

iBioSim: User's Manual

Chris J. Myers, Nathan Barker, Kevin Jones, Hiroyuki Kuwahara, Curtis Madsen, Nam Nguyen

Created: August 6th, 2008
Last Revised: March 1st, 2009

Contents

1	Introduction	3
2	Project Management	3
2.1	Creating and Opening Projects	4
2.2	Creating Models and Graphs	5
2.3	Importing Models	6
2.4	Editing Project Objects	7
2.5	Viewing Project Objects	8
2.6	Creating Tool Views	9
3	SBML Editor	10
3.1	SBML Math Formulas	11
3.2	Main Elements	12
3.2.1	Compartments	13
3.2.2	Species	14
3.2.3	Reactions	15
3.2.4	Global Parameters	17
3.3	Definitions/Types	18
3.3.1	Function Definitions	18
3.3.2	Unit Definitions	19
3.3.3	Compartment Types	20
3.3.4	Species Types	20
3.4	Initial Assignments/Rules/Constraints/Events	21
3.4.1	Initial Assignments	21
3.4.2	Rules	22
3.4.3	Constraints	22
3.4.4	Events	23
4	GCM Editor	24
4.1	Promoters	25
4.2	GCM Species	25
4.3	Influences	26
4.4	GCM Parameters	27
4.5	GCM Components	28
5	Analysis View	29
5.1	Simulation Options	29
5.2	Abstraction Options	31
5.3	Parameter Editor	32
6	Learn View	33
6.1	Data Manager	33
6.2	Learn Tool	33
7	TSD Graph Editor	36
8	Probability Graph Editor	39

9	Preferences	41
10	Genetic Circuit Model Format	42
11	Time Series Data Format	44
12	Tutorial	44
13	Reporting Bugs and Feature Requests	44
14	Credits	44

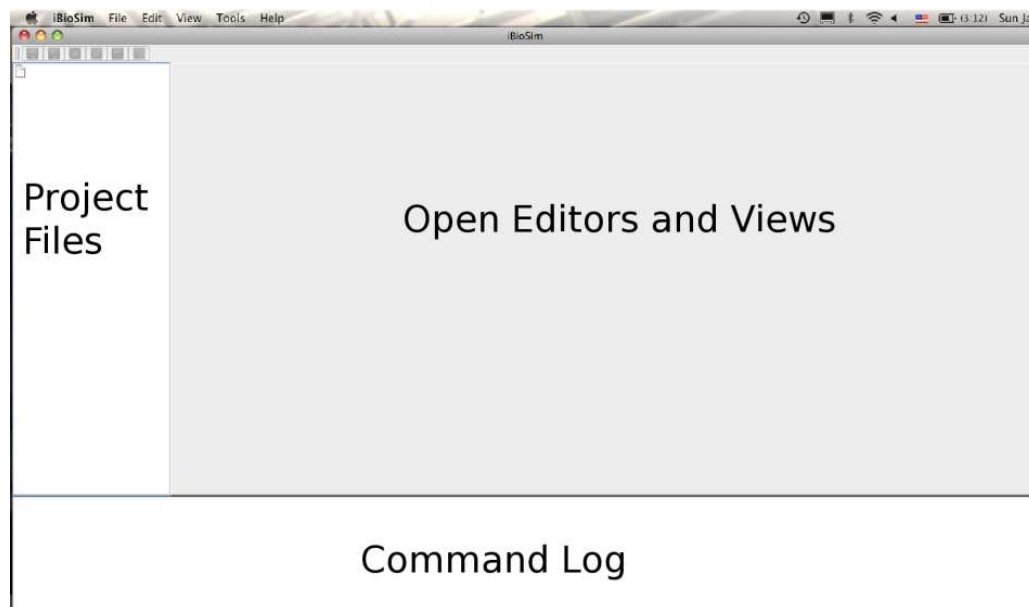
1 Introduction

iBioSim has been developed for the analysis of biochemical reaction network models. While the primary target of iBioSim is models of genetic circuits, models representing metabolic networks, cell-signaling pathways, and other biological and chemical systems can also be analyzed. iBioSim includes the following tools:

- GCM Editor - a tool to create a model using the *Genetic Circuit Model* (GCM) format. The GCM format is described in Nguyen's MS Thesis (UofUtah 2008).
- SBML Editor - a tool to create a model using the *Systems Biology Markup Language* (SBML).
- reb2sac - an abstraction-based ODE, Monte Carlo, and Markov analysis tool. This tool is described in Kuwahara's PhD Dissertation (UofUtah 2007).
- GeneNet - a tool to learn a GCM from *time series data* (TSD). This tool is described in Barker's PhD Dissertation (UofUtah 2007).
- TSD Graph Editor- a tool to visualize TSD files.
- Probability Graph Editor - a tool to visualize probability data.

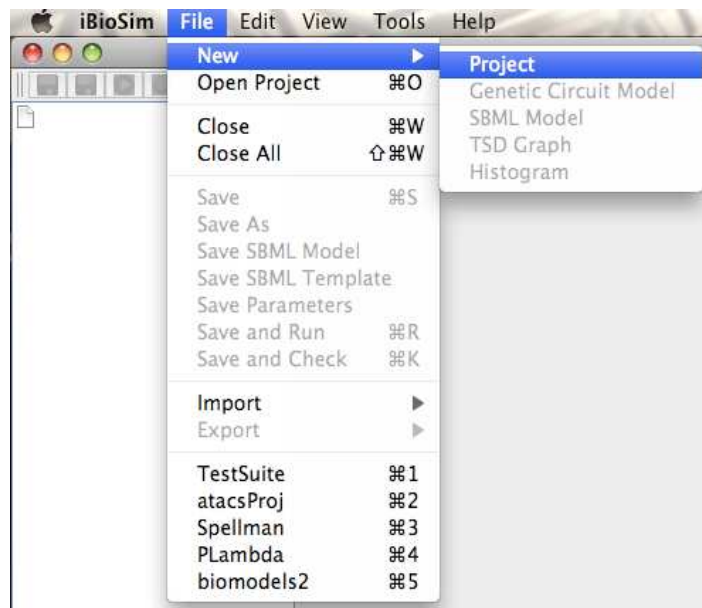
2 Project Management

A project is a collection of models, analysis views, learn views, and graphs. As shown below, iBioSim displays all project files on the left, the open models, views, and graphs on the right, and a log of all external commands on the bottom. The menu bar is located on the top of the window in the Windows and Linux versions. It is located on the top of the screen in the MacOS version.



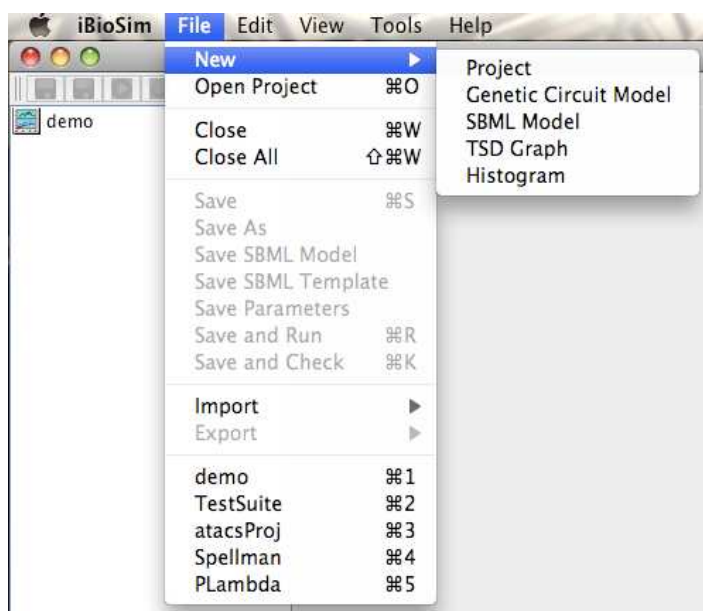
2.1 Creating and Opening Projects

To create a new project, select New → Project from the File menu as shown below. You will then be prompted to browse to a desired location and to give a name to the project directory. After you do this, click the new button and a new project directory will be created. To open a project, select Open → Project from the File menu. You will then be prompted to browse to a project directory to open, and clicking open will open the project. You may also open a project by selecting one of your five most recently opened projects by selecting the project name shown in the File drop down menu shown below.



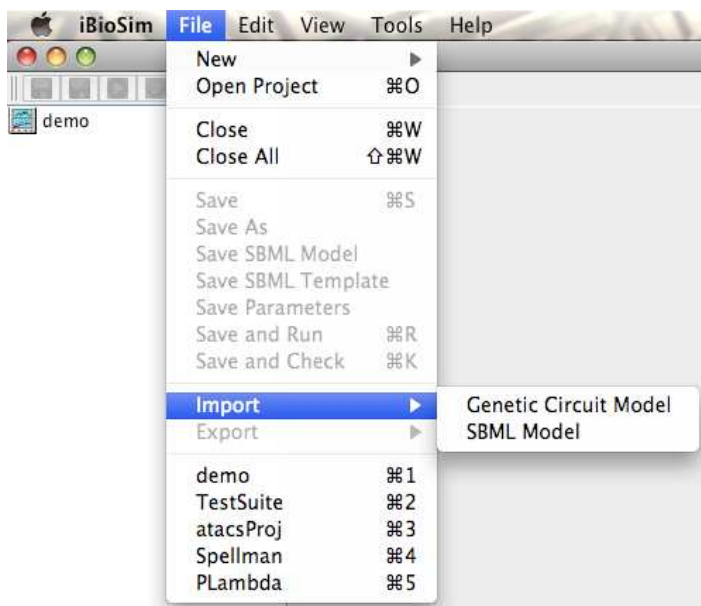
2.2 Creating Models and Graphs

After you have created or opened a project, you can create a new model or graph to add to the project. To create a new Genetic Circuit Model (see Section 10), select New → Genetic Circuit Model from the File menu as shown below. You will then be prompted to give a model id. At this point, a GCM editor (see Section 4) will open in a new tab. To create a new SBML model, select New → SBML Model from the File menu. You will then be prompted to give a model id. At this point, an SBML editor (see Section 3) will open in a new tab. To create a new TSD graph, select New → TSD Graph from the File menu. You will then be prompted to give a name to the TSD graph. At this point, a TSD graph editor (see Section 7) will open in a new tab. To create a new probability graph, select New → Probability Graph from the File menu. You will then be prompted to give a name to the probability graph. At this point, a probability graph editor (see Section 8) will open in a new tab. Once a model or graph is created, it can be opened again later by right clicking on the object in the project window and selecting “Edit”, or alternatively double-clicking on the object.



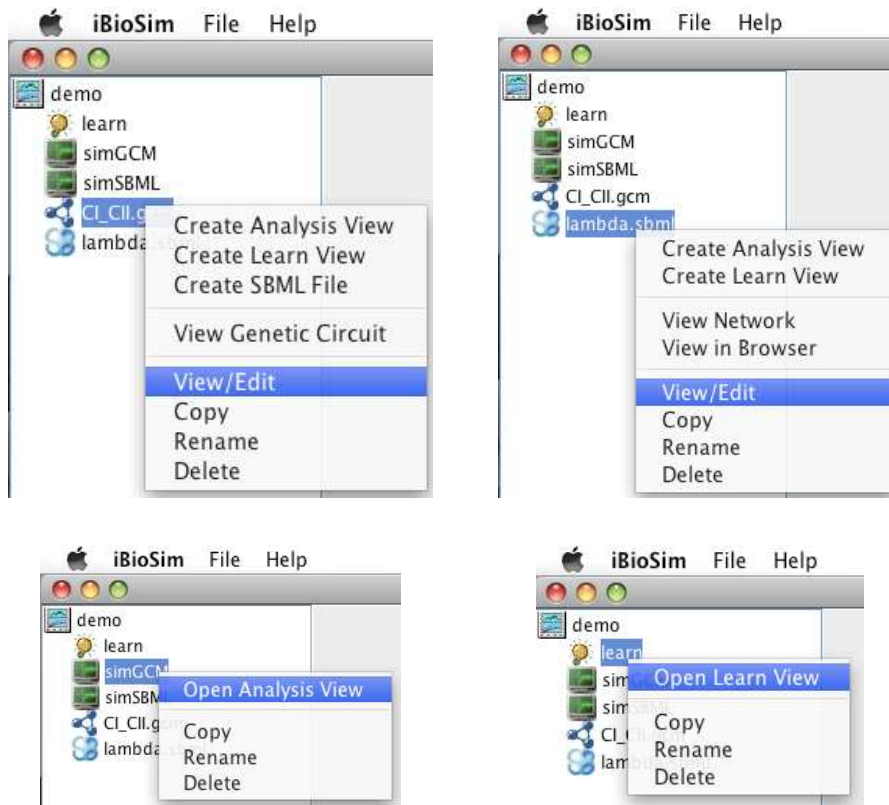
2.3 Importing Models

You can import into the current working project GCMs or SBML Models created by other programs or stored in other projects. To import a GCM, select Import → Genetic Circuit Model from the File menu as shown below. You will then be able to browse to find a model to import. After selecting the desired model, click the import button to bring the GCM into the project. Before bringing the model into the project, it will be checked to see if it is a valid GCM file. To import an SBML model, the procedure is the same except use the Import → SBML Model option. Before bringing the model into the project, it will be checked to see if it is a valid SBML file. The model will also be checked for consistency, and any errors or warnings will be reported. These should be corrected before analysis of the model is performed.



