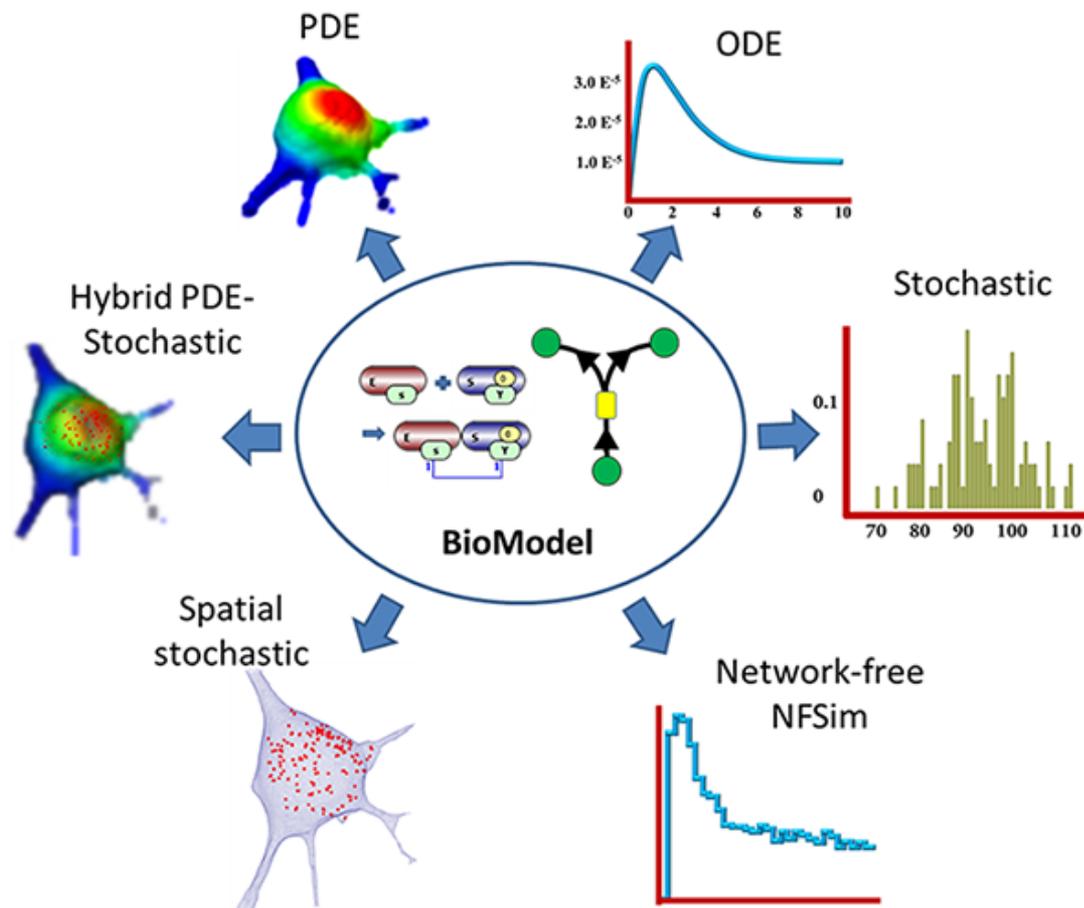


# VCell

To run VCell go to:  
[vcell.org](http://vcell.org)



VCell is developed  
at CCAM

Center for Cell Analysis & Modeling



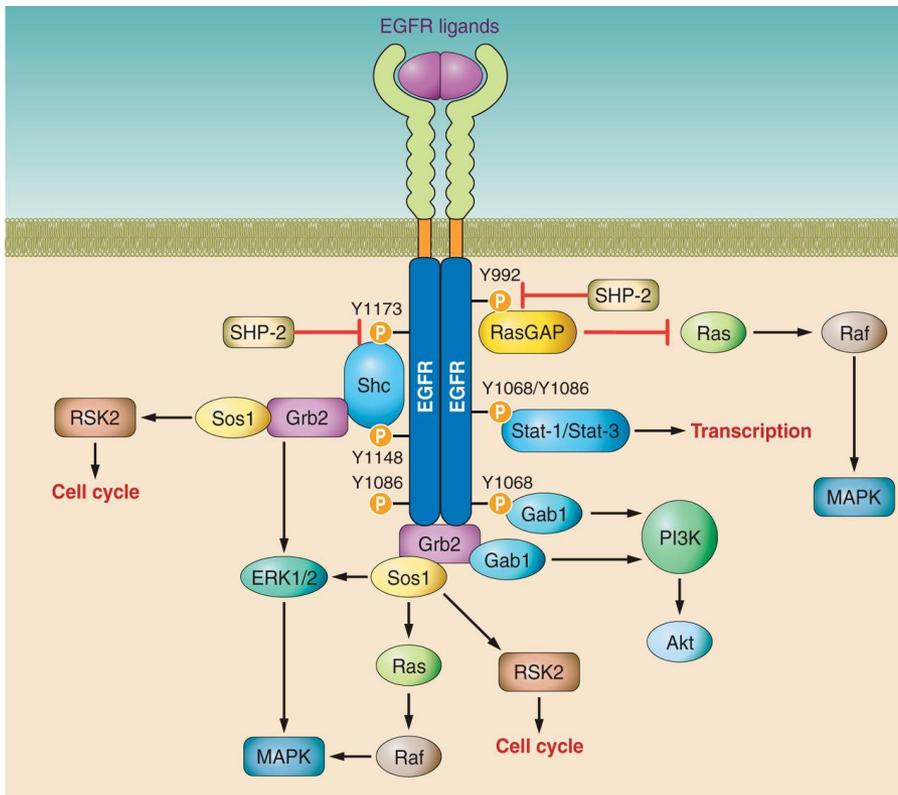
National Institute of  
General Medical Sciences

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 transphosphorylation, 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.

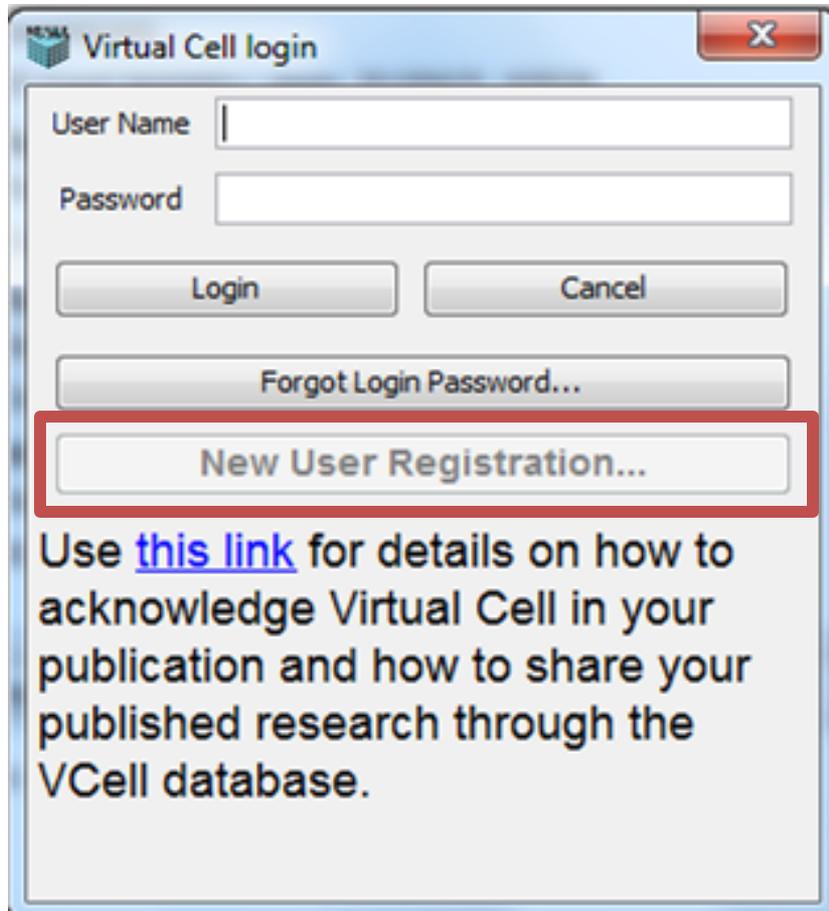
General familiarity with VCell software is recommended. Although this tutorial can be followed by a VCell novice, it is recommended that novice users first look through the VCell tutorials available at [http://vcell.org/vcell\\_software/user\\_guide.html](http://vcell.org/vcell_software/user_guide.html).

Model building can be matched to the BioModel *RB\_egfr\_tutorial* in the Tutorial folder in the VCell Database.

# Table of contents

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- [Physiology: Molecules](#)
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- [Physiology: Observables](#)
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- [Physiology: Reactions](#)
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- [Application: Deterministic Network Generation](#)
- [Application: Stochastic](#)
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# Opening VCell for the First Time



Virtual Cell login

User Name

Password

Login Cancel

Forgot Login Password...

**New User Registration...**

Use [this link](#) for details on how to acknowledge Virtual Cell in your publication and how to share your published research through the VCell database.

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.

The screenshot displays the VCell software interface. The top menu bar includes 'File', 'Server', 'Window', 'Tools', and 'Help'. The left sidebar shows a project tree for 'BioModel1' with categories like 'Physiology', 'Reaction Diagram', 'Reactions (0)', 'Structures (1)', 'Species (0)', 'Molecules (1)', and 'Observables (1)'. The main workspace is titled 'Molecules' and contains a table with columns 'Name', 'Depiction', and 'BioNetGen Definition'. A single entry 'MTO' is shown with a small circle icon and the definition 'MTO()'. Below the table, a 'New Molecule' button is highlighted. The 'Object Properties' panel shows 'Anchor Molecule' with 'No restrictions' selected. A context menu is open over a molecule shape labeled 'MTO', with options 'Rename' and 'Add Site' highlighted. The 'Annotation' panel is visible at the bottom.

Name	Depiction	BioNetGen Definition
MTO		MTO()

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

The screenshot displays the VCell software interface. On the left is a tree view of the model structure, with 'Molecules (1)' selected. The main window shows the 'Molecules' tab with a table of molecules:

Name	Depiction	BioNetGen Definition
EGF		EGF(Site0)

Below the table are buttons for 'New Molecule', 'Delete', and 'Pathway Links', along with a search field. The 'Object Properties' panel shows 'Anchor Molecule' with 'No restrictions' selected. A context menu is open over the 'Site0' site, with 'Rename' selected. Two red arrows point from text boxes to the 'Site0' site and the 'Rename' menu item.

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

2. Select **Rename**, and change the name to "Site". Press **Enter**.

**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.

File Server Window Tools Help

BioModel1

- Physiology
  - Reaction Diagram
  - Reactions (0)
  - Structures (1)
  - Species (0)
  - Molecules (2)**
  - Observables (2)
- Applications (0)
- Parameters, Functions and Units
- Pathway

VCeIl DB BMDB Pathway Comm Sabio

BioModels MathModels Geometries

Search

- Biological Models
  - My BioModels (2018nathans751) (16)
  - Shared BioModels (0)
  - Public BioModels (601)
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  - Education (34)

Name	Depiction	BioNetGen Definition
EGF		EGF(Site)
<b>EGFR</b>		<b>EGFR(ecd,tmd,Y1,Y2)</b>

New Molecule Delete Pathway Links

Object Properties Problems (0 Errors, 0 Warnings)

Anchor Molecule

No restrictions

Only these:

c0

Annotation

EGFR ecd tmd Y1 Y2

- Move right
- Move left
- Rename
- Delete
- Add State**

1. Create a new Molecule by left clicking on “New Molecule”.
2. Rename the Molecule to “EGFR” either by **right clicking** on the shape below, or by entering it in the table.
3. **Right click** on the molecule to call up a menu. Add four sites.
4. **Right click** on the molecule’s sites, select **Rename**, and change the names to: “ecd”, “tmd”, “Y1”, “Y2”. Press **Enter** to save.
5. **Right click** on the sites “Y1” and “Y2”. Select **Add state** (twice for each site).

**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.

The screenshot shows the VCell software interface. On the left is a tree view of the model structure, with 'Molecules (2)' selected. The main window displays a table of molecules and a detailed view of the EGFR molecule.

Name	Depiction	BioNetGen Definition
EGF		EGF(Site)
EGFR		EGFR(ecd,tmd,Y1~u~p,Y2~u~p)

The 'Anchor Molecule' section shows a diagram of the EGFR molecule with sites 'ecd', 'tmd', 'Y1', and 'Y2'. Each site has two states: 'u' (unbound) and 'p' (bound). A context menu is open over the 'p' state of the Y2 site, with 'Rename' and 'Delete' options. Red arrows point from the text boxes to these menu items.

