

Using the Simmune Analyzer to explore the effect of molecular inhibitors on the behavior of a simple model of G-protein coupled signaling

This tutorial demonstrates how to use the Simmune Analyzer. The Model illustrates the usage of molecular inhibitors in a standard pathway (G protein coupled receptor). The model file is provided with the Simmune material:

'G_proteins_w_RK_and_Inhibitors.dbf'.

The underlying G protein model is discussed in detail in a tutorial in the Simmune documentation. This model extends the basic G protein model by adding three inhibitors that act by competing for binding site interactions with other (standard) model components: 'Gbg_inhib' competes with Gbetagamma for binding to the G protein recombination site on Galpha. Those Galpha molecules this inhibitor is bound to are not available for Gbetagamma units. Gbg_inhib thereby prolongs the duration of the unbound status of Gbetagamma. Thus, in terms of pathway activity, this inhibitor actually causes an extended signaling time. However, since heterotrimeric G-proteins (Galpha bound to Gbetagamma) are required for G protein activation this inhibitor reduces the response at the level of the G proteins.

'Rec_Inhib' competes with Galpha for association with the activated receptor. It therefore dampens the response of the pathway. 'RecKin_Inhib' binds to the Receptor kinase and prevents it from interacting with the receptor. It is, thus, protecting the pathway from the negative regulatory action of the receptor kinase.

We will vary the concentrations of these molecules over two orders of magnitude and explore how those variations affect the behavior of the signaling network with regard to 'output' observables, such as the time courses of free Gbetagamma.

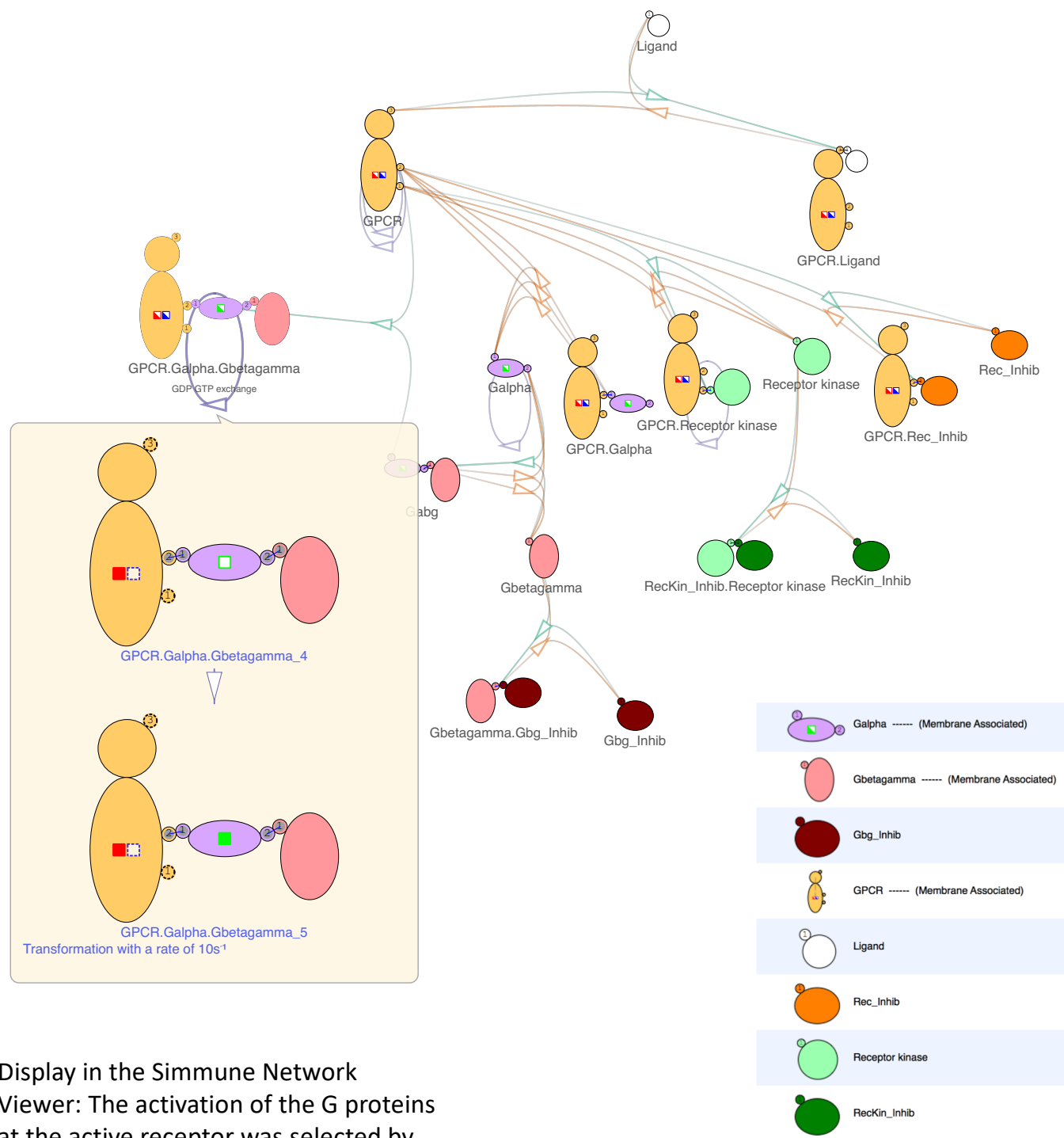
In the 'Documentation' folder of the Simmune distribution, there is a description of the Simmune Analyzer that addresses some of the more technical aspects of using the Analyzer.

If you encounter difficulties getting started with the Analyzer – in particular with regard to setting up the underlying MongoDB database – please also see the information provided in the release notes.

If questions persist or you want to send us comments you can contact us at:

simmune@niaid.nih.gov

The diagram below shows the signaling network with the receptor ligand ('Ligand') in the top level. It can be generated by loading the model into the Simmune Network Viewer. The Viewer will start with a default layout that can be edited to optimize the presentation of the displayed interactions. In the Simmune material, there is a pre-configured layout file that will reproduce exactly the layout shown here.



Display in the Simmune Network Viewer: The activation of the G proteins at the active receptor was selected by clicking on the arrow representing this reaction.

List of molecules for this model as seen in the Simmune Modeler

1. Start the Network Analyzer

Simmune Analyzer 2.3.1 -- Connected to @127.0.0.1

Model Initial State Protocol Rates Simulation **Status** Plot Simulation Groups Fit Global Sensitivity Data Export

Active Model

File Name: N/A

Name:

Note:

Save Annotation Delete Model

Available Models (click to activate)

Add New

Name	Model File	Note	Upload Time
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2. Load the model 'G_proteins_w_RK_and_Inhibitors' and activate it by clicking it (once).

