systems models, discrete systems models assume only a finite number of state changes within a time interval. Depending on the time base that underlies the model, discrete time stepped approaches and discrete event approaches are distinguished. The latter allows to associate arbitrary time spans with each state of the system and thus is based on a continuous notion of time, whereas the former is based on time that advances in equidistant steps. Regular, time-stepped PETRI NETS have been applied to qualitatively describe biochemical reaction networks [38]. The use of stochastic PETRI NETS marks the transition to discrete event simulation and the integration of quantitative and stochastic aspects [39]. In discrete event models state transition functions define into which state to change triggered by external events, e.g. the collisions of species like enzymes and metabolites in a biological model, or triggered by the flow of time, e.g. after the time required for intra-molecular rearrangements. In discrete event simulation, situation-based and time-based events can occur at any point in time and the resulting state and the time span needed for reaction can be randomly chosen. Thus, stochasticity comes natural to discrete event simulation (see section 2.2).

Continuous systems models can easily be translated into a set of differential equations, independently of being defined as bond graphs, as block diagrams, or as set of chemical reactions. The discrete modeling and simulation realm lacks such a common denominator that is widely accepted, even though general approaches exist. E.g. DEVS [40], PETRI NETS [41], and π -CALCULUS [42, 43] are formal and generally applicable approaches toward discrete event systems modeling. Each has been developed with a rather different objective in mind. The goal of DEVS has been to combine the functional, network and hierarchical perspective in describing systems, and thus stands in the tradition of general systems theory [44]. DEVS distinguishes between atomic models and coupled models. Whereas atomic models describe the behavior in terms of state transitions that

might be triggered by external events or the flow of time, and output functions, coupled models define how their components, which might be atomic or coupled, interact with each other and thus control the interaction between them. Thus, a hierarchical, modular construction of models is supported. An abstract simulator defines the execution semantics of typical DEVS models [40]. Extensions of DEVS support variable structure models [45], models that entail in their description the ability to change their own composition and interaction structure which is important in modeling and simulating biological systems [46]. DEVS models emphasize the definition of states and state transitions and therefore, are closely related to STATECHARTS, a model formalism that is widely applied in Systems Biology [47]. STATECHARTS can easily be transformed into DEVS. A transformation of DEVS models into the graphical notation of STATECHARTS facilitates the understanding of models [48].

PETRI NETS and π -CALCULUS have been developed for describing concurrent processes and are best known in the context of computer and engineering sciences. Whereas PETRI NETS focus on concurrent processes competing for resources, the π -CALCULUS is aimed at describing concurrent mobile processes, channels, locations, and interactions respectively. Thus, processes like protein to protein interactions can be described easily [49, 50, 51, 52]. Its extension in form of the stochastic π -CALCULUS, supports the definition of discrete event models and their execution by discrete event simulation. Thus, established approaches to transform existing continuous models into discrete event models [53], can be used to define and refine models in the stochastic π -CALCULUS. Openly available simulation systems like BIOSPI also push the application of the stochastic π -CALCULUS [54]. Recent developments like BIOAMBIENTS which is based on the stochastic π -CALCULUS, allow the description of spatial cell compartments, and entities moving from one compartment to the next and thus increase the expressiveness of the language [55]. The BRANE CALCULI [56, 57] addresses the need for modeling constructs of cellular coordination via membranes. It forms an application specific refinement of the general modeling and simulation approach. In the PROJECTIVE BRANE CALCULI the membrane actions become directed thereby, moving the calculi even closer to the perception of the activities within biological membranes [58]. These recent extensions are aimed at providing means and places for describing coordination and cooperation within biological cells [50], and lend additional structure and expressiveness to the modelling language. To specify the executional semantics of a model in a non-ambiguous manner an abstract simulator has been developed for the π -CALCULI [54], as has been done for DEVS like models [40]. One might note that when a continuous model is executed, numerical integration algorithms discretize the state and time base, and so diminish the conceptual distance toward discrete models. However, the assumption underlying continuous models is still that the system behaves continuously with an infinite number of infinitely close state transitions in each time interval. The numerical integration merely serves to approximate this behavior. In discrete event models in contrast, no continuity of behavior needs to be assumed. However, the situation becomes more interesting since recently it has

Table 1. Modeling formalisms: time and state space

	Discrete event	Discrete step-wise	Differential equation
input and output	arbitrary	arbitrary	real vector
state space	arbitrary	arbitrary	real vector
time base	real	discrete	real

been shown that discrete event models can be used to obtain approximations to the solutions of differential equation systems [17, 59]. This is done using a process of quantization in which events are scheduled based on predicted threshold crossings rather than time steps. Executed by a discrete event simulation engine it will reproduce the trajectories, in some cases in significantly less time [60].

Often systems can best be described by a combination of discrete and continuous models, e.g. if continuous processes exhibit discontinuities which require to switch from one continuous model to another one, or if leaving or entering a discrete phase depends on continuous processes that reach certain thresholds. Hybrid systems models combine continuous and discrete systems behavior. Many modeling and simulation approaches for discrete and continuous systems have been extended to support hybrid systems models. HYBRID PETRI NETS have been developed by adding continuous places and continuous transitions to the discrete places and transitions of regular PETRI NETS [61]. The continuous transitions of HYBRID PETRI NETS are used to describe kinetic reactions which are turned on and off by the marking of discrete places. These discrete places form the interface between continuous and discrete partitions of the PETRI NET [62]. HYBRID PETRI NETS as a graphical tool are well suited to describe metabolic processes, as they visualize chemical reactions and interdependencies. Similar arguments motivate the use of BLOCK DIAGRAMS, that allow to specify graphically continuous and hybrid models [63] and are supported by many simulation tools, e.g. [64]. The origin of block diagrams, unlike that of PETRI NETS, lies in the continuous realm. To allow the integration of discontinuities they have been extended by discrete elements, e.g. switching blocks. Both HYBRID PETRI NETS and BLOCK DIAGRAMS support the *mixed signal approach* in describing hybrid systems [65]. In contrast to that, HYBRID AUTOMATA [66] move the distinction of phases into the focus of modeling. State transitions of HYBRID AUTOMATA are triggered by continuous processes that are responsible for describing the continuous behavior of a system while being in one phase and determining the time and situation when to leave a phase and enter another one [67, 68, 69]. The growing need to integrate discontinuous behavior into Systems Biology models is reflected in extending existing simulation systems, e.g. GEPASI, or in the design of recent simulation systems for Systems Biology, e.g. the E-CELL simulation system.

2.2 Deterministic and Stochastic Systems Models

Modeling is the process of structuring our knowledge about a given system [40]. In this perspective, stochastic processes represent one means to express the uncertainty of our knowledge. A plethora of methods are dedicated to the problems

of stochastic modeling, e.g. to estimate suitable distributions for random variates, and to interpret the results of the simulation runs [70]. From the view of the modelled system, integrating stochasticity into the models might also serve a slightly different purpose: randomness or "noise" arising from small numbers of molecules involved in processes like gene expression and regulation can directly be represented in the model [71, 72]. Although stochastic elements are often associated with discrete event models, they are also applicable to continuous system models. In Systems Biology, inclusions of stochastic elements for modeling continuous processes have gained ground recently. E.g. chemical reaction equations are described by so called stochastic differential equations [73]. These equations determine the probability with which a combination of molecules will react in a given time interval.

