CELL BIOLOGY SIMULATION USING DEVS COMBINED WITH SBML

By

Zhijie Wang

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STATEMENT BY AUTHOR

SIGNED:______________________________
Zhijie Wang

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

________________________________ ________________________________
Dr. Bernard P. Zeigler Date
Professor of
Electrical and Computer Engineering

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Dedicated to my father and my mother

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for their selfless love and affection

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ABSTRACT

Computational cell biology is a relatively new branch of computational sciences which sets computer simulation, as well as molecular biology, biochemistry and biophysics, at the center of its disciplines.

This thesis first inspects this new field of science, cell biology, especially on its computational aspects, and identifies some computational techniques in the design of simulation algorithms and software platforms. One important characteristic of the computational cell biology is that it is highly heterogeneous in terms of the modeling formalisms and computational methods. Different simulation techniques are used for different types of sub-systems in the cell, and these sub-systems are often represented by different timescales. We found that the scientific necessity to integrate these computational subcomponents to form the multi-formalism and composite models is becoming increasingly important.

Secondly, this thesis proposes a novel computational framework based on discrete event and differential equation combined with System Biology Markup Language (SBML) to simulate the cell biological processes, which realizes efficient multi-algorithm simulations. It is demonstrated that this framework can give a significant speed-up to a real biological simulation model of *E. coli* heat-shock response by Discrete Event System Specifications (DEVS) Ordinary Differential Equations (ODEs) solver combined with SBML. It is also shown that this framework can boost the simulation of the single-gene

oscillatory circuit model. Some results of the numerical analysis and discussions for the heat-shock and single-gene oscillatory circuit model are presented.

Lastly, this thesis describes the layered architecture, design and implementation issues of DEVS-SBML Platform, a newly developed software environment for modeling, simulation and analysis in computational cell biology, in which this computational framework will be implemented as a future work.

CHAPTER 1 INTRODUCTION

1.1 Introduction

The advent of molecular biology in the twentieth century has led to exponential growth in our knowledge about the inner workings of life. Dozens of completed genomes are now at hand, and equipped with an array of high throughput methods to characterize the way in which encoded genes and their products operate, we find ourselves asking exactly how to assemble the various pieces. The question is whether we can predict the behavior of a living cell given sufficient information about its molecular components. As with any network of interacting elements, the overall behavior becomes non-intuitive as soon as their number exceeds three. Computers have proven to be an invaluable tool in the analysis of these systems, and many biologists are turning to the keyboard. It is worth noting that the motivation of the biologist here is somewhat different from that of the biologist who turns to computing for bioinformatics (such as sequence analysis). Bioinformatics delivers additional information about the biopolymers being studied, and may provide clues as to their function. What is sought in the analysis of cellular systems is a reconstruction of experimental phenomena from the known properties of the individual molecules, and more importantly, the interactions between them. Such a model, if sufficiently detailed and accurate, serves as a reference, a guide for interpreting experimental results, and a powerful means of suggesting new hypotheses.

Modeling, simulation, and analysis are therefore perfectly positioned for integration into the experimental cycle of cell biology. In addition to demystifying non-intuitive phenomena, simulation allows experimentally unfeasible scenarios to be tested, and has the potential to seriously reduce experimental costs. Although "real" experiments will always be necessary to advance our understanding of biological processes, conducting "*in silico*" experiments using computer models can help to guide the wet lab process, effectively narrowing the experimental search space.

The process of building in *silico* models of cells contrasts with the traditional hypothesis-driven research process in biology. Modeling can be described as a holistic approach that opposes the reductionism so widespread in biology. Molecular and cellular biologists have been overwhelmingly successful in identifying, purifying, and characterizing molecules crucial to specific cellular functions. However, results from genome projects reveal that most organisms contain a surprisingly small number of genes, at least relative to the complexity of the phenotype. This provides a striking demonstration of the nontrivial nature of molecular interactions in the cell - the whole, quite simply, is much more than the mere sum of its parts.

1.2 Contributions

The main contribution of this thesis is the introduction of a new technique, Discrete Event System Specification (DEVS) framework combined with Systems Biology Markup Language (SBML), to simulate biological processes. On this framework, virtually any, discrete/continuous, stochastic/deterministic, simulation algorithms can be implemented in biological simulations. More efficient method in biological simulation, DEVS framework, is found.

Secondly, biological models can be shared between DEVS and SBML by DEVS-SBML Platform, a Simulation Platform for computational cell biology. This simulation software is under development in a fully object-oriented fashion based on the DEVS algorithm framework combined with SBML.

As the rationale of development of these new computational frameworks and software, this thesis also argues that computational cell biology has some major features those distinguish itself from conventional computational sciences such as computational physics and biochemical simulations.

1.3 Organization

Chapter 2 discusses a domain analysis of computational cell biology from the viewpoint of simulation algorithm design and software engineering, and we identify some ’desirable features’ of cellular simulation software. In Chapter 3, we reviewed the basic DEVS concept and DEVS formalism. Multi-algorithm framework, the combination of the DEVS and the Differential Equation System Specifications (DESS) formalism, was introduced in this Chapter. The implementation of the integrator and Instantaneous Functions to solve Differential Equations is given in DEVSJAVA Continuous package. Chapter 4 defines a new computational framework to simulate biological processes, called DEVS-SBML Platform. The architecture, design and some features of DEVS-SBML Platform is shown. In chapter 5, we use two demonstration models, an E. coli heat-shock model and a single-gene oscillatory circuit model, and find that the DEVS algorithm can successfully combine SBML yielding considerable performance improvements to the biological field. This chapter also has some discussions on DEVS framework combined with SBML. The DEVS-SBML Platform will continue to be implemented as a future work, a generic software suite for modeling, simulation and analysis of cellular systems. Chapter 6 will give some conclusions for this thesis and future works.

CHAPTER 2 CELL BIOLOGY BACKGROUND AND SIGNIFICANCE

This chapter investigates the cell as a target of simulation, and discusses computational challenges that it poses. Some simulation methods in the computational cell biology were reviewed. We identify required features of cell biology simulators. Multi-algorithm simulation was introduced in the computational cell biology. Existing software platforms and projects also were reviewed. The definition of SBML was shown.

2.1 Simulation of cellular processes

The cell is a big system in terms of the number and the diversity of physical phenomena that constitutes its internal dynamics. The small number of genes in most organisms implies that molecular phenomena in the cell are, to say the least, nontrivial. Collective knowledge of parts of the system itself does not directly lead to understanding of the cell as a whole. Necessity and importance of cell simulation as a research method arises here; putting the data into databases is not enough, only a more constructive approach with computer modeling and simulation can provide a way to understand the cell as a system.

In this section, we review such chemical and physical phenomena and discuss some possible computational approaches to simulate these systems.

2.1.1 Metabolism

Energy metabolism is the best characterized part of all cellular behavior, and is particularly well understood in human red blood cells. Given that an erythrocyte is devoid of nuclei and other related features, it serves as an ideal model system for studying metabolism in isolation — this cell is, essentially, “a bag of metabolism”. Biochemists have succeeded in collecting enough quantitative data to allow kinetic models of the entire cell to be constructed, and there is a long history of computer simulations dating back to the 1960s [1]. These metabolic models, and many others, typically comprise a set of ordinary differential equations (ODE) that describe the rates of enzymatic reactions. These can be solved by numerical integration, for which several well-established algorithms exist.

Modern metabolic pathway models typically consist of primary state variables for molecular species concentrations, one ODE for each enzyme reaction, and a stoichiometric matrix. The rate equations of most modern enzyme kinetics models are derived using the King-Altman method [2], which is a generalized version of the classical formulation of Michaelis and Menten, the Michaelis-Menten equations [3]. Additional algebraic equations are commonly employed as constraints on the system. Thus, most metabolism models are described as differential-algebraic equations (DAE). The origin of numerical methods for solving ODEs can be traced back to Euler’s work in the eighteenth century. Variations of the Runge-Kutta method are generally used for simulations. Implicit variations of the methods, are often used for ’stiff’ systems which involve a wide range of time constants. See the following section for discussions about stiffness of ODEs. Although there have been certain major advances in the last few decades, the essence of the numerical algorithms for solving initial value problems of ODE systems was described by Gear in 1971 [4]. Press *et al.* also give an introduction to the topic in *Numerical Recipes in C* [5]. The theoretical and practical bases of simulating metabolic pathways are therefore quite well grounded. However, the design and implementation of simulation software and model construction methods, which this thesis attempts to highlight, are still under active discussion.

Metabolism, of course, is not the only function of the cell, and we must not forget that cells have other important roles such as gene expression, signal transduction, motility, vesicular transport, cell division and differentiation. Although quantitative data on these processes are still relatively sparse compared to erythrocyte energy metabolism, certain systems have been modeled with considerable success. Examples include the simulation of gene expression in phage-λ [6] and the signal transduction pathway controlling bacterial chemotaxis [7].

2.1.2 Signal transduction

Signal transduction pathways constitute an example of systems with characteristics that prevent the simple application of ODE-based modeling. These pathways are ordinarily composed of much fewer numbers of reactant molecules than metabolic systems, and the underlying stochastic behavior of the molecular interactions becomes evident. Accordingly, there have been attempts to model signal transduction pathways with stochastic computation [8] [9] instead of deterministic methods (*e.g.* use of ODEs).

Recently, it has been revealed that two-dimensional stochastic coupled lattice model of receptor localization of *E. coli* chemotaxis signaling better explains the system's hyper sensitivity to stimuli that could not be reproduced by conventional compartmental simulations [10].

2.1.3 Gene expression

Gene expression systems, like signaling pathways, tend to be composed of a small number of molecular entities, which include transcription factors, polymerases, and genes. These low copy number molecules orchestrate gene expression in a highly stochastic fashion. For example, the initial phase of gene expression is remarkable because its stochastic behavior has binary consequences: binding of a rare transcription factor and a single gene in a cell can determine whether the gene is turned on or off. It seems that in many cases, gene expression systems might best be modeled with stochastic equations, although there are many other ways to model these phenomena, depending on the modeler's interests. Examples include ODE models (*e.g.* linear models and mass action models), S-System models [11], and binary and multi-reaction models [12].

Another characteristic of gene expression systems is the richness of interaction with other cellular processes. These systems can control metabolic flux by changing the concentrations of enzymes, and at the same time being regulated by signaling proteins. Chromosomal structure is dynamically regulated by DNA binding factors, which are in turn derived from other genes. When a whole cell is modeled, elements in the gene expression system sometimes need information about elements in other systems, in order to allow cross-system interactions. Integration of gene expression and other systems at the whole cell level might best be accommodated by object-oriented data structures, as previously described in Hashimoto *et al.* [13].

2.1.4 Biophysical phenomena

All of the above simulation examples treat the properties of protein binding and enzyme kinetics reactions. However, certain cellular processes such as cytoskeletal movement and cytoplasmic streaming need to be modeled at the biophysical level. Cytoplasmic streaming involves diffusion of relatively heavy proteins, whereas the movement of the cytoskeleton causes structural changes, including cell division and cell differentiation. Simulations of these phenomena have been carried out since the 1970s [14]. Studies have become more precise with time, concomitant with the increase in our depth of understanding at the molecular level [15].

2.1.5 Summary

A few types of cellular processes and typical computational approaches are shown in Table 2.1. The interested reader is referred to two recent reviews which address some of the issues raised thus far: Tyson *et al.* provide an excellent review of computational cell biology with an emphasis on cell-cycle control [16], while Phair and Misteli review the application of kinetic modeling methods to biophysical problems [17].

Table 2.1 some cellular processes and typical computational approaches

Process type	Dominant phenomena	Typical computation schemes
Metabolism	Enzymatic reaction	DAE, PL, FBA, CA
Signal transduction	Molecular binding	DAE, stochastic algorithms (StochSim, Gillespie), diffusion-reaction
Gene expression	Molecular binding, polymerization, degradation	OOM, PL, DAE, Boolean networks, stochastic algorithms
DNA replication	Molecular binding, polymerization	OOM, DAE
Cytoskeletal dynamics	Polymerization, mechanical forces	DAE (including mechanical models), particle dynamics, OOM
Cytoplasmic streaming	Streaming	CA (e.g. lattice Boltzman), PDE

Abbreviations: CA, Cellular Automata; DAE, differential-algebraic equations (rate equation-based systems); FBA, flux balance analysis; OOM, object-oriented modeling; PL, power-law, such as S-System and Generalized Mass Action.

2.2 Computational cell biology

2.2.1 Differences from conventional computational sciences

Software development for non-trivial cell simulation projects is a notably expensive process. For research projects in the traditional computational sciences, where brute force computation remains operative, it is reasonable to develop new software for each project, sometimes in a disposable fashion. Most of the traditional computational science fields like computational physics are characterized by numerous uniform components and a limited number of simple principles. Cell simulation, in contrast, involves numerous and various components with different properties, interacting in diverse, complicated manners. Typical characteristics of several simulation targets are summarized in Table 2.2. The design and implementation of simulation software inevitably reflects the complexity of the problem.

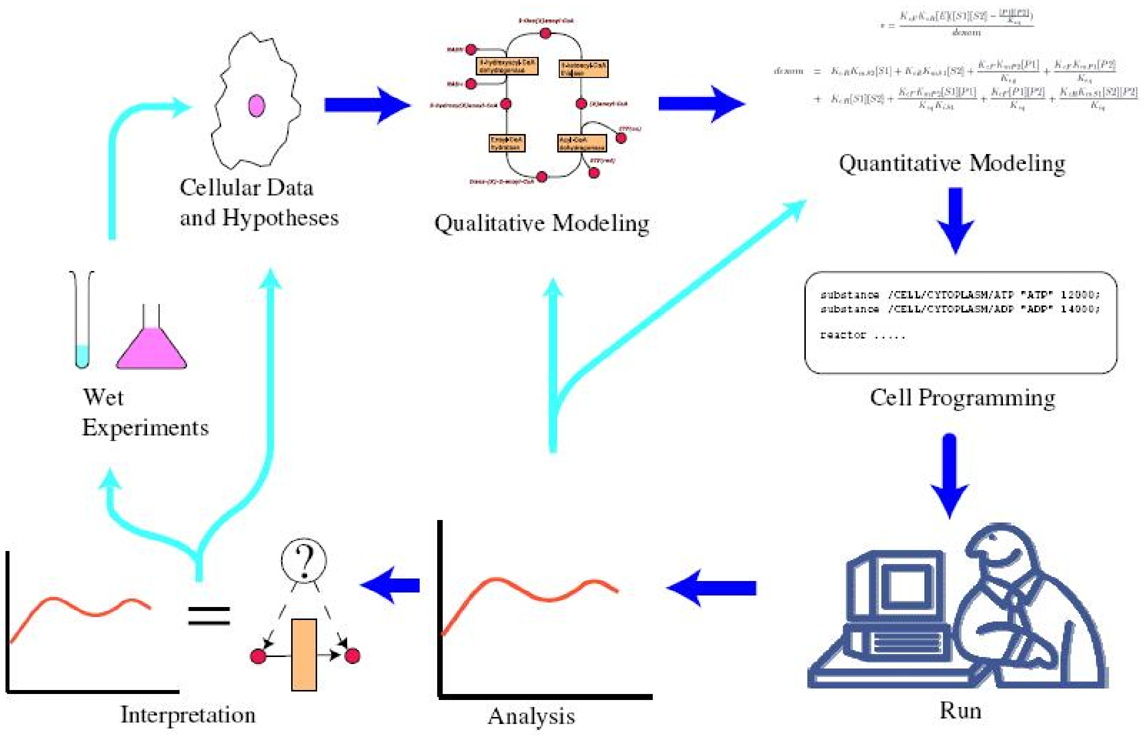
Table 2.2 Rough comparison of typical numbers characterizing various simulation targets

Target	Compartments	Components	Component types	Interaction modes
Prokaryotes (*E. coli*)	$\sim 10^1$	$\sim 10^{13-14}$ molecules $\sim 10^{3-4}$ species	$\sim 10^1$(1)	$\sim 10^{1-3}$(2)
Eukaryotes (*H. sapiens*)	$\sim 10^{3-4}$	$\sim 10^{17-18}$ molecules $\sim 10^{4-5}$ species	$\sim 10^1$(1)	$\sim 10^{1-4}$(2)
LSI (Electronic circuit)	usually 1	$\sim 10^{6-7}$	a few	1
CFD (Fluid dynamics)	usually 10^{0-1}	$\sim 10^{5-6}$	1	1
MD (Molecular dynamics)	1	$\sim 10^{2-6}$	a few	a few

Some typical numbers, which determine computational hardware and software requirements, are compared among several simulation targets. Large numbers of compartments, component types and interaction modes characterize cell simulation. Notes: (1) Component types which require different data structures or object classes are counted. For example, a ’membrane’ object needs a different object class from protein molecules. Different molecular species, however, are not counted. (2) This number depends on whether or not different enzyme kinetics equations, which are roughly proportional to the number of enzyme encoding genes, are counted as different interaction modes. Interaction modes other than enzymatic reactions include molecular bindings (complex formations), molecular collisions, DNA replication, cytoplasmic streaming, cytokinesis, and vesicular trafficking.

2.2.2 Computational cell biology research cycle

We envision a research cycle of cell biology that incorporates bio-simulation technology (Figure 2.1). Every step of the cycle completed outside of the wet lab depends upon sound methods in software engineering. Consider the storage, processing, and utilization of massive amounts of biological knowledge: only through integration of an intelligent modeling environment with sophisticated data and knowledge bases can the challenge of modeling a very large and complex system be accommodated. Although this thesis mainly considers the first half of the cycle (from ’Qualitative Modeling’ to ’Run’), a technological stagnancy in any one of the steps may form a bottleneck, and thus threaten the evolution of computational cell biology.



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substance /CELL/CYTOPLASM/ATP "ATP" 12000;
substance /CELL/CYTOPLASM/ADP "ADP" 14000;

reactor .....
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Figure 2.1 Research cycle of computational cell biology

The wet lab process is extended to include simulation software for a computational cell biology research project. Qualitative models (*e.g.* pathway maps) are built from *in vivo* and *in vitro* data and hypotheses, or a reference model (Qualitative Modeling). Then, quantitative characterization of cellular properties facilitates the transition to a mathematical system model (Quantitative Modeling). The numerical and discrete properties of the quantitative model are translated into a modeling language (Cell Programming), and the systemic behavior is predicted (Run). Results are then analyzed to suggest new hypotheses (Analysis and Interpretation). Any acquired hypothesis is subsequently tested by wet experiments, and the cycle begins a new.

2.3 Simulation methods in computational cell biology

As we have seen, simulation of the cell requires heterogeneous approaches according to the levels of abstraction, scales, and types of information available to construct simulation models. Here we briefly review some commonly used numerical simulation techniques.

2.3.1 Ordinary differential equation solvers

Ordinary differential equations (ODEs) are one of the most popular ways of describing continuous dynamical systems. A distinct strength of this formalism is that, with its well-established theory in numerical treatments and availability of high-performance generic solvers described in the following, it can represent virtually any continuous and deterministic dynamics elegantly.

In computational cell biology, the most popular use of ODE formalism is the macroscopic representation of chemically reacting systems by a means of kinetic rate equations. Elementary and some simple reactions are represented in a form of mass-actions, for example,

$$X_1 + X_2 \rightarrow X_3, \tag{2.1}$$

and it can be formulated by using the following differential equation

$$-\frac{d}{dt}[X_1] = -\frac{d}{dt}[X_2] = \frac{d}{dt}[X_3] = k \cdot [X_1][X_2], \tag{2.2}$$

Where X_n is the n-th chemical species ($[\cdot]$ denotes concentration), and k is the rate constant. Complex enzymatic reactions are often modeled by using Michaelis-Menten and King-Altman types of rate equations. The Michaelis-Menten equation corresponding to the following simplest enzymatic reaction mechanism

$$E + S \leftrightarrows ES \rightarrow E + P, \tag{2.3}$$

is

$$-\frac{d}{dt}S = \frac{d}{dt}P = \frac{K_{cat} \cdot E_T \cdot [S]}{K_m + [S]}, E_T = E + ES, \tag{2.4}$$

Where S, P, and E are the substrate, product, and enzyme, respectively, and K_{cat} is the catalytic constant (the turnover number of the enzyme), and K_m is the Michaelis constant.

Unlike some of other specialized formalisms introduced in the following sections, most differential equation solvers are generic, and can handle a variety of linear and nonlinear equations employed in cell biology. Some examples of representations of those phenomena other than rate equations include dynamic changes in environmental factors such as thermodynamic parameters temperature, pH, and volume

Initial value problems of ODEs

A system of ODE has a general form like this:

$$\frac{d}{dt}X = f(t,x), X(t_0) = x_0 \tag{2.5}$$

Where x is a vector of dependent variables, f is a vector of derivative functions, and t is an independent variable. In time-driven simulations, the independent variable t is usually time. The system is *autonomous* if the system does not explicitly depend on the independent variable t;

$$\frac{d}{dt}X = f(x) \tag{2.6}$$

Time simulation of ODE systems is equivalently reformulated as solving (2.5) for *X*(*t*), where $t \in \mathrm{R}^{+,\,0}$.

Taylor expansion of *X* in (2.5) at time t_0 gives

$$X(t) = X(t_0) + \sum_{i \in \mathrm{N}} \frac{\Delta t^i}{i!} \cdot \frac{d^i}{dt^i} X(t_0), \Delta t = t - t_0 \tag{2.7}$$

or,

$$X(t) = X(t_0) + \sum_{i \in \mathrm{N}} \frac{\Delta t^i}{i!} \cdot \frac{d^{(i-1)}}{dt^{(i-1)}} f(t_0), \Delta t = t - t_0 \tag{2.8}$$

If analytical differentiation of the system *f* to arbitrary high order can be derived, this Taylor expansion immediately gives the solution, or the simulation trajectory, *X*(*t*). Practically, however, ODE representations of biological problems often make use of nonlinear equations, and it is very hard to construct a general method of solving these equations analytically. Thus use of iterative numerical methods is the norm. This class of the problem is called the *initial value problem* (IVP) of ODEs.

Numerical solution of ODEs has a considerable history, and the oldest and simplest method was given by Euler in 1768 [63]. From the definition of derivatives,

$$\frac{d}{dt}X = \lim_{\Delta t \to 0} \frac{X_{n+1} - X_n}{t_{n+1} - t_n}, \tag{2.9}$$

Where

$$\Delta t = t_{n+1} - t_n, \tag{2.10}$$

Now for sufficiently small Δt we can assume that this formula gives an approximation of the derivatives at the time point $t_{n,}$

$$\frac{dX}{dt}\Big|_{t_n} (= f_n = f(t_n, X_n)) \cong \frac{X_{n+1} - X_n}{t_{n+1} - t_n} \tag{2.11}$$

Comparing this with (2.5),

$$X_{n+1} = X_n + \Delta t \cdot f\,(t_n, X_n) \tag{2.12}$$

We now get the *explicit Euler method*. It is called explicit because no unknown appears in the right hand side (RHS) of the equation (2.12).