Simmune Analyzer 2.3.1 -- Connected to @127.0.0.1 -- G_proteins_w_RK_and_Inhibitors.dbf

Model Initial State Protocol Rates Simulation **Status** Plot Simulation Groups Fit Global Sensitivity Data Export

Active Model

File Name: G_proteins_w_RK_and_Inhibitors.dbf

Name: G_proteins_w_RK_and_Inhibitors

Note:

Save Annotation Delete Model

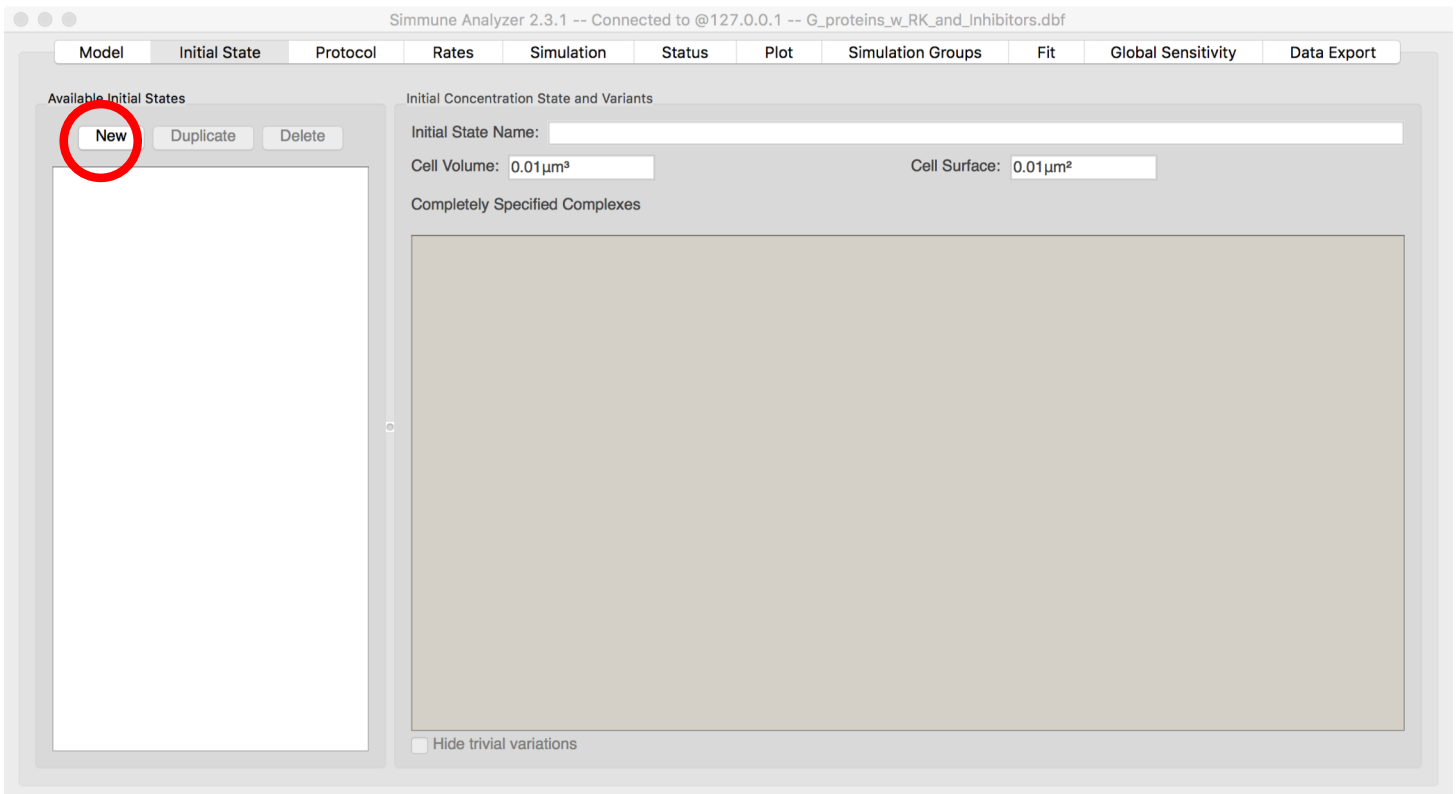
Available Models (click to activate)

1 Add New

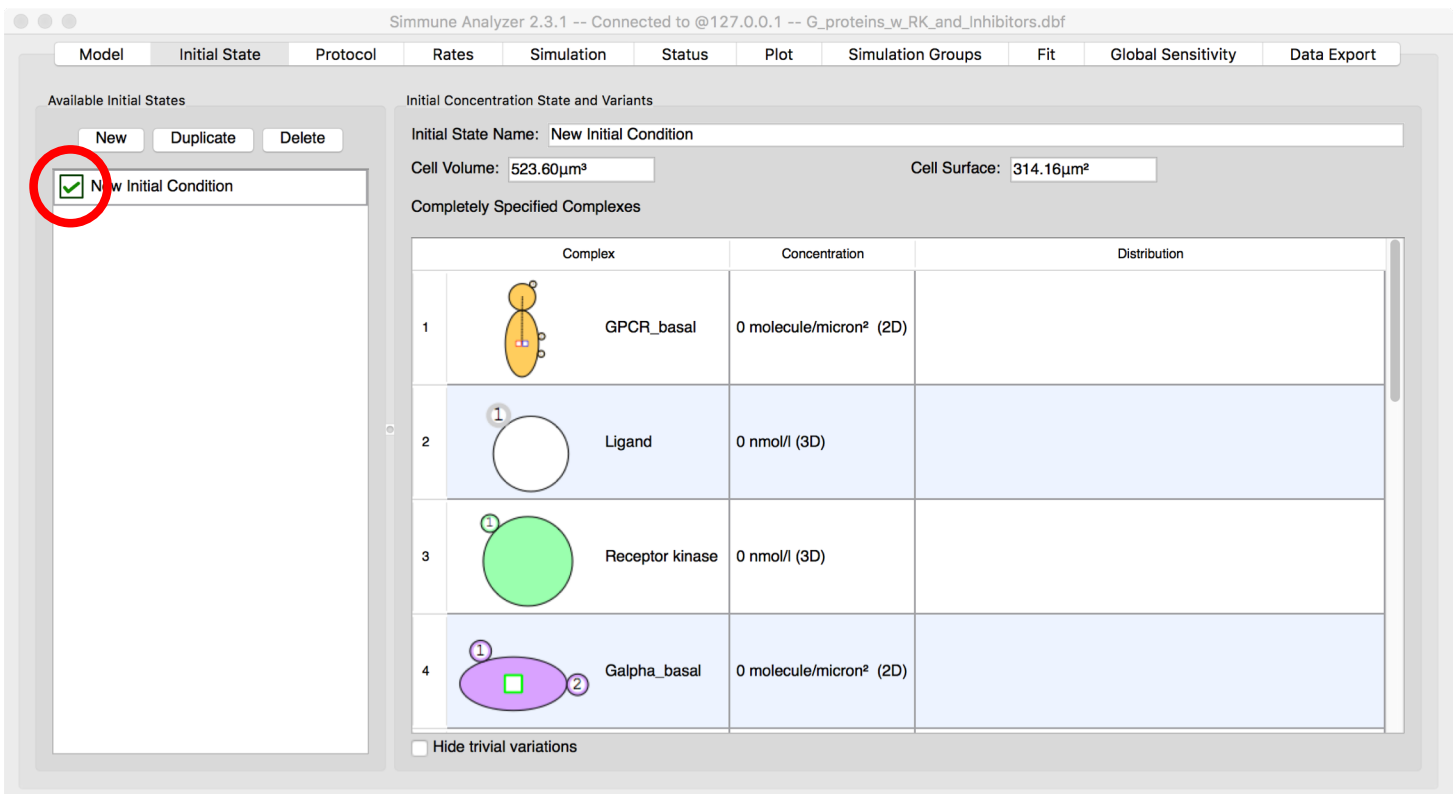
2

Name	Model File	Note	Upload Time
☒ G_proteins_w_RK_and_In...	G_proteins_w_RK_and_Inhibitors.dbf		3/30/18 5:33 PM

3. Go to the 'Initial State' tab.



4. Create a new initial state. Note the default cell volume and surface selection. Here, you can, for example, adjust for cells with a large nucleus, i.e. a small cytoplasm.