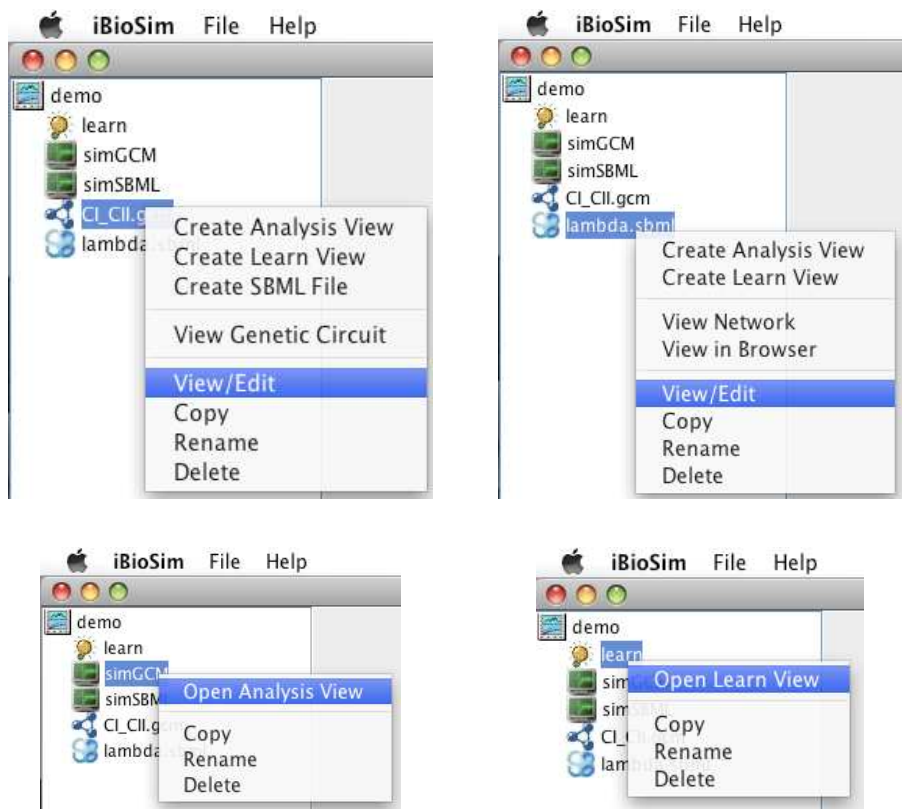
2.4 Editing Project Objects

All project objects can be modified by highlighting the object and using a right mouse click to open a menu of options as shown below. Using this menu, every type of object can be copied, renamed, or deleted (these actions can also be done from the Edit menu). For a GCM, the “View/Edit” option opens the model in a GCM editor (see Section 4). For an SBML model, the “View/Edit” option opens the model in an SBML editor (see Section 3). For a TSD graph, the “View/Edit” option opens the TSD graph in a TSD graph editor (see Section 7). For a probability graph, the “View/Edit” option opens the probability graph in a probability graph editor (see Section 8). For an analysis view, the “Open Analysis View” option opens the analysis view (see Section 5). For a learn view, the “Open Learn View” option opens the learn view (see Section 6).



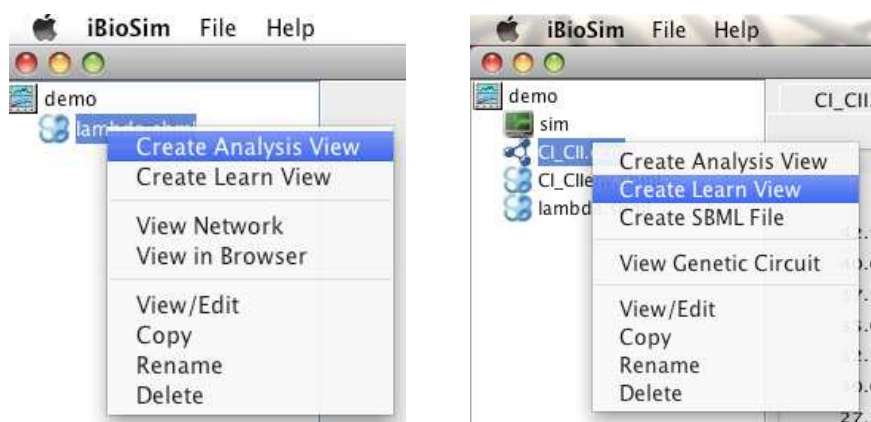
2.5 Viewing Project Objects

A GCM can also be viewed using GraphViz's dot program by right clicking on the model you want to view and selecting the "View Genetic Circuit" option. There are two additional ways to view an SBML model. You can either select the "View Network" option or the "View in Browser" option. The "View Network" option converts the model to a GraphViz file and then will open that file with GraphViz's dot program. The "View in Browser" option converts the model to an xhtml file and opens that file with your default xhtml browser. These options can also be reached using the View → Model sub-menu.



2.6 Creating Tool Views

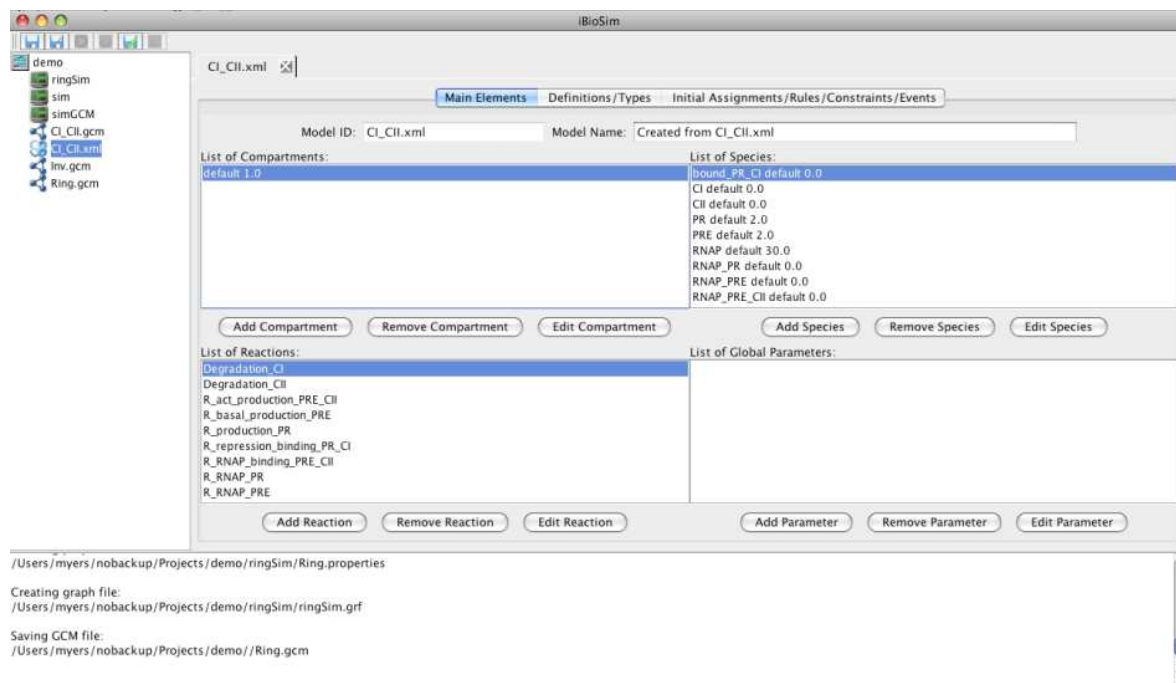
To perform analysis or learning, right click on a model and select “Create Analysis View” (see Section 5) to perform analysis or “Create Learn View” (see Section 6) to perform learning. You will then be prompted to give a name to your analysis or learn view. After a name is entered, a tab with the newly created view will open. Views can also be created using the Tools menu. Once a view is created, it can be opened again later by right clicking on an analysis directory and selecting “Open Analysis/Learn View” or alternatively double-clicking on the view.



When you create an analysis view from a GCM, an SBML model is automatically created for simulation and analysis. Within the analysis view, you can edit the initial concentrations and parameters. However, if you wish to be able to edit the structure, you should first create an SBML model using Create SBML Model option in the right click menu or the Save as SBML button in the GCM Editor (see Section 4). You can then open and edit this model using an SBML editor (see Section 3) and create an analysis view from this edited model.

3 SBML Editor

The SBML editor as shown below allows the user to create or modify an SBML model of a biochemical reaction network. An SBML model includes compartments (see Section 3.2.1), species (see Section 3.2.2), reactions (see Section 3.2.3), parameters (see Section 3.2.4), function definitions (see Section 3.3.1), unit definitions (see Section 3.3.2), compartment types (see Section 3.3.3), species types (see Section 3.3.4), initial assignments (see Section 3.4.1), rules (see Section 3.4.2), constraints (see Section 3.4.3), and events (see Section 3.4.4). Each of these items can be added, removed, or edited. To add a new item, click on the appropriate add button. You will then be prompted to provide a unique id and some properties for this new item (as described below). After you have filled out all of the required fields, click add and the new item will be added to the SBML model. To remove an item from the model, select that item and click the remove button. The item will then be removed from the model. However, if you try to remove an item that is being used (for example, a species that is used in a reaction), you will first have to remove its use. To edit an existing item, select that item from the list and click the edit button. An editing window will open and you will be able to change the properties of that item. When you are done editing this item, click save to save the changes to the item. After the model is complete, press disk icon to save the SBML model. The disk with a check mark icon saves and checks the model for consistency. Note that many checks are done on the fly, so it should be difficult to create models with consistency problems. However, if a user does not wish to be warned about undeclared units or does not wish to have units checked at all can set preferences to turn this off (see Section 9). The save and check button though will still present all the unit warnings and errors. Finally, the disk with a pencil icon can be used to store the model, but in this case, a new model ID will be requested and the model will be saved using that ID.



3.1 SBML Math Formulas

Math formulas appear in many SBML constructs. These formulas are expressed as text strings using a simple C-like syntax. SBML math formulas can include:

- Variables (compartment, species, parameter IDs, and reaction IDs)
- Real Numbers
- Built-in constants: `exponentiale`, `pi`, `true`, and `false`.
- Special variable `time` or `t` which returns the current simulation time.
- Mathematical operators including add (+), subtract (-), multiply (*), divide (/), and power (x^y) which is equivalent to `pow(x,y)`.
- A function defined in the list of function definitions.
- Logical functions: `and`, `or`, `xor`, `not`.
- Relational functions: `eq`, `neq`, `geq`, `gt`, `leq`, and `lt`.
- Unary functions: `abs`, `ceiling`, `exp`, `factorial`, `floor`, `ln`, `log`, `sqr`, and `sqrt`.
- Trigonometric functions: `cos`, `cosh`, `sin`, `sinh`, `tan`, `tanh`, `cot`, `coth`, `csc`, `csch`, `sec`, `sech`, `arccos`, `arccosh`, `arcsin`, `arsinh`, `arctan`, `arctanh`, `arccot`, `arccoth`, `arccsc`, `arccsch`, `arcsec`, and `arcsech`.
- The `delay(expr1,expr2)` function which returns the value of `expr1` at a time `expr2` time units earlier (not currently supported by analysis).
- The `piecewise(value1, case1, value2, case2, ..., otherwise)` function returns `value1` if `case1` is true, `value2` if `case2` is true, etc. If no cases are true, it returns `otherwise` value.

iBioSim's simulators also support several random functions which are added by default to any SBML file created with its SBML editor. The following random functions, therefore, can also be used in SBML math formula:

- Continuous random functions: `uniform(a,b)`, `normal(m,s)`, `exponential(mu)`, `gamma(a,b)`, `log-normal(z,s)`, `chisq(nu)`, `laplace(a)`, `cauchy(a)`, and `rayleigh(s)`.
- Discrete random functions: `poisson(mu)`, `binomial(p,n)`, and `bernoulli(p)`.

3.2 Main Elements

This Main Elements tab shown below is used to specify compartments (see Section 3.2.1), species (see Section 3.2.2), reactions (see Section 3.2.3), and parameters (see Section 3.2.4). This tab also includes the Model ID which is fixed to be the same as the filename as well as the Model Name which can be used to provide an arbitrary string description of the model.

The screenshot shows a software window titled 'CI_CII.xml' with a tabbed interface. The 'Main Elements' tab is active, showing fields for 'Model ID' (CI_CII.xml) and 'Model Name' (Created from CI_CII.xml). Below these are four lists: 'List of Compartments' (containing 'default 1.0'), 'List of Species' (containing 'bound_PR_CI default 0.0', 'CI default 0.0', 'CII default 0.0', 'PR default 2.0', 'PRE default 2.0', 'RNAP default 30.0', 'RNAP_PR default 0.0', 'RNAP_PRE default 0.0', and 'RNAP_PRE_CII default 0.0'), 'List of Reactions' (containing 'Degradation_CI', 'Degradation_CII', 'R_act_production_PRE_CII', 'R_basal_production_PRE', 'R_production_PR', 'R_repression_binding_PR_CI', 'R_RNAP_binding_PRE_CII', 'R_RNAP_PR', and 'R_RNAP_PRE'), and 'List of Global Parameters' (empty). Each list has associated 'Add', 'Remove', and 'Edit' buttons.

Model ID	Model Name
CI_CII.xml	Created from CI_CII.xml

List of Compartments:

default 1.0

Add Compartment Remove Compartment Edit Compartment

List of Species:

bound_PR_CI default 0.0
CI default 0.0
CII default 0.0
PR default 2.0
PRE default 2.0
RNAP default 30.0
RNAP_PR default 0.0
RNAP_PRE default 0.0
RNAP_PRE_CII default 0.0

Add Species Remove Species Edit Species

List of Reactions:

Degradation_CI
Degradation_CII
R_act_production_PRE_CII
R_basal_production_PRE
R_production_PR
R_repression_binding_PR_CI
R_RNAP_binding_PRE_CII
R_RNAP_PR
R_RNAP_PRE

Add Reaction Remove Reaction Edit Reaction

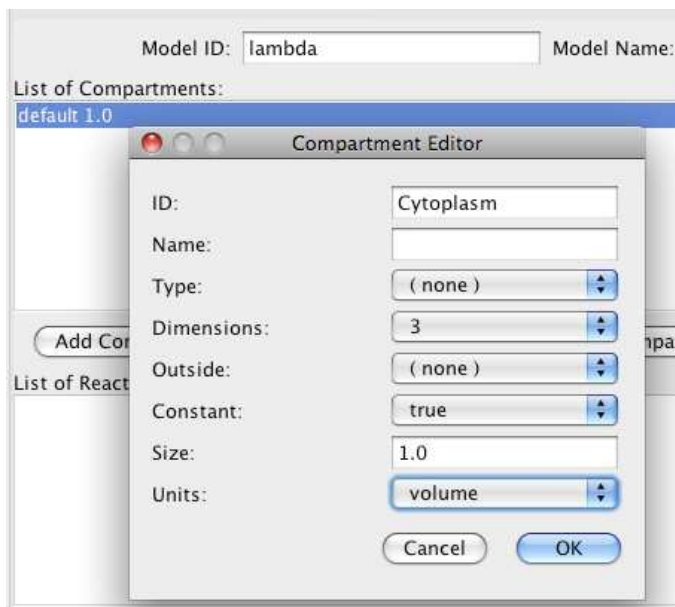
List of Global Parameters:

Add Parameter Remove Parameter Edit Parameter

3.2.1 Compartments

Compartments are used to specify locations where species are found. Every model must include at least one compartment. A new model includes a compartment named “default” that cannot be removed unless a new compartment is provided. A compartment to which species have been assigned also cannot be removed. As shown below, a compartment has the following fields:

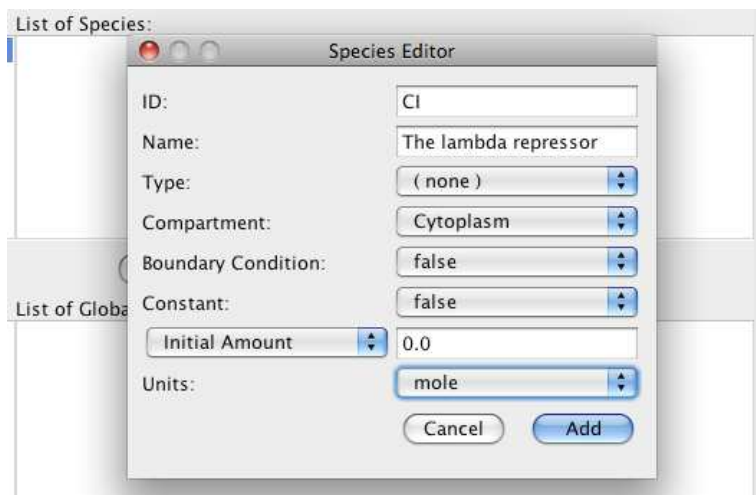
- ID: a unique ID composed of only alphanumeric characters and underscores.
- Name: an arbitrary string description (optional).
- Type: selected from the list of compartment types (default=none).
- Dimensions: number of spatial dimensions (default=3).
- Outside: the compartment that is outside this compartment (default=none).
- Constant: Boolean indicating if the size is constant (default=true).
- Size: initial size of the compartment (default=1.0).
- Units: the units for the size (default=none).



