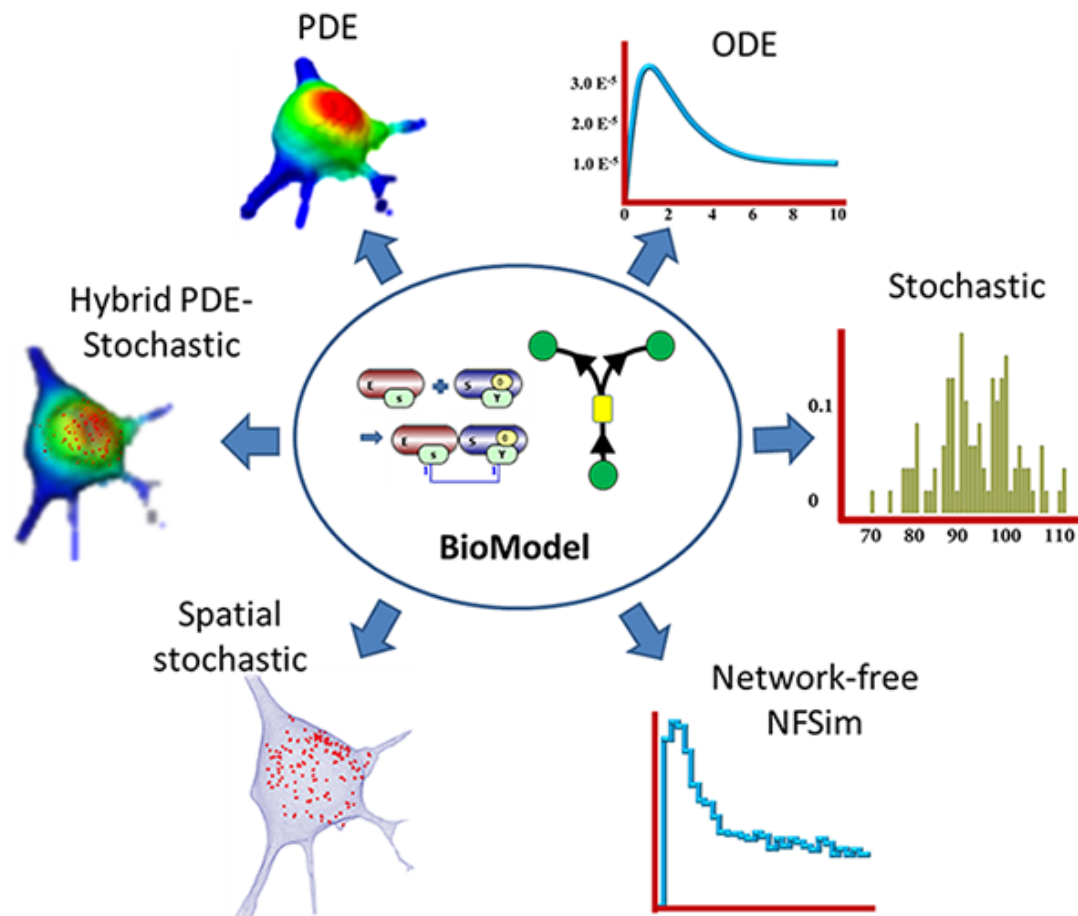


# VCell

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



VCell is developed  
at CCAM

Center for Cell Analysis & Modeling



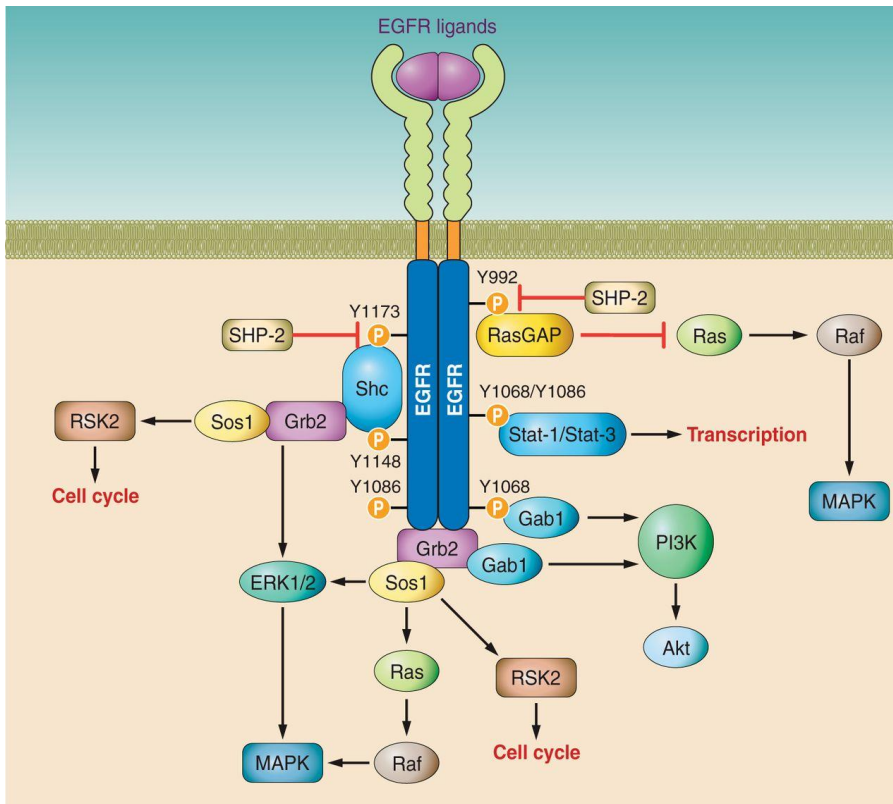
National Institute of  
General Medical Sciences

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

- [Opening VCell](#)
- [Physiology: Molecules](#)
- [Saving a VCell BioModel](#)
- [Physiology: Observables](#)
- [Physiology: Species](#)
- [Physiology: Reaction Rules](#)
- [Model Unit System](#)
- [Physiology: Reaction kinetics](#)
- [Physiology: Reactions](#)
- [Physiology: Visualization](#)
- [Application: Deterministic Network Generation](#)
- [Application: Stochastic](#)
- [Application: Network-Free](#)



# 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**.

The screenshot shows the VCell software interface. On the left is a 'BioModel1' tree view under 'Physiology', with 'Molecules (1)' selected. Below this is a 'Search' panel showing 'Biological Models' with subfolders like 'My BioModels (2018nathans751) (17)'. The main window has tabs for 'Reaction Diagram', 'Reactions', 'Structures', 'Species', 'Molecules', and 'Observables'. The 'Molecules' tab is active, showing a table with one molecule: 'MT0' with a depiction of a red circle and the definition 'MT0()'. Below the table are buttons for 'New Molecule', 'Delete', and 'Paste'. A 'New Molecule' button is highlighted with a red box. Below the buttons is an 'Object Properties' panel with 'Anchor Molecule' options: 'No restrictions' (selected) and 'Only these:' with a checkbox for 'c0'. A molecule shape 'MT0' is shown in the center. A right-click context menu is open over the 'MT0' shape, with options 'Rename' and 'Add Site'. The 'Rename' option is highlighted with a red box. Below the menu is an 'Annotation' panel. Red arrows point from numbered text boxes to these specific UI elements.

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.

File Server Window Tools Help

**BioModel1**

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


VCe ll DB BMD B Pathway Comm Sabio

BioModels MathModels Geometries

**Search**

- Biological Models
  - My BioModels (2018nathans751) (17)
  - Shared BioModels (0)
  - Public BioModels (601)
  - Tutorials (8)
  - Education (34)

Reaction Diagram Reactions Structures Species **Molecules** Observables

Name	Depiction	BioNetGen Definition
EGF		EGF(Site0)

New Molecule Delete Pathway Links Search

Object Properties Problems (0 Errors, 0 Warnings)

**Anchor Molecule**

☒ No restrictions

☐ Only these:

☐ c0

**Annotation**

EGF Site0

Move right

Move left

**Rename**

Delete

Add State

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



VCe1l DB BMDB Pathway Comm Sabio

BioModels MathModels Geometries

Search

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

Reaction Diagram Reactions Structures Species **Molecules** Observables

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

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).