5. Set initial concentrations as shown below.

Simmune Analyzer 2.3.1 -- Connected to @127.0.0.1 -- G_proteins_w_RK_and_Inhibitors.dbf

Model Initial State Protocol Rates Simulation Status Plot Simulation Groups Fit Global Sensitivity Data Export

Available Initial States

New Duplicate Delete

☒ New Initial Condition

Initial Concentration State and Variants

Initial State Name: New Initial Condition

Cell Volume: 523.60 μm^3 Cell Surface: 314.16 μm^2

Completely Specified Complexes

	Complex	Concentration	Distribution
1	 GPCR_basal	50 molecule/micron ² (2D)	Delta distribution at 1
2	 Ligand	0 nmol/l (3D)	
3	 Receptor kinase	100 nmol/l (3D)	Delta distribution at 1
4	 Galpha_basal	0 molecule/micron ² (2D)	
5	 Gbetagamma	0 molecule/micron ² (2D)	
6	 Gabg_GDP_u...	0 molecule/micron ² (2D)	

☐ Hide trivial variations

Simmune Analyzer 2.3.1 -- Connected to @127.0.0.1 -- G_proteins_w_RK_and_Inhibitors.dbf

Model Initial State Protocol Rates Simulation Status Plot Simulation Groups Fit Global Sensitivity Data Export

Available Initial States

New Duplicate Delete

☒ New Initial Condition

Initial Concentration State and Variants

Initial State Name: New Initial Condition

Cell Volume: 523.60 μm^3 Cell Surface: 314.16 μm^2

Completely Specified Complexes

	Complex	Concentration	Distribution
3	 Receptor kinase	100 nmol/l (3D)	Delta distribution at 1
4	 Galpha_basal	0 molecule/micron ² (2D)	
5	 Gbetagamma	0 molecule/micron ² (2D)	
6	 Gabg_GDP_u...	100 molecule/micron ² (2D)	Delta distribution at 1
7	 Gabg_GTP_u...	0 molecule/micron ² (2D)	
8	 Rec_Inhib	0 nmol/l (3D)	

☐ Hide trivial variations

6. Initial concentrations continued...

Simmine Analyzer 2.3.1 -- Connected to @127.0.0.1 -- G_proteins_w_RK_and_inhibitors.dbf

Model Initial State Protocol Rates Simulation Status Plot Simulation Groups Fit Global Sensitivity Data Export

Available Initial States

New Duplicate Delete

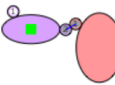
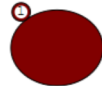
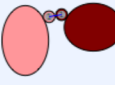
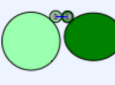
☒ New Initial Condition

Initial Concentration State and Variants

Initial State Name: New Initial Condition

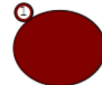
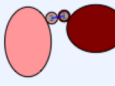
Cell Volume: 523.60 μm^3 Cell Surface: 314.16 μm^2

Completely Specified Complexes

	Complex	Concentration	Distribution
7	 Gabg_GTP_u...	0 molecule/micron ² (2D)	
8	 Rec_Inhib	100 nmol/l (3D)	Delta distribution at 1
9	 Gbg_Inhib	100 nmol/l (3D)	Delta distribution at 1
10	 Gbetagamma....	0 molecule/micron ² (2D)	
11	 RecKin_Inhib	100 nmol/l (3D)	Delta distribution at 1
12	 RecKin_Inhib....	0 nmol/l (3D)	

☐ Hide trivial variations

7. Now, we define the ranges for the variation of the ‘inhibitor’ concentrations as well as the distributions from which they will be drawn during the simulations. We vary the concentrations by applying factors between 0.1 and 10. To make sure the 0.1-1.0 range will be used just as frequently as the 1.0-10.0 range, we specify a Log uniform distribution.

8	 Rec_Inhib	100 nmol/l (3D)	Log Uniform: min=0.1 max=10
9	 Gbg_Inhib	100 nmol/l (3D)	Log Uniform: min=0.1 max=10
10	 Gbetagamma....	0 molecule/micron ² (2D)	
11	 RecKin_Inhib	100 nmol/l (3D)	Log Uniform: min=0.1 max=10

8. Change the name to ‘Initial Condition’ (not necessary but to be consistent with later sections of this tutorial).

The screenshot shows the 'Initial State' configuration window. On the left, under 'Available Initial States', there is a 'New' button, a 'Duplicate' button, and a 'Delete' button. Below these buttons is a list with one item: 'Initial Condition' with a green checkmark. On the right, under 'Initial Concentration State and Variables', the 'Initial State Name' is set to 'Initial Condition'. The 'Cell Volume' is set to '523.60 μm³'. There is a checkbox for 'Completely Specified Complexes' which is currently unchecked.

9. Go to the ‘Protocol’ tab, click ‘New’ and rename the protocol to ‘Protocol’.

The screenshot shows the 'Protocol' configuration window. The top navigation bar has tabs: Model, Initial State, Protocol (selected), Rates, Simulation, Status, Plot, Simulation Groups, Fit, Global Sensitivity, and Data Export. On the left, under 'Available Protocols', there is a 'New' button, a 'Duplicate' button, and a 'Delete' button. Below these buttons is a list with two items: 'Protocol' and 'Protocol'. On the right, under 'Protocol', the 'Name' is set to 'Protocol'. The 'Duration' is set to '100.00 s'. Below this, there is a table for 'Biochemical Stimuli' with columns: Time, Complex, and Concentration.

10. Click ‘Add Step’ and add a Ligand stimulus at 50 seconds. Set the total simulated (biological) time to 200 seconds.

The screenshot shows the 'Protocol' configuration window. The 'Name' is 'Protocol' and the 'Duration' is '200.00 s'. The 'Biochemical Stimuli' table now has two rows:

	Time	Complex	Concentration
	50 s	☐ Ligand	100 nmol/l

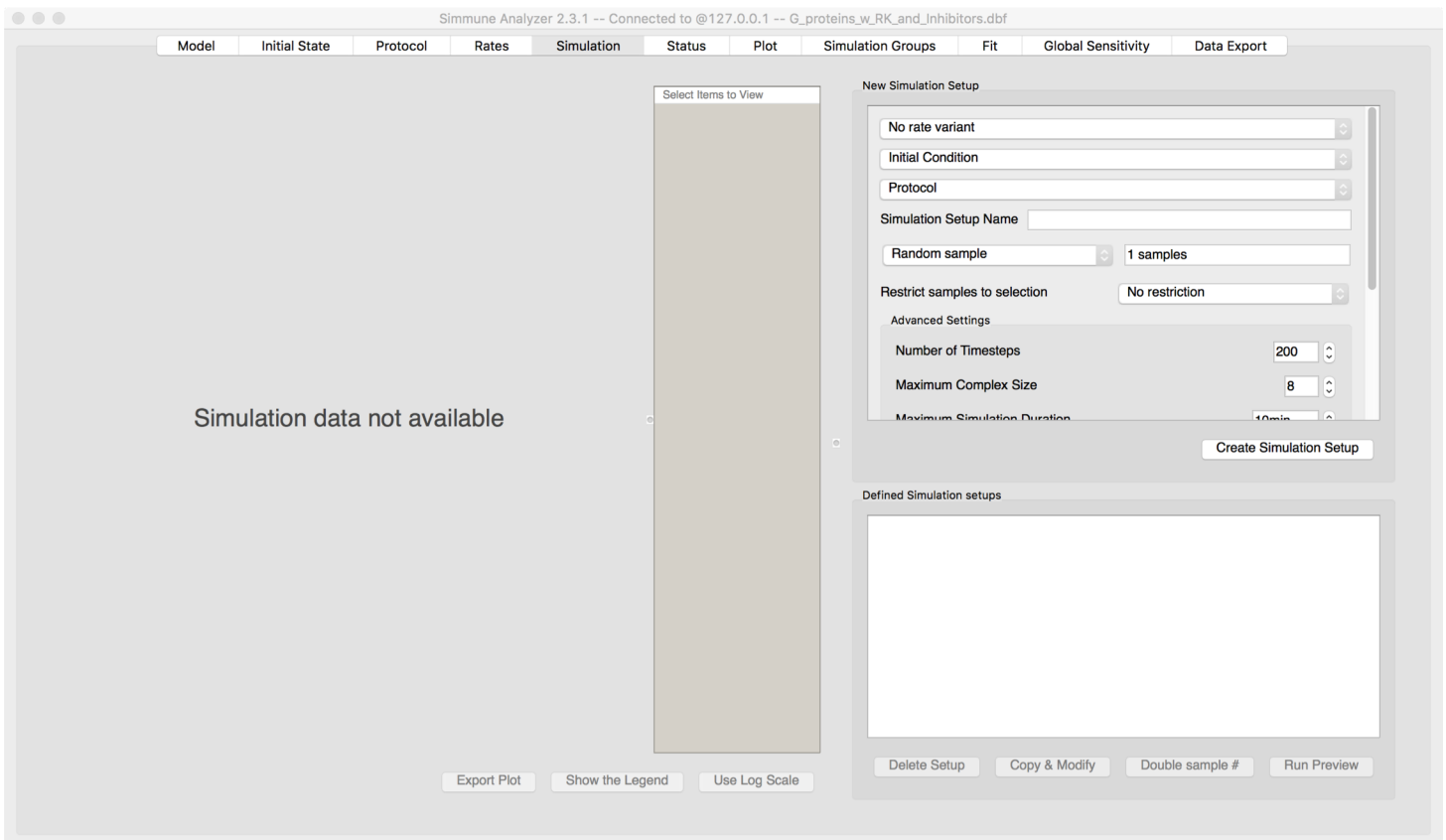
11. Click ‘Add Step’ and set the Ligand concentration to 0 at 120 seconds to wash out the Ligand.