**1. Right click on the site to call up a menu.**

**2. Select **Rename**, and change states "state1" and "state0", to "p" and "u" respectively. Press **Enter** to save. Do this for both sites "Y1" and "Y2".**

**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.

File Server Window Tools Help

- BioModel1
  - Physiology
    - Reaction Diagram
    - Reactions (0)
    - Structures (1)
    - Species (0)
    - Molecules (4)**
    - Observables (4)
  - Applications (0)
  - Parameters, Functions and Units
  - Pathway

Name	Depiction	BioNetGen Definition
EGF		EGF(Site)
EGFR		EGFR(ecd,tmd,Y1~u~p,Y2~u~p)
Grb2		Grb2(sh2)
Shc		Shc(sh3,Y~u~p)

You can use the search box to display only elements fitting the search pattern. You can search by Name or BNGL string.

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BioModels MathModels Geometries

**Search**

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  - My BioModels (2018nathans751) (16)
  - Shared BioModels (0)
  - Public BioModels (601)
  - Tutorials (8)
    - Membrane Frap
    - Tutorial\_FRAP
    - Tutorial\_FRAPbinding
    - Tutorial\_MultiApp
    - Tutorial\_PathwayCommons
    - Rule-based\_egfr\_tutorial**
    - Rule-based\_egfr\_compart
    - Rule-based\_Ran\_transport
  - Education (34)

New Molecule Delete Pathway Links Search

Object Properties Problems (0 Errors, 0 Warnings) Database File Info

**Anchor Molecule**

No restrictions

Only these:

c0

**Annotation**

Adaptor protein Shc. Binds to EGFR phosphotyrosines through sh2 domains, can be phosphorylated at phosphite Y.

**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.

File Server Window Tools Help

BioModel1

- Physiology
  - Reaction Diagram
  - Reactions (0)
  - Structures (1)
  - Species (0)
  - Molecules (4)**
  - Observables (4)
- Applications (0)
- Parameters, Functions and Units
- Pathway

VCell DB BMDB Pathway Comm Sabio

BioModels MathModels Geometries

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    - Tutorial\_MultiApp
    - Tutorial\_PathwayCommons
    - Rule-based\_egfr\_tutorial**
    - Rule-based\_egfr\_compart
    - Rule-based\_Ran\_transport
  - Education (34)

Reaction Diagram Reactions Structures Species **Molecules** Observables

Name	Depiction	BioNetGen Definition
EGF		EGF(Site)
EGFR		EGFR(ecd,tmd,Y1~u~p,Y2~u~p)
Grb2		Grb2(sh2)
Shc		Shc(sh3,Y~u~p)

1. Add molecule "Grb2" with a site "sh2". Add molecule "Shc" with sites "sh3" and "Y," with "Y" having two states, "u" and "p".

Check with the specification of Molecules in the *RB\_egfr\_tutorial* model in VCell 6.1 (Rule-based) folder.

New Molecule Delete Pathway Links Search

Object Properties Problems (0 Errors, 0 Warnings) Database File Info

**Anchor Molecule**

No restrictions

Only these:

c0

**Annotation**

Adaptor protein Shc. Binds to EGFR phosphotyrosines through sh2 domians, can be phosphorylated at phosphite Y.

2. Annotations can be entered here.

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

The screenshot shows the VCell software interface. The 'File' menu is open, with 'Save As...' highlighted. A red arrow points from the 'Save As...' option to a yellow callout box. The callout box contains the text: 'When ready to save, click on **File** and **Save As....** If you work locally (no internet connection), choose **Save As Local....**'

Name	Depiction	BioNetGen Definition
EGF		EGF(Site)
EGFR		EGFR(ecd,tmd,Y1~u~p,Y2~u~p)
Grb2		Grb2(sh2)
Shc		Shc(sh3,Y~u~p)

Below the table, there are buttons for 'New Molecule', 'Delete', and 'Pathway Links'. A search bar is also present. At the bottom, there is an 'Anchor Molecule' section with radio buttons for 'No restrictions' (selected) and 'Only these:'. Below this is a diagram of the Shc molecule with labels 'sh3', 'Y', 'u', and 'p'. An 'Annotation' section contains the text: 'Adaptor proten Shc. Binds to EGFR phosphotyrosines through sh2 domians, can be phosphorylated at phosphite Y.'

**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.

The screenshot shows the VCell software interface. On the left is a tree view of the model hierarchy, including 'BioModel1', 'Physiology', 'Reaction Diagram', 'Reactions (0)', 'Structures (1)', 'Species (0)', 'Molecules (4)', 'Observables (4)', 'Applications (0)', 'Parameters, Functions and Units', and 'Pathway'. Below this is a search bar and a list of folders like 'Biological Models', 'My BioModels (2018nathans751) (16)', 'Shared BioModels (0)', 'Public BioModels (601)', 'Tutorials (8)', and 'Education (34)'. The main window has a menu bar (File, Server, Window, Tools, Help) and a toolbar with tabs for 'Reaction Diagram', 'Reactions', 'Structures', 'Species', 'Molecules', and 'Observables'. The 'Structures' tab is active, displaying a table with columns 'Name', 'Type', and 'Electrical (Membrane Polarity)'. The table contains one row: 'Cell' (Type: 'Compartment'). Below the table are buttons for 'New Compartment', 'New Membrane', 'Delete', and 'Pathway Links', along with a search field. At the bottom, the 'Object Properties' panel is open, showing fields for 'Structure Name' (Cell), 'Size Variable Name' (Cell [ $\mu\text{m}^3$ ]), and 'Annotation'. A red box with arrows points to the 'Structures' tab and the 'Cell' entry in the table, with a text box containing instructions.

Name	Type	Electrical (Membrane Polarity)
Cell	Compartment	

To specify or edit the name of the compartment in which the reactions are taking place, click on the **Structures** tab, double click on the name of the compartment that is to be edited (do not create a new structure), and type in the new name. Press **Enter** to save.

**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.

File Server Window Tools Help

BioModel1

- Physiology
  - Reaction Diagram
  - Reactions (0)
  - Structures (1)
  - Species (0)
  - Molecules (4)
  - Observables (4)
- Applications (0)
- Parameters, Functions and Units
- Pathway

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BioModels MathModels Geometries

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Reaction Diagram Reactions Structures Species Molecules **Observables**

Name	Structure	Depiction	BioNetGen Definition	Count
O0_EGF_tot	Cell		EGF()	Molecules
O0_EGFR_tot	Cell		EGFR()	Molecules
O0_Grb2_tot	Cell		Grb2()	Molecules
O0_Shc_tot	Cell		Shc()	Molecules

1. Right click on **Observables** tab. You'll see a set of observables corresponding to the total number of Molecules of each type.

2. This observable selects species that have EGFR molecules in any state and any complex. Question marks and grey color mean that the state and whether sites are bound or unbound are not important for counting.

New Observable Duplicate Delete Pathway Links Search

Object Properties Problems (0 Errors, 0 Warnings) Database File Info

Add Pattern

Multimolecular

Polymer of

length = 2

length > 1

EGFR

Cell

ecd tmd Y1 Y2

3. The default setting will count "Molecules", meaning that a species is counted as many times as it has this Molecule. This means that dimers of EGFR are counted twice, and tetramers (if any) are counted four times.

Annotation

**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.

File Server Window Tools Help

The screenshot shows the VCell software interface. On the left is a tree view of the model structure, including 'Physiology', 'Reaction Diagram', 'Reactions (0)', 'Structures (1)', 'Species (0)', 'Molecules (4)', and 'Observables (5)'. The main window displays the 'Observables' table with the following data:

Name	Structure	Depiction	BioNetGen Definition	Count
O0_EGF_tot	Cell		EGF()	Molecules
O0_EGFR_tot	Cell		EGFR()	Molecules
O0_Grb2_tot	Cell		Grb2()	Molecules
O0_Shc_tot	Cell		Shc()	Molecules
O0	Cell			Molecules

Below the table are buttons for 'New Observable', 'Duplicate', and 'Delete'. A red arrow points from the 'New Observable' button to a text box. The graphics editor shows a dashed circle shape with a right-click context menu open, listing 'Delete Species Pattern', 'Add Molecule', and 'Specify structure (for all)'. A red arrow points from the 'Add Molecule' option to another text box. The 'Add Molecule' menu shows icons for EGF, EGFR, Grb2, and Shc.

1. A new Observable can be added by pressing the **New Observable** button below. The name can be edited in the table or in the graphics editor by **right clicking** on the shape. Rename the observable to Dimers.

2. When a dashed shape appears in the graphics editor, **right click** on the shape and choose **Add Molecule**. Select "EGFR".

**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.

File Server Window Tools Help

BioModel1

- Physiology
  - Reaction Diagram
  - Reactions (0)
  - Structures (1)
  - Species (0)
  - Molecules (4)
  - Observables (6)
- Applications (0)
- Parameters, Functions and Units
- Pathway

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BioModels MathModels Geometries

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Reaction Diagram Reactions Structures Species Molecules Observables

Name	Structure	Depiction	BioNetGen Definition	Count
O0_EGF_tot	Cell		EGF()	Molecules
O0_EGFR_tot	Cell		EGFR()	Molecules
O0_Grb2_tot	Cell		Grb2()	Molecules
O0_Shc_tot	Cell		Shc()	Molecules
Dimers	Cell		EGFR(tmd!+)	Molecules
Dimers_s	Cell		EGFR(tmd!+)	Species

Dimers are characterized by site "tmd" being in a bound state. **Right click** on the site shape (it will become white), and select "Site has external bond".

Create an Observable named Dimers\_s, identical to Dimers but set Count to "Species" (**double left click** on Molecules and select "Species").

New Observable Duplicate Delete Pathway Links Search

Object Properties Problems (0 Errors, 0 Warnings) Database File Info

Add Pattern

Multimolecular

Polymer of

length = 2

length > 1

EGFR

Cell

ecd tmd Y1 Y2

- Site is unbound
- + **Site has external bond**
- ? Site may be bound
- Site bond specified

Annotation

**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.

File Server Window Tools Help

BioModel1

- Physiology
  - Reaction Diagram
  - Reactions (0)
  - Structures (1)
  - Species (0)
  - Molecules (4)
  - Observables (7)**
- Applications (0)
- Parameters, Functions and Units
- Pathway

VCell DB BMDB Pathway Comm Sabio

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Reaction Diagram Reactions Structures Species Molecules **Observables**

Name	Structure	Depiction	BioNetGen Definition	Count
O0_EGF_tot	Cell		EGF()	Molecules
O0_EGFR_tot	Cell		EGFR()	Molecules
O0_Grb2_tot	Cell		Grb2()	Molecules
O0_Shc_tot	Cell		Shc()	Molecules
Dimers	Cell		EGFR(tmd!+)	Molecules
Dimers_s	Cell		EGFR(tmd!+)	Species
Y1	Cell			Molecules

New Observable Duplicate Delete Pathway Links Search

Object Properties Problems (0 Errors, 0 Warnings) Database File Info

Add Pattern

Multimolecular

Polymer of

length = 2

length > 1

EGFR Cell ecd tmd Y1 Y2

- ~ State: not specified
- ~ State: u
- ~ State: p

Annotation

To specify an Observable counting all phosphorylated sites "Y1", **right click** on the white state shape and select the desired state "p". Similarly, create an Observable counting phosphorylated sites "Y2".

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

File Server Window Tools Help

BioModel1

- Physiology
  - Reaction Diagram
  - Reactions (0)
  - Structures (1)
  - Species (0)
  - Molecules (4)
  - Observables (9)
- Applications (0)
- Parameters, Functions and Units
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BioModels MathModels Geometries

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Reaction Diagram Reactions Structures Species Molecules **Observables**

Name	Structure	Depiction	BioNetGen Definition	Count
O0_EGF_tot	Cell		EGF()	Molecules
O0_EGFR_tot	Cell		EGFR()	Molecules
O0_Grb2_tot	Cell		Grb2()	Molecules
O0_Shc_tot	Cell		Shc()	Molecules
Dimers	Cell		EGFR(tmd!+)	Molecules
Dimers_s	Cell		EGFR(tmd!+)	Species
Y1	Cell		EGFR(Y1~p!?)	Molecules
Y2	Cell		EGFR(Y2~p!?)	Molecules
Y_total	Cell		EGFR()	Molecules

New Observable Duplicate Delete Pat

Object Properties Problems (0 Errors, 0 Warnings) Database File Info

Add Pattern

Multimolecular

Polymer of

length = 2

length > 1

EGFR

Cell

ecd tmd Y1 Y2

Annotation

To specify an Observable counting all phosphorylated sites "Y1" and "Y2", first specify a pattern for "Y1", then click below and select **Add Species Pattern**. Then, specify a similar pattern but with site "Y2" in the phosphorylated state.

To have more space, **right click** on a line; keep the **right button pressed** and drag it down.

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

The screenshot shows the VCell software interface. On the left is a tree view of the model structure, including 'Physiology', 'Reaction Diagram', 'Reactions (0)', 'Structures (1)', 'Species (1)', 'Molecules (4)', and 'Observables (9)'. The main window is in the 'Species' tab, displaying a table with columns: Name, Structure, Depiction, Link, and BioNetGen Definition. The table contains one entry with Name 'R' and Structure 'Cell', and a row below it with '(add new here)'. Below the table are buttons for 'New Species', 'Duplicate', 'Delete', and 'Pathway Links', along with a search box. At the bottom, the 'Object Properties' panel shows 'Species Name' as 'R'. A context menu is open over a green sphere icon, with options: 'Specify Molecule', 'EGF', 'EGFR', 'Grb2', and 'Shc'. Two red arrows point from text boxes to the '(add new here)' text and the green sphere icon.