New Molecule Delete Pathway Links

Object Properties Problems (0 Errors, 0 Warnings)

Anchor Molecule

☒ No restrictions

☐ Only these:

☐ c0

EGFR ecd tmd Y1 Y2

Annotation

Move right  
Move left  
Rename  
Delete  
Add State

**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.

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

VCeell DB

BMDB

Pathway Comm

Sabio

BioModels

MathModels

Geometries

Search

Biological Models

My BioModels (2018nathans751) (16)

Shared BioModels (0)

Public BioModels (601)

Tutorials (8)

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)

New Molecule

Delete

Pathway Links

Search

Object Properties

Problems (0 Errors, 0 Warnings)

Anchor Molecule

☒ No restrictions

☐ Only these:

☐ c0

EGFR

ecd

tmd

Y1

Y2

p

u

p

u

Rename

Delete

Annotation

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

VCell DB

BMDB

Pathway Comm

Sabio

BioModels

MathModels

Geometries

Search

Biological Models

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)

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)

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

New MoleculeDeletePathway Links▼Search

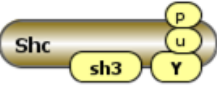
Object PropertiesProblems (0 Errors, 0 Warnings)Database File Info

Anchor Molecule

☒ No restrictions

☐ Only these:

☐ c0



Annotation

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

**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

Search

Biological Models

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)

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)

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.

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.

2. Annotations can be entered here.



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

File

Server Window Tools Help

New

Open

Close

Save

Save New Version

Save As...

Save As Local...

Revert to Saved

Compare with Saved

Permissions...

Model Annotation ...

Edit Annotation...

Field Data...

Import...

Export...

Exit

my biomodels (2016nattrans/51) (16)

Shared BioModels (0)

Public BioModels (601)

Tutorials (8)

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)

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.

When ready to save, click on **File** and **Save As....** If you work locally (no internet connection), choose **Save As Local....**



**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.

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

Search

Biological Models

My BioModels (2018nathans751) (16)

Shared BioModels (0)

Public BioModels (601)

Tutorials (8)

Education (34)

Reaction Diagram

Reactions

Structures

Species

Molecules

Observables

Name	Type	Electrical (Membrane Polarity)
Cell	Compartment	

New Compartment

New Membrane

Delete

Pathway Links

Search

Object Properties

Problems (0 Errors, 0 Warnings)

Database File Info

Select only one structure to edit properties

Structure Name

Cell

Size Variable Name

Cell [μm³]

Annotation

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

VCell DB

BMDB

Pathway Comm

Sabio

BioModels

MathModels

Geometries

Search

Biological Models

My BioModels (2018nathans751) (16)

Shared BioModels (0)

Public BioModels (601)

Tutorials (8)

Education (34)

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

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

BioModel1

Physiology

Reaction Diagram

Reactions (0)

Structures (1)

Species (0)

Molecules (4)

Observables (5)

Applications (0)

Parameters, Functions and Units

Pathway

VCe ll DB BMD B Pathway Comm Sabio

BioModels

MathModels

Geometries

Search

Biological Models

My BioModels (2018nathans751) (16)






Shared BioModels (0)

Public BioModels (601)

Tutorials (8)

Education (34)

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
O0	Cell			Molecules

New Observable Duplicate Delete Pathway Links Search

Object Properties Problems (0 Errors, 1 Warnings) Database

Add Pattern

Multimolecular

Polymer of

length = 2

length > 1

Delete Species Pattern

Add Molecule

Specify structure (for all)

EGF

EGFR

Grb2

Shc

Annotation

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

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

EGFR

Cell

ecd

tmd

Y1

Y2

Multimolecular

Polymer of

length = 2

length > 1

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

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

Pathway

VCell DB

BMDB

Pathway Comm

Sabio

BioModels

MathModels

Geometries

Search

Biological Models

My BioModels (2018nathans751) (16)

Shared BioModels (0)

Public BioModels (601)

Tutorials (8)

Education (34)

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.

File Server Window Tools Help

**BioModel1**

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


VCell DB BMDB Pathway Comm **Sabio**

BioModels MathModels Geometries

**Search**

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

Reaction Diagram Reactions Structures **Species** Molecules Observables

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”.


New Species Duplicate Delete Pathway Links Search

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

Species Name R

Linked Pathway Object(s)

Annotation

 Specify Molecule

- EGF
- EGFR**
- Grb2
- Shc

**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.

File Server Window Tools Help

BioModel1

Physiology

Reaction Diagram

Reactions (0)

Structures (1)

Species (1)

Molecules (4)

Observables (9)

Applications (0)

Parameters, Functions and Units

Pathway

VCell DB

BMDB

Pathway Comm

Sabio

BioModels

MathModels

Geometries

Search

Biological Models

My BioModels (2018nathans751) (16)

Shared BioModels (0)

Public BioModels (601)

Tutorials (8)

Education (34)

Reaction Diagram

Reactions

Structures

Species

Molecules

Observables

Name	Structure	Depiction	Link	BioNetGen Definition
<div><div>x</div><div>p</div></div> <div>(add new here)</div>	Cell			EGFR(ecd,tmd,Y1,Y2)

New Species

Duplicate

Delete

Pathway Links

Search

Object Properties

Problems (2 Errors, 0 Warnings)

Database File Info

Species Name

R

Linked Pathway Object(s)

Annotation

EGFR

Cell

ecd

tmd

Y1

Y2

?

?

~

State: u

~

State: p

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.

VCe ll DB

BMDB

Pathway Comm

Sabio

BioModels

MathModels

Geometries

Search

Biological Models

My BioModels (2018nathans751) (16)

Shared BioModels (0)

Public BioModels (601)

Tutorials (8)

Education (34)

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		->

VCell DB BMDB Pathway Comm Sabio

BioModels MathModels Geometries

Search

- Biological Models
- 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 Differe...

Delete

Specify Molecule

Specify structure

- EGF
- EGFR
- Grb2
- Shc

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.

**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>

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		@Cell:EGFR() ->

VCell DB BMDB Pathway Comm Sabio

BioModels MathModels Geometries

Search

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

New Reaction New Rule Duplicate Delete Pathway Links

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

ecd tmd Y1 Y2

->

Add Reactant

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**.

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.

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

VCe1l DB BMDB Pathway Comm Sabio

BioModels MathModels Geometries

Search

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

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()

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.

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 ecd tmd Y1 Y2 Cell

EGF Site Cell

EGFR ecd tmd Y1 Y2 Cell

Delete

Specify Molecule

Specify structure

EGF

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.

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		@Cell:EGFR(ecd,tmd)+@Cell:EGF(Site) -> @C

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.

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 Differe...

EGFR (ecd, tmd, Y1, Y2) + EGF (Site) -> EGFR (ecd, tmd, Y1, Y2) - EGF (Site)

**Site is unbound**

- Site has external bond
- Site may be bound
- Site bond specified

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





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

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


VCell DB BMDB Pathway Comm Sabio

BioModels MathModels Geometries

Search

- Biological Models
- My BioModels (2018nathans751) (16)
- Shared BioModels (0)
- Public BioModels (601)
- Tutorials (8)
- 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) -> @C

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

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

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

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 Differe... ☐

EGFR ecd tmd Y1 Y2 + EGF Site -> EGFR ecd tmd Y1 Y2 EGF Site

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

Kinetics

Editor

Reversible ☐

+

-

Add Reactant

Add Product

☐ Single Row Viewer

☐ Show Molecule Color

☐ Show Non-trivial

☐ Show Differe...

