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SIMULATION OF BIOCHEMICAL NETWORKS USING COPASI – A COMPLEX PATHWAY SIMULATOR

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ABSTRACT

Simulation and modeling is becoming one of the standard approaches to understand complex biochemical processes. Therefore, there is a big need for software tools that allow access to diverse simulation and modeling methods as well as support for the use of these methods. Here, we present a new software tool that is platform independent, user friendly and offers several unique features. In addition, we discuss numerical considerations and support for the switching between simulation methods.

1 INTRODUCTION

Simulation and modeling is becoming one of the standard approaches to understand complex biochemical processes. Therefore, there is a growing need for software tools that allow access to diverse simulation and modeling methods as well as support for the use of these methods. These software tools should be compatible, e.g., via file standards, platform independent and user friendly to avoid time-consuming conversions and learning procedures. In addition, they should be maintained by groups which reliably guarantee this for at least a number of years.

In order to meet this need for software in the field, several tools have been developed and released recently (see a list of SBML compatible tools at <http://www.sbml.org/index.psp/>). Most tools offer specific functionalities, e.g., stochastic simulations of reaction networks (see, for example, StochSim: Le Novère and Shimizu 2001) or flux analysis (FluxAnalyzer: Klamt et al. 2003). However, some tools contain whole suites of functionalities, e.g., simulation, flux and control analysis (ECell, SWB, Meng 2004).

Standard methods for simulations in this field comprise, e.g., the integration of ODEs and the stochastic simulation of trajectories on discrete particles basis (e.g., using Gillespie's algorithm (Gillespie 1976)).

In the following we would like to present simulation methods and topics related to this as implemented in a new tool - COPASI (COmplex PAthway SImulator) which combines different methods for the simulation, as well as for the analysis of biochemical reaction networks and is available for all major platforms (Linux, Mac OS, Windows, Solaris). As described below, COPASI supports non-expert users by, e.g., automatically converting reaction equations into the corresponding mathematical formalism, i.e., ODEs. Deterministic as well as stochastic simulations can be run on the same model by just selecting the respective method. In addition, in order to support the decision for the appropriate simulation method (Wolf et al. 1985), Lyapunov exponents can be computed for the model system.

2 GENERAL FEATURES

After the installation the user has the choice between two versions of COPASI: a full version with a graphical user interface (CopasiUI) and a smaller command line version (CopasiSE) that only contains the calculation engine. CopasiSE is useful in a number of situations. CopasiUI is the complete version of the program and is the one that we expect users to run most often as it provides a full graphical interface. In terms of execution of numerical procedures the two versions are almost equal, with CopasiUI being slightly slower if graphical output is requested; in practice the two share the same source code and are expected to produce exactly the same results.

COPASI's graphical interface is similar in operation to Windows Explorer, where there is a vertical window on the left with a set of functions organized in a hierarchical way; on the right there is a larger window that contains all of the controls to operate the function selected on the left. The major group of functions in the program are:

- Model, where the model can be edited and viewed according to a biochemical or mathematical perspective.
- Tasks, consisting of the major numerical operations on the model: steady state, time course, stoichiometry, and metabolic control analysis. Below each task an entry with results will appear after the task has been run.
- Multiple Tasks, which are operations repeating elementary tasks: parameter scanning, optimization, and parameter estimation.
- Output is where plots and reports are defined and listed.
- Functions containing the mathematical functions available such as the rate laws.

Model editing is done through tables (see Figure 4) and specialized widgets and the program provides various ways of editing the model items.

There are two major views of the model: a set of widgets provides a view from the biochemical perspective, where the model is composed of reactions, compartments, metabolites, etc.; while another provides a mathematical view, where the model is composed of variables and differential equations. The objective with this is that we expect different users to have different backgrounds and be more comfortable with one view or the other. This also provides a common software tool that may act as a translator of concepts for collaborators from different backgrounds.