The screenshot shows the 'Biochemical Stimuli' table with two rows:

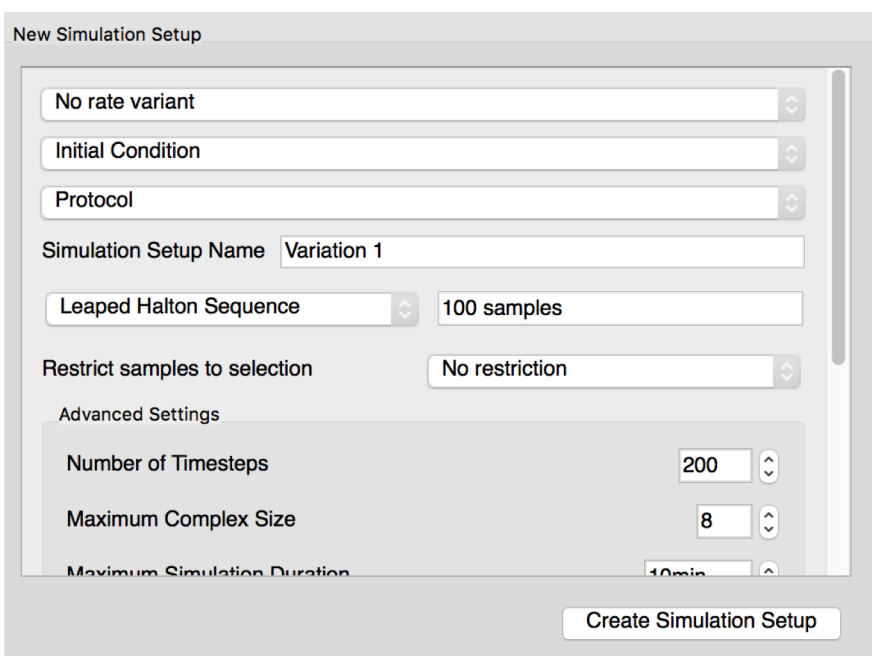
	Time	Complex	Concentration
	50 s	☐ Ligand	100 nmol/l
	120 s	☐ Ligand	0 nmol/l

12. We will skip the specification of variations of parameter values. (In the ‘Rates’ tab.) This could be used to explore how a model behaves when (potentially unknown) parameters such as associations etc. are being varied.

13. Go to the 'Simulations' tab.



14. Select the 'Initial Condition' and the 'Protocol' and set 100 samples and a 'Leaped Halton' Sequence for the parameter variation strategy. Choosing a Halton sequence instead of a random distribution has will allow you to add simulations to existing runs (by clicking 'double sampling #').



15. Click 'Create Simulation Setup'. This will start the simulations.

16. In the 'Status' tab you should see the simulation processes communicating with the database that collects the results.

Database Status

Name	Storage size
Simmune	3.08 GiB

Objects	Data size
72736	2.70 GiB

Simulations

Setup	Pending	Running	Failed	Finished	med o
1 Variation 1	68	3	0	30	0

Note that there is one more process listed than specified (in our case: 101 instead of 100). The last process will be started at the end to analyze the results and prepare them for inspection.

Detailed status for G_proteins_w_RK_and_Inhibitors.dbf :

Setup	Pending	Running	Failed	Finished	med o
1 Variation 1	0	0	0	101	0

17. Go to the 'Plot' tab and click 'New Plot'. Select 'Single simulation setup'.

Select the type of data you want to plot

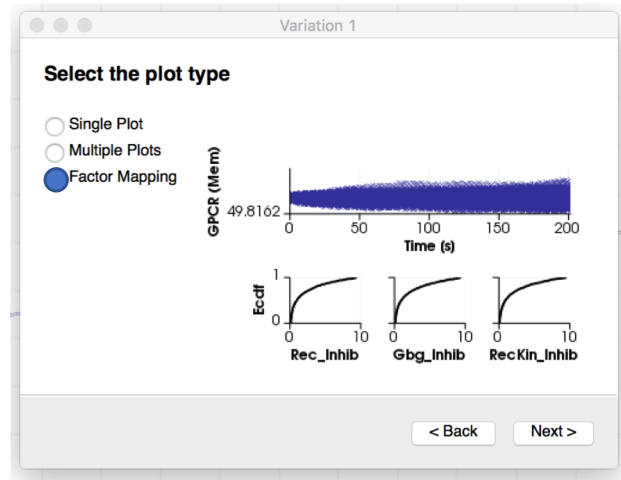
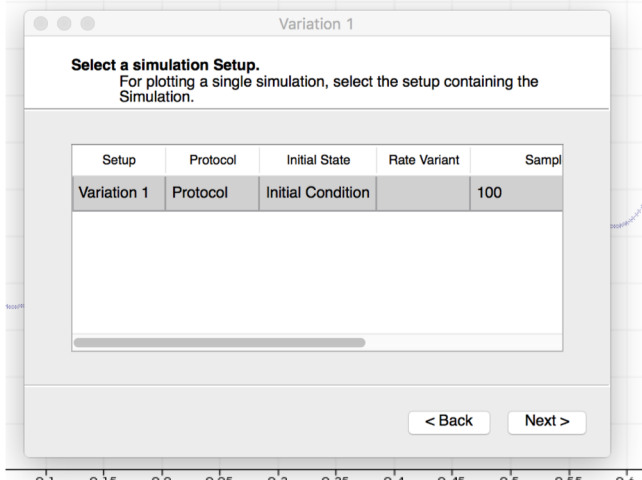
☒ Single simulation setup
Generate a plot from a single simulation setup.

☐ Groups of simulation setups
Use several simulation setups to create a plot. This can be used for plotting dose-response curves.