Interestingly, to solve these equations Gillespie [74, 75] suggested an algorithm that transforms the set of equations into a discrete event stochastic model. The representation in discrete event form is particularly striking in more recent implementations of the algorithm. E.g. the simulation system STOCHASTIRATOR is a discrete event simulator with the typical event queues and the handling of time and situation triggered events [73]. STODE [53] transforms automatically reaction rates and model parameters of a deterministic differential equation model internally into a stochastic discrete event model. The probabilities of single reactions depend on the number of reactants which again is subject to change via occurrence of reactions [73]. The stochastic discrete event models address specific constraints of continuous, deterministic models: concentrations do not necessarily change continuously, particularly if the dynamics of a small amount of entities, like DNA molecules and plasmids, shall be modeled [76]. In addition, sometimes, the dynamics of biological systems can be best approached in a stochastic manner, e.g. if the gene regulation is to be described [77], where stochastic fluctuations are abundant [78]. The exact stochastic simulation approach is not practical for the simulation of metabolic processes, in which large numbers of molecules of the same kind are involved, due to the computational cost for the calculation of all individual molecular collisions. Extensions of the approach overcome these difficulties and allow the stochastic simulation of systems composed of both intensive metabolic reactions and regulatory processes involving small numbers of molecules [79, 80, 81]. The combination of stochastic discrete with continuous sub-models has stimulated the desire for an easy integration of stochastic aspects into continuous models. One common approach is to assume a normal distribution for key parameters of the differential equation system. The result is that stochasticity can now permeate the entire model [16].

2.3 Qualitative, Quantitative and Semi-quantitative Systems Models

Continuous models are usually associated with quantitative models, i.e. models whose variables are numerically scaled, in the case of differential equations the state space is given by real values vectors. However, continuous behavior can

also be described qualitatively. E.g. QSIM [82] assumes a continuous, respectively hybrid behavior of a system and describes this in qualitative terms, e.g. rising trends, falling trends, landmarks, etc. This approach has also been used in Systems Biology, e.g. to describe the development of a λ -Phage in an eukaryotic cell [83]. Often the lack of quantitative data motivates the use of qualitative methods. Qualitative methods are often used as a first step to develop a quantitative model [84]. E.g. one obtains useful structural information by determining what variables play a role for certain kinetics and whether there exists a positive or negative influence between variables [85, 86]. Another motivation for the application of qualitative methods is that they are aimed at answering different kinds of questions than quantitative methods and offer different possibilities for analysis, for example whether certain states can be reached by the system and under which conditions. On the other hand, if not only the existence but also the degree, and the effect of opposite regulations are of interest purely qualitative models will not prove to be sufficiently expressive [32].

It is one advantage of discrete event simulation that its models combine easily qualitative and quantitative aspects of the system [12] (see also table 1), even though the assumption, that discrete event simulation requires less data than continuous one, as stated in [12], has to be inspected critically. In hybrid systems models, the “qualitatively scaled variables” come into play to describe the different phases or to initiate switching from one differential equation system to another.

3 Micro, Macro, and Multi-level Systems Models

Traditionally, two dichotomous views on systems prevail. “With individualism, macroscopic processes are either emergents or totally reducible aggregates, while with holism microscopic actions occur as local manifestations of system-wide processes” [87]. In sociology the distinction between micro, macro, and, to mediate between both, multi-level models is comparatively well established [88, 89]. Macro models describe a system as one entity. Variables and their interdependencies, which can be expressed as rules, equations, constraints etc., are attributed to this entity. Typical representatives of this class are differential equation models, which describe e.g. a biochemical system based on concentrations and reaction rates.

Micro models are models that represent systems as comprising huge numbers of rather homogeneously structured entities. Only the behavior of the individuals is explicitly modeled. The macro level of the system exists only as it aggregates results of the activities at micro level and is used for reflecting emergent phenomena, e.g. the development of specific spatial patterns. They do not have any behavior of their own. Typical representatives of this class are cellular automata and Lindenmeyer systems which are also applied for reconstructing spatial biochemical processes in Systems Biology [90, 91, 92, 93, 94].

Micro models often form only a transition to multi-level models which describes a system at least at two different levels. Interactions are taking place

within and between those levels. The description of systems at different levels of abstraction and different time scales facilitates taking spatial and temporal structured processes into consideration, e.g. [95].

Multi-level models allow us to explicitly describe “upward”, - and “downward causation”, i.e. “the whole is to some degree constrained by the parts (upward causation), but at the same time the parts are to some degree constrained by the whole (downward causation).” [18]. Their importance has been emphasized for systems in general [19] and biological systems in particular [20, 21]. The relevance of interrelating micro and macro models has also been raised recently for Systems Biology [22, 23, 24, 25].

The structure of multi-level models typically reveals whether they originated from macro or micro models. If the latter is the case we find a multiplicity of homogeneously structured entities, that describe e.g. different population of enzymes and proteins. If a macro model has been successively extended and refined to describe a system at different levels of organization, then comparatively few sub-models typically exist and those are heterogeneously structured with different patterns of behavior.

Individual-based models, which describe systems at two levels of organization, i.e. a micro and macro level [96, 97], belong to the class of multi-level models. They reveal their close relationship to micro models. In individual-based models the individual entities and the macro level are explicitly modeled. The individuals typically do not interact directly but via the macro level. Individual-based approaches are also increasingly being applied in cellular biology [98, 99, 100].

In the following we will shortly discuss the relationships of multi-level modeling and the previously discussed dimensions.

Continuous and Discrete Modeling. Multi-level models are neither restricted to discrete models nor to continuous ones. If multiple levels are formed by a successive extension and refinement of macro models they might be both, continuous or discrete. Continuous models can easily be structured into different cellular compartments, e.g. [101, 102], each of which behaves continuously. Even if multi-level models contain many homogeneously interacting and structured submodels, these might form continuous systems models [103], although in the case of many homogeneously interacting entities discrete models of the individuals prevail. They allow to combine a qualitative discrete perception of individuals and their behavior with a quantitative, concentration-oriented view at macro level and thus a comparison with measured concentration changes.

Deterministic and Stochastic Modeling. Again multi-level models might work deterministically or stochastically. The question whether stochastics plays a major role in multi-level models is closely related to the question whether discrete event models are part of the multi-level model. Most discrete event models consider stochastic effects in determining when and what will happen. In this case a simulation run turns into a random experiment and has to be treated as such [70].

When Gillespie suggested the transformation of deterministic continuous models into stochastic discrete event models, he also prepared the way for a micro perspective of cells. Although most implementations of the Gillespie algorithm, e.g. [73], record the number of molecules being in certain states and determine the time of next event and the most likely reaction to occur based on this “macro view”, they consider the molecules as atomic entities to be added or deleted from the bulk solution. A next step has been taken in STOCHSIM which attributes properties to these entities and thereby, allows to observe individual molecules over time. The model is based on a discrete step-wise execution [104]. The dominance of discrete approaches in multi-level modeling and single individuals being represented motivates the integration of stochastic aspects. So most multi-level models are stochastic models.

Quantitative and Qualitative Modeling. A multi-level model might be qualitative, quantitative, or semi-quantitative. If only continuous differential equation models are considered the state space presents itself as a vector of real numbers. Discrete event models of individuals support the representation of the modeled system by arbitrarily scaled variables. To allow a discrete event simulation to jump from one event to the next after some pre-defined time interval has elapsed, the time base of discrete event models is continuous and introduces typically some quantitative information - when does the next event occur or how long does a state persist per se. Purely qualitatively scaled variables are also perceivable. However, the combination of discrete, qualitative models at individual level and quantitative (discrete or continuous) models at macro level holds probably the most appeal to biologists as they allow to re-unify two perspectives in dealing with natural systems.