EGFR

Cell

ecd

tmd

Y1

Y2

+

EGF

Cell

Site

->

EGFR

Cell

ecd

tmd

Y1

Y2

EGF

Site

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

Kinetics

Editor

Reversible ☐

+

-

Add Reactant

Add Product

☒ Single Row Viewer

☐ Show Molecule Color

☐ Show Non-trivial

☐ Show Differe...

EGFR

Cell

ecd

tmd

Y1

Y2

+

EGF

Cell

Site

->

EGFR

Cell

ecd

tmd

Y1

Y2

EGF

Site



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

Kinetics Editor

Reversible ☐ + -

Add Reactant

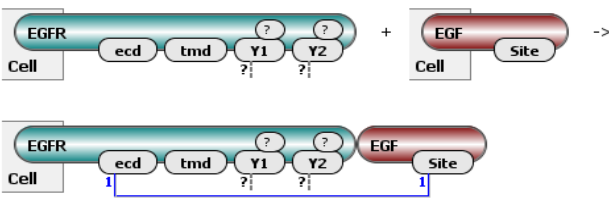
Add Product

☐ Single Row Viewer

☒ Show Molecule Color

☐ Show Non-trivial

☐ Show Differ...



Kinetics Editor

Reversible ☐ + -

Add Reactant

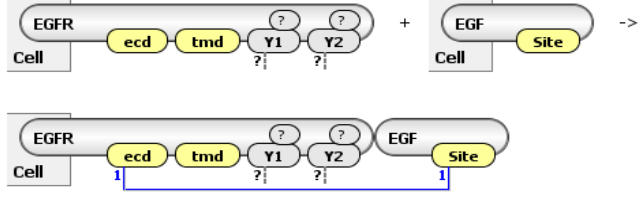
Add Product

☐ Single Row Viewer

☐ Show Molecule Color

☒ Show Non-trivial

☐ Show Differ...



Kinetics Editor

Reversible ☐ + -

Add Reactant

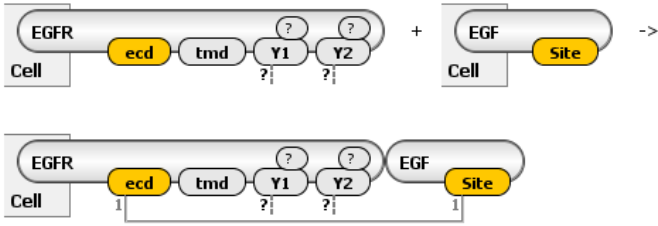
Add Product

☐ Single Row Viewer

☐ Show Molecule Color

☐ Show Non-trivial

☒ Show Differ...



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

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

VCell DB BMDB Pathway Comm Sabio

BioModels MathModels Geometries

Search

- Biological Models
  - My BioModels (2018nathans751) (16)
  - Shared BioModels (0)
  - Public BioModels (601)
  - Tutorials (8)
  - 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(ec

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.

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:  $K_f \cdot \prod \text{reactants} - K_r \cdot \prod \text{products}$  ) Convert units

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	$\text{s}^{-1}$

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.

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

VCell DB BMDB Pathway Comm Sabio

BioModels MathModels Geometries

Search

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

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

Model Unit System

Unit	Category
$\mu\text{m}$	primary
$\mu\text{m}^2$	primary
$\mu\text{m}^3$	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

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

select new unit system

☐ default

☐ sbml compatible

☒ **general**

type	unit	VCell default
length	<input type="text" value="um"/>	[um]
area	<input type="text" value="um2"/>	[um2]
volume	<input type="text" value="um3"/>	[um3]
time	<input type="text" value="s"/>	[s]
volume species substance	<input type="text" value="nM.um3"/>	[uM.um3]
membrane species substance	<input type="text" value="molecules"/>	[molecules]
lumped reaction substance	<input type="text" value="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)

Species (5)

Molecules (4)

Observables (9)

Applications (0)

Parameters, Functions and Units

Pathway

VCe

DB

BMDB

Pathway Comm

Sabio

BioModels

MathModels

Geometries

Search

Biological Models

My BioModels (2018nathans751) (16)

Shared BioModels (0)

Public BioModels (601)

Tutorials (8)

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(ec

New Reaction

New Rule

Duplicate

Delete

Pathway Links

Search

Object Properties

Problems (0 Errors, 0 Warnings)

Database File Info

Kinetics

Editor

Reaction Name

Reversible ☒ Kinetic Type

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 Differe...

EGFR<sub>1</sub> ecd tmd Y1 Y2 + EGFR<sub>2</sub> ecd tmd Y1 Y2 <->

EGFR<sub>1</sub> ecd tmd Y1 Y2 EGFR<sub>2</sub> ecd tmd Y1 Y2

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.

File Server Window Tools Help

BioModel1

- Physiology
  - Reaction Diagram
  - Reactions (3)
  - 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 Defir
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) -> @
Reaction Rule	ligand_bind	Cell		MassAction		@Cell:EGFR(ecd,tmd)+@Cell:EGF(Site) <-> @Cell: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).

VCell DB BMDB Pathway Comm Sabio

BioModels MathModels Geometries

Search

- Biological Models
  - 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 (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

Cell

ecd tmd Y1 Y2

EGFR

Cell

ecd tmd Y1 Y2

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!

File Server Window Tools Help

BioModel1

Physiology

- Reaction Diagram
- Reactions (4)
- Structures (1)
- Species (5)
- Molecules (4)
- Observables (9)

Applications (0)

Parameters, Functions and Units

Pathway

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)+@Cell:EGF(Site)

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

Cell

EGFR ecd tmd Y1 Y2

Cell

Single Row Viewer ☐

Show Molecule Color ☒

Show Non-trivial ☒

Show Differences ☐

1. Select a rule to duplicate and click on **Duplicate** button

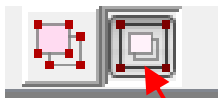
2. The Identical rule will appear with the name *oldname\_0*.

3. Rename the new rule and introduce any needed changes.

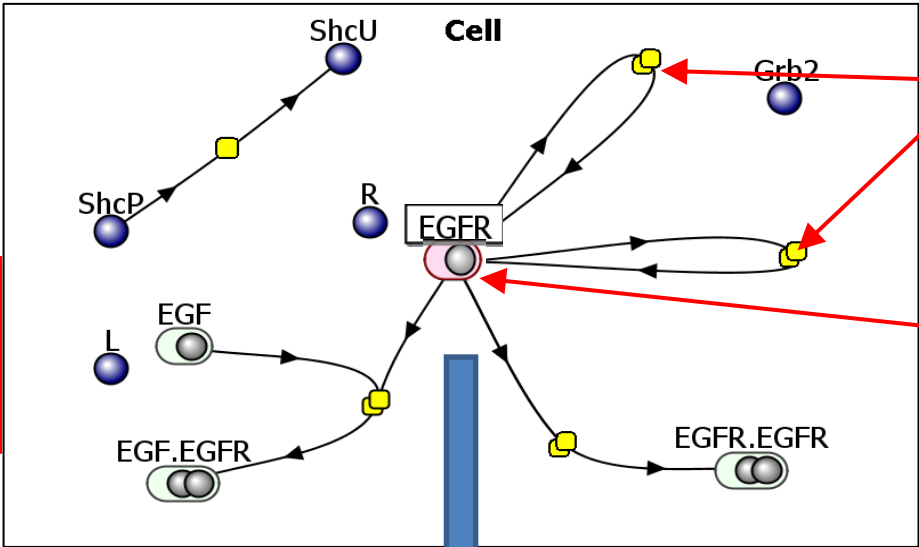
Note that the rule is irreversible.