Consistency and Convergence

Two requisite properties of a numerical method to be useful are *consistency* and *convergence*.

A numerical method is *consistent* when the local truncation error,

$$e \equiv |X_{<tn>}\,(t_{n+1}) - X_{n+1}|, \tag{2.13}$$

diminishes to zero as the step size decreases:

$$\lim_{\Delta t \to 0} e = 0, \tag{2.14}$$

where e is a vector of the local truncation errors of each variable of the system $x_i \in$ x. Here x < t_n > (t_n+1) is the exact solution with the initial condition x(t_n) at time t_n. Proof of consistency of the explicit Euler method is trivial. Using (2.10), (2.11), and assuming x$_n$ = x (x_{t_n}), the local truncation error of the method is obtained from (2.12) as

$$\begin{aligned} e^{euler} &= | X(t_{n+1}) - X_{n+1}^{euler} | \\ &= | X(t_n) + \sum_{i \in \mathrm{N}} \frac{\Delta t^i}{i!} \cdot \frac{d^{(i-1)}}{dt^{(i-1)}} f(t_n) - X(t_n) - \Delta f(t_n) | \qquad (2.15) \\ &= | \sum_{\{i: i \in N \wedge i \geq 2\}} \frac{\Delta t^i}{i!} \cdot \frac{d^{(i-1)}}{dt^{(i-1)}} f(t_n) | \qquad (2.16) \end{aligned}$$

It can be verified that $e^{euler} \rightarrow 0$ as $\Delta t \rightarrow 0$, hence the explicit Euler method is consistent. Third and higher components are ignorable when Δt is sufficiently small, thus:

$$e^{euler} \cong \Delta t^2 \left| \frac{1}{2} \cdot \frac{d}{dt} f(t_n) \right| \tag{2.17}$$

Consequently, it can be seen the error of the method has an order of Δt^2. Thus, a more formal formulation of the method comes with the local error term of $O(\Delta t^2)$:

$$X(t_{n+1}) = X_n + \Delta t \cdot f(t_n, X_n) + O(\Delta t^2) \tag{2.18}$$

In simulation, the *global truncation error*, which indicates accumulation of the local error after certain period of time, is of practical importance. The global truncation error of a method is given by

$$\mathrm{E} \equiv |X(t_i) - X_i| \tag{2.19}$$

For simplicity, assuming the step size Δt is constant, the simulation requires $(\Delta t)^{-1}$ iterations for a unit of time. Thus, the accumulation of the error can be denoted as

$$\mathrm{E} = (\Delta t)^{-1} \cdot \mathrm{e} \tag{2.20}$$

It can be read that generally the global truncation error is of order p with respect to the step size Δt, when the local truncation error is $O(\Delta t^{p+1})$. If a method has the global truncation error of $O(\Delta t^p)$, then it is said to be consistent with an order of accuracy p. The explicit Euler method therefore has a consistency of the first order.

One of the most important design goals of numerical methods is to get a good *convergence* with as small as possible computation cost. A method is said to be convergent if

$$\forall i : \lim_{\Delta t \to 0} | X(t_i) - X_i | = 0 \tag{2.21}$$

Non-convergent methods are of no use because qualities of outcomes of simulations are not assured.

Higher order methods

Computation cost of numerical methods is proportional to the inverse of the step size, $1/\Delta t$. One way of increasing the step size without a directly proportional increase in the error is use of methods of higher order consistency. Many algorithms of the second, higher, and variable consistency order have been proposed and being used, and most of them can be classified into two categories: single- and multiple-step methods. Multi-step methods make use of the information calculated in some past steps to conduct the current simulation step. Single-step methods are 'closed' in this sense; these methods utilizes only the current state of the system. Although historically the multi-step methods once had been a standard, and many popular software packages including LSODE and DASSL are based on this class of methods, recent advancements in single-step methods, especially variants of the Runge-Kutta method [63], is changing the picture. When the applications in computational cell biology simulation is under consideration, single-step methods have some favorable features over the multiple-step methods; it is (1) easier to implement the real-time user interaction efficiently, and (2) better suited in uses in conjunction with other algorithms in multi-formalism simulations (see the next section). Also, it often results in simpler implementation, and unlike multi-step methods, no special procedure is necessary in simulation start up. For those reasons, here mainly discusses about the Runge-Kutta methods.

The general form of an *s*-stage, single-step Runge-Kutta method is:

$$\begin{aligned} k_j &= f(t_n + c_j \Delta t, y_n + \Delta t \cdot \sum_{i=1}^{s} a_{j,i} k_i), j = 1 \ldots s \\ y_{n+1} &= y_n + \Delta t \cdot \sum_{j=1}^{s} b_j k_j \end{aligned} \qquad (2.22)$$

Where *a*, *b*, and *c* are called Runge-Kutta coefficients, or collectively Butcher array. Setting $s = 2$, a second-order explicit Runge-Kutta method can be derived in this way:

$$X_{n+1} = X_n + \Delta t \cdot (b_1 k_1 + b_2 k_2), \qquad (2.23)$$

$$k_1 = f(t_n, X_n),\ k_2 = f(t_n + c_2 \Delta t, X_n + \Delta t\, k_1\, a_{2,1}) \qquad (2.24)$$

where $a_{2,1}$, b_1, b_2, and c_2 are the Runge-Kutta coefficients to be decided. Now Taylor series expansion of k_2 to the first order gives

$$k_2 = f_n + c_2 \Delta t \frac{\partial f}{\partial t}|_{t_n} + \Delta t k_1 a_{2,1} \frac{\partial f}{\partial t}|_{t_n} \qquad (2.25)$$

Putting (2.25) into (2.23),

$$X_{n+1} = X_n + (b_1 + b_2)\Delta t f_n + \Delta t^2 (b_2 c_2 \frac{\partial f}{\partial t}|_{t_n} + b_2 a_{2,1} f_n \frac{\partial f}{\partial t}|_{t_n}) \qquad (2.26)$$

Now we want to compare this with the Taylor series (2.8), which, curtailing the third and higher order terms, becomes:

$$X_{n+1} = X_n + \Delta t f + \frac{\Delta t^2}{2} \frac{d^2}{dt^2} f \qquad (2.27)$$

$$= X_n + \Delta t f + \frac{\Delta t^2}{2} (\frac{\partial f}{\partial t} + f \frac{\partial f}{\partial x}) \qquad (2.28)$$

Then we now have three equations for the four coefficients;

$$b_1 + b_2 = 1,\ b_2 c_2 = ½,\ b_2 a_{2,1} = ½ \qquad (2.29)$$

These relations are under determined, and there can be infinite number of second-order explicit Runge-Kutta methods. For example, setting $b_1 = 0$ gives the *midpoint method*

$$X_{n+1} = X_n + \Delta t \cdot f(t_n + \frac{\Delta t}{2}, X_n + \Delta t \frac{f(t_n, X_n)}{2}) \tag{2.30}$$

In this way, higher order methods of arbitrary high order can be derived from comparison between (2.22) and the Taylor series. Use of higher order methods is preferable. As it can be understood from the equation (2.8) of the Taylor series, the scale of error term decreases rapidly with regard to the order of the method, and we can take exponentially large step sizes with the same level of truncation error. This in turn means that we can complete the simulation with less number of steps, and therefore less accumulation of round-off error.

It is known, however, the fourth order is a kind of optimum and most frequently used. Up to the fourth order it just requires the same number of stages of right-hand side evaluations as the consistency order, while fifth and higher order methods involve more stages than the order of the method. For example, at minimum 6 stages are necessary for the fifth order method, $s = 7$ for sixth order, $s = 9$ for seventh, and $s = 11$ for eighth.

Error control

A commonly used error control scheme of numerical methods is based on the local truncation error as follows:

$$\forall i : |e_i| \le safety \cdot (\sigma_{abs} + \sigma_{rel} \cdot (a\,|x_i| + b\Delta t\,|f_i|)), \tag{2.31}$$

Where $e_i \in$ e is the error of this step of the variable x_i, *safety* is a safety factor (usually ~ 110%), σ_{abs} and σ_{rel} are absolute and relative error tolerances, *a* and *b* are scaling factors for the value and derivative of the variable, respectively. In each step, numerical methods control the error by means of the step size and other parameters such as the order of the calculation. If the error of at least one variable violates the criteria (2.31), the solver rejects the step and redoes the calculation with a shrunken step size. Conversely, if the error is sufficiently smaller (say, ~ 50%) than the right hand side of (2.31), the step size can be elongated to reduce the computation cost. There can be many strategies of deciding step sizes. Two most frequently used methods are step halving/doubling and variations of the following basic equation

$$\Delta t_{new} = \Delta t \left| \frac{Tolerance \cdot x_n}{e_n} \right|^{1/s}, \tag{2.32}$$

where *Tolerance* is the right hand side of (2.31), and *n* is the index of the variable which gave the maximum error in the current step. It must be noted that generally it is impossible to obtain the exact values of the local truncation errors, and an integrator must somehow numerically estimate the e_n.

A frequently used strategy to estimate the local error is to have a pair of Runge-Kutta calculations of orders *p* and *p* + 1. The difference between solutions from these calculations gives a good approximation of the local error, because as the Taylor series expansion implies, the value of error term diminishes rapidly to the order. A neat trick to obtain this estimation of the local truncation error efficiently is the *embedded Runge-Kutta* method, devised by Fehlberg [18]. The underlying idea is to embed a Runge-Kutta calculation of the order *p* into that of the order *p* + 1. Table 2.3 is the Butcher array of a method called Fehlberg 2(3), which specifies Runge-Kutta coefficients. Calculating the equation (2.22) using the coefficient in the b_i and b^*_i columns give the second and the third order solutions, respectively, and it requires only three RHS evaluations.

Table 2.3 Butcher array for Runge-Kutta Fehlberg 2(3)

	0			a_{ij}
c_j	1	1		
	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{4}$	
	b_i	$\frac{1}{2}$	$\frac{1}{2}$	0
	b^*_i	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{2}{3}$

Stability and stiffness

Some differential systems are *stiff*. Although stiffness is not defined in a mathematically rigid way, here we give it a casual definition as follows: when the system has at least two very different time scales, *and* the trajectory is dominated by the slow movement, then it is stiff. To put this in a bit more formally, if the fast mode of the system has a stable manifold, and the state point of the system is captured by it, the system is stiff. Thus a system can become stiff and non-stiff according to where the state point is. For example, when the system is making a transition from one state point to a distant stable manifold, it is non-stiff, while as long as the trajectory is on the manifold, it is said to be stiff.

Stiffness can be understood in terms of the *Jacobian* matrix which is

$$J = \frac{\partial f}{\partial x} \tag{2.33}$$

Stiffness is sometimes defined as

$$\left| \frac{\partial f}{\partial x} \right| \Delta t \cdot C >> 1 \tag{2.34}$$

where C is a positive constant which represent a typical number of simulation steps (say, 10^6 or 10^{12}).

When the system is stiff, all the explicit methods explained so far experience hardship. Consider the exact solution at time t_n, $x(t_n)$, on the slow manifold. All the explicit methods primarily uses values of the derivative functions at the current state point of the numerical solution, x_n, to estimate the state of the system after the some amount of time Δt. Any numerical computation involves some amount of error ε, and it puts the numerical solution slightly off the slow manifold, thus, $x_n = x(t_n) + \varepsilon$. When the Jacobian is very large, this error ε magnifies the error in the next numerical solution point x_{n+1}, and the assumption behind (2.20), that is, the global error is a simple accumulation of the local error, no longer holds. Although the convergence of the computation (2.21) itself is not necessarily affected, the effectiveness of the error control scheme in (2.31) is destroyed. Because slow manifolds are often stable, the fast flows toward the center of that manifold appear in values of Jacobian around there, and in this case the trajectories disastrously show diverging oscillations. Even if the solver managed to detect the error in the step, it results in frequent step rejections, and forces the solver to take extremely small step sizes to converge.

A measure of tolerance against stiffness is *stability* of numerical methods. Consider a scalar system

$$\frac{dx}{dt} = f(x) \tag{2.35}$$

If the system is linear,

$$\frac{dx}{dt} = \lambda x \tag{2.36}$$

the stability of a method is defined by the stability function $R(\cdot)$, which is defined as

$$x_{n+1} = R(\Delta t \cdot \lambda) x_n \tag{2.37}$$

In the case of the explicit Euler method it is,

$$x_{n+1} = (1 + \Delta t \cdot \lambda) x_n \tag{2.38}$$

Setting $\Delta t \cdot \lambda = z$,

$$x_{n+1} = (1 + z) x_n \tag{2.39}$$

Thus the stability function of the method is

$$R(z) = 1 + z \tag{2.40}$$

Now the stability region of the method is given by

$$|R(z)| = |1 + z| \leq 1 \tag{2.41}$$

and it is shown that the explicit Euler method is stable only in a very narrow range $-2 \leq \Delta t \cdot \lambda \leq 0$. More generally, when it is a linear vector system, the constant λ is an eigen value of the constant coefficient matrix A in

$$\frac{dX}{dt} = A \cdot X \tag{2.42}$$

and thus a complex number.

Use of implicit methods overcomes this stability problem. The implicit Euler method, in a scalar form, has the following form:

$$x_{n+1} = x_n + f(t, x_{n+1}) \tag{2.43}$$

Putting the linear scalar system (2.36) into this and rearranging to the form of the stability function (2.37) gives the stability function of this method:

$$x_{n+1} = (\frac{1}{1-z})x_n \tag{2.44}$$

Therefore this method is stable in the region $|1 - z| \leq 1$, or, it is stable in an open region except for the domain $0 < \Delta t \cdot \lambda < 2$ in the case of the scalar system.

If the stability region of a method includes the whole left-half of the complex plane of z, which is the case of the implicit Euler method, it is called absolutely stable, or *A-stable*. That is, when the real part of λ is zero or negative, the method is guaranteed to be stable. Similarly, if the same condition is satisfied for general non-linear systems, it is said to be *B-stable*. The order of the global error of the method for the general non-linear systems is called *B-convergence*.

The exact solution of the linear problem (2.36) at the next time point t_{n+1} is easily derived as $e^{\lambda \Delta t}\, x_n$. Now recall the definition of the local error (2.13) again, and using (2.37):

$$e_{n+1} \equiv |(e^z - R(z))x_n| \tag{2.45}$$

A method is called *stiffly accurate* if

$$\lim_{|z| \to -\infty} |(e^z - R(z))x_n| = 0 \tag{2.46}$$

Now check if the implicit Euler method is stiffly accurate:

$$\lim_{|z|\to-\infty} | e^{z} - \frac{1}{1-z} | = 0 \tag{2.47}$$

If a method is both A-stable and stiffly accurate, it is said to be *L-stable* or stiffly A-stable or strongly A-stable. The point here is that no matter how large the damping force of the system is (even in the case it is infinite), the numerical solution does not, at least, diverge. This property is important in very stiff problems and some DAE systems. It is desirable that all numerical methods used are L-stable, but only a few A-stable methods are known to be L-stable.

Implicit methods

Implicit Euler method imposes solution of non-linear equations to conduct a step of computation. Newton's method [19] is most popularly used for this purpose. A Newton iteration to get x_{n+1} of (2.43) using the first order Taylor series expansion is:

$$X_{n+1}^{m} = X_n + \Delta t f(t_{n+1}, X_{n+1}^{m-1}) + \Delta t \frac{\partial f}{\partial X}|_{(t_{n+1}, X_{n+1}^{m-1})} (X_{n+1}^{m} - X_{n+1}^{m-1}) \tag{2.48}$$

or in a programmable form,

$$X_{n+1}^{m} = [X_n + \Delta t f(t_{n+1}, X_{n+1}^{m-1}) - \Delta t \frac{\partial f}{\partial X}|_{(t_{n+1}, X_{n+1}^{m-1})} X_{n+1}^{m-1}] \cdot (I - \Delta t \frac{\partial f}{\partial X}|_{(t_{n+1}, X_{n+1}^{m-1})})^{-1} \tag{2.49}$$

where *m* is the counter of the Newton iteration, and ***I*** is an identity matrix. The iteration is terminated when the difference $|\mathrm{x}^{m}{}_{n+1} - \mathrm{x}^{m-1}{}_{\mathrm{n+1}}|$ goes below a pre-defined threshold. In the same way, it is possible to derive implicit variations of higher order methods.

Despite their good stability of implicit methods that is necessary to solve stiff systems efficiently, a drawback is computation cost. In addition to that of its explicit counterpart, it requires m iterations of (2.49) involving a calculation of the Jacobian matrix and a matrix inversion. Generally, in non-linear computational cell biology problems, Jacobian cannot be obtained analytically, and numerical differentiation is necessary. Although the precision of this calculation of Jacobian does not affect the accuracy of the simulation itself as long as the Newton iteration converges, the cost of this computation is not negligible. The computation cost of a matrix inversion is in the order of $O(N^3)$, where N is the size of the matrix. N becomes proportionally bigger when higher order methods such as implicit Runge-Kutta are used.

Therefore, a good ODE simulator is supposed to be able to adaptively switch between explicit and implicit methods automatically detecting stiffness of the system. The best combination that this thesis suggests is a pair of the fourth order Runge-Kutta with adaptive step-sizing such as Dormand-Prince [20] or Runge-Kutta Fehlberg [18] method and Radau IIA [21] methods. Radau IIA is the best implicit Runge-Kutta equation ever known, and is L-stable, and B-convergent of the order s for the consistency of $2s - 1$. Three-stage ($s = 3$) version of Radau IIA with the fifth order consistency, sometimes called Radau 5, is often used.

2.3.2 Special types of differential system solvers

In cellular and biochemical simulations, specialized differential equation solvers are sometimes used. For example, power-law canonical differential equations are often used to model various types of cellular phenomena such as gene expression and metabolic systems. Two of these formalisms are S-System:

$$\frac{d}{dt}x_i = \alpha_i \prod_{j=1}^{n} x_i^{g_{i,j}} - \beta_i \prod_{j=1}^{n} x_i^{h_{i,j}}, i = 1,..., N, \tag{2.50}$$

where N is the size of the system, and α_i, β_i, $g_{i,j}$, $h_{i,j}$ are S-system coefficients, and Generalized Mass Action (GMA):

$$\frac{d}{dt}x_i = \gamma_i \prod_{j=1}^{n} x_i^{f_{i,j}}, i = 1,..., N, \tag{2.51}$$

where γ_i and $f_{i,j}$ are GMA coefficients.

A distinct feature of this kind of formalisms is that it does not distinguish the structure of the system from its dynamics. The whole properties of a system can be described by a single S-System or GMA matrix. When used in simulation, it provides good means of estimating the network structure of the system as well as kinetic orders. In other words, by using S-System and GMA formalisms, the difficult problem of network structure determination can be converted to a matter of numerical parameter estimation, which is compatible with well established technique of non-linear optimization with numerous powerful algorithms such as Genetic Algorithms and the modified Powell method. A special algorithm called ESSYNS method can be used to solve these power-law systems efficiently [22].

2.3.3 Stochastic algorithms for chemical systems

All numerical treatments explained above are continuous and deterministic, which means that those descriptions of chemical processes are macroscopic and approximate. Chemical systems are, at the bottom, composed of discrete molecules, and the assumption behind differential formalisms that state variables change continuously and their trajectories are differentiable does not hold, and thus, the differential descriptions fail to correctly reproduce the stochastic fluctuation in the number of molecules that becomes evident when copy numbers of involving molecular species of reactions are small.

Some variations of stochastic simulation algorithms for chemically reacting systems in some exact consequences of the chemical master equation are introduced here. Also stochastic simulation methods can be approximate using many possible ways. One of the best known procedures is given in Gillespie and Petzold [23].

2.3.3.1 Chemical master equation

Consider a finite and fixed volume **Ω** in which M reaction channels connect N molecular species. Temperature and other physical parameters that affect the reaction rate are all constant. In meso-scopic representation of the system, we do not track motion of each molecule. Then the state of the system is a vector of random variables $X(t) \in N^N$. It also assumes that occurrences of non-reactive collisions are so frequent that (1) it stirs the system between any two reactive collisions, and (2) each occurrence of reaction is a Markov process. Strictly speaking, in cell biology, most systems are in liquid phase and, unlike gas phase, once two solute molecules encounter, it is highly probable that these molecules experience numerous successive collisions because of the existence of solvent, making reactions non-Markovian. However, here we neglect this in the following discussions because experiences so far have shown that we can construct precise simulation methods without taking this non-Markovian argument into account.

Now the *propensity function*

$$a_j(X)dt \tag{2.52}$$

is defined as the probability that the *j*-th reaction will occur in **Ω** in an infinitesimal time interval [*t, t* + *dt*), given $X = X(t)$. The state change vector

$$\nu_j, \tag{2.53}$$

whose each element $\nu_{j,i}$ specifies the number of molecules of the *i*-th species changed by an occurrence of the *j*-th reaction.

If the reaction is elementary, the propensity function has the following form:

$$a_j(X) = c_j \eta_j, \tag{2.54}$$

where $c_j dt$ gives the probability that a pair of reactant molecules will collide and react in a unit time interval, and η_j is the number of distinct combinations of such reactant molecules in the state *X*. If the reaction has the form of (1) $X_1 \rightarrow X_2 \cdots$, $\eta_j = X_1$, if it is (2) $X_1 + X_2 \rightarrow X_3 \cdots$, then $\eta_j = X_1 X_2$, and if (3) $2X_1 \rightarrow \cdots$, then $\eta_j = max(X_1(X_1 - 1)/2, 0)$. The macroscopic rate constant k_j of the reaction is related with c_j in: (1) $k_j = c_j$, (2) $k_j/\Omega = c_j$, (3) $2k_j/\Omega = c_j$.

Given the propensity function and the state change vector, an exact and complete description of the chemically reacting system evolving from the initial condition X_0 at time t_0 can be derived:

$$\frac{\partial}{\partial t} P(X,t \mid X_0,t_0) = \sum_k^M [a_k(X - v_k)P(X - v_k \mid X_0,t_0) - a_k(X)P(X \mid X_0,t_0)] \tag{2.55}$$

This is called the *chemical master equation* (CME). This formalism was initially proposed as a simple stochastization of macroscopic rate equations [24], but has recently given a rigid micro-physical ground [25].

By definition of ***X***, (that is $\in N^N$), CME (2.55) is a system of a huge number of differential equations, and cannot be solved in any way (analytical or numerical) unless it represents a very simple system (for example, a single ion channel whose state is either open or close).

2.3.3.2 Exact stochastic simulation algorithms

Although the CME describes everything about a chemical system, it cannot be used in simulations directly. What we need is a method of trajectory realization based on the CME, which does not require evaluation of the whole state space defined in the system. One way to reduce the computation cost to a point where we can handle with our digital computers is a kind of lazy evaluation, which is, at each simulation step, calculating the values of propensity functions at neighboring state points to the current state point.