COPASI's native file format is based on XML and documentation of its schema is available so that other tools can write (or read) it. COPASI can also read Gepasi files, providing backwards compatibility with its predecessor. Finally COPASI is able to import SBML either level 1 or level 2, and thus it can obtain models from many sources, such as other simulators, model databases, pathway databases, and so on (for a list of SBML compatible software see <http://www.sbml.org/>).

In terms of model output, COPASI is obviously capable of saving its own file format, which contains the model as well as all defined tasks (active or inactive). Models can be exported in SBML and the program can also write the ordinary differential equations in plain C files (ready to be included in other C/C++ programs) and in Berkeley Madonna's format (<http://www.berkeleymadonna.com/>), which is a popular program for nonlinear dynamics that does not import SBML.

COPASI can also output results of its various functions in two ways: report files and plots. Report files are defined by the user and can take arbitrary form. Plotting support is built-in and plots, like reports, can be defined in very flexible ways. COPASI supports x-y line plots and distribution histograms (a feature we do not find in other simulators), scales can be linear or log-transformed, and it allows zooming and panning. There could be an unlimited number of plots defined for a single work session and their definitions are saved with the rest of the tasks.

COPASI is able to calculate simple time courses either using a deterministic or a stochastic framework or using a hybrid method that we developed. For deterministic solutions, the LSODA integrator (Petzold 1983) is used, while for stochastic it is the Gibson-Bruck version (Gibson and Bruck 2000) of the Gillespie method (Gillespie 1976) (see Figure 1). The user can easily switch between one method and the other by a simple choice from a menu. Note that COPASI automatically converts chemical kinetic rate laws into their appropriate discrete stochastic equivalent versions (this is an optional feature that can be disabled). Another basic simulation function is the calculation of steady states, which is carried out by a combination of the damped Newton method and forward or backward integration (using LSODA). The steady state can also be characterized with linear stability analysis and metabolic control analysis (Fell 1997).

COPASI determines structural (stoichiometric) properties of the biochemical network. Mass conservation is calculated using the algorithm described by Vallabhajosyula, Chickarmane, and Sauro (2006) that uses Householder reflections (the cited paper actually states that COPASI uses Gauss elimination, but since its publication we have switched to this more efficient algorithm). Elementary flux modes, a unique set of the smallest possible sub-networks that still allow a steady state (Heinrich and Schuster 1996), are calculated using our implementation of the METATOOL algorithm (Pfeiffer et al. 1999).

Finally, COPASI is equipped with a number of optimization algorithms of various types, which can be used to minimize or maximize any variable (or function of variables) in the model. The optimization algorithms are also used for estimating parameter values that best fit a set of data provided by the user. To this end, COPASI allows the experimental data to be a mixture of time courses and steady states.

COPASI is available from its dedicated web site at <http://www.copasi.org/>. The program can be downloaded in executable format for four different architectures: MS Windows, OS X, Linux (Intel), and Solaris (SPARC). Since the first stable release the source code is also available on the web site and so the program can be compiled for several other architectures. COPASI is a stand-alone program and runs on computers without need