To support the modeling of complex systems, many formalisms, languages and tools allow to hierarchically compose models. Supporting a hierarchical structure of a model helps realizing multi-level models however not all hierarchically composed models are designed as multi-level models. They do not necessarily describe a system at different organizational levels, they use the model hierarchy for modularization.

If we categorize a model as being quantitative, stochastic, discrete and multi-level, it is therefore interesting to ask what this means not only for the model itself but for potential sub- and super-models: what can be deduced along a compositional model hierarchy, which might or might not reflect the different organizational levels. A quantitative model implies that all sub-models are quantitative, the supermodel might be semi-quantitative or quantitative. If one sub-model contains stochastic aspects the entire model becomes a stochastic one. If a model is continuous all sub-models are continuous and all supermodel will be either hybrid or continuous. If a model is a multi-level model, its sub-models might be micro, macro, or multi-level models, its super-model will definitely be a multi-level model. If we have a micro model, we will have many homogeneously structured sub-models each of which describes an individual at macro level, its super-model will be a micro or multi-level model. A macro model might not have

any sub-models. In case it has components, its components are all macro models; the macro model itself can be part of a micro, macro, and multi-level model.

4 Biological Example: Diverse Models for the Tryptophan Synthase

After these theoretical considerations about the different modeling dimensions and their interdependencies, we will illustrate our exploration based on a biological example.

The Tryptophan Synthase is the last enzyme of the reaction cascade, which is responsible for the synthesis of the aromatic amino acid Tryptophan. The whole enzyme is a homo-dimer, whereas each monomer consists of two subunits, the α - and the β -subunit, which are connected by a largely hydrophobic tunnel. The enzyme has been isolated from microbial cells in [105] and characterized by *in-vitro* experiments using radiolabelled substrates. For the description of the *in-vitro* determined kinetics a quantitative, deterministic macro-model was developed [106]. The deduced reaction mechanism of the enzyme is shown in figure 1.

For each binding-state of the enzyme during the conversion from Indoleglycerol-3-phosphate (IGP) and Serine to Tryptophan and Glyceraldehyde-3-phosphate (G3P) at the α - and the β - subunit, respectively, a distinct variable was introduced, so that a system of ordinary differential equations could be derived (Figure 4).

After numerical integration and parameter fitting the trajectories resulting from several *in-vitro* experiments could be reproduced by the corresponding simulation experiments.

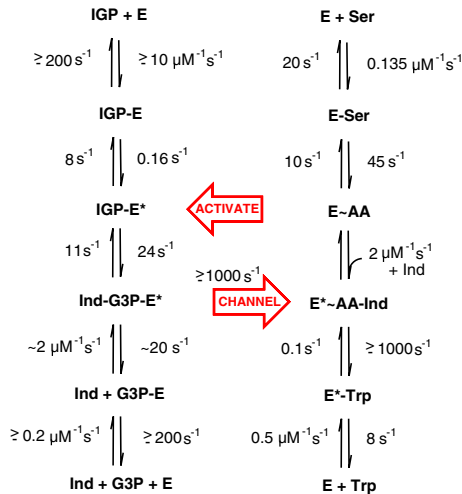


Fig. 1. Reaction scheme of tryptophan synthase [29]

$$\begin{aligned}
\dot{IGP} &= 200IGP-E - 10IGP \cdot E \\
IGP-E &= 10IGP \cdot E + 8IGP-E^* - 200IGP \cdot E - 0.16IGP-E \\
IGP-E^* &= 0.16IGP-E + 11Ind-G3P-E^* - 8IGP-E^* - 24IGP-E^* \\
Ind-G3P-E^* &= 24IGP-E^* + 2G3P-E \cdot Ind - 20Ind-G3P-E^* - 11Ind-G3P-E^* \\
G3P-E &= 20Ind-G3P-E^* + 0.2G3P-E \cdot Ind - 2G3P-E \cdot Ind - 200G3P-E \cdot Ind \\
G3P &= 200G3P-E - 0.2G3P \cdot E \\
\dot{Ind} &= 20Ind-G3P-E^* - 2G3P-E \cdot Ind - 2EAA \cdot Ind \\
\dot{Ser} &= 20E-Ser - 0.135Ser \cdot E \\
E-Ser &= 0.135Ser \cdot E + 10EAA - 20E-Ser - 45E-Ser \\
EAA &= 45E-Ser - 10EAA - 2EAA \cdot Ind \\
E^*AA-Ind &= 2EAA \cdot Ind + 0.1E^*-Trp - 1000E^*AA-Ind \\
EsTrp &= 1000E^*AA-Ind + 0.5E \cdot Trp - 0.1E^*-Trp - 8E^*-Trp \\
Trp &= 8E^*-Trp - 0.5E \cdot Trp
\end{aligned}$$

Fig. 2. Continuous macro model of the tryptophan synthase

Summarizing the model it is clear that:

- it is a continuous systems model since the equation system 4 describes continuous change rates of the metabolite concentrations via balancing the reaction velocity terms for the building and decay of the different entities;
- it is a macro-model – as the model contains only one level and no individual entities are modeled, but homogeneous populations of the entities are regarded as one variable and all variables are attributed to the same entity: the tryptophan synthase system;
- it is deterministic – as equations 4 do not contain stochastic elements, like distribution functions for inter-arrival times or the different enzyme-metabolite populations (IGP-E, etc.);
- it is quantitative - as the state space is a real-valued vector (IGP-E, etc.);

As mentioned above, the model is particularly suited for the description of the experimentally determined concentration changes. Nevertheless some known structural characteristics of the enzyme Tryptophan Synthase are not reflected equally well. Especially the macro-models' description of the hydrophobic channel is strongly simplified: it is known from independently performed X-Ray experiments for the structural analysis, that the tunnel can store up to four indole molecules. This could imply a time delay for tunneling the indole from the α - to the corresponding β -subunit, that was not taken into account by the macro-model. Integrating the tunnel's capacity into the described macro model would significantly complicate the model's execution, since time-delayed differential equations have to be defined. In addition, it would burden the model structure reducing the transparency of the model.

Therefore a discrete-event stochastic multi-level model was generated [100] to allow a more detailed description of the individual enzymes including additional structural (qualitative) information about the enzymes and to allow at the same time the reproduction of the *in-vitro* experiments. In spite of the additional com-

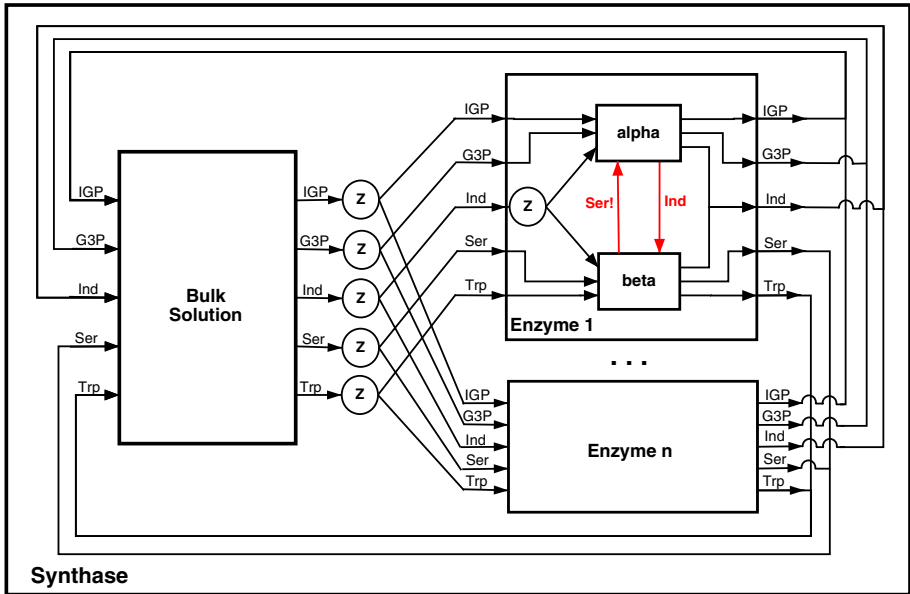


Fig. 3. A multi-level model of tryptophan synthase [29]

plexity the multi-level model should remain transparent for the experimentalists, see figure 3.