Gillespie proposed the *next reaction density function* [26] [25],

$$p(\tau, j \mid X, t) = a_j(X) \exp(-\tau \sum_{k}^{M} a_k(X)) \tag{2.56}$$

which defines the probability that the next reaction in the system occurs in the infinitesimal time interval $[t + \tau, t + \tau + d\tau)$, and the reaction is the *j*-th reaction.

With this next reaction density function, a step of simulation is defined as a procedure of generating a pair of numbers (τ, μ), that indicates the step size and the state transition difference function $X(t + \tau) = X(t) + \nu_j$. There can be some possible ways of generating this real-integer pair of random numbers, and one of the straightforward ways is called the Direct method. The joint density function (2.56) can be rewritten as a composition of two simple functions for τ and j, respectively.

$$p(\tau, \mu \mid X, t) = p_1(\tau \mid X, t) \cdot p_2(\mu \mid X, t) \tag{2.57}$$

where $p_1(\tau \mid X, t)$ is the probability that the first firing of any of the reaction channels occurs at time $t + \tau$, and $p_1(\mu \mid X, t)$ is the probability that μ is the reaction that fired. These two functions are defined as:

$$P(\tau \mid X, t) = \sum_k^M a_k(X) \exp(-\tau \sum_k^M a_k(X)), \tag{2.58}$$

$$\Pr(\mu \mid X, t) = a_\mu(X) / \sum_k^M a_k(X), \tag{2.59}$$

In numerical computer simulations, a type of random numbers that is available in the most efficient way is the uniform, unit-interval random distribution $U(0, 1)$. To generate τ according to (2.58) from a sample of the uniform distribution, u_1, the following Monte Carlo inversion function of (2.58) is used:

$$\tau = (\sum_k^M a_k(X))^{-1} \cdot \ln(\frac{1}{u_1}), \tag{2.60}$$

Determining μ is then a simple task. Using another number u_2 taken from the uniform random number generator,

$$\mu = \min\{n : n \in N \wedge \sum_{k}^{n} a_k(\mathrm{X}) > u_2 \sum_{l} a_l(\mathrm{X})\} \tag{2.61}$$

Main portion of the computation cost of the Direct method comes from two random number generations and calculation of M propensity functions per a simulation step.

Here is a procedural definition of the Gillespie's Direct method:

1. Initialize: set initial number of molecules, and reset t ($t \leftarrow 0$).
2. Calculate the values of the propensities (a_i) for all the reactions.
3. Choose τ according to (2.60).
4. Determine the next reaction μ according to (2.61).
5. Change the number of molecules: $X \leftarrow X + \nu_\mu$.
6. $t \leftarrow t + \tau$.
7. Go to (2).

Another approach to the generation of the (τ, μ) pair is the *First Reaction method*, also devised by Gillespie [26]. It generates tentative τ s for all reaction channels,

$$\tau_l = \frac{1}{a_l(\mathrm{X})} \cdot \ln(\frac{1}{u}), \tag{2.62}$$

where u is a unit-interval uniform random number. Adopt the smallest τ_l as τ:

$$\tau = \min_{l} \tau_l \tag{2.63}$$

μ is then the reaction which got the smallest τ_l:

$$\mu = l \quad s.t. \quad \tau_l = \tau \tag{2.64}$$

This algorithm requires M random numbers and M calculations of the propensity functions, thus runs slower than the Direct method. However, it gives a basis for the recently proposed Next Reaction method, which we will discuss next.

The First Reaction method in a procedural appearance is like this:

1. Initialize: set initial numbers, and $t \leftarrow 0$.
2. Calculate the values of the propensities (a_i) for all the reaction channels.
3. For each of the reactions, calculate a putative time τ_i of the next occurrence of the reaction, according to the propensity calculated in (2).
4. Pick a reaction whose τ_μ is the least, $\tau \leftarrow \tau_\mu$.
5. Change the number of molecules: $X \leftarrow X + \tau_\mu$.
6. $t \leftarrow t + \tau$.
7. Go to (2).

Twenty four years after Gillespie's original work [26], Gibson published a paper that proposes a vastly improved version of the First Reaction method, called the *Next Reaction method*, in 2000 [27]. The core idea of the new method is to limit recalculation of τ_l to really necessary cases. In the First Reaction method, τ_l for each reaction is calculated in each simulation step. But with the Markovian assumption of the meso-scopic formalism, the recalculations are not necessary for reactions not affected by the current reaction μ. To take full advantage of this good opportunity of optimization, this method (1) uses absolute time, rather than relative time in the Direct and the First reaction methods, (2) dependencies between reactions are pre-calculated at simulation start-up, and (3) the absolute putative time τ_l of each reaction is stored in a dynamic priority queue data structure to speed up the operation of changing the value of τ_l.

The cost of this algorithm is just one random number generation and evaluations of the propensity functions affected by the current reaction. This is supposed to be near or perhaps on the theoretical limit as long as the simulation precisely tracks each occurrence of reaction events. This method has to, however, maintain the priority queue, of which cost is typically $O(\log_2(M))$ integer operations. Because the numbers of other operations such as the random number generation and propensity function evaluations grows proportionally to the density of the stoichiometry matrix, this logarithmic term can form a bottleneck in computation speed for large *M*s. The point of equilibrium between costs of the priority queue and other operations is implementation and platform dependent. It is worth noting, however, that recent advancements in pseudo random number generation methods such as the Mersenne Twister algorithm [28] and integration of multiple high-performance floating-point operation units into CPU chips have been lowering the equilibrium point of *M*. Vasudeva and Bhalla [29] has some practical performance comparisons of these methods.

Below are the step-by-step instructions for the Next Reaction method:

1. Initialize: set initial numbers, $t \leftarrow 0$, and for each reaction *i*, calculate a putative time τ_i.
2. Pick the reaction with the least putative time τ_μ.
3. Change the number of molecules: $X \leftarrow X + \tau_\mu$.
4. $t \leftarrow \tau_\mu$.

5. for each affected reaction α,

 (a) Calculate new a_α, new.

 (b) If $\alpha = \mu$, calculate new τ_α.

 (c) If $\alpha \neq \mu$,

$$\tau_a \leftarrow \frac{a_{\alpha,old}}{a_{\alpha,new}}(\tau_a - t) + t$$

6. Go to 2.

The scaling operation in step 5(c) is an optimization to avoid using extra random numbers, which is effectively equivalent as reusing the random numbers generated in the previous step. Legitimacy of this random number reusing is discussed in detail in Gibson and Bruck [27]. Of course, generating a new random number here and doing the same as 5(b) yields the same result.

To summarize, as long as the number of reactions is sufficiently large, and the computation needs to be exact (each occurrence of a reaction must be counted), Gibson's Next Reaction method is the best known way of realizing simulation trajectories according to CME.

Stochastic simulation methods also can be approximate using many possible ways. Gillespie in 2001 [30] defined a simulation procedure called as *tau-leaping* method. Continuously one of the best known approximate procedures is given in Gillespie and Petzold [23] later.

2.3.4 Other methods

We reviewed some simulation methods in computational cell biology, and there are several more schemes those are already in popular use or still under development.

Cellular automata (CA) is an important structure. The origin of CA traces back to John von Neuman's work [31] in theoretical computer science. Subsequently, it became a major way of investigating qualitative simulations of complex phenomena in diffusion-reaction systems, such as for excitable media and Turing patterns. Recently it is becoming popular as a means of quantitative modeling and simulation of physical phenomena such as fluid dynamics and diffusion-reaction [32]. Related to computational cell biology is a type of CA for enzymatic reaction networks with spatial extent represented by molecular diffusion proposed in Weimar [33]. Patel et al. [34] has an application of CA to the model of tumor growth. Ermentrout and Edelstein-Keshet [35] has some more references.

Another instance of a recent development is a hybrid dynamic/static pathway simulation method that combines conventional ODE-based rate equations with static, flux-based subcomponents [36]. The static part is based on a time-less metabolic flux analysis (MFA), and its (re-)calculation is triggered by simulation steps of the dynamic, ODE sub-components. Some more examples are Monte-Carlo Brownian dynamics method originally developed for simulation of micro-scopic simulation of synaptic transmission [37], boolean network, coupled map lattice, and difference equations.

This diversity in computational approaches is a natural consequence of the heterogeneity and multiple scales of the cell as a dynamical system, and therefore is an obvious reason of the need for the multi-formalism modeling and multi-algorithm simulation discussed in the next section.

2.3.5 Multi-algorithm simulation

In the cell, as we have seen in the previous sections, various components with different properties interact in diverse manners. All cellular subsystems are highly non-linear, and couplings of the subsystems are often non-linear as well. The nonlinearity indicates that the whole system is not equivalent to the sum of the subsystems. Although a subsystem in isolation can be investigated to some extent by assuming steady and simplified boundary conditions, the real behavior and role of the subsystem cannot be elucidated unless it is considered as part of the whole.

Cell simulators must therefore allow simulation of cell subsystems in both isolated and coupled forms. Simulation of coupled subsystems requires performing computations on mutually interacting subsystems with different computational properties on a single platform. There is, however, no universal algorithm which can efficiently conduct simulation of all the subsystems at once, and so simulators must allow multiple computation algorithms to coexist in a single model.

In order to support multi-algorithm computation, the scheme of the computing must therefore provide a single abstract programming interface, which allows indiscriminate interaction among the modules, and gives the front-end programs a standard means of visualizing and manipulating these modules. This also means that the implementations of the algorithm modules must be isolated from the system-provided common interface.

Related to this necessity of the multi-algorithm simulator are the representation schemes of the target physical entity (the cell) in the computer. The primary state variables of a cell model are the quantities of species, and these can be modeled using two different approaches. In the first, each state variable is a positive real number, and the fractional parts are not discarded; this number format is suitable for working with the empirical rate equations of biochemistry. The second approach keeps molecular quantities as natural numbers, and a variety of methods may be used to maintain the semantics of the fractional part, and these can be either stochastic or deterministic. In addition, realistic cell models usually require a mixture of continuous state variables like temperature, electric potential, and free energy, and discrete variables like the state flags of multi-state molecules (e.g. transiently modified proteins). Therefore, the simulator needs to handle types of positive, non-zero molecular quantities, real negative or positive numbers, and discrete states of molecules.

As discussed design options, there are two design schemes of multi-formalism simulation frameworks: embedding and combining approach.

A major source of strengths of the embedding approach is that it enforces modularity, or context-independent design, on implemented modules. Each simulation algorithm module and thus sub-model is required to have a well-defined interface of time-scheduling and communications with other modules. Good outcomes of the modularity of the embedding algorithm are:

- Once an algorithm is implemented in a modular way, it can be used in combination with any other algorithm modules.
- Implementations of algorithm modules are often simpler and more maintainable than combined forms of the same set of algorithms.

In fact, the embedding framework supports combining by allowing algorithms in combined forms to be implemented as algorithm modules. Also the embedding framework itself could be extended to support adaptive switching between algorithms by providing a mechanism of ’Process migration’, which allows Process objects to migrate among multiple Steppers of different algorithms during a simulation. This is one of our future works.

On the other hand, one drawback in this modular design is that, because of the inherent ’interface barriers’ between sub-models, dynamic switching between algorithms implemented in different modules is still a future work, and currently not available. This is immediately an advantage of the combined approach. For example, Gillespie’s tau-leaping method can be viewed as a combination of an exact method and the approximate tau-leaping scheme, and it switches to the exact method when the approximation becomes inappropriate. These methods connect smoothly to each other in a natural way as the scales of concentration and propensity changes.

Let’s take a closer look at this problem. A combination of two completely different simulation algorithms treats different formalisms. An example is a combination of a stochastic elementary reaction sub-model, for which Gillespie algorithm is often used, and an ODE sub-model of Michaelis-Menten-type enzymatic complex reactions. In a biochemical sense, these two sub-models require different types of kinetic parameters and modeling approaches, and these are not inter-convertible. Another example is when we have a model of metabolism as diffusion-reaction cellular automata, and want to co-simulate this with a boolean-network model of gene expression. This case calls the multiple modeling formalisms combined different simulation algorithms. Generally, this approach only occasionally results in a good simulator design, as it requires a new simulation algorithm to be developed for each distinct combination of modeling formalisms, unless there is an existing algorithm generic enough to run these formalisms simultaneously. Zeigler's *DEV&DESS* [61] is an exceptionally useful example here, because it combines two very general formalisms: discrete event and differential equation. In this thesis we will introduce DEVS framework to solve system biological processes. In the next chapter we will review some basic DEVS concepts, formalisms and the implementation of the quantized integrator to Differential Equation models in DEVSJAVA.

2.4 Existing software platforms and projects

Cell simulation is a relatively new area of computational science, but attempts to numerically simulate biochemical pathways has a considerable history. This section briefly reviews available software related to this work.

2.4.1 General purpose simulation software

One of the oldest publicly available software packages specialized for rate equation based numerical simulation of cellular processes is KINSIM [38]. It is a DOS application which is still actively used by biochemists for kinetic analysis of enzyme reaction systems. GEPASI [39] is also a rate equation based simulator with an integrated and easy to use interactive Windows GUI, and is widely used by biochemists for both research and education. GEPASI's successor, COPASI, is being developed with a focus on large scalability and distributed parallel computing. DBSolve [40] is another ODE-based simulator. ProMoT [41] is a Lisp based object-oriented modeling environment that uses the DIVA numeric's solver as a simulation backend. A commercial tool for block-oriented generic simulation of complex systems, SCoP [42] is used to run differential and difference equation based cell models. A-Cell [43] is a GUI-based piece of software used to construct biochemical reactions and electro-physiological models of neurons and other types of cell. Bio/Spice [44] was initially intended for genetic circuit simulation, and is now being developed as a generic modeling and simulation environment linked to object-relational databases. Jarnac, or SCAMP II, is a successor of the SCAMP simulator [45], and equipped with a powerful and flexible scripting language that enables users to program a dynamic object-oriented cell model. Virtual Cell [46] provides an intuitive WWW Java applet based modeling environment which allows modelers to construct spatial and biochemical models in both biological and mathematical semantic planes, and has support for empirical 3D data from microscopy. PLAS [47] is a simple yet powerful tool for modeling, simulation, and analysis of S-Systems. And still many groups use a generic modeling package such as Mathematica or MatLab, although these tools are not customized to support bio-simulation *per se*.

2.4.2 Specialized simulation software

StochSim [48] is a stochastic biochemical simulator in which individual molecules and molecular complexes are represented as individual software objects. Its unique algorithm makes for effective simulation of biochemical systems like the bacterial chemotaxis signaling pathway, where only a small number of molecules are involved and some of them are multi-state. MCell [37] is another simulator in which individual molecules are treated not statistically, but individually, with a Monte-Carlo type random-walk algorithm for Brownian dynamics. MCell is designed for the simulation of interactions between ligands and binding sites on receptors, enzymes, and transporters (amongst other molecules). Both simulators, StochSim and MCell, are somewhat infrequently used for simulation of sub-cellular dynamics because of high computational costs, yet the algorithms employed are likely to become indispensable if given appropriate roles in a multi-algorithm whole cell model.

2.4.3 Software platforms and languages

The Caltech ERATO Kitano Systems Biology Project is developing an XML-based Systems Biology Markup Language (SBML) as a means of interchanging bio-simulation models [49]. The group has set out to encompass all the features of Bio/Spice, DBSolve, E-Cell, Gepasi, Jarnac, StochSim, and Virtual Cell. A similar modeling language being developed by the University of Auckland and Physiome Sciences is CellML [50]. The Systems Biology Workbench (SBW) is also being developed by the Caltech ERATO team [51]. SBW is a distributed object computing environment for biological modeling and simulation. It provides an infrastructure that unifies various software components like model editors, simulators and data analyzers/visualizers. Using SBML as its language, it may well become the standard platform of model exchange, data exchange, and inter-operation.

2.5 Systems Biology Markup Language

Systems biology is characterized by synergistic integration of theory, computational modeling, and experiment [52]. Many contemporary research initiatives demonstrate the growing popularity of this kind of multidisciplinary work (e.g. Abbott, 1999 [53]). There now exists a variety of computational tools for the budding systems biologist (see below); however, the diversity of software has been accompanied by a variety of incompatibilities, and this has lead to numerous problems. For example:

- Users often need to work with complementary resources from multiple simulation/analysis tools in the course of a project. Currently this involves manually re-encoding the model in each tool, a time-consuming and error-prone process.
- When simulators are no longer supported, models developed in the old systems can become stranded and unusable. This has already happened on a number of occasions, with the resulting loss of usable models to the community. Continued

innovation and development of new software tools will only aggravate this problem unless the issue is addressed.

- Models published in peer-reviewed journals are often accompanied by instructions for obtaining the model definitions. However, because each author may use a different modeling environment (and model representation language), such model definitions are often not straightforward to examine, test and reuse.

The current inability to exchange models between different simulation and analysis tools has its roots in the lack of a common format for describing models. To address this, a *Software Platforms for Systems Biology* forum under the auspices of the ERATO Kitano Systems Biology Project was formed. The forum initially included representatives from the teams developing the biological software packages.

The forum decided at the first meeting in April 2000 to develop a simple, XML-based language for representing and exchanging models between simulation/analysis tools: the *Systems Biology Markup Language* (SBML). They chose XML, the eXtensible Markup Language [54], because of its portability and increasingly widespread acceptance as a standard data language for bioinformatics [55]. SBML is formally defined using UML, the Unified Modeling Language (Object Management Group, 2002), and this in turn is used to define a representation in XML. The base definition, *SBML Level 1*[56], is the result of analyzing common features in representation languages used by several ODE-, DAE- and stochastic-based simulators, and encompasses the minimal information required to support non-spatial biochemical models. Subsequent releases of SBML (termed *levels*) will add additional structures and facilities to Level 1 based on features requested and prioritized by the SBML community. By freezing sets of features in SBML definitions at incremental levels, they hope to provide software authors with stable standards and allow the simulation community to gain experience with the language definitions before introducing new elements.

2.5.1 Structure of Model Definitions in SBML

A chemical reaction can be broken down into a number of conceptual elements: reactant species, product species, reactions, stoichiometries, rate laws, and parameters in the rate laws. To analyze or simulate a network of reactions, additional components must be made explicit, including compartments for the species, and units on the various quantities. A definition of a model in SBML simply consists of lists of one or more of these various components (Figure 2.2, Figure 2.3):

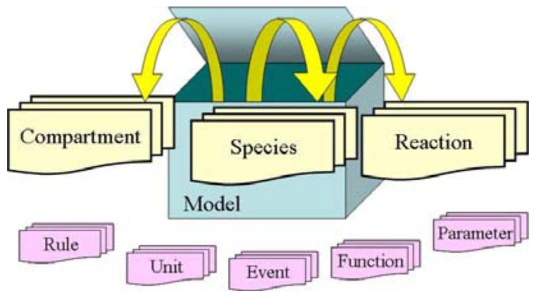


Figure 2.2 an SBML Document Structured

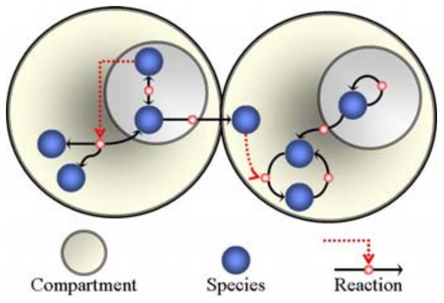


Figure 2.3 a model according to SBML

Compartment: A container of finite volume for well-stirred substances where reactions take place.

Species: A chemical substance or entity that takes part in a reaction. Some example species are ions such as calcium ions and molecules such as ATP.

Reaction: A statement describing some transformation, transport or binding process that can change one or more species. Reactions have associated rate laws describing the manner in which they take place (Figure 2.4).

Parameter: A quantity that has a symbolic name. SBML provides the ability to define parameters that are global to a model, as well as parameters that are local to a single reaction.

Unit definition: A name for a unit used in the expression of quantities in a model. This is a facility for both setting default units and for allowing combinations of units to be given abbreviated names.

Rule: A mathematical expression that is added to the model equations constructed from the set of reactions. Rules can be used to set parameter values, establish constraints between quantities, etc.

Function: A named mathematical function that may be used throughout the rest of a model.

Event: A statement describing an instantaneous, discontinuous change in a set of variables of any type (species quantity, compartment size or parameter value) when a triggering condition is satisfied.

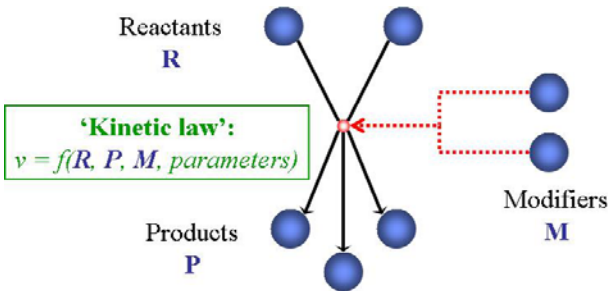
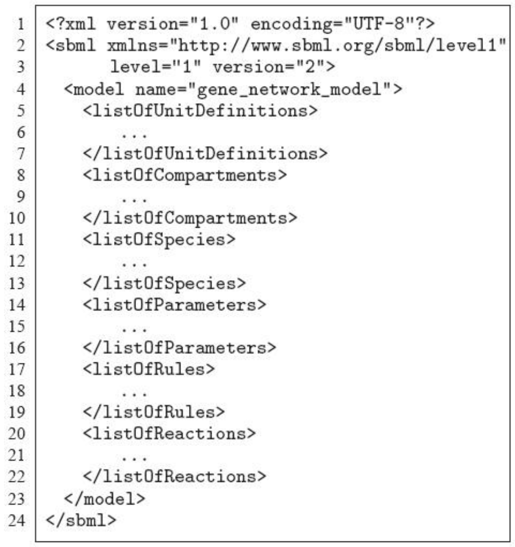


Figure 2.4 Reactions According to SBML



```
<?xml version="1.0" encoding="UTF-8"?>
<sbml xmlns="http://www.sbml.org/sbml/level1"
       level="1" version="2">
  <model name="gene_network_model">
    <listOfUnitDefinitions>
        ...
    </listOfUnitDefinitions>
    <listOfCompartments>
        ...
    </listOfCompartments>
    <listOfSpecies>
        ...
    </listOfSpecies>
    <listOfParameters>
        ...
    </listOfParameters>
    <listOfRules>
        ...
    </listOfRules>
    <listOfReactions>
        ...
    </listOfReactions>
  </model>
</sbml>
```

Figure 2.5 the skeleton of a model definition expressed in SBML, showing some possible top-level elements.

Figure 2.5 shows the skeleton of an SBML model description. It exhibits the standard characteristics of an XML data stream [54]: it is plain text, each element consists of a matched pair of start/end tags enclosed by '<' and '>' characters, some elements can contain attributes of the form *attribute* ='*value* ', and the first line contains a particular sequence of characters (beginning with '<?xml') declaring the rest of the data stream as conforming to the XML encoding standard.