Condition for the dephosphorylation: "Y1" site is phosphorylated and unbound.

Reaction rule outcome: "Y1" site becomes unphosphorylated.

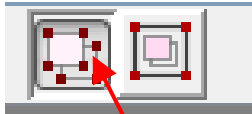


In the collapsed reaction diagram, the reaction rule participants are grouped by molecular structure.



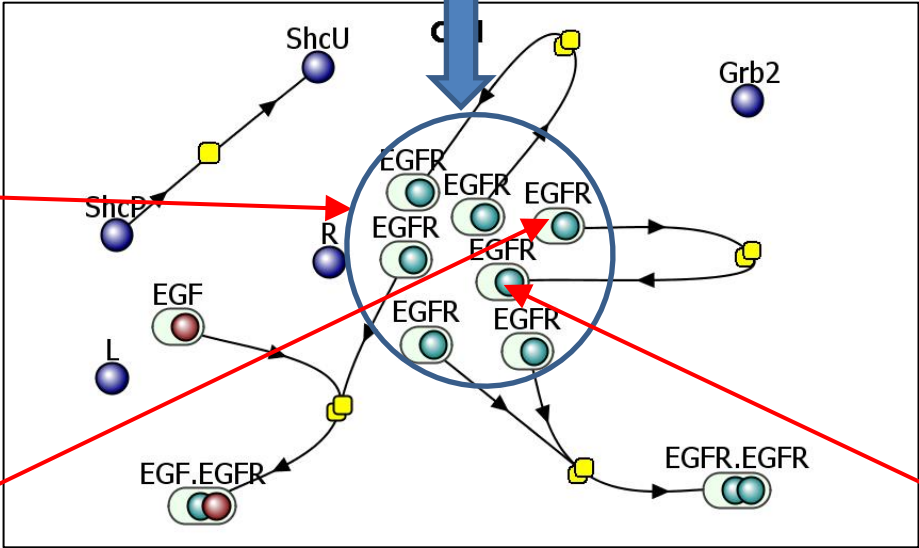
These are Reaction rules transforming EGFR molecule.

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

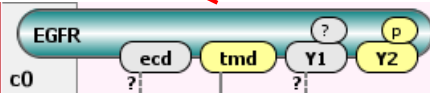
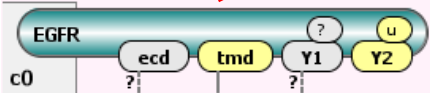


In the full reaction diagram, every reactant and product species pattern is shown individually if they are distinct in details.

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.

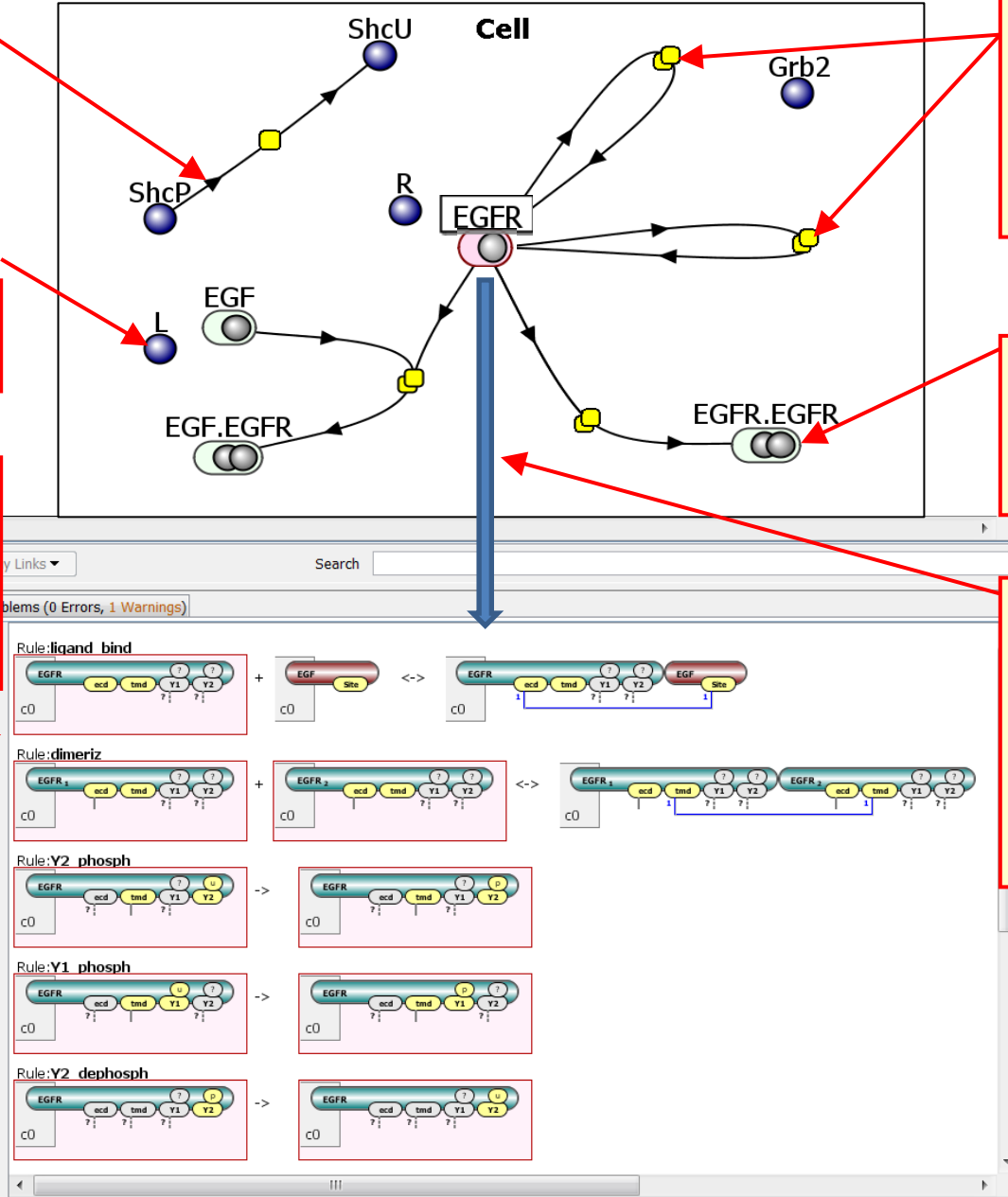
Species are depicted as blue spheres

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 molecular pattern is used.



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.

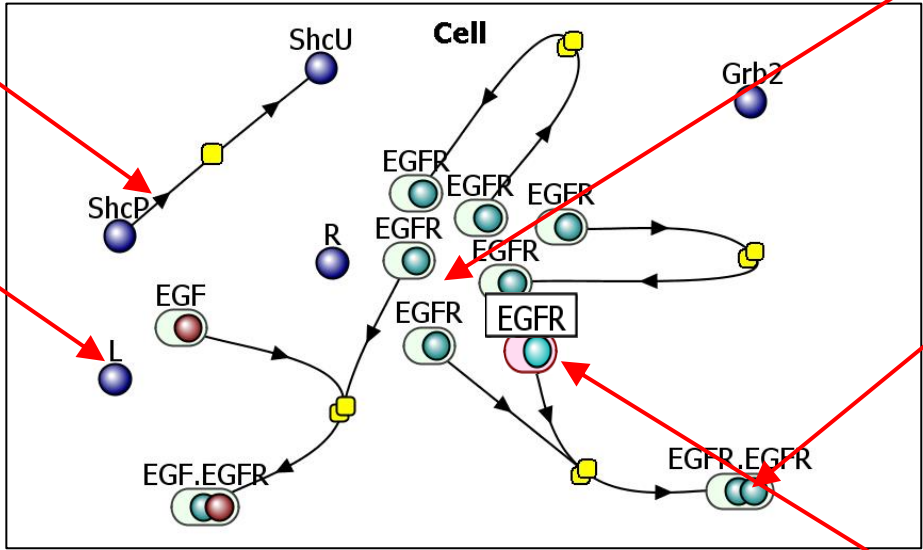
Colors within shapes correspond to molecular colors.