In the following the conversion of the continuous deterministic macro-model into the discrete-event stochastic multi-level model is discussed in more detail step by step:

1. Transformation of a *continuous* deterministic macro-model to a *discrete-event* stochastic macro-model:
 - this is e.g. done by the Gillespie Algorithm [74], that was deduced to exactly simulate a stochastic differential equation system describing chemical reactions systems. Depending on the actual numbers of each molecule and enzymes in each binding-state at a discrete time, the algorithm deduces, when the next reaction will take place and what reaction it will be. After that time the number of molecules and enzymes with different binding-states is updated and the next reaction time is determined;
2. Transformation of a discrete-event stochastic *macro model* to a step-wise-discrete stochastic *micro model*:
 - to form a micro model from the discrete stochastic macro-model individual entities with their properties, i.e. in our case mainly the different binding-states of the enzymes, have to be modeled;
 - corresponding simulations can be realized in STOCHSIM: at each time step of the simulation, which is determined by the fastest reaction step,

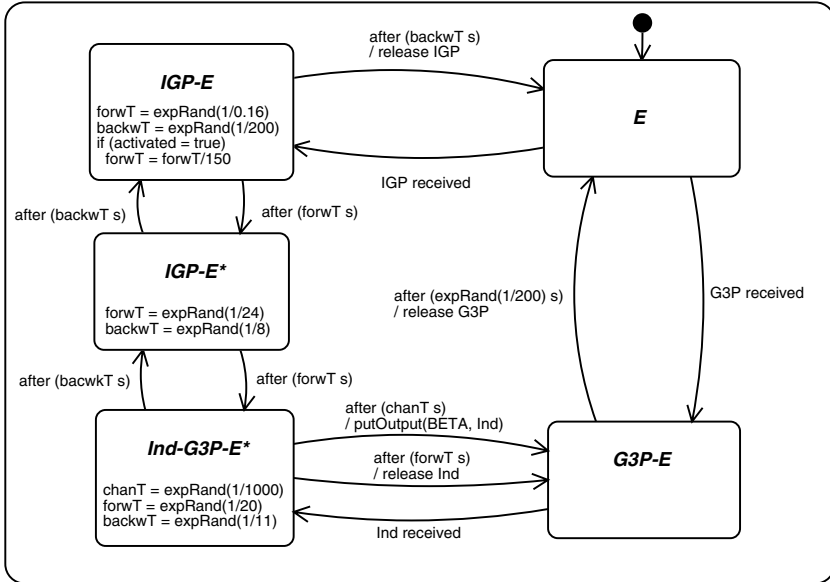


Fig. 4. Phases and transitions for an α -subunit of a single enzyme [29]

i.e. in our case the tunneling reaction, two molecules were randomly chosen. Their current properties, i.e. binding-states, decide whether a reaction can take place at all. According to the probability defined for this reaction (which is correlated to the reaction's velocity) the reaction will actually be executed;

- the simulation is time-consuming due to the many time-steps, at which no reaction takes places;

3. Transformation of a *step-wise-discrete* stochastic *micro model* to a *discrete-event* stochastic *multi-level model*:

- micro to multi-level: The individual enzyme with its different binding-states is subdivided into an α - and a β -subunit, which communicate via the tunnel.

In addition to the individual enzymes, a macro-level is introduced which records the IGP, Ser, Trp, Ind, G3P molecules and the α - β -models in the bulk solution. It has the function to distribute the substrate and product molecules to and from the individual enzymes. The frequency is determined by the current concentration of metabolites and enzymes and the velocity of the different reactions. It offers a macro perspective on the system, where the bulk solution is the system of interest having concentrations and equations for describing the change of concentrations attributed to it. As the bulk solution contains only metabolites that are of interest for the tryptophan synthase, each change of concentration is translated into forwarding metabolites to individual enzymes. In addi-

tion, the macro level records the trajectories of the metabolite concentrations over time and can be used for validating the model based on the *in-vitro* experiments.

- step-wise-discrete to discrete-event: the internal state, i.e. the binding-state, of the α/β -model determines whether a reaction will take place and when, this is defined according to the Gillespie algorithm (Figure 4).

5 Different Perspectives of Multi-level Models

By adopting the object metaphor, the number of discrete and continuous simulation systems that integrate the different traditional views in modeling systems, i.e. as functional models, as networks of interactions, and as hierarchical composition of models is steadily increasing, e.g. JAMES [107], GENOMICOBJECTNET, [108], E-CELL [109], and BIOSPI [110]. Thereby, composition and interaction determine the overall structure of a model in general and of a multi-level model in particular.

At the lowest layer we find functional models of individuals. They might be represented as quantitative, or qualitative, continuous or discrete model, integrating stochastic aspects or describing the system’s behavior deterministically. To define the interaction between models, interfaces have to be defined. To let models interact it is important to distinguish between so called “value couplings” that support a direct exchange of values, so each change in one submodel is directly reflected to a change in another submodel, and an exchange of values by events. Whereas the former supports the coupling of continuous models the latter is used to support the coupling of discrete systems models. A combination of both allows to support the coupling of hybrid models. To facilitate the interaction, often the interaction of hybrid submodels is restricted to exchanging discrete events at discrete times [111, 112].

Grouping strongly interacting submodels into one model supports a hierarchical composition of models. Thus, a compositional hierarchy is introduced bottom up. Similarly we can assume that a hierarchy is introduced top down by starting with the coupled or composite model and asking for its components. Most modeling formalisms assume a strong composition, i.e. one model component belongs only to one coupled model. Often coupled models or composite models simply frame a group of models so that they can be treated as one model, e.g. as it is the case in DEVS, in composite hierarchical Petri Nets, like the GENOMICOBJECTNET [113], and in BIOAMBIENTS [55]. To belong to the components of such a coupled model can easily be interpreted as residing in one space. This view is emphasized in BIOAMBIENTS [55], which, based on the stochastic π -CALCULUS, is directed toward supporting higher level abstractions and the description of complex, spatial phenomena in Systems Biology.