2.5.2 CellDesigner: a structured diagram editor

CellDesigner [57] is a structured diagram editor for drawing gene-regulatory and biochemical networks. Networks are drawn based on the process diagram, with graphical notation system proposed by Kitano [52], and are stored using SBML, a standard for representing models of biochemical and gene-regulatory networks.

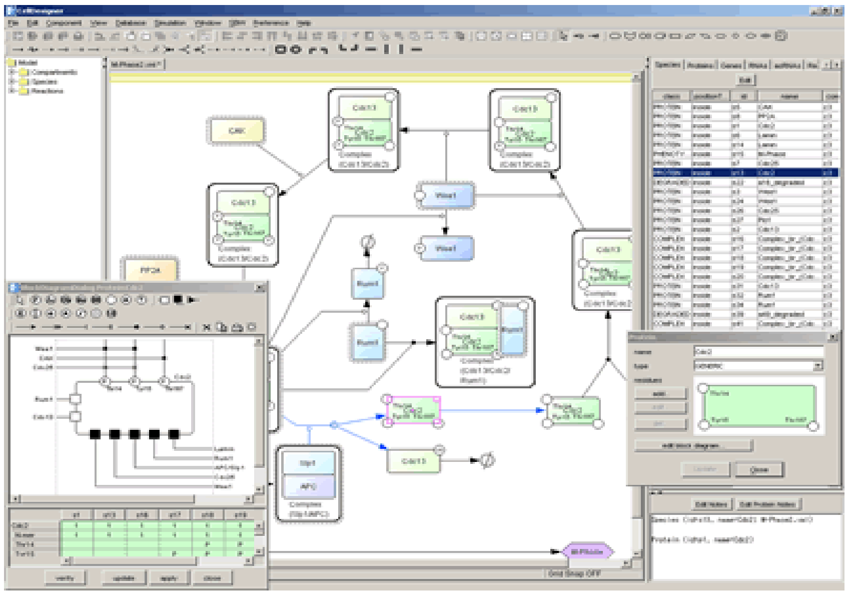


Figure 2.6 the user interface of CellDesigner ver.3.5.2

2.5.3 LibSBML

LibSBML [58] is a library designed to help you read, write, manipulate, translate, and validate SBML files and data streams. It is not an application itself (though it does come with many example programs), but rather a library you can embed in your own applications.

LibSBML is written in ISO C and C++ but as a library it may be used from all the programming languages C++, C, Java, Python, Perl, Lisp, Matlab, and Octave. In fact, they strive to adhere to the natural idioms of each particular language to make the libSBML programming experience seamless. For example, SBML <listOf> elements behave like lists and sequences in Python, but vectors in Matlab. Also, the C and C++ interfaces are completely distinct (it's possible to program in pure C), but in C++ the C APIs may be called without sacrificing type safety.

The LibSBML code is very portable and is supported on Linux, Windows (native), and Mac OS X.

CHAPTER 3 DEVS AND DEVSJAVA

In this chapter, firstly we review some basic DEVS concepts, formalisms and algorithms. Secondly Multi-algorithm framework and Differential Equation models were introduced. And lastly the implementation of the Quantized integrator to Differential Equations was given in DEVSJAVA.

3.1 DEVS

3.1.1 DEVS and DEVS Formalism

Discrete Event System Specification (DEVS) is a mathematical formalism to describe real-world system behavior in an abstract and rigorous manner. Compared with traditional methodology for modeling and simulation, DEVS formalism describes and specifies a modeled system as a mathematical object, and such object based representation of the targeted system can then be implemented using different simulation languages, especially modern object-oriented ones. In general, a system has a set of key parameters when being modeled in a modeling framework, which include time base, inputs, states, outputs, and functions for determining state transitions. Discrete event systems in general encapsulate these parameters as object entities, and then use modern object oriented simulation languages to describe the relationship among the specified entities. As a pioneering formal modeling and simulation methodology, DEVS provides a concrete simulation theoretical foundation, which promotes fully object-oriented modeling and simulation techniques for solving today’s simulation problems required by other science and engineering discipline. The insight provided by the DEVS formalism is in the simple way that it characterizes how discrete event simulation languages specify discrete event system parameters [59]. Having such an abstraction, it is possible to design new simulation languages with sound semantics that are easier to understand than traditional ones. Figure 3.1 presents a DEVS concept framework to show the basic objects and their relationships in a DEVS modeling and simulation world. These basic objects include [60]:

- The *real system*, in existence or proposed, which is regarded as fundamentally a source of data
- *model*, which is a set of instructions for generating data comparable to that observable in the real system. The structure of the model is its set of instructions. The behavior of the model is the set of all possible data that can be generated by faithfully executing the model instructions.
- *simulator*, which exercises the model's instructions to actually generate its behavior.
- *experimental frame*, which captures how the modeler’s objectives impact on model construction, experimentation and validation. As implemented in DEVSJAVA, such experimental frames are formulated as model objects in the same manner as the models of primary interest. In this way, model/experimental frame pair’s form coupled model objects with the same properties as other objects of this kind. It will

become evident later, that this uniform treatment yields immediate benefits in terms of modularity and system entity structure representation.

These basic objects are then related by two relations [60]:

• *modeling relation*: linking real system and model, defines how well the model represents the system or entity being modeled. In general terms a model can be considered valid if the data generated by the model agrees with the data produced by the real system in an experimental frame of interest.

• *simulation relation*, linking model and simulator, represents how faithfully the simulator is able to carry out the instructions of the model.

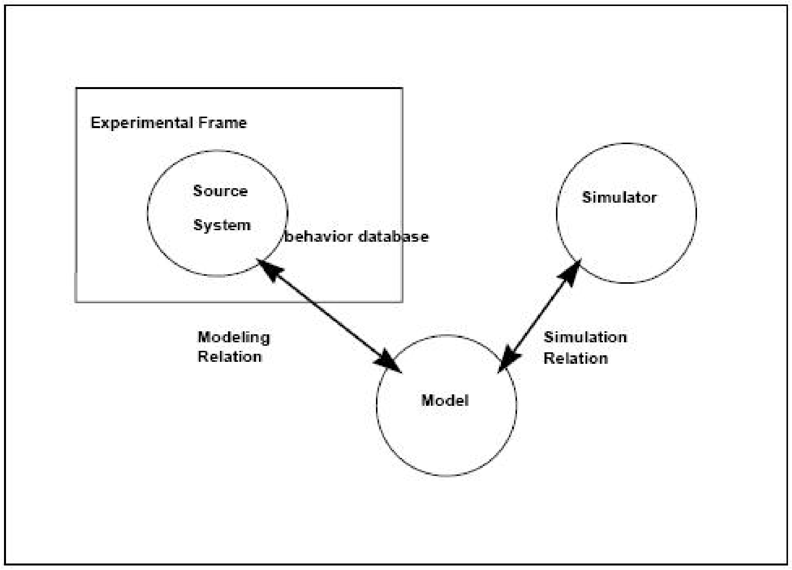


Figure 3.1 Basic Entities and Relations [60]

In the view from DEVS, the basic items of data produced by a system or model are time segments. These time segments are mappings from intervals defined over a specified time base to values in the ranges of one or more variables [60]. These variables can either be observed or measured. An example of a data segment is shown in Figure 3.2, where X is inputs, S is states, e is time elapsed, and Y is outputs.

In fact, DEVS formalism provides a formal definition to describe the data segment depicted above in Figure 3.2, and the history of DEVS can be traced back to decades ago.

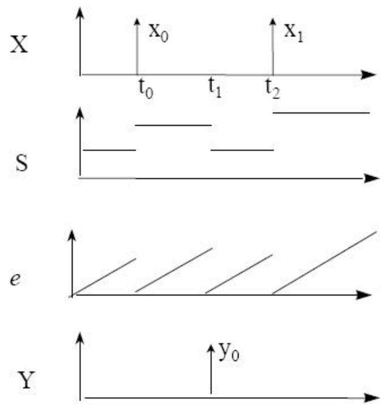


Figure 3.2 Discrete Event Time Segments [61]

A standard and classic DEVS formalism is defined as a structure [61]:

$$DEVS = (X, Y, S, \delta_{ext}, \delta_{int}, \lambda, ta)$$

Where

X is the set of inputs;

Y is the set of outputs;

S is the set of *sequential* states;

δ_{ext}: $Q \times X \rightarrow S$ is the *external state transition function*;

δ_{int}: $S \rightarrow S$ *is* the *internal state transition function*;

λ: $S \rightarrow Y$ is the output function;

ta: $S \rightarrow \Re_0^+ \cup \infty$ is the *time advance function*;

With $Q = \{(s, e) \mid s \in S, 0 \leq e \leq ta(s)\}$ is the set of *total states*.

Figure 3.3 illustrates the key concept of above classic DEVS formalism. Assuming the system is in state *S* after a previous state transition, it will stay in state *S* for a duration determined by *ta(s)*. When this resting time of *ta()* expires (or say the elapsed time *e=ta(s)*), the system gives output $\lambda(s)$ and changes its state from *s* to *s'*. This state transition is exactly determined by the internal transition function δ_{int} as mentioned in the formalism. However, if an external event occurs through the input *X* before the duration specified by *ta(s)* (or say, the system is in total state (*s, e*) with *e* = *ta(s)*, the system will change to a state determined by δ_{ext} *(s, e, x)*. After the system changes its state to a new state, the same rules in the formalism are applied to govern how the system responses to discrete events. DEVS makes an explicit difference between internal and external state transitions, where the internal transition function determines the system's new state when no events have occurred since the last transition, while the external transition function determines the system's new state when an external event occurs between *0* and *ta(s)*. It is worth to note that *ta(s)* is a real number including *0* and, where "*0*" means that the system is a so-called "*transitory*" state that no external events can intervene, and "∞" means that the system is in a so-called "*passive*" state that is unchanged forever until an external event wakes it up.

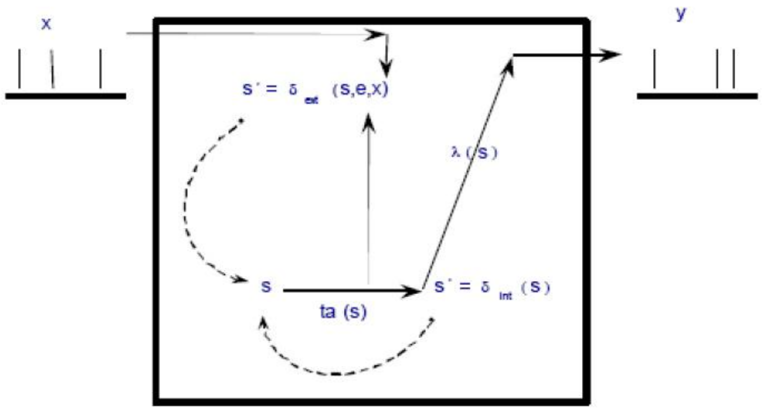


Figure 3.3 an Illustration for Classic DEVS Formalism [61]

The above classic DEVS formalism does not take into account of concurrent events, and therefore, has relatively limited usage for real-world application. With the consideration of concurrent events and parallel processing on a discrete event system, parallel DEVS system specification is developed from classic DEVS. The key capabilities of Parallel DEVS beyond the classical DEVS are [61]:

- Ports are represented explicitly – there can be any of input and output ports on which values can be received and sent.
- Instead of receiving a single input or sending a single output, basic parallel DEVS models can handle bags of inputs and outputs. It should be noted here that a bag can contain many elements with possibly multiple occurrences of its elements.
- A transition function, called confluent, is added, which decides the next state in cases of collision between external and internal events.

Such parallel DEVS formalism consists of two parts: basic and coupled models. A basic model of a standard parallel DEVS is a structure [61]:

$$DEVS = (X, Y, S, \delta_{ext}, \delta_{int}, \delta_{con}, \lambda, ta)$$

With X is the set of input events;

S is the set of sequential states;

Y is the set of output events;

$\delta_{int}: S \rightarrow S$ is the internal transition function;

$\delta_{ext}: Q \times X^b \rightarrow S$ is the external transition function,

Where X^b is a set of bags over elements in X,

$\delta_{con}: S \times X^b \rightarrow S$ is the confluent transition function,

Subject to $\delta_{con}(s, \phi) = \delta_{int}(s)$

$\lambda : S \rightarrow Y^b$ is the output function;

$ta: S \rightarrow \Re_0^+ \cup \infty$ is the time advance function,

Where $Q = \{(s, e) \mid s \in S, 0 < e < ta(s)\}$, and e is the elapsed time since last state transition.

Such basic model as defined in parallel DEVS captures the following information from a discrete event system:

- the set of input ports through which external events are received
- the set of output ports through which external events are sent
- the set of state variables and parameters
- the time advance function which controls the timing of internal transitions
- the internal transition function which specifies to which next state
- the system will transit after the time given by the time advance function has elapsed

- the external transition function which specifies how the system changes state when an input is received. The next state is computed on the basis of the present state, the input port and value of the external event, and the time that has elapsed in the current state
- the confluent transition function which decides the next state in cases of collision between internal and external events
- the output function which generates an external output just before an internal transition takes place

Basic model is a building block for a more complex coupled model, which defines a new model constructed by connecting basic model components. Two major activities involved in coupled models are specifying its component models and defining the couplings which create the desired communication networks. A coupled model is defined as follows [61]:

$$DN = <X, Y, D, \{M_i\}, \{I_i\}, \{Z_{i,j}\}>$$

Where X is a set of external input events;

Y is a set of outputs;

D is a set of components names;

for each i in D,

M_i is a component model

I_i is the set of influences for i

for each j in I_i,

$Z_{i,j}$ is the i-to-j output translation function

A coupled model template captures the following information:

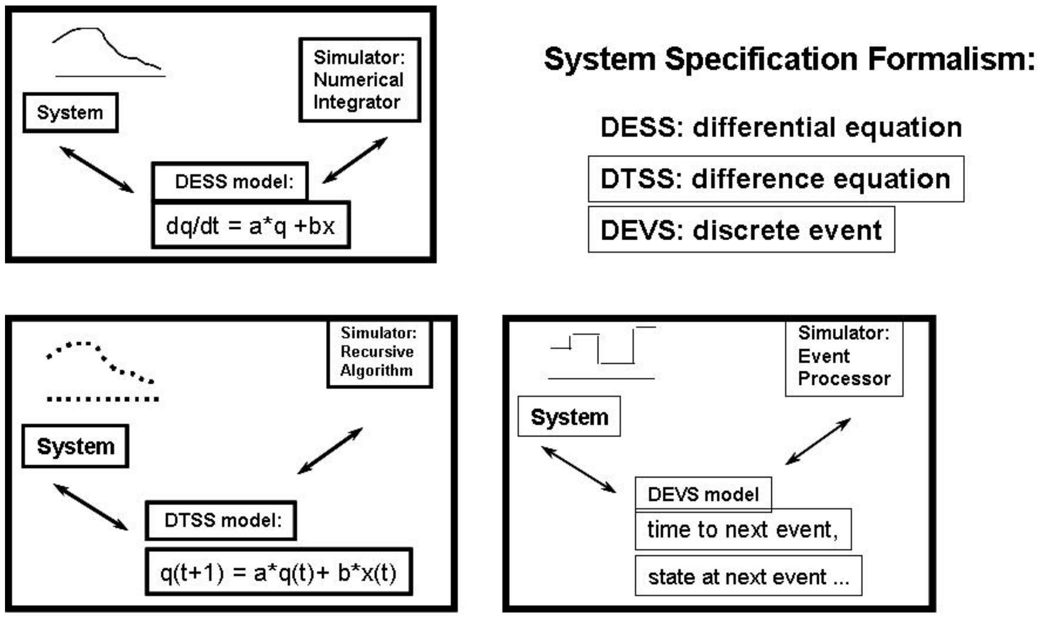
- the set of components
- for each component, its influences
- the set of input ports through which external events are received
- the set of output ports through which external events are sent
- the coupling specification consisting of:
    - the external input coupling (EIC) connects the input ports of the coupled to one or more of the input ports of the components
    - the external output coupling (EOC) connects the output ports of the components to one or more of the output ports of the coupled model
    - internal coupling (IC) connects output ports of components to input ports of other components

As we have seen in this section, DEVS formalisms are strictly defined and it evolves continuously to satisfy the requirement of today's large and complex system modeling and simulation. It has been extended by a lot of researcher around world; however, its core concept is unchanged as we can see from the classic and parallel formalisms. In the next section, we will discuss DEVS modeling framework.

3.1.2 Basic systems modeling formalisms

The basic systems modeling formalisms can be classified into three categories: Differential Equation System Specifications (DESS), Discrete Time System Specifications (DTSS), and Discrete Event System Specifications (DEVS) (Figure 3.4)

[61]. However, many real world phenomenons cannot be fit into the Procrustean bed of one formalism at a time. More generally, the ambitious systems now being designed, such as an automated highway traffic control system, cannot be modeled by a pure discrete or continuous paradigm. Instead, they require a combined discrete/continuous modeling and simulation methodology that supports a *multi-formalism* modeling approach and the simulation environments to support it.


System
Simulator: Numerical Integrator
DESS model:
dq/dt = a*q +bx
System Specification Formalism:
DESS: differential equation
DTSS: difference equation
DEVS: discrete event
System
Simulator: Recursive Algorithm
DTSS model:
q(t+1) = a*q(t)+ b*x(t)
System
Simulator: Event Processor
DEVS model
time to next event,
state at next event ...
Figure 3.4 Basic Systems Specification Formalisms

3.1.3 Multi-algorithm framework

Skipping many years of accumulating developments, the next major advance in systems formalisms was the combination of discrete event and differential equation formalisms into one, the DEV&DESS. This formalism subsumes both the DESS and the DEVS and thus supports the development of coupled systems whose components are expressed in any of the basic formalisms. Such *multi-formalism* modeling capability is important since the world does not usually lend itself to using one form of abstraction at a time. For example, a chemical plant is usually modeled with differential equations while its control logic is best designed with discrete event formalisms.

Two approaches to multi-formalism modeling are depicted in Figure 3.5. To *combine* formalisms we have to come up with a new formalism that subsumes the original ones. This is the case with DEV&DESS, which combines DEVS and DESS, the *discrete event* and *differential equation* system specification formalisms. Another approach to multi-formalism modeling is to *embed* one formalism into another.

Embedding the other formalisms (DESS and DTSS) into DEVS is attractive since then both discrete and continuous components are naturally included in the same environment. But, with continuous and discrete components interacting, we still need the DEV&DESS formalism to provide the basics of how such interaction should occur and to provide formalism in which embedding DESS into DEVS can be rigorously analyzed. Moreover, for combined models that cannot be feasibly embedded into DEVS, we must employ the DEV&DESS formalism.

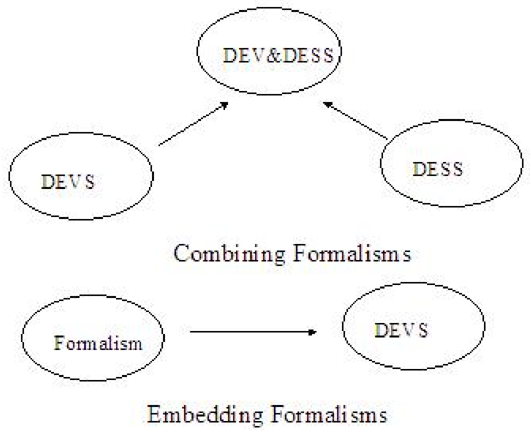


Figure 3.5 Multi-formalism Approaches

Figure 3.6 illustrates the DEVS and DESS combined model concept which has both DEVS and DESS elements working together. Input ports of X^{discr} accept event segments and input ports of X^{cont} accepts piecewise continuous or piecewise constant segments. The latter influences both model parts, while the event input only influences the DEVS part. Each part produces its own corresponding outputs, Y^{discr} and Y^{cont}, respectively. The parts can also influence each other's states.

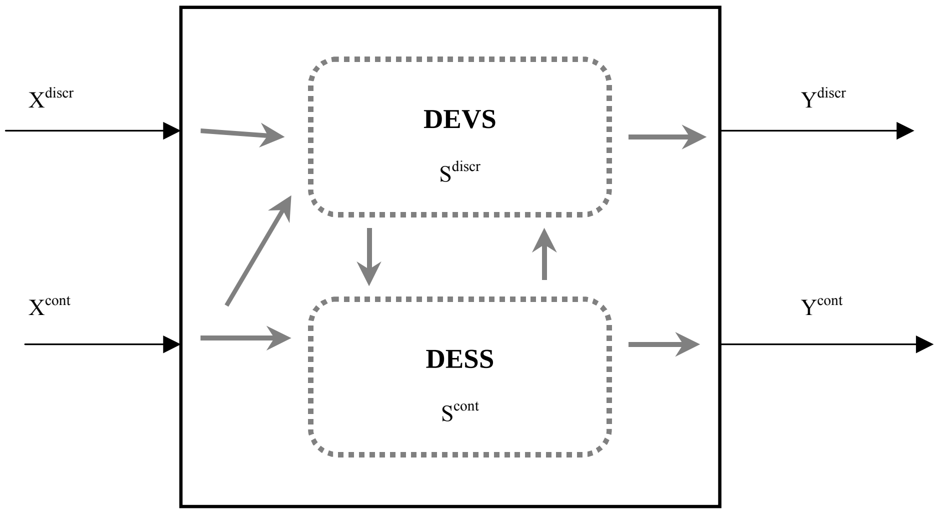


Figure 3.6 DEVS and DESS combined model

Fundamental for the understanding of combined modeling is how the discrete part is affected by the continuous part, that is, how the DESS part causes events to occur. Figure 3.7 illustrates this. In the intervals between events, the DESS input, state, and output values change continuously. In a combined model, events occur whenever a condition specified on the continuous elements becomes true. Typically, the condition can be viewed as a continuous variable reaching and crossing a certain threshold or whenever two continuous variables meet (in which case, their difference crosses zero). In such situations, an event is triggered and the state is changed discontinuously. An event which is caused by the changes of continuous variables is called a *state event* (in distinction to internal events of pure DEVS which are scheduled in time and are called *time events*).

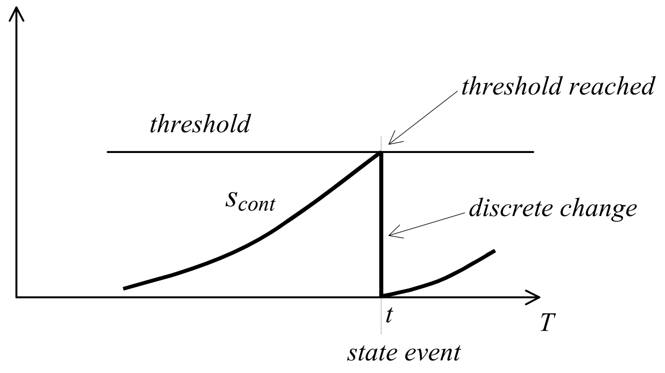


Figure 3.7 state event

In the subsequent section, DEVSJAVA, a known implementation of parallel DEVS formalism, is reviewed with the focus on how to solve the Differential Equation using DEVS view.