Name	Structure	Depiction	Link	BioNetGen Definition
R	Cell			
(add new here)				

1. To add species, **left double click** on (add new here) and change the name to R. Alternatively, use the “New Species” button below.

2. By default, a species is created without a molecular structure (green shape). To specify molecular composition, **right click** on the green shape, **Specify Molecule**, and select “EGFR”.

**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.

The screenshot shows the VCell software interface. On the left is a tree view of the model structure. The main window has tabs for Reaction Diagram, Reactions, Structures, Species, Molecules, and Observables. The Species tab is active, displaying a table with one species: 'Cell' with a depiction of a cell and a BioNetGen definition 'EGFR(ecd,tmd,Y1,Y2)'. A red 'x' icon in the Name column indicates an error. Below the table are buttons for 'New Species', 'Duplicate', 'Delete', and 'Pathway Links'. At the bottom, the 'Object Properties' panel shows 'Species Name' as 'R'. A 'Problems' tab is selected, showing '2 Errors, 0 Warnings'. A diagram of the EGFR molecule is shown with states 'ecd', 'tmd', 'Y1', and 'Y2'. A context menu is open over the 'Y1' state, showing options for 'State: u' and 'State: p'. Three text boxes with red arrows provide instructions: one points to the red 'x' icon, another points to the 'Y1' state, and a third points to the 'State: u' option in the menu.

Name	Structure	Depiction	Link	BioNetGen Definition
<span style="color:red">x</span> P (add new here)	Cell			EGFR(ecd,tmd,Y1,Y2)

Red color indicates an error.

1. After the EGFR molecule is assigned to a species, an error is generated because sites "Y1" and "Y2" must be in a specific state (a species must have a unique state).
2. Specify the state by **right click** on a state shape and selecting a required state ("u").

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

File Server Window Tools Help

BioModel1

- Physiology
  - Reaction Diagram
  - Reactions (0)
  - Structures (1)
  - Species (5)**
  - Molecules (4)
  - Observables (9)
- Applications (0)
- Parameters, Functions and Units
- Pathway

Reaction Diagram Reactions Structures **Species** Molecules Observables

Name	Structure	Depiction	Link	BioNetGen Definition
R	Cell			EGFR(ecd,tmd,Y1~u,Y2~u)
L	Cell			EGF(Site)
Grb2	Cell			Grb2(sh2)
ShcP	Cell			Shc(sh3,Y~p)
ShcU	Cell			Shc(sh3,Y~u)
(add new here)				

Complete the specification of all Species. You may check the list in the *RB\_egfr\_tutorial* model in VCell 6.1 (Rule-based) folder.

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New Species Duplicate Delete Pathway Links Search

Object Properties Problems (0 Errors, 0 Warnings) Database File Info

Select only one object (e.g. species, reaction, simulation) to view/edit properties.

**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).

File Server Window Tools Help

BioModel1

Physiology

- Reaction Diagram
- Reactions (1)
- Structures (1)
- Species (5)
- Molecules (4)
- Observables (9)
- Applications (0)
- Parameters, Functions and Units
- Pathway

Reaction Diagram Reactions Structures Species Molecules Observables

Reaction	Name	Structure	Depiction	Kinetics	Link	BioNetGen Definition
Reaction Rule	r0	Cell	->	MassAction		->

In the next few slides we will define a rule for the ligand binding to the receptor.

1. Click the **New Rule** button to generate a new rule.
2. Errors and warnings are generated immediately. They will disappear as the rule is being specified.
3. **Right click** on a dashed shape to specify the molecule to be included in a reactant pattern.

VCell DB BMDB Pathway Comm Sabio

BioModels MathModels Geometries

Search

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  - My BioModels (2018nathans751) (16)
  - Shared BioModels (0)
  - Public BioModels (601)
  - Tutorials (8)
  - Education (34)

New Reaction New Rule Duplicate Delete Pathway Links Search

Object Properties Problems (2 Errors, 2 Warnings) Database File Info

Kinetics Editor

Reversible  + -

Add Reactant

Add Product

Single Row Viewer

Show Molecule Color

Show Non-trivial

Show Differ...

Delete

Specify Molecule

Specify structure

EGF

EGFR

Grb2

Shc

**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>

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VCell DB BMDB Pathway Comm Sabio

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Reaction Diagram Reactions Structures Species Molecules Observables

Reaction	Name	Structure	Depiction	Kinetics	Link	BioNetGen Definition
Reaction Rule	r0	Cell	 ->	MassAction		@Cell:EGFR() ->

Here we define the EGFR molecule acting as a reactant.

1. Note that the number of errors and warnings decreased as the rule was specified.

2. To add the next reactant, click on the **Add Reactant** button. Alternatively, one can **right click** on a white space after -> and choose **Add Reactant**.

New Reaction New Rule Duplicate Delete Pathway Links Search

Object Properties Problems (1 Errors, 1 Warnings) Database File Info

Kinetics Editor

Reversible

Add Reactant

Add Product

Single Row Viewer

Show Molecule Color

Show Non-trivial

Show Differ...

EGFR  ->

Cell 

Add Reactant

3. After a dashed shape for a new reactant appears, **right click** on it to add a molecule as the second reactant as before.

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

After reactants are defined, products are specified.

To specify a reactant or product pattern consisting of several molecules, **right click** on the white space next to an existing Molecule.

File Server Window Tools Help

BioModel1

- Physiology
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Reaction Diagram | Reactions | Structures | Species | Molecules | Observables

Reaction	Name	Structure	Depiction	Kinetics	Link	BioNetGen Definition
Reaction	r0	Cell		MassAction		@Cell:EGFR()+@Cell:EGF()-> @Cell:EGFR()

VCe11 DB | BMDB | Pathway Comm | Sabio

BioModels | MathModels | Geometries

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New Reaction | New Rule | Duplicate | Delete | Pathway Links | Search

Object Properties | Problems (0 Errors, 0 Warnings) | Database File Info

Kinetics | Editor

Reversible   

Add Reactant

Add Product

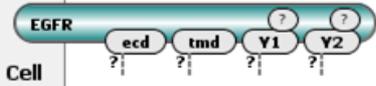
Single Row Viewer

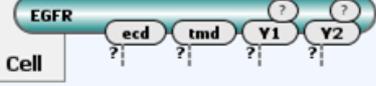
Show Molecule Color

Show Non-trivial

Show Differ...

EGFR + EGF ->

Cell  + Cell 

Cell 

Delete

Specify Molecule  EGF

Specify structure  EGFR

 Grb2

 Shc

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

The screenshot displays the VCell software interface for editing a reaction rule. On the left, a tree view shows the project structure under 'BioModel1', including 'Physiology', 'Reaction Diagram', 'Reactions (1)', 'Structures (1)', 'Species (5)', 'Molecules (4)', and 'Observables (9)'. Below this is a search bar and a list of biological models.

The top panel shows a table of reactions:

Reaction	Name	Structure	Depiction	Kinetics	Link	BioNetGen Definition
Reaction Rule	r0	Cell		MassAction		@Cell:EGFR(ecd,tmd)+@Cell:EGF(Site) -> @C

The main workspace shows the reaction rule editor. The reactant side features an EGFR molecule with sites 'ecd', 'tmd', 'Y1', and 'Y2', and an EGF molecule with a 'Site' site. The product side shows the EGFR-EGF complex. A context menu is open over the 'Site' property, listing options: 'Site is unbound', 'Site has external bond', 'Site may be bound', and 'Site bond specified'. Red arrows point from the text boxes to the 'Site' property and the 'ecd'/'tmd' sites.

We define conditions under which reactions may happen. Here, EGF binds if no ligand is bound (ecd is unbound) and the receptor is not in a dimer (tmd is unbound).

To select features of reactants, right click on the site shape and select its state and/or binding status.

All changes in Reactant patterns are propagated down to the same molecules in product patterns.

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

File Server Window Tools Help

BioModel1  
Physiology  
Reaction Diagram  
Reactions (1)  
Structures (1)  
Species (5)  
Molecules (4)  
Observables (9)  
Applications (0)  
Parameters, Functions and Units  
Pathway

Reaction Diagram Reactions Structures Species Molecules Observables

Reaction	Name	Structure	Depiction	Kinetics	Link	BioNetGen Definition
Reaction	r0	Cell		MassAction		@Cell:EGFR(ecd,tmd)+@Cell:EGF(Site) -> @C

Site "tmd" of the reactant pattern is unbound, so the only possible change is to make it bound to another site: it may not have implicit external bond ("has external bond") or be in an uncertain status ("may be bound").

To specify how product patterns differ from reactant patterns, **right click** on the shape and select features. For a binding reaction rule, specify how molecules in the product pattern are connected.

New Reaction New Rule Duplicate Delete Pathway Links Search

Object Properties Problems (0 Errors, 0 Warnings) Database File Info

Kinetics Editor

Reversible  + -

Add Reactant Add Product

EGFR ecd tmd Y1 Y2 + EGF Site ->

Cell Cell

Cell

- Site is unbound  
+ Site has external bond  
? Site may be bound  
Site bond specified

EGFR(ecd!1,tmd!1).EGF  
EGFR(ecd!1,Y1!1).EGF  
EGFR(ecd!1,Y2!1).EGF  
EGFR(ecd!1).EGF(Site!1)

Single Row Viewer  
 Show Molecule Color  
 Show Non-trivial  
 Show Differ...

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

File Server Window Tools Help

BioModel1

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    - Molecules (4)
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VCell DB BMDB Pathway Comm Sabio

BioModels MathModels Geometries

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Reaction Diagram Reactions Structures Species Molecules Observables

Reaction	Name	Structure	Depiction	Kinetics	Link	BioNetGen Definition
Reaction Rule	ligand_bind	Cell		MassAction		@Cell:EGFR(ecd,tmd)+@Cell:EGF(Site) -> @C

1. Change a reaction rule name by **double left click** on the rule name.

2. Note that by default a rule is created irreversible.

3. To make a rule reversible and to enter kinetics, **left click** on Kinetics.

New Reaction New Rule Duplicate Delete Pathway Links Search

Object Properties Problems (0 Errors, 0 Warnings) Database File Info

Kinetics Editor

Reversible  + -

Add Reactant

Add Product

EGFR ecd tmd Y1 Y2 + EGF Site ->

Cell Cell

EGFR ecd tmd Y1 Y2 EGF Site

Cell

Single Row Viewer

Show Molecule Color

Show Non-trivial

Show Differe...

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

Reversible  + -

Add Reactant

Add Product

Single Row Viewer

Show Molecule Color

Show Non-trivial

Show Differe...

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

Reversible  + -

Add Reactant

Add Product

Single Row Viewer

Show Molecule Color

Show Non-trivial

Show Differe...

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.

The screenshot shows the VCell Editor interface. On the left, the 'Editor' tab is active, and the 'Show Molecule Color' checkbox is checked, indicated by a red arrow. The main window displays a reaction rule: EGFR (with ecd, tmd, Y1, and Y2 domains) + EGF (with Site domain). The product shows the EGFR and EGF molecules bound together. The components are color-coded: ecd is light blue, tmd is light green, Y1 and Y2 are light yellow, and Site is light red.

**TIP:** Any combination of viewing buttons can be used.

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

The screenshot shows the VCell Editor interface. On the left, the 'Show Non-trivial' checkbox is checked, indicated by a red arrow. The main window displays the same reaction rule as the previous screenshot. The components are highlighted in yellow: ecd, tmd, Y1, Y2, and Site. The 'Show Molecule Color' checkbox is unchecked.

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

The screenshot shows the VCell Editor interface. On the left, the 'Show Differ...' checkbox is checked, indicated by a red arrow. The main window displays the same reaction rule as the previous screenshots. The components are highlighted in orange: ecd, tmd, Y1, Y2, and Site. The 'Show Molecule Color' and 'Show Non-trivial' checkboxes are unchecked.

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

The screenshot shows the VCell software interface. On the left is a tree view of the model structure. The main window is titled 'Reaction Diagram' and shows a table of reaction rules. Below this is a 'Kinetics' editor for the selected rule 'ligand\_bind'. The editor includes a 'Reversible' checkbox (checked), a 'Kinetic Type' dropdown set to 'Mass Action', and a table of kinetic parameters. Four red callout boxes provide instructions on how to configure these settings.

Reaction	Name	Structure	Depiction	Kinetics	Link	BioNetGen Definition
Reaction Rule	ligand_bind	Cell		MassAction		@Cell:EGFR(ecd,tmd)+@Cell:EGF(Site) <-> @Cell:EGFR(ec

Name	Description	Global	Expression	Units
ruleRate	rate of reactions generated by rule	<input type="checkbox"/>	Variable	$\mu\text{M}\cdot\text{s}^{-1}$
Kf	microscopic forward rate	<input type="checkbox"/>	0.0	$\text{s}^{-1}\cdot\mu\text{M}^{-1}$
Kr	microscopic reverse rate	<input type="checkbox"/>	0.0	$\text{s}^{-1}$

1. To make a rule reversible, check the **Reversible** button.

2. Note that the only allowable kinetic type is **Mass Action**, where every reaction selected by a Reaction Rule has a rate law of forward rate times the product of reactant amounts minus the reverse rate times the product of product amounts.

