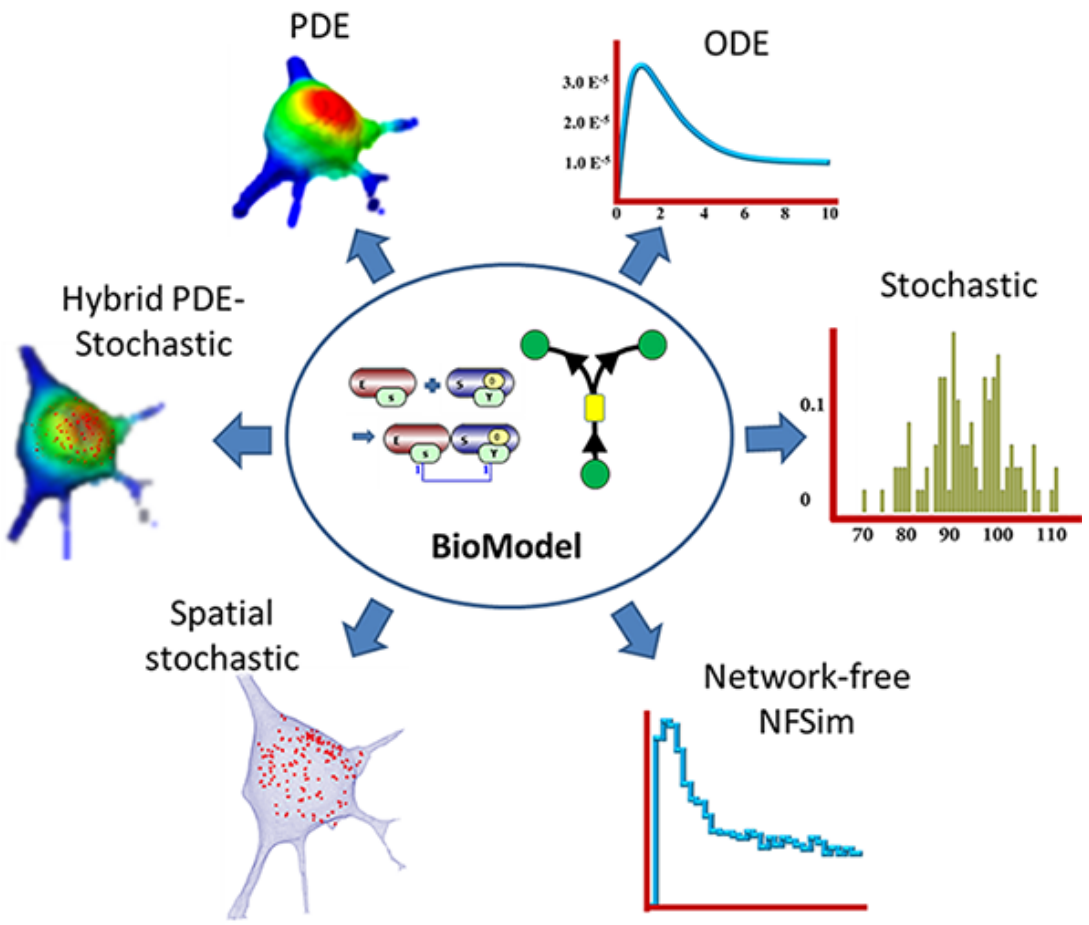
Contents

VCell

To run VCell go to:

vcell.org



UNIVERSITY OF CONNECTICUT HEALTH CENTER

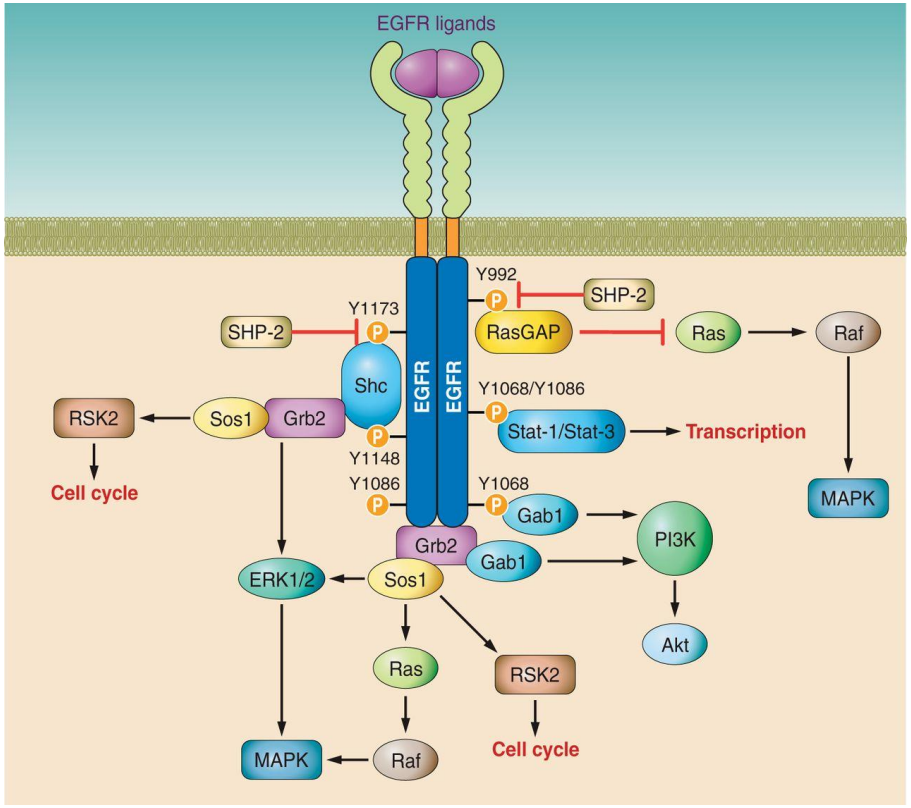
CCAM
VCell is developed at CCAM
Center for Cell Analysis & Modeling

NIH
National Institute of General Medical Sciences
VCell is funded by the NIGMS

VCell Tutorial

Building a Rule-Based Model

We will demonstrate how to create a rule-based model of EGFR receptor interaction with two adapter proteins Grb2 and Shc. A Receptor-monomer reversibly binds a ligand at the extracellular domain, triggering dimerization through transmembrane domains. The receptor kinase transphosphorylates two receptor phosphotyrosines that independently recruit two adapter proteins, Grb2 and Shc. Shc itself is subject to transhphosporylation, where the phosphorylated form has a lower affinity to a receptor phosphotyrosine.



http://physrev.physiology.org/content/96/3/1025

In this tutorial you will learn how to:

- Create a rule-based **Physiology** with Molecules, Species, Rules and Observables.
- Simulate a model using **Deterministic application** that expands rules into a reaction network using the **BioNetGen** engine.
- Simulate a model using a **Stochastic application** that simulates the reaction network generated by **BioNetGen**.
- Simulate a model using **Network-Free** application that skips network generation and directly computes Observables using **NFSim** engine.

General familiarity with VCell software is recommended. Although this tutorial can be followed by a VCell novice, it is recommended that novice users first look through the VCell tutorials available at http://vcell.org/vcell_software/user_guide.html .

Model building can be matched to the BioModel *RB_egfr_tutorial* in the Tutorial folder in the VCell Database.

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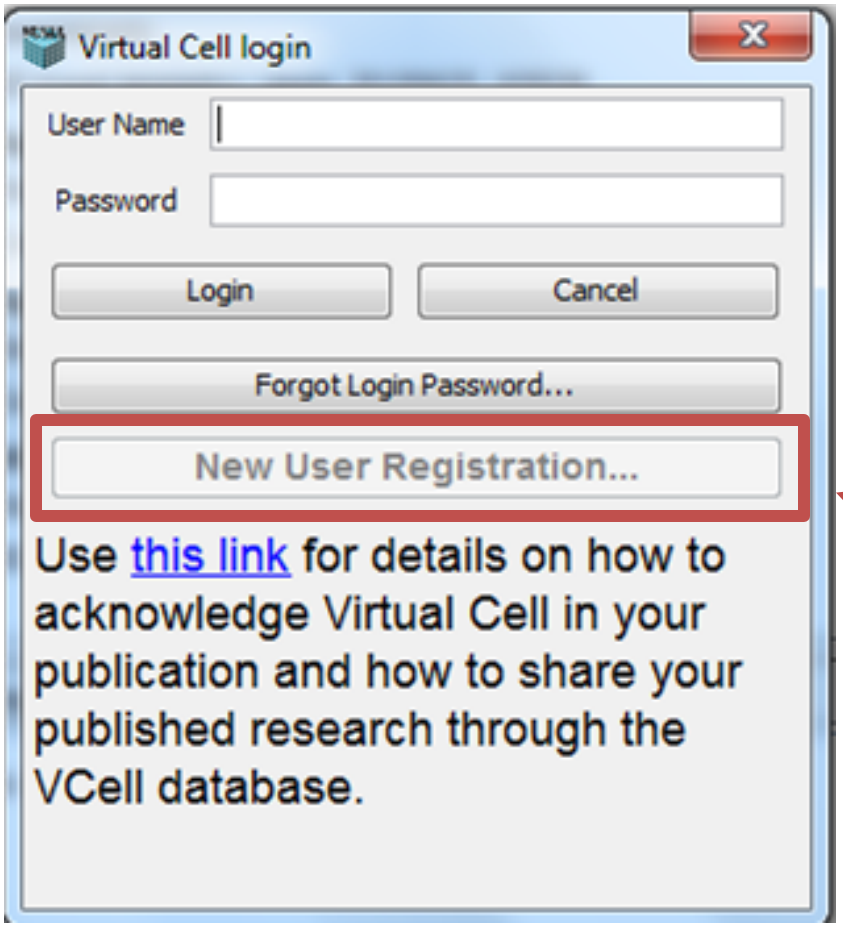
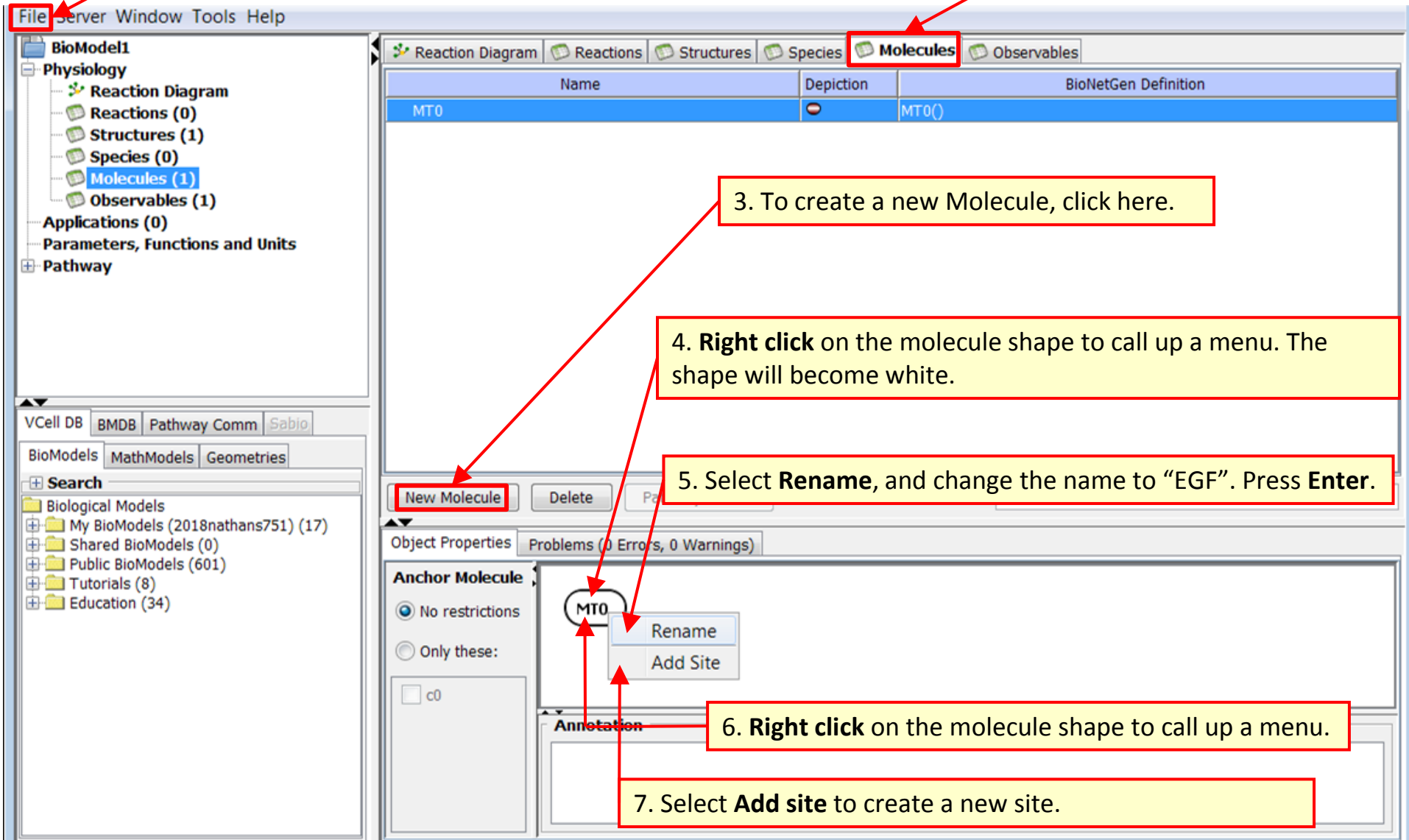
Opening VCell for the First Time



You need to register as a new user if you want to run simulations on VCell, compute resources, or use the VCell database to view and store models that can be shared with collaborators.

1. To create a new VCell model, click File > New > BioModel.

2. To start creating Molecules, click on **Molecules.**



3. To create a new Molecule, click here.

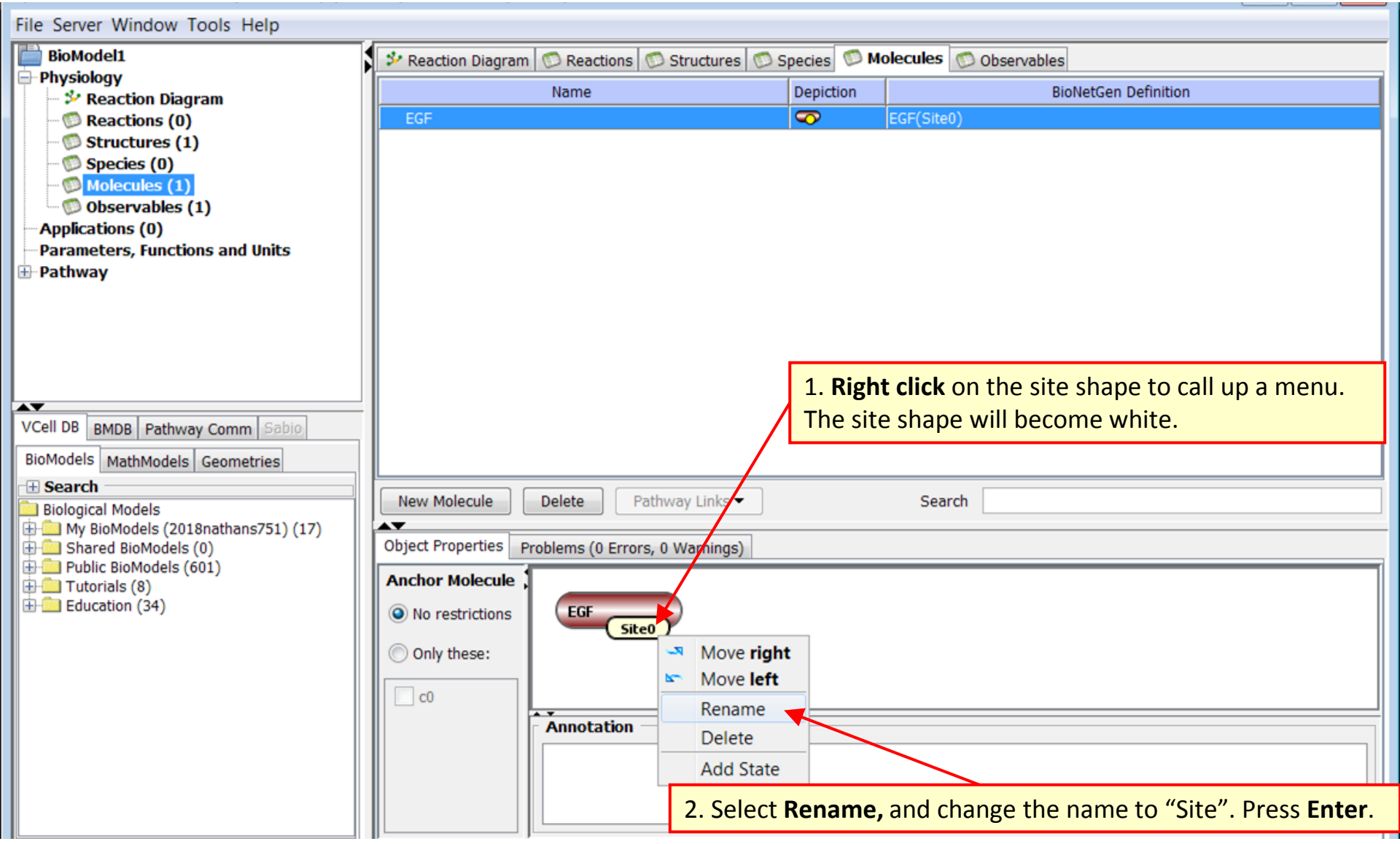
4. **Right click** on the molecule shape to call up a menu. The shape will become white.

5. Select **Rename**, and change the name to “EGF”. Press **Enter**.

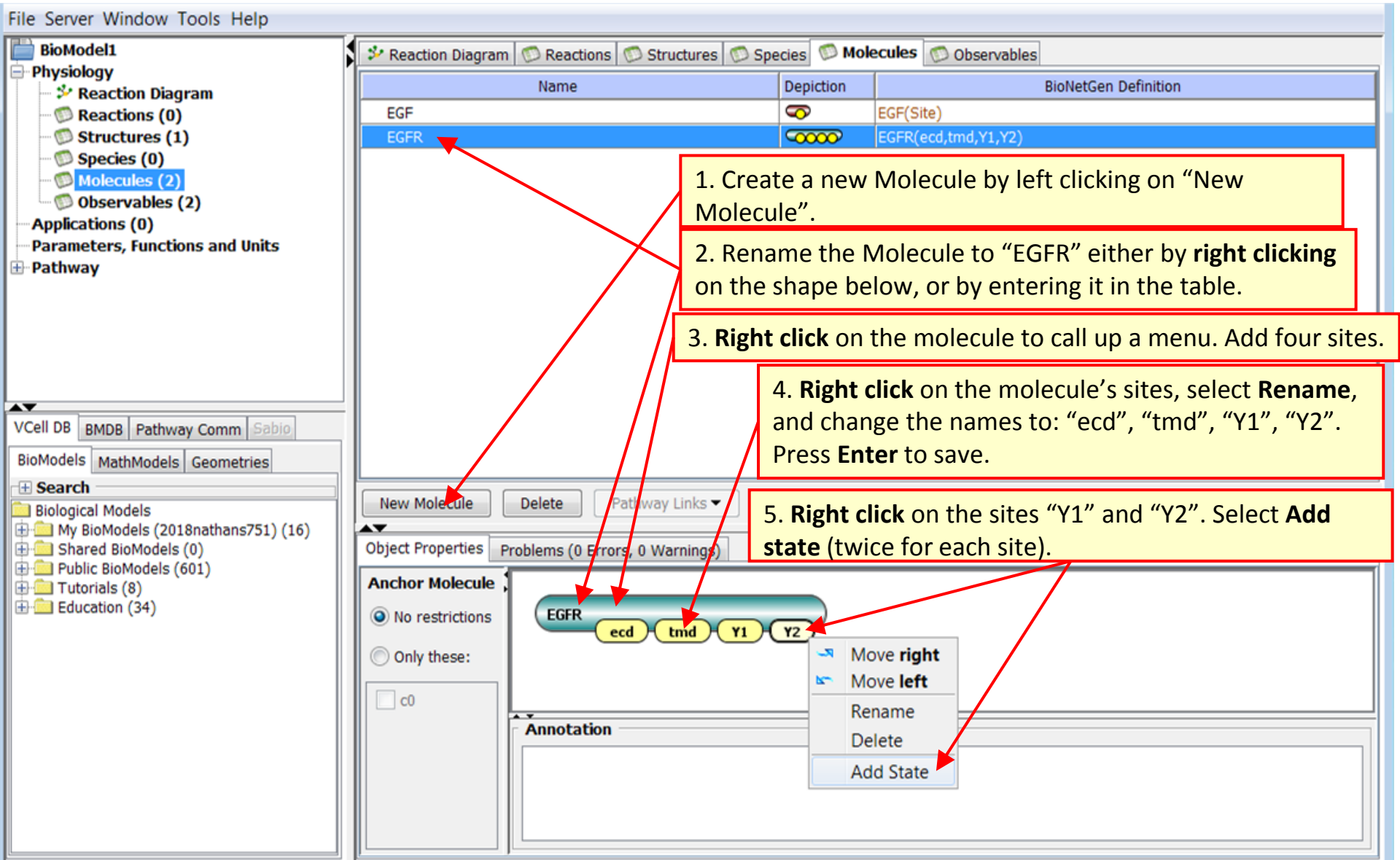
6. **Right click** on the molecule shape to call up a menu.

7. Select **Add site** to create a new site.

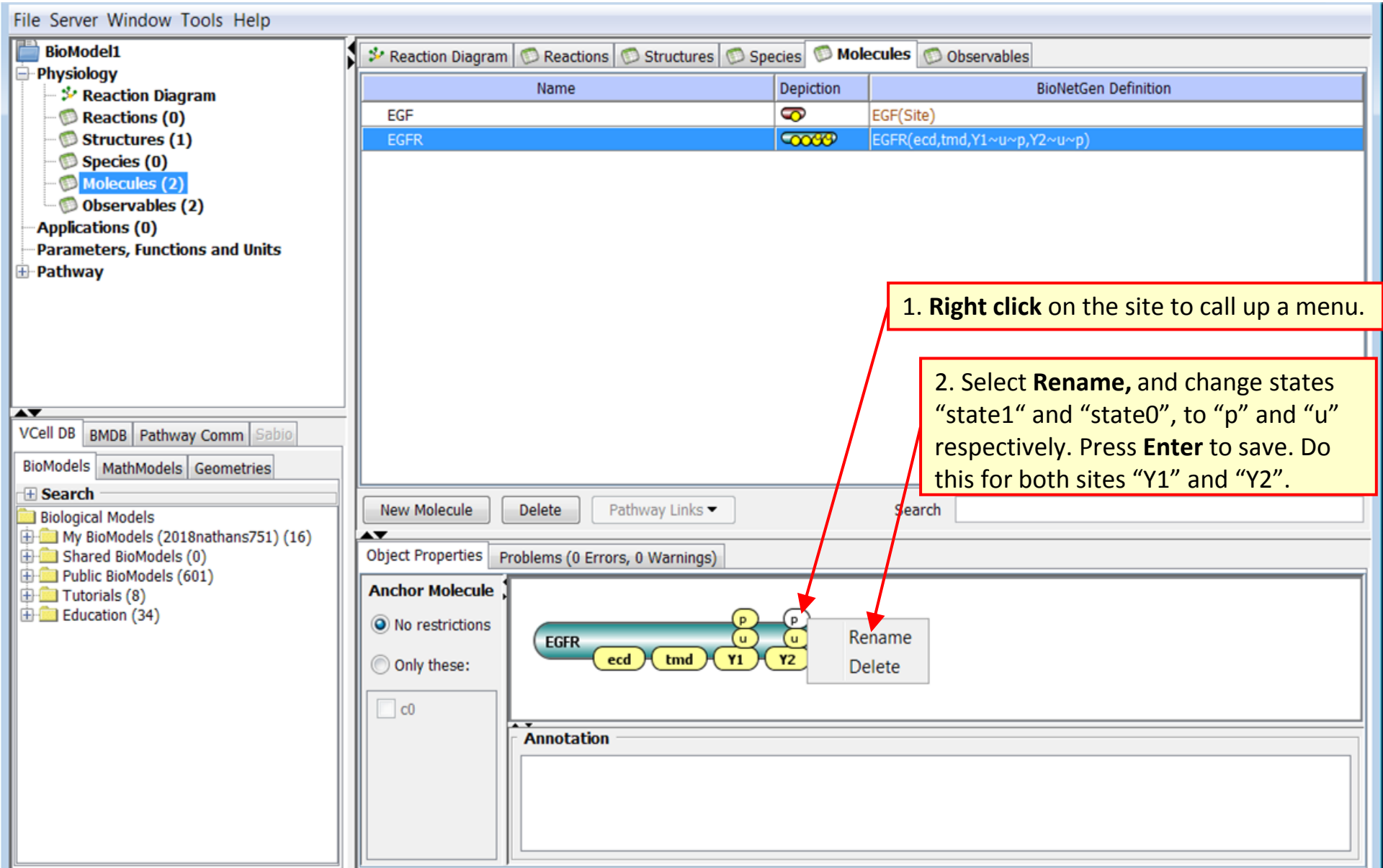
TIP: If something goes wrong, press **ESC** on the keyboard.



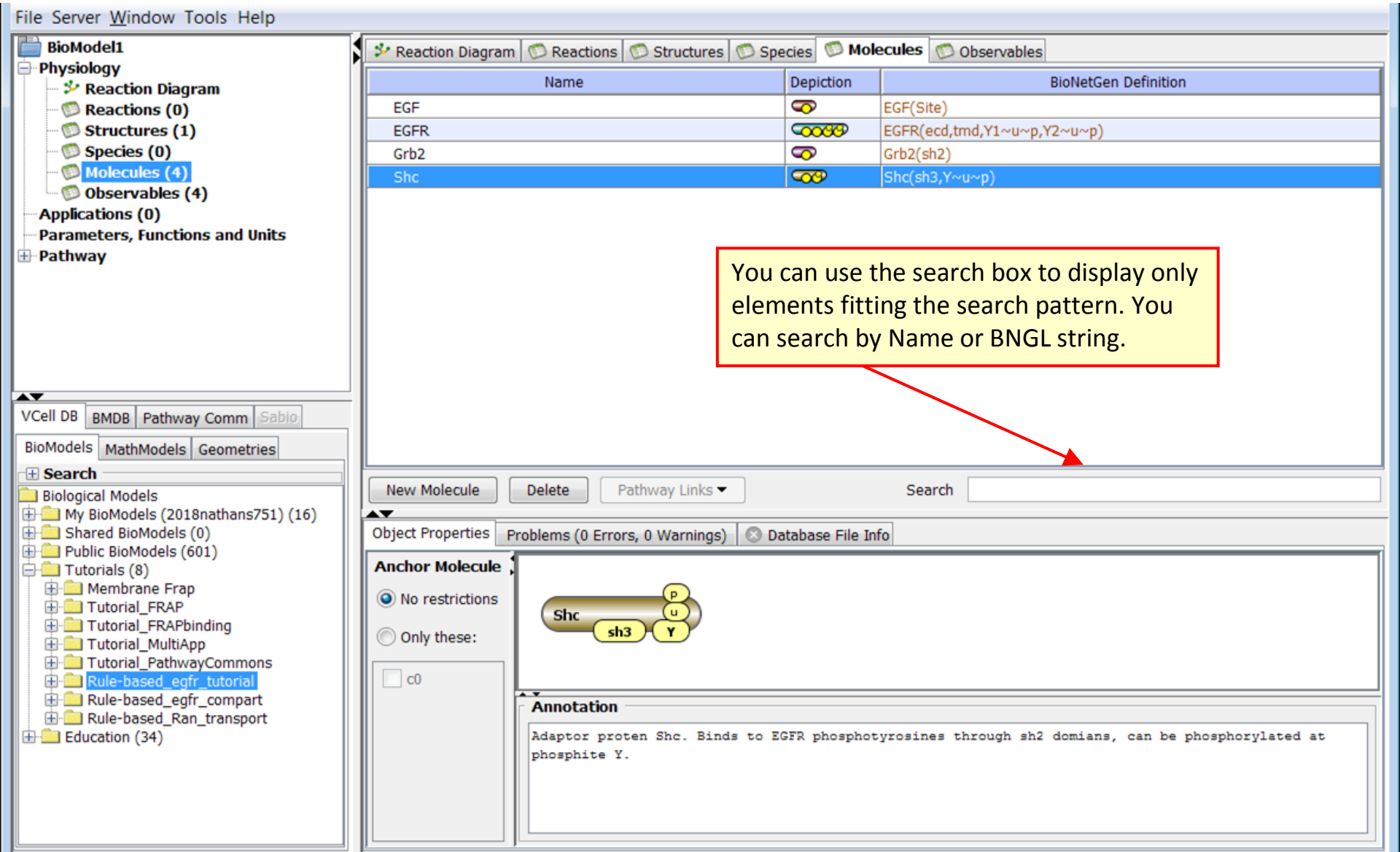
TIP: A Molecule name can always be changed by double clicking in Name field, editing, and pressing **ENTER**. It does not matter if the molecule is already used elsewhere – the change will be propagated everywhere in the model.