3.2.2 Species

Species are the molecules that appear as reactants, products, or modifiers in the reactions in the biochemical reaction network. As shown below, a species has the following fields:

- ID: a unique ID composed of only alphanumeric characters and underscores.
- Name: an arbitrary string description (optional).
- Type: selected from the list of species types (default=none).
- Compartment: location of the species (default=default).
- Boundary Condition: Boolean indicating if the species amount/concentration cannot be changed by reactions (default=false).
- Constant: Boolean indicating if the species amount/concentration is constant (default=false).
- Initial Amount/Concentration: initial value of the amount or concentration of the species. Whether it is an amount or concentration can also be selected (default=amount/0.0).
- Units: the units for the amount/concentration (default=none).



3.2.3 Reactions

Reactions are used to create or destroy molecular species in a biochemical reaction network. As shown below, a reaction is composed of the following:

- ID: a unique ID composed only of alphanumeric characters and underscores.
- Name: an arbitrary string description (optional).
- Reversible: a Boolean indicating if the reaction is reversible (default=false).
- Fast: a Boolean indicating if the reaction is fast (default=false).
- List of Reactants: species that are consumed by this reaction.
- List of Products: species that are produced by this reaction.
- List of Modifiers: species that are neither produced or consumed by this reaction.
- List of Local Parameters: symbolic values that can be used in the kinetic law or stoichiometry math formulas for this reaction.
- Kinetic law: an SBML math formula (see Section 3.1) describing the rate or probability for this reaction.

When adding a reactant or product, the user must specify a species ID and the stoichiometry (i.e., the number of molecules produced or consumed by the reaction). The stoichiometry can also be expressed as a stoichiometry math formula. Each parameter is composed of an ID, Name, Value, and Units. The list of parameters begins with a default forward reaction rate (kf) and reverse reaction rate (kr). These names and their values should likely be edited. The kinetic law can either be automatically generated using the Use Mass Action button or manually entered. The “Use Mass Action” button creates a rate law using the law of mass action. It assumes that the first parameter in the list is the forward reaction rate and the second parameter in the list is the reverse reaction rate. The “Clear” button clears the kinetic law editor. The stoichiometry math and kinetic law formulas can only include those species that appear as reactants, products, or modifiers.

Reaction Editor

ID: Dimerize_Cl Name: Reaction to dimerize Cl Reversible: true Fast: false

List Of Reactants: List Of Products: List Of Modifiers:

Add Reactant Remove Reactant Edit Reactant Add Product Remove Product Edit Product Add Modifier Remove Modifier Edit Modifier

List Of Local Parameters: Kf 0.1 kr 1.0

Kinetic Law:

Add Parameter Remove Parameter Edit Parameter Use Mass Action Clear Cancel Add

List Of Reactants: Reactants Editor

Species: Cl

Stoichiometry Math: nd

Cancel Add

Add Reactant Remove Reactant Edit Reactant

List Of Products: Products Editor

Species: Cl2

Stoichiometry: 1

Cancel Add

Add Product Remove Product Edit Product

Parameter Editor

ID: k2f

Name: Dimerization rate

Value: 0.1

Units: per_second_mole

Cancel OK

Add Parameter Remove Parameter Edit Parameter

Kinetic Law:

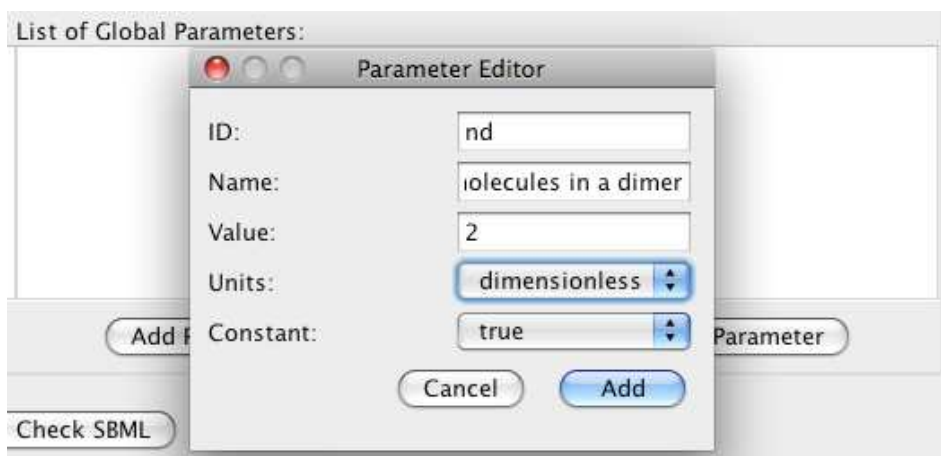
$$k2f * \text{pow}(\text{Cl}, \text{nd}) - k2r * \text{Cl2}$$

Use Mass Action Clear

3.2.4 Global Parameters

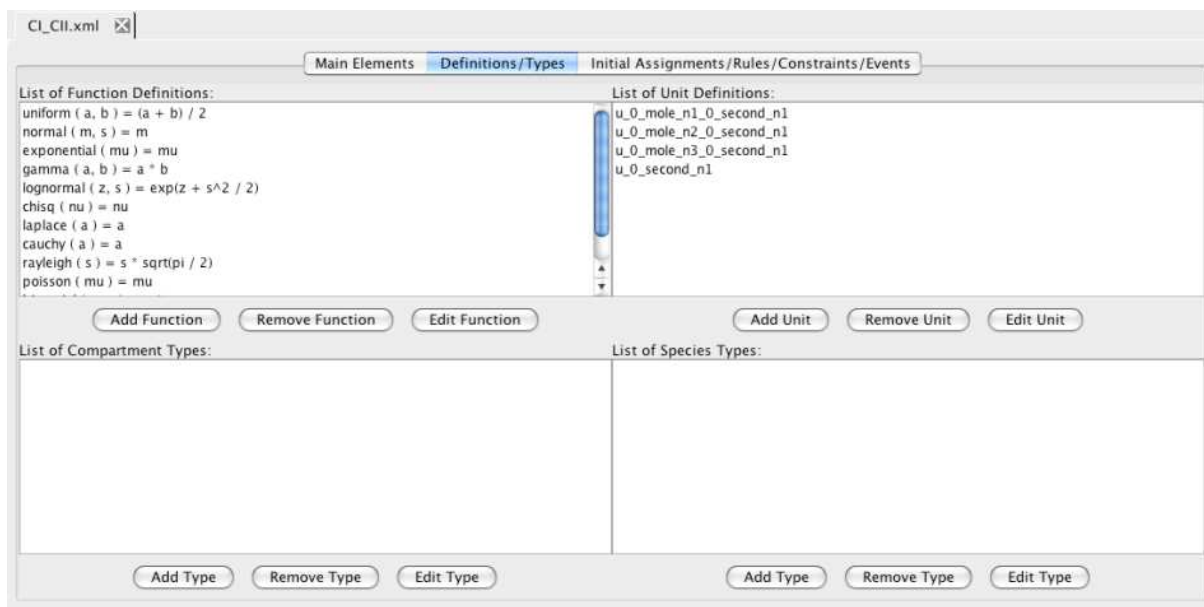
Global parameters are variables that can be used in SBML math formulas (see Section 3.1). As shown below, a parameter includes the following:

- ID: a unique ID composed only of alphanumeric characters and underscores.
- Name: an arbitrary string description (optional).
- Value: initial value for the parameter.
- Units: the units for the parameter value (default=none).
- Constant: Boolean indicating if the parameter value is constant (default=true).



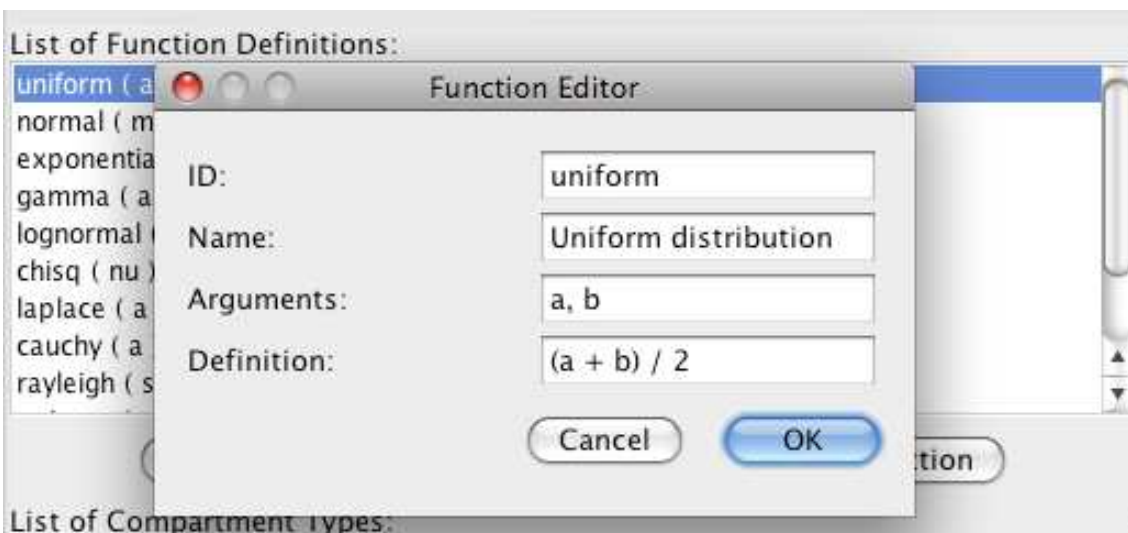
3.3 Definitions/Types

The Definitions/Types tab shown below allows users to provide function definitions (see Section 3.3.1), unit definitions (see Section 3.3.2), compartment types (see Section 3.3.3), and species types (see Section 3.3.4).



3.3.1 Function Definitions

Function definitions are used to create user defined functions that can then be used in SBML math formulas (see Section 3.1). As shown below, function definitions include an ID, an optional name field, a comma-separated list of arguments, and its definition. The definition is an SBML math formula though it is restricted to only use variable names which are arguments to the function. While functions can call other functions, they cannot be recursive (i.e., call themselves) either directly or indirectly (i.e., through a cycle of function calls). As mentioned earlier, several random functions supported by iBioSim's simulators are added automatically.



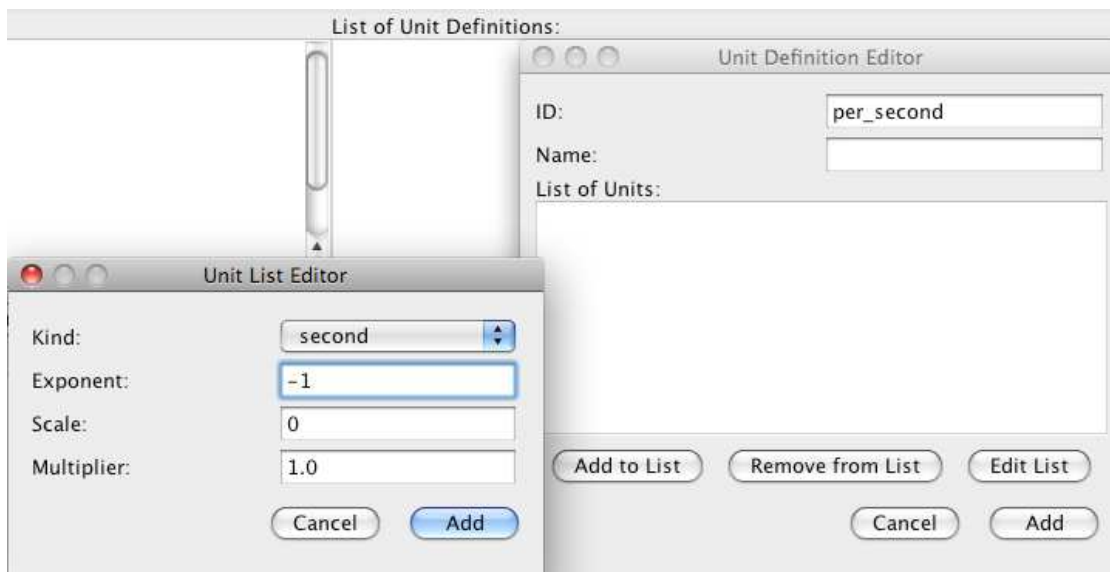
3.3.2 Unit Definitions

Unit definitions are used to construct user-defined units which are derived from the set of base units. As shown below, a unit definition includes an ID, an optional name, and a list of units that define it. There are buttons to add, remove, and edit elements in the list of units. Each unit is composed of a kind, exponent, scale, and multiplier. The kind is selected from the list of base units in the table below:

ampere	gram	katal	metre	second	watt
bacquerel	gray	kelvin	mole	siemens	weber
candela	henry	kilogram	newton	sievert	
coulomb	hertz	litre	ohm	steradian	
dimensionless	item	lumen	pascal	tesla	
farad	joule	lux	radian	volt	

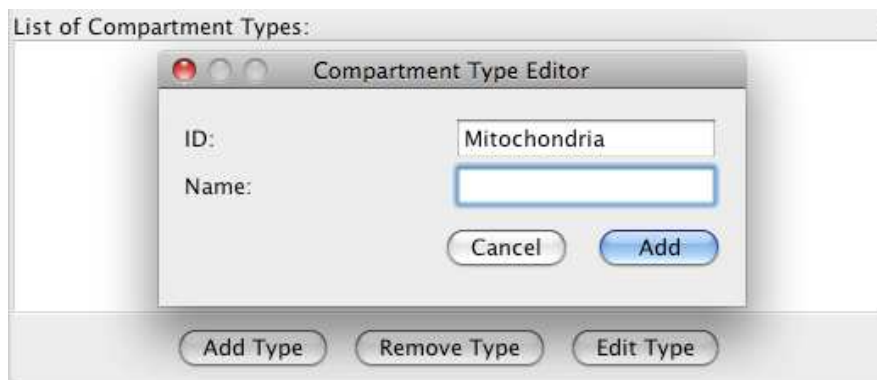
The exponent and scale are integers, and the multiplier is a real number that specifies the relationship between the derived unit and the base unit using the relation below:

$$\text{unit} = (\text{multiplier} * 10^{\text{scale}} * \text{baseUnit})^{\text{exponent}}$$



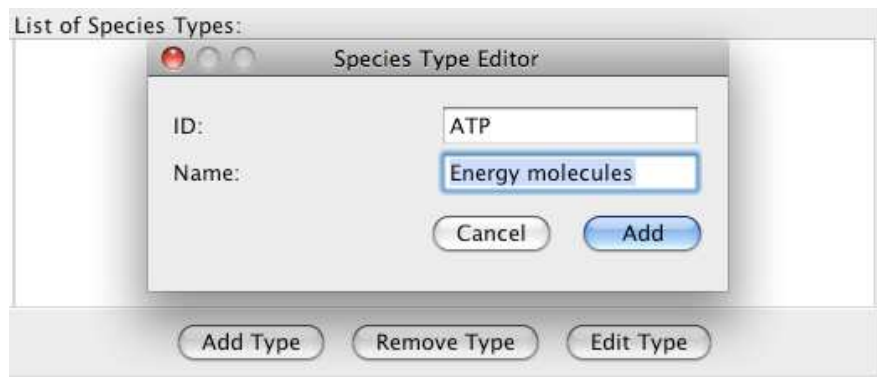
3.3.3 Compartment Types

Compartment types are used to relate multiple compartments. As shown below, a compartment type includes an ID and an optional name field.