< Back Next >

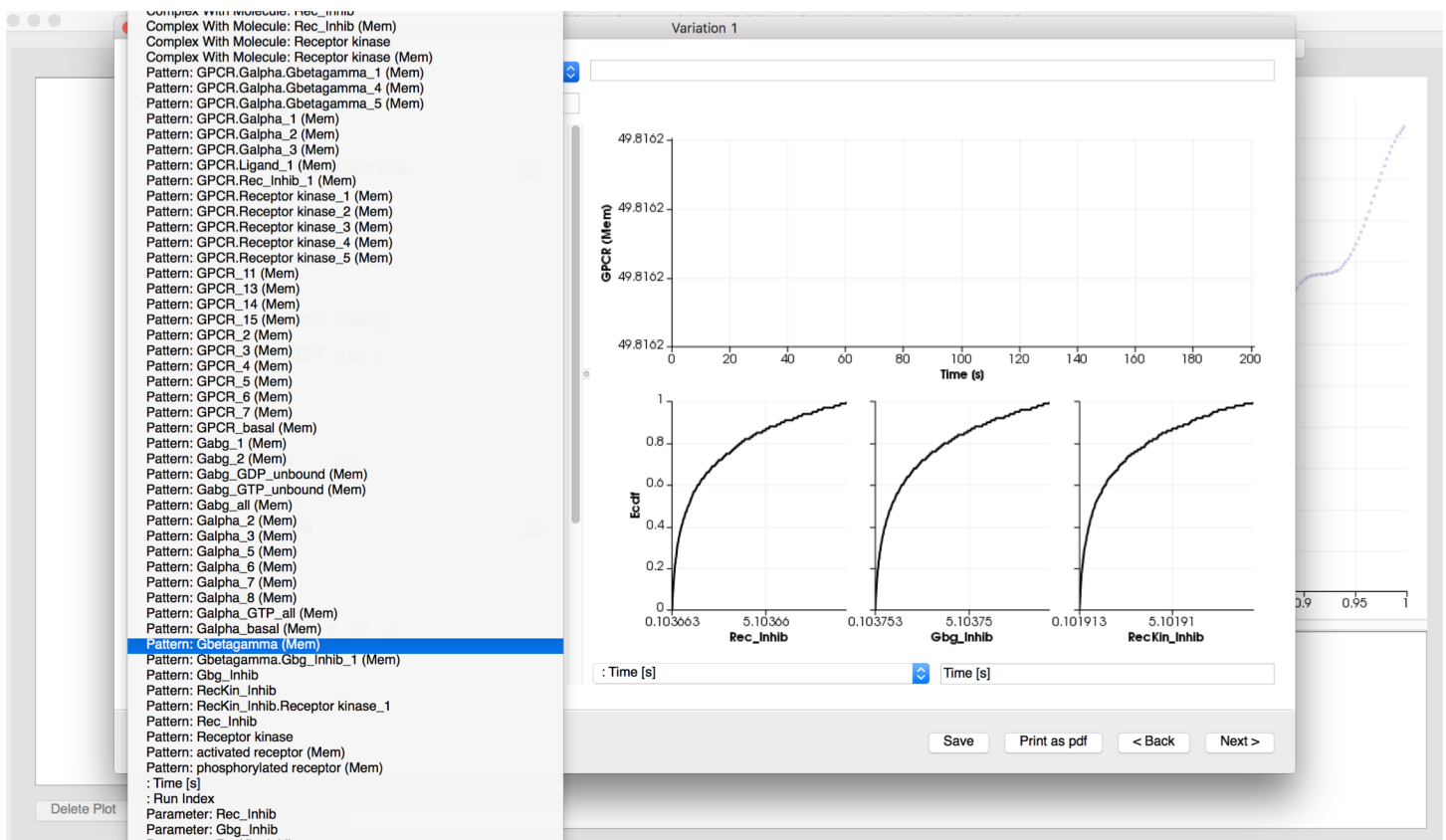
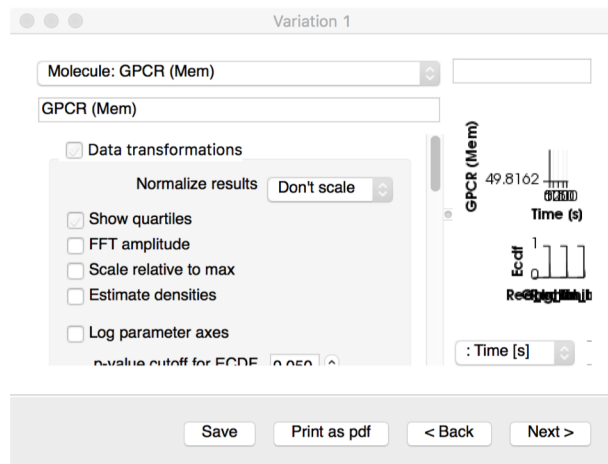
Delete Plot Print as pdf **New Plot**