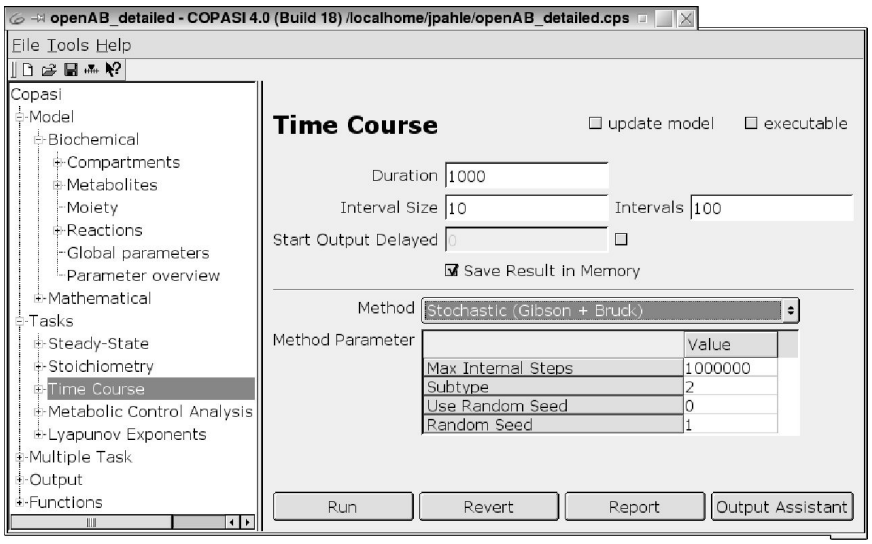

Figure 1: COPASI Trajectory Task Widget

for network connections once it has been installed (i.e., it is not a server nor requires connection to servers).

In the following we will discuss several issues concerning the simulation of biochemical networks, especially with the unique ability of Copasi to switch between different formalisms. We will exemplify these using a simple and abstract model for a biochemical reaction.

3 HYBRID SIMULATION ALGORITHM

A number of hybrid methods have been proposed that combine the accuracy of the stochastic simulations for parts of the system where needed and the speed of the deterministic simulation for the rest (see Haseltine and Rawlings 2002; Puchalka and Kierzek 2004; Kiehl, Mattheyses, and Simmons 2004; Salis and Kaznessis 2005; Alfonsi et al. 2005). They partition the reaction network into subnetworks and use appropriate stochastic or deterministic simulation methods on each of those subnets.

Our hybrid method combines the stochastic simulation algorithm by Gibson and Bruck (2000) (Next Reaction Method) with a numerical integration of ODEs (4th order Runge-Kutta, LSODA — see Petzold 1983). The biochemical network is dynamically partitioned into a deterministic and a stochastic subnet depending on the current particle numbers in the system. The stochastic subnet contains the reactions, where at least one low numbered species is involved. The remaining reactions, i.e., all those that only affect high numbered species, form the deterministic subnet. The user can define the limits for which particle numbers should be considered low or high. The two subnets are then simulated in parallel using the stochastic and deterministic solver respectively.

We settled on the simple partitioning criterion using particle numbers for three reasons. First, the amplitude of relative fluctuations of particle numbers are high in low-numbered species. Single reaction events can have a significant impact here. Reactions involving those species should therefore be handled stochastically. Second, most of the computational effort of stochastic simulation algorithms is spent on fast reactions. In order to speed up the simulation, fast reactions should be taken out of the stochastic subsystem and simulated deterministically. The higher the substrates' particle numbers the faster the reaction in mass action reactions and for some parts of the phase space for enzyme kinetics. Third, if only reactions involving high numbered species are simulated deterministically, the relative changes in particle numbers are minimal. For this reason, the change in reaction propensities in the stochastic subnet caused by the fast subnet during one stochastic step can be neglected. Our algorithm therefore approximates the influence of the deterministic subnet on the stochastic subnet during one step as constant, that means that the reaction propensities in the stochastic subnet are constant during this time interval.

Our hybrid algorithm is able to simulate models faster than pure stochastic methods, while taking random effects in the stochastic subnet into account. The dynamical partitioning is vital, e.g., for oscillating systems. However, the speed-up is very model-dependent. Therefore, we want to emphasize, that our algorithm should be considered experimental. Because of the computational overhead for partitioning the system the hybrid method can take even longer than pure stochastic methods in some cases. In addition, low-numbered species, which take part in fast reactions, slow the simulation down by forcing the fast reactions to be simulated stochastically. By using two distinct user-defined

limits for the particle numbers and a hysteresis-like updating scheme for the partitioning we avoid unnecessary and time consuming swaps, if particle numbers are fluctuating in a medium range.

ODEs describing biochemical networks are often stiff. In one variant of our hybrid method we therefore use the LSODA method (Petzold 1983), which is adequate for stiff systems, for the numerical integration of the deterministic subnetwork. We also implemented a hybrid solver, which uses a simple 4th order Runge Kutta method. Since the hybrid calculation requires many separate integrations of small time intervals, using a simple one-step solver, which is lacking the computational overhead of more sophisticated stiff-solvers, can be advantageous.