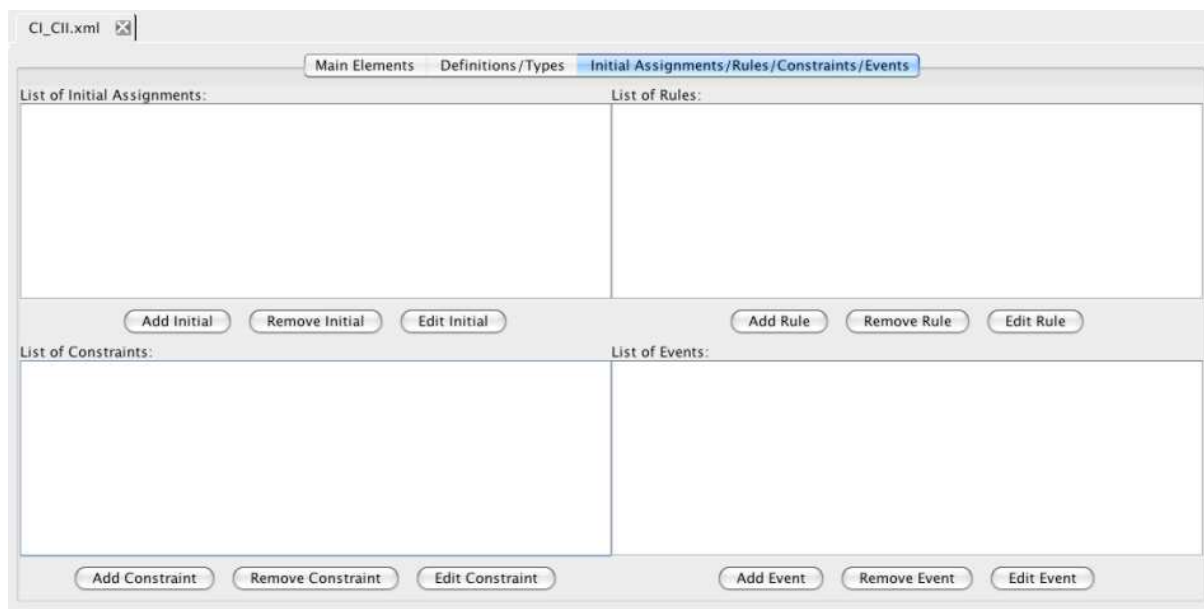
3.3.4 Species Types

Species types are used to relate multiple species. As shown below, a species type includes an ID and an optional name field.



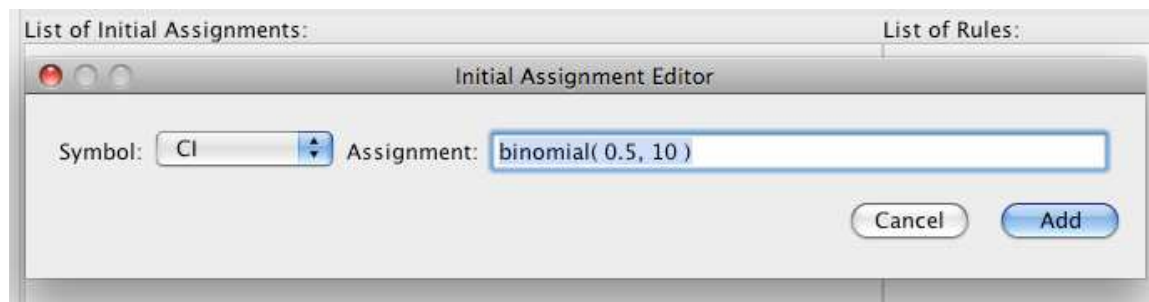
3.4 Initial Assignments/Rules/Constraints/Events

The Initial Assignments/Rules/Constraints/Events tab as shown below allows users to provide initial assignments (see Section 3.4.1), rules (see Section 3.4.2), constraints (see Section 3.4.3), and events (see Section 3.4.4).



3.4.1 Initial Assignments

Initial assignments as shown below are used to provide an SBML math formula (see Section 3.1) that is evaluated at time 0 to determine the initial value of a compartment size, species amount or concentration, or parameter. The value of this formula takes precedence over the initial value specified in the object.



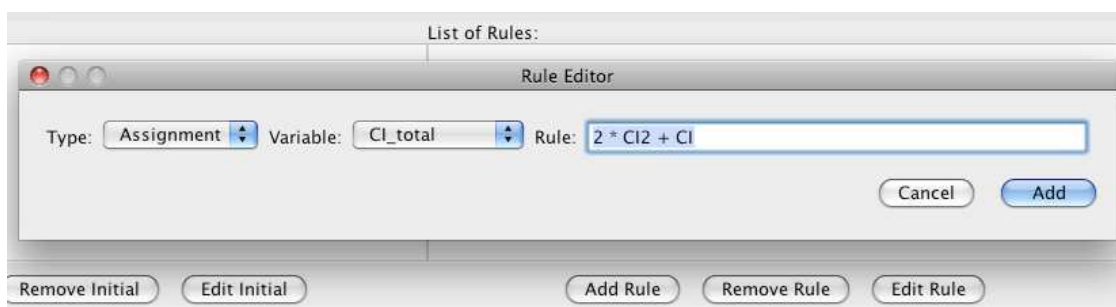
3.4.2 Rules

There are three types of rules: algebraic, assignment, and rate rules which are in the following form:

Algebraic	left-hand side is zero	$0 = f(W)$
Assignment	left-hand side is a scalar	$x = f(W)$
Rate	left-hand side is a rate-of-change	$\frac{dx}{dt} = f(W)$

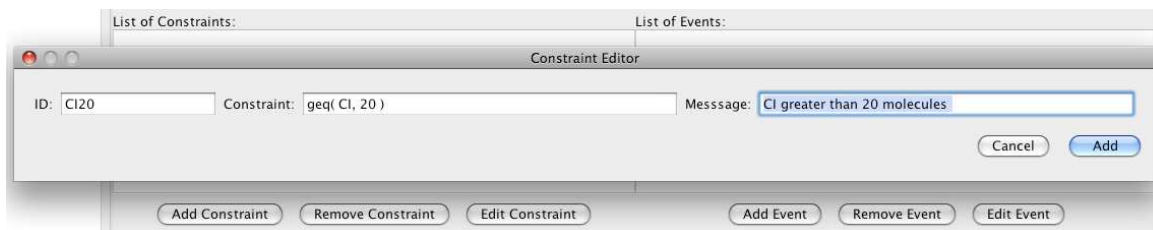
Algebraic rules specify relationships which must be maintained (not currently supported by analysis). Assignment rules specify the value of a compartment size, species amount or concentration, or parameter in terms of an SBML math formula (see Section 3.1). A variable cannot be determined by both an assignment rule and initial assignment. Rate rules specify the rate of change of a compartment size, species amount or concentration, or parameter in terms of an SBML math formula (see Section 3.1). A variable cannot be determined by both an assignment rule and a rate rule. A species that is a reactant or a product of any reaction cannot be updated by either an assignment rule or rate rule.

When adding a rule, the user first selects the type of rule as shown below. This will automatically restrict the set of variables available for the left-hand side to those that are valid. The user should then select a variable, and enter an SBML math formula (see Section 3.1) for the rule. When editing a rule, the user cannot modify the rule type.



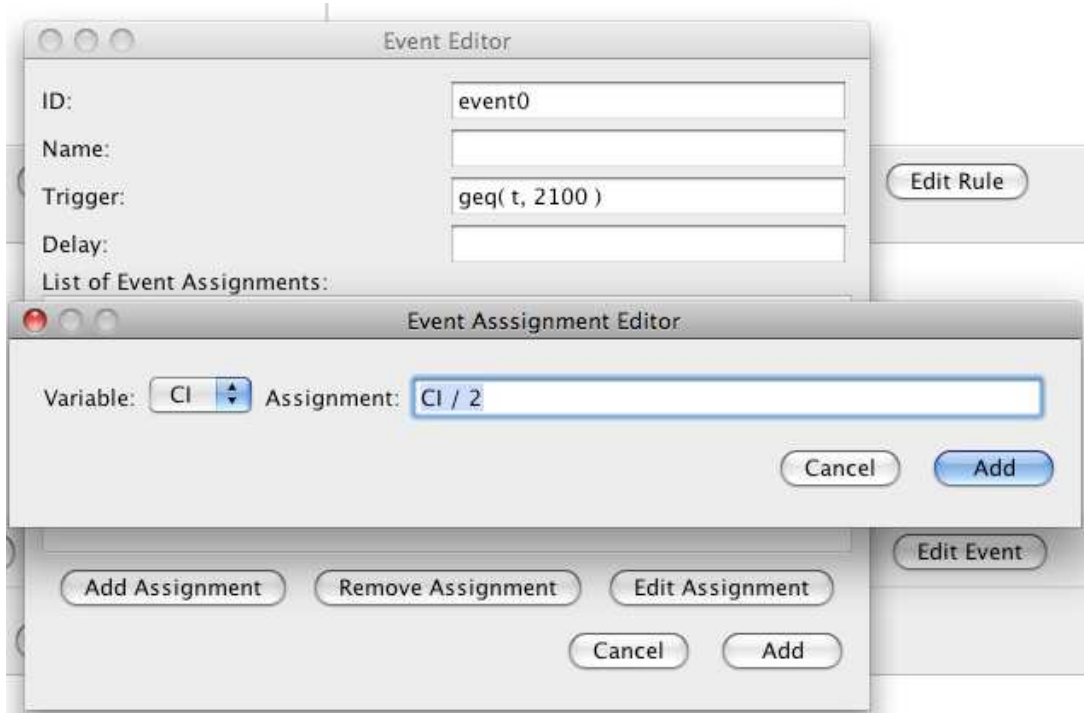
3.4.3 Constraints

Constraints are used to specify properties that should cause simulation to terminate. Our analysis method can provide histograms that show the proportion of simulations that are terminated due to each possible constraint. As shown below, each constraint is composed of an ID which is used to identify it in these histograms, a constraint given as an SBML math formula (see Section 3.1), and a message describing the constraint. A default id is automatically generated when a new constraint is created.



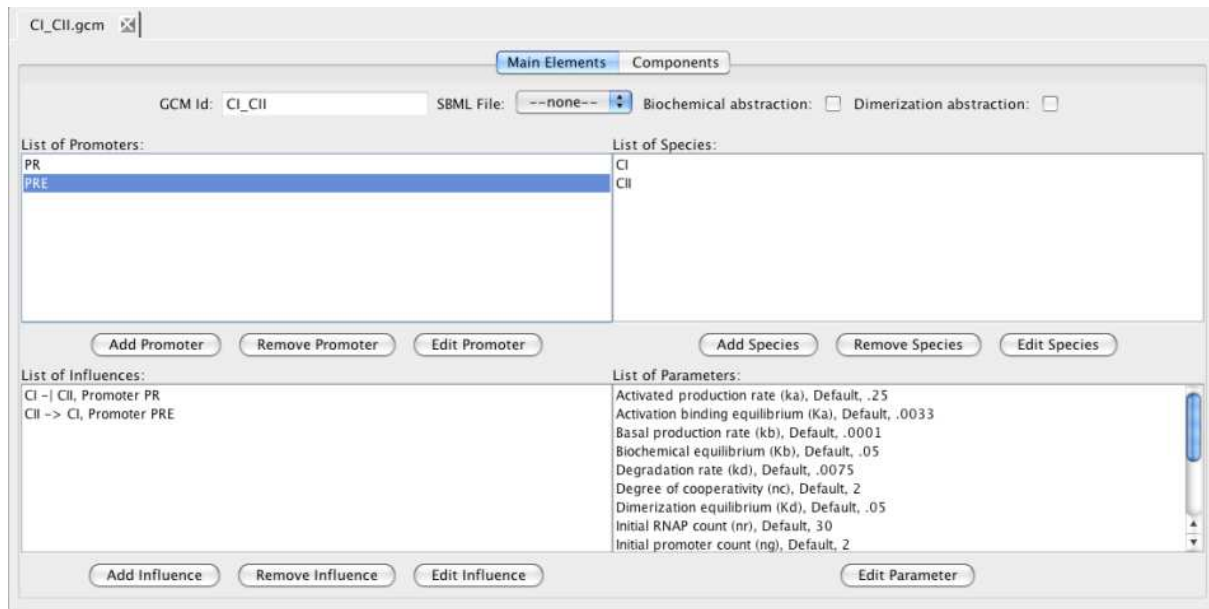
3.4.4 Events

Events are used to specify discrete changes of compartment sizes, species amounts or concentrations, and parameter values. As shown below, each event is composed of an ID, an optional name, a trigger formula, an optional delay formula, and a list of event assignments. When adding a new event, a default ID is provided. The behavior of an event is that during each simulation cycle, the trigger formula is evaluated. If it was false in the previous simulation cycle, and it is now evaluating to true, the event is scheduled to occur at a time in the future specified by the delay formula or immediately if no delay formula is provided. It should be noted that since the trigger value must change from false to true, no event is scheduled if the trigger evaluates to true at the start of simulation. When an event occurs, it executes all the event assignments. Each event assignment sets a compartment size, species amount or concentration, or parameter value to the value specified by the SBML math formula (see Section 3.1) provided with the event assignment.