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

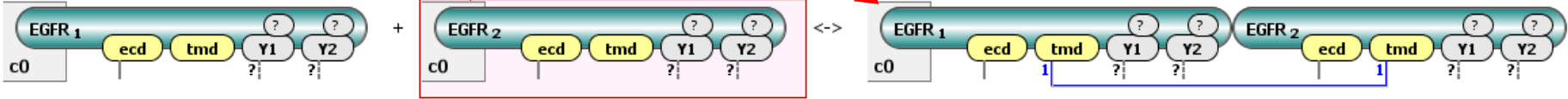
The black arrows indicate the direction of the reactions and reaction rules.

Species are depicted as blue spheres.

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



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 displays the VCell software interface. On the left, the 'BioModel1' tree shows 'Reaction Diagram' selected. The main workspace shows a reaction diagram with species 'ShcP' and 'ShcU' connected by a reaction 'r0'. The 'Object Properties' panel at the bottom shows the reaction 'r0' with its kinetic type set to 'Mass Action [μM/s]'. The table below lists the reaction parameters and species concentrations.

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.

File Server Window Tools Help

BioModel1

Physiology

Reaction Diagram

Reactions (11)

Structures (1)

Species (5)

Molecules (4)

Observables (9)

Applications (0)

Parameters, Functions and Units

Pathway

VCeL DB

BMDB

Pathway Comm

Sabio

BioModels

MathModels

Geometries

Search

Biological Models

My BioModels (2018nathans751) (2)

Shared BioModels (0)

Public BioModels (601)

Tutorials (10)

Education (34)

Reaction Diagram

Reactions

Structures

Species

Molecules

Observables

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

New Reaction

New Rule

Duplicate

Delete

Pathway Links

Search

Object Properties

Problems (0 Errors, 0 Warnings)

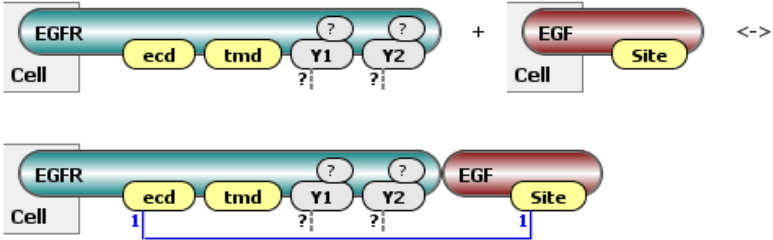
Database File Info

Show Warnings

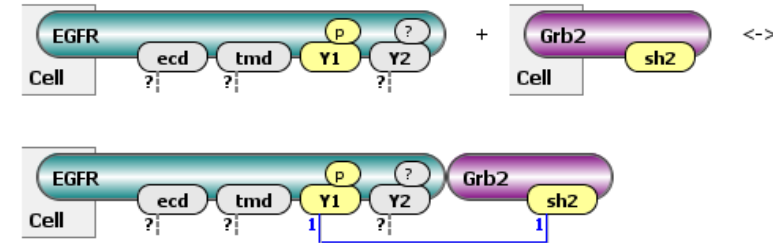
Refresh

Description	Url	Source	Defined In:
-------------	-----	--------	-------------

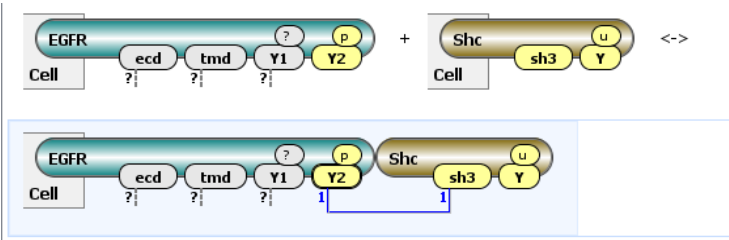
Complete reaction rule as in the following two slides, or in the *RB\_egfr\_tutorial* model in the VCell 6.1 (Rule-based) folder. Pay attention to reversibility of rules and kinetic rates.



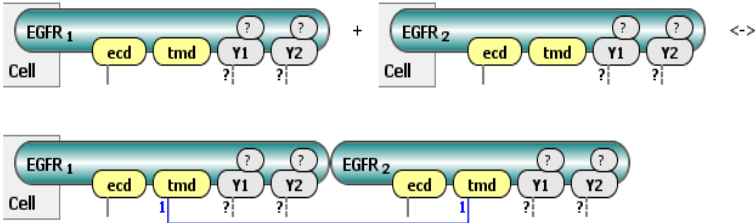
**Ligand\_Bind** (receptor must be in monomeric form (tmd is unbound) and not bound to ligand (ecd is unbound) for reaction to happen.)



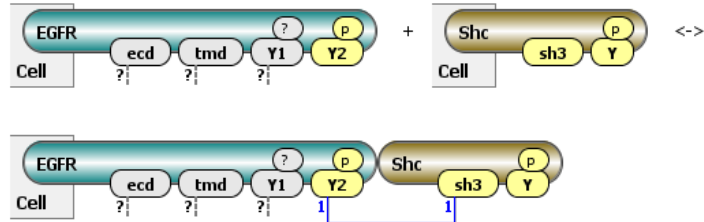
**R\_Grb2\_interaction** (EGFR does not have to be in monomeric form. Y1 has to be phosphorylated, for it to bind to sh2).



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

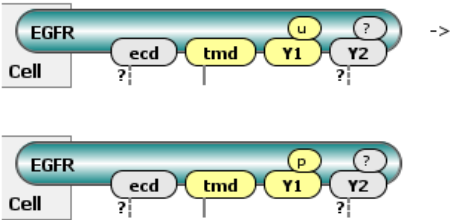


**Dimeriz** (tmd must be unbound and ecd has to be bonded externally for the two tmd sites to bond and form a dimer).

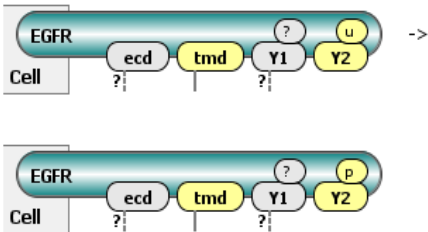


**R\_ShcP\_interaction** (for this reaction to occur, the Y site on Shc has to be unbound and phosphorylated. The unphosphorylated Y2 binds with sh3).

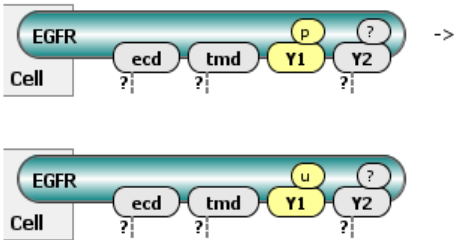
Reaction	Reversible?	Kf	Kr
ligand_bind	Yes	0.003 1/(nM s)	0.06 1/s
Dimeriz	Yes	0.001 1/(nM s)	0.1 1/s
R_Grb2_interaction	Yes	0.001 1/(nM s)	0.05 1/s
R_ShcP_interaction	Yes	4.5E-04 1/(nM s)	0.3 1/s
R_ShcU_interaction	Yes	0.045 1/(nM s)	0.6 1/s



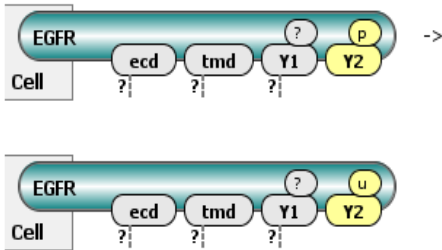
Y1\_Phosph (for phosphorylation to occur, tmd must be externally bound, implying a dimeric form).