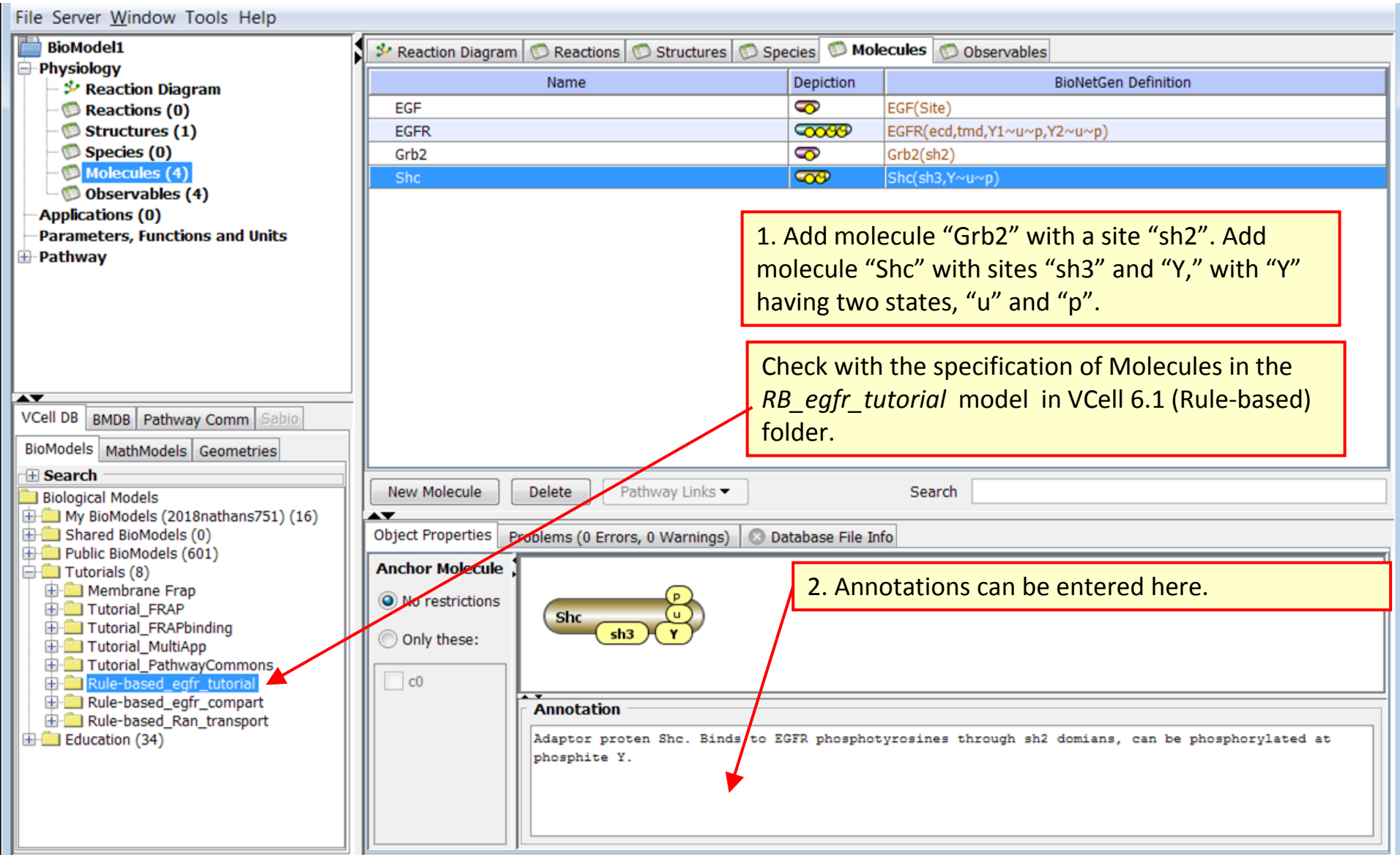
TIP: Sites can always be moved right and left among the Molecule length and renamed, states can always be renamed. To delete a state, you must first eliminate all places where this site is used, e.g. in reaction rules that change the site.



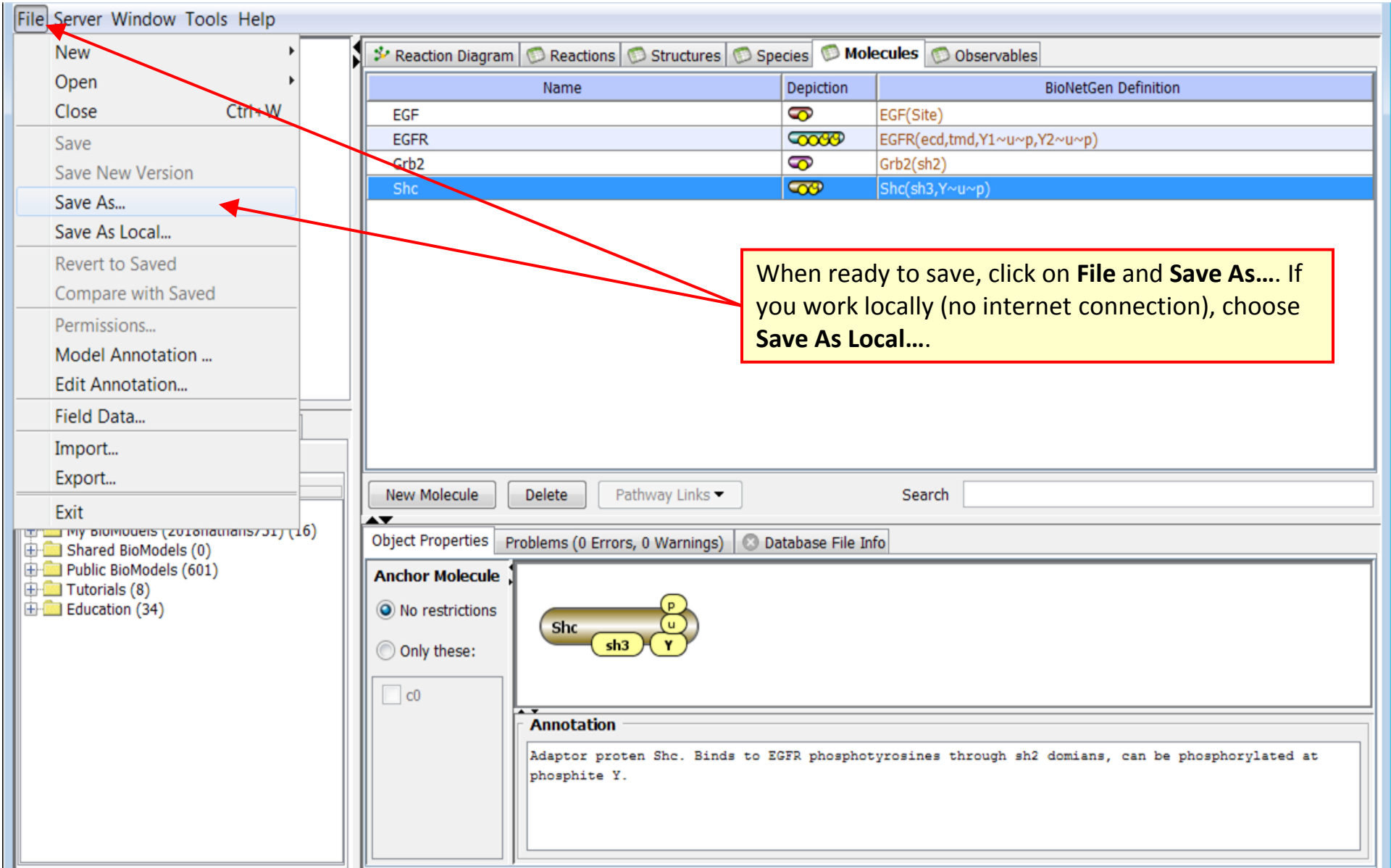
TIP: BioNetGen definition displays the test strings that encodes elements of a rule-based model in the BioNetGen language (BNGL). In BNGL, molecular states are listed after site name with ~ appended.



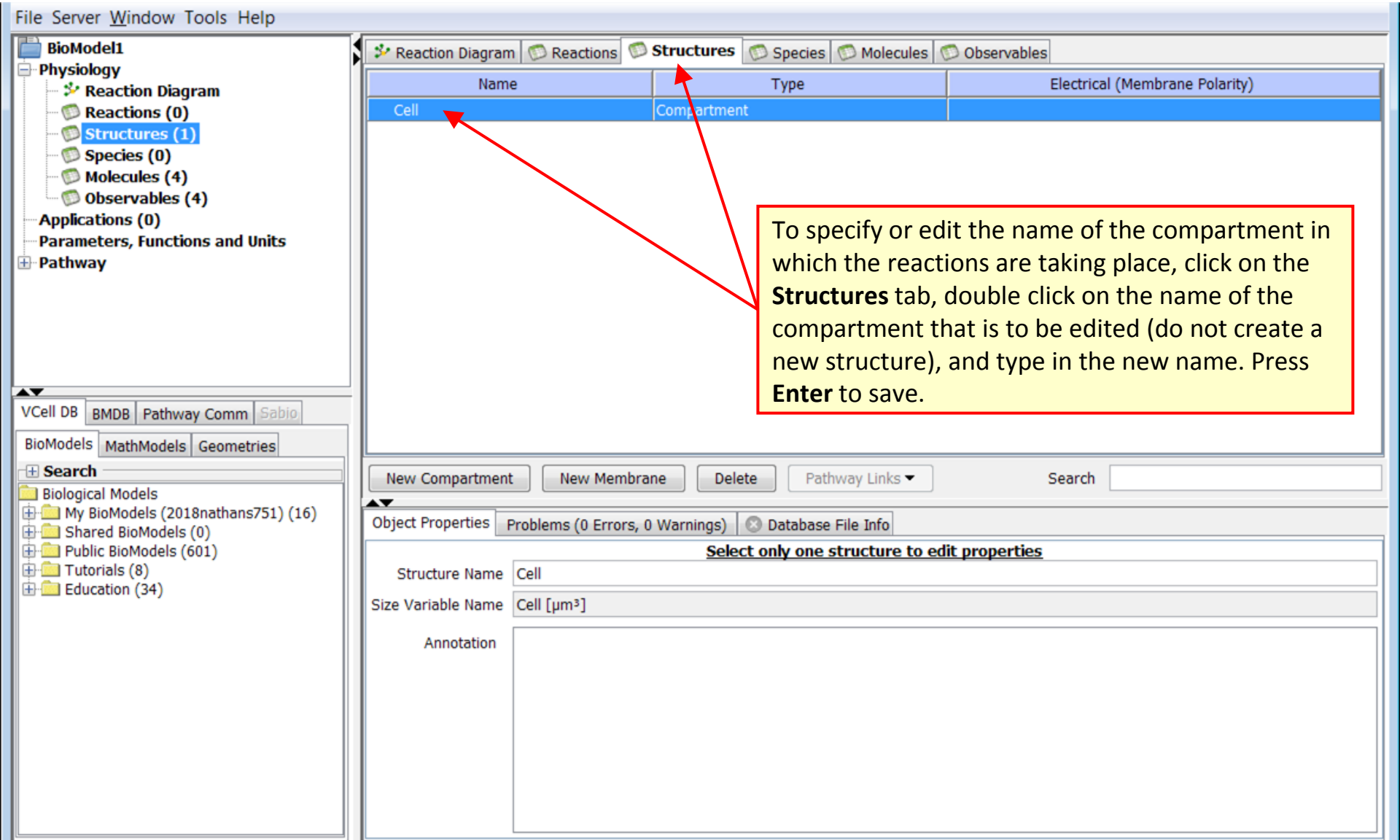
TIP: Molecule colors are ordered and cannot be changed. Molecules can be added and/or deleted at any time, but reaction rules, species and observables that use these molecules must be deleted first. A warning will appear if deletion is not allowed.



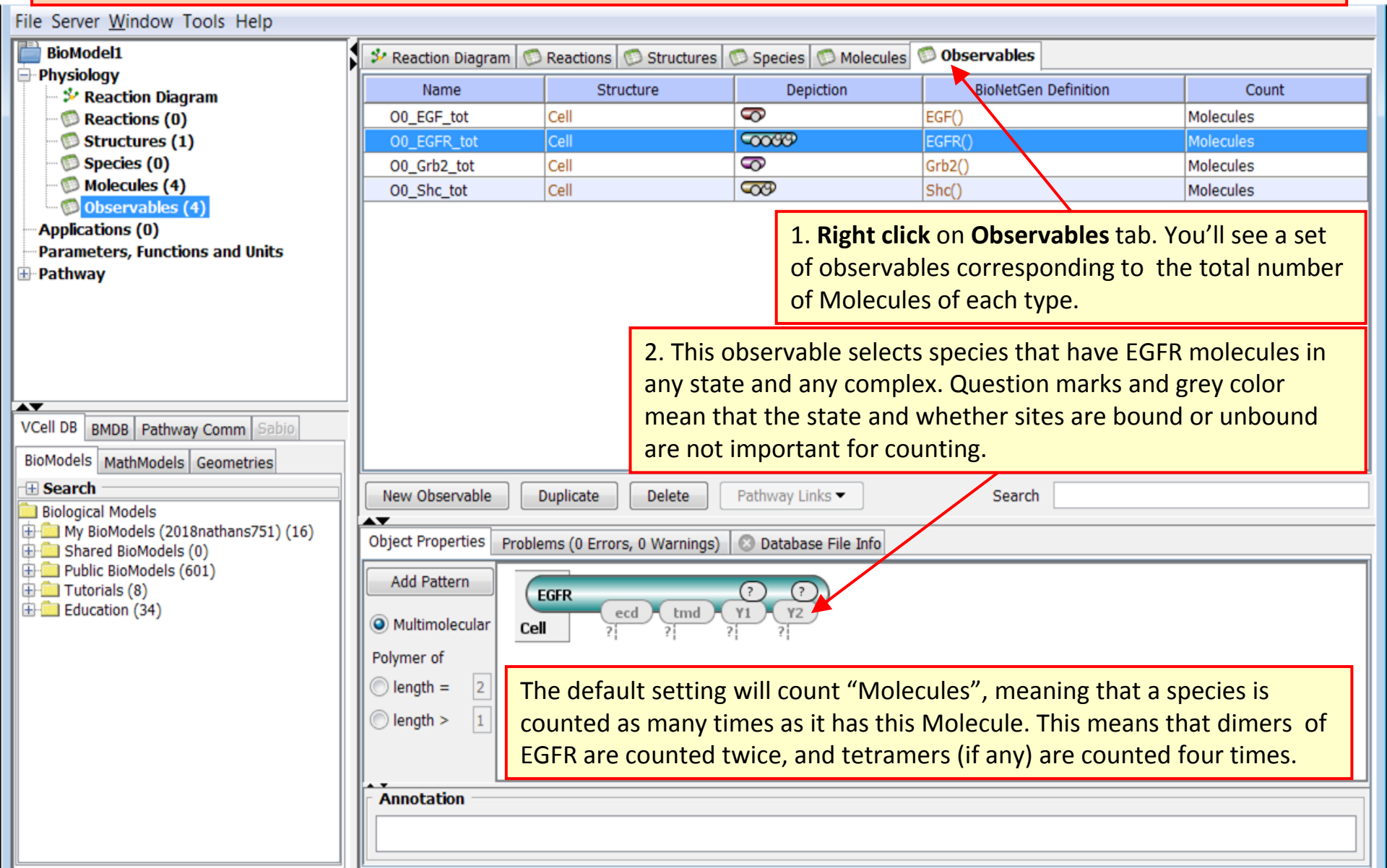
TIP: Save your model as often as you can, so you don't lose any changes!



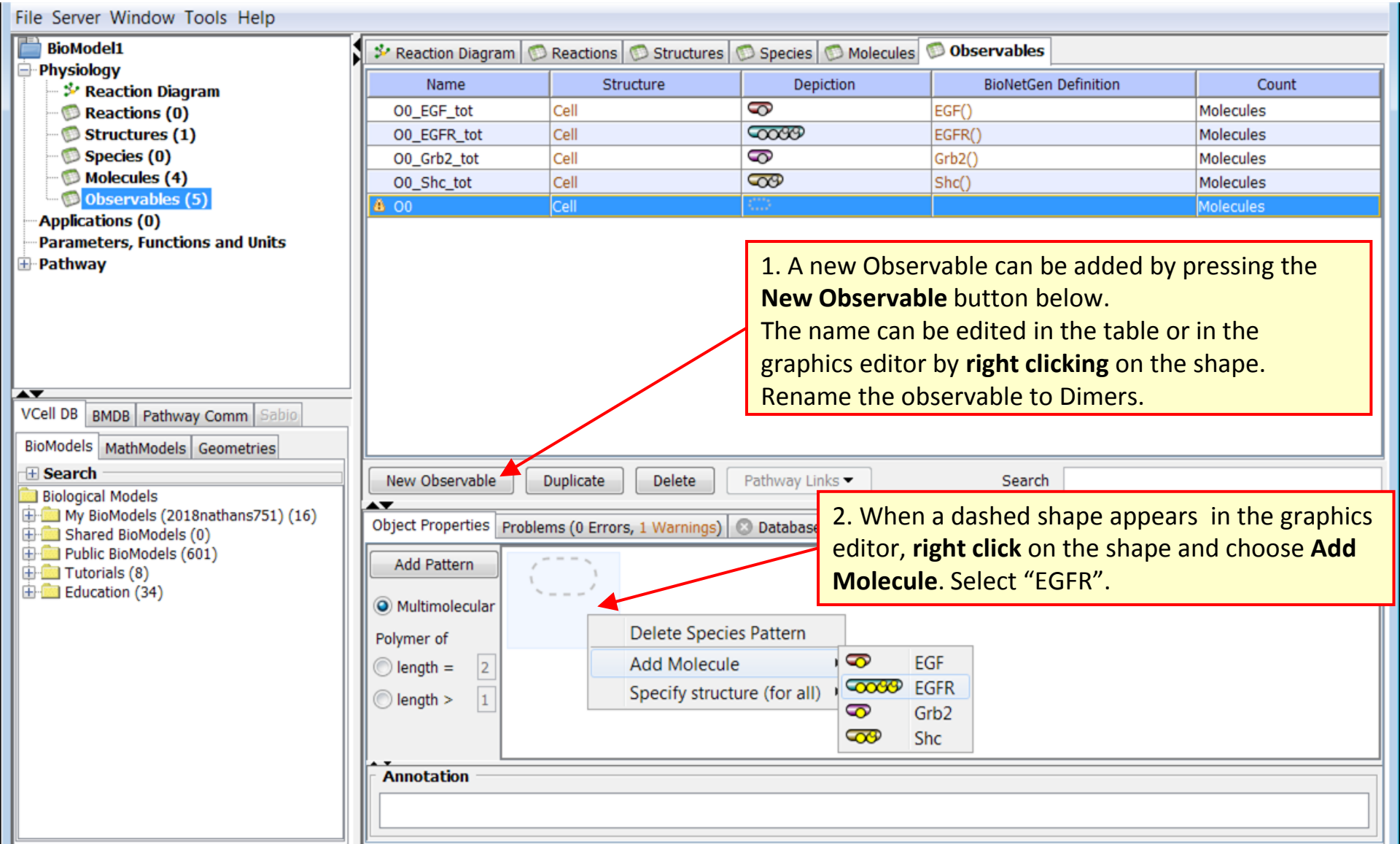
TIP: Compartments can be volumetric (3D) and membranes (2D). They can be added any time, but all species defined before compartments are introduced will be located in volume and cannot be moved to membranes.



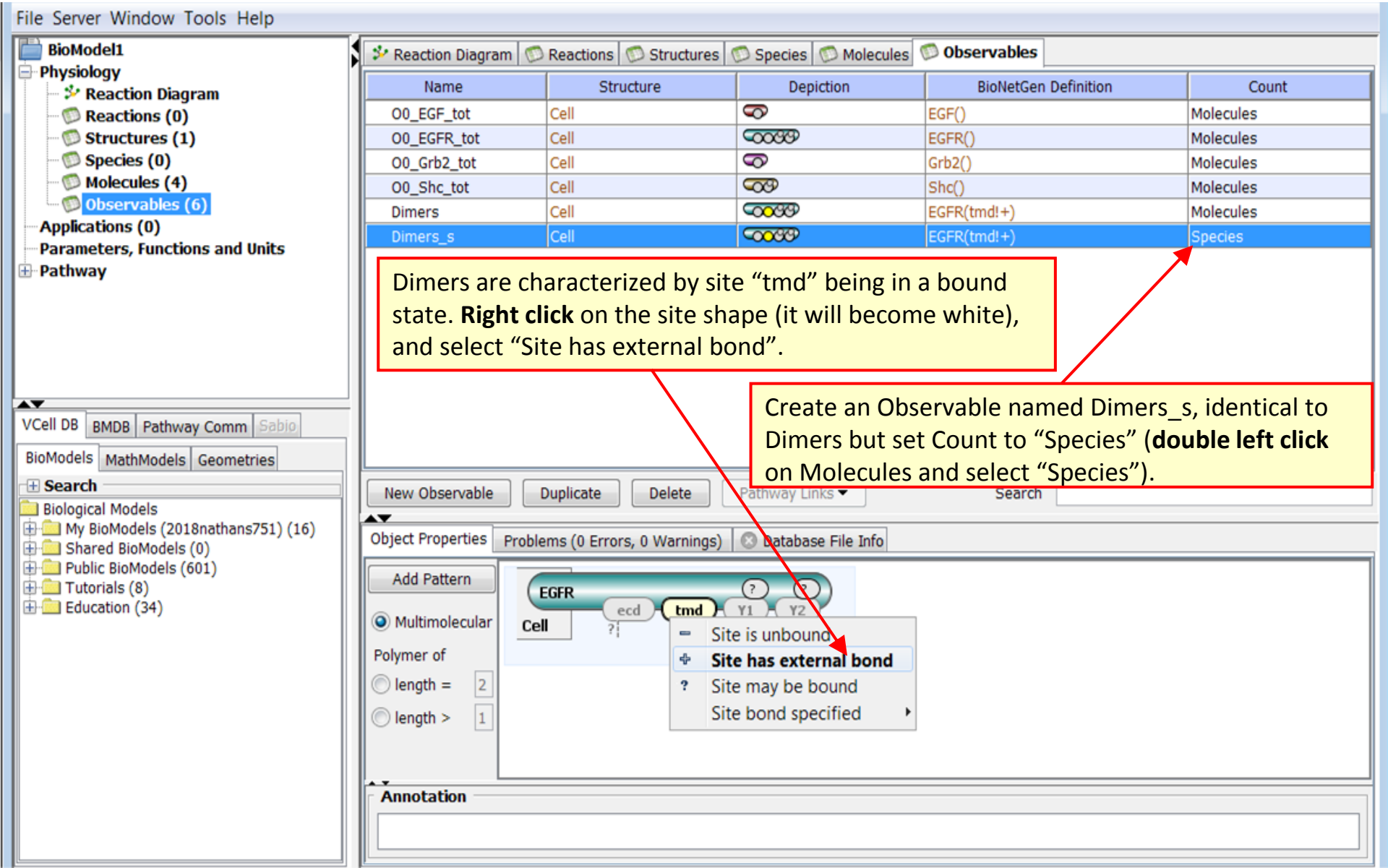
TIP: Each Observable corresponds to a sum of species selected by species patterns. Specific species are identified the network is generated using reaction rules. An observable corresponding to the total amount of all species that include this molecule is automatically generated for every molecule.



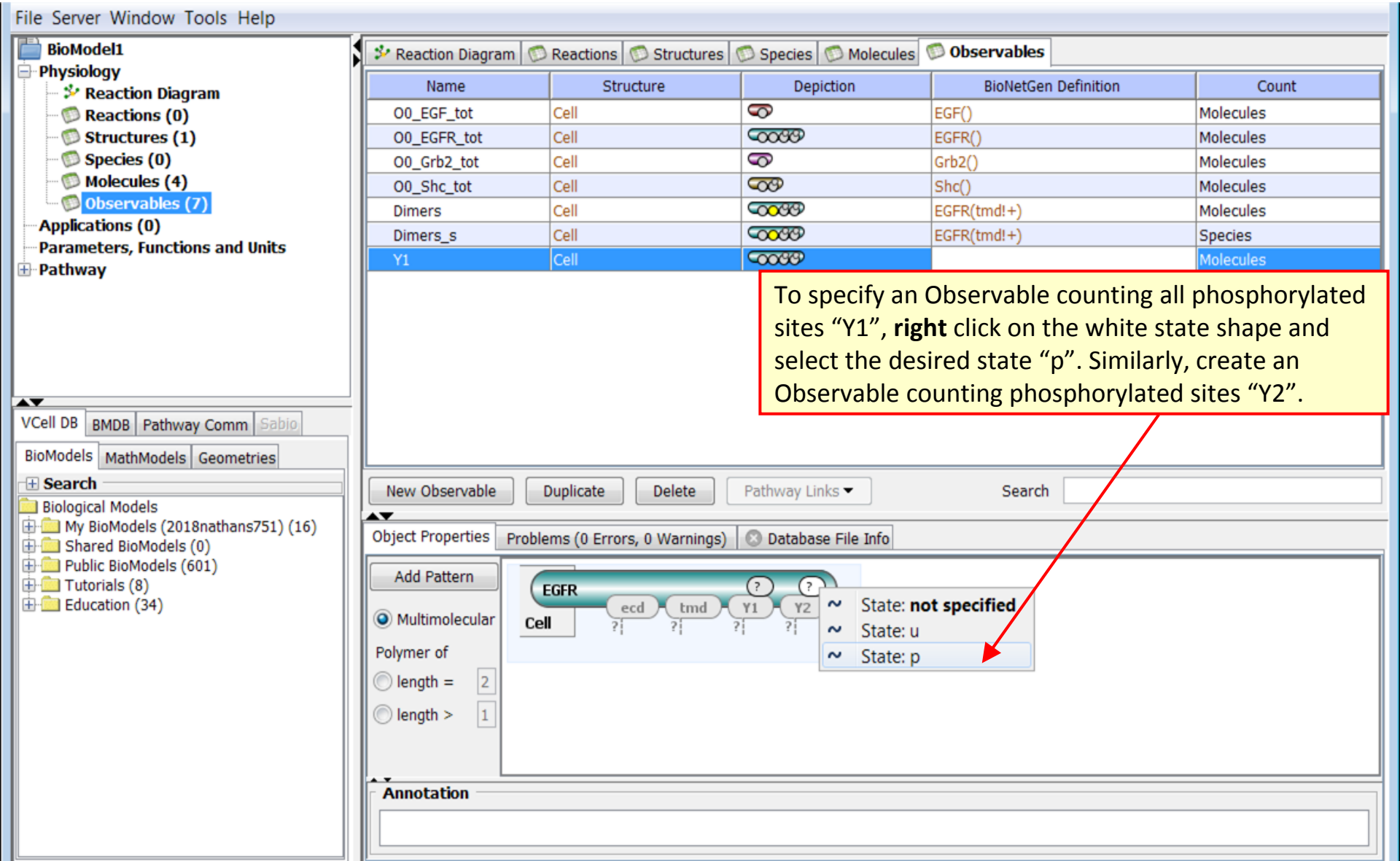
TIP: Every table has a column BioNetGen definition. It can be edited *only once* –the first time an object is specified. It is useful if you have separate BNGL code you want to paste, but do not want to import for some reason. If you paste in BNGL code, once you click enter it cannot be further edited unless you export back out as BNGL.