4 GCM Editor

The GCM editor shown below allows the user to create or modify a GCM (see Section 10). A GCM is a compact graphical representation of a genetic circuit which can later be synthesized into an SBML model. A GCM includes promoters (see Section 4.1), GCM species (see Section 4.2), influences (see Section 4.3), GCM parameters (see Section 4.4), and an optional SBML file. GCM species, influences, and promoters can be added, removed, or edited. Parameters can only be edited. An SBML file can also be selected to merge with the SBML generated from a GCM. This allows either customization of the SBML model or the addition of SBML constructs such as Initial Assignments, Rules, Constraints, and Events (see Section 3.4). Finally, a GCM can be constructed out of other GCM components which are listed under the Components tab.



To add a new item to the GCM, click on the appropriate add button. You will then be prompted to input information regarding the new item. After you have filled out the required fields, click on ok and the new item will be added into the GCM. To remove an item from the GCM, select that item and click the remove button. The item will then be removed from the GCM. However, if you try to remove species or promoters that are used in an influence, you will first have to remove the influence in order to remove the species or promoter from the model. To edit an existing item, select that item from the list and click the edit button. An editing window will open and you will be able to change the properties of that item. When you are done editing this item, click save to save the changes to the item. To merge an existing SBML file with the GCM output, click on the SBML file and select the SBML file to use. This will merge the contents of the selected SBML file with the SBML file that is generated from the GCM. Finally, there are two abstractions that can be selected to be performed when generating SBML, the biochemical and dimerization abstractions.

Once a GCM is completed, the user can save the GCM or save the GCM using a new name. The user can also save the GCM as SBML which creates an SBML file of the same name as the GCM. Finally, the user can save an SBML template which creates a blank SBML file with the same species as the GCM. This is useful for creating an SBML file which will be attached to the GCM, and includes rules, constraints, or events.

4.1 Promoters

Promoters are special species which represent the region of the DNA from which transcription is initiated. When adding or editing promoters, the user must supply a unique ID. An optional name can also be provided which is an arbitrary string description for the promoter. If desired, the user can then modify the initial promoter count (ng), the RNAP binding equilibrium (K_o), the open complex production rate (ko), the stoichiometry of production (i.e., the number of transcripts per mRNA, np), the basal production rate (kb), or the activated production rate (ka).

Promoter Editor

List of Promoters: | List of Species:

ID: PR

Name:

Initial promoter count (ng): default 2

RNAP binding equilibrium (K_o): default .033

Open complex production rate (ko): default .05

Stoichiometry of production (np): default 10

Basal production rate (kb): default .0001

Activated production rate (ka): default .25

Cancel Ok

4.2 GCM Species

GCM species are the molecules (usually proteins) produced by genes. When adding or editing a species, the user must provide a unique ID. The user can also select the type of the species to be normal, constant, or unconstrained. A normal species will result in gene production and degradation reactions being produced. A constant species will not generate any production or degradation reactions. An unconstrained species will produce a constant production and degradation reaction. The user can also specify an initial species count (ns), a Dimerization equilibrium (K_d), and a degradation rate (kd).

Species Editor

List of Species: | List of Species:

ID: CI

Name: The lambda repressor

Type: normal

Initial species count (ns): default 0

Dimerization equilibrium (K_d): default .05

Degradation rate (kd): default .0075

Cancel Ok

4.3 Influences

Influences describe the relationships between the GCM species. When adding or editing an influence, the user must select an input and output species, as well as the type of influence. If the type is repression, then the input species represses the production of the output species. If the type is activation, then the input species activates the production of the output species. The user can also specify whether the influence has a promoter. If a promoter is selected, then this groups all influences using the same promoter together.

For example, if there are two influences:

A -| C, Promoter P1

B -| C, Promoter P2

this will create two reactions, where in the presence of A and B, C is repressed. This would behave roughly like a NAND gate. If, on the other hand, there are two influences:

A -| C, Promoter P1

B -| C, Promoter P1

this creates one reaction, where in the presence of A or B, C is repressed. This would behave roughly like a NOR gate.

Users can also specify if the influence is a biochemical influence. A biochemical influence requires all input species belonging to the same promoter to be present in order to affect transcription.

For example, if there are two biochemical influences:

A +| C, Promoter P1

B +| C, Promoter P1

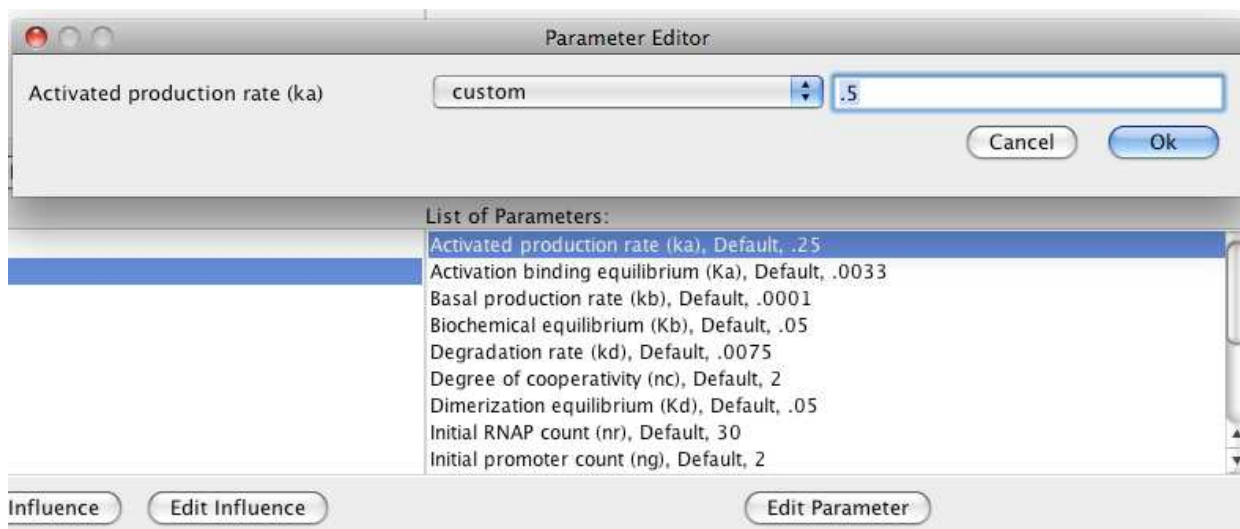
this will create two reactions, A and B combines into a complex, and the complex represses the production of C. This is a NAND gate. If, on the other hand, if biochemical is not selected, this behaves as a NOR gate.

The user can also set the value for degree of cooperativity (nc) which is the number of binding sites for transcription factors. The N-mer as transcription factor (nd) field determines how many monomers of the input species must be bound together in order to affect transcription. The user can also set the value of the repression or activation binding equilibrium (Kr and Ka). Finally, the user can specify the biochemical equilibrium (Kb).

4.4 GCM Parameters

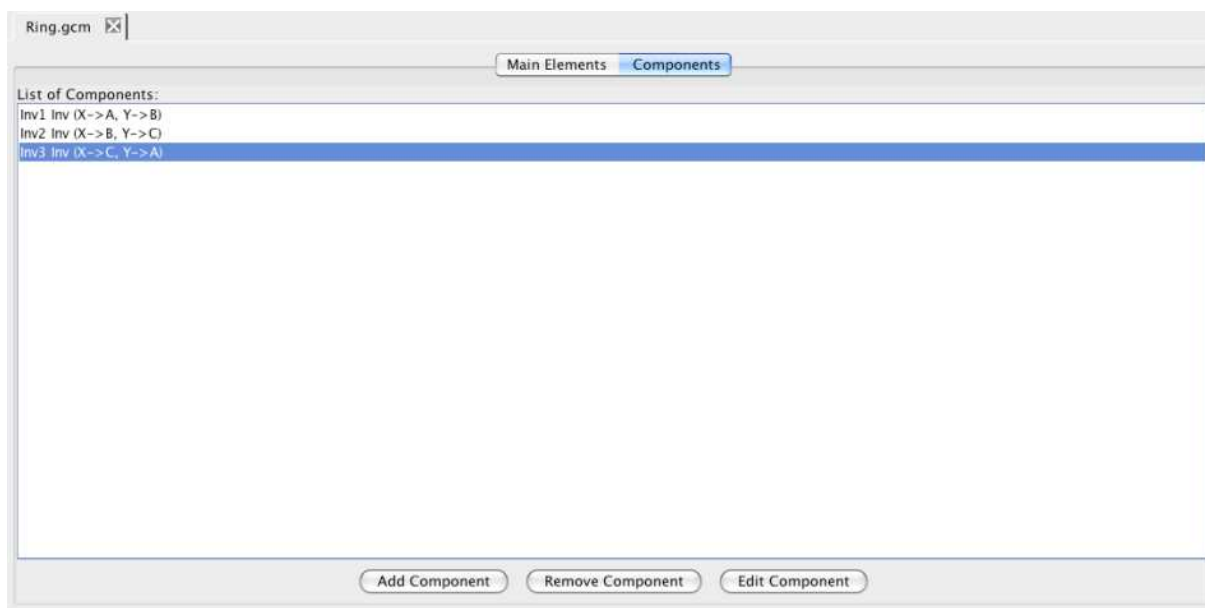
GCM parameters are a list of global parameters that are used when generating the SBML model for the GCM. The parameter list allows the user an easy way to change all the parameter values in a convenient location. If a parameter in the GCM is set to default, it will use the value found in the GCM parameter list. These defaults can be modified in the user preferences (see Section 9). The GCM parameters are listed below:

ID	Default Value	Units	Structure	Description
nr	30	molecule	model	Initial RNAP count
ns	0	molecule	species	Initial species count
Kd	0.05	$\frac{1}{\text{molecule}}$	species	Dimerization equilibrium
kd	0.0075	$\frac{1}{\text{sec}}$	species	Degradation rate
ng	2	molecule	promoter	Initial promoter count
np	10	molecule	promoter	Stoichiometry of production
nc	2	molecule	promoter	Degree of cooperativity
Ko	0.033	$\frac{1}{\text{molecule}}$	promoter	RNAP binding equilibrium
ko	0.05	$\frac{1}{\text{sec}}$	promoter	Open complex production rate
kb	0.0001	$\frac{1}{\text{sec}}$	promoter	Basal production rate
ka	0.25	$\frac{1}{\text{sec}}$	promoter	Activated production rate
nd	1	molecule	influence	N-mer as transcription factor
Kr	0.5	$\frac{1}{\text{molecule}^{nc}}$	influence	Repression binding equilibrium
Ka	0.0033	$\frac{1}{\text{molecule}^{(nc+1)}}$	influence	Activation binding equilibrium
Kb	0.05	$\frac{1}{\text{molecule}}$	influence	Biochemical equilibrium

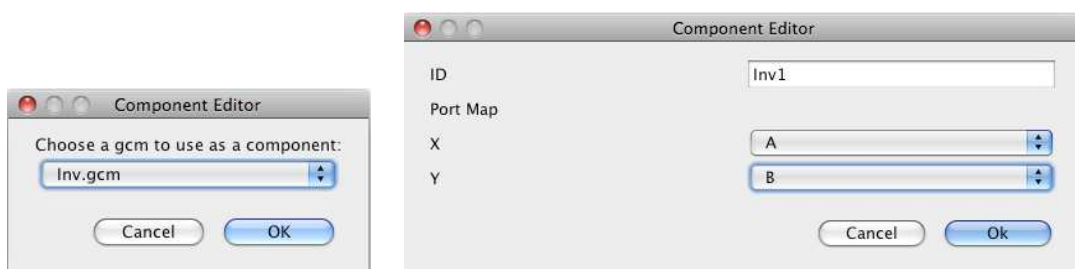


4.5 GCM Components

A GCM can be constructed hierarchically out of other GCM components. The list of GCM components are shown under the Components tab as shown below. The items in the list show the unique component instance ID, the component name, and the port map (i.e., the association of species in the component to species in the current GCM model). This is also where components can be added, removed, or edited.

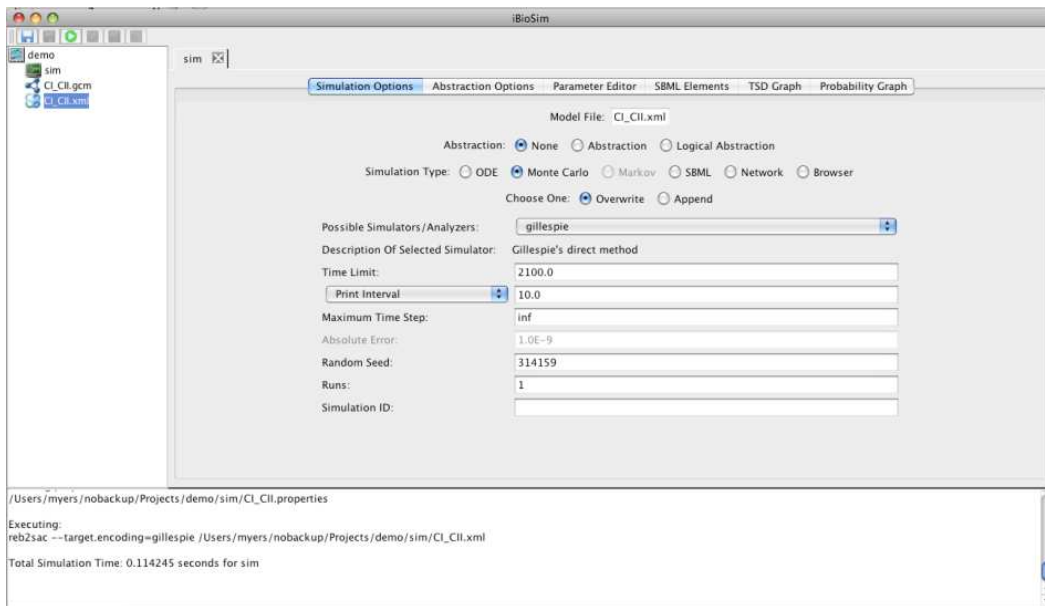


As shown below, when adding a new component, it first asks the name of the GCM component file. Then, it asks you to give a unique name to the component instantiation. Finally, it asks for a port map. Namely, one can associate each species in the component GCM with a species in the current GCM model. Note that not all species need to be associated.



5 Analysis View

The analysis view is used to analyze biochemical reaction network models. The analysis view as shown below includes tabs for simulation options (see Section 5.1), abstraction options (see Section 5.2), a parameter editor (see Section 5.3), a TSD graph editor (see Section 7), and a probability graph editor (see Section 8). The disk icon in the upper-left is used to save the simulation options while the play button icon is used to run the simulation.