If a coupled model is interpreted as representing a spatial cell compartment, the ability to support variable structure models, i.e., models that are able to change their own composition and interaction structure [45], becomes a prerequisite to describe phenomena of proteins joining and leaving cell compartments. Composite models have no behavior of their own, their behavior is spec-

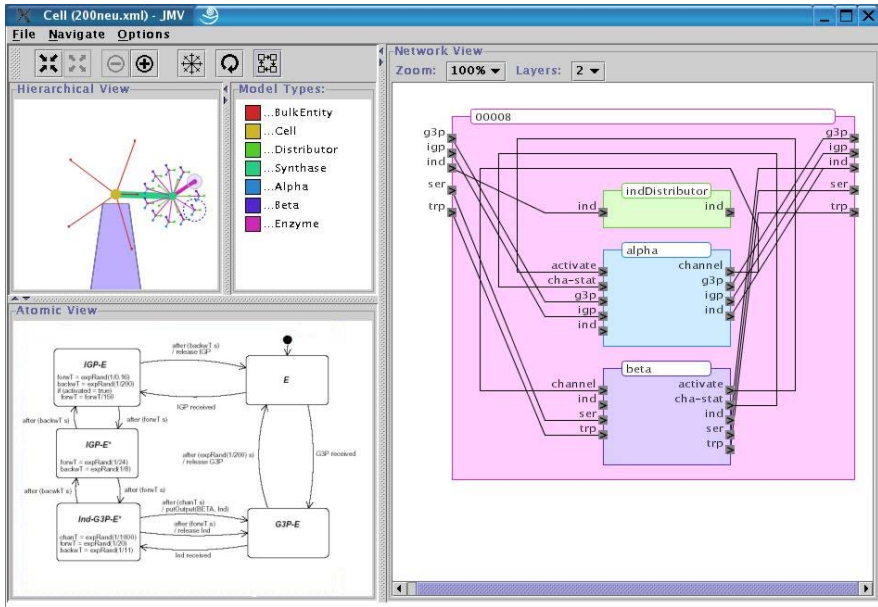


Fig. 5. Screen shot explicating the atomic, the network and the hierarchy perspective in multi-level modeling [114]

ified by their components and their interactions. This lack of own state and behavior does not hamper to use them to introduce a notion what does and what does not belong to a single cell compartment. However, to explicitly describe a macro view, a separate model has to be introduced to describe state and dynamic at the macro level, as has been done to model the Tryptophan synthase in JAMES (Fig. 5). The multi-level model contains sub-models that describe enzymes as micro models, and sub-models that describe the behavior of entire enzyme populations as macro models.

Figure 5 represents the different perspectives in modeling. The macro level contains models that describe the state and dynamics of the different populations of the bulk solution (see also figure 3). The macro models responsible for the indole, the serine, the IGP, and the G3P interact with the “micro model” responsible for the synthase. The former keeps track of the amounts of substrates, products and enzymes and defines the behavior at the level of concentrations and collision probability.

The micro model synthase contains thousands of models each of which describes a single enzyme, (figure 5 in upper left corner). Thus, the overall composition tree is highly unbalanced, one of the children has more than 800 children. As we are interested in the role the channel plays in the tryptophan synthase, we define the enzyme model to consist of two different subunits, i.e. α and β , which communicate via the channel (see figure 5 on the right hand side). The

behavior of each subunit is modeled as discrete transitions from one state to the other. State changes might be triggered by the arrival of metabolites or by the flow of time (see figure 5 in the lower, left corner).

Multi-level models promise a flexible approach toward the understanding of cellular systems. However, they also provide new challenges for modeling, simulation, and visualization techniques, alike – which is illustrated in the above figure. Different perspectives on the model, that can be interactively selected and refined, are needed to visualize the model structure in a compact manner and to enable users to rapidly manipulate the model structure [114].

6 Discussion

Thinking about variables and their continuous change rates appears closely related to a macro perception rather than a micro perception of a system. Continuous models reflect the observation of experiments in cellular biology nicely. The starting point of multi-level models seems somehow different. It is focused on the active entities of the processes. Their states, behavior, and interaction with others are directly described. In continuous models the structural information are indirectly deducible from the model parameter and the structure of the differential equations. Though continuous models can easily be structured into components or objects to describe a system as being comprised of interacting subsystems, often the focus is on the global scheme of reaction mechanisms.

Discrete modeling approaches prevail if single entities and their dynamics shall be described. Since many phenomena can only be measured on population level, models of single enzymes are typically only checked for plausibility. For validation model populations can be created, thus turning to micro models embracing many individuals. To consider the slight deviation between individuals stochastic effects are introduced supporting realistic phenomena on macro level. Individual-based models combine a macro and micro view on the system under study. They form a first step toward multi-level models, where different description levels of systems are integrated. Interaction and coordination are taking place within and between levels of organization. Modularity, hierarchical structure, and flexible modeling come natural to multi-level models, however at the cost of simulation efficiency requiring special solutions [115]. There however, appears to be no silver bullet for modeling cellular systems. The objective of the simulation study should drive the level of resolutions chosen, structure, and the formalism employed. The background of the modelers might bias the choice of approach unintentionally. Therefore they should educate themselves to appreciate the variety of choices that have become available in the last several years.

The multi-level modeling approach offers a way of bridging between micro and macro level constructs. The concept of homomorphism has been proposed as the way to express macro level constructs in terms of micro level ones in a way that preserves their behavior. Although several examples have been developed to illustrate this approach [44, 116, 117], more research and more attempts to apply the research results are sorely needed. Advances in modeling, simulation,

and computational biology in general, may well hinge on achieving better ways to include multiple levels of resolution. Multi-level models move the focus of modeling and simulation from seeking the most simplistic model able to reproduce the observed data, to a flexible, easily refinable and re-usable “middle-out” model-design that suits the structure of our knowledge and the current question of interest best.

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References

1. Kitano, H.: Systems Biology: A Brief Overview. *Science* **295** (2002) 1662–1664
2. Wolkenhauer, O.: Systems biology: the reincarnation of systems theory applied in the biology? *Briefings in Bioinformatics* **2** (2001) 258–270
3. Chabrier-Rivier, N., Fages, F., Soliman, S.: The Biochemical Abstract Machine Biocham. In: *Proceedings of the 2nd International Workshop on Computational Methods in Systems Biology*. (2004)
4. Hucka, M., Finney, A., Sauro, H., Bolouri, H.: The erato systems biology workbench: Architectural evolution. In Yi, T.M., Hucka, M., Morohashi, M., Kitano, H., eds.: *The Proceedings of the 2nd International Conference on Systems Biology*. (2001)
5. Hucka, M., Finney, A., Sauro, H.M., Bolouri, H., Doyle, J.C., Kitano, H., Arkin, A., Bornstein, B.J., Bray, D., Cornish-Bowden, A., Cuellar, A.A., Dronov, S., Gilles, E.D., Ginkel, M., Gor, V., Goryanin, I.I., Hedley, W.J., Hodgman, T., Hofmeyr, J.H., Hunter, P.J., Juty, N., Kasberger, J.L., Kremling, A., Kummer, U., Le Novere, N., Loew, L.M., Lucio, D., Mendes, P., Minch, E., Mjolsness, E.D., Nakayama, Y., Nelson, M.R., Nielsen, P.F., Sakurada, T., Schaff, J.C., Shapiro, B., Shimizu, T.S., Spence, H.D., Stelling, J., Takahashi, K., Tomita, M., Wagner, J., Wang, J.: The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. *Bioinformatics* **19** (2003) 524–531
6. Cuellar, A., Lloyd, C., Nielsen, P., Bullivant, D., Nickerson, D., Hunter, P.: An overview of CellML: 1.1, A Biological Model Description Language. *Simulation - Transactions of the SCS* **79** (2003) 740–747
7. Domach, M.M., Leung, S.K., Cahn, R.E., Cocks, G.G., Shuler, M.L.: Computer model for glucose-limited growth of a single cell of *Escherchia coli* B/r-A. *Biotechnology and Bioengineering* **26** (1984) 203–216
8. Teusink, B., Passarge, J., Reijenga, C.A., Esgalhado, E., van der Weijden, C.C., Schepper, M., Walsh, M.C., Bakker, B.M., van Dam, B., van Dam, K., Westerhoff, H.V., Snoep, J.L.: Can yeast glycolysis be understood in terms of *in vitro* kinetics of the constituent enzymes? Testing biochemistry. *European Journal of Biochemistry* **267** (2000) 5313–5329
9. Hynne, F., Donø, S., Sørensen, P.G.: Full-scale model of glycolysis in *Saccharomyces cerevisiae*. *Biophysical Chemistry* **94** (2001) 121–163