3.2 Differential Equation Models

Recall that in discrete time modeling there is a state transition function which computes the state at the next time instant given the current state and input. In the classical modeling approach of differential equations, the state transition relation is quite different. For differential equation models we do not specify a next state directly but instead, use a *derivative function* to specify the rate of change of the state variables. At any particular time instant on the time axis, given a state and an input value, we only know the rate of change of the state. The state at any point in the future must compute from this information.

3.2.1 Basic Model: The Integrator

To see how this form of modeling works, let us consider the most elementary continuous system – the simple integrator (Figure 3.8). The integrator has one input variable x and one output variable y. One can imagine it as a reservoir with infinite capacity. Whatever is put into the reservoir is accumulated – with a negative input value meaning outflow. The state of the reservoir is its current contents. When we want to express this in equation form we need a variable to represent the current contents. This is our state variable q. The current input x represents the rate of current change of the contents which we express by equation

$$\mathrm{d}q(t) / \mathrm{d}t = x(t)$$

and the output y is equal to the current state

$$y(t) = q(t)$$

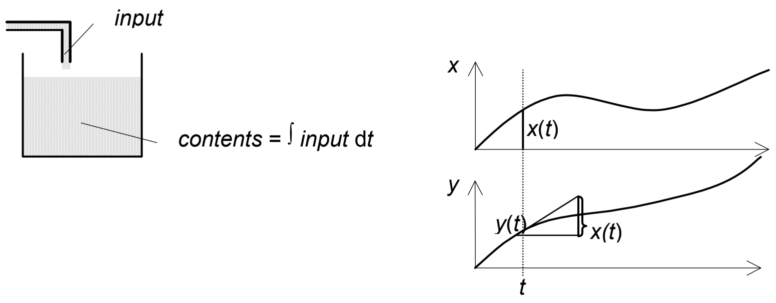


Figure 3.8 Basic Integrator Concepts

Usually continuous systems are expressed by using several state variables. Derivatives are then functions of some, or all, the state variables. Let q_1, q_2, ..., q_n be the state variables and x_1, x_2, ..., x_m be the input variables, and then a continuous model is formed by a set of first-order differential equations

$$d\,q_1(t)/dt = f_1(q_1(t), q_2(t), ..., q_n(t), x_1(t), x_2(t), ..., x_m(t))$$

$$d\,q_2(t)/\mathrm{dt} = f_2(q_1(t), q_2(t), ..., q_n(t), x_1(t), x_2(t), ..., x_m(t))$$

...

$$d\,q_n(t)/\mathrm{dt} = f_n(q_1(t), q_2(t), ..., q_n(t), x_1(t), x_2(t),..., x_m(t))$$

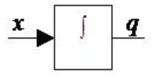


Figure 3.9 the Integrator

Note that the derivatives of the state variables q_i are computed respectively, by functions f_i which have the state and input vectors as arguments. Representing the integrator in diagrammatic form as in Figure 3.10, we can represent a set of first order differential equations in the coupled model form of Figure 3.10. The state and input vector are input to the rate of change functions f_i. Those provide as output the derivatives dq_i/dt of the state variables q_i which are forwarded to integrator blocks. The outputs of the integrator blocks are the state variables q_i.

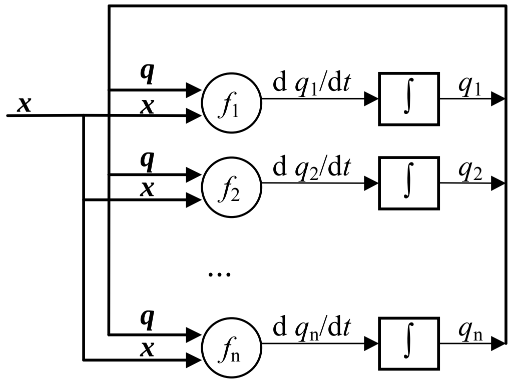


Figure 3.10 Structure of differential equation specified systems

3.2.2 Continuous system simulation

The diagram above reveals the fundamental problem that occurs when a continuous system is simulated on a digital computer. In the diagram we see that given a state vector q and an input vector x for a particular time instant t_i we only obtain the derivatives dq_i/dt. But how do we obtain the dynamic behavior of the system over time? In other words, how do we obtain the state values after this time? This problem is depicted in Figure 3.11. In digital simulation, there is necessarily a next computation instant t_{i+1} and a nonzero interval $[t_i, t_{i+1}]$. The model is supposed to be operating in continuous time over this interval, and the input, state and output variables change continuously during this period. The computer program has available only the values at t_i and from those it must estimate the values at t_{i+1} without knowledge of what is happening in the gaps between t_i and t_{i+1}. This means it must do the calculation without having computed the input, state, and output trajectories associated with the interval (t_i, t_{i+1}).

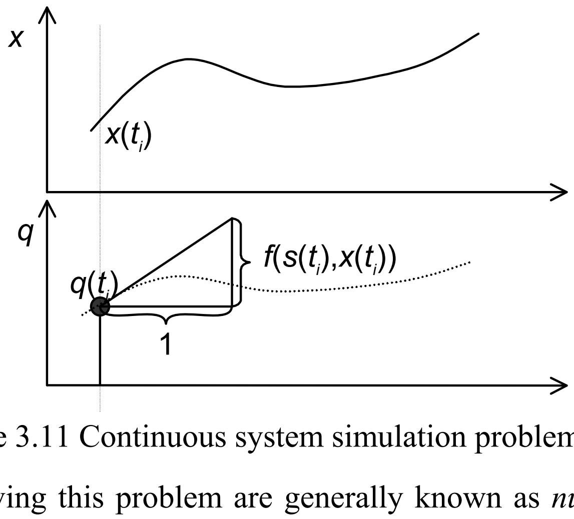


Figure 3.11 Continuous system simulation problems

Schemes for solving this problem are generally known as *numerical integration methods*. A whole literature deals with design and analysis of such methods [62] [63].

The basic idea of numerical integration is easily stated − an integration method employs estimated past and/or future values of states, inputs and derivatives in an effort to better estimate a value for the present time (*0*) (Figure 3.12). Thus to compute a state value $q(t_i)$ for a present time instance t_i, may involve computed values of states, inputs and derivatives at prior computation times $t_{i\text{-}1}$, $t_{i\text{-}2}$, ... and/or predicted values for the current time t_i and subsequent time instants t_{i+1}, t_{i+2}, ...

Notice that the values of the states and the derivatives are mutually interdependent – the integrator itself causes a dependence of the states on the derivatives and through the derivative functions f_i the derivatives are dependent on the states. This situation sets up an inherent difficulty which must be faced by every approximation method - the propagation of errors.

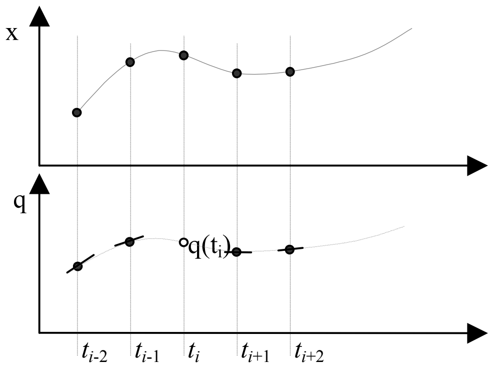


Figure 3.12 Computing state values at time t_i based on estimated values at time instants prior and past time t_i

We consider the simplest integration method, generally known as the *Euler* or *rectangular* method. The idea underlying the Euler method is that for a perfect integrator

$$\frac{dq(t)}{dt} = \lim_{h \to 0} \frac{q(t+h) - q(t)}{h}$$

thus for small enough *h*, we should be able to use the approximation

$$q(t+h) = q(t) + h \cdot \frac{dq(t)}{dt}$$

Now the input to an integrator, as in Figure 3.9, is the derivative $x(t) = dq/dt$. So we have

$$q(t+h) = q(t) + h \cdot x(t)$$

With h fixed, we can iterate to compute successive states at time instants $0, h, 2h, 3h, \ldots$ given the initial state at time 0 and the input values $x(0), x(h), \ldots$ Although straightforward to apply, Euler integration has some drawbacks. On one hand, to obtain accurate results the step size *h* has to be sufficiently small. But the smaller the step size the more iterations are needed and hence the greater the computation time for a given length of run. On the other hand the step size also cannot be decreased indefinitely, since the finite word size of digital computers introduces round-off and truncation errors. Therefore, a host of different integration method have been developed which often show much better speed/accuracy tradeoffs than the simple Euler method. However, these methods introduce stability problems as we discuss in a moment.

We now describe briefly the principles of some frequently employed integration methods. We can distinguish methods according to whether or not they use estimated future values of variables to compute the present values. We call a method *causal* if it only employs values at prior computation instants. A method is called *non-causal* if in addition to prior values it also employs estimated values at time instants at and after the present time. The order of a method is the number of pairs of derivative/state values it employs to compute the value of the state at time t_i. For example, a method employing the values of q and its derivative at times t_{i-2}, t_{i-1}, t_i, and t_{i+1} are of order 4. Table 3.1 summarizes the pros and cons of integration methods.

Table 3.1 Summary of Integration Method Speed-Accuracy Tradeoffs

Integration Method	Efficiency	Start-up Problems	Stability Problems	Robustness
Euler	low	none	not for sufficiently small step sizes	high
Causal methods	high	yes	yes	low
Non-Causal methods	high	no, if only future values used	yes	low

3.3 Differential Equation Models in DEVSJAVA

3.3.1 DEVSJAVA

DEVSJAVA [64] is an implementation in Java of DEVS framework that has been used for solving real-world simulation problem as well as serving as an openly available teaching tool. It is a fully object orient implementation of standard parallel DEVS formalism, and therefore, provides a very dynamic and flexible modeling and simulation framework. DEVSJAVA has relatively complex class hierarchical structure. The base classes of the DEVS sub-hierarchy are *Atomic* and *Coupled* as the main derived classes of it [64]. Class *digraph* is a main subclass of class coupled to define coupled model as described in previous subsection. For DEVS model developers, the user-defined model classes should derive from these basic classes and such model classes then become new components in DEVS for later reuse. The implementation of DEVSJAVA supports the fundamental concept of DEVS hierarchical construction and makes it easier to build complex model.

3.3.2 DEVS representation of discrete event integrators

It is useful to have a compact representation of the integration scheme that is readily implemented on a computer, can be extended to produce new schemes, and provides an immediate support for parallel computing. DEVS satisfies this need. A detailed treatment of DEVS can be found in [61]. Several simulation environments for DEVS are readily available online (e.g. PowerDEVS [65], adevs [66], DEVSJAVA [67], CD++ [68], and JDEVS [69] to name just a few).

DEVS uses two types of structures to describe a discrete event system. Atomic models describe the behavior of elementary components. Here, an atomic model will be used to represent individual integrators and differential functions. Coupled models describe collections of interacting components, where components can be atomic and coupled models. In this application, a coupled model describes a system of equations as interacting integrators and function blocks.

An atomic model is described by a set of inputs, set of outputs, and set of states, a state transition function decomposed into three parts, an output function, and a time advance function. Formally, the structure sees the basic model of a standard parallel DEVS in section 3.1.1. The external transition function describes how the system changes state in response to input. When input is applied to the system, it is said that an external event has occurred. The internal transition function describes the autonomous behavior of the system. When the system changes state autonomously, an internal event is said to have occurred. The confluent transition function determines the next state of the system when an internal event and external event coincide. The output function generates output values at times that coincide with internal events. The output values are determined by the state of the system just prior to the internal event. The time advance function determines the amount of time that must elapse before the next internal event will occur, assuming that no input arrives in the interim.

Coupled models are described by a set of components and a set of component output to input mappings. For our purpose, we can restrict the coupled model description to a flat structure (i.e., a structure composed entirely of atomic models) without external input or output coupling (i.e., the component models can not be affected by elements outside of the network). With these restrictions, a coupled model is described by the structure

$$N =< \{M_k\}, \{z_{ij}\} >$$

Where

$\{M_k\}$ is a set of atomic models, and

$\{z_{ij}\}$ is a set of output to input maps z_{ij}: $Y_i \rightarrow X_j \cup \Phi$

Where the i and j indices correspond to M_i and M_j in $\{M_k\}$ and Φ is the non-event:

The output to input maps describe which atomic models can affect one another. The output to input map is, in this application, somewhat over generalized and could be replaced with more conventional descriptions of computational stencils and block diagrams. The non-event is used, in this instance, to represent components that are not connected. That is, if component i does not influence component j, then $z_{ij}(x_i) = \Phi$, where $x_i \in X_i$.

These structures describe what a model can do. A canonical simulation algorithm is used to generate dynamic behavior from the description. The algorithm is given in the following DEVS algorithm. Algorithm assumes a coupled model *N*, with a component set $\{M_1, M_2, \ldots, M_n\}$, and a suitable set of output to input maps. For every component model M_i, there is a time of last event and time of next event variable tL_i and tN_i, respectively. There are also state, input, and output variables s_i, x_i, and y_i, in addition to the basic structural elements (i.e., state transition functions, output function, and time advance function). The variables x_i and y_i are bags, with elements taken from the input and output sets X_i and Y_i, respectively. The simulation time is kept in variable *t*.

Each of the *x* variables is associated with an atomic model called an integrator. The input to the integrator is the value of the differential function, and the output of the integrator is the appropriate y variable. The integrator has four state variables

- q_l, the last output value of the integrator,
- *q*, the current value of the integral,
- *dq/dt*, the last known value of the derivative, and
- σ, the time until the next output event.

The integrator's input and output events are real numbers. The value of an input event is the derivative at the time of the event. An output event gives the value of the integral at the time of the output.

DEVS simulation algorithm

```
$t \leftarrow 0$ {Initialize the models}
```

```
for all $i \in [1, n]$ do
    tL$_i \leftarrow 0$
    set $s_i$ to the initial state of $M_i$
end for
while terminating condition not met do
    for all $i \in [1, n]$ do
        $tN_i \leftarrow tL_i + ta(s_i)$
        Empty the bags $x_i$ and $y_i$
    end for
    $t \leftarrow \min\{tN_i\}$
    for all $i \in [1, n]$ do
        if $tN_i = t$ then
            $y_i \leftarrow \lambda_i(s_i)$
            for all $j \in [1, n]$ & $j \neq i$ & $z_{ij}(y_i) \neq \Phi$ do
                Add $z_{ij}(y_i)$ to the bag $x_j$
            end for
        end if
    end for
    for all $i \in [1, n]$ do
        if $tN_i = t$ & $x_i$ is empty then
            $s_i \leftarrow \delta_{int,i}(s_i)$
            $tL_i \leftarrow t$
        else if $tN_i = t$ & $x_i$ is not empty then
            $si \leftarrow \delta_{con,i}(s_i, x_i)$
            $tL_i \leftarrow t$
        else if $tN_i \neq t$ & $x_i$ is not empty then
            $s_i \leftarrow \delta_{ext,i}(s_i, t - tL_i, x_i)$
            $tL_i \leftarrow t$
        end if
    end for
end while
```

3.3.3 Mapping Differential Equation Models into DEVS Integrator Models

Figure 3.13 shows the mapping Differential Equation Systems to DEVS Simulators in DEVSJAVA Simulation of Continuous Systems.

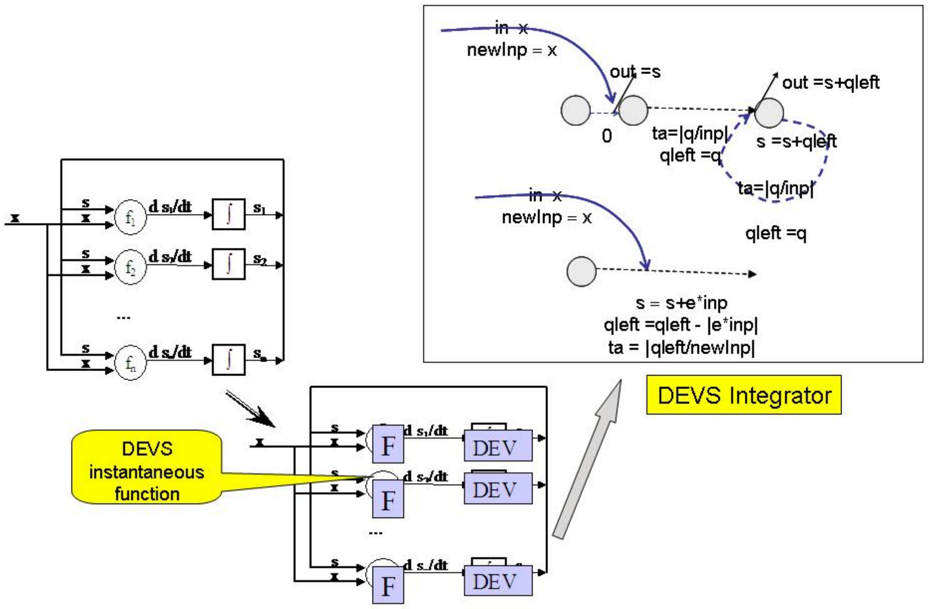


Figure 3.13 Mapping Differential Equation Models into DEVS Integrator Models

3.3.4 DEVS Representation of Quantized Integrator in DEVSJAVA

The DEVS realization of the quantized integrator has the simple definition:

$$M = (X, Y, S, \delta_{ext}, \delta_{ext}, \delta_{int}, \lambda, ta).$$

Where $X = Y = R$, $S = R \times R \times I$ and

- $\delta_{ext}((q, x, n), e, x') = (q + x*e, x', n)$
- $\delta_{int}(q, x, n) = (n + D*sign(x), x, n + sign(x))$
- $\delta_{con}((q, x, n), x') = (n + D*sign(x), x', n + sign(x))$
- $\lambda(q, x) = (n + sign(x))*D$
- $ta(q, x, n) = | ((n+1)D - q)/x |$ *if* $x > 0$ and $(n+1)D - q > 0$

  $= | (q - nD)/x |$ *if* $x < 0$ and if $|q-nD| > 0$

  $= | D/x |$ *if* $x \neq 0$ and none of the above

  $= \infty$ otherwise *(i.e., $x = 0$)*

Here we keep track of the boundary below (or at) the current state q, *i.e*, the integer floor *(q/D)*. Recall that even if we start on a boundary, the state may eventually be inside a block (hence not a multiple of D) as a result of an external transition. If, as in Figure 3.14(a), we are on a boundary, the time advance computation merely divides D by the current input x (which is the derivative, or slope, after all). If we reach the upper boundary ($n+1$) or lower boundary ($n–1$), we output and update the state accordingly. Note that so long as the input remains the same, the time to cross successive boundaries will be the same. Figure 3.14 (b) shows that when a new input is received, we update the state using the old input and the elapsed time. From this new state, *q*, the new time to reach either the upper or lower boundary is computed.

The Quantized DEVS integrator greatly reduces the number of transitions and output messages needed to simulate an integrator. It also reduces the message size from double to integer. Actually since the only possible transitions are to the upper or lower boundaries, only one bit to represent the binary valued set +1, -1 need be sent.

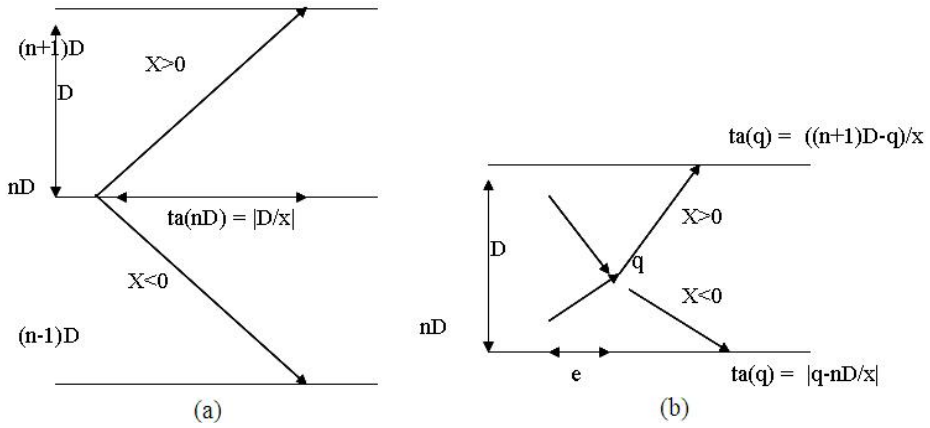


Figure 3.14 DEVS simulator of a Quantized Integrator

The implementation of the Quantized Integrator and Instantaneous Functions is given in DEVSJAVA Continuous package [70].

CHAPTER 4 DEVS COMBINED WITH SBML SYSTEM IN CELL BIOLOGY

In the previous chapters, an implication of the ’ontological complexity’ of the cell that leads to need for a sophisticated software platform has been discussed. Unlike some of conventional approaches to physical and biochemical systems, such complicated nature of the cell as a target system is inevitably reflected by the design and implementation of the software.

In this chapter, we introduce a new technique DEVS to solve the Cell Biology Processes. We will discuss design and implementation of DEVS-SBML Platform, a new simulation platform based on DEVS and SBML to enable sharing of biological models. This platform will be compatible DEVS model with SBML model to solve Biology Processes using DEVS ODE solver. We put an emphasis on architecture and implementation of DEVS-SBML Simulation Platform, a part of the DEVS-SBML Platform of which core is an object-oriented simulation engine that implements the cell simulation using DEVS algorithm discussed in the previous chapter.

4.1 Introduction

Computational cell biology is a rapidly growing simulation-oriented research field that has been greatly stimulated by the array of high throughput methods developed in recent years. In a previous chapter, we have argued that cell simulation poses many significant computational challenges that are distinct from those encountered in problems of other disciplines such as molecular dynamics, or computational physics, and even conventional biochemical simulations (see also in [71]). A vast array of molecular processes occurs simultaneously in the cell. The involved physical and chemical processes include molecular diffusion, molecular binding, enzymatic catalysis and higher order phenomena such as cytoplasmic streaming, complex macromolecular interactions (such as the dynamics of RNA polymerase on the DNA molecule), and global structural changes caused by cell division and cell differentiation. Many different kinds of simulation algorithms have been proposed and are currently being used for simulation of cellular processes. Different algorithms have different strengths, and are often suited to different spatial and temporal scales. In here we will use DEVS views to solve system biological processes, a new simulation platform, DEVS-SBML Platform. In the next section we will introduce the layer architecture of DEVS-SBML Platform.