5.1 Simulation Options

iBioSim comes with a number of simulation methods, ranging from continuous-deterministic simulation methods to discrete-stochastic simulation methods. In order to perform efficient temporal behavior analysis, various model abstraction can also be automatically applied. These routines are implemented within the reb2sac tool described in Kuwahara's PhD Dissertation (UofUtah 2007).

The first set of radio buttons in this tab specifies the levels of abstraction. "None" means to use no abstraction, "Abstraction" means to perform reaction-based abstraction, and "Logical Abstraction" means to perform both reaction-based and logical abstractions.

The second set of radio buttons specify the type of analysis. "ODE" is for continuous-deterministic simulation, "Monte Carlo" is for discrete-stochastic simulation, "Markov" performs temporal probability distribution analysis on finite-state Markov chain models, "sbml" outputs the model in SBML format, "Network" outputs the structure of the model in the GraphViz format for display by dotted, "Browser" outputs the model in xhtml format for display in a web browser.

The last set of radio buttons asks if you want to "Overwrite" the simulation runs or if you want to "Append" more simulation runs. If you have not yet performed any simulation, this option is disabled.

The next field specifies the simulation method you want to use based on the simulation type you specified. The methods available are:

Type	Method ID	Description
ODE	Euler	The forward Euler Method
ODE	gear1	Gear Method M=1
ODE	gear2	Gear Method M=2
ODE	rk4imp	Implicit 4th order Runge-Kutta at Gaussian points
ODE	rk8pd	Embedded Runge-Kutta Prince-Dormand (8,9) method
ODE	rkf45	Embedded Runge-Kutta-Fehlberg (4, 5) method
Monte carlo	Gillespie	Gillespie's SSA direct method
Monte carlo	emc-sim	Use jump count as next reaction time
Monte carlo	bunker	Uses mean for next reaction time
Monte carlo	nmc	Uses normally distributed next reaction time

There are some properties that need to be set for simulation. The table below specifies these:

Field	Description
Time Limit	The simulation time limit
Print Interval	The print time interval for each simulation run
Maximum Time Step	The maximum time step allowed (also minimum time step for the Euler method)
Absolute Error	Used by the adaptive time step ODE methods
Random Seed	An integer number as a seed to generate random numbers
Runs	The number of Monte Carlo simulation runs to perform
Simulation ID	Creates a simulation directory with the ID name

5.2 Abstraction Options

This tab as shown below allows the user to set the properties of rapid equilibrium, QSSA, and operator site abstraction methods.

- Rapid Equilibrium Condition 1 specifies threshold $T1$ such that the rapid equilibrium condition fails when $T1 > E0/(S0 + k - 1/k1)$.
- Rapid Equilibrium Condition 2 specifies threshold $T2$ such that the rapid equilibrium condition fails when $T1 > k2/k - 1$.
- The QSSA condition specifies threshold T used by the QSSA abstraction method where $T > E0/(S0 + KM)$.
- The Max concentration threshold specifies the maximum number of molecules that a species can have initially and still be considered an operator site by the operator site reduction.

This tab also allows the user to select the interesting species. Interesting species are the ones that are used in the analysis, and hence are those which should never be abstracted away. This tab shows all available species, and to make a species (or set of species) interesting, highlight the species and press the Add Species button. There is also a button to remove interesting species and to clear all interesting species.

The screenshot shows a software window titled 'sim' with a tabbed interface. The 'Abstraction Options' tab is active. It contains four input fields for thresholds: 'Rapid Equilibrium Condition 1' (0.1), 'Rapid Equilibrium Condition 2' (0.1), 'QSSA Condition' (0.1), and 'Max Concentration Threshold' (15). Below these are two lists: 'Available Species' and 'Interesting Species'. The 'Available Species' list includes 'bound_PR_CI', 'CI', 'CII', 'PR', 'PRE', 'RNAP', 'RNAP_PR', 'RNAP_PRE', and 'RNAP_PRE_CII'. The 'Interesting Species' list includes 'CI' and 'CII'. At the bottom are three buttons: 'Add Species', 'Remove Species', and 'Clear Species'.

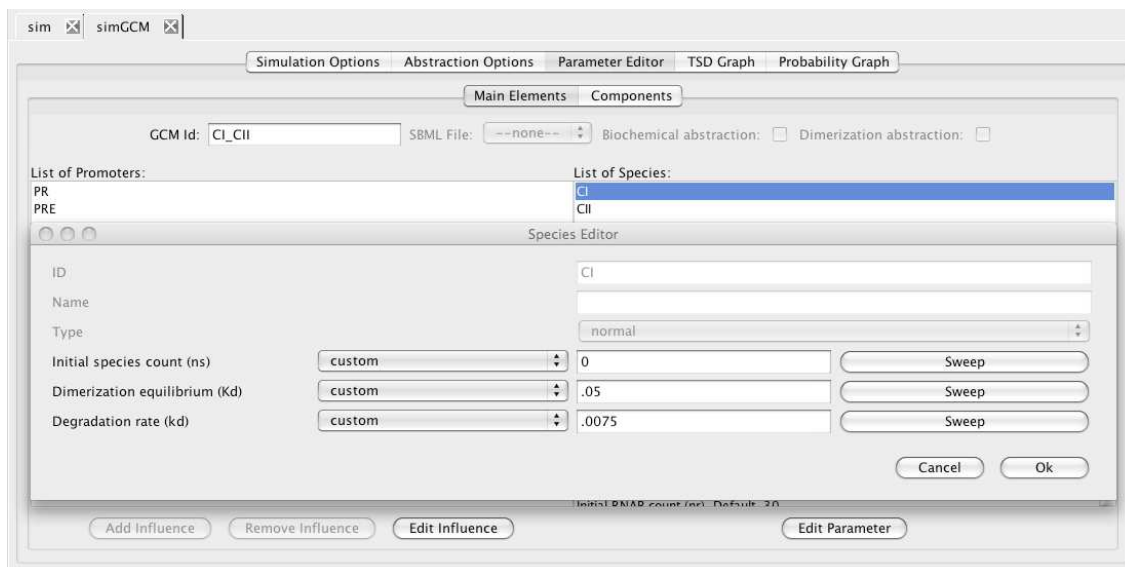
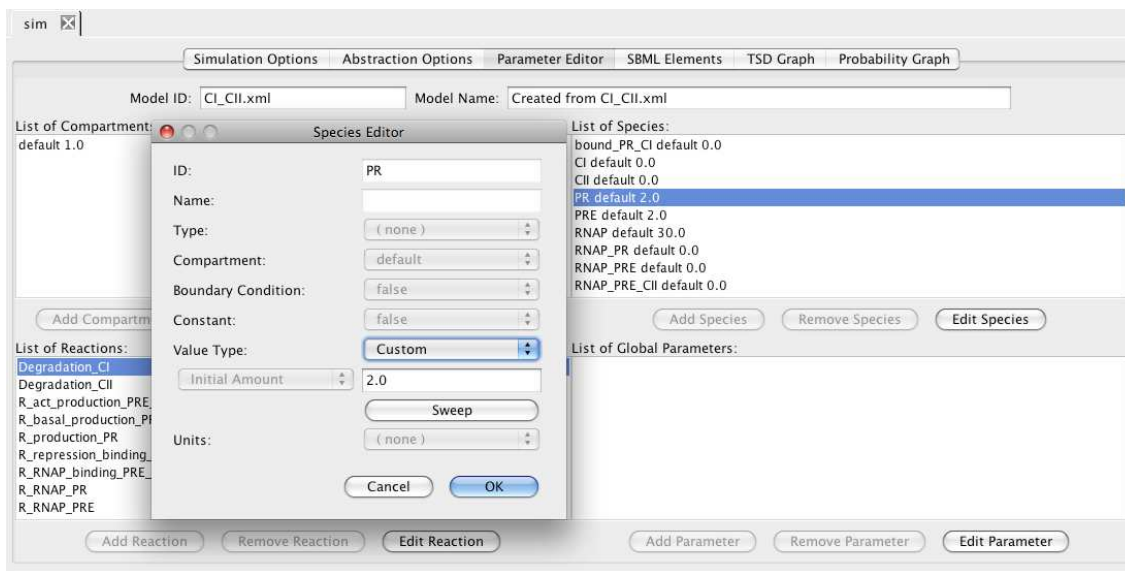
Parameter	Value
Rapid Equilibrium Condition 1:	0.1
Rapid Equilibrium Condition 2:	0.1
QSSA Condition:	0.1
Max Concentration Threshold:	15

Available Species:	Interesting Species:
bound_PR_CI	CI
CI	CII
CII	
PR	
PRE	
RNAP	
RNAP_PR	
RNAP_PRE	
RNAP_PRE_CII	

Buttons: Add Species, Remove Species, Clear Species

5.3 Parameter Editor

The parameter editors as shown below are similar in form to the SBML and GCM editors, but it only allows initial concentrations and parameters to be adjusted. Each of these parameters starts with the original value specified in the SBML or GCM associated with this analysis view. By changing the type to “Custom”, a new value can be entered. Changing the type back to “Original”, restores the original value. These values can also be swept by pressing the “Sweep” button. In this case, a menu will pop-up which allows you to provide a start value, a stop value, a step amount, and a level (1 or 2). When analyzing using sweep parameters, one analysis run is produced for each value stepped through from start to stop. The parameters at level 2 are changed first. When they have all reached their stop value, the parameters at level 1 are stepped once, and the parameters at level 2 are stepped through again. This process repeats until all parameters at level 1 have stepped to their stop value.



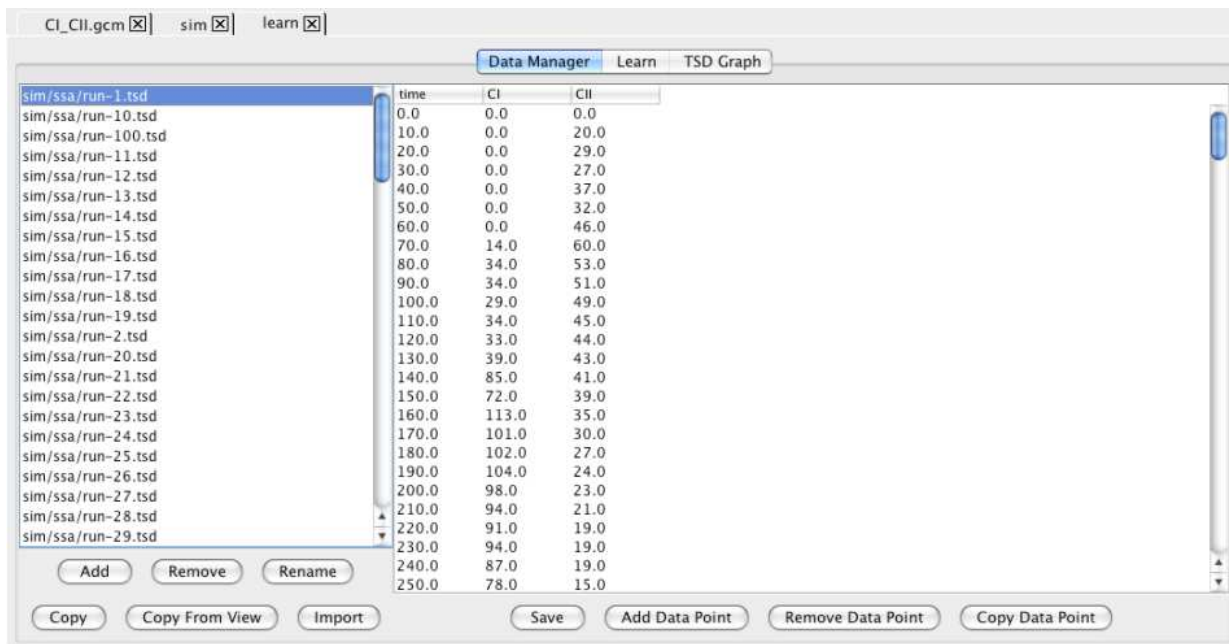
6 Learn View

The learn view is used to discover genetic circuit connectivity from time series data. The learn view includes tabs for a data manager (see Section 6.1), a learn tool (see Section 6.2), and a TSD graph editor (see Section 7).

6.1 Data Manager

The data manager as shown below is used to both enter time series experimental data as well as bring data into the learn view. The Add button is used to create a new data file. After pressing this button, enter the name of the new data file, and then enter the data for this file using the data editor to the right. The Remove button deletes all highlighted files. Note that after highlighting one file, you can use the ctrl key to highlight additional files or the shift key to highlight a range of files. The Rename button is used to change the name of a data file. The Copy button copies a data file. The Copy From View button brings up a list of all analysis and learn views in the current project, and data from the selected view will be copied into this learn view. Finally, the Import button brings up a file browser, and it allows you to import a data file from outside this project. These files can be in time series data (TSD) format (see Section 11), comma separated value (CSV) format, or tab delimited format (DAT).

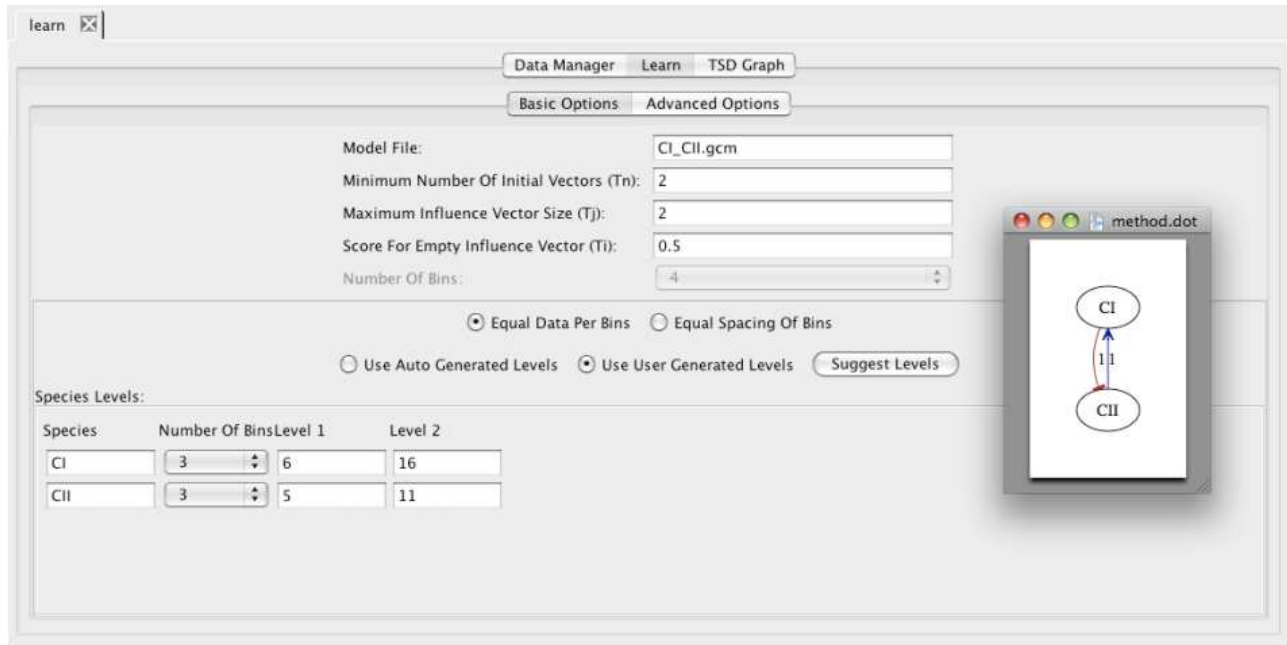
The contents of the data file highlighted on the left appear in the data editor on the right. Individual data entries can be modified, new data points can be added using the Add Data Point button, data points can be removed using the Remove Data Point button, and data points can be copied using the Copy Data Point button. When you are satisfied with all your changes, you should press the Save button to record your changes.