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



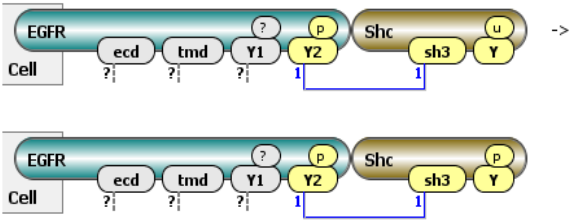
Y1\_Dephosph (the Y1 site changes states from phosphorylated to unphosphorylated).



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).



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).

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
ShcDephosph	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.

File Server Window Tools Help

BioModel1

Physiology

Reaction Diagram

Reactions (11)

Structures (1)

Species (5)

Molecules (4)

Observables (9)

Applications (0)

Parameters

Pathway

VCell DB

BMDB

Pathway Comm

Sabio

BioModels

MathModels

Geometries

Search

Biological Models

My BioModels (2018nathans751) (16)

Shared BioModels (0)

Public BioModels (601)

Tutorials (8)

Education (34)

Name	Math Type	Annotation
------	-----------	------------

New Application ▾

Expand All

Collapse All

Deterministic

Stochastic

Network-Free

New Application ▾

Delete

More Copy Actions ▾

Compare...

Search

Object Properties

Problems (0 Errors, 0 Warnings)

Database File Info

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.

File Server Window Tools Help

BioModel1

Physiology

Reaction Diagram

Reactions (11)

Structures (1)

Species (5)

Molecules (4)

Observables (9)

Applications (1)

Application0

Geometry

Specifications

Protocols

Simulations

Parameter Estimation

Parameters, Functions and Units

Pathway

VCeDB BMDb Pathway Comm Sabio

BioModels MathModels Geometries

Search

Biological Models

My BioModels (2018nathans751) (16)

Shared BioModels (0)

Public BioModels (601)

Tutorials (8)

Education (34)

Geometry Specifications Protocols Simulations Parameter Estimation

Species Reaction Network

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]

Search

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

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.

File Server Window Tools Help

BioModel1

Physiology

- Reaction Diagram
- Reactions (11)
- Structures (1)
- Species (5)
- Molecules (4)
- Observables (9)

Applications (1)

- Application0
  - Geometry
  - Specifications**
  - Protocols
  - Simulations
  - Parameter Estimation

Parameters, Functions and Units

VCell DB BMDB Pathway Comm Sabio

BioModels MathModels Geometries

Search

- Biological Models
  - My BioModels (2018nathans751) (2)
  - Shared BioModels (0)
  - Public BioModels (601)
  - Tutorials (10)
  - Education (34)

Geometry Specifications Protocols Simulations Parameter Estimation

Species Reaction Network

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"/>

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

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

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, including 'BioModel1', 'Physiology', 'Reactions (11)', 'Structures (1)', 'Species (5)', 'Molecules (4)', 'Observables (9)', 'Applications (1)', and 'Application0'. Under 'Application0', 'Geometry' is selected. The main window has tabs for 'Species', 'Reaction', and 'Network'. The 'Network' tab is active, showing a table of 'Network Constraints' with columns 'Name', 'Type', and 'Value'. The table contains two rows: 'Max Iterations' with value '3' and 'Max Molecules / Species' with value '10'. Below the table is a 'Generated Network' section. A dialog box titled 'Edit / Test Constraints' is open, showing input fields for 'Max. Iterations' (set to 3) and 'Max. Molecules / Species' (set to 11). The dialog has buttons for 'Test / Run', 'Apply', and 'Cancel'. Red arrows point from numbered text boxes to specific UI elements: 1. 'Left click on Network.' points to the 'Network' tab. 2. 'Left click on Edit/Test Constraints.' points to the 'Edit / Test Constraints' button in the main window. 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.' points to the input fields in the dialog box. 4. 'Left click on Test/Run.' points to the 'Test / Run' button in the dialog box.

File Server Window Tools Help

Geometry Specifications Protocols Simulations Parameter Estimation

Species Reaction Network

Network Constraints

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

Generated Network

Species: unavailable  
Reactions: unavailable  
Warning: none

Search

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

View Edit / Test Constraints  
View Create new VCell BioModel from Network

Max. Iterations 3  
Max. Molecules / Species 11

Test / Run Apply Cancel

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.

File Server Window Tools Help

BioModel1

Physiology

- Reaction Diagram
- Reactions (11)
- Structures (1)
- Species (5)
- Molecules (4)
- Observables (9)

Applications (1)

- Application0
  - Geometry
  - Specifications**
  - Protocols
  - Simulations
  - Parameter Estimation

Parameters, Functions and Units

Pathway

Geometry Specifications Protocols Simulations Parameter Estimation

Species Reaction **Network**

Network Constraints

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

Generated Network

Species: unavailable

Reactions: unavailable

Warning: none

Search

View Edit / Test Constraints

View Create new VCell BioModel from Network

Apply the new constraints?

Max. Iterations 3

Max. Molecules / Species 11

Warning: Max Iterations number may be insufficient.

Apply Cancel

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.

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.

File Server Window Tools Help

BioModel1

Physiology

Reaction Diagram

Reactions (11)

Structures (1)

Species (5)

Molecules (4)

Observables (9)

Applications (1)

Application0

Geometry

Specifications

Protocols

Simulations

Parameter Estimation

Parameters, Functions and Units

Pathway

VCell DB

BMDB

Pathway Comm

Sabio

BioModels

MathModels

Geometries

Search

Biological Models

My BioModels (2018nathans751) (16)

Shared BioModels (0)

Public BioModels (601)

Tutorials (8)

Education (34)

Geometry Specifications Protocols Simulations Parameter Estimation

Species Reaction Network

Network Constraints

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

Generated Network

Species: unavailable

Reactions: unavailable

Warning: none

View

Edit / Test Constraints

View

Create new VCell BioModel from Network

Apply the new constraints?

Max. Iterations 12

Max. Molecules / Species 12

Warning: none

Apply

Cancel

Object Properties

Problems (0 Errors, 0 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

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.

2. Click **Apply** to prepare network for simulation.

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.*

File Server Window Tools Help

BioModel1

Physiology

Reaction Diagram

Reactions (11)

Structures (1)

Species (5)

Molecules (4)

Observables (9)

Applications (1)

Application0

Geometry

Specifications

Protocols

Simulations

Parameter Estimation

Parameters, Functions and Units

Pathway

VCeell DB BMDB Pathway Comm Sabio

BioModels

MathModels

Geometries

Search

Biological Models

My BioModels (2018nathans751) (16)

Shared BioModels (0)

Public BioModels (601)

Tutorials (8)

Education (34)

Geometry Specifications Protocols Simulations Parameter Estimation

Species Reaction Network

Network Constraints

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

Generated Network

Species: 106

Reactions: 684

Warning: none

View

Edit / Test Constraints

View

Create new VCell BioModel from Network

Search

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

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.

1. Click to see all species in a separate pop-up window.

2. Click to see all reactions in a separate pop-up window.

3. See a reaction network in a separate window (may take a long time).



**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.



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.