4.2 Layered Architecture of Cell Biology with DEVS and SBML

The layered architecture of DEVS-SBML Platform is shown in Figure 4.1 and 4.2. At the top is the application layer that contains model in SBML model and DEVSJAVA or DEVSML. Generally, biology processes can be described by chemical reactions. Chemical reactions can be represented by kinetic rate equations or Michaelis-Menten and King-Altman rate equations to Complex enzymatic reactions. SBML is a language to describe the biological processes. So the designer can start with SBML model in the system cell biology. The SBML representation of the system biology model, which is essentially XML validated by the standardized Document Type Definition (DTD) (shown in section 2.5 of Chapter 2), can now participate in model composition. The system biology model can also verily be stored in the Library for reuse; this is one of the main goals of the creation of SBML. Then the SBML model can be translated into the DEVS model by the translator based on the development tool, libSBML (described in the section 2.5.3 of Chapter 2). The DEVS integrator is ready for simulating DEVS model in DEVSJAVA. The DEVS model also can be translated into the DEVS Modeling Language (DEVSML) [72] model.

SBML ←→ DEVSJAVA ←→ DEVSML ←→ JAVAML / CPlusML

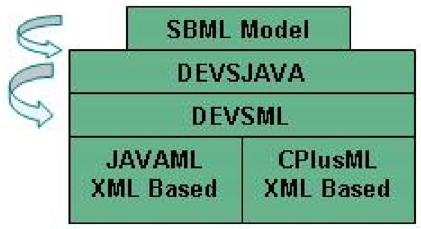


Figure 4.1 Layered Architecture of System Cell Biology processes with DEVS and SBML

The DEVSML model is then sent to various remote locations or specific Server, wrapped in Simple Object Access Protocol (SOAP) message to the destination host (Server in our case). Based on the information contained in the DEVSML model description, corresponding simulator is called for to instantiate the model and executes the simulation with the designated simulator.

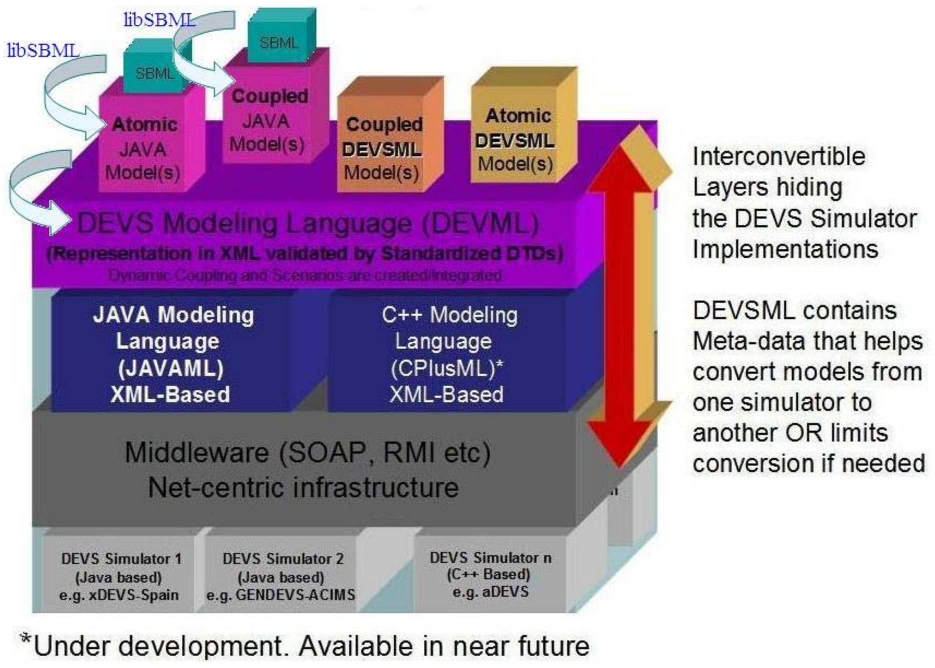

libSBML
libSBML
SBML
SBML
Atomic JAVA Model(s)
Coupled JAVA Model(s)
Coupled DEVSML Model(s)
Atomic DEVSML Model(s)
DEVS Modeling Language (DEVML)
(Representation in XML validated by Standardized DTDs)
Dynamic Coupling and Scenarios are created/integrated
JAVA Modeling Language (JAVAML) XML-Based
C++ Modeling Language (CPlusML)* XML-Based
Middleware (SOAP, RMI etc) Net-centric infrastructure
DEVS Simulator 1 (Java based) e.g. xDEVS-Spain
DEVS Simulator 2 (Java based) e.g. GENDEVS-ACIMS
DEVS Simulator n (C++ Based) e.g. aDEVS
Interconvertible Layers hiding the DEVS Simulator Implementations
DEVSML contains Meta-data that helps convert models from one simulator to another OR limits conversion if needed
*Under development. Available in near future

Figure 4.2 the Architecture of System Cell Biology processes with DEVS and SBML

DEVSML

The second layer is the DEVSML layer itself that provides seamless integration, composition and dynamic scenario construction resulting in portable models in DEVSML that are complete in every respect. These DEVSML models can be ported to any remote location using the net-centric infrastructure and be executed at any remote location. Another major advantage of such capability is total simulator *'transparency'*. The simulation engine is totally transparent to model execution over the net-centric infrastructure. The DEVSML model description files in XML contains meta-data information about its compliance with various simulation *'builds'* or versions to provide true interoperability between various simulator engine implementations. This has been achieved for at least two independent simulation engines as they have an underlying DEVS protocol to adhere to. This has been made possible with the implementation of a single atomic DTD and a single coupled DTD that validates the DEVSML descriptions generated from these two implementations. Such run-time interoperability provides great advantage when models from different repositories are used to compose bigger coupled models using DEVSML seamless integration capabilities.

JavaML / CPlusML

JavaML [73] is an XML-Based source code representation for Java programs. The JAVAML DTD specifies various elements of a valid JavaML document. It is well-suited to be used as canonical representation of Java source code for tools. It comes with an XSLT-based back-converter that translates a JavaML document back into java source code. More details about JavaML can be found at [73]. CPlusML also is an XML-Based source code representation for C++ programs. CPlusML is still under development.

4.3 DEVS with SBML Platform

We want to develop a platform for the DEVS with SBML (DEVS-SBML) to model and simulate biological processes. Through this modeling and simulation platform, we hope to increase the productivity of biologists.

4.3.1 DEVS-SBML Platform

DEVS-SBML Platform is a chemical kinetics simulation software platform implemented in Java. It provides a model definition environment and various simulation engines for evolving a dynamical model from specified initial data. A model consists of a biological process of interacting chemical species, and the reactions through which they interact. The software can then be used to simulate the reaction kinetics of the biological system of interacting species. The software will consist of the following elements:

- A scripting engine to define a model and run simulations on the model, and to export a model defined in the Systems Biology Markup Language
- An implementation of the translator from the SBML model to the DEVS model
- An implementation of the DEVS algorithm for simulating chemical reaction kinetics
- An implementation of the simulation engine to invoke the different algorithms such as Stochastic algorithms, Deterministic Algorithms etc., for simulating chemical reaction kinetics
- A graphical user interface (GUI) application that can be used to run simulations and export a model defined in the Systems Biology Markup Language

Models are defined in text files that you can edit, or generate using an external tool (e.g., Celldesigner or Jdesigner). The DEVS-SBML Platform can read and write SBML Level 1 specification. The file extension of the SBML language is: “.xml”.

4.3.2 The design of DEVS-SBML Platform

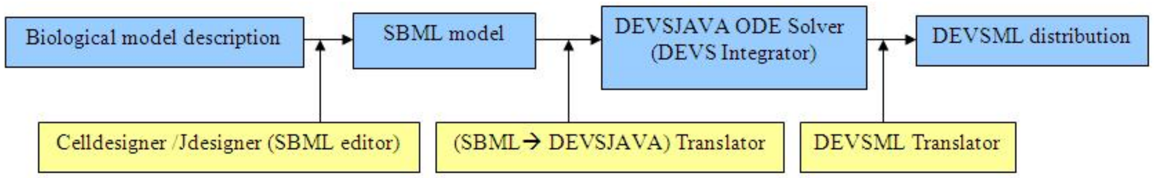

Biological model description
SBML model
DEVSJAVA ODE Solver (DEVS Integrator)
DEVSML distribution
Celldesigner /Jdesigner (SBML editor)
(SBML→ DEVSJAVA) Translator
DEVSML Translator

Figure 4.3 Overview DEVS with SBML Platform Development

This DEVS-SBML platform will be described by Figure 4.3. First part, SBML editor will be described by Celldesigner or Jdesigner free software (shown in section 2.5.2 of Chapter 2), a structured diagram editor. We also already implemented a text-based editor of the SBML model. Second part, the translator of SBML model to DEVS model reads all of information such as the species (reactants and products of the chemical reactions), the parameters and the whole chemical reactions, from the SBML model files, then translates the chemical reactions into the differential equations based on the kinetic law by the translator based on libSBML. We can calculate these differential equations by using the DEVS ODE solver. Third part, the DEVSML translator will be performed from the DEVS model to DEVSML model, then DEVSML model will be distributed by the web service. The DEVSML translator will be implemented by Saurab [72].

4.3.3 The translator from SBML model to DEVS Model

The translator modular is described in Figure 4.4. Model Builder (depicting the

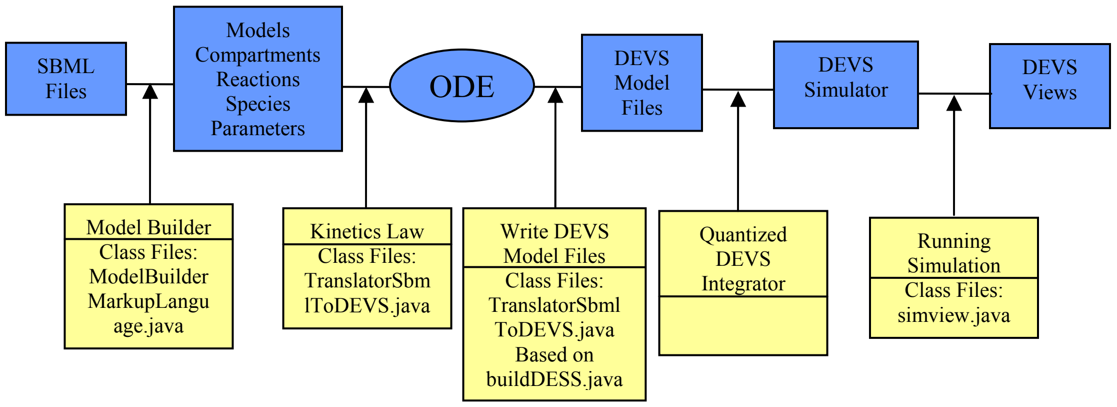

SBML Files
Models Compartments Reactions Species Parameters
ODE
DEVS Model Files
DEVS Simulator
DEVS Views
Model Builder
Class Files: ModelBuilder MarkupLanguage.java
Kinetics Law
Class Files: TranslatorSbmlToDEVS.java
Write DEVS Model Files
Class Files: TranslatorSbmlToDEVS.java Based on buildDESS.java
Quantized DEVS Integrator
Running Simulation
Class Files: simview.java

Figure 4.4 Translator Modular from SBML model to DEVS model

classes related to ModelBuilderMarkupLanguage.java) builds a model from SBML input (SBML files). The SBML must be valid SBML level 1 (version 1 or 2). ModelBuilderMarkupLanguage.java class uses the SBMLValidate.SBMLReader class (based on the development tool, libSBML) to parse and query an SBML document contained in a String. This String includes all biological process information such as models, compartments, reactions, species, parameters etc. TranslatorSbmlToDEVS.java class will manipulate this string and translate this information into Ordinary Differential Equations (ODEs) by chemical reaction kinetics law. Then based on buildDESS classes, TranslatorSbmlToDEVS.java class writes a DEVS model file of biological process. And then DEVS simulator can run the DEVS model using Quantized DEVS Integrator. DEVS simview.java class can display this biological process.

The translator modular can process the SBML model to DEVS model file, and display the biological process using DEVS view.

4.3.4 DEVS-SBML GUI framework

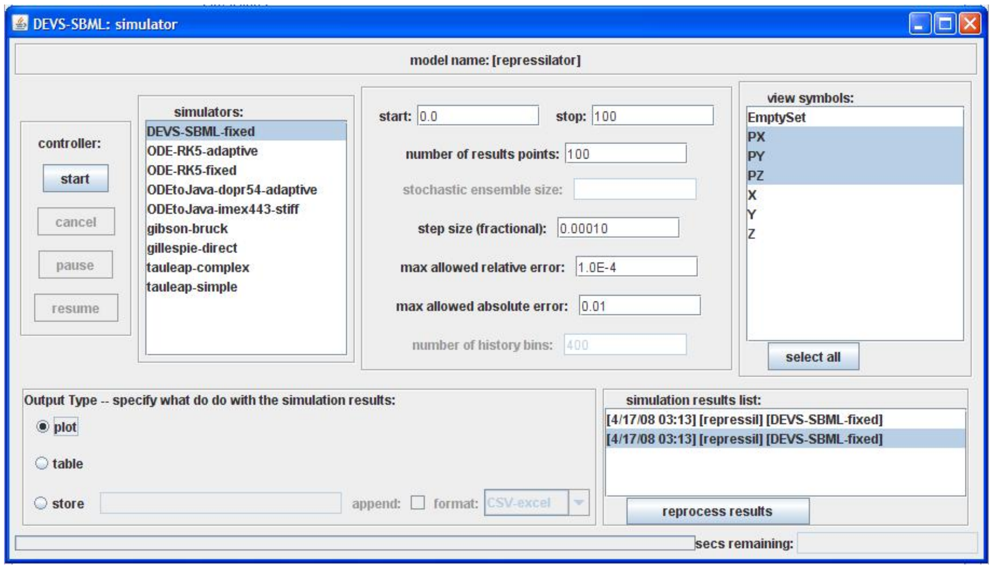
The notable software features of DEVS-SBML are: portability across many computer architectures, integration with external software programs, and a graphical user interface (GUI). In this section, these software features are described.

DEVS-SBML is implemented in the Java programming language, which enables DEVS-SBML to execute on any computer platform for which a Java 2 Runtime Environment of version 1.4.1 or newer, is available. DEVS-SBML has been optimized for efficient numerical computation in the Java Runtime Environment.

DEVS-SBML is capable of simulating models expressed in the Systems Biology Markup Language (SBML) Level 1 [49], [56]. DEVS-SBML can also export a model into SBML.

Several simulation algorithms within DEVS-SBML may be invoked, such as the DEVS ODE, Runge-Kutta ODE, Gibson-Bruck, and Gillespie Direct simulators etc. DEVS-SBML employs a modular design in which each simulator is a software unit that conforms to a simple, well-defined interface specification. This feature will be described in next section.

DEVS-SBML provides a menu-driven graphical user interface. This user interface includes screens for simulation control, model editing, and plotting simulation results. Also we can output the results of the simulation by plotting, a tabular format or the stored files. A screen capture of the DEVS-SBML graphical user interface is implemented to solve the biology processes in Figure 4.5.



model name: [repressilator]

simulators:
DEVS-SBML-fixed
ODE-RK5-adaptive
ODE-RK5-fixed
ODEtoJava-dopr54-adaptive
ODEtoJava-imex443-stiff
gibson-bruck
gillespie-direct
tauleap-complex
tauleap-simple

start: 0.0 stop: 100
number of results points: 100
stochastic ensemble size:
step size (fractional): 0.00010
max allowed relative error: 1.0E-4
max allowed absolute error: 0.01
number of history bins: 400

view symbols:
EmptySet
PX
PY
PZ
X
Y
Z

Output Type -- specify what do do with the simulation results:
[✓ plot]
table
store append: format: CSV-excel

simulation results list:
[4/17/08 03:13] [repressil] [DEVS-SBML-fixed]
[4/17/08 03:13] [repressil] [DEVS-SBML-fixed]

Figure 4.5 the GUI of the DEVS-SBML Simulation platform

4.3.5 Modular Simulation Framework

DEVS-SBML has a modular design in which a *simulator* is a plug-in that conforms to a well-defined software interface. Each simulator is implemented as a self-contained unit that creates all of the internal data structures it needs to function. This allows for a variety of simulation techniques to be applied to a single model description, and for the clean separation of the simulation method from the model description. The model definition is focused on the biochemical semantics of defining chemical species and reactions. The technique and parameters for simulating the model are specified in the simulation controller, and do not require changes to the model.

DEVS-SBML will include both stochastic and deterministic simulators. The stochastic simulators will use discrete-event or multiple-event Monte Carlo algorithms. The deterministic simulators model the dynamics as a set of ODEs which are solved numerically.

One benefit of this modular design is that one may use a deterministic ODE-based solver for optimization and parameter fitting, and switch to a stochastic simulation technique for exploring the stochastic dynamics, once the model parameters have been established. This modularity also simplifies the task of implementing a new simulator and integrating it into the system. The simulators described in this section will be available in our software system.

CHAPTER 5 RESULTS AND DISCUSSIONS

In the last chapter, we have been described a new simulation platform based on DEVS and SBML, also called DEVS-SBML Platform. Some features of DEVS-SBML Platform were reviewed.

In this chapter we will analyze the efficiency and performance of DEVS-SBML Platform by comparing the different simulation algorithms to biological processes. And we also show the simulation procedure of DEVS-SBML Platform, and how to work using the translator from SBML model to DEVS model. Two simple biological processes examples were given using DEVS-SBML Platform.

5.1 Introduction

Two relatively simple models have been constructed. The first is a heat-shock response to examine the performance and efficiency using DEVS solver with SBML by comparing the different deterministic algorithms, Deterministic and Stochastic algorithm respectively. The second example is a Hypothetical single-gene oscillatory circuit in a eukaryotic cell to show how to work using DEVS framework with SBML and how to translate SBML model to DEVS model in programming level. The accuracy and error analysis of DEVS-SBML Platform also were discussed in the single-gene oscillatory circuit model.

All the timings and results given in this work have been taken on a PC running RedHat Linux 9 operating system with a dual Hyper-Threading-enabled Intel Xeon 2.8GHz CPU and 2 GB of RAM. This implementation is a single-thread application.

5.2 The efficiency analysis of DEVS-SBML Platform

5.2.1 Heat-shock model

When cells are exposed to high temperature, the synthesis of a small number of proteins known as heat-shock proteins becomes selective and rapid [74]. This process is called the heat-shock response. σ^{32}, a variation of the σ subunit of RNA polymerase, has been implicated as the global regulator for this system [75]. DnaJ is a molecular chaperon that plays an important role in regulating the activity and stability of σ^{32} [76]. Many molecular species involved in this process are present in small numbers, most of which relate to gene expression processes, and require stochastic simulation. In contrast, protein folding involves large quantities of molecules, making stochastic simulation computationally challenging.

To evaluate the performance of a DEVS simulation scheme, we set up an ODE model and a stochastic model based on the *E. coli* heat-shock model [77]. Protein folding/unfolding processes have been added to the model, and modeled as differential equations in the ODE and using the Gillespie algorithm in the stochastic model (Figure 5.1). The list of reactions and initial values of this model are shown in Table 5.1 and 5.2, respectively. The difference between Srivastava's original model and the model used in this work is also described in the legend for Figure 5.1. All parameter settings in the two variations of the model are identical.

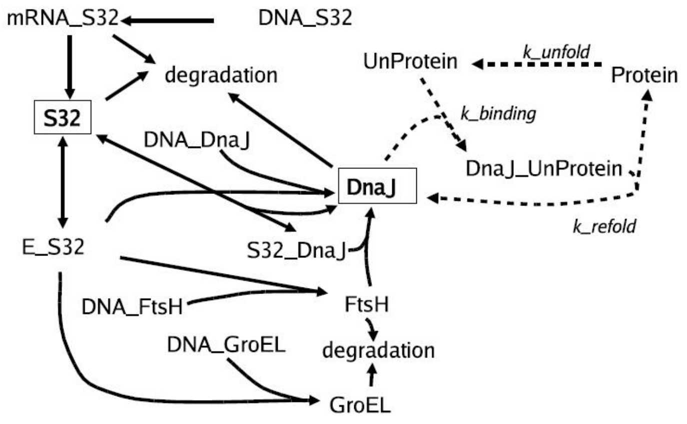


Figure 5.1 the heat-shock demonstration model

Model scheme for the heat-shock model. S32 means σ^{32}; E_S32 means the RNAP core enzyme with σ^{32}; Protein means folded protein and UnProtein means unfolded protein. Dashed lines in the upper-right corner represent reaction modeled using Gillespie in the stochastic model. Rate constants for σ^{32} transcription and translation are 1.4×10^{-3} and 7×10^{-2}, respectively, which are the only different parameters from the Srivastava's model. *k_unfold* = *k_binding* = 0.2, and *k_refold* = 9.73×10^{6}.

Table 5.1 Reactions list of heat-shock model

Reaction	Parameter
$DNA.\sigma^{32} \rightarrow mRNA.\sigma^{32}$	$1.4 \times 10^{-3}s^{-1}$
$mRNA.\sigma^{32} \rightarrow \sigma^{32} + mRNA.\sigma^{32}$	$0.07s^{-1}$
$mRNA.\sigma^{32} \rightarrow degradation$	$1.4 \times 10^{-6}s^{-1}$
$\sigma^{32} \rightarrow RNAP\sigma^{32}$	$0.7s^{-1}$
$RNAP\sigma^{32} \rightarrow \sigma^{32}$	$0.13s^{-1}$
$DNA.DnaJ + RNAP\sigma^{32} \rightarrow DnaJ + DNA.DnaJ + \sigma^{32}$	$4.41 \times^{6} M^{-1}s^{-1}$
$DnaJ \rightarrow degradation$	$6.4 \times 10^{-10}s^{-1}$
$\sigma^{32} + DnaJ \rightarrow \sigma^{32}.DnaJ$	$3.27 \times 10^{5}M^{-1}s^{-1}$
$\sigma^{32}.DnaJ \rightarrow \sigma^{32} + DnaJ$	$4.4 \times 10^{-4}s^{-1}$
$DNA.FtsH + RNAP\sigma^{32} \rightarrow FtsH + DNA.FtsH + \sigma^{32}$	$4.41 \times 10^{6}M^{-1}s^{-1}$
$FtsH \rightarrow degradation$	$7.4 \times 10^{-11}s^{-1}$
$\sigma^{32}.DnaJ + FtsH \rightarrow DnaJ + FtsH$	$1.28 \times 10^{3}M^{-1}s^{-1}$
$DNA.GroEL + RNAP\sigma^{32} \rightarrow GroEL + DNA.GroEL + \sigma^{32}$	$5.69 \times 10^{6}M^{-1}s^{-1}$
$GroEL \rightarrow degradation$	$1.8 \times 10^{-8}s^{-1}$
$^{*}Protein \rightarrow UnfoldedProtein$	$0.2s^{-1}$
$^{*}DnaJ + UnfoldedProtein \rightarrow DnaJ.UnfoldedProtein$	$9.7256 \times 10^{6}M^{-1}s^{-1}$
$^{*}DnaJ.UnfoldedProtein \rightarrow Protein + DnaJ$	$0.2s^{-1}$

Except the parameters for σ^{32} transcription, translation, and the last three reactions, all the parameters are from Srivastava's model. The partitioning of this heat-shock model for the composite run was based on the propensities of the reactions. When reactants are in small numbers, the propensity of the reaction is smaller and the fluctuation or noise is bigger, which make stochastic method essential. Reactions with asterisks (*), which consume protein molecules, have at least two order higher numbers of reactant molecules than most of the other reactions. All the reactions were modeled using ODE method in the deterministic model and Gillespie in the stochastic model.