3. Expressions for forward and reverse rates can be any complicated functions.

4. Note that default units are  $\mu\text{M}$ . The unit system must be changed to use other units like nM or molecules.

**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.

1. To change the unit system, left click on Parameters, functions, and units.

2. Left click on **Model Unit System**.

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- Parameters, Functions and Units**
- Pathway

---

VCell DB BMDB Pathway Comm Sabio

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Model Unit System

Unit	Category
µm	primary
µm <sup>2</sup>	primary
µm <sup>3</sup>	primary
s	primary
Volume	primary
Time	primary
Volume Substance	primary
Membrane Substance	primary
LumpedReactionSubstance	primary
Voltage	electrical
Current	electrical
Capacitance	electrical
Conductance	electrical
Stochastic Substance	stochastic

select new unit system

default

sbml compatible

general

type	unit	VCell default
length	um	[um]
area	um2	[um2]
volume	um3	[um3]
time	s	[s]
volume species substance	nM.um3	[uM.um3]
membrane species substance	molecules	[molecules]
lumped reaction substance	molecules	[molecules]

OK Cancel

3. Click on **Change Unit System**.

4. Select **general**.

5. Enter new units.

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

File Server Window Tools Help

BioModel1

- Physiology
  - Reaction Diagram
  - Reactions (1)
  - Structures (1)
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VCeell DB BMDB Pathway Comm Sabio

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- Education (34)

Reaction Diagram Reactions Structures Species Molecules Observables

Reaction	Name	Structure	Depiction	Kinetics	Link	BioNetGen Definition
Reaction Rule	ligand_bind	Cell		MassAction		@Cell:EGFR(ecd,tmd)+@Cell:EGF(Site) <-> @Cell:EGFR(ecd,tmd):EGF(Site)

New Reaction New Rule Duplicate Delete Pathway Links Search

Object Properties Problems (0 Errors, 0 Warnings) Database File Info

**Kinetics** Editor

Reaction Name ligand\_bind

Reversible  Kinetic Type Mass Action ( for each reaction:  $K_f \cdot \Pi$  reactants -  $K_r \cdot \Pi$  products ) Convert units

Name	Description	Global	Expression	Units
ruleRate	rate of reactions generated by rule	<input type="checkbox"/>	Variable	nM.s <sup>-1</sup>
Kf	microscopic forward rate	<input type="checkbox"/>	0.003	s <sup>-1</sup> .nM <sup>-1</sup>
Kr	microscopic reverse rate	<input type="checkbox"/>	0.06	s <sup>-1</sup>

Annotation and Pathway Links

Linked Pathway Object(s):

Set values in proper units. Match all values to the *RB\_egfr\_tutorial* model in the VCell 6.1 (Rule-based) folder. Values are also listed in a table on the next slide.

**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.

File Server Window Tools Help

BioModel1

- Physiology
  - Reaction Diagram
    - Reactions (2)
    - Structures (1)
    - Species (5)
    - Molecules (4)
    - Observables (9)
  - Applications (0)
  - Parameters, Functions and Units
  - Pathway

Reaction Diagram | Reactions | Structures | Species | Molecules | Observables

Reaction	Name	Structure	Depiction	Kinetics	Link	BioNetGen Definition
Reaction Rule	ligand_bind	Cell		MassAction		@Cell:EGFR(ecd,tmd)+@Cell:EGF(Site) <-> @Cell:EGFR(ec
Reaction Rule	dimeriz	Cell		MassAction		@Cell:EGFR(ecd!+,tmd)+@Cell:EGFR(ecd!+,tmd) -> @Cell:

Similarly, set dimerization reaction rule as in the *RB\_egfr\_tutorial* model in the VCell 6.1 (Rule-based) folder.

Conditions for the rule to happen: both receptors are bound at "ecd" and unbound at "tmd" sites.

Note the rule is reversible.

Reversible  + -

Add Reactant

Add Product

Single Row Viewer

Show Molecule Color

Show Non-trivial

Show Differ...

Reaction rule outcome: a new bond between "tmd" sites.

**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.

The screenshot displays the VCell software interface. On the left is a tree view of the model structure, including 'Physiology', 'Reaction Diagram', 'Structures', 'Species', 'Molecules', and 'Observables'. The main window shows a 'Reactions' table with three entries: 'Y1\_Phosph', 'dimeriz', and 'ligand\_bind'. Below the table is a 'Kinetics' editor for the 'Y1\_Phosph' reaction, showing a diagram of an EGFR molecule with sites 'ecd', 'tmd', 'Y1', and 'Y2'. The 'Y1' site is shown with a 'P' (phosphorylation) and a vertical line underneath. The 'Reversible' checkbox is unchecked. A search panel on the bottom left shows a list of biological models.

Reaction	Name	Structure	Depiction	Kinetics	Link	BioNetGen Defn
Reaction Rule	Y1_Phosph	Cell		MassAction		@Cell:EGFR(tmd!+,Y1~u) -> @Cell:EGFR(tmd!+,Y1~p)
Reaction Rule	dimeriz	Cell		MassAction		@Cell:EGFR(ecd!+,tmd)+@Cell:EGFR(ecd!+,tmd) -> @Cell:EGFR(ecd!+,tmd,tmd)
Reaction Rule	ligand_bind	Cell		MassAction		@Cell:EGFR(ecd,tmd)+@Cell:EGF(Site) -> @Cell:EGFR(ecd,tmd,EGF)

**Set the irreversible phosphorylation reaction rule as in the *RB\_egfr\_tutorial* model in the VCell 6.1 (Rule-based) folder.**

**Conditions for the phosphorylation: "Y1" site is unbound and unphosphorylated, "tmd" site is bound (which means that the receptor is a part of aggregate).**

**Note that the rule is irreversible.**

**Reaction rule outcome: "Y1" site becomes phosphorylated.**

**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!

The screenshot displays the VCell software interface. On the left is a navigation tree for 'BioModel1' containing 'Physiology', 'Reaction Diagram', 'Reactions (4)', 'Structures (1)', 'Species (5)', 'Molecules (4)', 'Observables (9)', 'Applications (0)', 'Parameters, Functions and Units', and 'Pathway'. The main window is divided into several panes:

- Reaction Diagram:** A table listing reaction rules. The 'Y1\_dephosph' rule is selected.
 

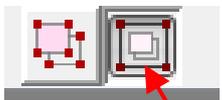
Reaction	Name	Structure	Depiction	Kinetics	Link
Reaction Rule	Dimerization	Cell		MassAction	@Cell:EGFR(ecd!+,tmd)+@Cell:EGFR(ecd!+,tmd) <->
Reaction Rule	Y1_phosph	Cell		MassAction	@Cell:EGFR(tmd!+,Y1~u) -> @Cell:EGFR(tmd!+,Y1~p)
Reaction Rule	Y1_dephosph	Cell		MassAction	@Cell:EGFR(tmd!+,Y1~p) -> @Cell:EGFR(tmd!+,Y1~u)
Reaction Rule	ligand_bind	Cell		MassAction	@Cell:EGFR(ecd,tmd)+@Cell:EGF(Site) <-> @Cell:EGFR(ecd,tmd,EGF)
- Buttons:** 'New Reaction', 'New Rule', 'Duplicate', 'Delete', 'Pathway Links', and a search field.
- Object Properties:** 'Kinetics' and 'Editor' tabs. The 'Reversible' checkbox is unchecked.
- Editor:** Shows the 'Y1\_dephosph' reaction rule. The reactant is an EGFR molecule with a phosphorylated Y1 site (Y1~p) and an unbound Y2 site (Y2). The product is an EGFR molecule with an unphosphorylated Y1 site (Y1~u) and an unbound Y2 site (Y2). The reaction arrow is a single arrow pointing right, indicating it is irreversible.

Three numbered steps are provided in yellow boxes:

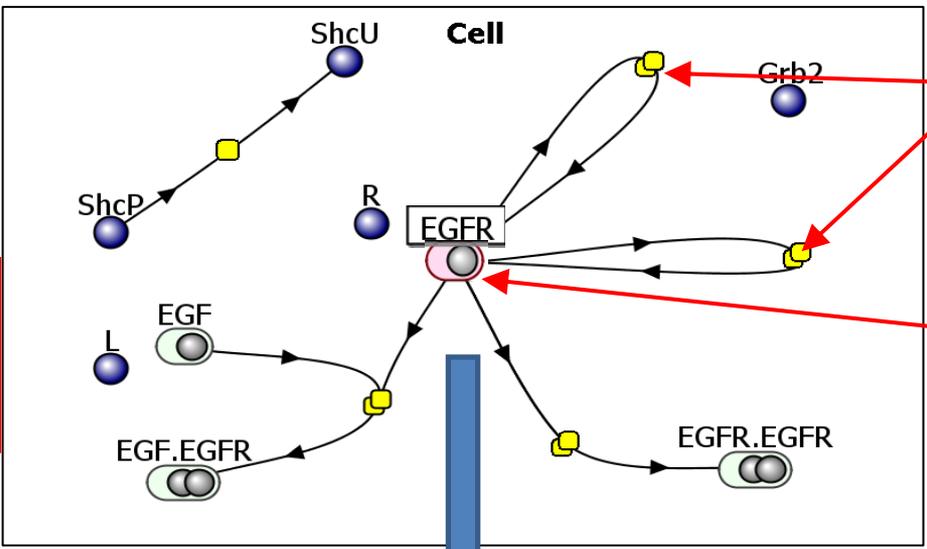
1. Select a rule to duplicate and click on **Duplicate** button
2. The Identical rule will appear with the name *oldname\_0*.
3. Rename the new rule and introduce any needed changes.

Additional annotations in yellow boxes:

- Note that the rule is irreversible.
- Condition for the dephosphorylation: "Y1" site is phosphorylated and unbound.
- Reaction rule outcome: "Y1" site becomes unphosphorylated.

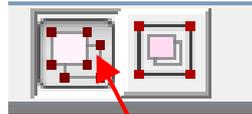


In the collapsed reaction diagram, the reaction rule participants are grouped by molecular structure.

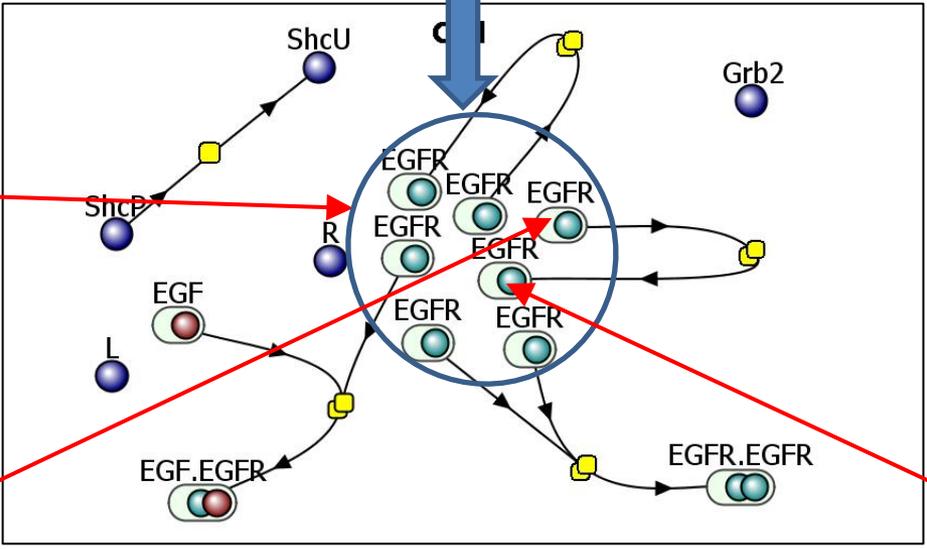


These are Reaction rules transforming EGFR molecule.

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

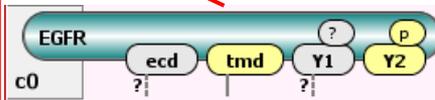
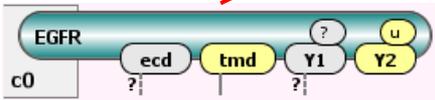


These are distinct species patterns corresponding to the same molecular structure in the collapsed reaction diagram.