File Server Window Tools Help

Rule\_Based\_Egrf

Physiology

Reaction Diagram

Reactions (11)

Structures (1)

Species (5)

Molecules (4)

Observables (9)

Applications (1)

Application0

Geometry

Specifications

Protocols

Simulations

Parameter Estimation

Parameters, Functions and Units

Pathway

VCeell DB BMDB Pathway Comm Sabio

BioModels MathModels Geometries

Search

Biological Models

My BioModels (2018nathans751) (17)

Shared BioModels (0)

Public BioModels (601)

Tutorials (8)

Education (34)

Geometry Specifications Protocols Simulations Parameter Estimation

Simulations Output Functions Generated Math

+

+

+

+

Name	End Time	Output Option	Solver	Running Status	Results
Simulation0	60.0	keep every 1 sample	Combined IDA/CVODE	not saved	no

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).

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

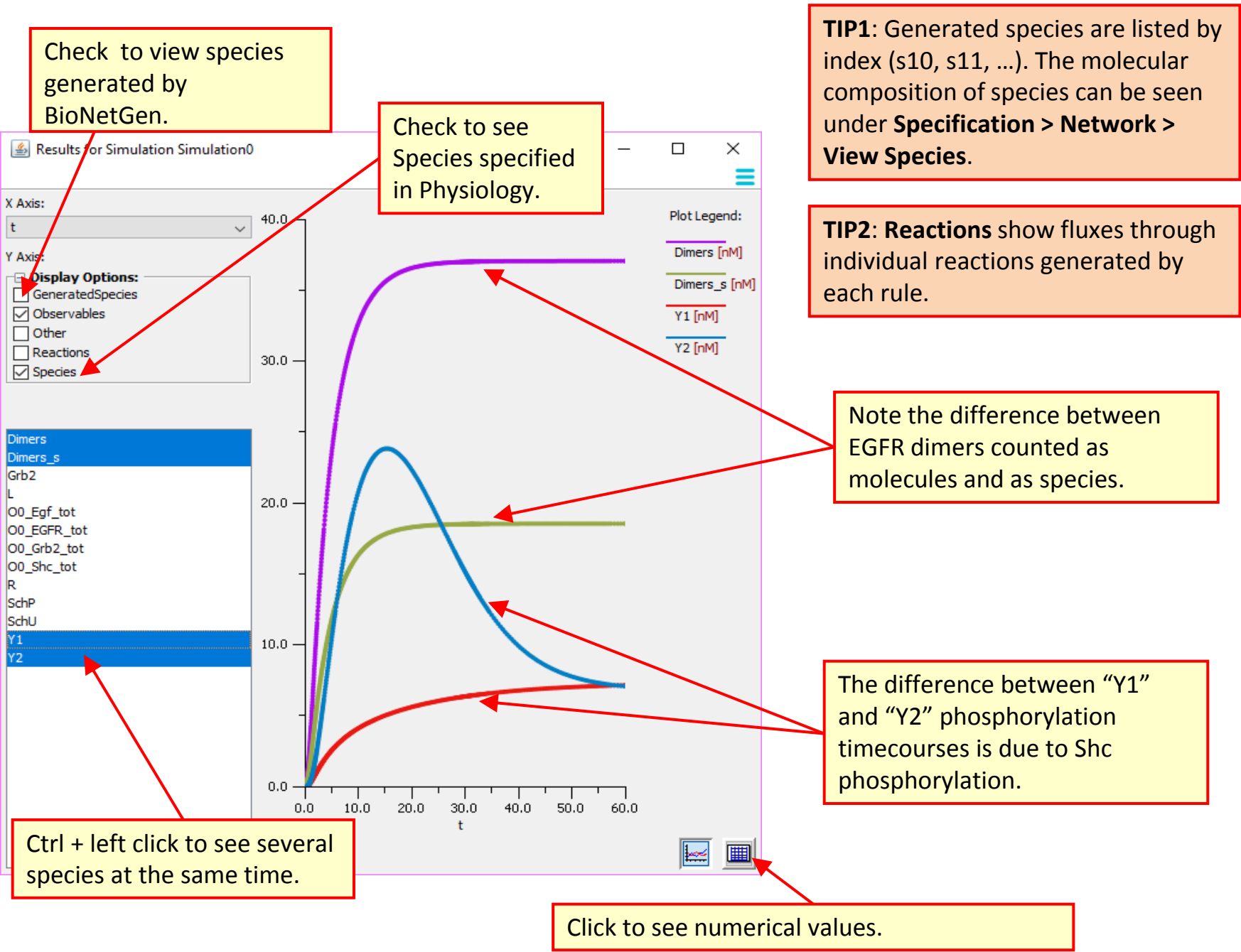
Annotation:

Settings:

Max timestep	Output	Rel tol	Abs tol	Sensitivity Analysis
1.0s	keep every 1 sample, at most 1000	1.0E-9	1.0E-9	no

Parameters with values changed from defaults

Parameter Name	Default	New Value/Expression	Scan
----------------	---------	----------------------	------



**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.

File Server Window Tools Help

**Rule\_Based\_Egrf**

- Physiology
  - Reaction Diagram
  - Reactions (11)
  - Structures (1)
  - Species (5)
  - Molecules (4)
  - Observables (9)
- Applications (1)
  - Application0
    - Geometry
    - Specifications
    - Protocols
    - Simulations
    - Parameter Estimation
- Parameters, Functions
- Pathway

VCCell DB BMDDB Pathway

BioModels MathModels Geometries

Search

- Biological Models
  - My BioModels (2018nathans751) (17)
  - Shared BioModels (0)
  - Public BioModels (601)
  - Tutorials (8)
  - Education (34)

Geometry Specifications Protocols Simulations Parameter Estimation

Species Reaction Network

Network Constraints

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

Copy As

- Deterministic
- Stochastic
- Network-Free

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

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.

Geometry Specifications Protocols Simulations

Structure Mapping Geometry Definition

Physiology (structures)

Cell

Cell

Geometry (subd...)

Compartment

☐ Volume/Surface Calculator

Structure	Size
Cell	10 [ $\mu\text{m}^3$ ]

Geometry Specifications Protocols Simulations

Species Reaction Network

Initial Condition: ☐ Concentration ☒ Number of Particles ☐ Randomize Initial Condition

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).

Check to see Species specified in Physiology.