As an example, we investigate a simple open biochemical system where A reacts to B catalyzed by E. The corresponding reaction system is the following:

$$\begin{aligned} \rightarrow \quad & A \\ A + E \rightleftharpoons \quad & AE \\ AE \rightarrow \quad & B + E \\ B \rightarrow \quad & \end{aligned} \tag{1}$$

This corresponds to the following systems of equations:

$$\begin{aligned} A' &= k_1 - k_2 \cdot A \cdot E + k_3 \cdot AE \\ E' &= -k_2 \cdot A \cdot E + k_3 \cdot AE + k_4 \cdot AE \\ AE' &= k_2 \cdot A \cdot E - k_3 \cdot AE - k_4 \cdot AE \\ B' &= k_4 \cdot AE - k_5 \cdot B \end{aligned} \tag{2}$$

In Figure 2 we simulated this system using our hybrid algorithm and low particle numbers. The individual reactions are simulated stochastically or deterministically according to the actual particle numbers and the user defined limits.

4 HANDLING MICHAELIS-MENTEN TERMS IN STOCHASTIC SIMULATIONS

When stochastically simulating a reaction network which has been described by a set of ODEs all reaction rates have to be transferred to a corresponding reaction probability. This is rather simple and straightforward if mass action kinetics is assumed (Gillespie 1976). However, if kinetic terms like Michaelis-Menten kinetics are involved which in principal represent a lumping of terms each of which corresponds to an elementary reaction, the big question is if it is justifiable to use such a rate expression with stochastic simulations. There has been quite some discussions about this topic recently. However, several authors showed (Cao, Gillespie, and Petzold 2005; Rao and Arkin 2003) that as long as the initial assumptions for the assumed kinetics hold (e.g., excess substrate, fast reversible enzyme-substrate-complex formation), it is indeed justified to assume the enzymatic reaction to constitute one single step with the respective rate law.

Basically, the rate law which consists of a mass action part and a kinetic part, the latter of which also depends on reactant amounts and other factors, is assumed to freeze and to be constant for the single reaction event that is computed in each step of the stochastic computation. This rate then has to be computed anew for the next round of stochastic simulation.

Taking our example from above, the same system could also be described using the Michaelis-Menten form as long as the formation of AE is fast and reversible compared to product release and as long as there is a substantial surplus of substrate compared to enzyme. In this case the equations lump to the following ones:

$$\begin{aligned} A' &= k_1 - V_{max} \cdot \frac{A}{K_m + A} \\ B' &= -k_5 \cdot B + V_{max} \cdot \frac{A}{K_m + A} \end{aligned} \tag{3}$$

In Figure 3 we compare time series of the stochastically simulated elaborate system with the lumped system. We made sure that the assumptions for lumping the system hold. As can be easily seen, both trajectories correspond to each other.

5 REVERSIBLE REACTIONS IN STOCHASTIC SIMULATIONS

Generally stochastic simulation requires that reversible reactions are considered as separate forward and backward reactions, each irrevesible. In the deterministic modeling framework forward and backward flows can cancel each other out so that the reversible reaction rate can be given as a single mathematical expression (usually containing a difference between a forward and a backward term). In the stochastic modeling framework however each single forward or backward reaction event needs to be considered so that a reversible reaction has to be treated as two irreversible reactions. COPASI provides a tool that helps the user to do the necessary adjustments to the model. If this tool is used every reversible reaction is replaced by two corresponding irreversible reactions. If the reaction had a kinetic law of mass action type also the kinetic laws and the parameters of the newly created reactions are automatically adjusted. For other kinds of reactions the user will have to adjust the kinetics afterwards.

In Figure 4 we show the conversion of the reversible mass-action kinetics assumed for the formation of AE in the above example to the individual forward and backward reaction for use in the stochastic simulation.