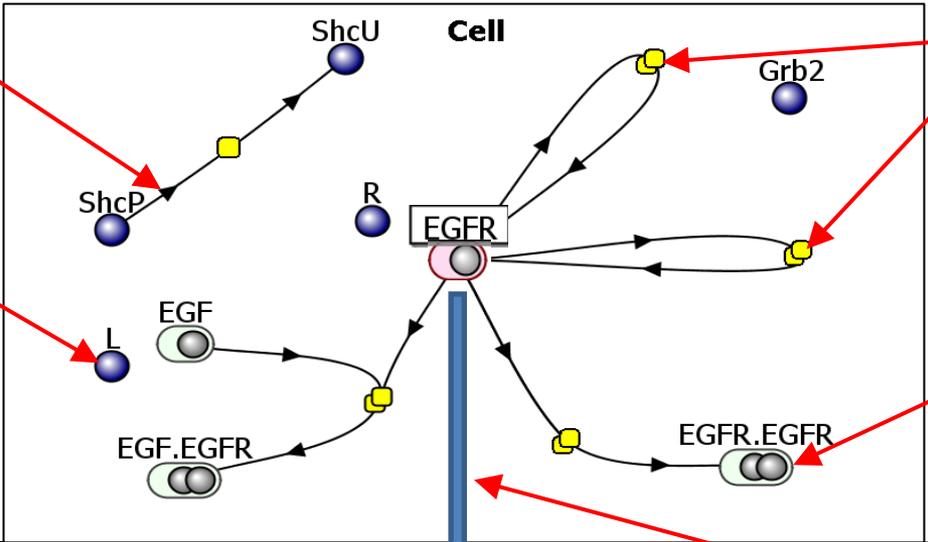


In the full reaction diagram, every reactant and product species pattern is shown individually if they are distinct in details.

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 molecular pattern is used.

Search

Problems (0 Errors, 1 Warnings)

Rule: **ligand bind**

Rule: **dimeriz**

Rule: **Y2 phosph**

Rule: **Y1 phosph**

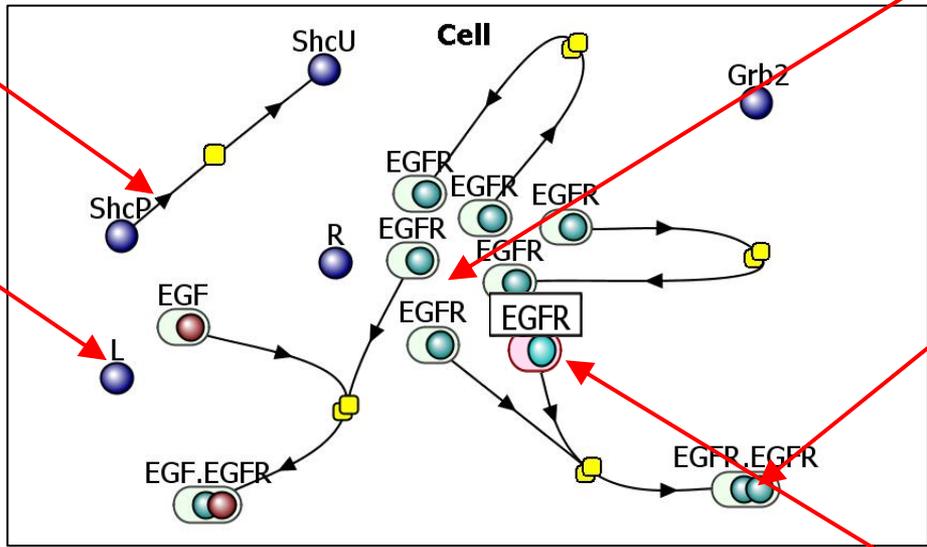
Rule: **Y2 dephosph**

This is the full reaction diagram.

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.

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

Species are depicted as blue spheres.

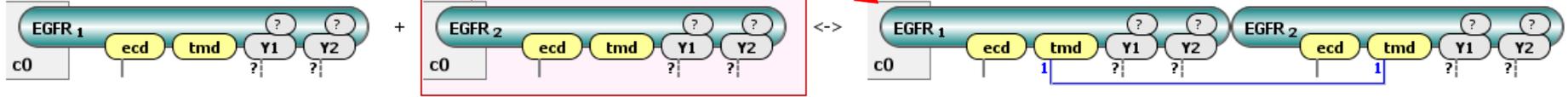


Colors within shapes correspond to molecular colors.

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

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

Rule: dimeriz



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

The screenshot shows the VCell software interface. On the left is a tree view of the model structure. The main window displays a reaction diagram with species like ShcP, ShcU, EGFR, and EGF. A reaction 'r0' is highlighted. Below the diagram is a table for defining the reaction's kinetics.

**1. Click on Reaction Diagram.** (Points to the 'Reaction Diagram' button in the left sidebar)

**2. Select RX Connection tool.** (Points to the 'RX' tool icon in the top toolbar)

**3. Connect required species.** (Points to the connection between ShcP and ShcU in the diagram)

**4. Specify reaction kinetics.** (Points to the 'Kf' value in the kinetics table)

Name	Description	Global	Expression	Units
J	reaction rate	<input type="checkbox"/>	$(K_f \cdot ShcP - K_r \cdot ShcU)$	nM.s <sup>-1</sup>
Kf	forward rate constant	<input type="checkbox"/>	0.005	s <sup>-1</sup>
Kr	reverse rate constant	<input type="checkbox"/>	0.0	s <sup>-1</sup>
ShcP	Species Concentration	<input checked="" type="checkbox"/>	Variable	nM
ShcU	Species Concentration	<input checked="" type="checkbox"/>	Variable	nM

**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.

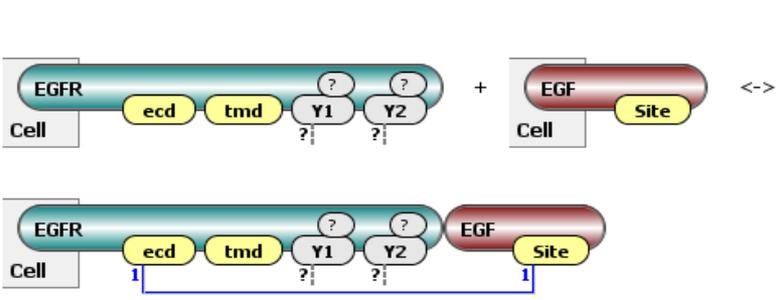
The screenshot displays the VCell 6.1 interface with the 'Reactions' tab selected. The main window shows a table of reaction rules. The table has the following columns: Reaction, Name, Structure, Depiction, Kinetics, and Link. The reactions listed are:

Reaction	Name	Structure	Depiction	Kinetics	Link
Reaction Rule	R_Grb2_interaction	Cell		MassAction	@Cell:EGFR(Y1~p)+@Cell:Grb2(sh2) <-> @C
Reaction Rule	ligand_bind	Cell		MassAction	@Cell:EGFR(ecd,tmd)+@Cell:EGF(Site) <-> @
Reaction Rule	Y2_phosph	Cell		MassAction	@Cell:EGFR(tmd!+,Y2~u) -> @Cell:EGFR(tmd
Reaction Rule	Y2_dephosph	Cell		MassAction	@Cell:EGFR(Y2~p) -> @Cell:EGFR(Y2~u)
Reaction Rule	Y1_phosph	Cell		MassAction	@Cell:EGFR(tmd!+,Y1~u) -> @Cell:EGFR(tmd
Reaction Rule	Y1_dephosph	Cell		MassAction	@Cell:EGFR(tmd!+,Y1~p) -> @Cell:EGFR(tmd
Reaction Rule	Sch_phosph	Cell		MassAction	@Cell:EGFR(Y2~p!1).Shc(sh3!1,Y~u) -> @Ce
ShcP -> ShcU	Sch_Dephosph	Cell		MassAction	ShcP -> ShcU
Reaction Rule	R_SchU_interaction	Cell		MassAction	@Cell:EGFR(Y2~p)+@Cell:Shc(sh3,Y~u) <->
Reaction Rule	R_SchP_interaction	Cell		MassAction	@Cell:EGFR(Y2~p)+@Cell:Shc(sh3,Y~p) <->
Reaction Rule	Dimerization	Cell		MassAction	@Cell:EGFR(ecd!+,tmd)+@Cell:EGFR(ecd!+,t

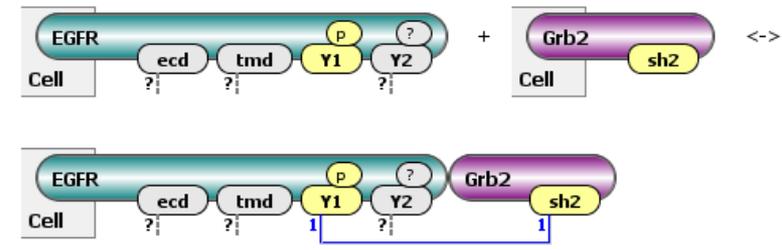
At the bottom of the table area, there is a search bar with the text 'Search' and a dropdown menu for 'Pathway Links'. Below the table, there are buttons for 'New Reaction', 'New Rule', 'Duplicate', 'Delete', and 'Pathway Links'. The 'Object Properties' section shows 'Problems (0 Errors, 0 Warnings)' and 'Database File Info'. A 'Show Warnings' checkbox is checked, and a 'Refresh' button is present. The 'Warnings' table has columns for Description, Url, Source, and Defined In.

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.

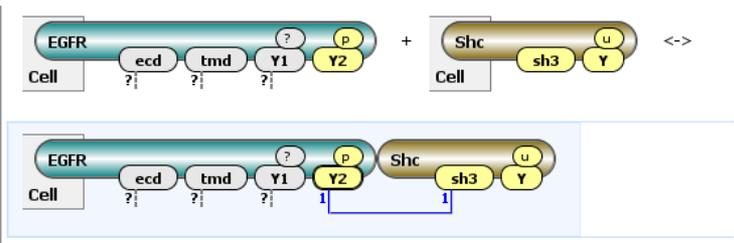
Rule-Based Tutorial VCell 6.1: Review of Rules



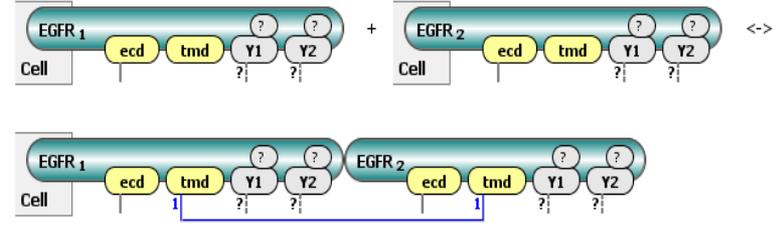
Ligand\_Bind (receptor must be in monomeric form (tmd is unbound) and not bound to ligand (ecd is unbound) for reaction to happen.)



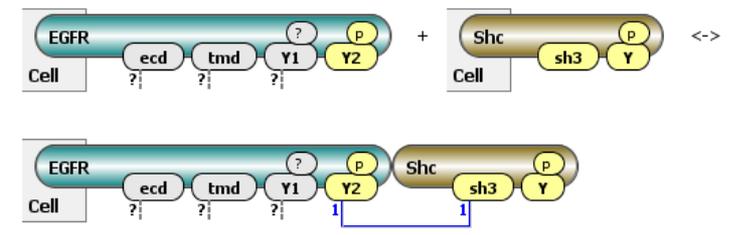
R\_Grb2\_interaction (EGFR does not have to be in monomeric form. Y1 has to be phosphorylated, for it to bind to sh2).



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

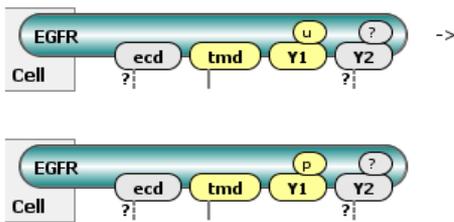


Dimeriz (tmd must be unbound and ecd has to be bonded externally for the two tmd sites to bond and form a dimer).

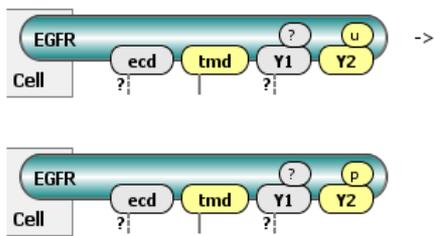


R\_ShcP\_interaction (for this reaction to occur, the Y site on Shc has to be unbound and phosphorylated. The unphosphorylated Y2 binds with sh3).

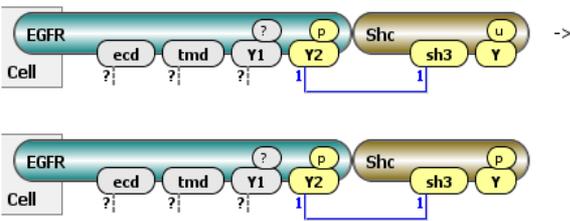
Reaction	Reversible?	Kf	Kr
ligand_bind	Yes	0.003 1/(nM s)	0.06 1/s
Dimeriz	Yes	0.001 1/(nM s)	0.1 1/s
R_Grb2_interaction	Yes	0.001 1/(nM s)	0.05 1/s
R_ShcP_interaction	Yes	4.5E-04 1/(nM s)	0.3 1/s
R_ShcU_interaction	Yes	0.045 1/(nM s)	0.6 1/s



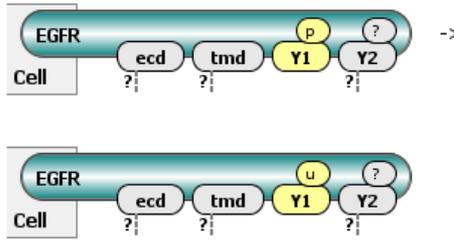
**Y1\_Phosph** (for phosphorylation to occur, tmd must be externally bound, implying a dimeric form).