Table 5.2 List of initial values used in heat-shock model

Variable	Initial Values	Unit
DNA.σ^{32}	1	number
mRNA.σ^{32}	17	number
σ^{32}	15	number
RNAPσ^{32}	76	number
DNA_DnaJ	1	number
DNA_FtsH	0	number
DNA_GroEL	1	number
DnaJ	464	number
FtsH	200	number
GroEL	4314	number
DnaJ_UnfoldedProtein	5e6	number
Protein	5e6	number
$\sigma^{32}.DnaJ$	2959	number
Cell volume	1.5e-15	liter
UnfoldedProtein	2e5	number

We ran the heat-shock model for 100 seconds and traced the quantities of σ^{32} and *DnaJ* to benchmark the performance. These two species were selected because σ^{32} is biologically important, as it controls the expression level of heat-shock proteins, and *DnaJ* is on the boundary of the algorithms in the DEVS composite model. The total number of protein molecules in this example model is of order $10^6 – 10^7$. Figure 5.2 (a) and (b) are the simulation results of the heat-shock model of (a) σ^{32} and (b) DnaJ time courses using the DEVS ODE solver and E-cell system. It can be seen that the results of the DEVS ODE solver and the E-cell system models almost match both in σ^{32} and DnaJ cases. This is shown that DEVS method also is a correct way to simulate the biological process.

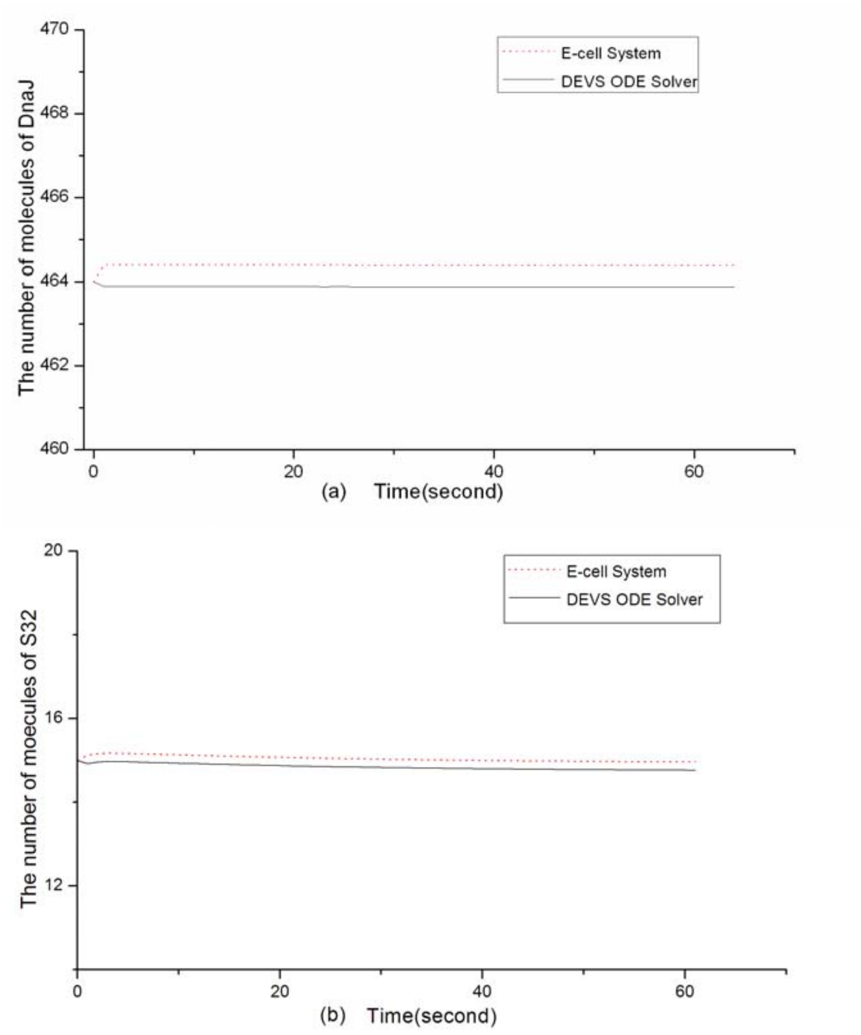


Figure 5.2 Simulation results of the heat-shock model using DEVS ODE solver (fine curve) and the E-cell system (dot curve). Comparisons of (a) DnaJ and (b) σ^{32} time courses of the first 65 seconds of the simulation for the DEVS ODE solver and the E-cell system.

5.2.2 Efficiency analysis of the DEVS-SBML platform

Table 5.3 shows some results using different software tools, E-cell [78], CVODE [79] and DEVSJAVA in the deterministic model. We can see DEVS integrator is faster and more efficient than other tools to calculate the biological process, the heat-shock response.

Table 5.3 the results from the different tools in the heat-shock response

Tools	Running Time(*sec.)*	σ^{32} Level	*DnaJ* Level
E-cell System[78]	3.703 ± 0.21	15.01	464.4
CVODE	2.952 ± 0.037	15.23	464.97
DEVSJAVA	2.673 ± 0.051	14.89	463.95

These models were run for 100 seconds. Each timing is an average of five runs. The results do not include times for program invocation and parsing of the XML model file.

In this heat-shock response model, DEVS integrator is more efficient method to simulate the heat-shock response process. This is immediately an advantage of the combined DEV&DESS approach. This method connect smoothly to DEVS and DESS each other in a natural way as the scales of concentration and propensity changes of chemical species, such as reactants or products of chemical reactions. In many instances, the discrete event approximation enjoys a computational advantage as well [80] [81].

The reason for the performance advantage can be understood intuitively in two related ways. The first is to observe that the time advance function determines the frequency with which state updates are calculated at a cell. The time advance at each cell is inversely proportional to the magnitude of the derivative, and so cells that are changing slowly will have large time advances relative to cells that are changing quickly. This causes the simulation algorithm to focus effort on the changing portion of the solution, with significantly less work being devoted to portions that are changing slowly. A second explanation can be had by observing that the number of quantum crossings required for the solution at a grid point to move from its initial to final state is, approximately, equal to the distance between those two states divided by the quantum size. This gives a lower bound on the number of state transitions that are required to move from one state to another. It can be shown that, in many instances, the number of state transitions required by the DEVS model will closely approximate this ideal number [80].

5.2.3 ODE Deterministic Algorithm and Gillespie Stochastic Algorithm

In the limit of large numbers of reactant molecules, stochastic and deterministic simulations are equivalent [82]. In contrast, if the system has low copy numbers of species, the deterministic law of mass action breaks down because the steady-state fluctuations in the number of molecules (which is proportional to the square root of the number of molecules) becomes a significant factor in the behavior of the system [83]. ODE models isolate the biochemical system into a group of deterministic and continuous reactions, and tacitly ignore fluctuations in the pathway [84]. With identical parameter settings, the stochastic and deterministic models can produce different results, and stochastic models are generally believed to be more accurate [85] [86]. Figure 5.3 shows the simulation results of the heat-shock model of (a) σ^{32} and (b) DnaJ using the DEVS ODE solver and Gillespie stochastic algorithm. The mean levels of DnaJ and σ^{32} were calculated over the simulation results, and trapezoid method was used for the mean calculation in the stochastic run. It can be seen that means of the DEVS ODE solver and the stochastic models agree well both in σ^{32} and DnaJ cases. The mean levels of σ^{32} and DnaJ were calculated: 15.02 ± 0.421 and 464.23 ± 0.452 in the stochastic run.

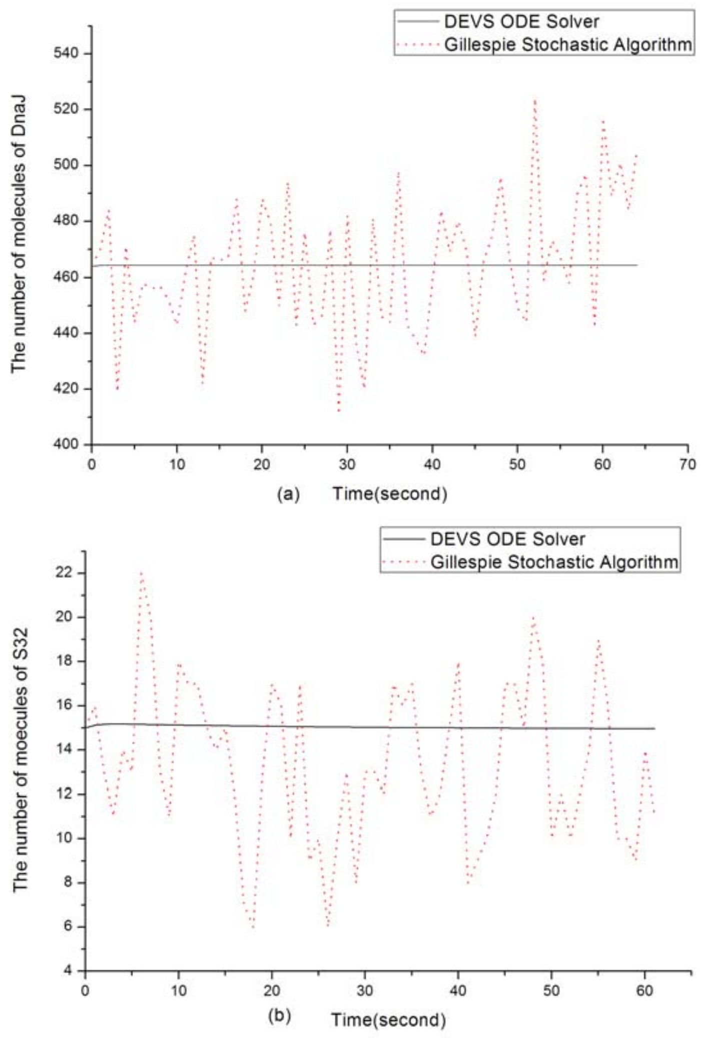


Figure 5.3 Simulation results of the heat-shock model using the DEVS ODE solver (fine curve) and Gillespie stochastic algorithm (dot curve). Comparisons of (a) DnaJ and (b) σ^{32} time courses of the first 65 seconds of the simulation for the DEVS model and the stochastic model. Each timing is an average of five runs.

Despite its advantages, however, Gillespie's scheme of exact stochastic simulation has limited utility due to its computational cost which is proportional to the number of molecules. Maybe the composition of the different simulation methods is a good way [78]. We can combine DEVS ODE model with the stochastic model together to form multi-formalism simulation frameworks to improve the performance of the original purely stochastic model. A logical extension to this "static" combination of the deterministic and stochastic schemes would be "dynamic" switching between these different simulation methods. This will be further explored in future work.

5.2.4 Performance analysis in the deterministic and stochastic schemes

The DEVS ODE heat-shock model runs faster than the Gillespie's stochastic model. The following crude approximation of computational costs of the ODE models withstands some discussion.

$$O_{ODE} \propto \gamma [\partial f / \partial x] N \tag{5.1}$$

$$O_{Gillespie} \propto [\sum a] \log N \tag{5.2}$$

Where $[\partial f / \partial x]$ is a measure of the degree of stiffness, N is the number of reactions, $[\sum a]$ denote the total propensity, and γ is a constant parameter to relate $[\partial f/\partial x]$ and $[\sum a]$. Computational cost of explicit ODE solvers is largely determined by the degree of stiffness, which is dominated by $[\partial f/\partial x]$ (where x is a dependent variable, and f is the derivative function for x). The large performance difference between the ODE and Gillespie implies the following:

$$\gamma [\partial f / \partial x] N \ll [\sum a] \log N \tag{5.3}$$

In fact, the step size of the ODE Stepper was about 10^{-3}, while the step size of the Gillespie-Gibson Stepper was typically in the range of $10^{-6} - 10^{-9}$.

The performance of the deterministic and stochastic simulation algorithms described above has been benchmarked using a variant of the heat-shock response model for *Escherichia coli* proposed by Srivastava *et al.* [77] and adapted by Takahashi *et al.*

for benchmarking the performance of the E-Cell simulator [78]. This model includes a large separation of dynamical time scales, which is typical of complex biochemical networks described in section 5.2.1. The benchmark results for the heat-shock model are summarized in Table 5.4. The results show large performance difference between the deterministic and stochastic algorithms, the same as what we discussed above in section 5.2.2. The results also show in the stochastic schemes, the efficiency of the Gibson-Bruck algorithm relative to the Gillespie Direct algorithm, and the significant speed improvement of Tau-Leap algorithms over the Gibson-Bruck and Gillespie algorithms.

Table 5.4 the results in the deterministic and stochastic schemes

Algorithm		*Running Time(sec.)*
Deterministic method	DEVS ODE	2.673 ± 0.051
Stochastic method	Gillespie Direct	514.6 ± 28.2
	Gibson-Bruck	404.9 ± 10.7
	Tau-Leap Simple	35.53 ± 0.43
	Tau-Leap Complex	9.55 ± 0.15

Benchmark results for solving the dynamics of the *E. coli* heat-shock model out to 100 seconds. The heat-shock model was solved for 100 seconds of simulation time, using five algorithms: DEVS ODE, Gillespie, Gibson-Bruck, Tau-Leap Complex, and Tau-Leap Simple. The ensemble size for the stochastic simulators was one. For each of the five simulators, the simulation was repeated five times, in order to obtain an average. The error tolerance for the Tau-Leap algorithms was 0.01. Both the relative and absolute error tolerances for the ODE solver were 10^{-4}.

It should be emphasized that no modifications of the model definition file were necessary in order to switch between the various simulation algorithms shown above. This is made possible because our model definition language is simulation algorithm-agnostic. Furthermore, the Tau-Leap method does not require an *ad hoc* partitioning of the model into stochastic and deterministic reaction channels. This is a potential advantage in analyzing a complex model for which the "*fast*" and "*slow*" degrees of freedom are not known a *priori*. This will be one of future research topics.

5.3 The simulation procedure of DEVS-SBML Platform

5.3.1 Hypothetical single-gene oscillatory circuit in a eukaryotic cell

Our example is a two-compartment model of a hypothetical single-gene oscillatory circuit in a eukaryotic cell [49]. The model is shown diagrammatically in Figure 5.4 and the list of chemical reactions for the model is given in Table 5.5. In this highly simplified model, the nucleus of the cell is represented as one compartment and the surrounding cell cytoplasm as another compartment. Let us suppose that there is a gene G which encodes its own repressor and is transcriptionally activated at a constant rate, V_i, by a ubiquitous transcription factor U. Transcriptional activation involves several enzymatic reactions summarized here as the production of active *RNAP* (from source material, *src*) and its degradation (to *waste*). The transcribed *mRNA* is then transported out of the nucleus and into the cytoplasm, where it is translated into the product (*P*) of the gene G from constituent amino acids (*AA*) and where it is also subject to degradation. *P* travels from the cytoplasm back into the nucleus to repress further transcription of G, but is itself also subject to degradation. Eventually, the concentration of *P* becomes so low that G can be reactivated by U, and the cycle repeats itself. To simulate the model, Table 5.6 also shows the initial value of all species.

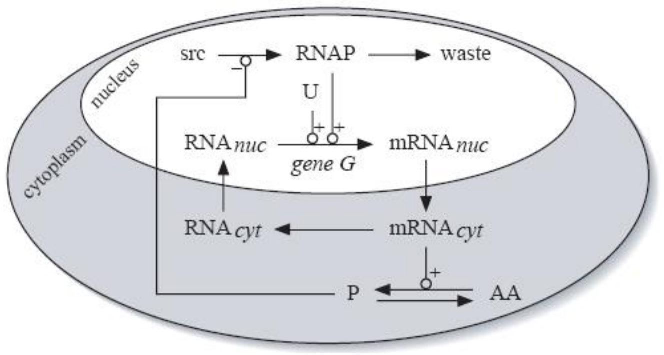


Figure 5.4 Schematic diagram of the hypothetical single-gene oscillatory circuit model

Table 5.5 Reactions list in the single-gene oscillatory circuit model.

Reaction	Rate
$src \rightarrow RNAP$	$V_i/(1+P/K_i)$
$RNAP \rightarrow waste$	$V_{kd} \cdot RNAP$
$RNA_{nuc} \rightarrow mRNA_{nuc}$	$\frac{V_{m1} \cdot RNAP \cdot RNA_{nuc}}{K_{m1} + RNA_{nuc}}$
$mRNA_{nuc} \rightarrow mRNA_{cyt}$	$k_1 \cdot mRNA_{nuc}$
$mRNA_{cyt} \rightarrow RNA_{cyt}$	$\frac{V_{m2} \cdot mRNA_{cyt}}{mRNA_{cyt} + K_{m2}}$
$RNA_{cyt} \rightarrow RNA_{nuc}$	$k_2 \cdot RNA_{cyt}$
$AA \rightarrow P$	$\frac{V_{m3} \cdot mRNA_{cyt} \cdot AA}{AA + K_{m3}}$
$P \rightarrow AA$	$(V_{m4} \cdot P)/(P + K_{m4})$

$mRNA_{nuc}$: mRNA in nucleus. *$mRNA_{cyt}$*: mRNA in cytoplasm. *RNA_{cyt}*, *RNA_{nuc}*: RNA constituents. The terms beginning with the letters '*K*' and '*V*' are parameters given values in Table 5.7.

Table 5.6 the initial value of Species list in the single-gene oscillatory circuit model

name	Compartment	Position To Compartment	quantity type	initial Quantity
src	C2:nucleus	inside	Amount	0
waste	C2:nucleus	inside	Amount	0
RNAP	C2:nucleus	inside	Amount	0.66
RNA_{nuc}	C2:nucleus	inside	Amount	96
$mRNA_{nuc}$	C2:nucleus	inside	Amount	0.003
$mRNA_{cyt}$	C1:cytoplasm	inside	Amount	3.8
RNA_{cyt}	C1:cytoplasm	inside	Amount	0.005
P	C1:cytoplasm	inside	Amount	20
AA	C1:cytoplasm	inside	Amount	90.465

Table 5.7 the parameters of the reactions in the single-gene oscillatory circuit model

name	V_i	K_i	V_{kd}	V_{m1}	K_{m1}	V_{m2}	K_{m2}	V_{m3}	K_{m3}	V_{m4}	K_{m4}	k_1	k_2
value	10	0.6	1	50	1	50	0.5	50	80	50	1	100	100

5.3.2 The simulation procedure of DEVS-SBML Platform

Base on the above of the hypothetical single-gene oscillatory circuit model's description and section 4.3 in last chapter, First step we can use the SBML specification [56] to describe the model, in detail refer to Appendix A: singleGene.xml (SBML model description file). Also we can use a structured diagram editor: CellDesigner^{Tm} [57] to draw this model diagram to get the SBML model description file. Figure 5.5 is the process diagram using software tool CellDesigner v3.5.2. The process diagram is a state transition diagram that represents transition of the state of each molecule using arrows that indicate transition and circle-headed arrows and bar-headed arrows to specify promotion and inhibition of such transitions. We can use this tool to edit or change the model, include initial value of the species, the parameters of reactions etc.

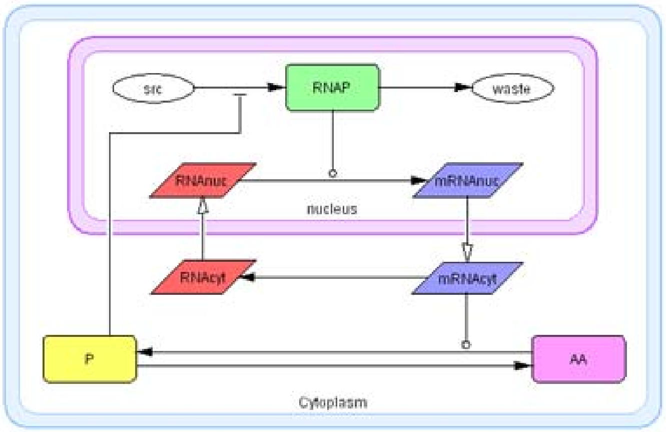


Figure 5.5 the process diagram of the single-gene oscillatory circuit model using software CellDesigner v3.5.2.

The following step we will translate SBML model into DEVSJAVA model, called translator based on the development tool of SBML, libSBML [58]. LibSBML can read, write, manipulate, and validate SBML files and data streams. So the translator reads all of information of the single-gene oscillatory circuit model from the SBML file, singGene.xml, to a string contained all of biological model information such as the species, chemical reactions etc. And all chemically reacting information can be represented by a means of kinetic rate equations or Michaelis-Menten and King-Altman types of rate equations (see section 2.3.1). Then by the translator class, we construct DEVS model files, package singleGene (refer to Appendix B). Then DEVSJAVA can solve these differential equations by DEVS quantization integrator. The Figure 5.6 is the viewer of DEVSJAVA ODE solver for the single-gene oscillatory circuit model.

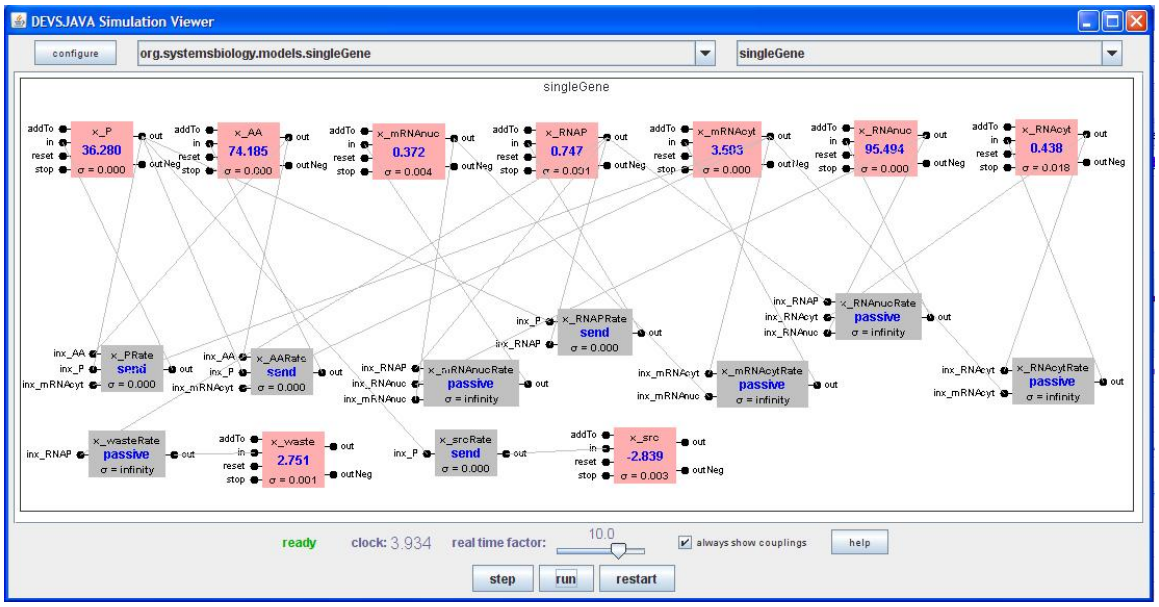


Figure 5.6 the DEVS viewer for the single-gene oscillatory circuit model

To evaluate the performance of a DEVS simulation, we set up a DEVS ODE model of the single-gene oscillatory circuit with the initial value of species in Table 5.6

and the parameters of the reactions in Table 5.7 respectively. We ran the single-gene oscillatory circuit model for the first 20 seconds to trace the quantities of all species, which is given in Figure 5.7.