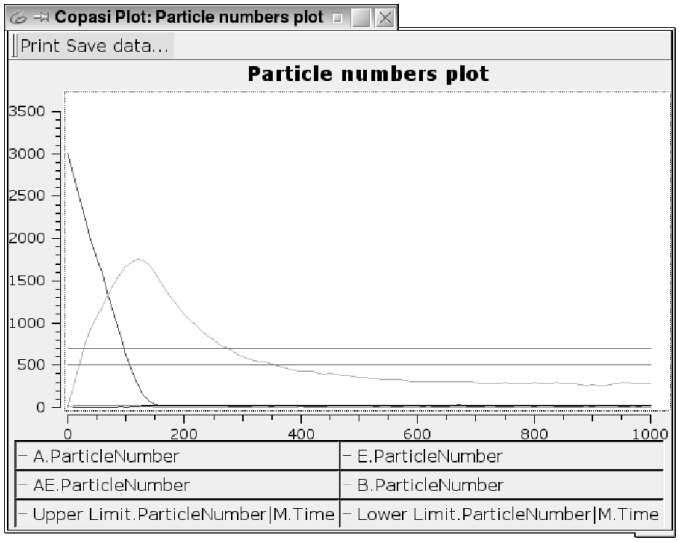

Figure 2: Result of the Hybrid Simulation of the System (2) in a Time Interval 10^3 *s*, for a Volume $5 \cdot 10^{-19}$ *ml*, Initial Concentrations $A(t_0) = 10$ *mMol/ml*, $E(t_0) = 0.1$ *mMol/ml*, $AE(t_0) = 0$ *mMol/ml*, $B(t_0) = 0$ *mMol/ml* and Parameters $k_1 = 0.01$ *mMol/(ml · s)*, $k_2 = 20$ *ml/(mMol · s)*, $k_3 = 10$ *1/s*, $k_4 = 1$ *1/s*, $k_5 = 0.01$ *1/s* (Lower and Upper Limits for the Particle Numbers are 500 and 700, Correspondingly).

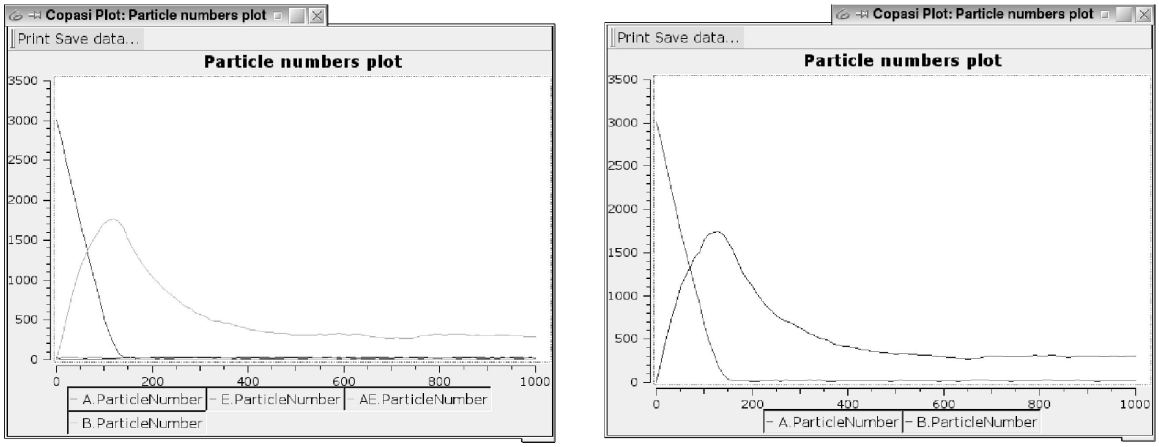

Figure 3: Results of Stochastic Simulations of the Detailed Equations (2)) and the lumped Equations (3)) System in the Time Interval 10^3 *s*, for the Volume $5 \cdot 10^{-19}$ *ml*, Initial Concentrations $A(t_0) = 10$ *mMol/ml*, $E(t_0) = 0.1$ *mMol/ml*, $AE(t_0) = 0$ *mMol/ml*, $B(t_0) = 0$ *mMol/ml* and Parameters $k_1 = 0.01$ *mMol/(ml · s)*, $k_2 = 20$ *ml/(mMol · s)*, $k_3 = 10$ *1/s*, $k_4 = 1$ *1/s*, $k_5 = 0.01$ *1/s*, $V_{max} = 0.1$ *mMol/(ml · s)* , $K_m = 0.1$ *mMol/ml*.