18. Select the simulations we just performed and then 'Factor Mapping'.

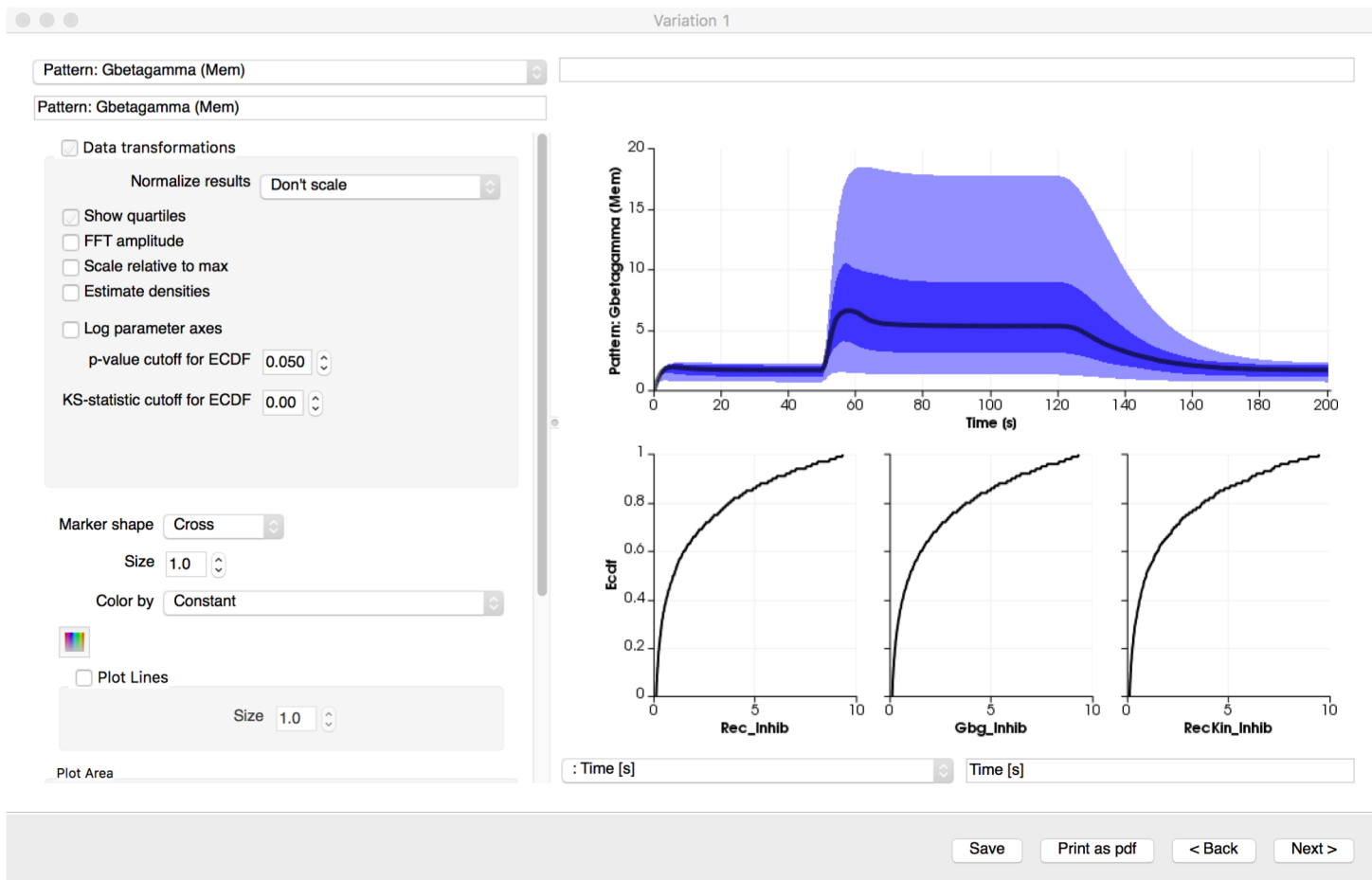


19. Resize the window to fill more of your screen.

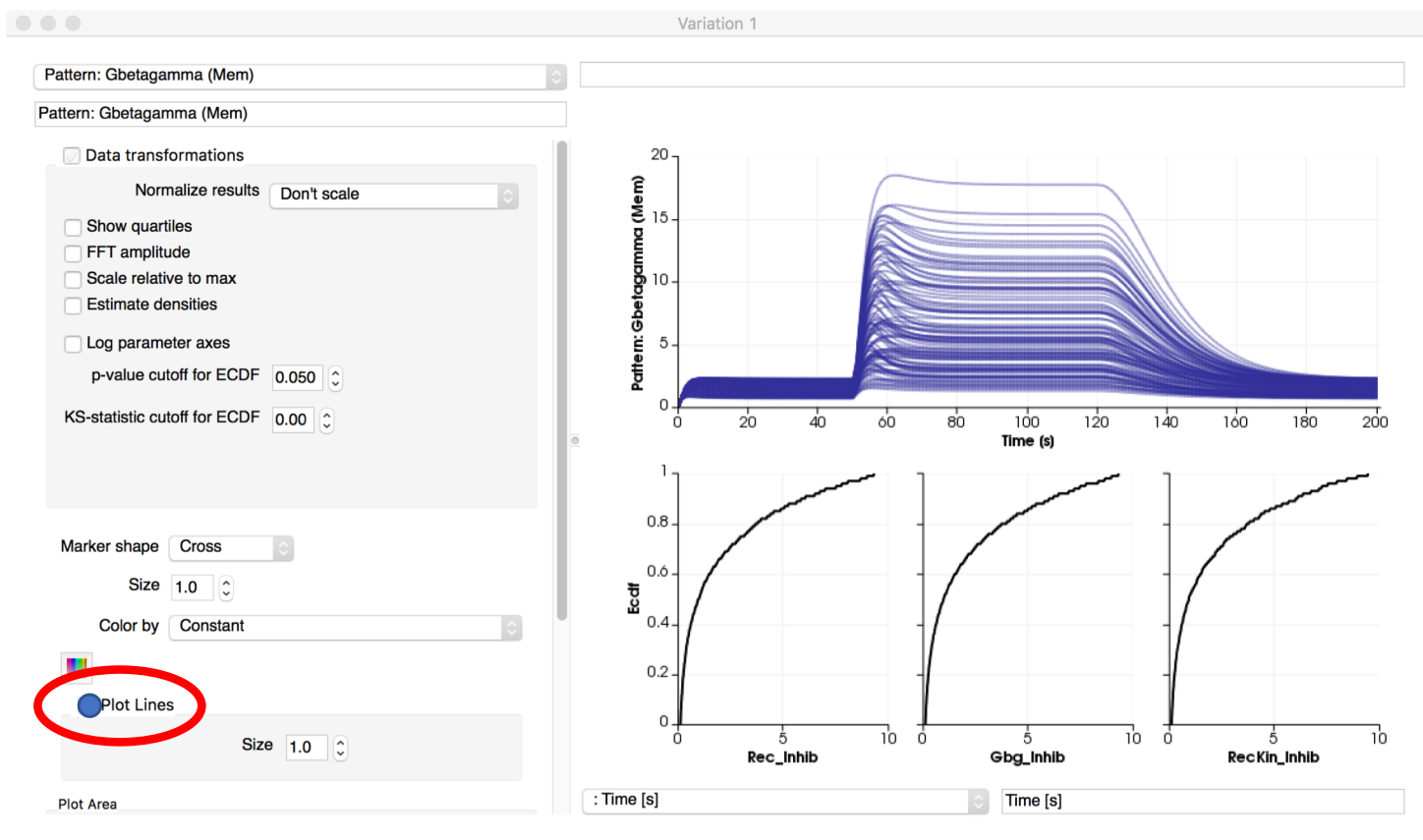
Select the pattern
Gbetagamma (Mem)
from the list of observables.



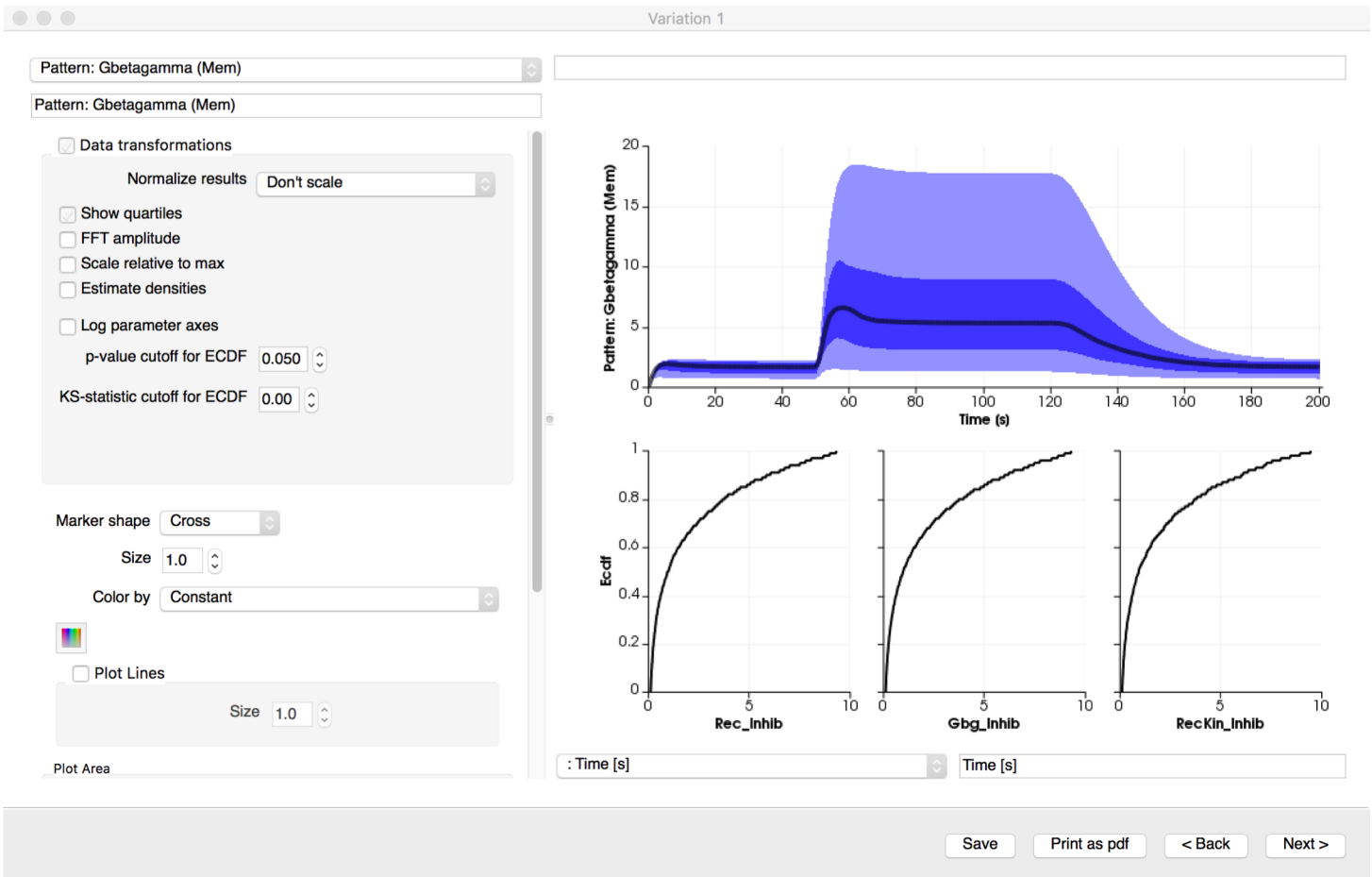
20. Per default, the quartiles of the simulated timecourses for the selected observable are shown:



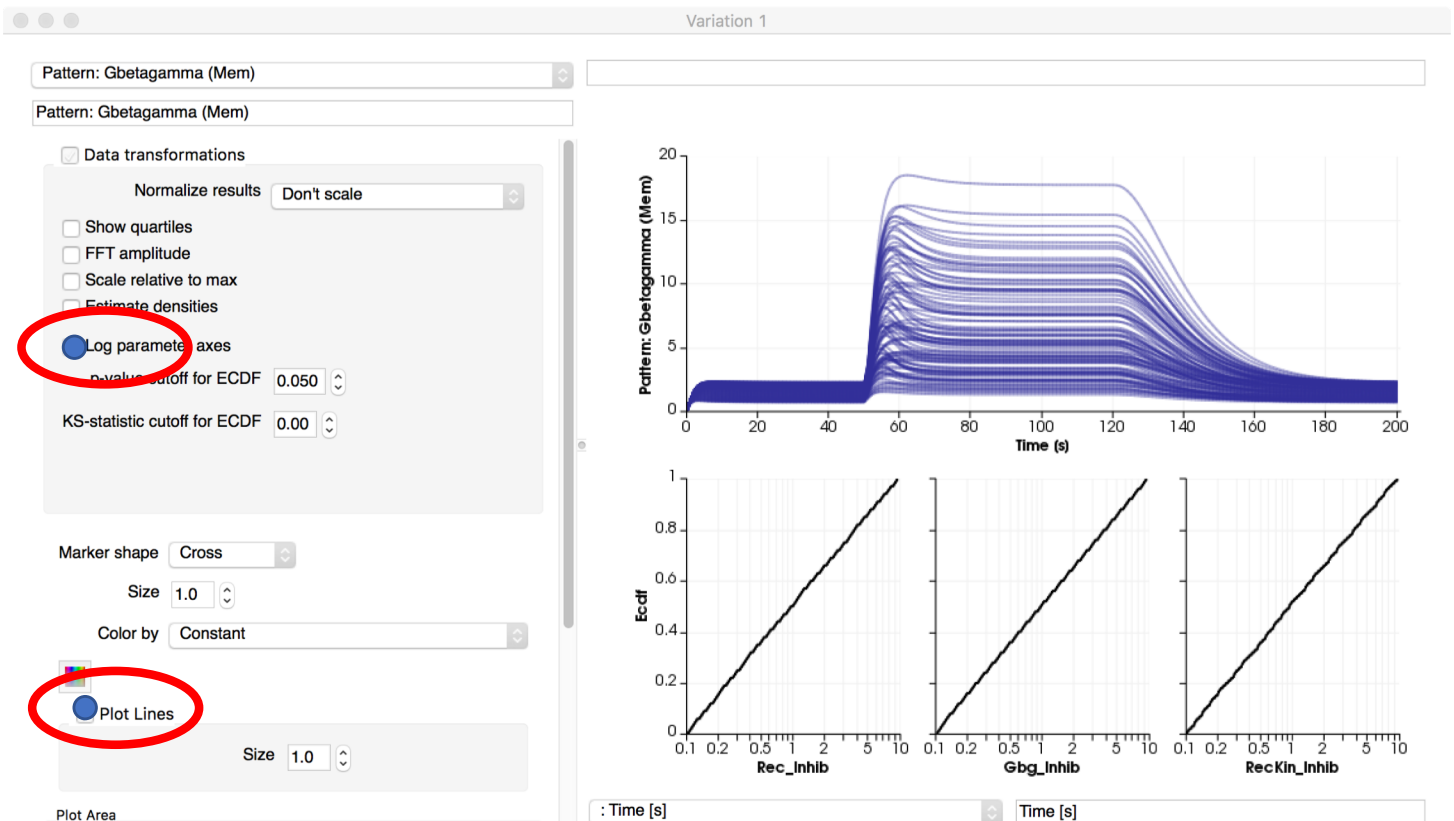
21. Switch the quartiles off and select 'Plot Lines' to see individual results.



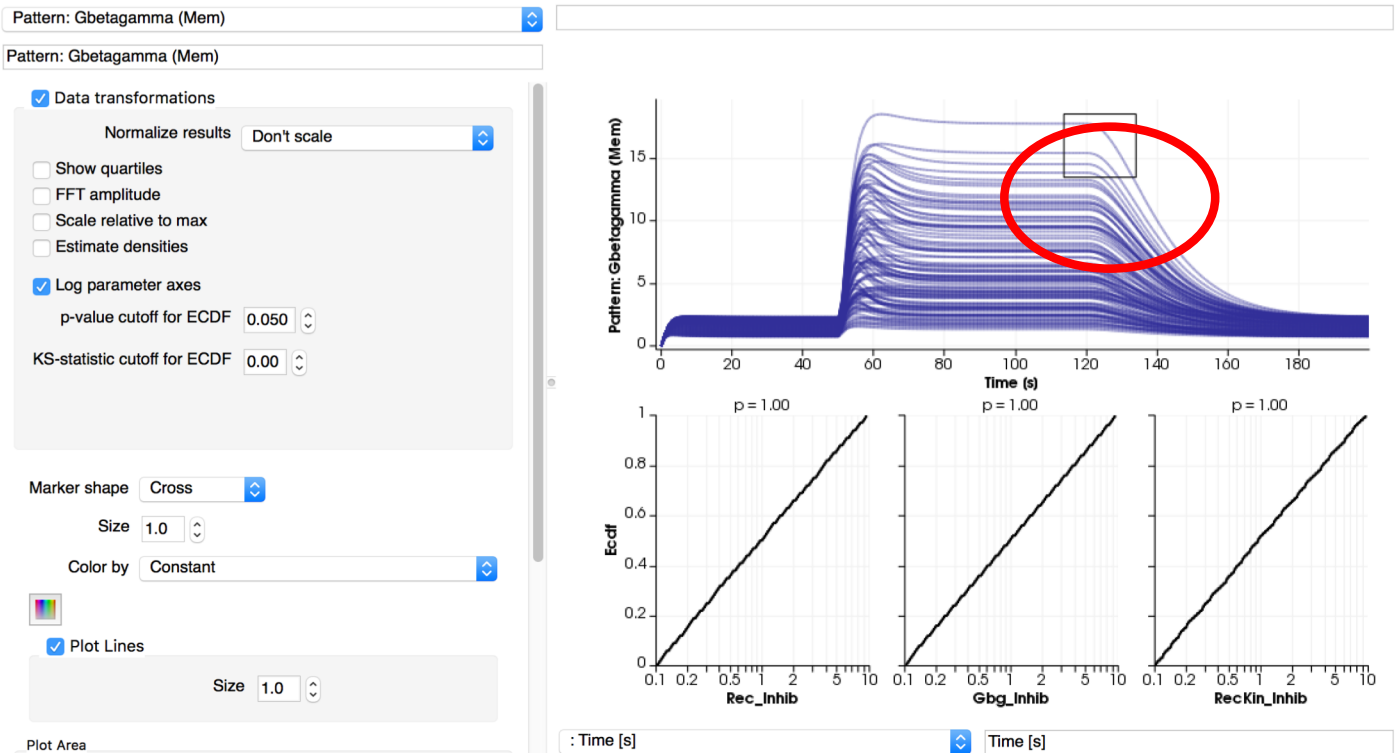
20. Per default, the quartiles of the simulated timecourses for the selected observable are shown:



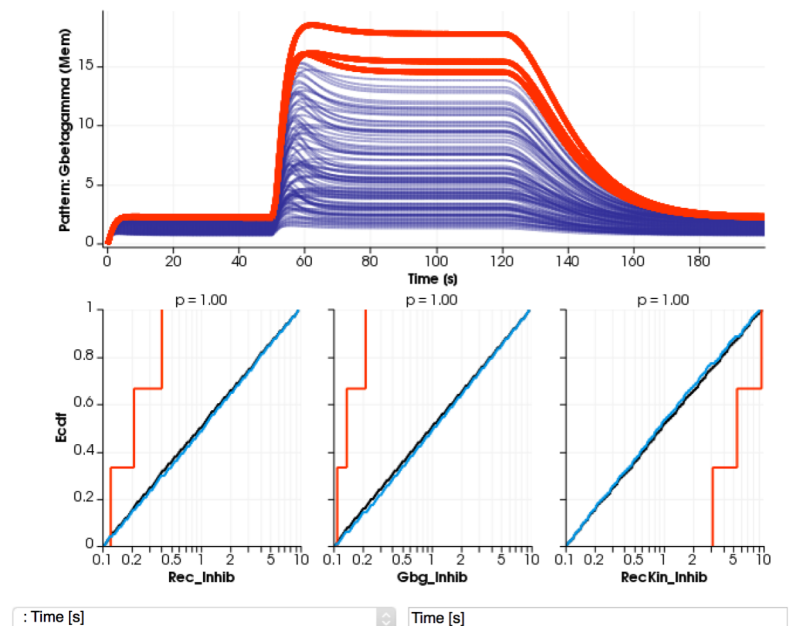
21. Switch the quartiles off and select 'Plot Lines' to see individual results. Set 'Log parameter axes'.



22. Using the right mouse button (Mac: <control>+mouse) you can select simulation curves.



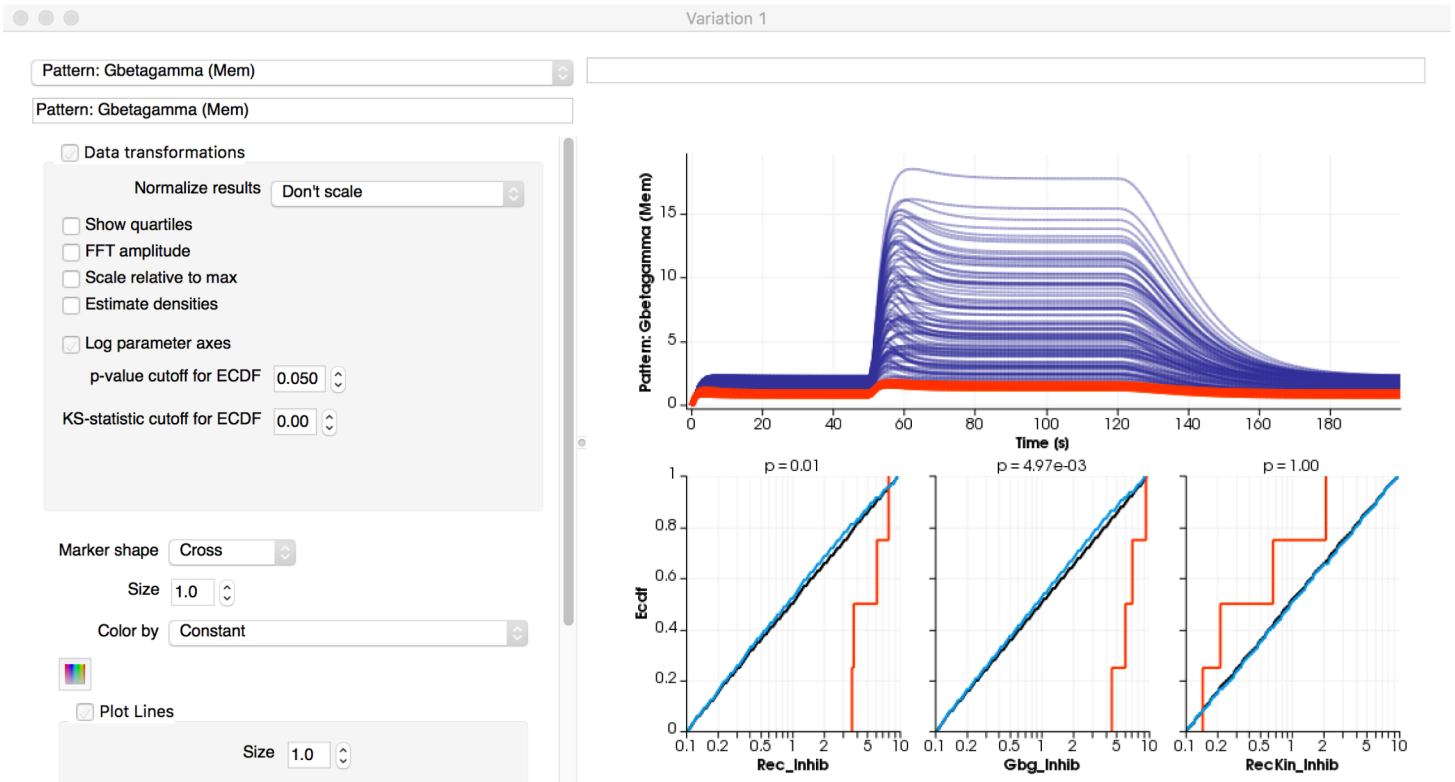
Below the curves, the analyzer will show which parameter ranges are compatible with the selected behavior. Empirical cumulative density functions (ecdf) of parameters are plotted. Selecting points in the primary display will cause a simultaneous selection of parameter values. Points at selected times, that themselves are not selected are shown in blue.



Note, that we have selected strong responses of Gbetagamma. The analysis windows displaying the Ecdf plots indicates that this requires low concentrations of Rec_Inhib and Gbg_inhib and high concentrations of RecKin_Inhib.

Using Shift+right (Mac: <control>+<command>+mouse) mouse button allows to select multiple regions by adding to the current selection, points can be de-selected using Ctrl+right mouse button. If points at different times are selected a parameter value will only be part of the selected set if it is selected at every time at which at least one point is selected.

23. Low output of the signaling pathway is compatible with high concentrations of the 'real' inhibitors (Rec_Inhib and Gbg_Inhib) but also with a variety of concentrations of the inhibitors for the receptor kinase. The fact that Rec_Inhib holds the signal down from the beginning means that the receptor kinase does not have a high impact.



24. Selecting first a broad low-middle response and then the peak region of that selection shows that an initially strong signal that subsequently decays requires low Rec_Inhib but pronounced activity of the receptor kinase (and thus low RecKin_Inhib) to limit the signal at later time points.