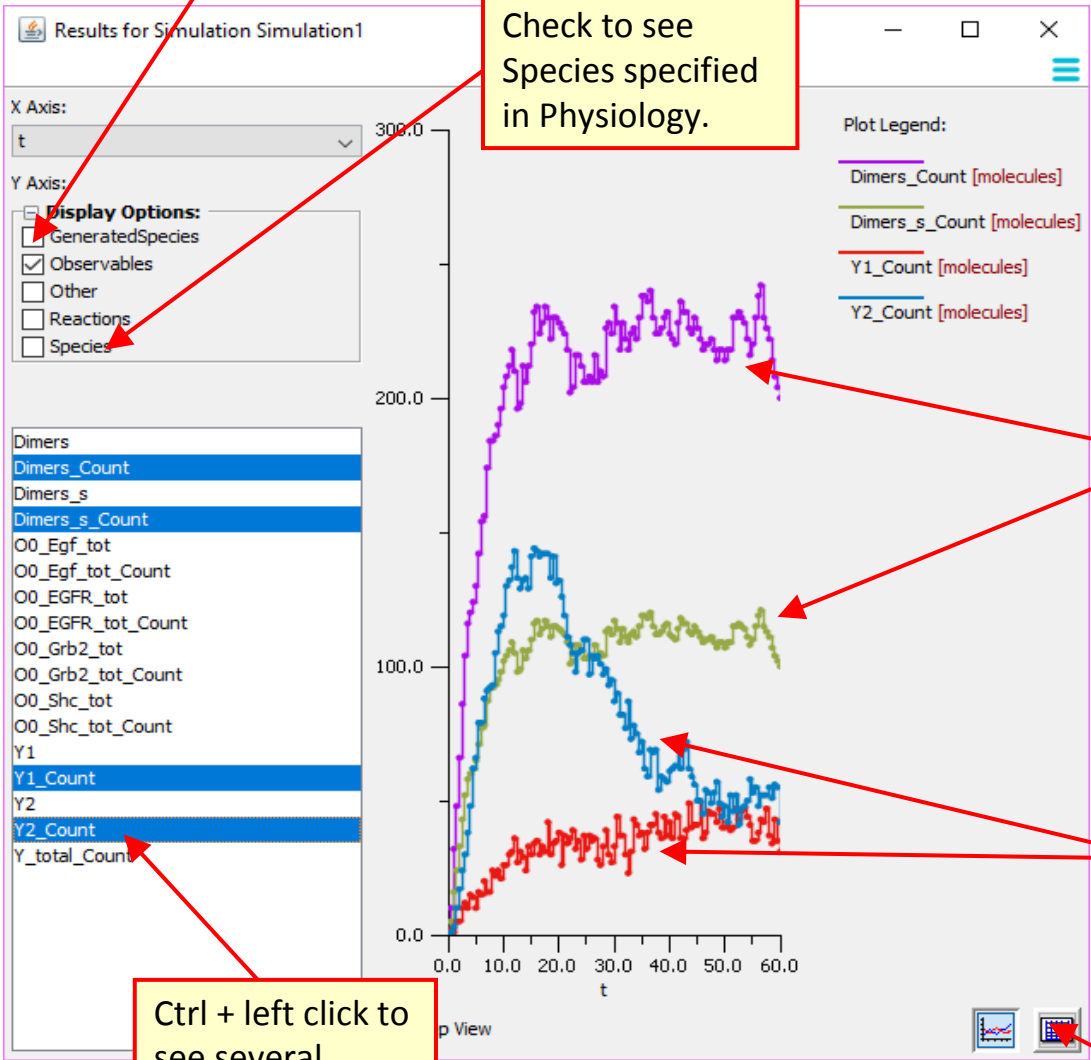
**TIP2:** Select **Other** to view show reaction rates (as `Kf_...`) and reaction firing events (as `P_...`) per second for each individual reaction generated by each rule.

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.

The screenshot shows the VCell software interface. On the left, a tree view under 'Rule\_Based\_Egrf' shows 'Applications (2)' with 'Application0' and 'Copy of Application0'. A right-click context menu is open over 'Copy of Application0', showing options: '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 a text box to the 'Copy As' option. At the bottom, a 'BioModels' search panel is visible, showing categories like 'Biological Models', 'My BioModels', 'Shared BioModels', 'Public BioModels', 'Tutorials', and 'Education'. The main workspace shows 'Structure Mapping' and 'Geometry Definition' tabs, with a 'Volume/Surface Calculator' button and a table with 'Size' and '10 [ μm³ ]'.

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.

The screenshot displays the VCell software interface. On the left is a project tree with folders like 'Rule\_Based\_Egrf', 'Physiology', and 'Applications'. In the center, the 'Edit Simulation1' dialog box is open, showing tabs for 'Parameters' and 'Solver'. The 'Parameters' tab is active, displaying 'Time Bounds' (Starting: 0.0, Ending: 60.0), 'Output Options' (Output Interval: 0.05 s), and 'Advanced Solver Options'. On the right, a 'Results' panel is visible with a 'no' status and a 'Scan' button. Five red callout boxes with arrows point to specific elements: 1. Points to the 'Ending' time field in the 'Time Bounds' section. 2. Points to the 'Run on server' button (a server icon) in the top right of the dialog. 3. Points to the 'Run locally' button (a green play icon) in the top right of the dialog. 4. Points to the 'Edit Simulation' button in the 'Simulations' list on the left. 5. Points to the '?' help icon in the 'Advanced Solver Options' section.

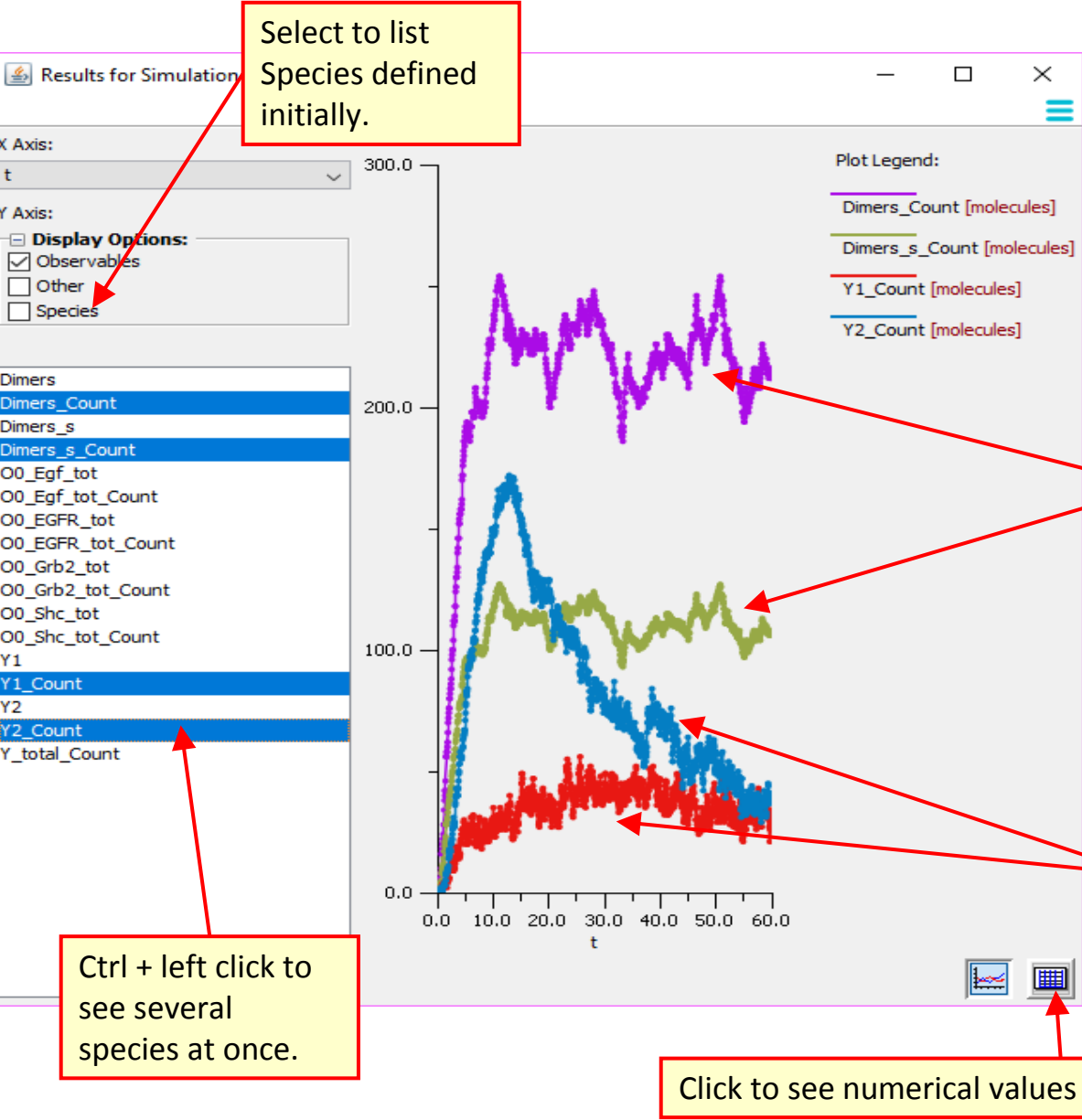
1. Set end time.

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.



**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.

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