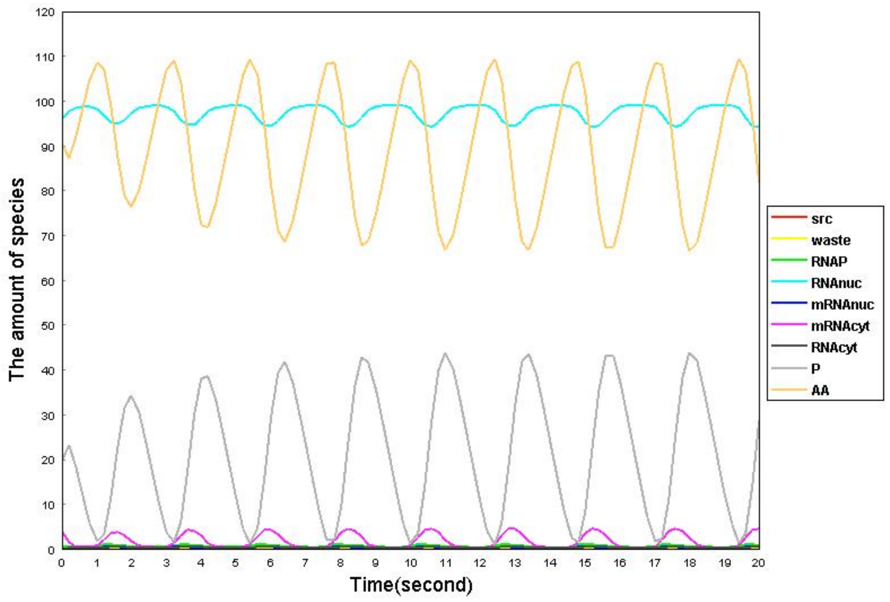


Figure 5.7 Simulation results with Quantum Size (D) = 0.001 of all species for the single-gene oscillatory circuit model

5.3.3 Accuracy of DEVS-SBML simulation

The specie amino acids (AA) were selected to analyze the single-gene oscillatory circuit model, because AA is biologically important, as it controls the expression level of the proteins directly.

For the discussing the accuracy of DEVS-SBML Platform, we like to introduce the result of CVODE as a benchmark. So the estimation and control of errors of CVODE is very important. In CVODE, local truncation errors in the computed values of y are estimated and the solver will control the vector e of estimated local errors in accordance with a combination of input relative and absolute tolerances. Specifically the vector *e* is made to satisfy an inequality of the form [87]

$$\| e \|_{WRMS,\, ewt} \le 1 \tag{5.4}$$

where the weighted root-mean-square norm $\| e \|_{WRMS,\, w}$ with weight vector *w* is defined as

$$\|e\|_{WRMS,w} = \sqrt{\frac{\sum_{i=1}^{N} (e_i w_i)^2}{N}} \tag{5.5}$$

The CVODE error weight vector *ewt* has components

$$ewt_i = \frac{1}{RTOL \cdot |y_i| + ATOL_i} \tag{5.6}$$

where the non-negative relative and absolute tolerances *RTOL* and *ATOL* are specified by the user. Here *RTOL* is a scalar, but *ATOL* can be either a scalar or a vector.

The CVODE integrator computes an estimate e_i of the local error at each time step and strives to satisfy the inequality (5.4). $\| e \|_{WRMS,\, w}$ is the weighted root-mean-square norm defined in terms of the user-defined relative and absolute tolerances (see (5.5) and (5.6)). Since these tolerances define the allowed error per step, they should be chosen conservatively. Experience indicates that a conservative choice yields a more economical solution than error tolerances that are too large. The error control mechanism in CVODE varies the step-size and order in an attempt to take minimum number of steps while satisfying the local error test (5.4). Therefore it is important to pick all the tolerance values conservatively, because they control the error committed on each individual time step. The final (global) errors are some sort of accumulation of those per-step errors. A good rule of thumb is to reduce the tolerances by a factor of .01 from the actual desired limits on errors. So if you want .01% accuracy (globally), a good choice is $RTOL = 10^{-6}$. But in any case, it is a good idea to do a few experiments with the tolerances to see how the computed solution values vary as tolerances are reduced. In the following calculation using CVODE solver, we setup $RTOL = 10^{-6}$ and $ATOL = 10^{-5}$, because the calculation results of single-gene oscillatory circuit model using CVODE method are no big change as $RTOL$ is reduced from 10^{-6}. So the final (global) errors of running single-gene model using CVODE method are controlled to .01%.

The single-gene oscillatory circuit model was run using the DEVS (fine curve) and CVODE (dot curve) methods, the simulation results for the first 10 time courses is shown in Figure 5.8. We can see the trajectory of AA in the DEVS model almost matches that of the CVODE method. No difference is by observing the simulation results between the DEVS and CVODE in the first and second moments of the time course. An analysis of higher moments showed a slight difference (Figure 5.8). However, as the overall behavior of AA exemplifies, it can be seen that the simulation results of the DEVS and the CVODE models agree well. It has been verified DEVS-SBML Platform is a right and accurate software tool to the simulation of the biological process.

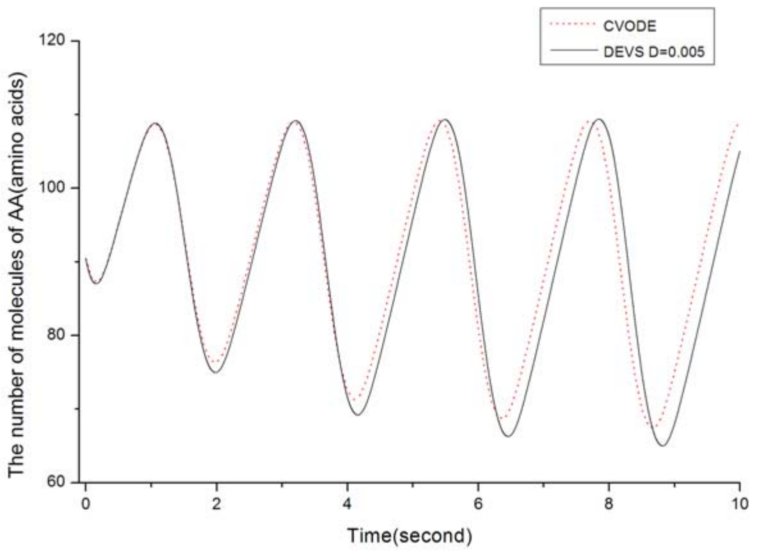


Figure 5.8 Simulation results of AA of the single-gene oscillatory circuit model for the DEVS model and the CVODE model. Comparison of AA time courses of the first 10 seconds of the simulation.

5.3.4 Error analysis in DEVS algorithm

Figure 5.9 (a) shows the simulation result with different values of Quantum Size D. Even with large values of D, it can be seen that the simulation result remains bounded. Figure 5.9 (b) shows the error in the simulation result for the more reasonable choices of D. From the figure, the correspondence between a reduction in D and a reduction in the computational error is readily apparent.

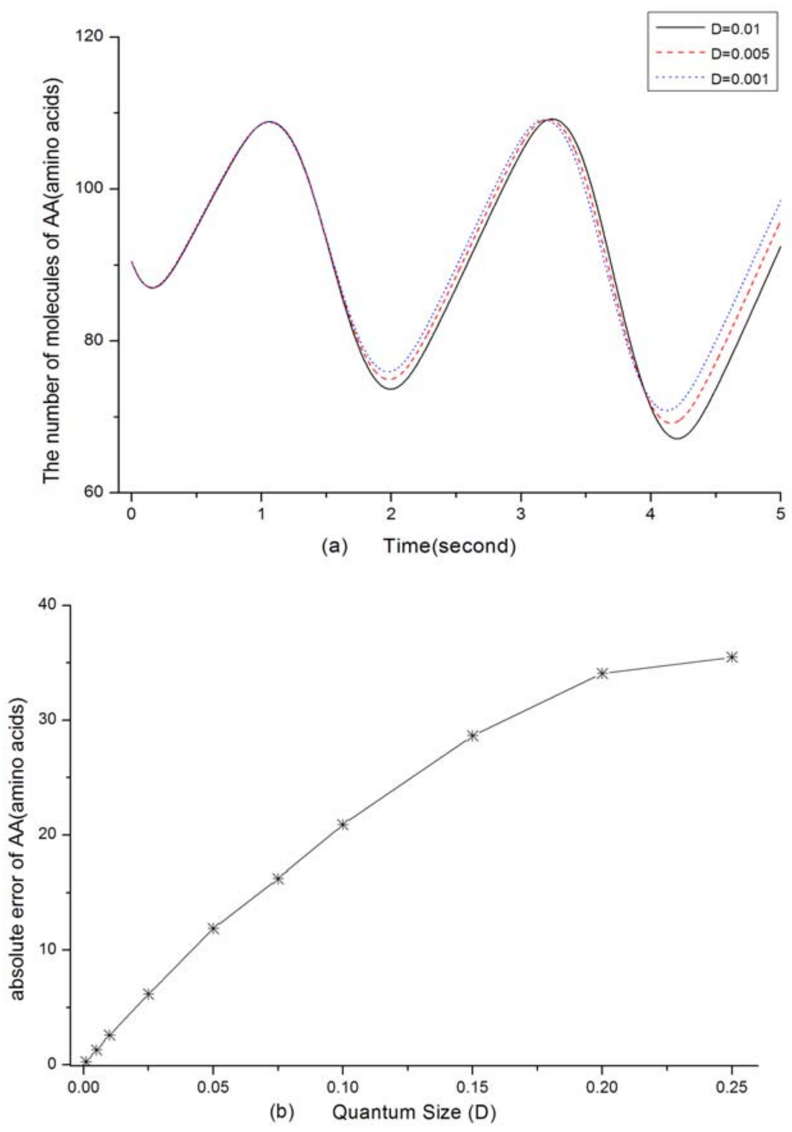


Figure 5.9 DEVS simulation results analysis of AA for the single-gene oscillatory circuit model (a) with different values of D. (b) absolute error of AA as a function of D.

Figure 5.10 demonstrates the execution time with Quantum Size D in DEVS framework for the single-gene oscillatory circuit model. It can be seen that as the quantum size D becomes bigger, the execution time becomes smaller. The execution time reduction highly depends on the quantum size D. In Figure 5.9 (b) we already see that as quantum size grows, the absolute error also grows. However, the model having bigger quantum size shows the better execution time as shown in the Figure 5.10. In other word, the model has a better execution time as the quantum size become bigger, but it triggers a bad error rate. So this is a trade off between execution time and accuracy.

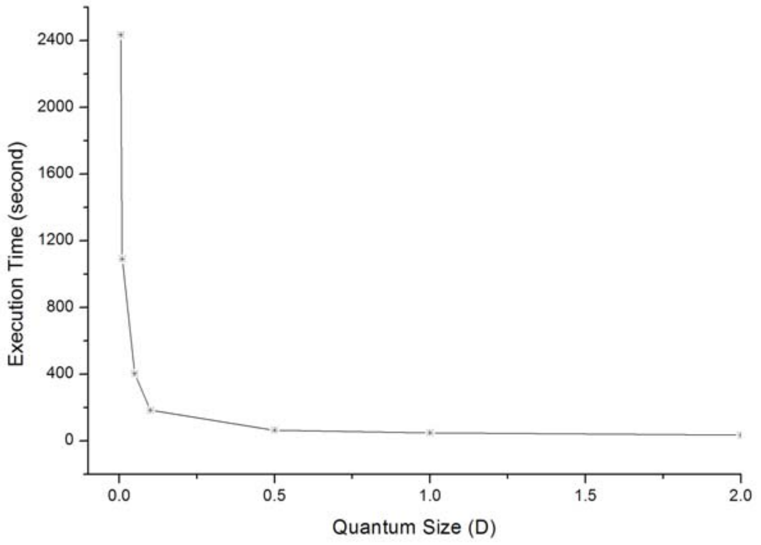


Figure 5.10 Execution time as a function of D for the single-gene oscillatory circuit model

5.4 Discussions of DEVS algorithm

Due to the non-linear nature of the sub-systems and the intimate couplings between them, simulation is crucial for cell biology research. In the past, however, it has been the norm to adopt different simulation algorithms for different sub-systems of the cell. This had made it difficult to combine the sub-cellular models, and in many cases this also limited the applications of the simulation to single-scale, sub-cellular problems.

In this chapter, we have provided a DEVS algorithm that incorporates the differential equations as a Multi-formalism approach. In the section 5.3 we had given two examples to analyze the DEVS algorithm combined with SBML. From the numerical experiments performed before, they were shown that this DEVS algorithm combined with SBML can efficiently drive simulation models. We already analyzed the error and execution time of DEVS combined with SBML which is shown that as quantum size D grows, the absolute error also grows, but it triggers a bad execution time. The effects also show that means of the DEVS ODE model and the stochastic Gillespie's model agree well. We also calculated the simulation result using the CVODE model and DEVS model; it can be shown the results almost match.

The best algorithm for a specific model is determined by the nature of the target system. Our DEVS algorithm combined with SBML provides a solution for situations in which it is necessary to simulate processes concurrently across multiple scales of time, space or concentration [84].

CHAPTER 6 CONCLUSIONS AND FUTURE WORKS

6.1 Conclusions

In a summary, this thesis discussed numerical simulations in more details in computational cell biology in three parts.

First we overviewed the area of research, cell biology, from the viewpoint of simulation studies, and commonly used modeling schemes and numerical methods have been reviewed. We performed an analysis of some computational techniques in the design of simulation algorithms and software platforms in this field of research.

Secondly, we reviewed the basic DEVS concept and DEVS formalism. We also have inspected three basic system modeling formalisms: DEVS, DTSS and DESS. Multi-algorithm framework, the combination of the DEVS and the DESS formalism, was reviews in this thesis. The implementation of the integrator and Instantaneous Functions to differential equations is given in DEVSJAVA Continuous package.

Lastly, a novel computational framework for cell biology simulation that can drive different simulation models with DEVS combined with SBML, DEVS-SBML Platform, has been introduced. This object-oriented framework based on DEVS defines discrete and continuous ’transition functions’, and can incorporate virtually any discrete and continuous simulation algorithms. By comparing the different deterministic algorithms, Deterministic and Stochastic algorithm respectively, it is shown that DEVS-

SBML Platform can efficiently handle the biological models. Also one example is given to show how to work using DEVS framework with SBML and how to translate SBML model to DEVS model. Two relatively simple models, (1) the *E. coli* heat-shock response model and (2) the single-gene oscillatory circuit model, are constructed. It was suggested that this framework will likely be scaled up to more realistic, complex and large-scale computational cell biology problems.

In a conclusion, we already have introduced new techniques to solve Biology Processes using DEVS algorithm in this thesis. We can share biological models described by DEVS-SBML Platform, which will be developed a new simulation platform. It was shown that it enable biological simulations more efficient and faster using DEVS than other methods, such as CVODE, the E-cell system etc. The performance measurement shows how the quantum size of DEVS integrator affects execution time and error rate. The numerical experimental result shows the followings clearly: the bigger quantum size the biological models have, the higher error rate and the better execution time they have. DEVS-SBML Platform, a generic software environment for computational cell biology, is being under development. We also show the simulation procedure of DEVS-SBML Platform, and how to work using the translator from SBML model to DEVS model. It has been verified that, owing to its object-oriented design, DEVS-SBML Platform will be implemented on a real software platform using Java language in an intuitive and efficient way.

6.2 Future works

We achieve both evaluating the efficiency of the simulation results of biological processes and performing the changes of the simulation results with Quantum Size D over system cell biology. In addition, we also accomplish some comparisons DEVS ODE models with stochastic models. However, there are further research works: developing more realistic, complex and large-scale computational cell biology problems such as metabolism, signal transduction pathways and gene expression systems etc., and further investigating how to use the stochastic algorithm in the DEVS framework, due to the stochastic models are generally believed to be more accurate to the real biological processes. For the future work, it will be implemented the combination of the deterministic and stochastic schemes into the DEVS framework to simulate the biological models.

Another future work need to be continuously developed and implemented DEVS-SBML Platform, a generic software environment. Design and implementation of a generic computational framework and scientific software demand a way of thinking that is completely different from ordinary virtues in many other scientific disciplines. Well defined and concrete problems often characterize good problem-driven scientific projects, whilst abstraction and generalization of the problems are the driving forces for the algorithm and software developers. Although at the time of writing this it is somewhat unclear that what extent of application domains are to be given to the algorithmic, computational and software frameworks developed in this work successfully, we strongly believe that, at least, necessity of the novel approach, DEVS combined with SBML, to the computational problems those characterize the new field of computational cell biology, including multi-formalism and multi-scale simulations, poses genuine challenges. We hope that the ideas described in this thesis stimulate further discussions and developments.

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https://computation.llnl.gov/casc/sundials/documentation/cv_guide.pdf
Example Programs for CVODE v2.6.0
https://computation.llnl.gov/casc/sundials/documentation/cv_examples.pdf

APPENDIX A: SingleGene.xml

```
<?xml version="1.0" encoding="UTF-8"?>
<sbml xmlns="http://www.sbml.org/sbml/level2" level="2" version="1">
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<listOfCompartments>
<compartment id="default" size="1"/>
<compartment id="c1" name="Cytoplasm" size="1" outside="default"/>
<compartment id="c2" name="nucleus" size="1" outside="c1"/>
</listOfCompartments>

<listOfSpecies>
<species id="s1" name="src" compartment="c2" initialAmount="0"
boundaryCondition="true" charge="0"/>
<species id="s2" name="waste" compartment="c2" initialAmount="0"
boundaryCondition="true" charge="0"/>
<species id="s3" name="RNAP" compartment="c2" initialAmount="0.66" charge="0"/>
<species id="s4" name="RNAnuc" compartment="c2" initialAmount="96" charge="0"/>
<species id="s5" name="mRNAnuc" compartment="c2" initialAmount="0.003"
charge="0"/>
<species id="s6" name="mRNAcyt" compartment="c1" initialAmount="3.8"
charge="0"/>
<species id="s7" name="RNAcyt" compartment="c1" initialAmount="0.005"
charge="0"/>
<species id="s8" name="P" compartment="c1" initialAmount="20" charge="0"/>
<species id="s9" name="AA" compartment="c1" initialAmount="90.465" charge="0"/>
</listOfSpecies>

<listOfReactions>

<reaction id="re1" reversible="false" fast="false">
<listOfReactants>
<speciesReference species="s1"/>
</listOfReactants>
<listOfProducts>
<speciesReference species="s3"/>
</listOfProducts>
<listOfModifiers>
<modifierSpeciesReference species="s8"/>
</listOfModifiers>
<kineticLaw>
<math xmlns="http://www.w3.org/1998/Math/MathML">
<apply>
```

```
<divide/>
<ci> Vi </ci>
<apply>
<plus/>
<cn type="integer"> 1 </cn>
<apply>
<divide/>
<ci> s8 </ci>
<ci> Ki </ci>
</apply>
</apply>
</apply>
</math>
<listOfParameters>
<parameter id="Vi" value="10"/>
<parameter id="Ki" value="0.6"/>
</listOfParameters>
</kineticLaw>
</reaction>

<reaction id="re2" reversible="false" fast="false">
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</listOfProducts>
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<ci> Vkd </ci>
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```

```
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<apply>
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<ci> Vm1 </ci>
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<ci> s4 </ci>
</apply>
<apply>
<plus/>
<ci> s4 </ci>
<ci> Km1 </ci>
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<parameter id="Km1" value="1"/>
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<listOfProducts>
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<kineticLaw>
<math xmlns="http://www.w3.org/1998/Math/MathML">
<apply>
<divide/>
<apply>
<times/>
```

```
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</apply>
<apply>
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</listOfProducts>
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<ci> s8 </ci>
<ci> Km4 </ci>
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</apply>
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<reaction id="re9" reversible="false" fast="false">
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```

```
<kineticLaw>
<math xmlns="http://www.w3.org/1998/Math/MathML">
<apply>
<times/>
<ci> k1 </ci>
<ci> s5 </ci>
</apply>
</math>
<listOfParameters>
<parameter id="k1" value="100"/>
</listOfParameters>
</kineticLaw>
</reaction>

<reaction id="re10" reversible="false" fast="false">
<listOfReactants>
<speciesReference species="s7"/>
</listOfReactants>
<listOfProducts>
<speciesReference species="s4"/>
</listOfProducts>
<kineticLaw>
<math xmlns="http://www.w3.org/1998/Math/MathML">
<apply>
<times/>
<ci> k2 </ci>
<ci> s7 </ci>
</apply>
</math>
<listOfParameters>
<parameter id="k2" value="100"/>
</listOfParameters>
</kineticLaw>
</reaction>

</listOfReactions>
</model>
</sbml>
```

APPENDIX B: Package singleGene using DEVS model

Package singleGene describe the hypothetical single-gene oscillatory circuit model in DEVS framework using Java language. Package singleGene is generated automatically by the translator from the SBML model to the DEVS model. Package singleGene include several Java source code files:

singleGene.java: display chemical reactions of the single-gene model using DEVS Integrators

x_AARate.java: the kinetic Law rate of the specie AA

x_mRNAcytRate.java: the kinetic Law rate of the specie mRNAcyt

x_mRNAnucRate.java: the kinetic Law rate of the specie mRNAnuc

x_PRate.java: the kinetic Law rate of the specie P

x_RNAcytRate.java: the kinetic Law rate of the specie RNAcyt

x_RNAnucRate.java: the kinetic Law rate of the specie RNAnuc

x_RNAPRate.java: the kinetic Law rate of the specie RNAP

x_srcRate.java: the kinetic Law rate of the specie src

x_wasteRate.java: the kinetic Law rate of the specie waste