10. Santillán, M., Mackey, M.C.: Dynamic regulation of the tryptophan operon: A modeling study a comparison with experimental data. *Proceedings of the National Academy of Sciences of the USA* **98** (2001) 1364–1369
11. Reddy, V.N., Liebman, M.N., Mavrovouniotis, M.L.: Qualitative analysis of biochemical reaction systems. *Computers in Biology and Medicine* **26** (1996) 9–24
12. Xia, X.Q., Wise, M.J.: DiMSim: A Discrete-Event Simulator of Metabolic Networks. *Journal of Chemical Information and Computer Science* **43** (2003) 1011–1019
13. Jones, M.E., Berry, M.N., Phillips, J.W.: Futile Cycles Revisited: A Markov Chain Model of Simultaneous Glycolysis and Gluconeogenesis. *Journal of Theoretical Biology* **217** (2002) 509–523
14. Arkin, A., Ross, J.: Computational functions in biochemical reaction networks. *Biophysical Journal* **67** (1994) 560–578
15. Hjemfelt, A., Ross, J.: Implementation of logic functions and computations by chemical kinetics. *Physica D* **84** (1995) 180–193
16. Bentele, M., Eils, R.: General stochastic hybrid method for the simulation of chemical reaction processes in cells. In: *Proceedings of the 2nd International Workshop on Computational Methods in Systems Biology*. (2004)
17. Zeigler, B., Praehofer, H., T.G., K.: *Theory of Modeling and Simulation*. Academic Press, London (2000)
18. Heylighen, F. In: *Downward Causation*. Principia Cybernetica Web, <http://pespmc1.vub.ac.be/DOWNCAUS.html> (access date: 12.05.2004)
19. Bunge, M.: *Ontology II: A World of Systems*. Volume 4 of *Treatise of Basic Philosophy*. Reidel, Dordrecht (1979)
20. Campbell, D.: *Downward causation in Hierarchically Organized Biological Systems*. In Ayala, F., Dobzhansky, J., eds.: *Studies in the Philosophy of Biology*. University of California Press, Berkeley (1974) 179–186
21. Salthe, S.: *Evolving Hierarchical Systems*. Columbia University Press (1985)
22. Strohmann, R.: Organization becomes cause in the matter. *Nature Biotechnology* **18** (2000) 575–576
23. Whitesides, G., Boncheva, M.: Beyond molecules: Self-assembly of mesoscopic and macroscopic components. *PNAS* **99** (2002) 4769–4774
24. Hartwell, L.H., Hopfield, J.J., Leibler, S., Murray, A.W.: From molecular to modular cell biology. *Nature* **402** (1999) C47–C52
25. Vilar, J.M.G., Guet, C.C., Leibler, S.: Modeling network dynamics: the lac operon, a case study. *The Journal of Cell Biology* **161** (2003) 471–476
26. Kremling, A., Jahreis, K., Lengeler, J.W., Gilles, E.D.: The Organization of Metabolic Reaction Networks: A Signal Oriented Approach to Cellular Models. *Metabolic Engineering* **2** (2000) 190–200
27. Kremling, A., Gilles, E.D.: The Organization of Metabolic Reaction Networks II. Signal Processing in Hierarchical Structured Functional Units. *Metabolic Engineering* **3** (2001) 138–150
28. Kremling, A., Bettenbrock, K., Laube, B., Jahreis, K., Lengeler, J.W., Gilles, E.D.: The Organization of Metabolic Reaction Networks: III. Application for Diauxic Growth on Glucose and Lactose. *Metabolic Engineering* **3** (2001) 362–379
29. Degenring, D., Röhl, M., Uhrmacher, A.: Discrete Event, Multi-Level Simulation of Metabolite Channeling. *BioSystems* **75** (2004) 29–41
30. Minsky, M.: *Models, Minds, Machines*. In: *Proc. IFIP Congress*. (1965) 45–49
31. Cellier, F.E.: *Continuous System Modeling*. Springer, New York (1992)

32. de Jong, H.: Modeling and Simulation of Genetic Regulatory Systems: A Literature Review. *Journal of Computational Biology* **9** (2002) 67–103
33. Mendes, P.: GEPASI: a software package for modelling the dynamics, steady states and control of biochemical and other systems. *Computer Applications in the Biosciences* **9** (1993) 563–571
34. Ginkel, M., A., K., Nutsch, T., Rehner, R., Gilles, E.: Modular modeling of cellular systems with ProMoT/Diva. *Bioinformatics* **19** (2003) 1169–1176
35. Sauro, H.: Jarnac: A system for interactive metabolic analysis. In: *Animating the cellular map: Proceedings of the 9th International Meeting on BioThermoKinetics*, Stellenbosch University Press (2000)
36. Goryanin, I., Hodgman, T., Selkov, E.: Mathematical simulation and analysis of cellular metabolism and regulation. *Bioinformatics* **15** (1999) 749–758
37. Shapiro, B.E., Levchenko, A., Meyerowitz, E.M., Wold, B.J., Mjolsness, E.D.: Cellerator: extending a computer algebra system to include biochemical arrows for signal transduction simulations. *Bioinformatics* **19** (2003) 677–678
38. Fuss, H.: Simulation of Biological Systems with PetriNets - Introduction to Modelling of Distributed Systems. In Moller, D., ed.: *Advances in System Analysis*. Vieweg, Braunschweig, Wiesbaden (1987) 1–12
39. Goss, P., Peccoud, J.: *Biochemistry Quantitative Modeling of Stochastic Systems in Molecular Biology by Using Stochastic Petri Nets*. Proceedings of National Academy of Sciences of the USA **95** (1998) 6750–6755
40. Zeigler, B.: *Multifaceted Modelling and Discrete Event Simulation*. Academic Press, London (1984)
41. : Petri Nets World. <http://www.daimi.au.dk/PetriNets/> (access date: 08.11.2004)
42. Milner, R.: *Communicating and Mobile Systems: The π Calculus*. Cambridge University Press (1999)
43. Priami, C.: The Stochastic pi-Calculus. *The Computer Journal* **38** (1995) 578–589
44. Zeigler, B.: A Note on System Modelling, Aggregation and Reductionism. *J. of Biomedical Computing* **2** (1971) 277–280
45. Uhrmacher, A.: Dynamic Structures in Modeling and Simulation - A Reflective Approach. *ACM Transactions on Modeling and Simulation* **11** (2001) 206–232
46. Uhrmacher, A.M.: Reasoning about Changing Structure: A Modeling Concept for Ecological Systems. *International Journal on Applied Artificial Intelligence* **9** (1995) 157–180
47. Kam, N., Harel, D., Kugler, H., Marelly, R., Pnueli, A., Hubbard, E., Stern, M.: Formal Modelling of *C. elegans* Development: A Scenario Based Approach. In C., P., ed.: *Computational Methods in Systems Biology*. Volume 2602 of Lecture Notes in Computer Science., Springer Verlag Heidelberg (2003) 3–20
48. Borland, S., Vangheluwe, H.: Transforming Statecharts to DEVS. In: *Summer Computer Simulation Conference*. (2003) 154–159
49. Danos, V., Laneve, C., eds.: *BioConcur - Workshop on Concurrent Models in Molecular Biology*, Electronic Notes in Theoretical Computer Science (2003)
50. Kuttler, C., Blossey, R., Niehren, J.: Gene Regulation in the Pi Calculus: Modelling Cooperativity at the Lambda Switch. In: *BioConcur 2004*, Elsevier (2004)
51. Regev, A., Shapiro, E.: Cells as computation. *Nature* **419** (2002) 343 www.wisdom.weizmann.ac.il/~aviv.
52. Lecca, P., Priami, C., Quaglia, P., Rossi, B., Laudanna, C., Constantin, G.: Language Modelling and Simulation of Autoreactive Lymphocytes Recruitment in Inflamed Brain Vessels. *SCS Simulation* (Submitted)