6 PARAMETER SCANS IN SIMULATIONS

Finally, we want to present the scanning feature of COPASI. A graphical interface is provided that allows easy access to the following features: parameter scans (a simulation is run several times, a parameter is changed for each run), repeated simulations (a simulation is repeated without any parameter changed, useful for stochastic simulations), or random parameter sampling (a parameter is set to a random value from a specified distribution, this allows Monte Carlo parameter scans).

Also these features can be nested arbitrarily. As an example it is possible to define a calculation where for each of 10 values of a kinetic parameter the stochastic simulation is repeated 100 times.

Figure 5 shows an example of COPASI scan task widget for the parameter k_4 of the model system (2). In Figure 6 we show multiple runs of the time course simulation of our

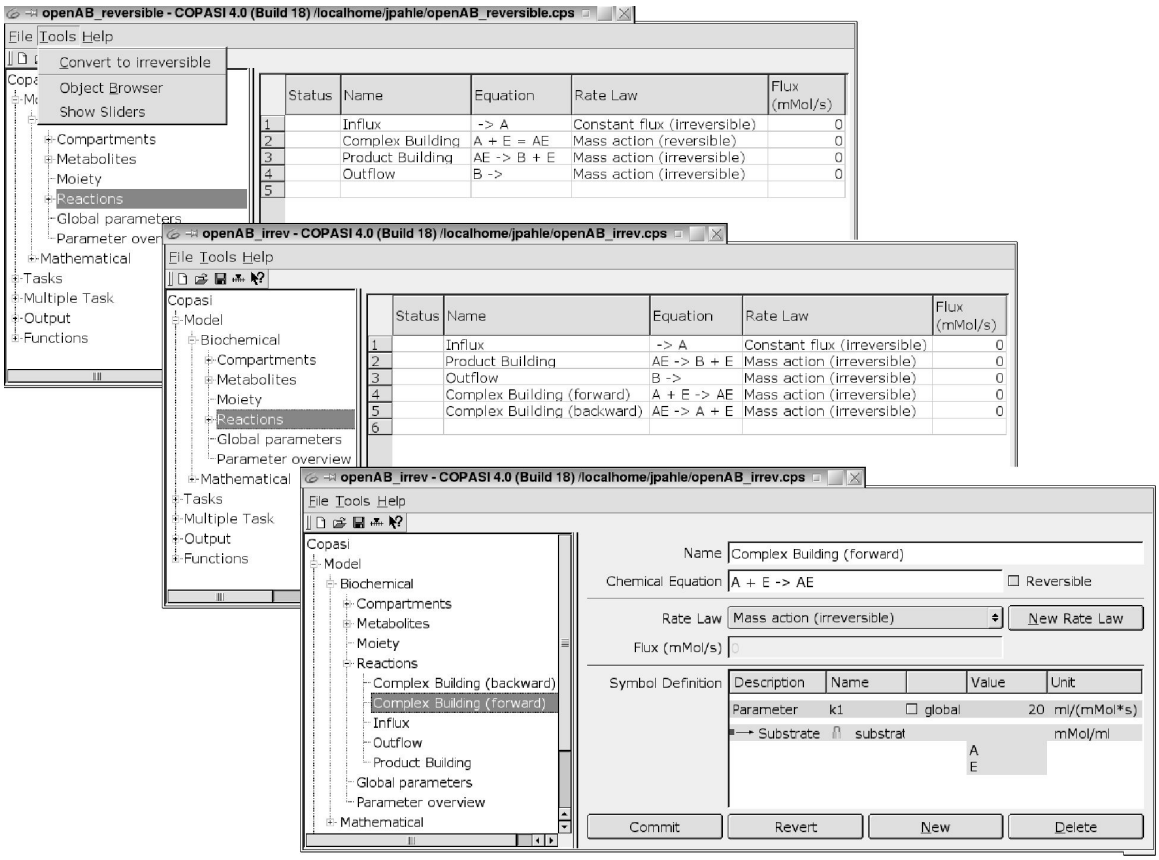

Figure 4: Application of the COPASI Menu Tool "Convert to Irreversible" to Our Model System (2).

simple example system which were performed due to one preset parameter scan.