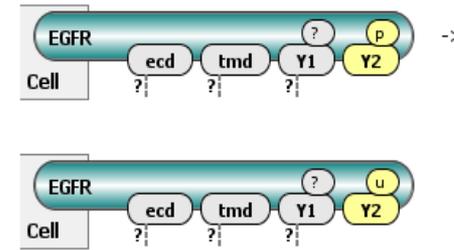
**Y2\_Phosph** (for phosphorylation to occur, tmd must be externally bound, implying a dimeric form).



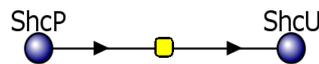
**Sch\_Phosph** (The Y site on Shc changes from unphosphorylated to phosphorylated. In order for this to happen, sh3 must be bound to the phosphorylated Y2 site).



**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
Y1_dephosph	No		4.5 1/s
Y2_phosph	No		0.5 1/s
Y2_dephosph	No		4.5 1/s
Shc_phosph	No		3.0 1/s
ShcDephosp	No		0.005 1/s

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

The screenshot displays the VCell software interface. On the left, a tree view shows the project structure under 'BioModel1', including 'Physiology', 'Reaction Diagram', 'Reactions (11)', 'Structures (1)', 'Species (5)', 'Molecules (4)', 'Observables (9)', 'Applications (0)', 'Parameters', and 'Pathway'. A context menu is open over the 'Applications (0)' folder, with 'New Application' selected, and a sub-menu showing 'Deterministic', 'Stochastic', and 'Network-Free'. A red arrow points from a text box to the 'Deterministic' option. The main workspace is a table with columns 'Name', 'Math Type', and 'Annotation'. Below the table are buttons for 'New Application', 'Delete', 'More Copy Actions', and 'Compare...', along with a search bar. At the bottom, there are tabs for 'Object Properties', 'Problems (0 Errors, 0 Warnings)', and 'Database File Info'. The 'Object Properties' tab is active, showing the instruction: 'Select only one object (e.g. species, reaction, simulation) to view/edit properties.'

1. **Right click** on Application, select New Application > Deterministic. A ***Deterministic application*** uses the BioNetGen engine to generate a reaction network that is solved as a system of ODEs.

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

The screenshot shows the VCell software interface. On the left is a tree view under 'BioModel1' with 'Physiology' expanded to show 'Applications (1)' containing 'Application0'. Under 'Application0', 'Specifications' is selected. The main workspace shows the 'Specifications' tab with a table of species. Below this is a search bar and an 'Object Properties' table. Three red arrows point from callout boxes to the 'Specifications' tab, the 'Species' column, and the 'Initial Condition' column.

Species	Structure	Clamped	Initial Condition
ShcU	Cell	<input type="checkbox"/>	150.0 [nM]
ShcP	Cell	<input type="checkbox"/>	0.0 [nM]
R	Cell	<input type="checkbox"/>	100.0 [nM]
L	Cell	<input type="checkbox"/>	680.0 [nM]
Grb2	Cell	<input type="checkbox"/>	58.0 [nM]

Description	Parameter	Expression	Units
initial concentration for Grb2	initConc	58.0	nM

**1. Left click on new Application, select Specifications.**

**2. Left click on Species.**

**3. Set initial values of species specified in the Physiology.**

**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.

Name	Type	Enabled	Fast
Sch_Dephosph	Reaction	<input checked="" type="checkbox"/>	<input type="checkbox"/>
ligand_bind	Reaction Rule	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Dimerization	Reaction Rule	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Y1_phosph	Reaction Rule	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Y1_dephosph	Reaction Rule	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Y2_phosph	Reaction Rule	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Y2_dephosph	Reaction Rule	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Sch_phosph	Reaction Rule	<input checked="" type="checkbox"/>	<input type="checkbox"/>
R_SchU_interaction	Reaction Rule	<input checked="" type="checkbox"/>	<input type="checkbox"/>
R_SchP_interaction	Reaction Rule	<input checked="" type="checkbox"/>	<input type="checkbox"/>
R_Grb2_interaction	Reaction Rule	<input checked="" type="checkbox"/>	<input type="checkbox"/>

1. Left click on Application, select **Specifications**.

2. Left click on Reaction.

3. Uncheck to disable (remove from network generation).

4. Reactions (not rules) can be declared to have fast kinetics. The scale separation will be used by ODE solver.

**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.

The screenshot shows the VCell software interface. On the left is a tree view of the model structure. The main window has tabs for 'Geometry', 'Specifications', 'Protocols', 'Simulations', and 'Parameter Estimation'. The 'Network' tab is active, showing a table of network constraints. A dialog box titled 'Edit / Test Constraints' is open, allowing the user to modify the 'Max. Iterations' and 'Max. Molecules / Species' values. Red arrows point from callout boxes to the 'Network' tab, the 'Test / Run' button, the input fields in the dialog, and the 'Edit / Test Constraints' button.

Name	Type	Value
Max Iterations	value	3
Max Molecules / Species	value	10

Max. Iterations	3
Max. Molecules / Species	11

1. Left click on Network.

2. Left click on Edit/Test Constraints.

3. Set **Max. Iterations** and **Max. Molecules/Species**. The simulation will be performed on your local computer, so speed will depend on your CPU power.

4. Left click on Test/Run.

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.

The screenshot shows the VCell software interface. On the left is a tree view of the model components. The main window is divided into several panels. The top panel shows tabs for Geometry, Specifications, Protocols, Simulations, and Parameter Estimation. Below this is the 'Network Constraints' table:

Name	Type	Value
Max Iterations	value	3
Max Molecules / Species	value	10

Below the table is a 'Generated Network' section with 'Species: unavailable', 'Reactions: unavailable', and 'Warning: none'. A dialog box titled 'Apply the new constraints?' is open, showing the current values and a warning: 'Warning: Max Iterations number may be insufficient.' The dialog has 'Apply' and 'Cancel' buttons. A red arrow points from the 'Cancel' button to a yellow callout box on the right. Another red arrow points from the 'Iteration 3' line in the console output to a yellow callout box on the left.

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.

```

Object Properties | Problems (0 Errors, 1 Warnings) | Database File Info | Network Generation Status

Running BioNetGen ...
Iteration 0: 5 species
Iteration 1: 6 species
Iteration 2: 7 species
Iteration 3: 9 species
Creating BNC output spec ...
Return BioNetGen output to requester...
Total run time: 2.9 s.
Warning: Max Iterations number may be insufficient.
Please go to the Specifications / Network panel and adjust the number of Iterations.

```

**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.

The screenshot displays the VCell software interface. On the left is a tree view of the model structure, including 'Physiology', 'Applications (1)', and 'Parameters, Functions and Units'. The 'Specifications' tab is selected. The main panel shows 'Network Constraints' with a table:

Name	Type	Value
Max Iterations	value	3
Max Molecules / Species	value	11

Below this is the 'Generated Network' section, which is currently empty. A dialog box titled 'Apply the new constraints?' is open, showing the current constraints: Max. Iterations: 12, Max. Molecules / Species: 12, and Warning: none. The 'Apply' button is highlighted with a red arrow. Another red arrow points to the 'Apply' button in the dialog box, with a callout box containing the text: '2. Click **Apply** to prepare network for simulation.'

At the bottom, the 'Network Generation Status' window shows the following output:

```
Running BioNetGen ...
Iteration 0: 5 species
Iteration 1: 6 species
Iteration 2: 7 species
Iteration 3: 9 species
Iteration 4: 18 species
Iteration 5: 35 species
Iteration 6: 60 species
Iteration 7: 87 species
Iteration 8: 106 species
Iteration 9: 106 species
Creating BNG output spec ...
Return BioNetGen output to requester...
Total run time: 12.8 s.
```

A red arrow points from the 'Apply' button in the dialog box to the 'Iteration 9' line in the status window, with a callout box containing the text: '1. Check generation progress. No warnings means that the network is fully generated.'

**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.*

The screenshot shows the VCell software interface. The left sidebar displays a tree view of the project 'BioModel1', with 'Specifications' selected. The main window has tabs for 'Species', 'Reaction', and 'Network', with 'Specifications' active. A table titled 'Network Constraints' is visible, showing 'Max Iterations' and 'Max Molecules / Species' both set to 'value' 12. Below this, the 'Generated Network' section shows 'Species: 106', 'Reactions: 684', and 'Warning: none'. Three callout boxes with red arrows point to specific buttons: '1. Click to see all species in a separate pop-up window.' points to a 'View' button; '2. Click to see all reactions in a separate pop-up window.' points to another 'View' button; and '3. See a reaction network in a separate window (may take a long time).' points to a 'Create new VCell BioModel from Network' button. At the bottom, the 'Network Generation Status' window shows a log of iterations from 0 to 9, with the number of species increasing from 5 to 106, and a total run time of 12.8 s.

Name	Type	Value
Max Iterations	value	12
Max Molecules / Species	value	12

Generated Network  
Species: 106  
Reactions: 684  
Warning: none

```
Iteration 0: 5 species
Iteration 1: 6 species
Iteration 2: 7 species
Iteration 3: 9 species
Iteration 4: 18 species
Iteration 5: 35 species
Iteration 6: 60 species
Iteration 7: 87 species
Iteration 8: 106 species
Iteration 9: 106 species
Creating BNG output spec ...
Return BioNetGen output to requester...
Total run time: 12.8 s.
Updating the network constraints with the test values.
```

**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.

View Generated Species

Index	Name	Structure	Depiction	Expression
1	R	Cell		EGFR(Y1~u,Y2~u,ecd,tmd)
2	L	Cell		EGF(Site)
3	Grb2	Cell		Grb2(sh2)
4	ShcP	Cell		Shc(Y~p,sh3)
5	ShcU	Cell		Shc(Y~u,sh3)
6	s5	Cell		EGF(Site!1).EGFR(Y1~u,Y2~u,ecd!1,tmd)
7	s6	Cell		EGF(Site!1).EGF(Site!2).EGFR(Y1~u,Y2~u,ecd!1,tmd!3).EGFR...
8	s7	Cell		EGF(Site!1).EGF(Site!2).EGFR(Y1~p,Y2~u,ecd!1,tmd!3).EGFR...
9	s8	Cell		EGF(Site!1).EGF(Site!2).EGFR(Y1~u,Y2~p,ecd!1,tmd!3).EGFR...
10	s9	Cell		EGF(Site!1).EGFR(Y1~p,Y2~u,ecd!1,tmd)
11	s10	Cell		EGF(Site!1).EGFR(Y1~u,Y2~p,ecd!1,tmd)
12	s11	Cell		EGF(Site!1).EGF(Site!2).EGFR(Y1~p,Y2~u,ecd!1,tmd!3).EGFR...
13	s12	Cell		EGF(Site!1).EGF(Site!2).EGFR(Y1~p,Y2~p,ecd!1,tmd!3).EGFR...
14	s13	Cell		EGF(Site!1).EGF(Site!2).EGFR(Y1~p,Y2~u,ecd!2,tmd!3).EGFR...
15	s14	Cell		EGF(Site!1).EGF(Site!2).EGFR(Y1~u,Y2~p,ecd!1,tmd!3).EGFR...
16	s15	Cell		EGF(Site!1).EGF(Site!2).EGFR(Y1~p!3,Y2~u,ecd!1,tmd!4).EG...
17	s16	Cell		EGF(Site!1).EGF(Site!2).EGFR(Y1~u,Y2~p!3,ecd!1,tmd!4).EG...