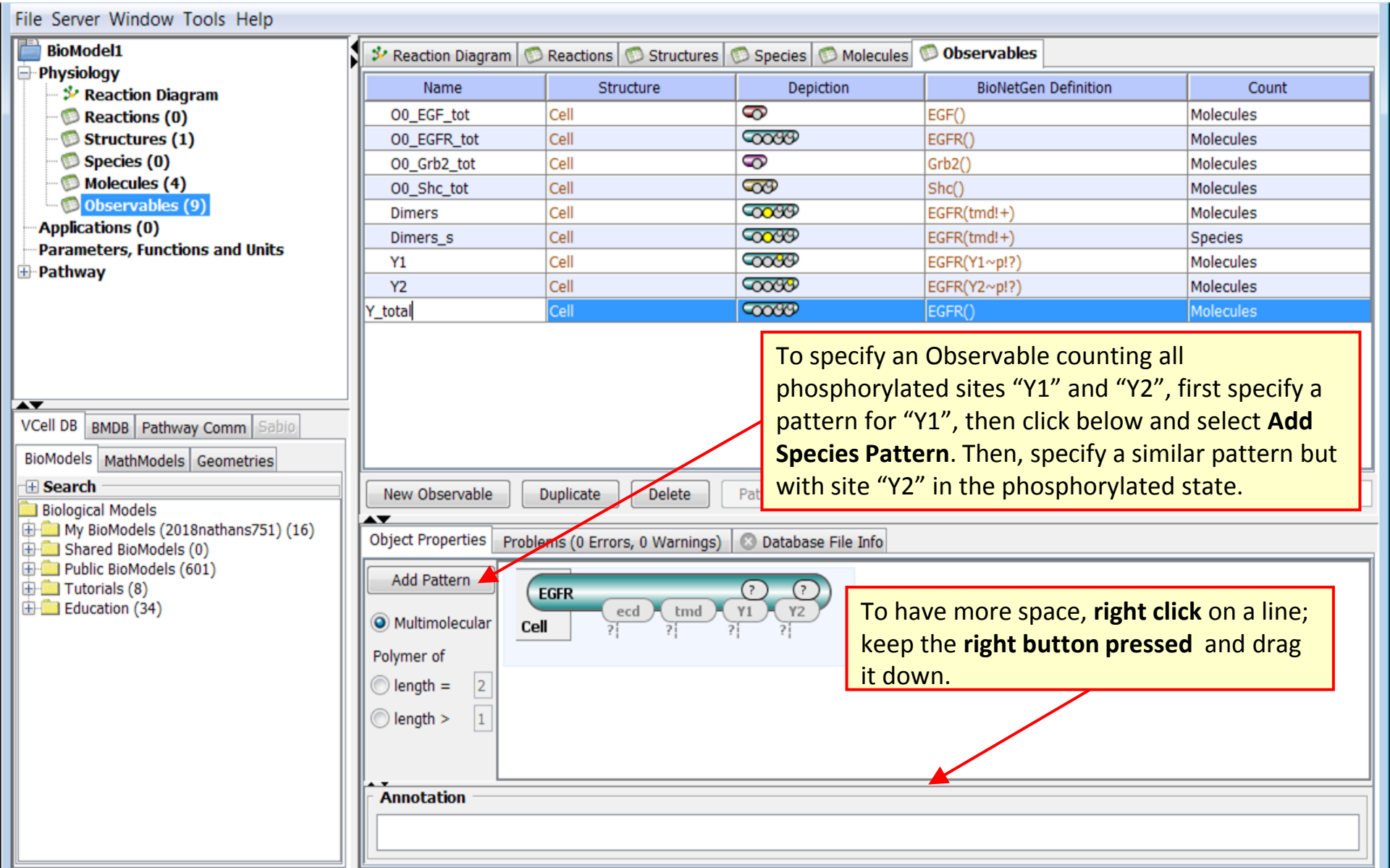
TIP: A yellow warning sign or red error sign may appear temporarily if something is wrong. After the error/warning is corrected, the sign will disappear within a few seconds.



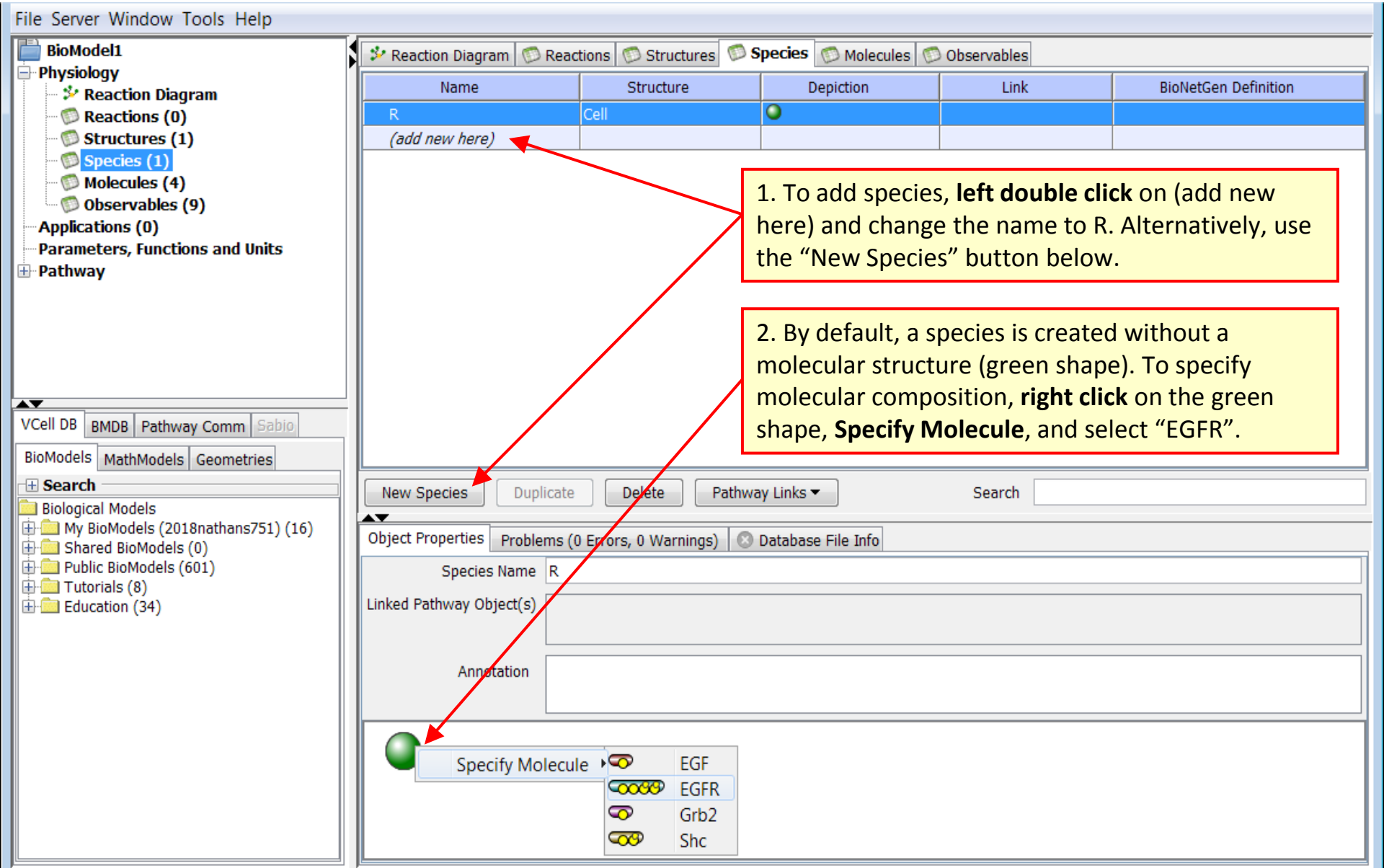
TIP: If you rename a Molecule, the Observable corresponding to its total will be renamed automatically as long as you do not change its name. For example, changing **_tot** to **_total** will decouple the Observable from the Molecule definition, and it will be no longer renamed automatically if you change the name of this molecule.



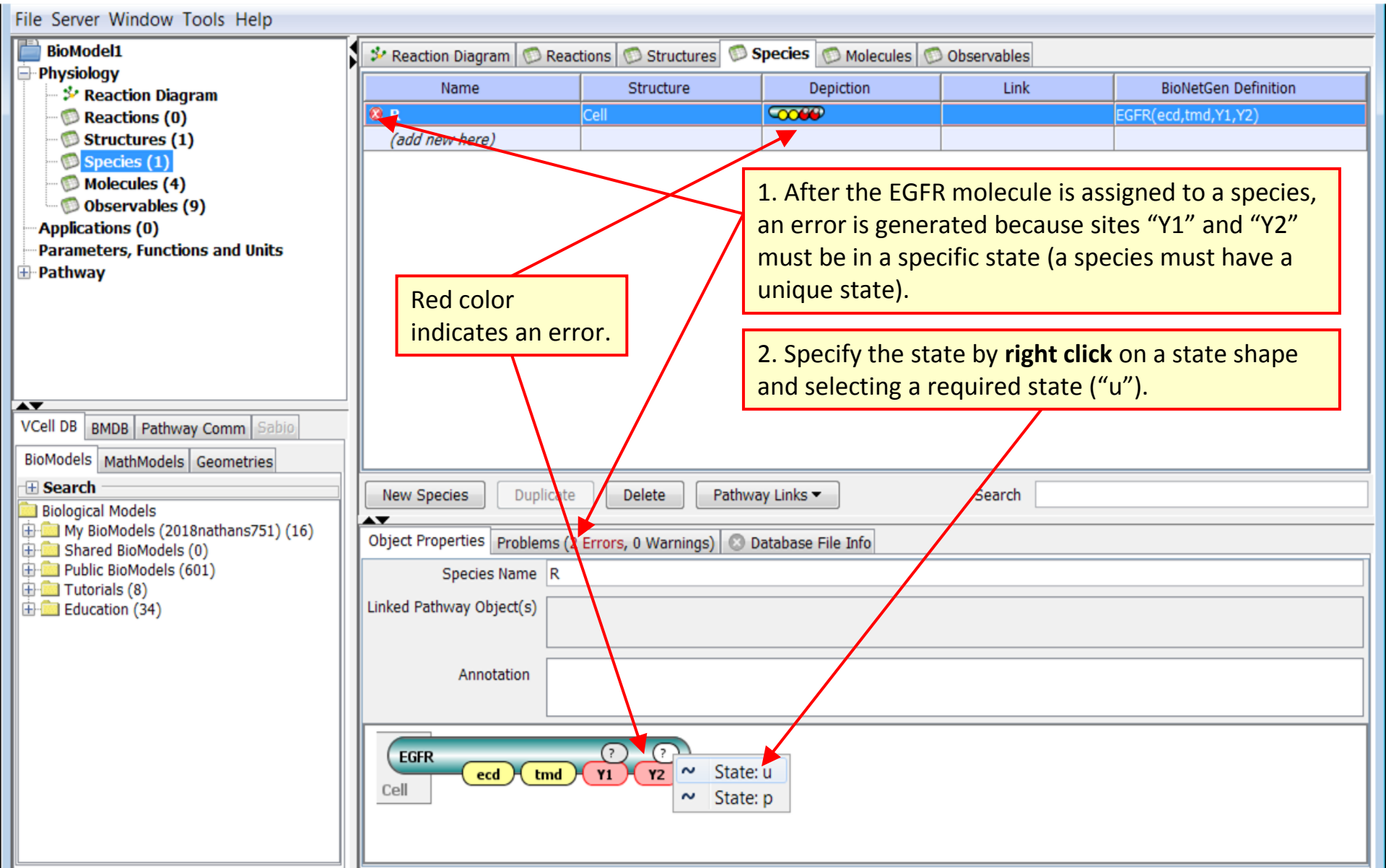
TIP: Species corresponding to each Observable can be seen after network generation under Application > Simulations > Generated Math > Math Description Language.



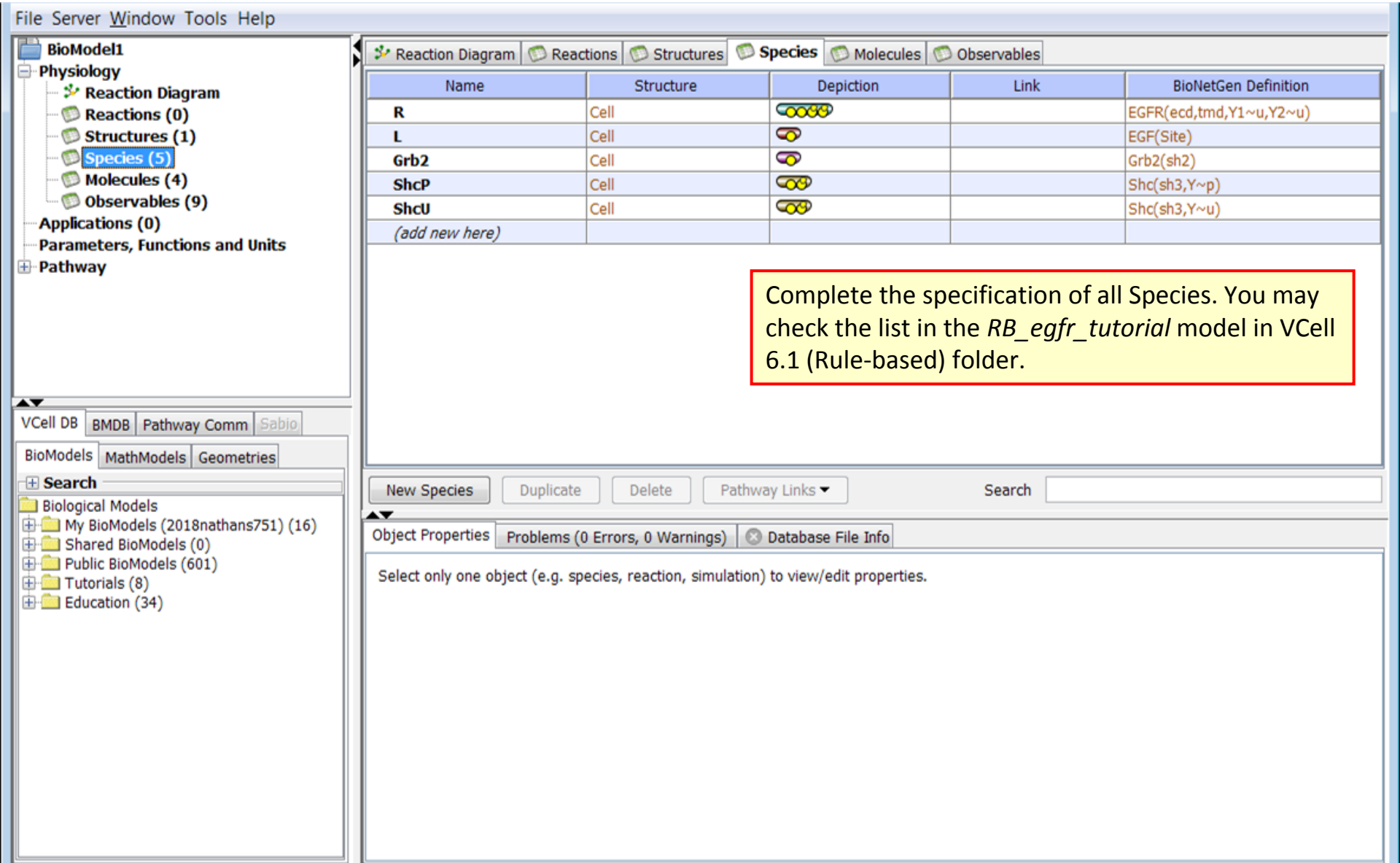
TIP: Species may consist of more than one molecule, but the molecules must be connected.



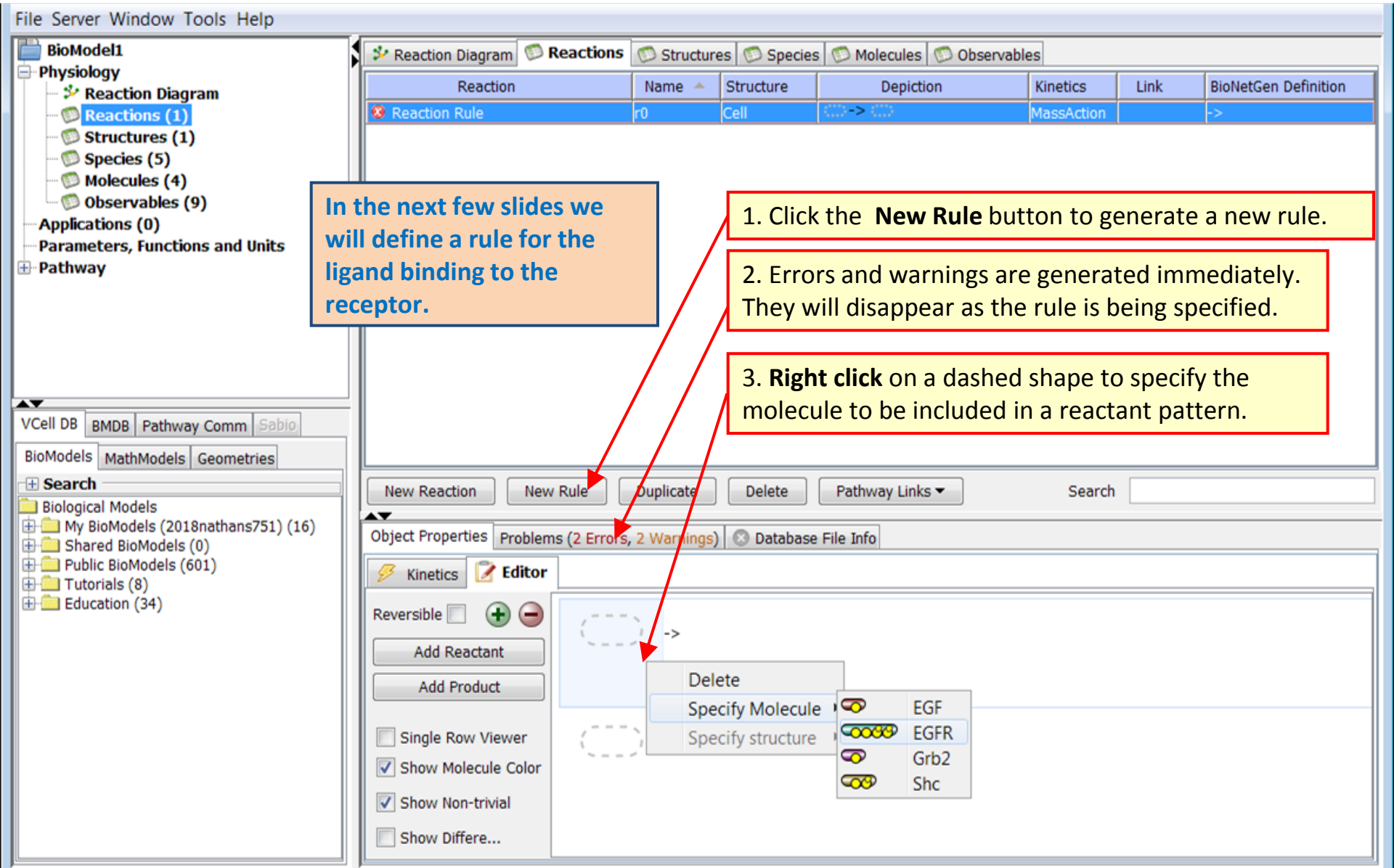
TIP: **Left click** on the Problems tab will show the list of errors and warnings. **Double left click** on a problem will bring up the issue.



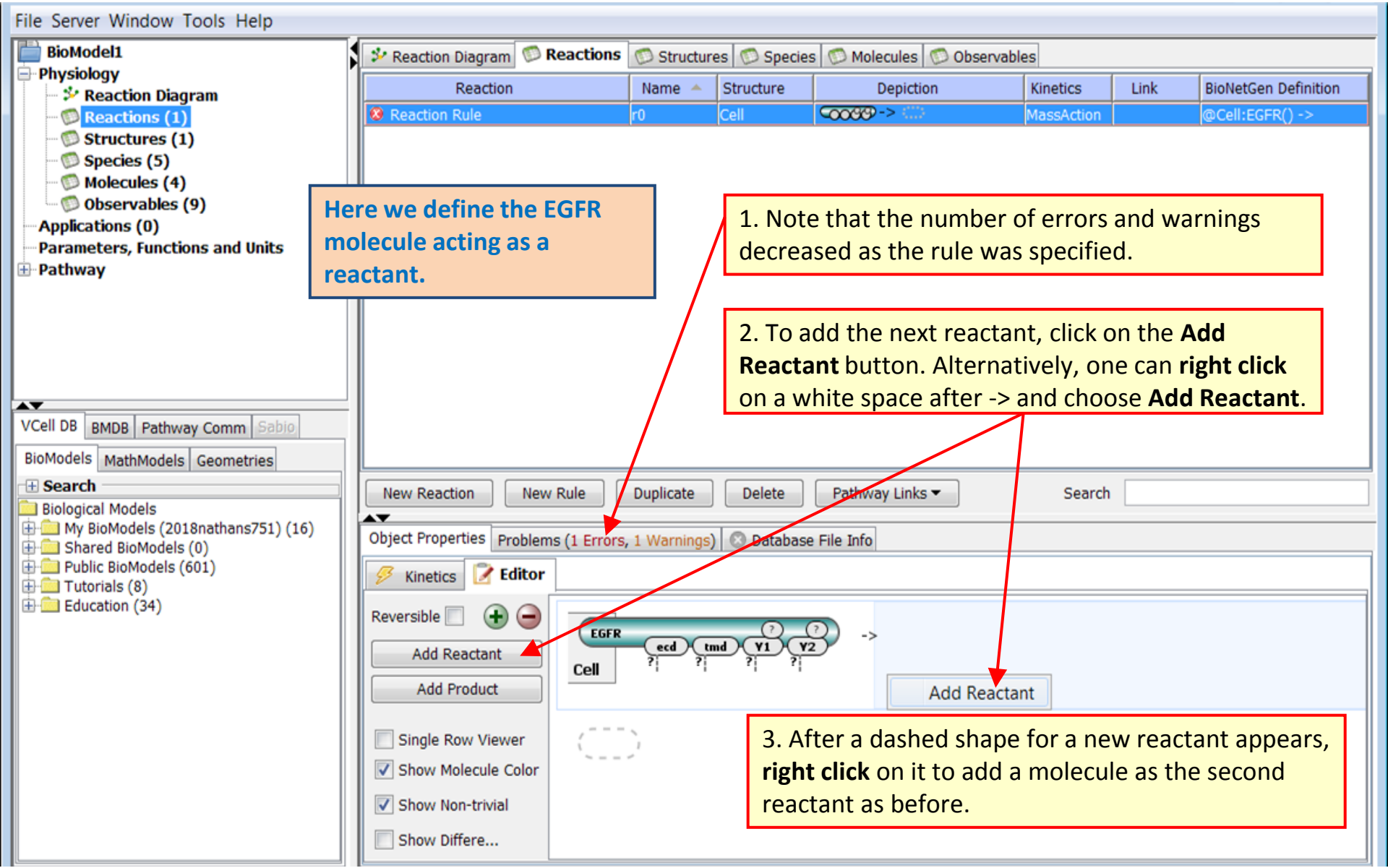
TIP: **Left click** on a Table column name (e.g. Name) will sort the table by this column.



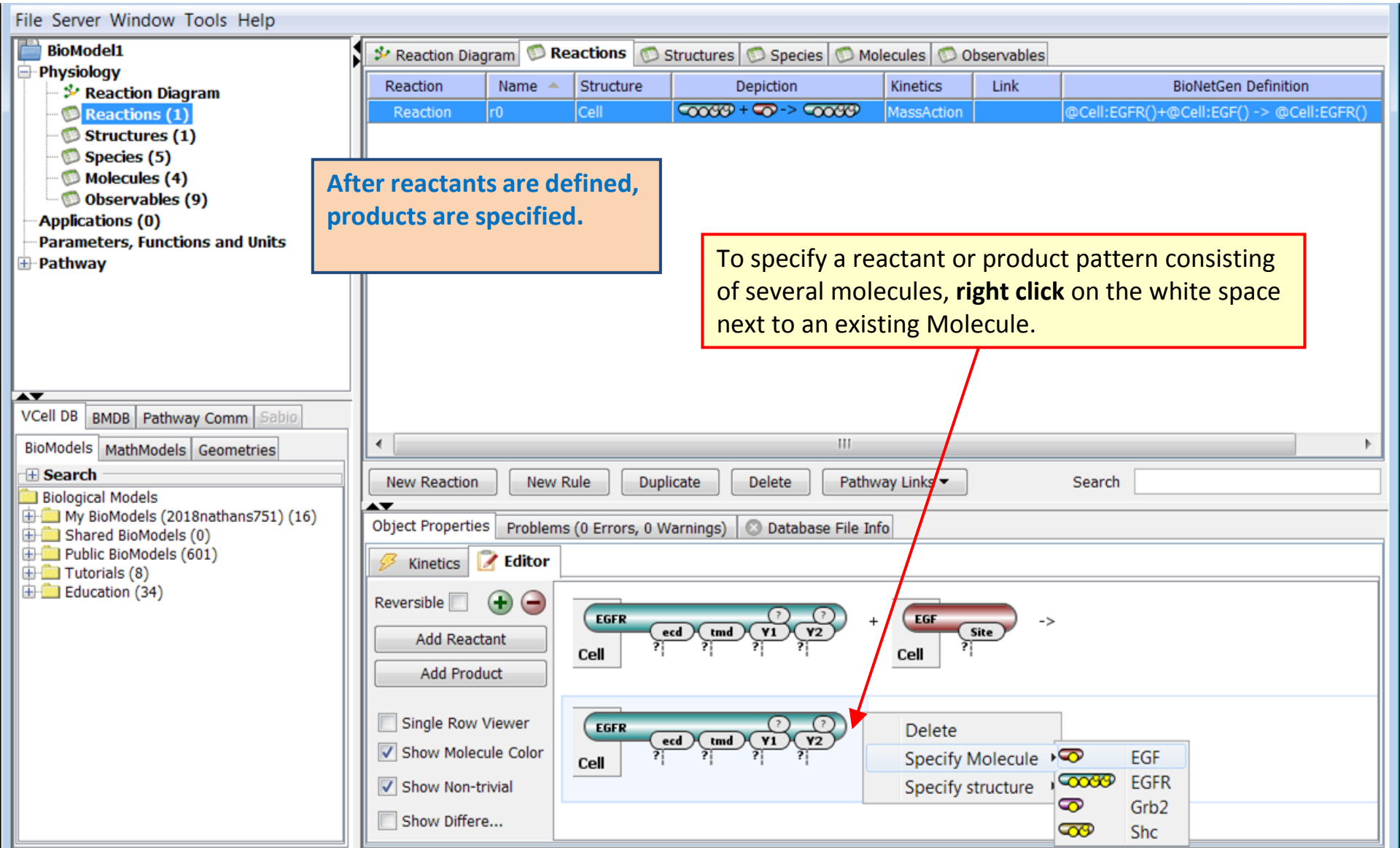
TIP: Reaction rules generate reactions by selecting species that serve as reactants and generating new species i.e. the products of these reactions. Thus, each reaction rule is defined with reactant patterns (that select species to be reactants) and products patterns (to define how reactant molecules are modified).