6.2 Learn Tool

The learn tool shown below uses the GeneNet algorithm described in Barker's PhD dissertation (UofUtah 2007) . To use this learn tool, adjust any options described below, if desired, then press the play button icon. The resulting genetic circuit is specified using our Genetic Circuit Model

(GCM) Format (see Section 10) and is shown graphically using GraphViz's Dotty tool. On this tab, one can also save the parameters without learning, view the last learned circuit, save the generated circuit into the project, and view the last run log.



Below are the basic learning options as shown above are as follows:

- Minimum Number of Initial Vectors (T_n) (default=2):
 T_n is a threshold value used in the CreateInfluenceVectorSet algorithm and represents the minimum number of influence vectors constructed in this algorithm.
- Maximum Influence Vector Size (T_j) (default=2):
 T_j is a threshold value used in the CombineInfluenceVectors algorithm to determine the maximal size of merged influence vectors.
- Score for Empty Influence Vector (T_i) (default=0.5):
The score for an influence vector with no influences in it.
- Number of Bins (default=4):
The number of bins value specifies how many values the encoded time series data can assume.
- Equal Data Per Bins / Equal Spacing of Bins:
This radio button selects whether the auto generated levels should be determined by equaling dividing the data between the bins or by equally dividing the range of the data.
- Use Auto Generated Levels / Use User Generated Levels:
This radio button allows the user to select whether they want the levels separating the bins to be auto generated or the user would like to provide them.
- When using user provided levels, the Suggest Levels button will provide the levels that would have been auto generated as a suggestion. These levels can then edited by the user. The number of bins for each species can also be individually adjusted.

The advanced learning options shown below are as follows:

- Ratio for Activation (Ta) (default=1.15):
A probability ratio above this value results in a vote for an influence vector that has a majority of activation influences.
- Ratio for Repression (Tr) (default=0.75):
A probability ratio above this value results in a vote for an influence vector that has a majority of repression influences.
- Merge Influence Vectors Delta (Tm) (default=0.0):
Two influence vectors cannot be merged unless the difference in their scores is less than this value.
- Relax Thresholds Delta (Tt) (default=0.025):
The values of Ta and Tr are modified by this amount when these thresholds are relaxed.
- Debug Level (default=0):
This controls how much information is displayed by the GeneNet algorithm when it runs.
- Successors / Predecessors / Both (default=Successors):
This radio button selects whether successor data point pairs, predecessor data point pairs, or both are used.
- Basic FindBaseProb (default=unchecked):
When selected, the basic FindBaseProb function is used.

learn

Data Manager Learn TSD Graph

Basic Options Advanced Options

Ratio For Activation (Ta): 1.15

Ratio For Repression (Tr): 0.75

Merge Influence Vectors Delta (Tm): 0.0

Relax Thresholds Delta (Tt): 0.025

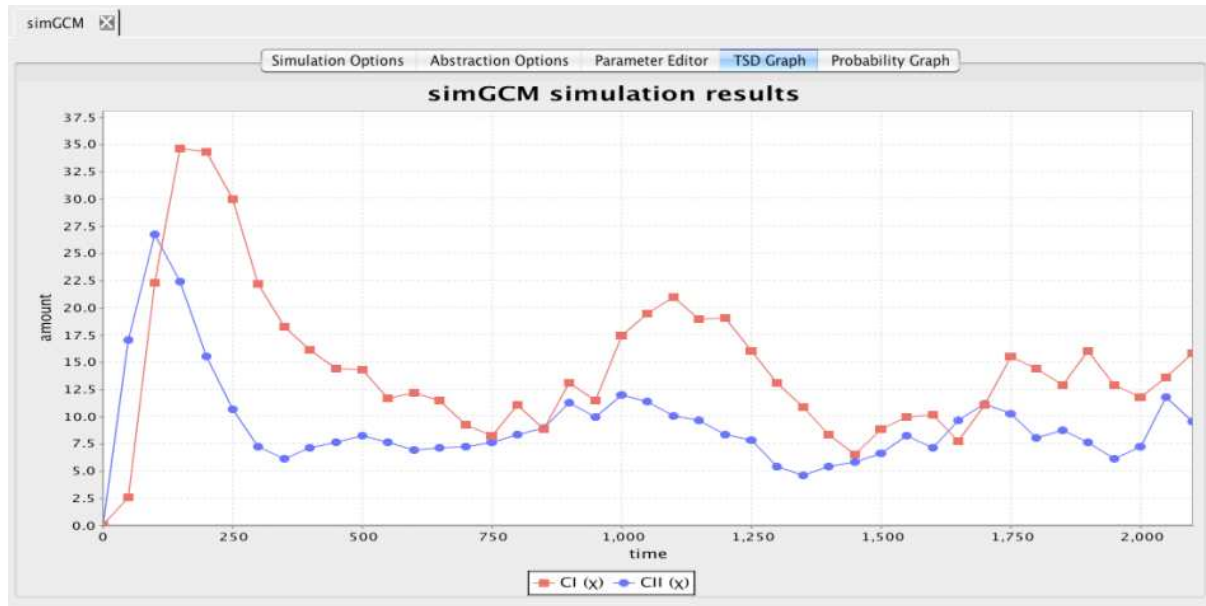
Debug Level: 0

☒ Successors ☐ Predecessors ☐ Both

☐ Basic FindBaseProb

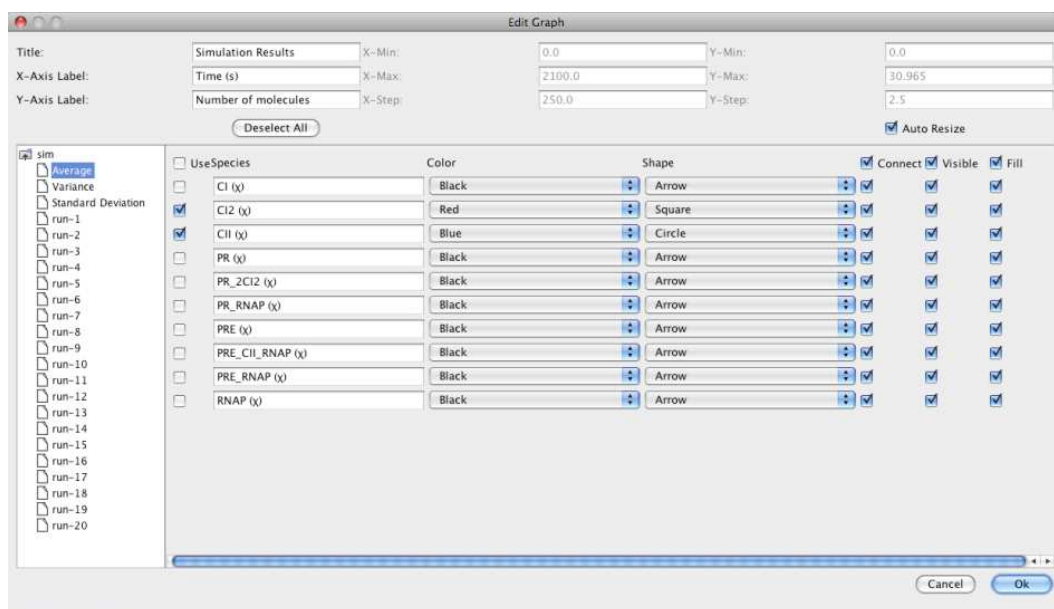
7 TSD Graph Editor

The TSD graph editor appears as a tab in both analysis and learn views. TSD graphs can also be created at the top-level of the project to allow you to integrate results from several analysis or learn views. These graphs can be created using the New → TSD Graph menu option. Once created, they can be viewed and edited by double clicking on the graph in the project window. An example graph is shown below.



In the TSD graph editor shown below, a graph is created by double clicking on the graph. You can then set various parameters and select what values you would like to have graphed. The parameters that you can select for a graph include:

- Title - The title of the graph.
- X-Axis Label - The label displayed for the x-axis.
- Y-Axis Label - The label displayed for the y-axis.
- X-Min - The starting value for the x-axis.
- X-Max - The ending value for the x-axis.
- X-Step - The increment for the x-axis.
- Y-Min - The starting value for the y-axis.
- Y-Max - The ending value for the y-axis.
- Y-Step - The increment for the y-Axis.
- Auto Resize Check Box - Determines whether to automatically resize the graph for best fit.



The data selection menu on the left displays all of the available sets of data that can be graphed. In particular, one can graph the average, variance, standard deviation, or results from individual simulation runs. For a top-level graph, these data sets will be organized hierarchically. Hierarchy is also introduced when simulations in an analysis view are given simulation IDs or after performing an analysis while sweeping parameter values. After selecting a data set, one can select individual species to graph and how they are to be displayed. In other words, for each species, there are the following options:

- Use Check Box - Determines whether or not this species is displayed on the graph. Checking or unchecking the box at the top changes the state for all species in the data set.
- Species Label - The name displayed in the legend.
- Drop Down Menu Of Colors - The color that is used for this species.
- Drop Down Menu Of Shapes - The shape that is used to mark the data points.
- Connect Check Box - Determines whether to connect the points with a line. Checking or unchecking the box at the top changes the state for all species in the data set.
- Visible Check Box - Determines whether shapes are visible on the line. Checking or unchecking the box at the top changes the state for all species in the data set.
- Fill Check Box - Determines whether shapes are filled on the line. Checking or unchecking the box at the top changes the state for all species in the data set.

Note that a check mark appears on a data set to indicate that some species have been selected in that data set. Also, all species can be deselected by pressing the Deselect All button.

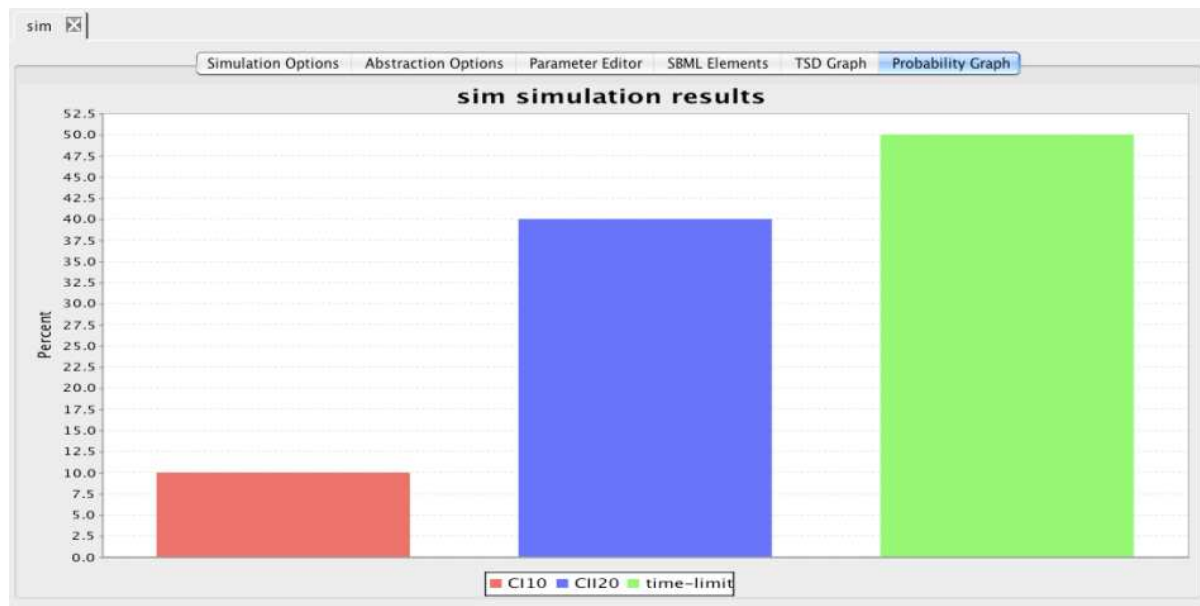
The disk icon button saves the settings for the graph to a file, so when you re-open the graph, it will reload this data and display in the same way as before. The disk icon with a pencil icon prompts for a filename and creates a new top-level graph with that name. Finally, the “Export” icon button prompts for a filename and exports the data to the given name. The extension provided for the filename is used to determine how the graph is to be exported. The supported file types are:

- csv - comma separated value data file.
- dat - column separated data file.
- eps - encapsulated postscript.
- jpg - JPEG (Joint Photographic Experts Group).
- pdf - portable document format.
- png - portable network graphics.
- svg - scalable vector graphics.
- tsd - time series data format (see Section 11).

If no extension is given, then the file type is the one specified in the file filter (default is pdf). For image (i.e., not data) file types, you will be prompted to give a desired pixel height and width for the file before the file is exported.