Search

+  
-

Close

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		$EGFR(Y1\sim u, Y2\sim u, ecd, tmd) + EGF(Site) \rightarrow EGF(Site!1).EGFR(Y1\sim u, Y2\sim u, ecd!1, tmd)$
2	ligand_bind	Cell		$EGF(Site) + EGFR(Y1\sim p, Y2\sim u, ecd, tmd) \rightarrow EGF(Site!1).EGFR(Y1\sim p, Y2\sim u, ecd!1, tmd)$
3	ligand_bind	Cell		$EGF(Site) + EGFR(Y1\sim u, Y2\sim p, ecd, tmd) \rightarrow EGF(Site!1).EGFR(Y1\sim u, Y2\sim p, ecd!1, tmd)$
4	ligand_bind	Cell		$EGF(Site) + EGFR(Y1\sim p, Y2\sim p, ecd, tmd) \rightarrow EGF(Site!1).EGFR(Y1\sim p, Y2\sim p, ecd!1, tmd)$
5	ligand_bind	Cell		$EGF(Site) + EGFR(Y1\sim p!1, Y2\sim u, ecd, tmd).Grb2(sh2!1) \rightarrow EGF(Site!1).EGFR(Y1\sim p!2, sh2!1)$
6	ligand_bind	Cell		$EGF(Site) + EGFR(Y1\sim u, Y2\sim p!1, ecd, tmd).Shc(Y\sim p, sh3!1) \rightarrow EGF(Site!1).EGFR(Y1\sim u, Y2\sim p!2, sh3!1)$
7	ligand_bind	Cell		$EGF(Site) + EGFR(Y1\sim u, Y2\sim p!1, ecd, tmd).Shc(Y\sim u, sh3!1) \rightarrow EGF(Site!1).EGFR(Y1\sim u, Y2\sim p!2, sh3!1)$
8	ligand_bind	Cell		$EGF(Site) + EGFR(Y1\sim p, Y2\sim p!1, ecd, tmd).Shc(Y\sim p, sh3!1) \rightarrow EGF(Site!1).EGFR(Y1\sim p, Y2\sim p!2, sh3!1)$
9	ligand_bind	Cell		$EGF(Site) + EGFR(Y1\sim p, Y2\sim p!1, ecd, tmd).Shc(Y\sim u, sh3!1) \rightarrow EGF(Site!1).EGFR(Y1\sim p, Y2\sim p!2, sh3!1)$
10	ligand_bind	Cell		$EGF(Site) + EGFR(Y1\sim p!1, Y2\sim p, ecd, tmd).Grb2(sh2!1) \rightarrow EGF(Site!1).EGFR(Y1\sim p!2, sh2!1)$
11	ligand_bind	Cell		$EGF(Site) + EGFR(Y1\sim p!1, Y2\sim p!2, ecd, tmd).Grb2(sh2!1).Shc(Y\sim p, sh3!2) \rightarrow EGF(Site!1).EGFR(Y1\sim p!2, sh2!1).Shc(Y\sim p, sh3!2)$
12	ligand_bind	Cell		$EGF(Site) + EGFR(Y1\sim p!1, Y2\sim p!2, ecd, tmd).Grb2(sh2!1).Shc(Y\sim u, sh3!2) \rightarrow EGF(Site!1).EGFR(Y1\sim p!2, sh2!1).Shc(Y\sim u, sh3!2)$
13	ligand_bin...	Cell		$EGF(Site!1).EGFR(Y1\sim u, Y2\sim u, ecd!1, tmd) \rightarrow EGF(Site) + EGFR(Y1\sim u, Y2\sim u, ecd, tmd)$
14	ligand_bin...	Cell		$EGF(Site!1).EGFR(Y1\sim p, Y2\sim u, ecd!1, tmd) \rightarrow EGF(Site) + EGFR(Y1\sim p, Y2\sim u, ecd, tmd)$
15	ligand_bin...	Cell		$EGF(Site!1).EGFR(Y1\sim u, Y2\sim p, ecd!1, tmd) \rightarrow EGF(Site) + EGFR(Y1\sim u, Y2\sim p, ecd, tmd)$
16	ligand_bin...	Cell		$EGF(Site!1).EGFR(Y1\sim p, Y2\sim p, ecd!1, tmd) \rightarrow EGF(Site) + EGFR(Y1\sim p, Y2\sim p, ecd, tmd)$
17	ligand_bin...	Cell		$EGF(Site!1).EGFR(Y1\sim p!2, Y2\sim u, ecd!1, tmd).Grb2(sh2!2) \rightarrow EGF(Site) + EGFR(Y1\sim p!1, Y2\sim u, ecd, tmd).Grb2(sh2!1)$
18	ligand_bin...	Cell		$EGF(Site!1).EGFR(Y1\sim u, Y2\sim p!2, ecd!1, tmd).Shc(Y\sim p, sh3!2) \rightarrow EGF(Site) + EGFR(Y1\sim u, Y2\sim p!1, ecd, tmd).Shc(Y\sim p, sh3!1)$
19	ligand_bin...	Cell		$EGF(Site!1).EGFR(Y1\sim u, Y2\sim p!2, ecd!1, tmd).Shc(Y\sim u, sh3!2) \rightarrow EGF(Site) + EGFR(Y1\sim u, Y2\sim p!1, ecd, tmd).Shc(Y\sim u, sh3!1)$
20	ligand_bin...	Cell		$EGF(Site!1).EGFR(Y1\sim p, Y2\sim p!2, ecd!1, tmd).Shc(Y\sim p, sh3!2) \rightarrow EGF(Site) + EGFR(Y1\sim p, Y2\sim p!1, ecd, tmd).Shc(Y\sim p, sh3!1)$
21	ligand_bin...	Cell		$EGF(Site!1).EGFR(Y1\sim p, Y2\sim p!2, ecd!1, tmd).Shc(Y\sim u, sh3!2) \rightarrow EGF(Site) + EGFR(Y1\sim p, Y2\sim p!1, ecd, tmd).Shc(Y\sim u, sh3!1)$
22	ligand_bin...	Cell		$EGF(Site!1).EGFR(Y1\sim p!2, Y2\sim n, ecd!1, tmd).Grb2(sh2!2) \rightarrow EGF(Site) + EGFR(Y1\sim p!1, Y2\sim n, ecd, tmd).Grb2(sh2!1)$

Search

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.

The screenshot shows the VCell software interface with the following components:

- Left Panel:** A tree view showing the model structure: Rule\_Based\_Egrf > Physiology > Reaction Diagram > Reactions (11), Structures (1), Species (5), Molecules (4), Observables (9). Under Applications (1), Application0 is expanded to show Geometry, Specifications, Protocols, Simulations (highlighted in blue), and Parameter Estimation. Below this are Parameters, Functions and Units, and Pathway.
- Top Panel:** Tabs for Geometry, Specifications, Protocols, Simulations (active), and Parameter Estimation. Sub-tabs include Simulations, Output Functions, and Generated Math.
- Simulations Table:**

Name	End Time	Output Option	Solver	Running Status	Results
Simulation0	60.0	keep every 1 sample	Combined IDA/CVODE	not saved	no
- Bottom Panel:** Object Properties section with tabs for Problems (0 Errors, 0 Warnings), Database File Info, and Network Generation Status. It includes an Annotation field, a Settings table, and a Parameters with values changed from defaults table.

Five numbered callouts with red arrows point to specific elements in the interface:

1. Click on Simulations. (Points to the 'Simulations' sub-tab in the top panel)
2. Set end time. (Points to the '60.0' value in the 'End Time' column of the Simulations table)
3. For advanced options; i.e. different solvers and outputs, click Edit. (Points to the 'keep every 1 sample' value in the 'Output Option' column)
4. Click to run locally (on user's computer). (Points to the blue play button icon in the top right of the Simulations table)
5. Click to run on a VCell server (will store simulation results). (Points to the green play button icon in the top right of the Simulations table)

1. Click on Simulations.

2. Set end time.

3. For advanced options; i.e. different solvers and outputs, click Edit.

5. Click to run on a VCell server (will store simulation results).

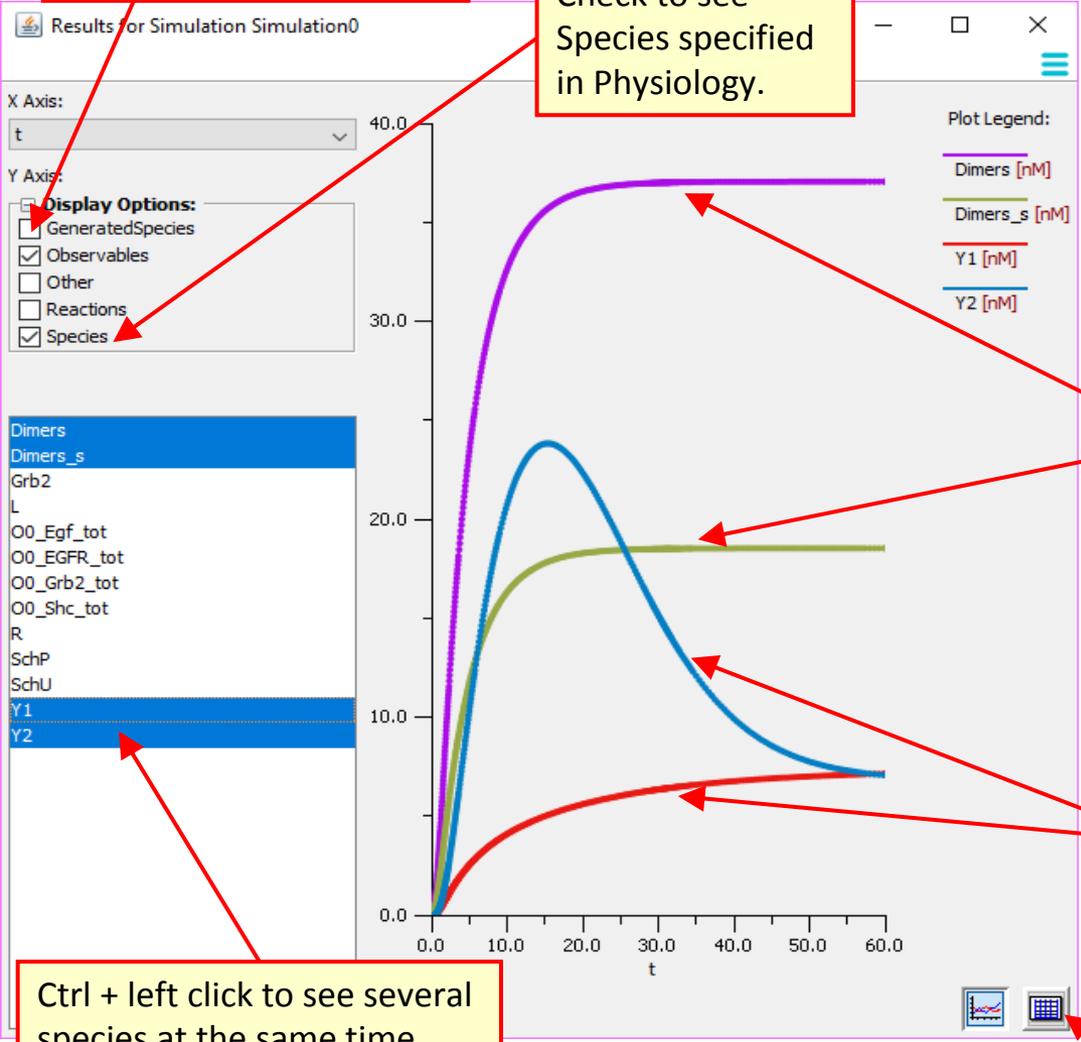
4. Click to run locally (on user's computer).

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.

The screenshot shows the VCell software interface. On the left, a tree view shows a project named 'Rule\_Based\_Egrf' with sub-items like 'Physiology', 'Reaction Diagram', 'Reactions (11)', 'Structures (1)', 'Species (5)', 'Molecules (4)', 'Observables (9)', and 'Applications (1)'. Under 'Applications (1)', 'Application0' is selected, and a context menu is open with 'Copy As' highlighted. A sub-menu is visible with 'Stochastic' selected. A red arrow points from the 'Stochastic' option to the first text box. The main window shows tabs for 'Geometry', 'Specifications', 'Protocols', 'Simulations', and 'Parameter Estimation'. The 'Network' tab is active, displaying 'Network Constraints' with a table:

Name	Type	Value
Max Iterations	value	12
Max Molecules / Species	value	12

At the bottom left, there is a 'BioModels' search panel with a search bar and a list of folders: 'Biological Models', 'My BioModels (2018nathans751) (17)', 'Shared BioModels (0)', 'Public BioModels (601)', 'Tutorials (8)', and 'Education (34)'. The bottom right panel shows 'Object Properties' with a search bar and a message: 'Select only one object (e.g. species, reaction, simulation) to view/edit properties.'

1. One can create a stochastic application by copying a deterministic application. **Right click** on Deterministic Application, select **Copy As> Stochastic**. Initial values of species will be copied to the new application. A **Stochastic application** uses the BioNetGen engine to generate a reaction network that is solved using direct or hybrid Gibson solvers.

2. Alternatively, a new application can be created by a **right click** on Applications, select **New Application > Stochastic**.

**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  $\mu\text{m}^3$  (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.

The screenshot displays the VCell software interface. The top panel, titled 'Geometry Definition', shows a 3D model of a cell labeled 'Cell' within a 'Physiology (structures)' environment. A red square in the 'Geometry (subd...)' panel is connected to the cell. Below this, a table lists the 'Cell' structure with a size of 10  $\mu\text{m}^3$ . The bottom panel, titled 'Species', shows the 'Initial Condition' set to 'Number of Particles' (selected with a radio button). The table below lists species and their initial conditions:

Species	Structure	Clamped	Initial Condition	Force Continuous
R	Cell	<input type="checkbox"/>	602.0 [molecules]	<input type="checkbox"/>
L	Cell	<input type="checkbox"/>	4094.0 [molecules]	<input type="checkbox"/>
Grb2	Cell	<input type="checkbox"/>	349.0 [molecules]	<input type="checkbox"/>
SchP	Cell	<input type="checkbox"/>	0.0 [molecules]	<input type="checkbox"/>
SchU	Cell	<input type="checkbox"/>	903.0 [molecules]	<input type="checkbox"/>

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.