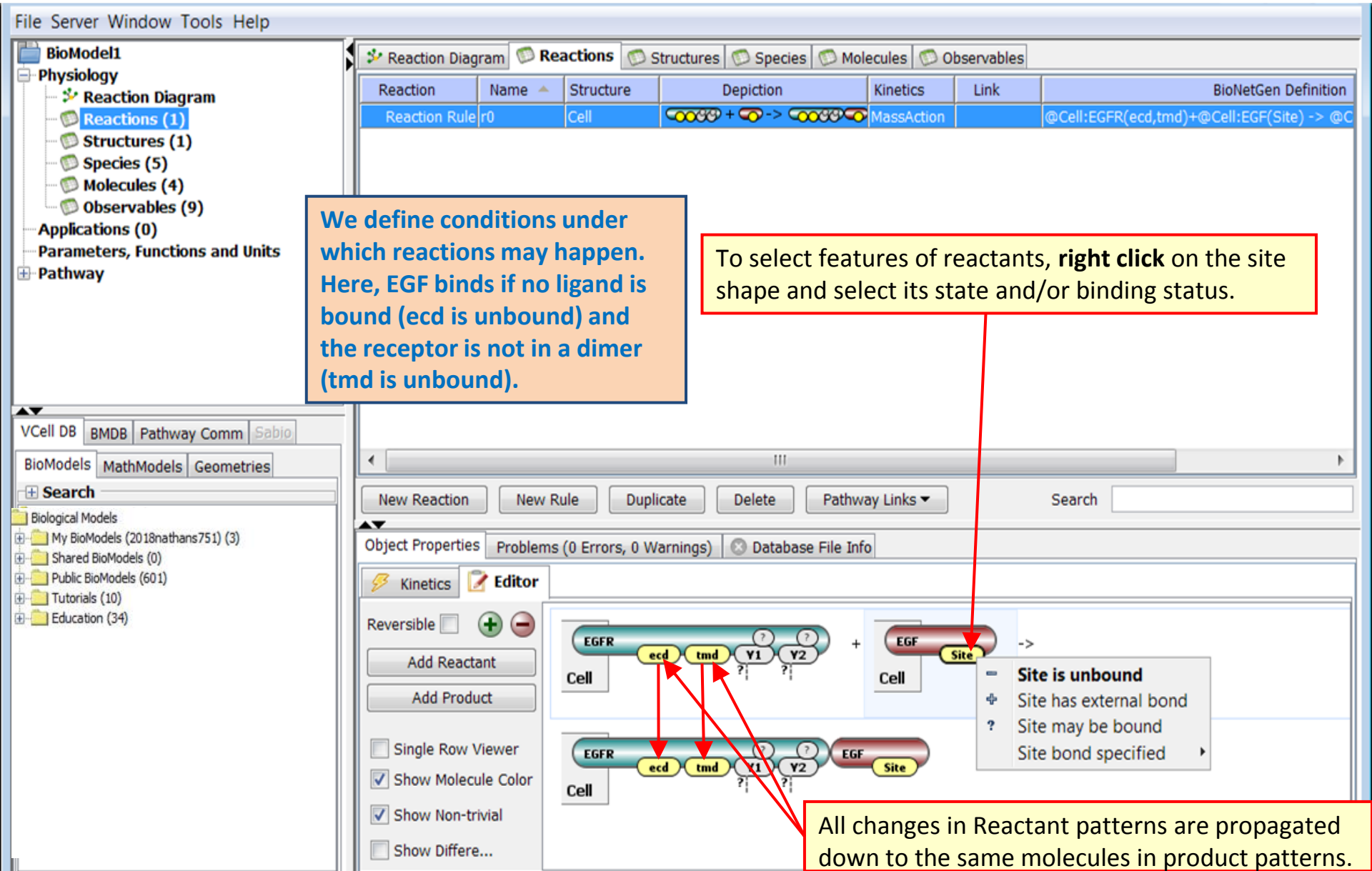
TIP: Always check errors and warnings until you understand the issue. If in trouble, use Help from the top menu. It is fully searchable. It can be printed from http://vcell.org/support



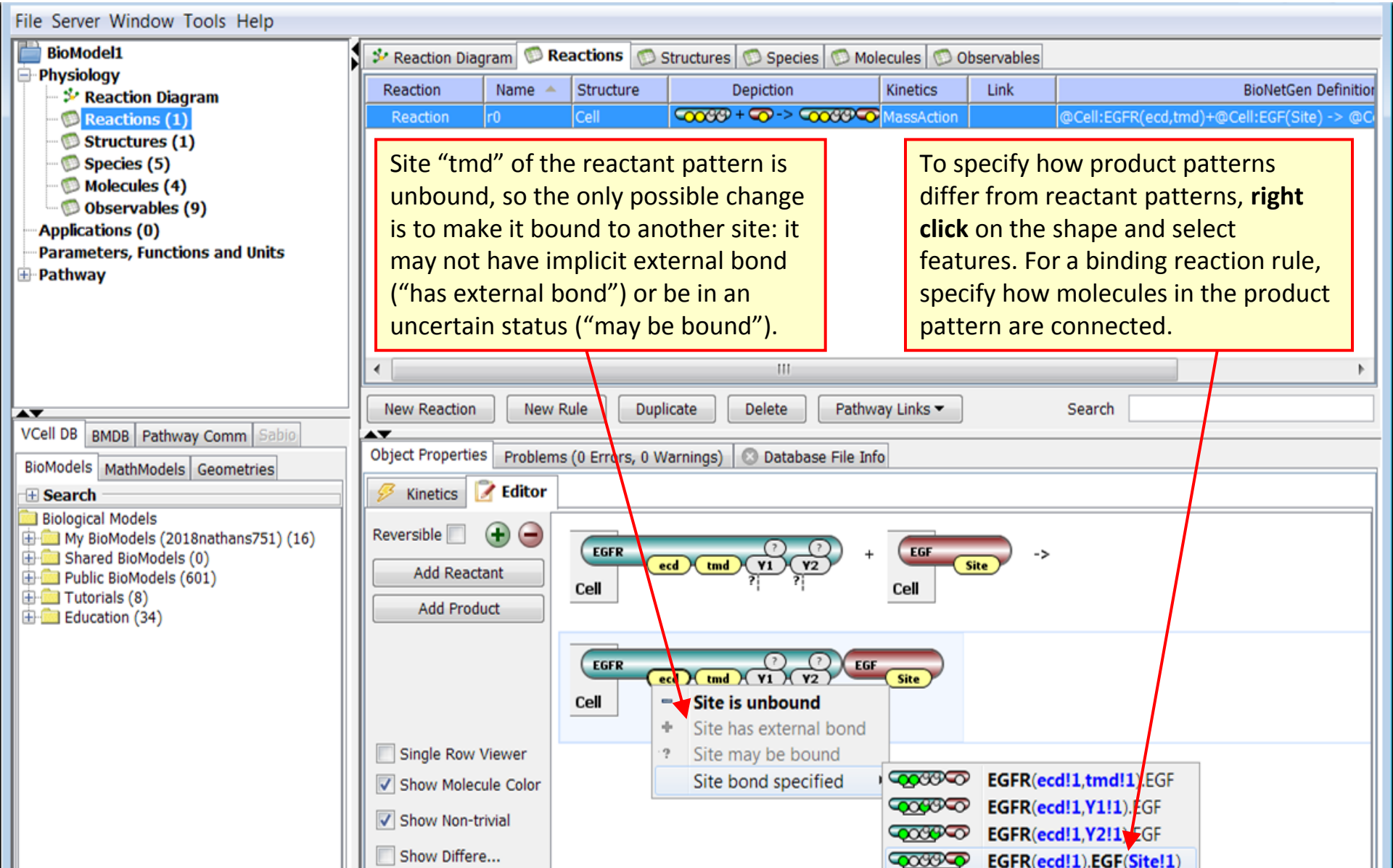
TIP: The search field can be used to filter all lists by an entered term, such as Molecule or site name.



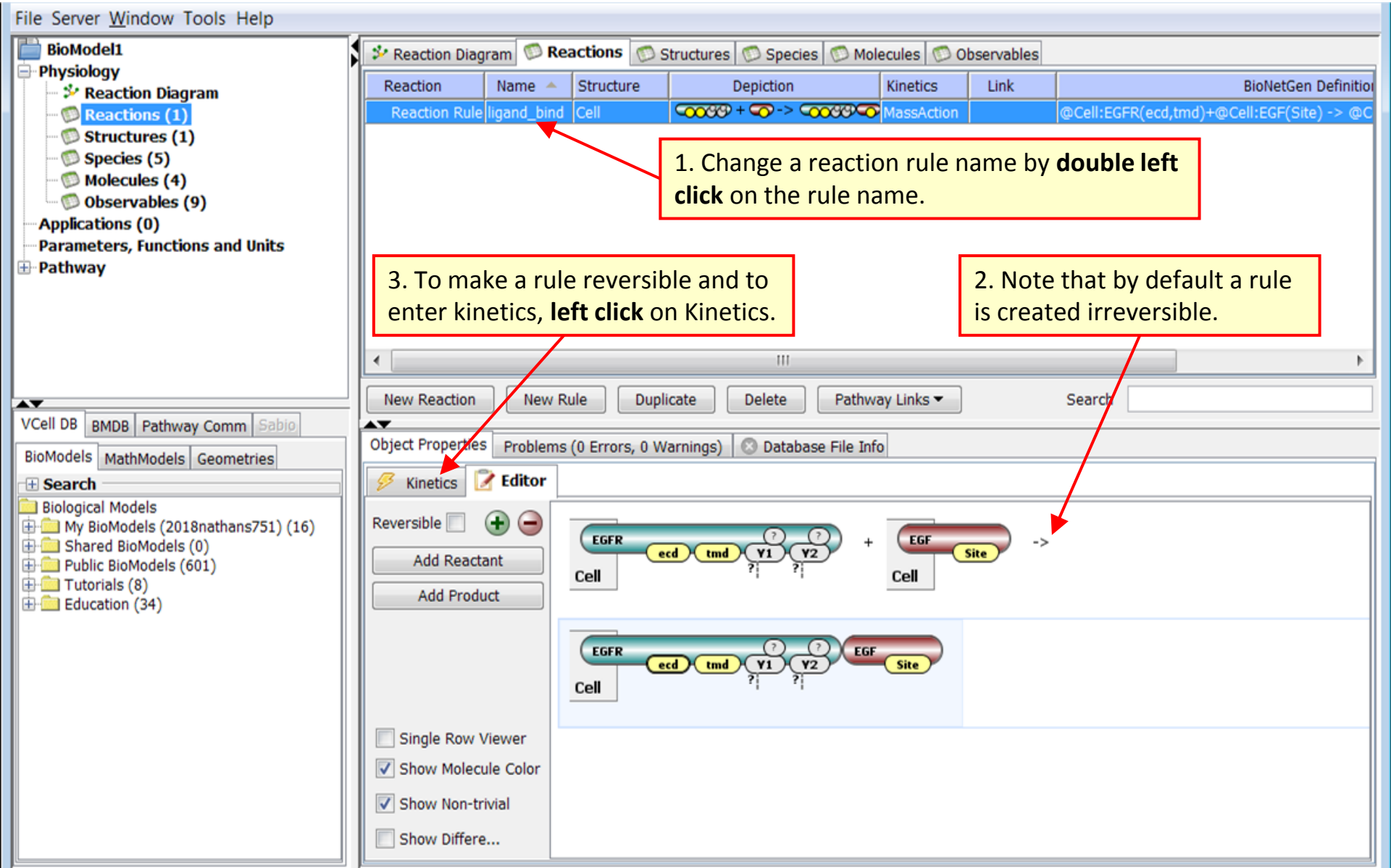
TIP: Molecules in reactant/product patterns can be rearranged by **right click** on the Molecule shape and choosing **Move right**/**Move left** actions.



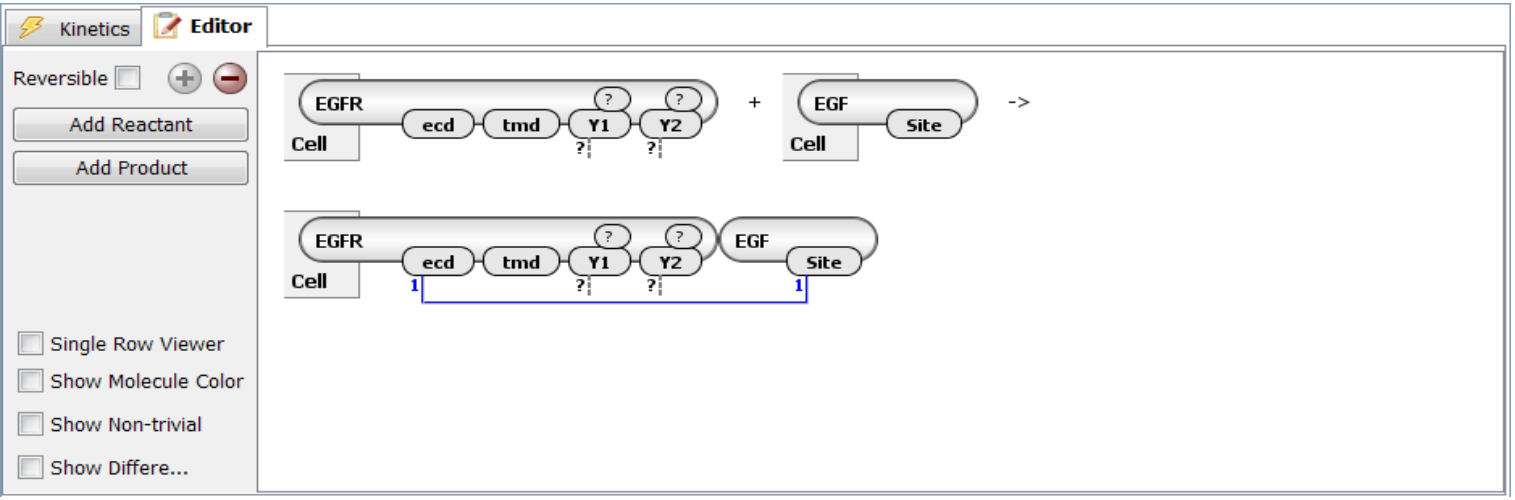
TIP: Note that some options for binding status are greyed out because they are impossible.



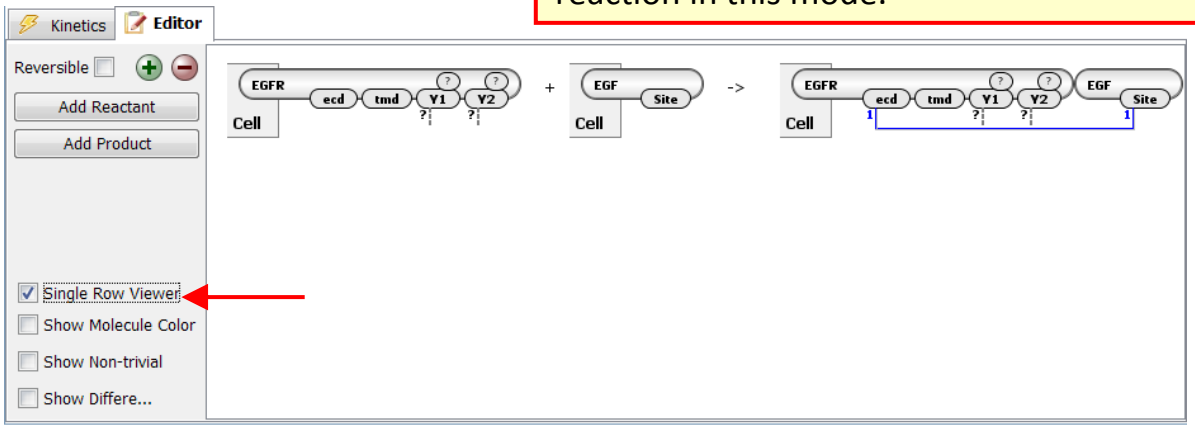
TIP: Sites in yellow without any symbols underneath are always unbound.



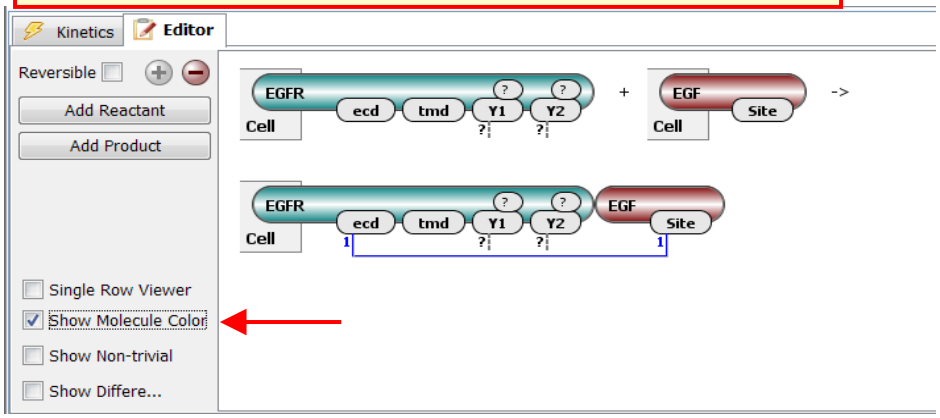
With no boxes checked, the reaction is shown in black and white, with only the site specific bonds indicated in color.



Checking the **Single Row Viewer** box aligns the entire reaction in one row. You can not edit the reaction in this mode.

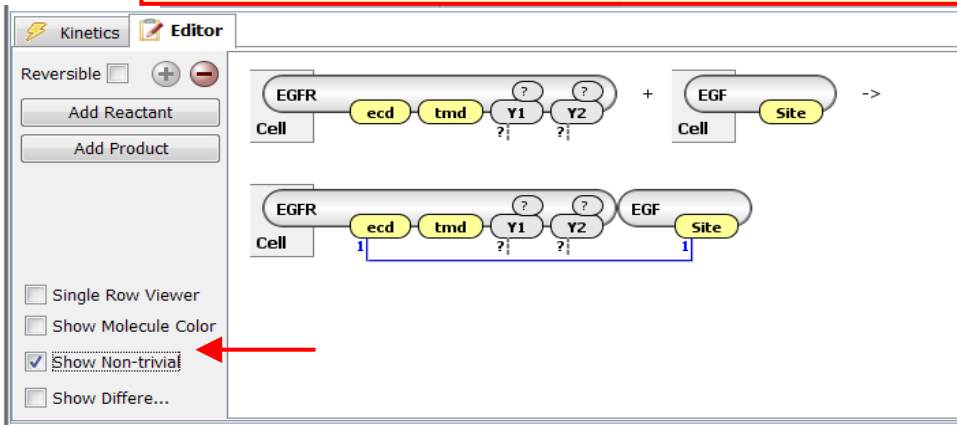


Checking the **Show Molecule Color** box adds an ordered color to the molecule to help with visual differentiation. The specific color can not be changed.

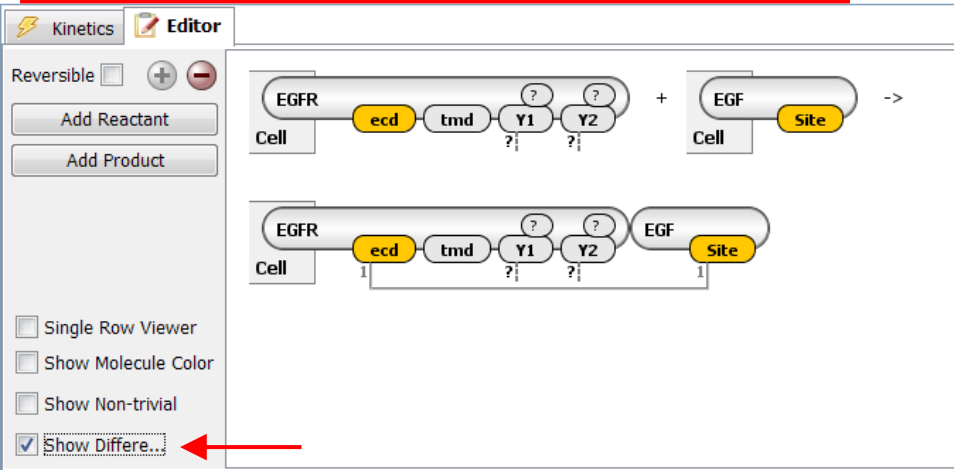


TIP: Any combination of viewing buttons can be used.

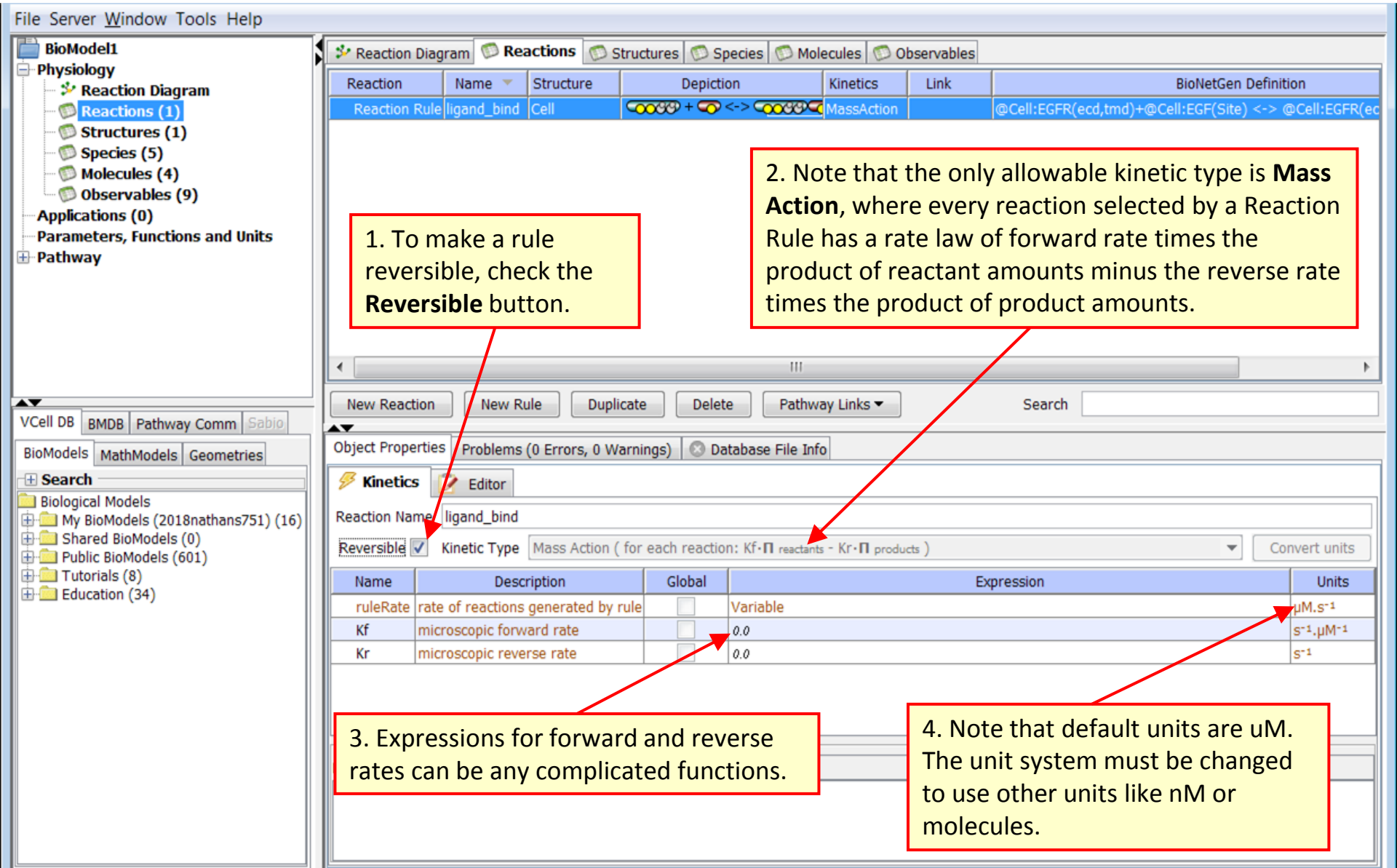
Checking the **Show Non-trivial** box highlights assigned sites and states in yellow.



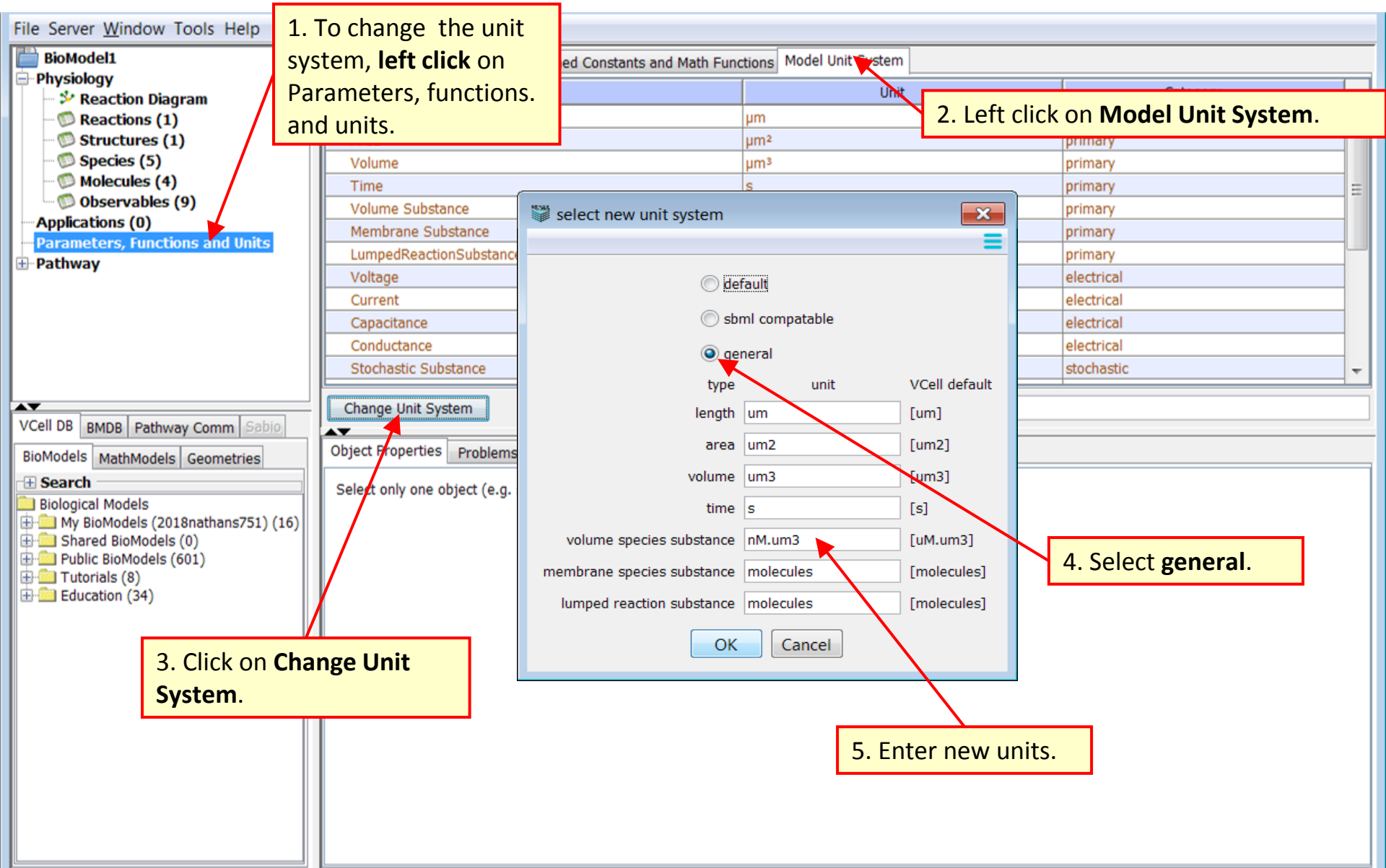
Checking the **Show Differe…** box highlights in orange the differences in bonds, sites, and states between the reactants and the products.



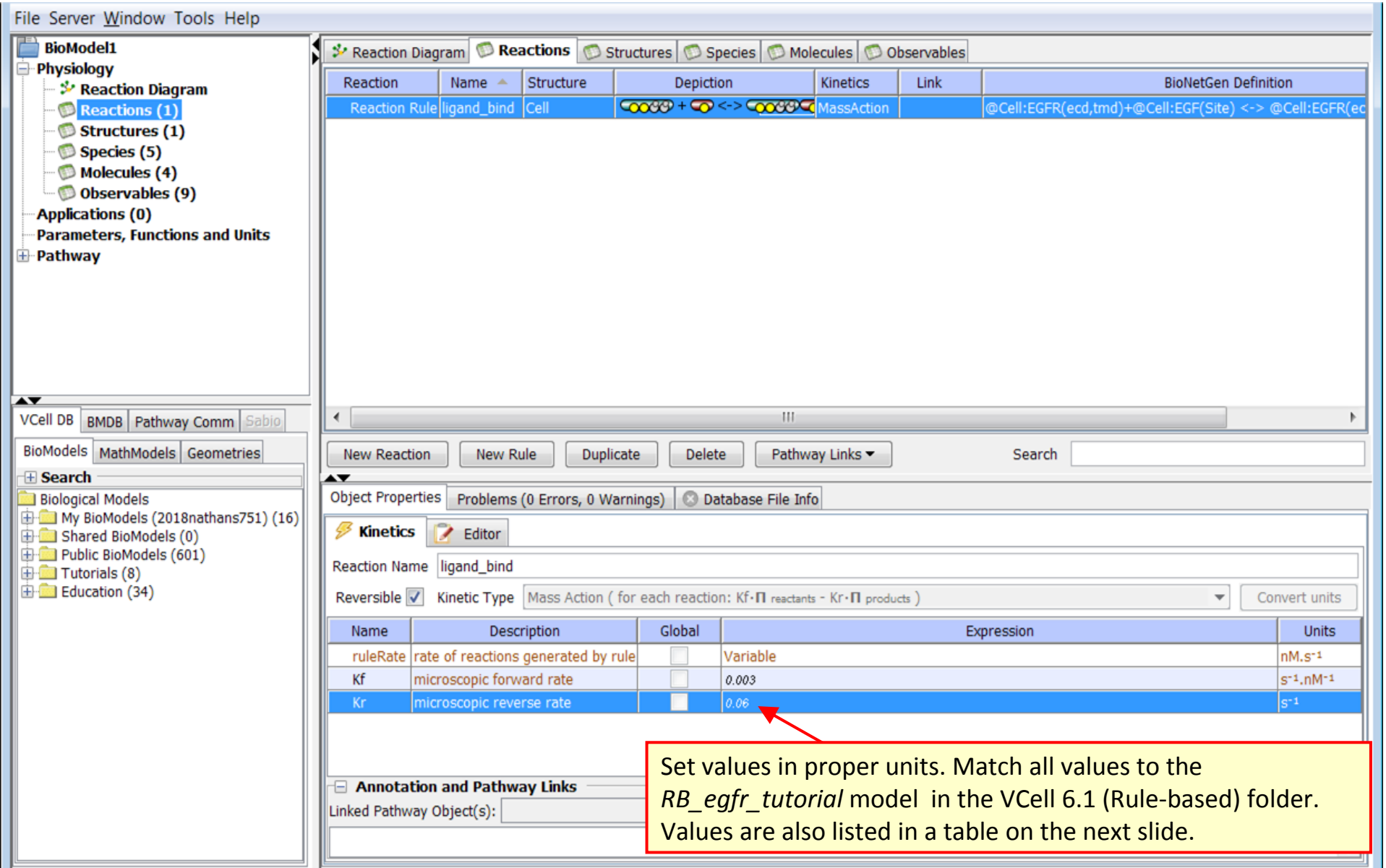
TIP: The numbers of specified Molecules, Species, Reactions and Observables are always displayed in the left panel.