53. Van Gend, K., U., K.: STODE - Automatic Stochastic Simulation of Systems Described by [differential equations]. In Yi, T.M., Hucka, M., Morohasi, M., Kitano, H., eds.: Proceedings of the 2nd International Conference on Systems Biology, Omnipress, Madison, USA (2001) 326–333
54. Philipps, A., Cardelli, L.: A correct abstract machine for the stochastic pi-calculus. In: Proc. of BIO-CONCUR'04. Electronic Notes in Theoretical Computer Science, Elsevier (2004)
55. Regev, A., Panina, E., Silverman, W., Cardelli, L., Shapiro, E.: BioAmbients: An Abstraction for Biological Compartments. Theoretical Computer Science (2004)
56. Cardelli, L.: Brane Calculi. In: Proc. of BIO-CONCUR'03. Electronic Notes in Theoretical Computer Science, Elsevier (2003)
57. Mc Collum, J., Cox, C., Simpson, M., Peterson, G.: Accelerating Gene Regulatory Network Modeling Using Grid-Based Simulation. Simulation - Transactions of the SCS (2004)
58. Danos, V., Pradalier, S.: Projective brane calculus. In: Proceedings of the 2nd International Workshop on Computational Methods in Systems Biology. (2004)
59. Zeigler, B.: Discrete Event Abstraction: An Emerging Paradigm For modeling complex adaptive systems. In Booker, L., ed.: Perspectives on Adaptation in Natural and Artificial Systems - Essays in Honor of John Holland,. Oxford University Press (2004)
60. Nutaro, J., Zeigler, B., Jammalamadaka, R., Akerkar, S.: Speeding-Up the Simulation of Continuous Systems with Parallel DEVS:A Gas Shock Wave Example. In Darema, F., ed.: Dynamic Data Driven Applications Systems. Academic Publishers (2004)
61. Chen, M., Hofestädt, R., Freier, A.: A Workable Approach for Modeling and Simulation of Biochemical Processes with Hybrid Petri Net System. In: 1st International MTBio Workshop on Function and Regulation of Cellular Systems: Experiments and Models,, Dresden (2001)
62. Matsuno, H., Fujita, S., Doi, A., Nagasaki, M., Miyano, S.: Towards biopathway modeling and simulation. In VanDerAalst, W., Best, E., eds.: Applications and Theory of Petri Nets. Volume 2679 of Lecture Notes in Computer Science. (2003) 3–22
63. Cho, K.H., Johansson, K., Wolkenhauer, O.: A Hybrid Systems Framework for Cellular Processes. submitted for publication (2004)
64. : Matlab Simulink. <http://www.mathworks.com> (access date: 08.11.2004)
65. Liu, J., Lee, E.: A component-based approach to modeling and simulating mixed-signal and hybrid systems. ACM Transactions on Modeling and Computer Simulation **12** (2002) 343–368
66. Henzinger, T.: The theory of hybrid automata. In: Proceedings of the 11th Annual Symposium on Logic in Computer Science (LICS), IEEE Computer Society Press (1996) 278–292
67. Alur, R., Belta, C., Ivancic, F., Kumar, V., Rubin, H., Schug, J., Sokolsky, O., Webb, J.: Visual programming for modeling and simulation of biomolecular regulatory networks. In: International Conference on High Performance Computing. (2002)
68. Mishra, B., Policriti, A.: Systems Biology and Automata. In: 3rd Workshop on Computation of Biochemical Pathways and Genetic Networks, Heidelberg, Springer Verlag (2003)

69. Belta, C., Finin, P., Habets, L., Halasz, A., Irieliniksi, M., Kurnar, V., Rubin, H.: Understanding the bacterial stringent response using reachability analysis of hybrid systems. In: *Lecture Notes in Computer Science*. Volume 2993. Springer (2004)
70. Law, A., Kelton, W.: *Simulation, Modeling, and Analysis*. MCGraw Hill International Editions, New York (1991)
71. Rao, C.V., Wolf, D.M., Arkin, A.P.: Control, exploitation and tolerance of intracellular noise. *Nature* **420** (2002) 231–237
72. Fedoroff, N., Fontana, W.: Small Numbers of Big Molecules. *Science* **297** (2002) 1129–1131
73. Gibson, M.A., Bruck, J.: EfficientExact Stochastic Simulation of Chemical Systems with Many Species and Many Channels. *Journal of Physical Chemistry A* **104** (2000) 1876–1889
74. Gillespie, D.T.: A General Method for Numerically Simulating the Stochastic Time Evolution of Coupled Chemical Reactions. *The Journal of Physical Chemistry B* **22** (1976) 403–434
75. Gillespie, D.T.: Exact Stochastic Simulation of Coupled Chemical Reactions. *The Journal of Physical Chemistry B* **81** (1977) 2340–2361
76. Kuo, D., Keasling, J.D.: A Monte Carlo simulation of plasmid replication during the bacterial division cycle. *Biotechnology and Bioengineering* **52** (1996) 633–647
77. Kierzek, A.M.: STOCKS: STOChastic Kinetic Simulations of biochemical systems with Gillespie algorithm. *Bioinformatics* **18** (2002) 470–481
78. Cowan, R.: Stochastic models for DNA replication. In Shanbhag, D., Rao, C., eds.: *Stochastic Processes*. Handbook of Statistics. (2003)
79. Gillespie, D.T.: Approximate accelerated stochastic simulation of chemically reacting systems. *The Journal of Chemical Physics* **115** (2001) 1716–1733
80. Gillespie, D.T., Petzold, L.R.: Improved leap-size selection for accelerated stochastic simulation. *The Journal of Chemical Physics* **119** (2004) 8229–8234
81. Puchulka, J., Kierzek, A.M.: Bridging the Gap between Stochastic and Deterministic Regimes in the Kinetic Simulations of the Biochemical Reaction Networks. *Biophysical Journal* **86** (2004) 1357–1372
82. Kuipers, B.: *Qualitative Reasoning: Modeling and Simulation with Incomplete Knowledge*. MIT Press (1994)
83. Heidtke, K.R., Schulze-Kremer, S.: Design and implementation of a qualitative simulation model of λ phage infection. *Bioinformatics* **14** (1998) 81–91
84. Ideker, T., Lauffenburger, D.: Building with a scaffold: emerging strategies for high- to low-level cellular modeling. *Trends in Biotechnology* **21** (2003) 255–262
85. Thomas, R., Kaufman, M.: Multistationarity, the basis of cell differentiation and memory. I. structural conditions of multistationarity and other nontrivial behavior. *Chaos* **11** (2001) 170–179
86. Thomas, R., Kaufman, M.: Multistationarity, the basis of cell differentiation and memory. II. Logical analysis of regulatory networks in terms of feedback circuits. *Chaos* **11** (2001) 180–195
87. Tilly, C.: Micro, Macro, or Megrim? Paper for the Göttinger Gespräch zur Geschichtswissenschaft, Microhistory - Macrohistory: Complementary or Incommensurable ? (1997)
88. Knorr-Cetina, K., Cicourel, A., eds.: *Advances in Social Theory and Methodology - Towards an Integration of Micro and Macro Sociologies*. Routledge and Kegan Paul, Boston (1981)