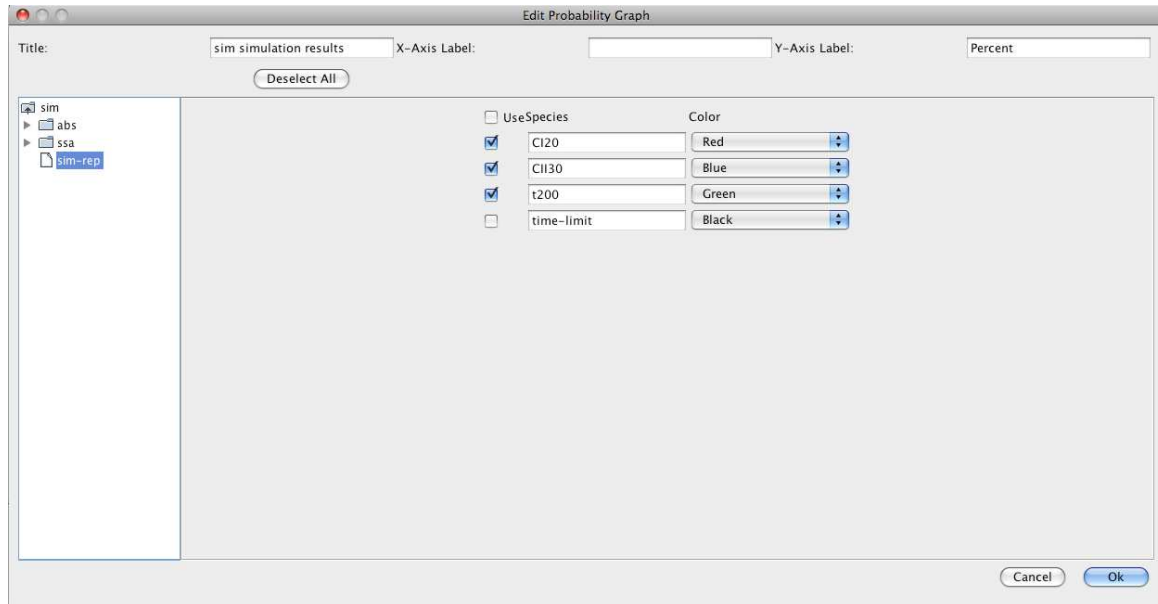
8 Probability Graph Editor

Probability graphs are used to display histograms for reasons that simulations terminated. This is used in conjunction with SBML constraints to determine the likelihood of various conditions. The probability graph editor appears as a tab in analysis views. Probability graphs can also be created at the top-level of the project to allow you to integrate results from several analysis views. These graphs can be created using the New → Probability Graph menu option. Once created, they can be viewed and edited by double clicking on the graph in the project window. An example probability graph is shown below.



In the probability graph editor, a graph is created by double clicking on the graph. You can then set various parameters and select what values you would like to have graphed. The parameters that you can select for a graph include:

- Title - The title of the graph.
- X-Axis Label - The label displayed for the x-axis.
- Y-Axis Label - The label displayed for the y-axis.



The data selection menu on the left displays all of the available sets of data that can be graphed. For a top-level graph, these data sets will be organized hierarchically. Hierarchy is also introduced when simulations in an analysis view are given simulation IDs or after performing an analysis while sweeping parameter values. After selecting a data set, one can select individual constraints to graph and how they are to be displayed. In other words, for each constraint, there are the following options:

- Use Check Box - Determines whether or not this constraint is displayed on the graph. Checking or unchecking the box at the top changes the state for all constraints in the data set.
- Constraints Label - The name displayed in the legend.
- Drop Down Menu Of Colors - The color that is used for this constraint.

Note that a check mark appears on a data set to indicate that some constraints have been selected in that data set. Also, all constraints can be deselected by pressing the Deselect All button.

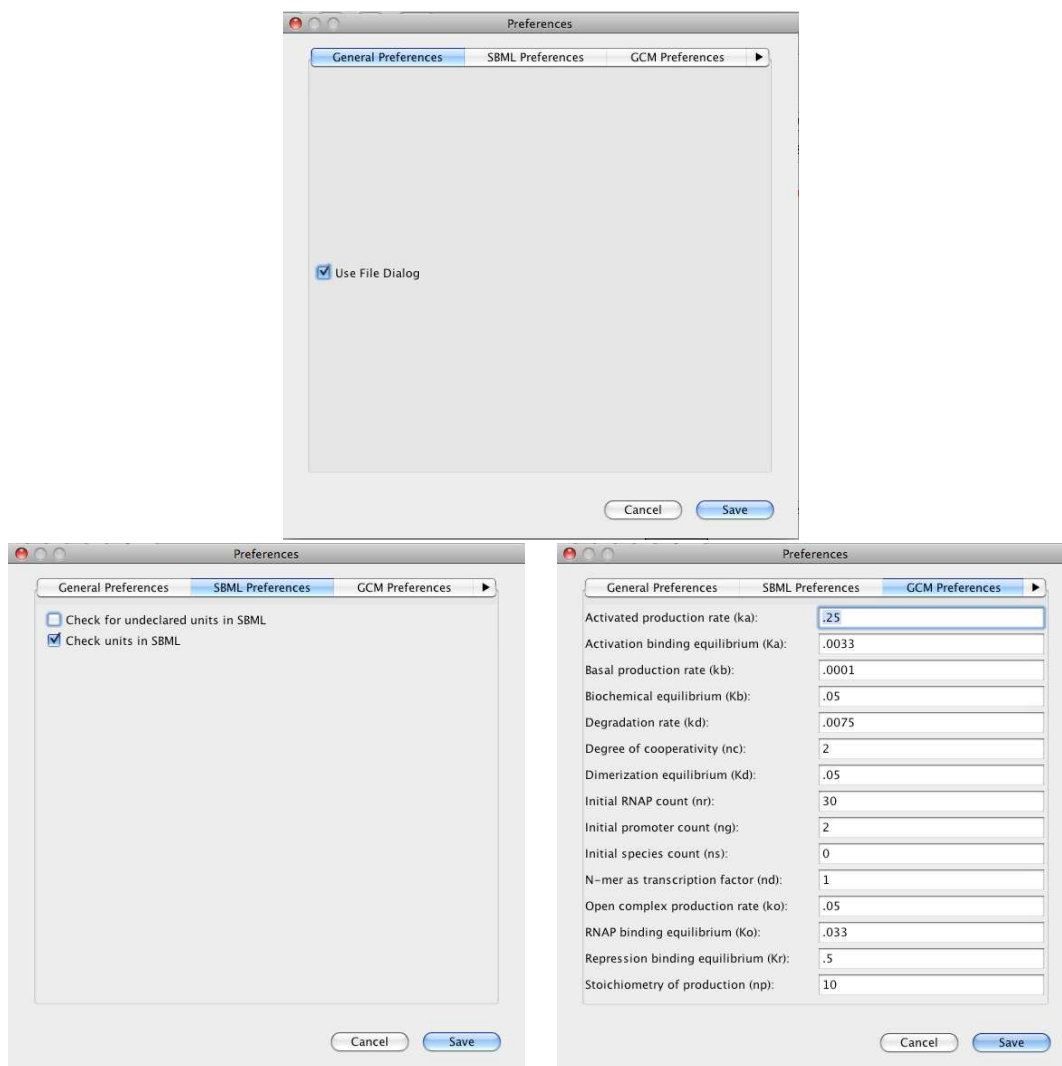
The disk icon button save the settings for the graph to a file, so when you re-open the graph, it will reload this data and display in the same way as before. The “Save As” icon button prompts for a filename and creates a new top-level graph with that name. Finally, the “Export” icon button prompts for a filename and exports the data to the given name. The extension provided for the filename is used to determine how the graph is to be exported. The supported file types are:

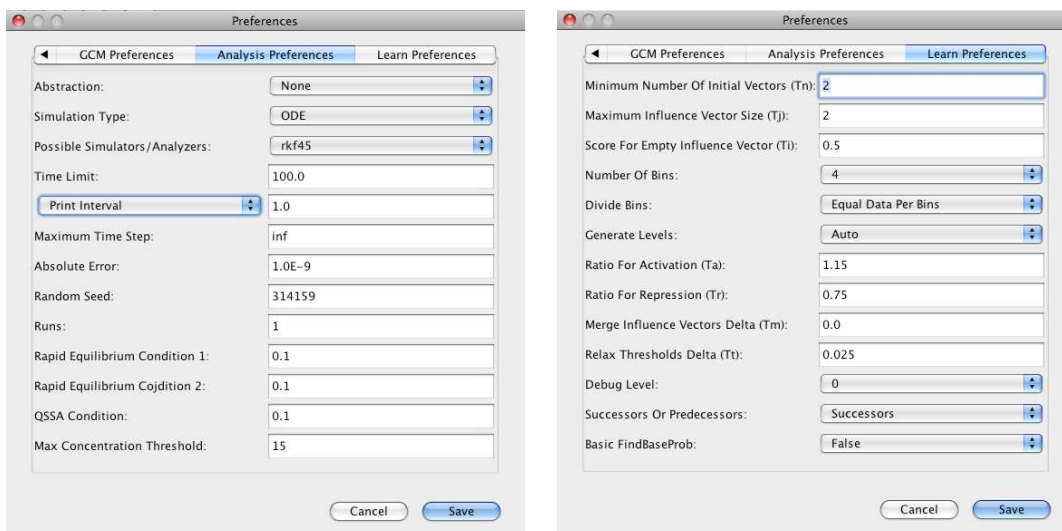
- eps - encapsulated postscript.
- jpg - JPEG (Joint Photographic Experts Group).
- pdf - portable document format.
- png - portable network graphics.
- svg - scalable vector graphics.

If no extension is given, then the file type is the one specified in the file filter (default is pdf). For image (i.e., not data) file types, you will be prompted to give a desired pixel height and width for the file before the file is exported.

9 Preferences

User preferences can be set by selecting the **Preferences** option under the **File** menu on Linux and Windows or the **iBioSim** menu on MacOS. As shown below, under the General Preferences tab, the user can decide whether they wish to use a File Dialog for selecting files. If this is not checked, files are selected using the default Java File Chooser. The SBML Preferences tab allows users to select whether they wish to see warnings about undeclared units in SBML and whether they wish to check units at all. The GCM Preferences tab allows users to change the GCM default parameter values. The Analysis Preferences tab allows users to change the default values used by the analysis tool. Finally, the Learn Preferences tab allows users to change the default values used by the learn tool.





10 Genetic Circuit Model Format

Our genetic circuit model (gcm) format specifies a genetic circuit using the same format used by the GraphViz graph drawing tool. The vertices in the graph are the species in the genetic circuit, and the edges in the graph represent the activation and repression relationships between the species. An activation relationship is shown with a blue (blue4) arrow (vee) and a repression relationship is shown with a red (firebrick4) tee. The label field in the species declaration is the name of the species. The arrowhead field in the relationship declaration represents the type of relationship between the species. Repression is labeled with a tee and activation is labeled with a vee. The label field in the relationship declaration represents how many molecules are necessary to activate or repress the production of the species. An example is shown below for a simple genetic circuit in which the species CI represses CII while CII activates CI production. The $s_1 \rightarrow s_2$ edge has a label field of "2" which means two molecules of CI are required to form a dimer to repress CII.

```
digraph G {
  CI [shape=ellipse,color=black,label="CI"];
  CII [shape=ellipse,color=black,label="CII"];
  CII -> CI [color="blue4",arrowhead=vee];
  CI -> CII [color="firebrick4",label="2",arrowhead=tee];
}
```

More advanced behavior can be modeled by using extra fields. The promoter field groups a set of species together. The examples below shows how the promoter field works. In the genetic circuit model below, species A represses the production of species B and C, independently. If there was exactly 1 molecule of species A, it would only be able to repress production of species B or C, but not both.

```
digraph G {
  A [shape=ellipse,color=black,label="A"];
  B [shape=ellipse,color=black,label="B"];
  C [shape=ellipse,color=black,label="C"];
  A -> B [color="blue4",arrowhead=tee];
```

```
A -> C [color="blue4",arrowhead=tee];
}
```

With the promoter field, one species A now represses the promoter “P1”, which produces both species B and C. This means that one molecule of species A will repress the production of both species B and C.

```
digraph G {
  A [shape=ellipse,color=black,label="A"];
  B [shape=ellipse,color=black,label="B"];
  C [shape=ellipse,color=black,label="C"];
  A -> B [color="blue4",arrowhead=tee,promoter="P1"];
  A -> C [color="blue4",arrowhead=tee,promoter="P1"];
}
```

The promoter field can also be used to separate production reactions. In the example below, both species A and B can repress the production of species C. If either is present, then very little C will be produced. This behavior is like a NOR gate.

```
digraph G {
  A [shape=ellipse,color=black,label="A"];
  B [shape=ellipse,color=black,label="B"];
  C [shape=ellipse,color=black,label="C"];
  A -> C [color="blue4",arrowhead=tee];
  B -> C [color="blue4",arrowhead=tee];
}
```

However, if there needs to be two different sources of production for species C, the promoter field can be used to accomplish this. In the example below, A represses the production of C by binding to the P1 promoter, and B represses the production of C by binding to the P2 promoter. Both A and B need to be present to fully repress the level of C. If either is at a low level, then the level of C will be high. This behavior is like a NAND gate.

```
digraph G {
  A [shape=ellipse,color=black,label="A"];
  B [shape=ellipse,color=black,label="B"];
  C [shape=ellipse,color=black,label="C"];
  A -> C [color="blue4",arrowhead=tee,promoter="P1"];
  B -> C [color="blue4",arrowhead=tee,promoter="P2"];
}
```

The example below shows how to model an AND gate. The species A and B have the constant flag set to true. This means that A and B have no production and degradation reactions. The reactions contain a promoter label “P1”. This means that the species C can be activated by both A and B. Combined with the type flag of biochemical, this creates a biochemical reaction where species A and B combine together to form a complex to activate production of species C.

```
digraph G {
  A [shape=ellipse,color=black,label="A",const=true];
```

```

B [shape=ellipse,color=black,label="B",const=true];
C [shape=ellipse,color=black,label="C"];
A -> C [color="blue4",arrowhead=vee,promoter="P1",type=biochemical];
B -> C [color="blue4",arrowhead=vee,promoter="P1",type=biochemical];
}

```

11 Time Series Data Format

The time series data (tsd) format is composed of a parenthesized and comma-separated set of time points. Each time point is composed of a parenthesized and comma-separated set of data for that time point. This first time point is composed of a set of strings that are the labels for the data entries. The first entry in each time point is by convention the time for that time point. Below is an example simulation of the species CI and CII from 0 to 1000 seconds with time points separated by 100 seconds.

```

(("time","CI","CII"), (0,0,0), (100,0,19), (200,20,25), (300,19,18), (400,17,20), (500,17,46),
(600,26,40), (700,43,43), (800,63,28), (900,72,34), (1000,72,28))

```

12 Tutorial

A detailed tutorial is available in the `docs` directory that comes with the distribution.

13 Reporting Bugs and Feature Requests

In order to report a bug or to request a change or feature, please send an email to:
atacs-bugs@vlsigroup.ece.utah.edu.

The subject line must begin with one of the following keywords or the mail will be filtered by our spam filters:

- BUG - error or crash of the software
- CHANGE - something which can be improved
- FEATURE - something new

14 Credits

The iBioSim tool is being developed at the University of Utah by Chris Myers, Nathan Barker, Kevin Jones, Hiroyuki Kuwahara, Curtis Madsen, and Nam Nguyen. Nathan Barker is now with Southern Utah University, and Hiroyuki Kuwahara is now with the Centre for Computational and System Biology in Trento, Italy.