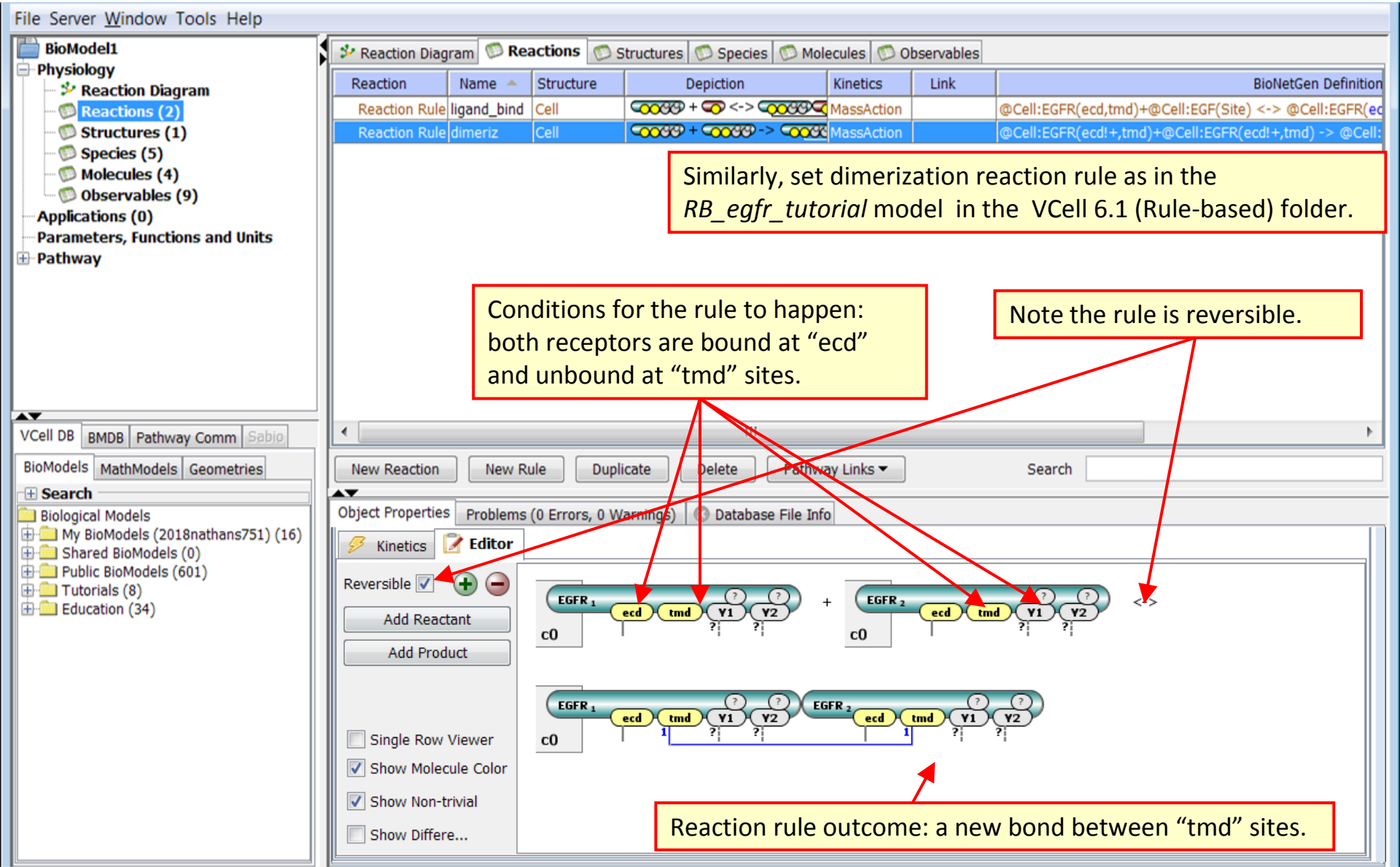
TIP: The unit system must be changed before entering any numeric values. Otherwise, all values will be converted from the old units to a new unit system.



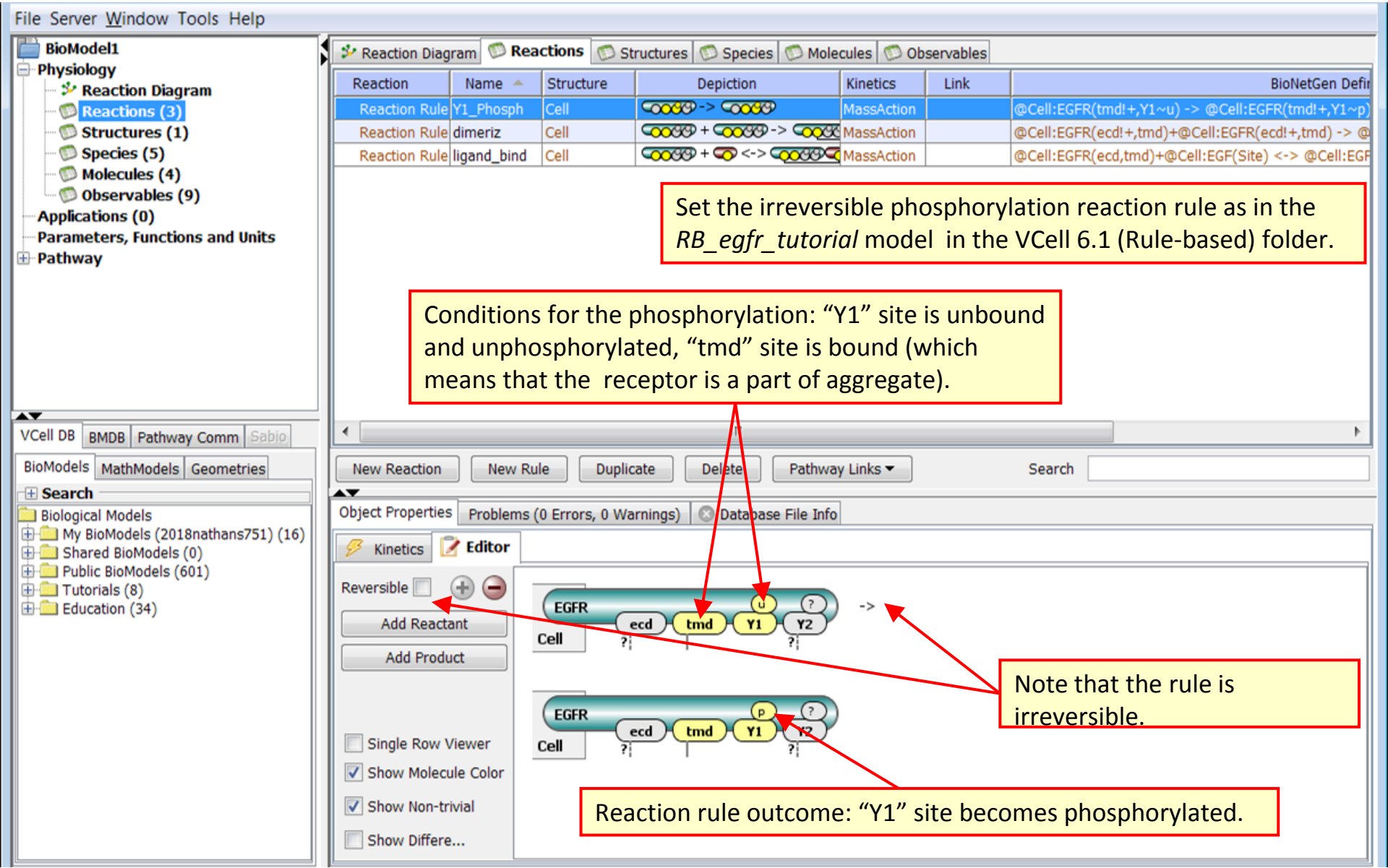
TIP: VCell has various kinetic types, but rule-based models in version 6.1 are limited to mass-action kinetic only.



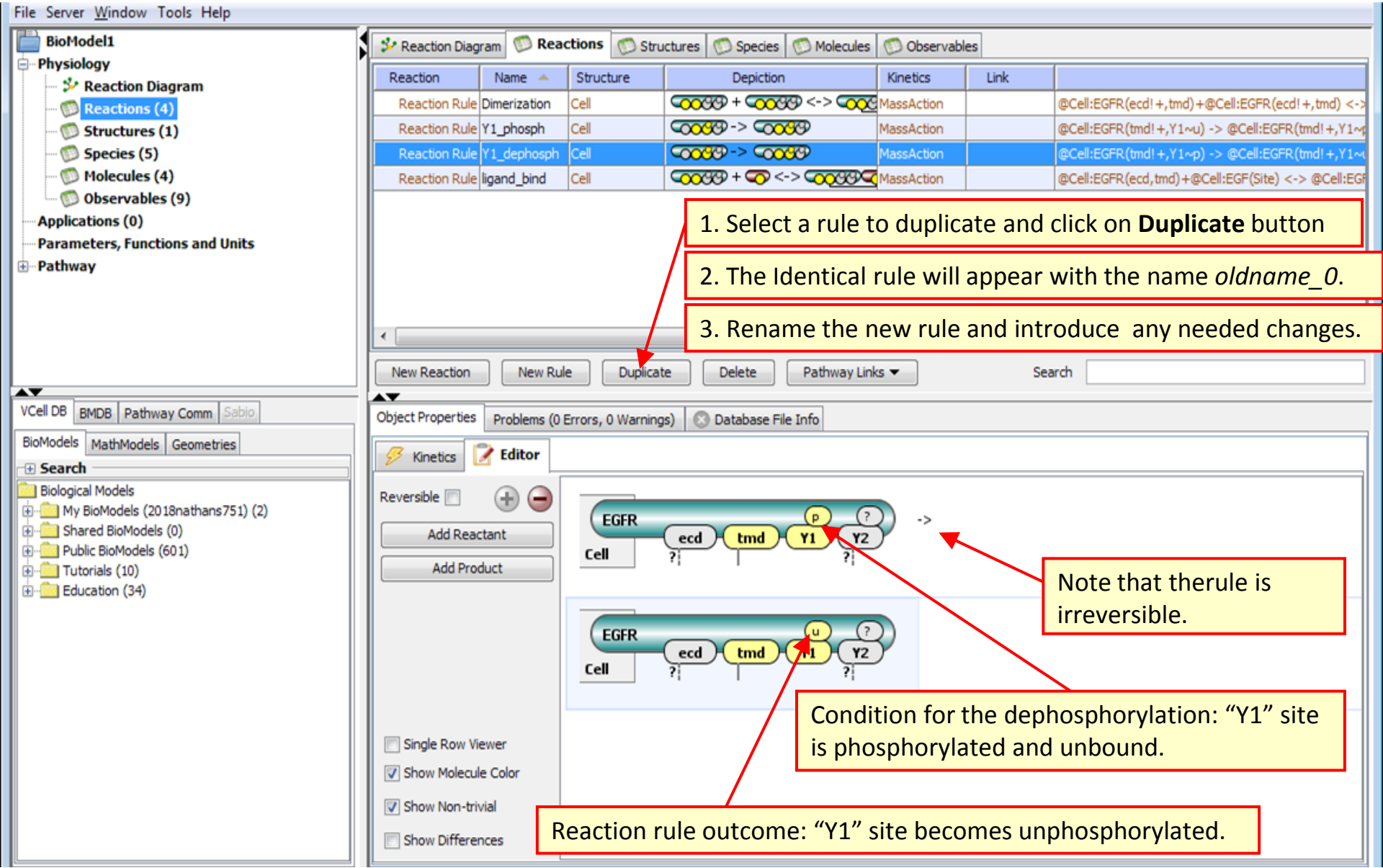
TIP: If reactants or products contain identical molecules, they are automatically numbered for the modeler's convenience, so the user can match reactants to products.

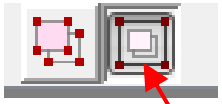


TIP: A site with a vertical line underneath means that the site is bound, but the binding partner is not explicitly specified and can be any molecule allowable by rules.

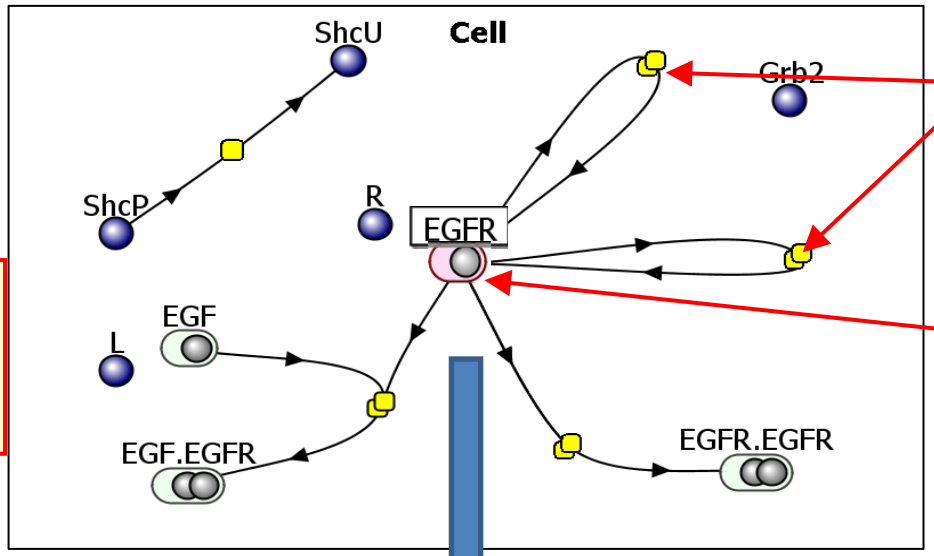


TIP: Using the **Duplicate** button can save a lot of time when a combination of multiple molecules participates in multiple reaction rules. Make sure you edit the copied rule and not the original one!



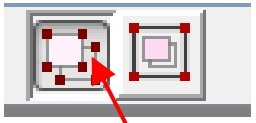


In the collapsed reaction diagram, the reaction rule participants are grouped by molecular structure.



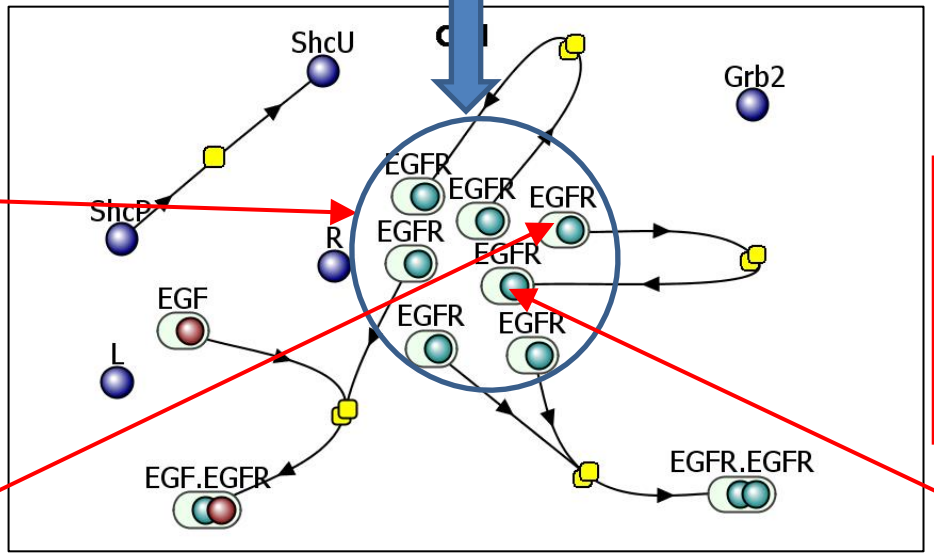
These are Reaction rules transforming EGFR molecule.

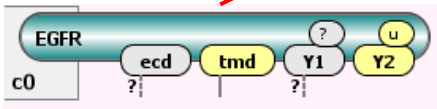
The reactant and product species patterns are distinct, but both contain the **EGFR** molecule, so they are shown as a single node.



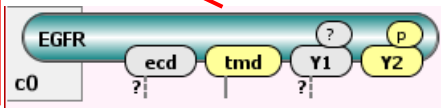
In the full reaction diagram, every reactant and product species pattern is shown individually if they are distinct in details.

These are distinct species patterns corresponding to the same molecular structure in the collapsed reaction diagram.





The two nodes, though they look alike, correspond to the two identical molecular structures, but are different in details; one is unphosphorylated at **Y1**, and another is phosphorylated at **Y1**.



This is the collapsed reaction diagram.

The black arrows indicate the direction of the reactions and reaction rules.

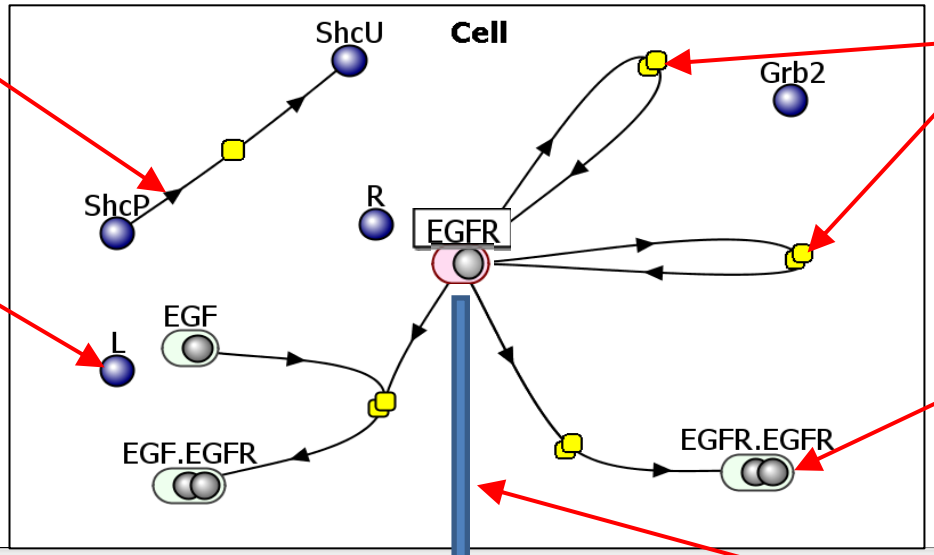
Species are depicted as blue spheres

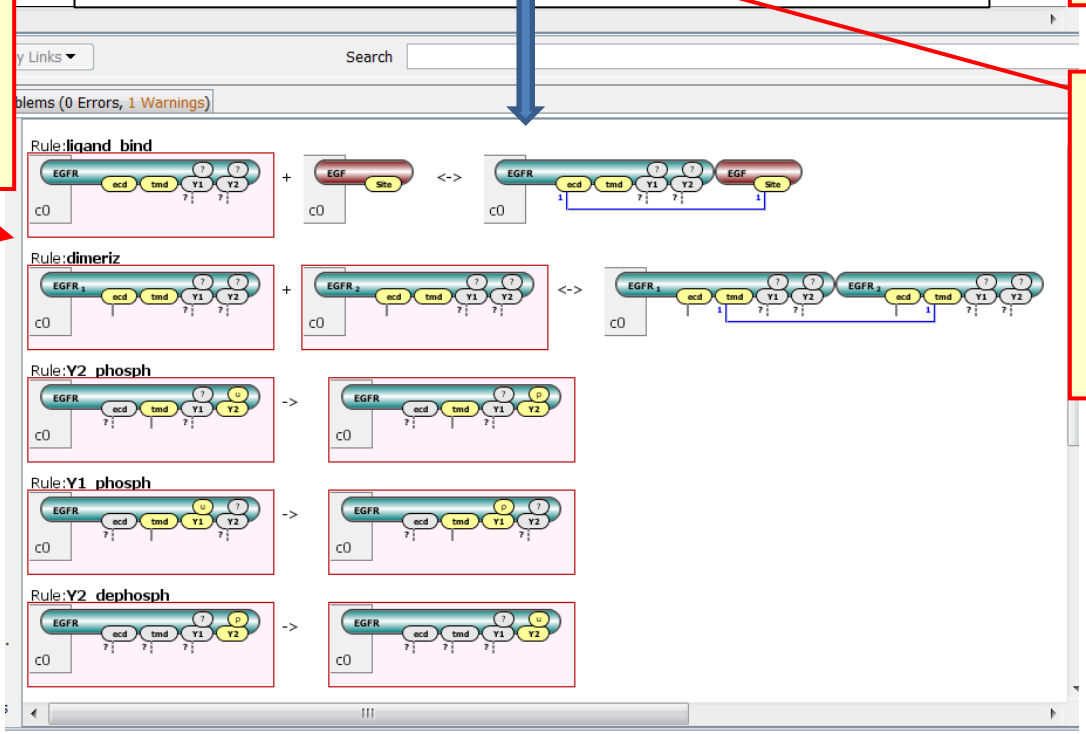
The molecular structure that was clicked on is highlighted in red in the displayed reaction rules.

Reaction rules (phosphorylation and dephosphorylation) where the product and reactant have identical molecular structures.

Molecular structures with 2 spheres instead of one are bimolecular. In this case it is an EGFR dimer.

By clicking on any molecular node in the reaction diagram, one can see all reaction rules in which this molelcular pattern is used.





This is the full reaction diagram.

The black arrows indicate the direction of the reactions and reaction rules.

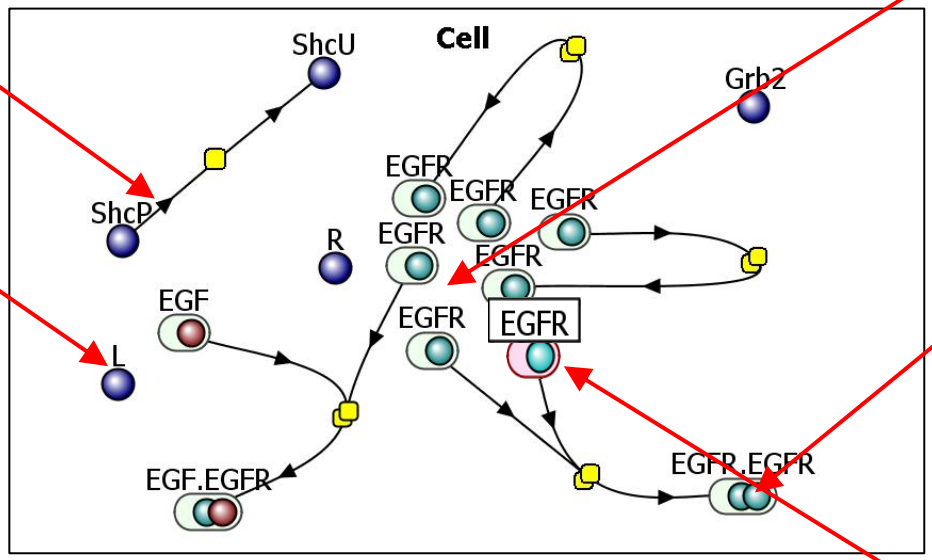
Species are depicted as blue spheres.

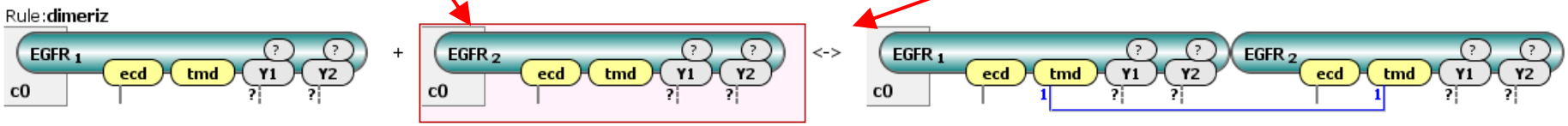
The molecular structure that was clicked on is highlighted in red in the displayed reactions.

Notice how in this version of the diagram there are no groupings. Each reaction, product, and reactant is shown separately as opposed to being combined by molecular structure.

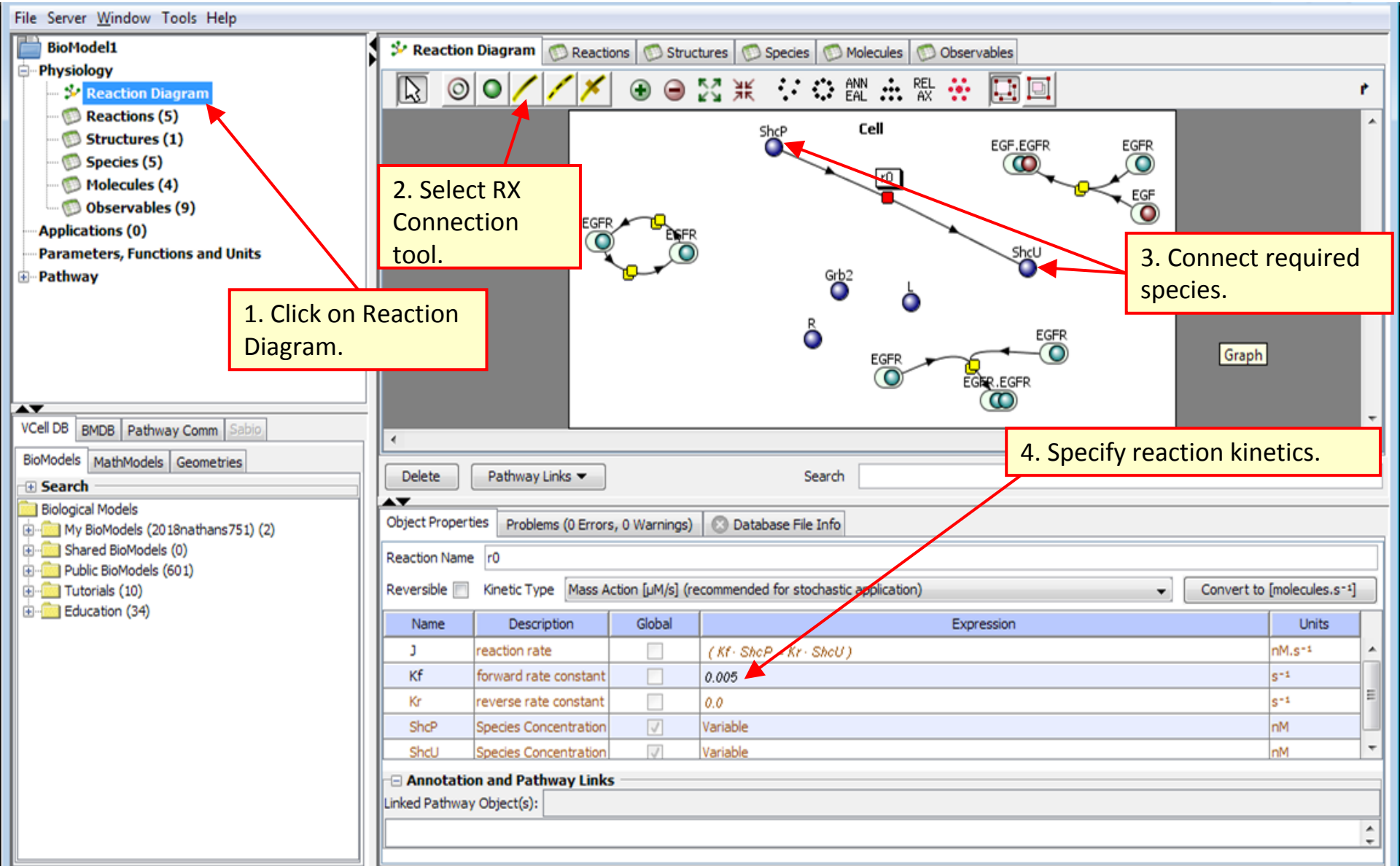
Colors within shapes correspond to molecular colors.