89. Troitzsch, K.: Multilevel Simulation. In Troitzsch, K., Mueller, U., Gilbert, G., Doran, J., eds.: *Social Science Microsimulation*. Springer (1996) 107–120
90. Kokai, G., Toth, Z., Vanyi, R.: Modelling blood vessels of the eye with parametric L-systems using evolutionary algorithms. In Horn, W., Shahar, Y., Lindberg, G., Andreassen, S., Wyatt, J., eds.: *Artificial Intelligence in Medicine*. Volume 1620 of *Lecture Notes in Artificial Intelligence*. (1999) 433–442
91. Garcia-Olivares, A., Villarroel, M., Marijuan, P.C.: Enzymes as molecular automata: a stochastic model of self-oscillatory glycolytic cycles in cellular metabolism. *Biosystems* **56** (2000) 121–129
92. Wurthner, J., Mukhopadhyay, A., Piemann, C.: A cellular automaton model of cellular signal transduction. *Computers in Biology and Medicine* **30** (2000) 1–21
93. Alber, M., Kiskowski, M., Glazier, J.A., Jiang, Y.: On cellular automaton approaches to modeling biological cells. In Rosenthal, J., Gilliam, D.S., eds.: *Mathematical Systems Theory in Biology, Communications, Computation and Finance*. Volume 134 of *IMA Volumes in Mathematics and its Applications*. (2003) 1–39
94. Kniemeyer, O., Buck-Sorlin, G.H., Kurth, W.: Representation of genotype and phenotype in a coherent framework based on extended L-systems. In Banzhaf, W., Christaller, T., Dittrich, P., Kim, J.T., Ziegler, J., eds.: *Advances in Artificial Life*. Volume 2801 of *Lecture Notes in Artificial Intelligence*. (2003) 625–634
95. Swameye, I., Müller, T., Timmer, J., Sandra, O., Klingmüller, U.: Identification of nucleocytoplasmic cycling as a remote sensor in cellular signaling by data-based dynamic modeling. *PNAS* **100** (2003) 1028–1033
96. Uhrmacher, A.M., Swartout, W.: *Agent-Oriented Simulation*. In Obaidat, M., Papadimitriou, G., eds.: *Applied System Simulation*, Amsterdam, Kluwer Academic Press (2003)
97. Uhrmacher, A., Degenring, D.: From macro- to Multi-Level Models in Systems Biology. In Gauges, R., Kummer, U., Pahle, J., Rost, U., eds.: *Proc. of the 3rd Workshop on Computation of Biochemical Pathways and Genetic Networks*. (2003)
98. Kreft, J., Booth, G., Wimpenny, J.: BacSim a simulator for individual based modelling of bacterial colony growth. *Microbiology* **144** (1998) 3275–3287
99. Gregory, R.: *An Individual Based Model for Simulating Bacterial Evolution*. In: *Evolvability and Individuality Workshop*, University of Hertfordshire (2002)
100. Degenring, D., Röhl, M., Uhrmacher, A.M.: Discrete event simulation for a better understanding of metabolite channeling- A system-theoretic approach. In C., P., ed.: *Computational Methods in Systems Biology*. Volume 2602 of *Lecture Notes in Computer Science*, Springer Verlag Heidelberg (2003) 114–126
101. Rizzi, M., Baltés, T., Theobald, U., Reuss, M.: In Vivo Analysis of Metabolic Dynamics in *Saccharomyces cerevisiae* II. Mathematical Model. *Biotechnology and Bioengineering* **55** (1997) 592–608
102. Takahashi, K., Yugi, K., Hashimoto, K., Yamada, Y., Pickett, C., Tomita, M.: Computational challenges in cell simulation. *IEEE Intelligent Systems* **17** (2002) 64–71
103. Henson, M., Müller, D., Reuss, M.: Cell Population Modelling of Yeast Glycolytic Oscillations. *Biochemical Journal* **368** (2002) 433–446
104. Morton-Firth, C.J., Bray, D.: Predicting Temporal Fluctuations in an Intracellular Signalling Pathway. *Journal of Theoretical Biology* **192** (1998) 117–128
105. Anderson, K., Miles, E., Johnson, K.: Serine Modulates Substrate Channeling in Tryptophan Synthase. *The Journal of the Biological Chemistry* **266** (1991) 8020–8033

106. Anderson, K., Kim, A., Quillen, J., Sayers, E., Yand, X., Miles, E.: Kinetic Characterization of Channel Impaired Mutants of Tryptophan Synthase. *The Journal of Biological Chemistry* **270** (1995) 29936–29944
107. Uhrmacher, A.M., Tyschler, P., Tyschler, D.: Modeling Mobile Agents. *Future Generation Computer System* **17** (2000) 107–118
108. Elmquist, H., Mattson, S.: Modelica - The Next Generation Modeling Language - An International Design Effort. In: *First World Congress of System Simulation, Singapore* (1997)
109. Takahashi, K., Kaizu, K., Hu, B., Tomita, M.: A multi-algorithm, multi-timescale method for cell simulation. *Bioinformatics* **20** (2004) 538–546
110. : Biospi simulator. <http://www.wisdom.weizmann.ac.il/~biospi/> (access date: Okt. 2004)
111. Lynch, N., Segala, R., Vaandraager, F.: Hybrid I/O automata. Technical Report MITLCS-TR-827d, MIT Laboratory for Computer Science (2003)
112. : Anylogic - Simulation Software. <http://www.xjtek.com/anylogic/> (access date: May 2004)
113. Nagasaki, M., Doi, A., Matsuno, H., Miyano, S.: Genomic Object Net: A platform for modeling and simulating biopathways. *Applied Bioinformatics* (2003)
114. Biermann, S., Uhrmacher, A., Schumann, H.: Supporting Multi-Level Models in Systems Biology by Visual Methods. In: *Proceedings of European Multi-Simulation Conference*. (2004)
115. Fujimoto, R.: *Parallel and Distributed Simulation Systems*. John Wiley and Sons (2000)
116. Zeigler, B.: Statistical Simplification of Neural Nets. *Intl. J. of Machine Studies* **7** (1975) 371–393
117. Zeigler, B.: Simplification of Biochemical Systems. In Segel, L., ed.: *Mathematical Models in Molecular and Cellular Biology*, Cambridge University Press (1981)