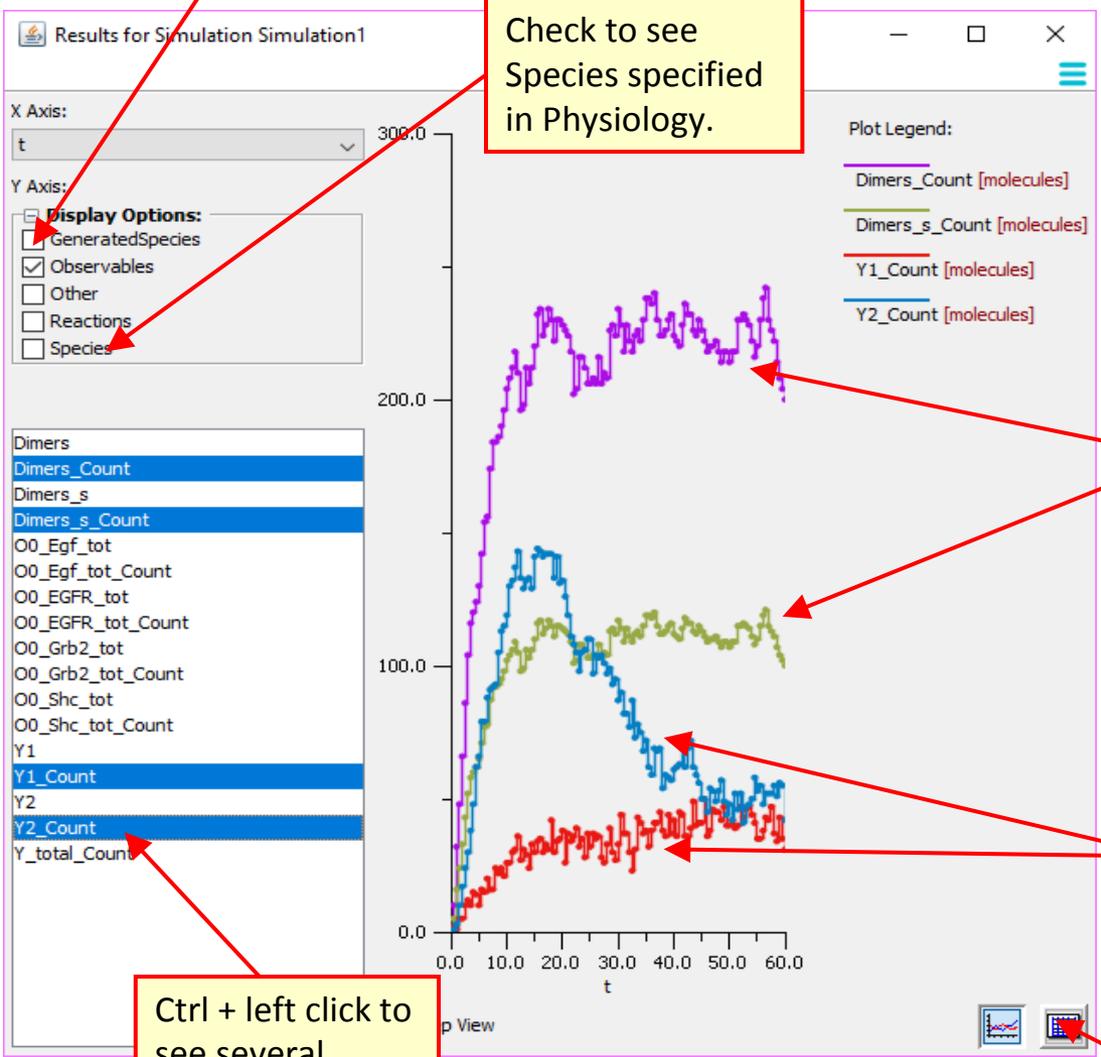
Check to see species generated by BioNetGen.

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).

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.

Check to see Species specified in Physiology.



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.

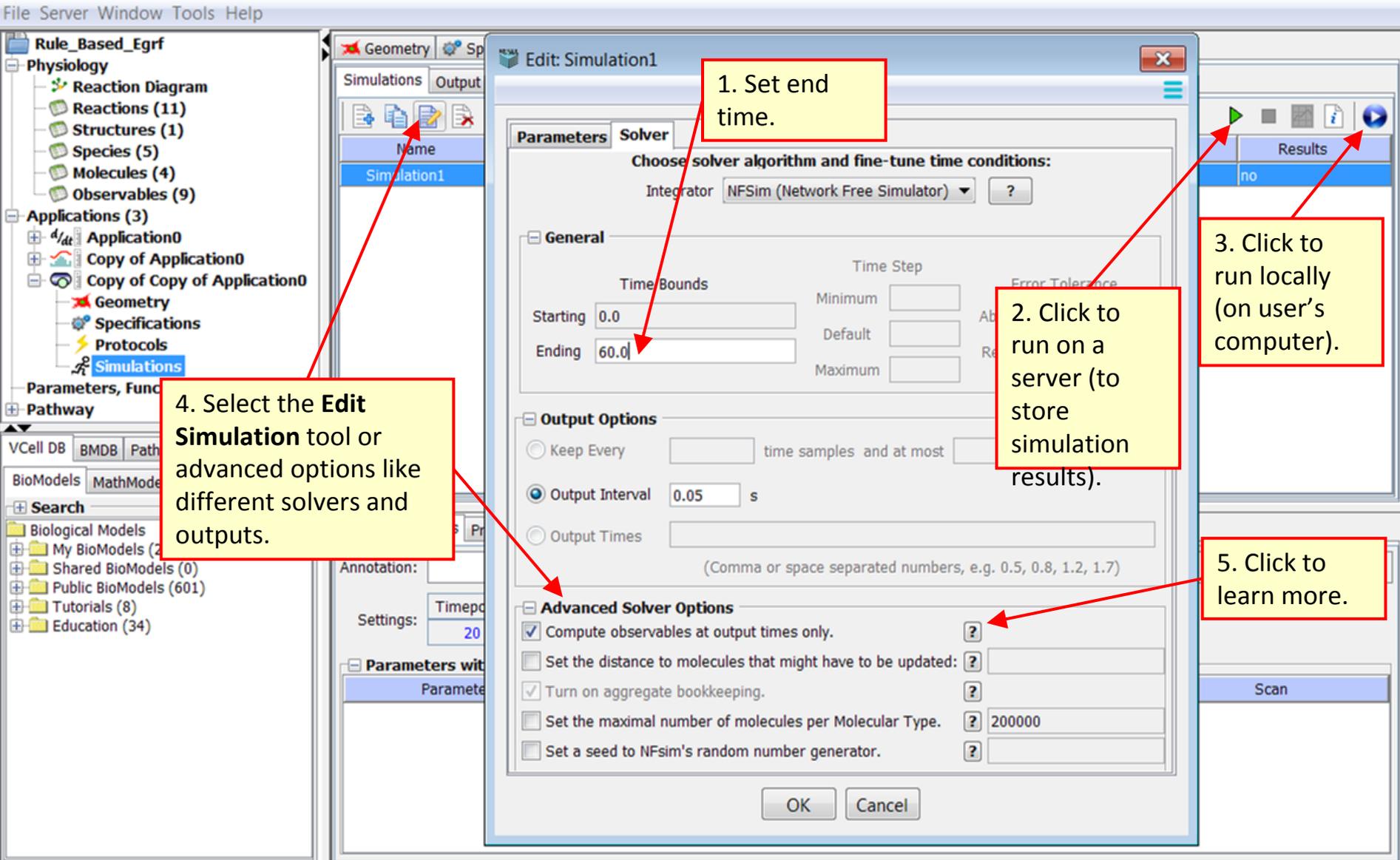
**TIP:** A Network-Free application simulates timecourses for observables without network generation. If the network size is too large or infinite, it is the only way to compute observables. However, individual species are not visible. To check whether a specific species is populated, it can be added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

The screenshot displays the VCell software interface. On the left, a tree view shows a project named 'Rule\_Based\_Egrf' with sub-items like 'Physiology', 'Reaction Diagram', 'Reactions (11)', 'Structures (1)', 'Species (5)', 'Molecules (4)', 'Observables (9)', 'Applications (2)', and 'Pathway'. Under 'Applications (2)', 'Application0' is selected, and a context menu is open over it. The menu options are: 'Rename', 'Delete', 'Copy', 'Copy As', 'New BioModel From App', 'Expand All', and 'Collapse All'. The 'Copy As' sub-menu is open, showing 'Deterministic', 'Stochastic', and 'Network-Free'. A red arrow points from the 'Copy As' menu item to a yellow text box. Below the tree view, there are tabs for 'VCell DB', 'BMDB', and 'Pathway Comm'. At the bottom left, there is a 'BioModels' search panel with a 'Search' field and a list of folders: 'Biological Models', 'My BioModels (2018nathans751) (17)', 'Shared BioModels (0)', 'Public BioModels (601)', 'Tutorials (8)', and 'Education (34)'. The main workspace is divided into 'Structure Mapping' and 'Geometry Definition' tabs. A table at the bottom right shows a 'Volume/Surface Calculator' with a 'Size' column containing the value '10 [ μm³ ]'. A second yellow text box is located at the bottom right, with a red arrow pointing from it to the 'New BioModel From App' menu item.

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.



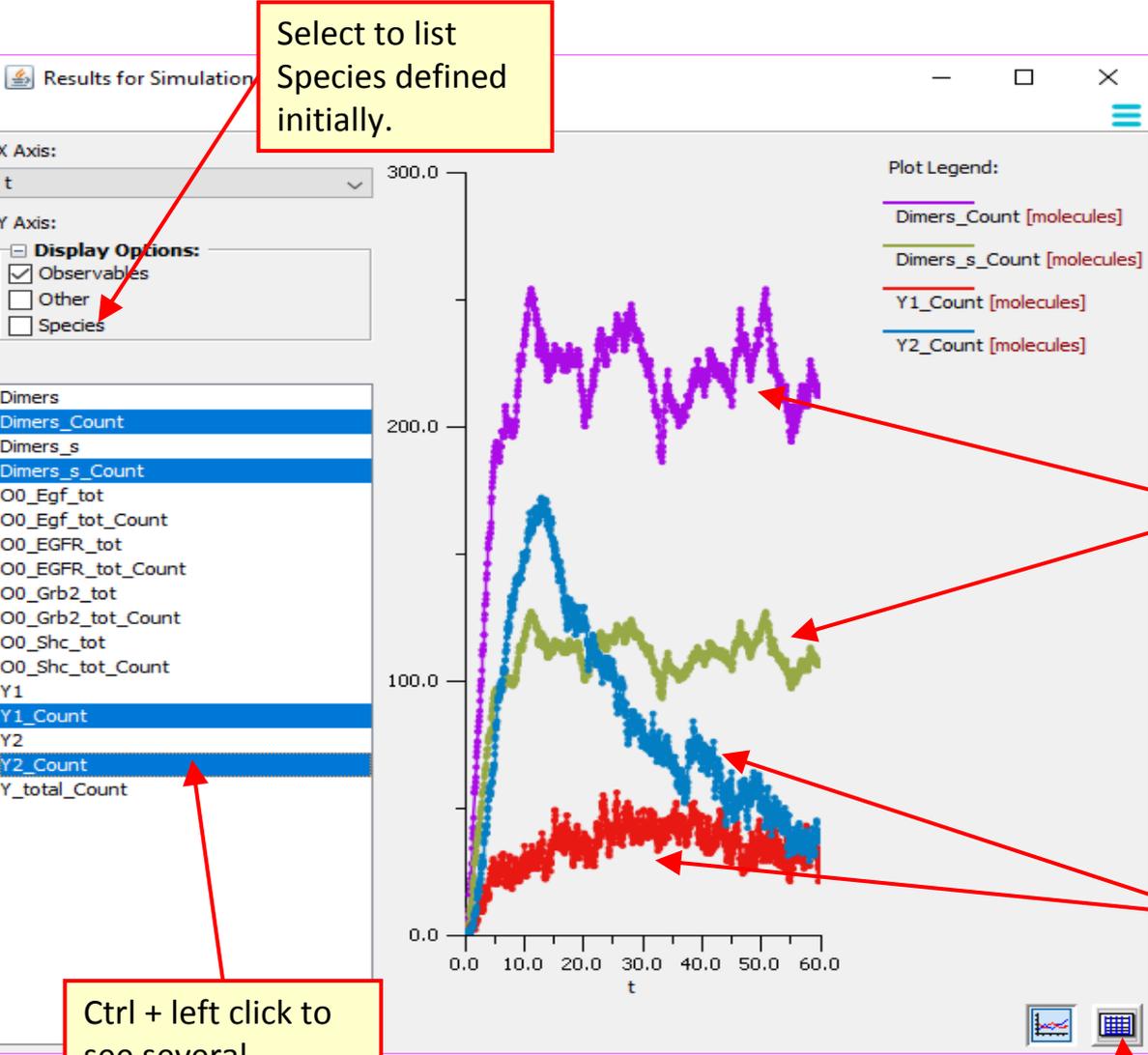
1. Set end time.

2. Click to run on a server (to store simulation results).

3. Click to run locally (on user's computer).

4. Select the **Edit Simulation** tool or advanced options like different solvers and outputs.

5. Click to learn more.



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

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