By clicking on an EGFR, the reaction rule in which it is implicated is shown.

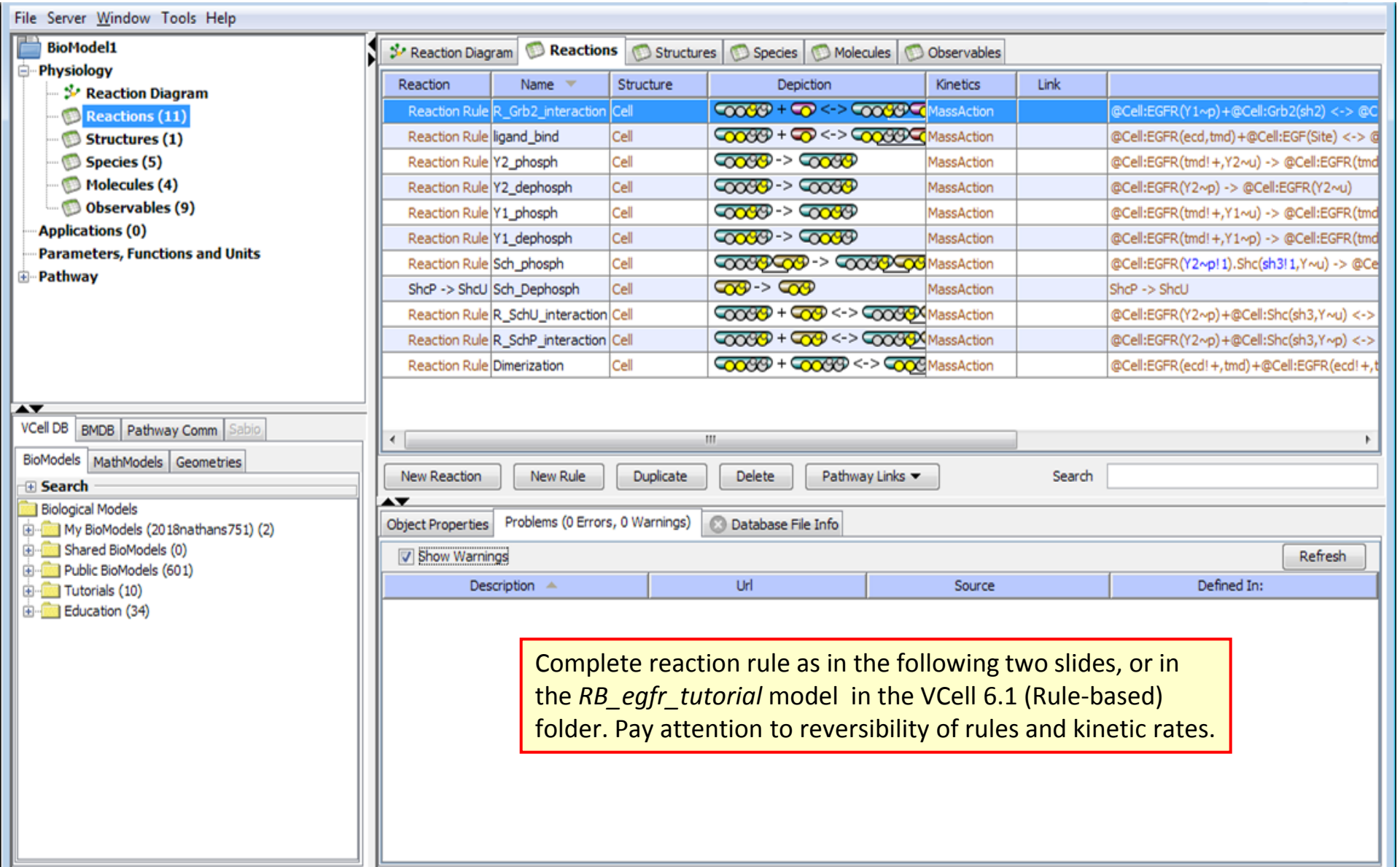




TIP. One can use VCell reaction tools to create non-rule based reactions among species (see other tutorials on VCell use).

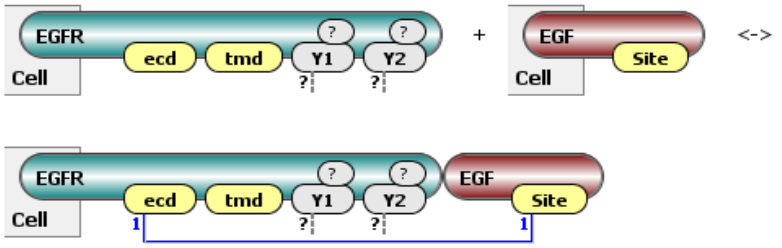


TIP: Enter a string (e.g. Molecule or Site name) in the Search field, and the table will be filtered to display only entries containing this string. You can enter any BNGL string as well.

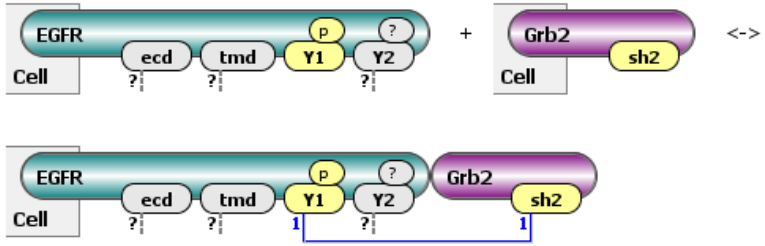


Complete reaction rule as in the following two slides, or in the *RB_egfr_tutorial* model in the VCell 6.1 (Rule-based) folder. Pay attention to reversibility of rules and kinetic rates.

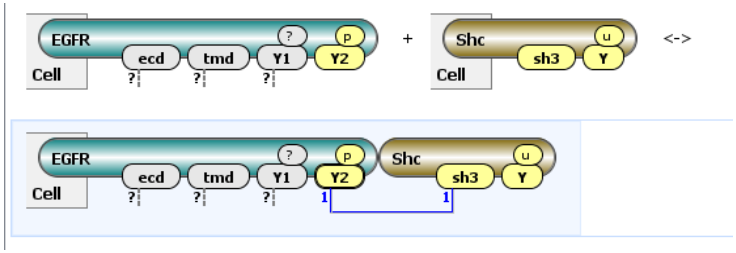
Contents



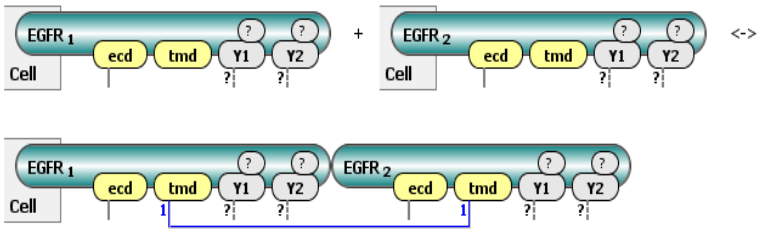
Ligand_Bind (receptor must be in monomeric form (tmd is unbound) and not bound to ligand (ecd is unbound) for reaction to happen.)



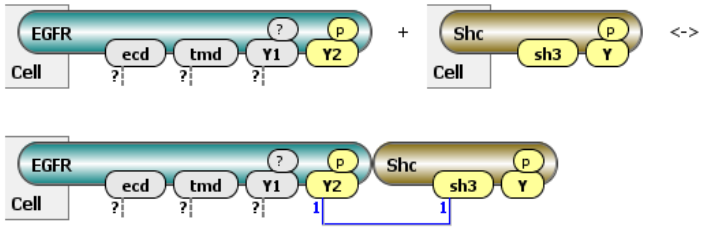
R_Grb2_interaction (EFGR does not have to be in monomeric form. Y1 has to be phosphorylated, for it to bind to sh2).



R_ShcU_interaction (Receptor is not necessarily in monomeric form. Y on Shc must be unphosphorylated. Phosphorylated Y2 binds with sh3).



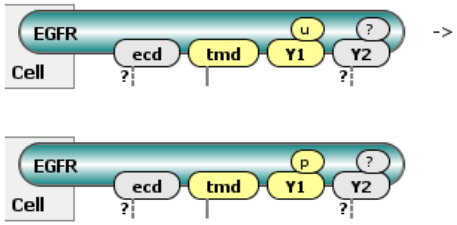
Dimeriz (tmd must be unbound and ecd has to be bonded externally for the two tmd sites to bond and form a dimer).



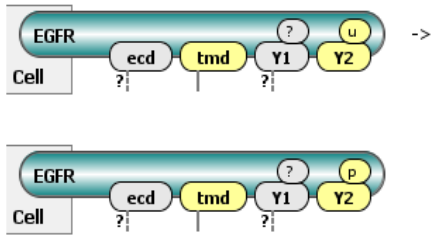
R_ShcP_interaction (for this reaction to occur, the Y site on Shc has to be unbound and phosphorylated. The unphosphorylated Y2 binds with sh3).

Reaction	Reversible?	Kf	Kr
ligand_bind	Yes	0.003 1/(nM s)	0.06 1/s
Dimeriz	Yes	0.001 1/(nM s)	0.1 1/s
R_Grb2_interaction	Yes	0.001 1/(nM s)	0.05 1/s
R_ShcP_interaction	Yes	4.5E-04 1/(nM s)	0.3 1/s
R_ShcU_interaction	Yes	0.045 1/(nM s)	0. 6 1/s

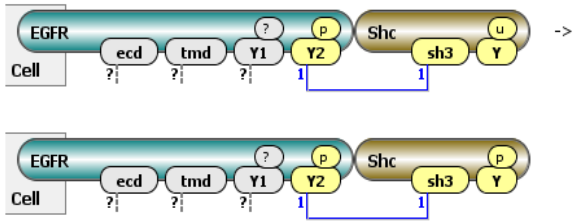
Contents



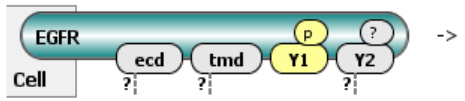
Y1_Phosph (for phosphorylation to occur, tmd must be externally bound, implying a dimeric form).



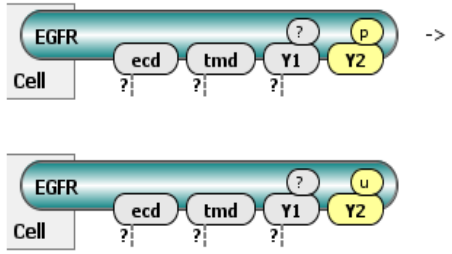
Y2_Phosph (for phosphorylation to occur, tmd must be externally bound, implying a dimeric form).



Sch_Phosph (The Y site on Shc changes from unphosphorylated to phosphorylated. In order for this to happen, sh3 must be bound to the phosphorylated Y2 site).



Y1_Dephosph (the Y1 site changes states from phosphorylated to unphosphorylated).

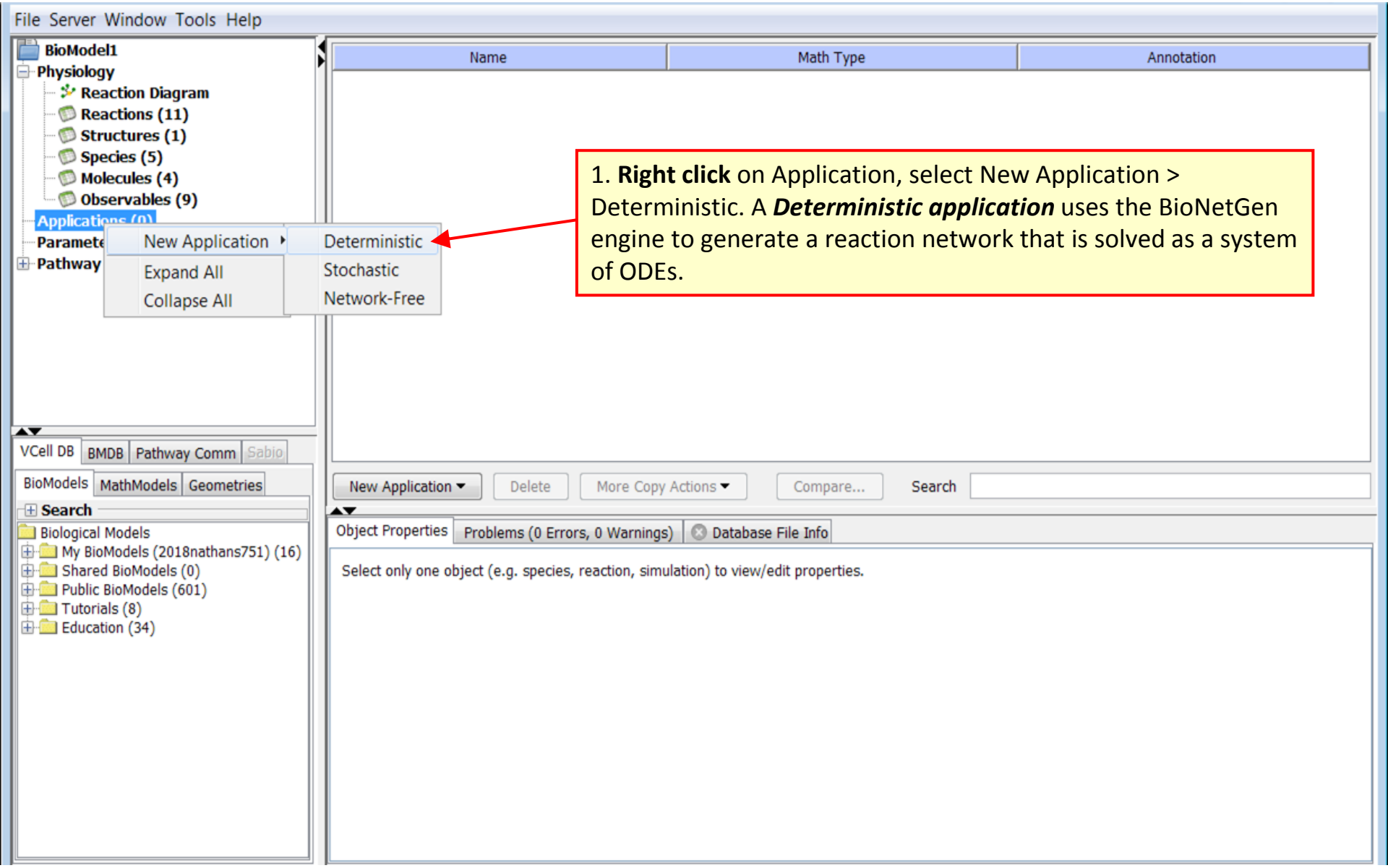


Y2_Dephosph (the Y1 site changes states from phosphorylated to unphosphorylated).

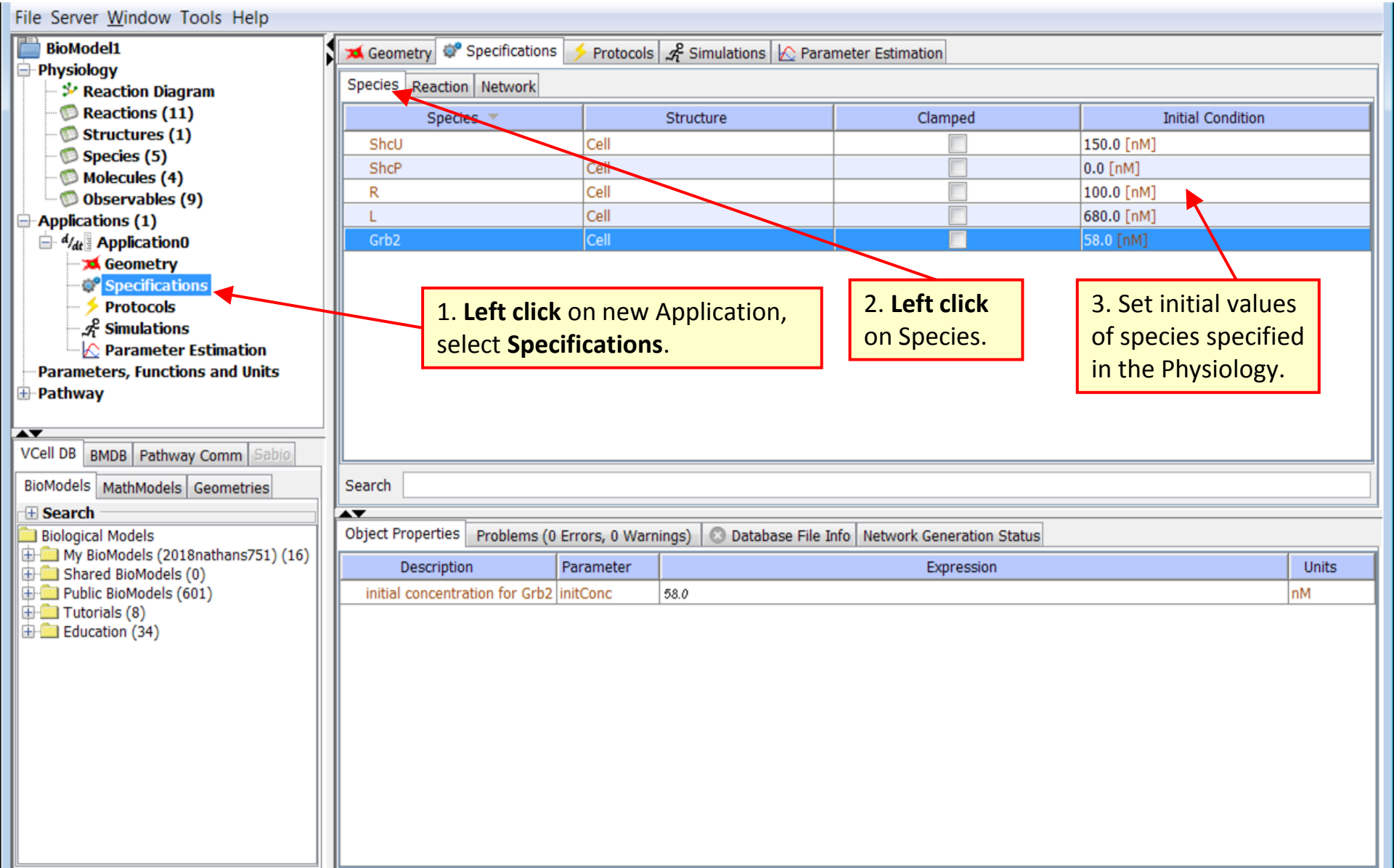
Sch_Dephosph (this is a reaction, not a reaction rule, meaning that it is a reaction that takes place between species instead of molecular patterns).

Reaction	Reversible?	Kf	Kr
Y1_phosph	No	0.5 1/s	0.0
Y1_dephosph	No	4.5 1/s	0.0
Y2_phosph	No	0.5 1/s	0.0
Y2_dephosph	No	4.5 1/s	0.0
Shc_phosph	No	3.0 1/s	0.0
ShcDephosp	No	0.005 1/s	0.0

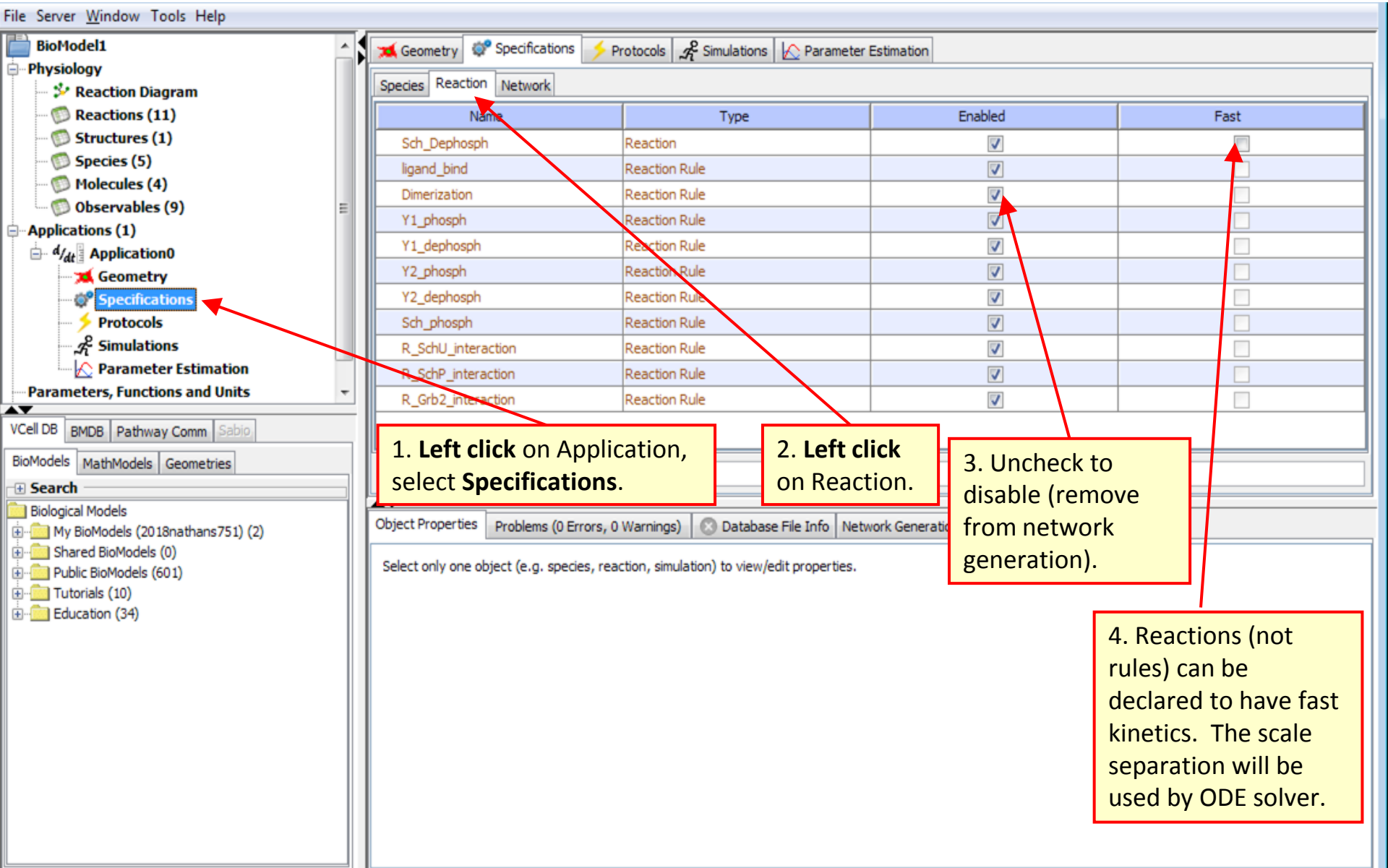
TIP: Check other VCell tutorials at http://vcell.org to learn about the use of Applications in VCell.



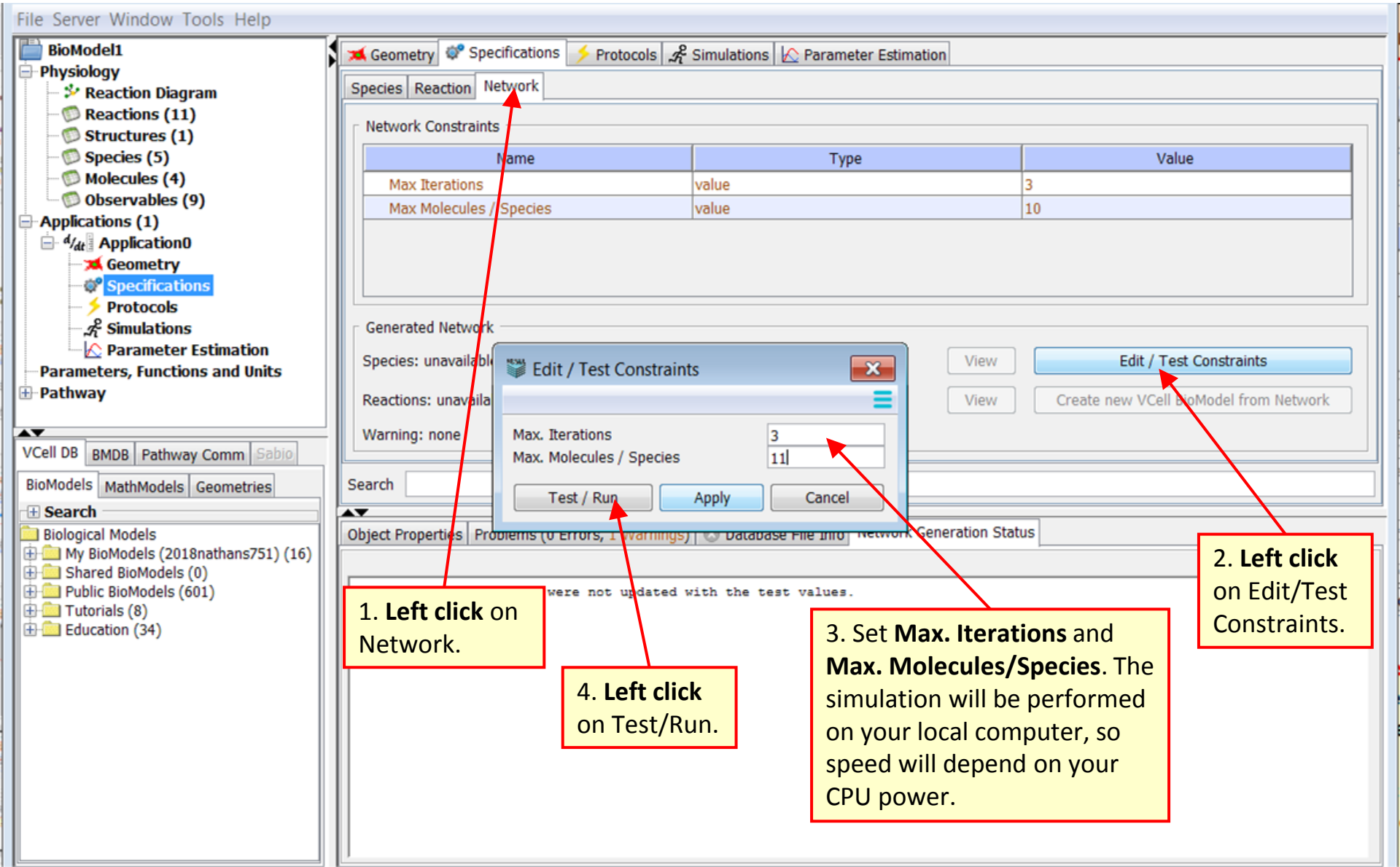
TIP: **Clamped** means that the value of species is kept constant during the simulation.