7 CONCLUSIONS

We have presented COPASI, a new software tool for simulating and analyzing biochemical networks. We specifically discussed the simulation abilities of COPASI. COPASI enables the user to simply switch between different simulation methods, the numerical integration of ODEs, the stochastic simulation on discrete particle basis and the hybrid algorithm.

This ability is facilitated by several developments which were discussed in this article. The hybrid algorithm is a new development. Conversion of deterministic to stochastic models is supported by investigating the role of Michaelis-Menten terms and a tool that automatically converts reversible to irreversible reactions. Additionally, parameter scans enable the user to perform multiple runs without changing parameters manually.

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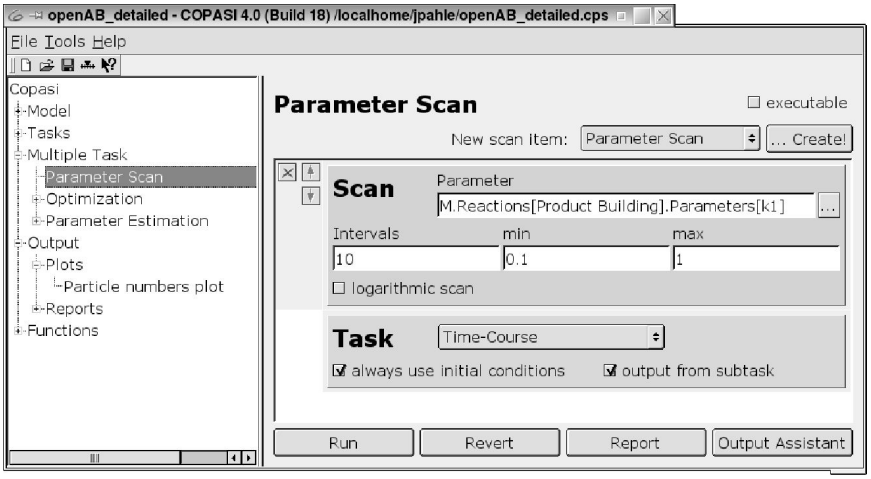

Figure 5: COPASI Scan Task Widget with Scan Item

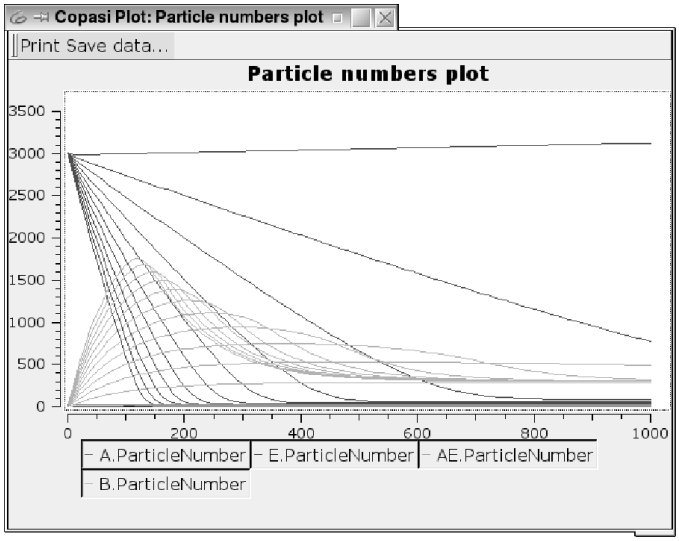

Figure 6: Result of a Parameter Scan for the Model System (2) for 10 Different Values of the Parameter k_4 within the Scan Interval $[0.1, 1]$.

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