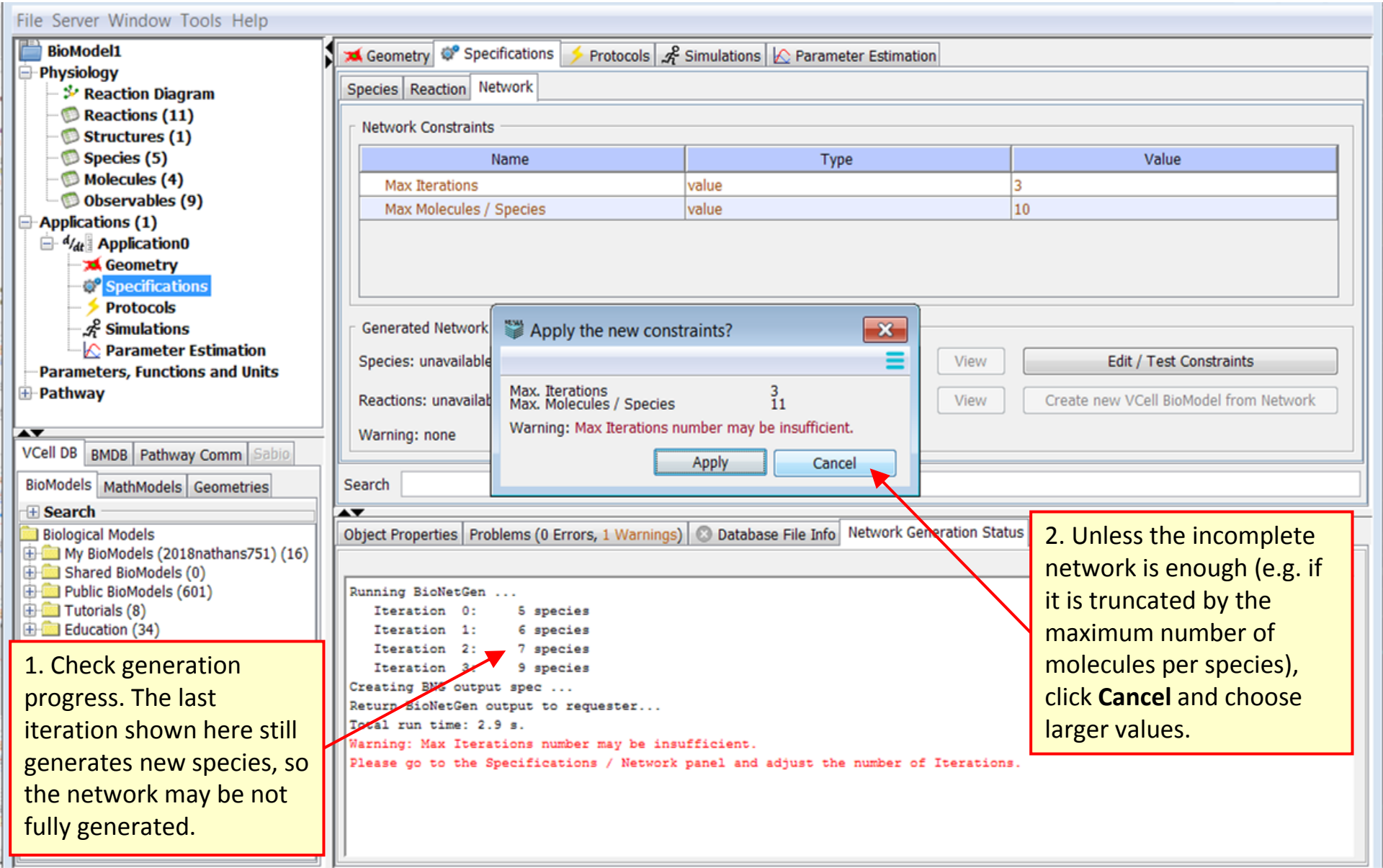
TIP: Enabling/disabling reactions is very useful for model validation: see how the network size is changing when upstream or downstream reaction rules are disabled.



TIP: Setting Max. Molecules/Species may be biologically relevant if, for example, it is known from experiments that complexes may have no more than a certain number of molecules.



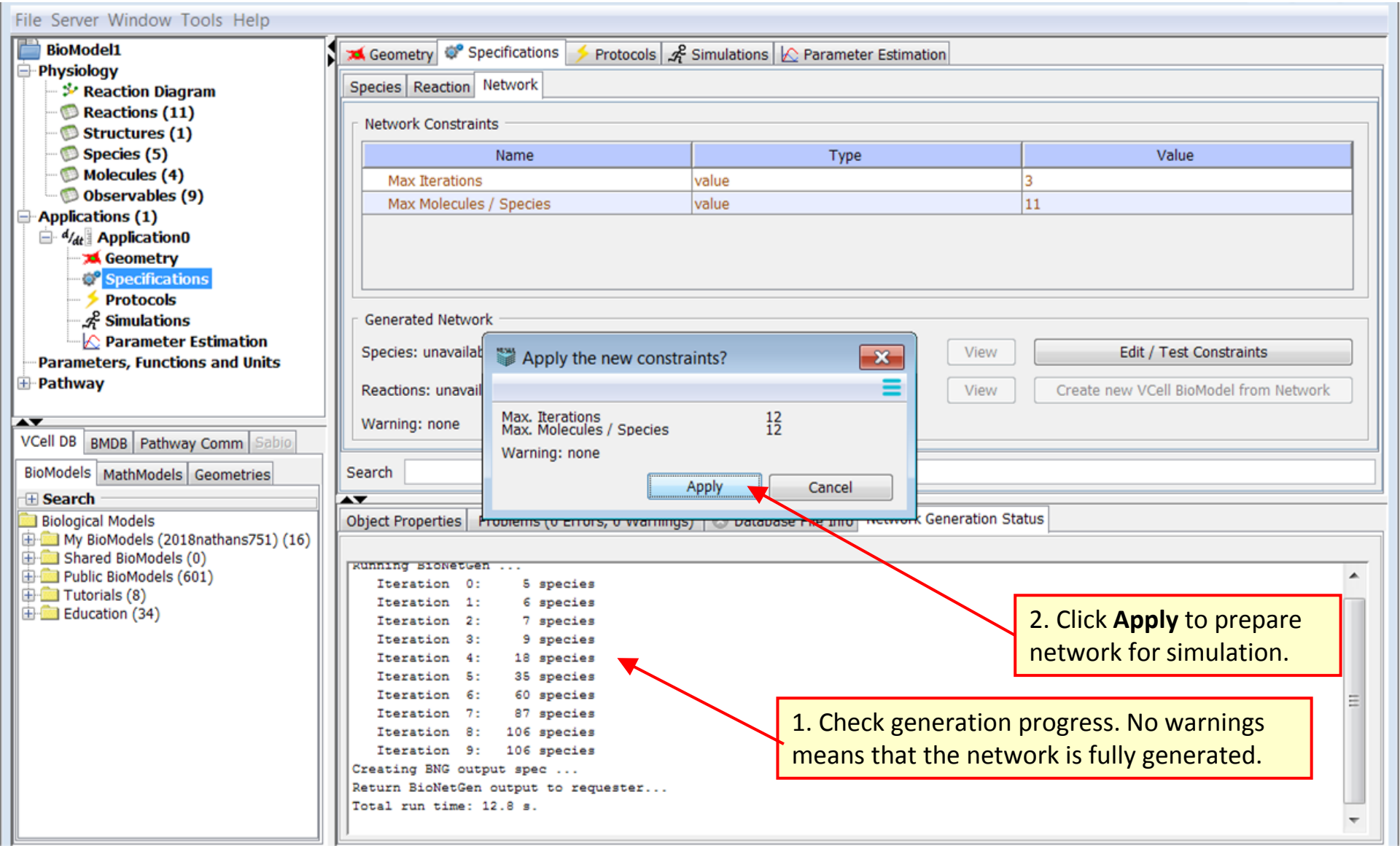
TIP: Network generation may take a long time, so the default values are set very low. Most likely, they are too low for the network to be generated fully, and you will need to increase them.



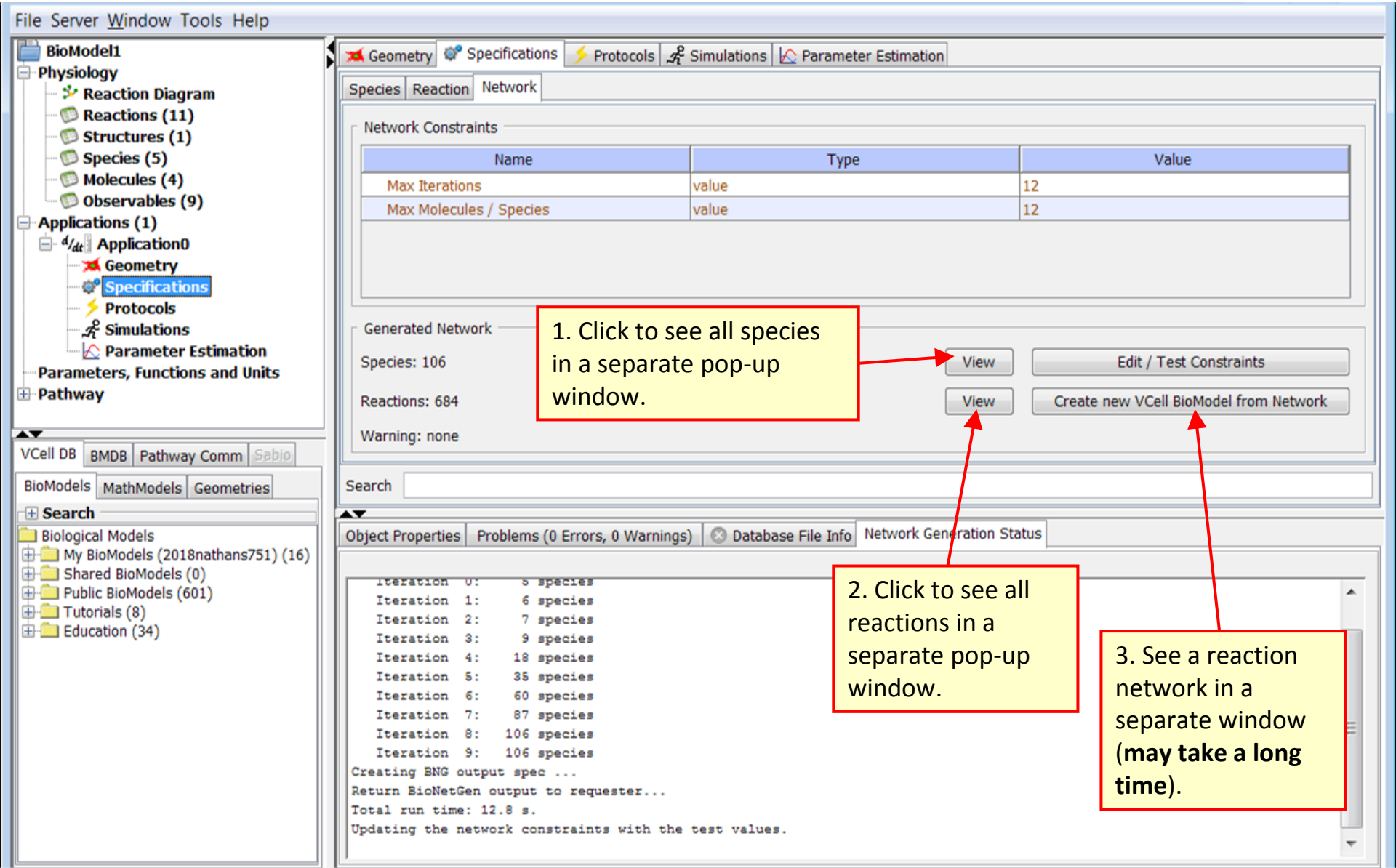
1. Check generation progress. The last iteration shown here still generates new species, so the network may be not fully generated.

2. Unless the incomplete network is enough (e.g. if it is truncated by the maximum number of molecules per species), click **Cancel** and choose larger values.

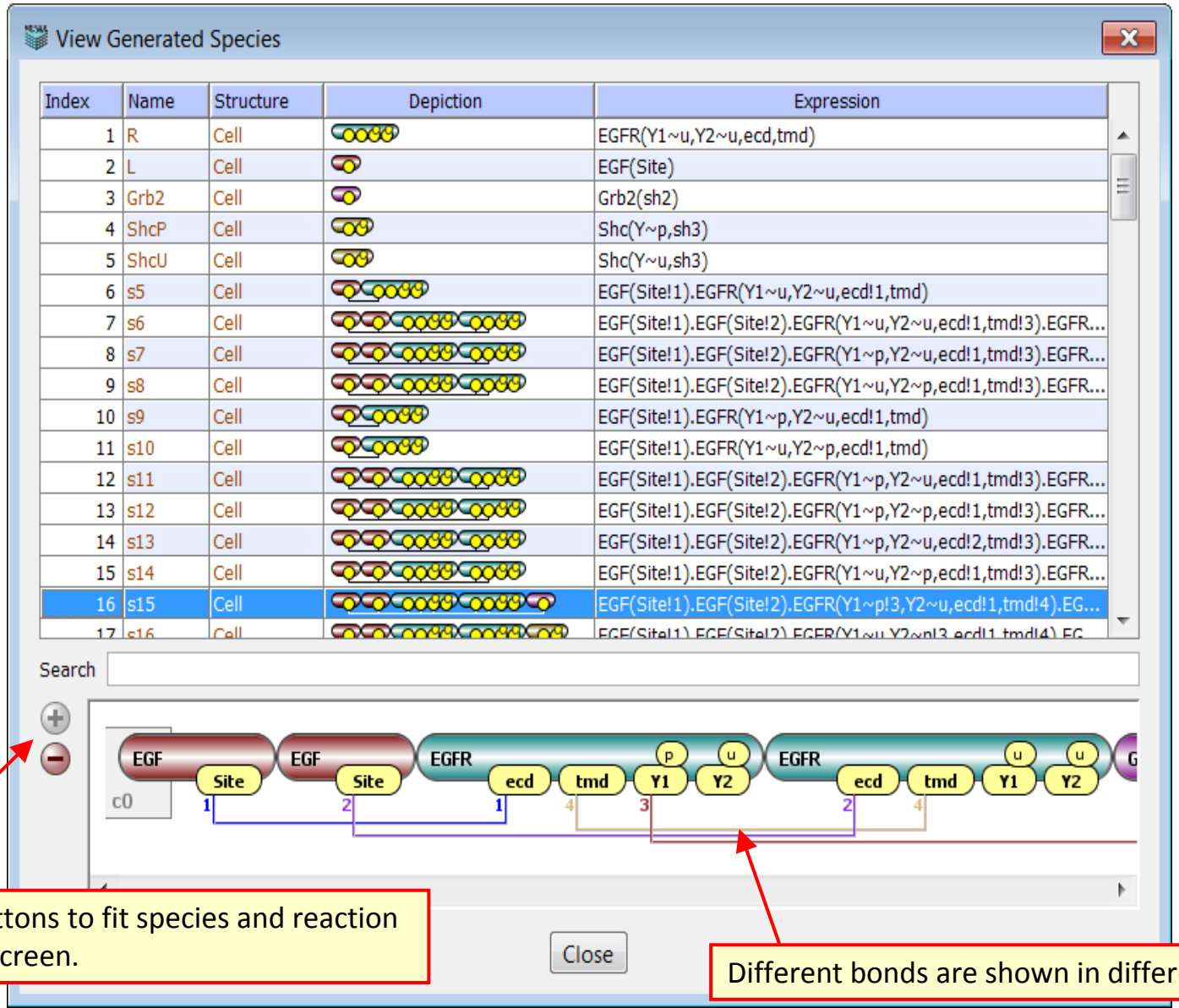
TIP: If network generation takes too long, it can be cancelled. VCell has a hard limit on the maximum number of species and reactions. If a generated network size exceeds this limit, constraints will not be applied, and the model should be adjusted to become smaller, or a ***Network-Free*** application used instead.



TIP: All actions on this page are optional but highly recommended to verify that the generated network contains all expected, and does not contain unexpected, species and reactions. *Creating a new BioModel may take a long time and is not recommended for large networks*.

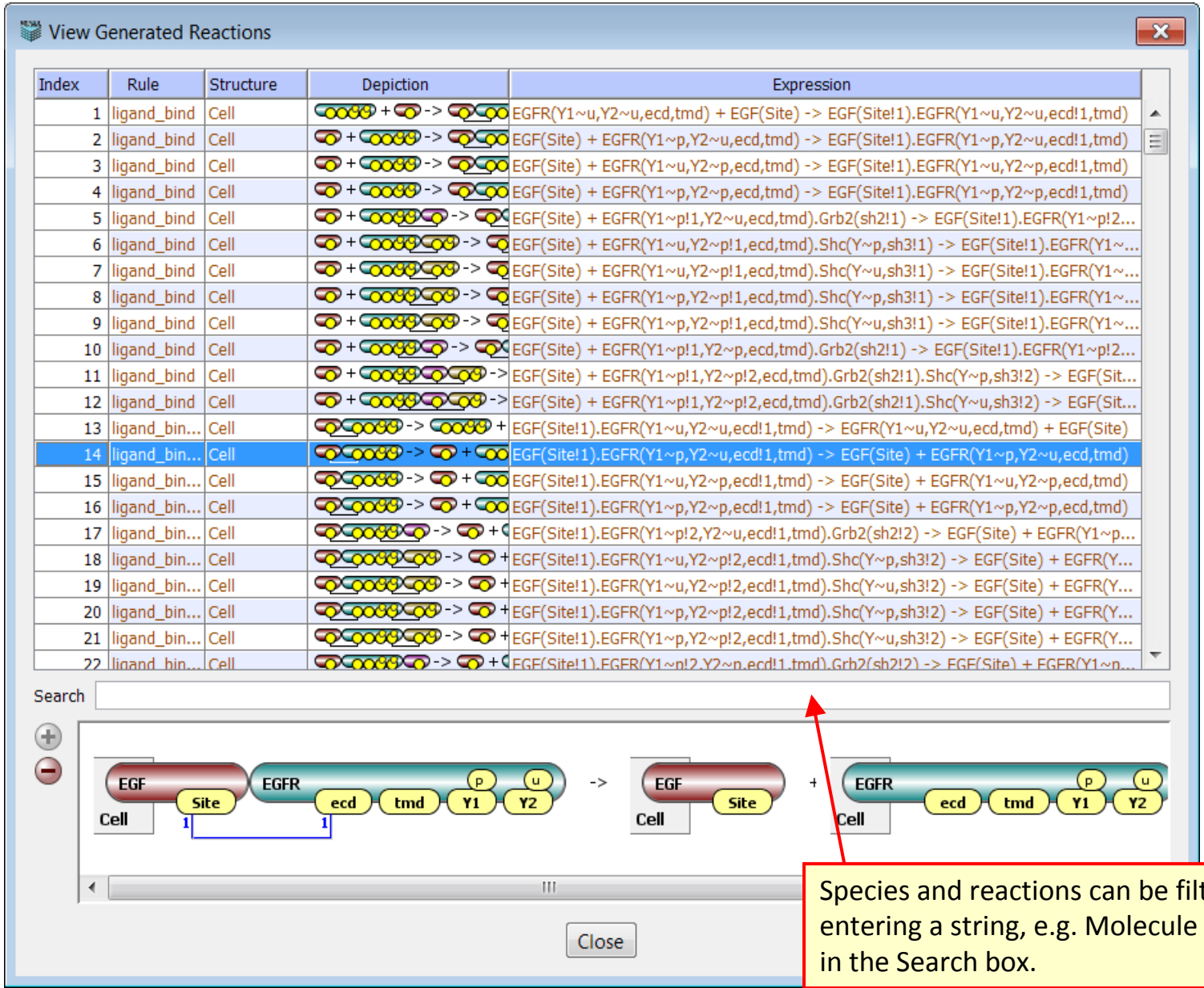


TIP: Filtering is very useful to verify the model. If you see that names of Molecules and Sites are too generic for efficient filtering – go back and change them. This is an easy and safe procedure, but you will need to rerun network generation. After the network is verified, it can be simulated.

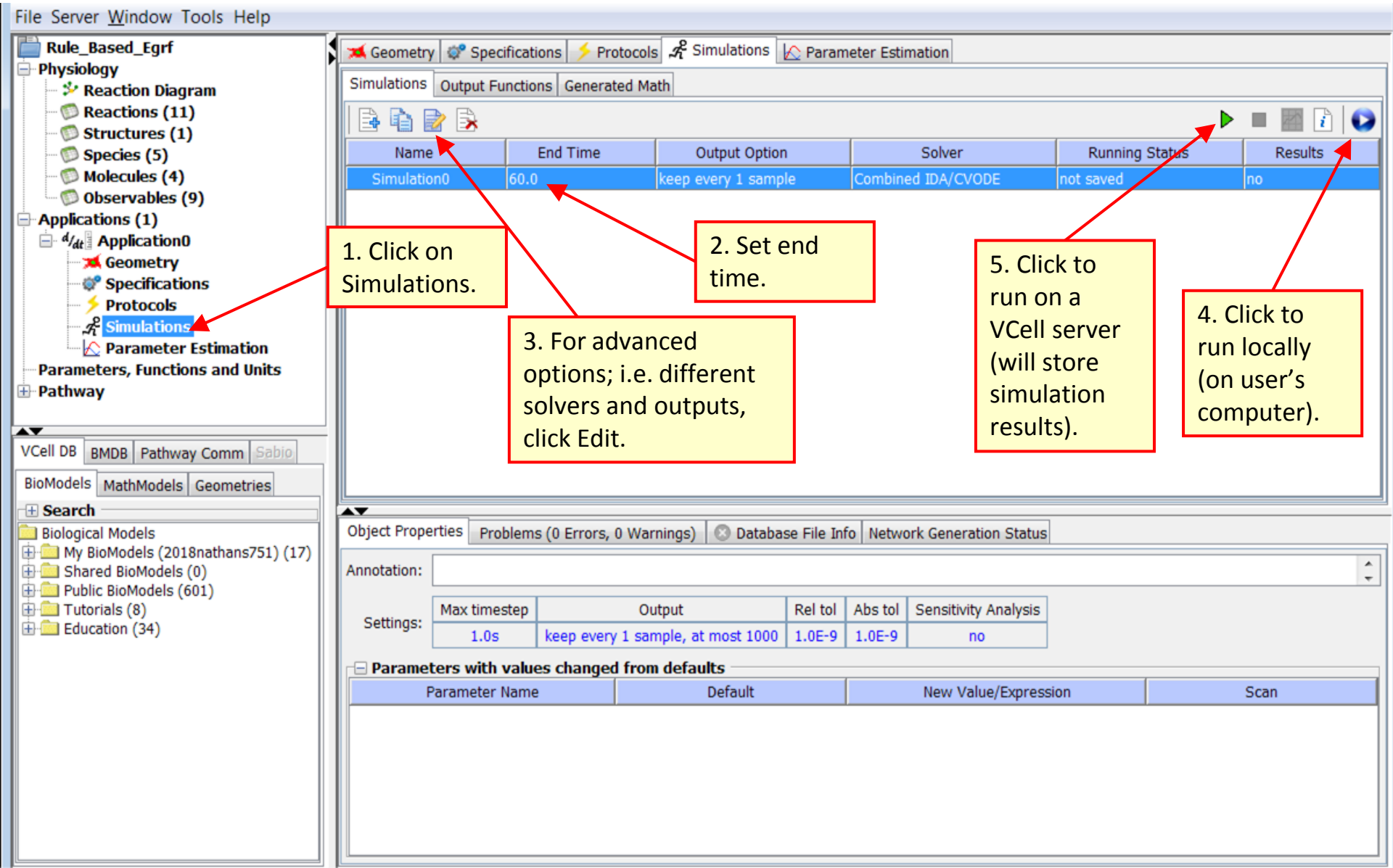


Use these buttons to fit species and reaction rules on the screen.

Different bonds are shown in different colors.


View Generated Reactions
Index
Rule
Structure
Depiction
Expression
1 ligand_bind Cell EGFR(Y1~u,Y2~u,ecd,tmd) + EGF(Site) -> EGF(Site!1).EGFR(Y1~u,Y2~u,ecd!1,tmd)
2 ligand_bind Cell EGF(Site) + EGFR(Y1~p,Y2~u,ecd,tmd) -> EGF(Site!1).EGFR(Y1~p,Y2~u,ecd!1,tmd)
3 ligand_bind Cell EGF(Site) + EGFR(Y1~u,Y2~p,ecd,tmd) -> EGF(Site!1).EGFR(Y1~u,Y2~p,ecd!1,tmd)
4 ligand_bind Cell EGF(Site) + EGFR(Y1~p,Y2~p,ecd,tmd) -> EGF(Site!1).EGFR(Y1~p,Y2~p,ecd!1,tmd)
5 ligand_bind Cell EGF(Site) + EGFR(Y1~p!1,Y2~u,ecd,tmd).Grb2(sh2!1) -> EGF(Site!1).EGFR(Y1~p!2...
6 ligand_bind Cell EGF(Site) + EGFR(Y1~u,Y2~p!1,ecd,tmd).Shc(Y~p,sh3!1) -> EGF(Site!1).EGFR(Y1~...
7 ligand_bind Cell EGF(Site) + EGFR(Y1~u,Y2~p!1,ecd,tmd).Shc(Y~u,sh3!1) -> EGF(Site!1).EGFR(Y1~...
8 ligand_bind Cell EGF(Site) + EGFR(Y1~p,Y2~p!1,ecd,tmd).Shc(Y~p,sh3!1) -> EGF(Site!1).EGFR(Y1~...
9 ligand_bind Cell EGF(Site) + EGFR(Y1~p,Y2~p!1,ecd,tmd).Shc(Y~u,sh3!1) -> EGF(Site!1).EGFR(Y1~...
10 ligand_bind Cell EGF(Site) + EGFR(Y1~p!1,Y2~p,ecd,tmd).Grb2(sh2!1) -> EGF(Site!1).EGFR(Y1~p!2...
11 ligand_bind Cell EGF(Site) + EGFR(Y1~p!1,Y2~p!2,ecd,tmd).Grb2(sh2!1).Shc(Y~p,sh3!2) -> EGF(Sit...
12 ligand_bind Cell EGF(Site) + EGFR(Y1~p!1,Y2~p!2,ecd,tmd).Grb2(sh2!1).Shc(Y~u,sh3!2) -> EGF(Sit...
13 ligand_bin... Cell EGF(Site!1).EGFR(Y1~u,Y2~u,ecd!1,tmd) -> EGFR(Y1~u,Y2~u,ecd,tmd) + EGF(Site)
14 ligand_bin... Cell EGF(Site!1).EGFR(Y1~p,Y2~u,ecd!1,tmd) -> EGF(Site) + EGFR(Y1~p,Y2~u,ecd,tmd)
15 ligand_bin... Cell EGF(Site!1).EGFR(Y1~u,Y2~p,ecd!1,tmd) -> EGF(Site) + EGFR(Y1~u,Y2~p,ecd,tmd)
16 ligand_bin... Cell EGF(Site!1).EGFR(Y1~p,Y2~p,ecd!1,tmd) -> EGF(Site) + EGFR(Y1~p,Y2~p,ecd,tmd)
17 ligand_bin... Cell EGF(Site!1).EGFR(Y1~p!2,Y2~u,ecd!1,tmd).Grb2(sh2!2) -> EGF(Site) + EGFR(Y1~p...
18 ligand_bin... Cell EGF(Site!1).EGFR(Y1~u,Y2~p!2,ecd!1,tmd).Shc(Y~p,sh3!2) -> EGF(Site) + EGFR(Y...
19 ligand_bin... Cell EGF(Site!1).EGFR(Y1~u,Y2~p!2,ecd!1,tmd).Shc(Y~u,sh3!2) -> EGF(Site) + EGFR(Y...
20 ligand_bin... Cell EGF(Site!1).EGFR(Y1~p,Y2~p!2,ecd!1,tmd).Shc(Y~p,sh3!2) -> EGF(Site) + EGFR(Y...
21 ligand_bin... Cell EGF(Site!1).EGFR(Y1~p,Y2~p!2,ecd!1,tmd).Shc(Y~u,sh3!2) -> EGF(Site) + EGFR(Y...
22 ligand_bin... Cell EGF(Site!1).EGFR(Y1~p!2,Y2~p,ecd!1,tmd).Grb2(sh2!2) -> EGF(Site) + EGFR(Y1~p...
Search
EGF
Site
Cell
EGFR
ecd
tmd
Y1
Y2
p
u
1
1
->
EGF
Site
Cell
+
EGFR
ecd
tmd
Y1
Y2
p
u
Cell
Close
Species and reactions can be filtered by entering a string, e.g. Molecule or Site name, in the Search box.

TIP: Most models can be efficiently simulated locally (blue button). But if you want to save simulation results in the database for quick retrieval later on, the server simulation (green button) is recommended.

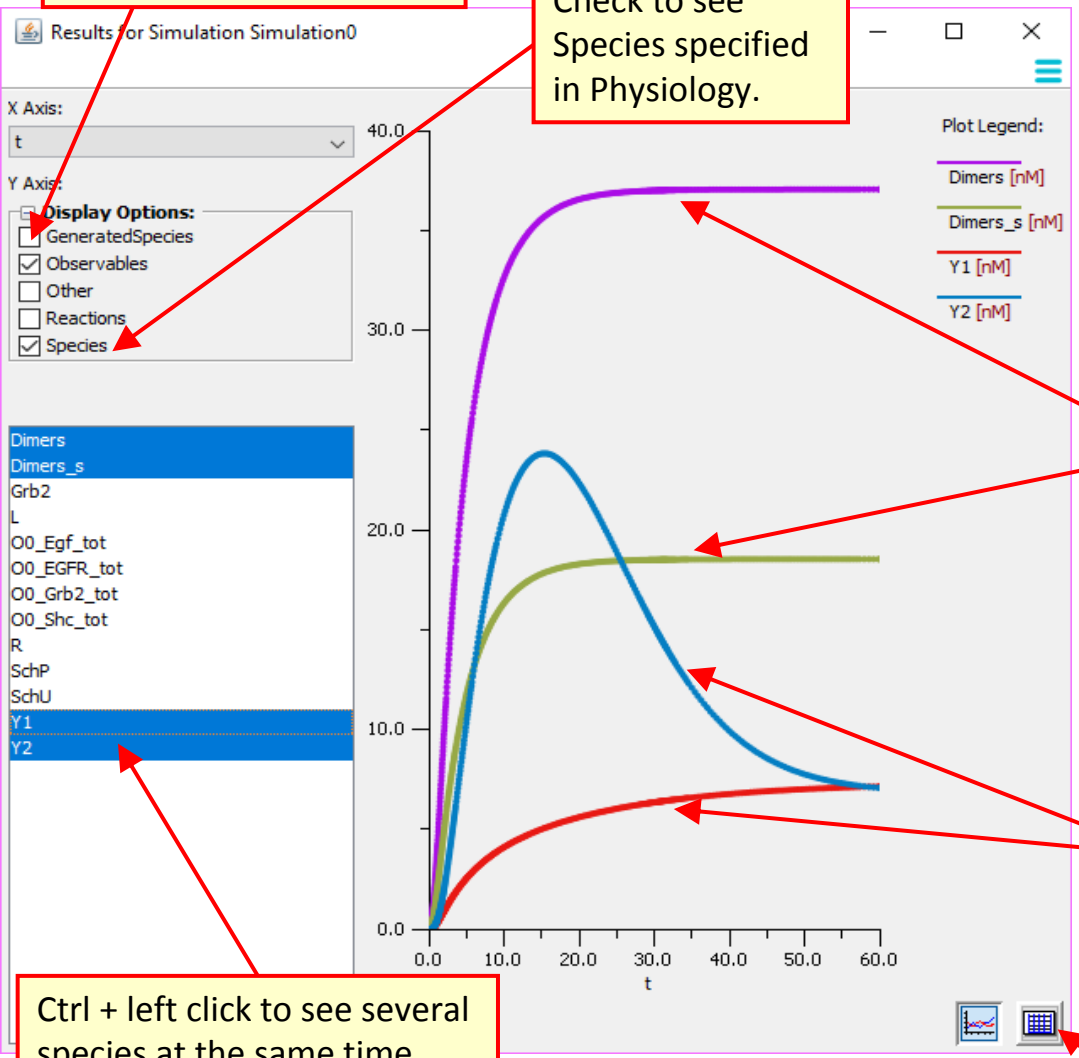


Check to view species generated by BioNetGen.

Check to see Species specified in Physiology.

TIP1: Generated species are listed by index (s10, s11, …). The molecular composition of species can be seen under **Specification > Network > View Species**.

TIP2: **Reactions** show fluxes through individual reactions generated by each rule.



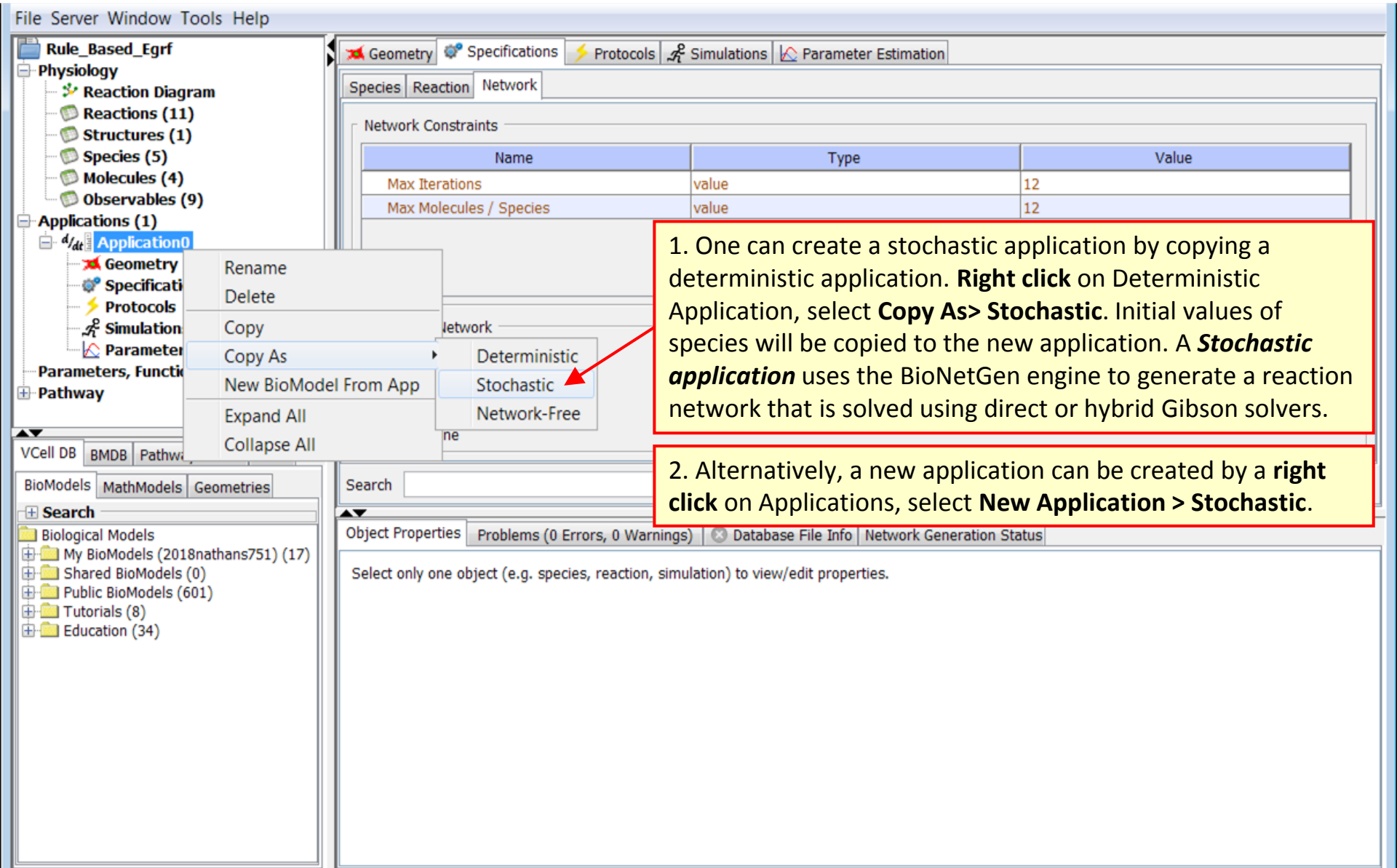
Note the difference between EGFR dimers counted as molecules and as species.

The difference between “Y1” and “Y2” phosphorylation timecourses is due to Shc phosphorylation.

Ctrl + left click to see several species at the same time.

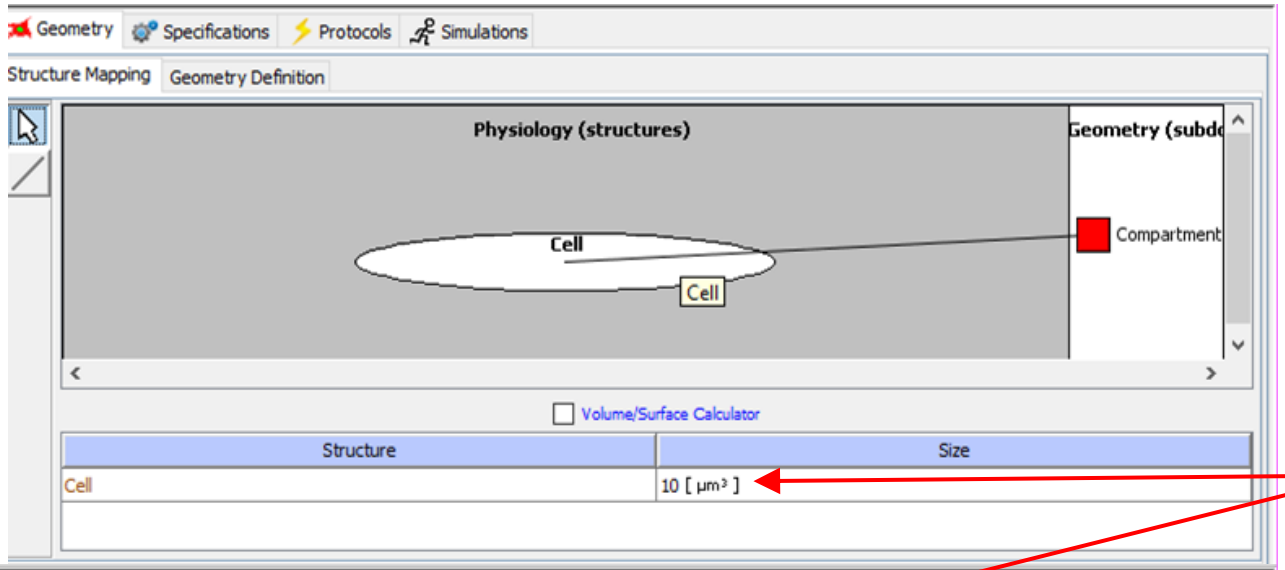
Click to see numerical values.

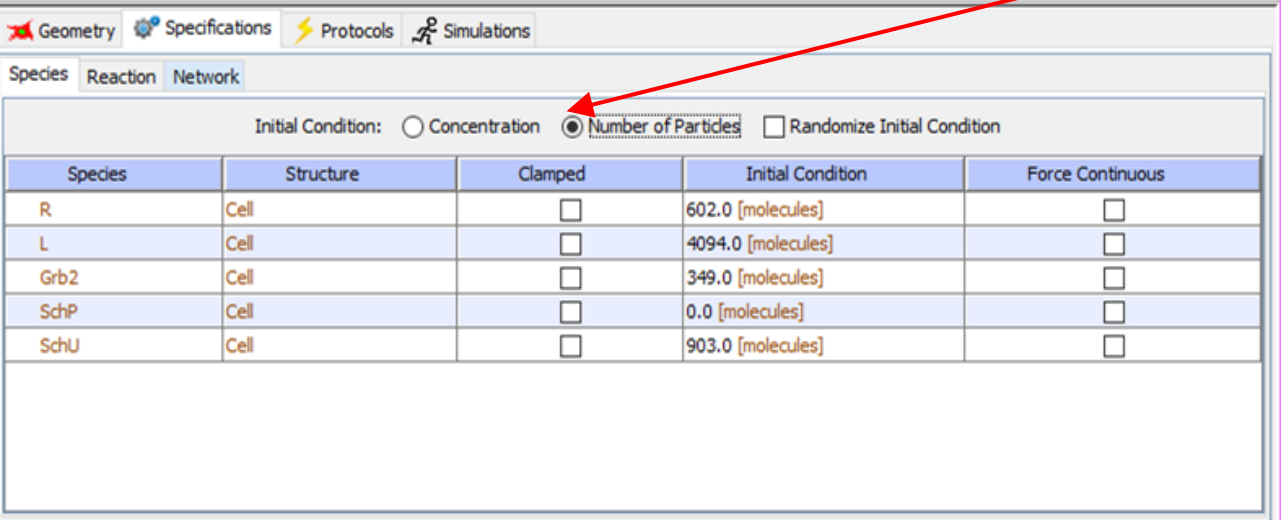
TIP: A stochastic application is recommended when the number of particles is low, and a deterministic simulation (using concentrations) may miss noise and fluctuations. It uses the same network generated by BioNetGen.



TIP1: If the model was defined in concentrations, concentrations are converted into particle numbers using the volumes specified under Geometry. The default size is 5000 um3 (average cell size), so the number of particles will be exceedingly large. You need to decrease Size to a small simulation volume.

TIP2: To keep concentrations fixed, check "Concentration" before switching to Geometry and changing its Size.





Switching back and forth between **Geometry > Structure Mapping** and **Specifications > Species**, make sure your simulation volume is sufficiently small, so that for given concentrations the number of particles is small enough for stochastic simulations.

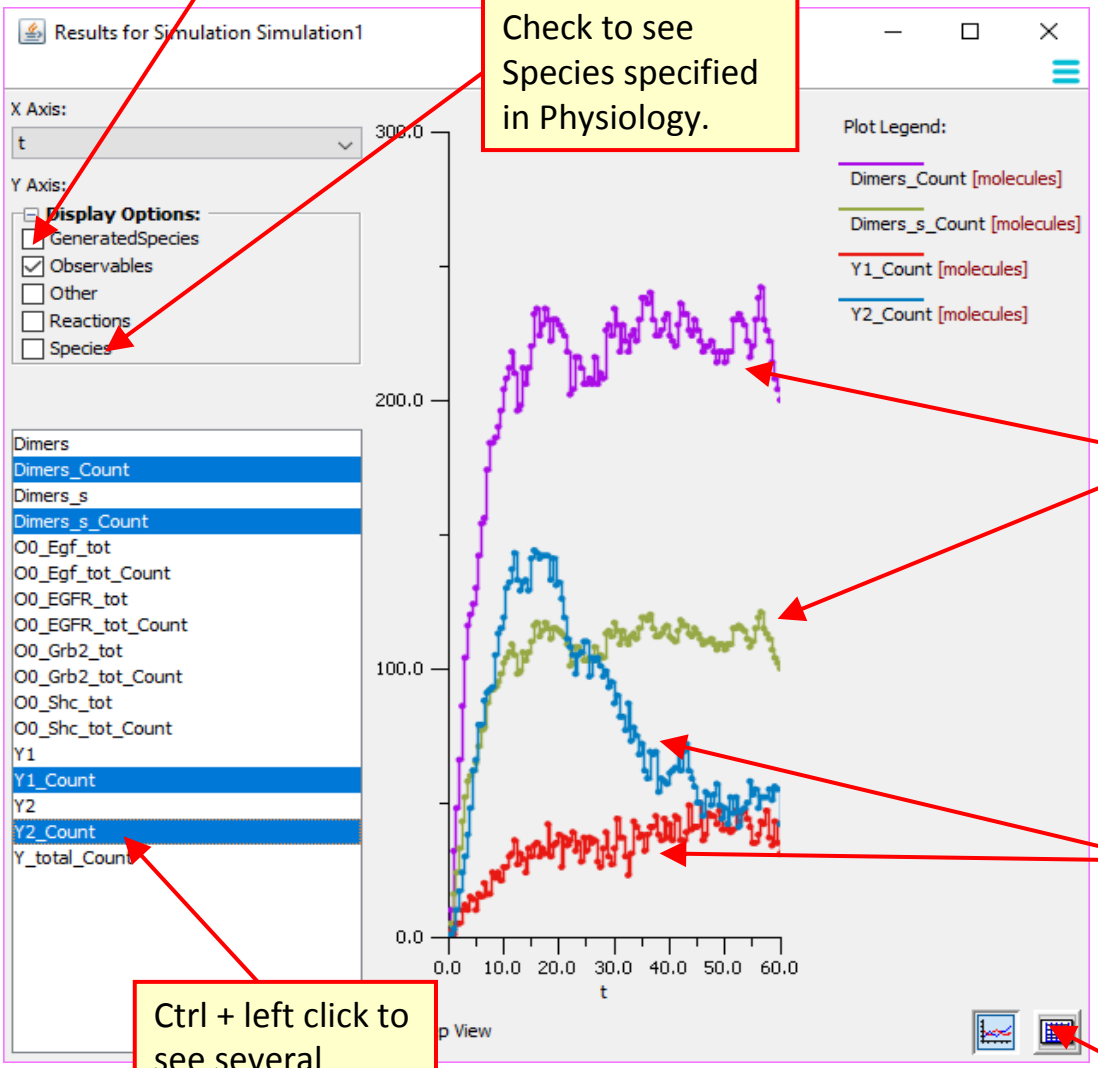
Check to see species generated by BioNetGen.

1. Create a new simulation in the stochastic application using the same settings as the previous simulation.

TIP1: Every species and observable is presented in two units – concentrations (to compare to deterministic results) and molecules (displayed with **_Count** appendix).

Check to see Species specified in Physiology.

TIP2: Select **Other** to view show reaction rates (as Kf_…) and reaction firing events (as P_...) per second for each individual reaction generated by each rule.



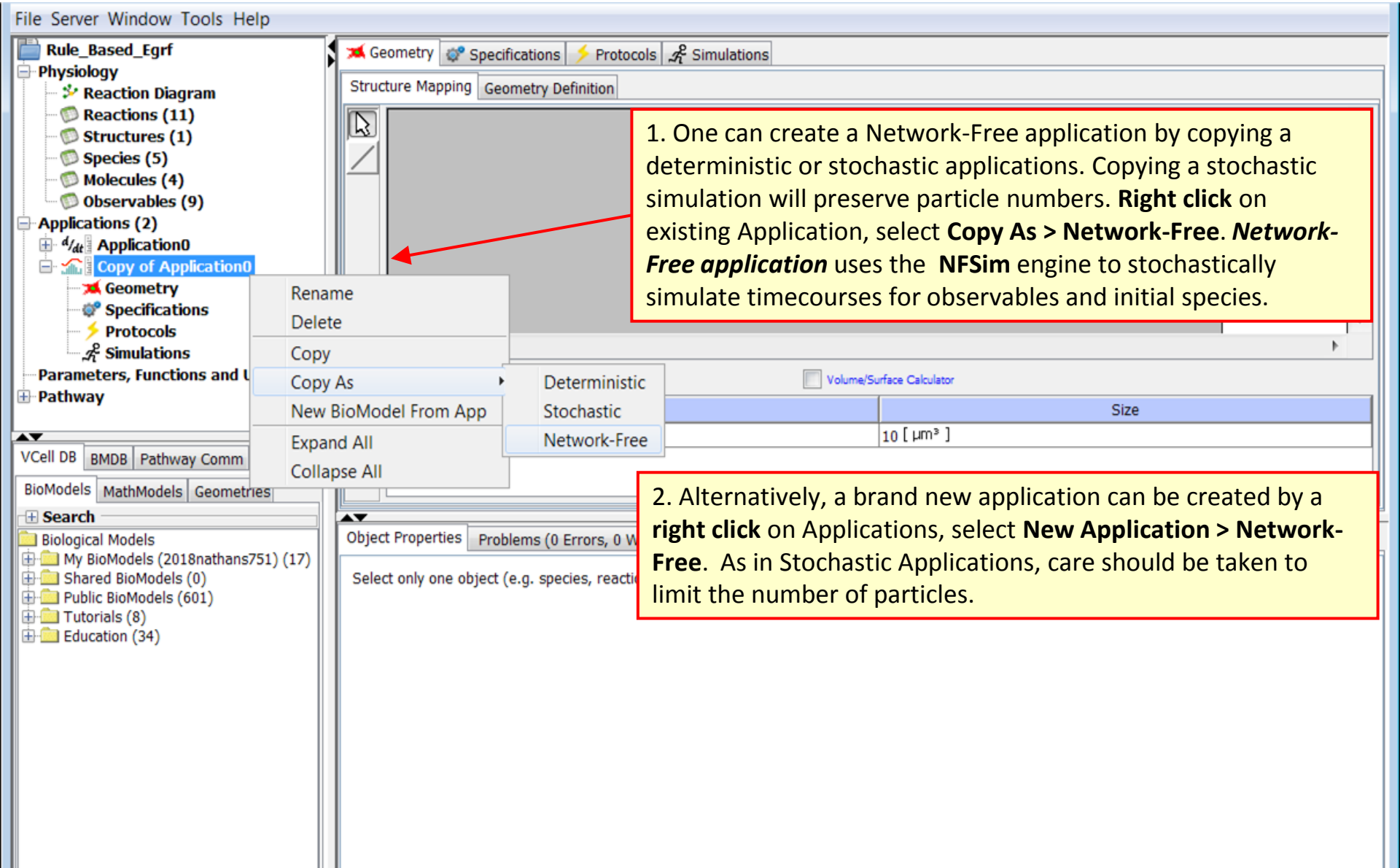
Note the difference between EGFR dimers counted as molecules and as species.

The difference between “Y1” and “Y2” phosphorylation timecourses is due to Shc phosphorylation.

Ctrl + left click to see several species at once.

Click to see numerical values.

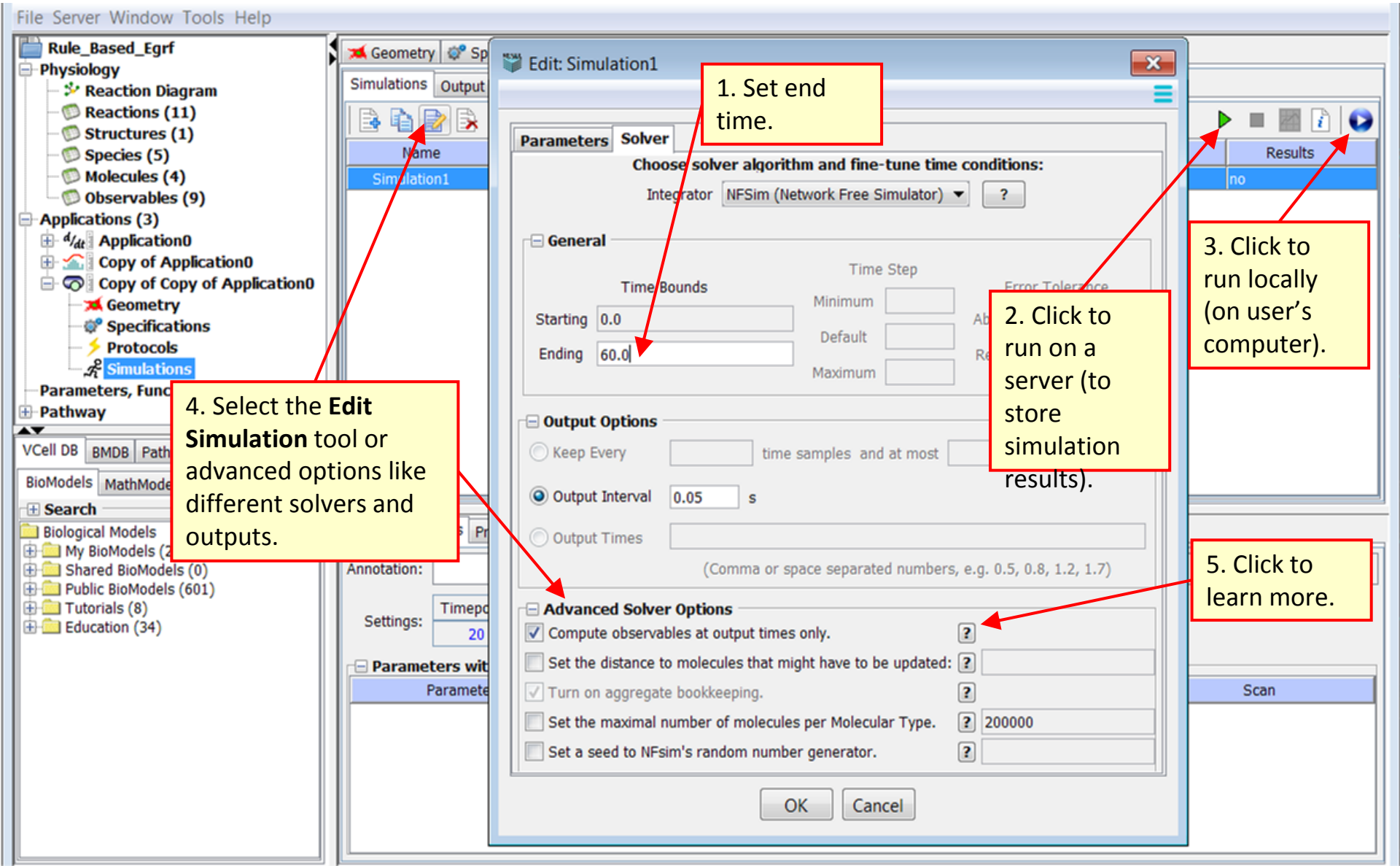
TIP: A Network-Free application simulates timecourses for observables without network generation. If the network size is too large or infinite, it is the only way to compute observables. However, individual species are not visible. To check whether a specific species is populated, it can be added to the list of Observables.

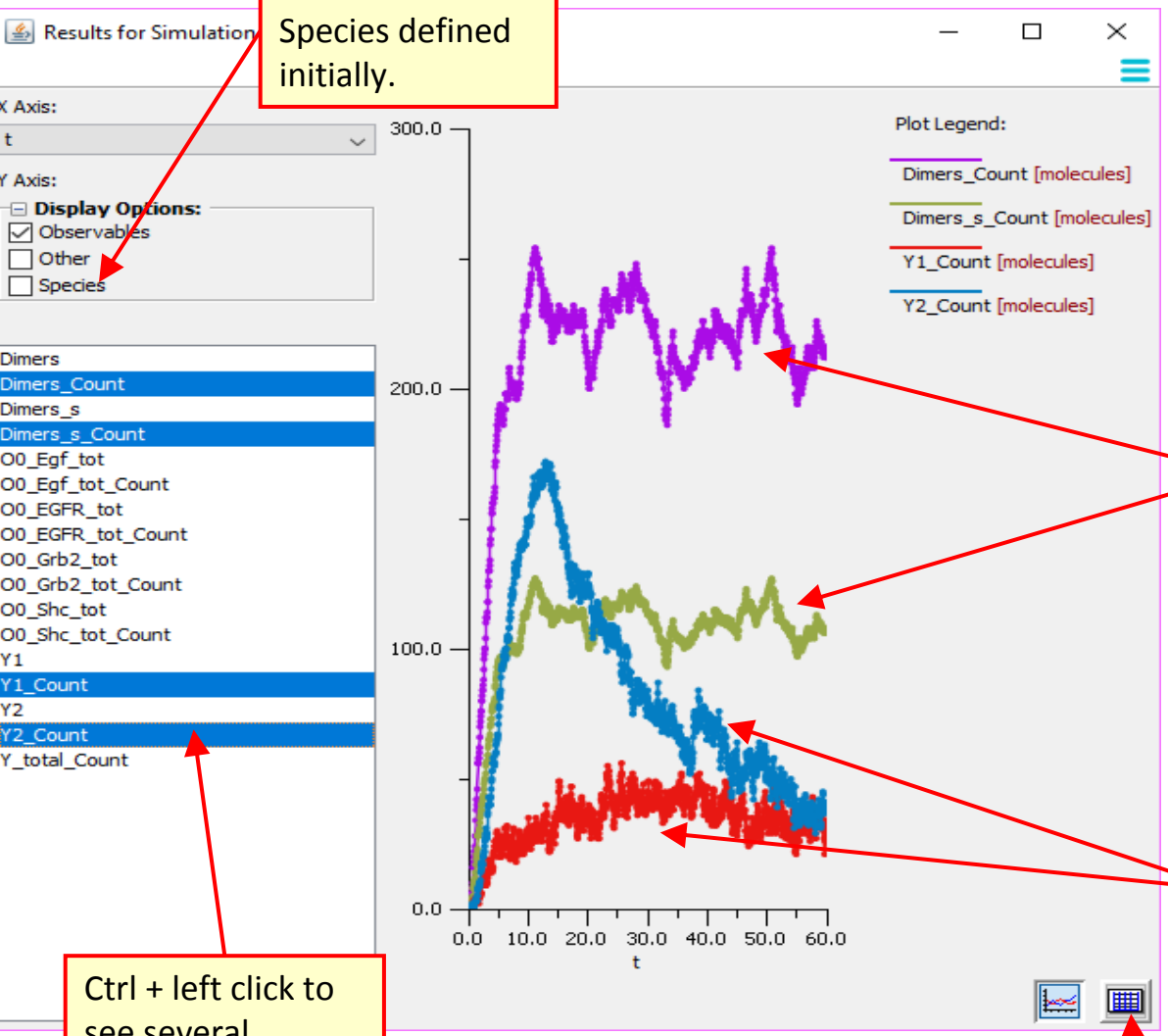


1. One can create a Network-Free application by copying a deterministic or stochastic applications. Copying a stochastic simulation will preserve particle numbers. **Right click** on existing Application, select **Copy As > Network-Free**. ***Network-Free application*** uses the **NFSim** engine to stochastically simulate timecourses for observables and initial species.

2. Alternatively, a brand new application can be created by a **right click** on Applications, select **New Application > Network-Free**. As in Stochastic Applications, care should be taken to limit the number of particles.

TIP: The NFSim engine has a large number of fine-tuning options. Generally, default options should be sufficient to simulate most models. If necessary, click on Edit. Options are documented under ? and in the Help menu.





Select to list Species defined initially.

TIP: Generally, deterministic, stochastic and NFSim simulation results should be similar (given noise and fluctuations). If NFSim results are very different from results from a network, it may mean that the network is truncated and *not exhaustively generated.*

See the difference between EGFR dimers counted as molecules and as species

The difference between "Y1" and "Y2" phosphorylations timecourses is due to Shc phosphorylation.

Ctrl + left click to see several species at once.

Click to see numerical values

Acknowledgements

The following students worked on this tutorial:

Tanya Miller (2015) – Pomperaug High School
Nathan Schaumburger (2017) – Hall High School, West Hartford

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