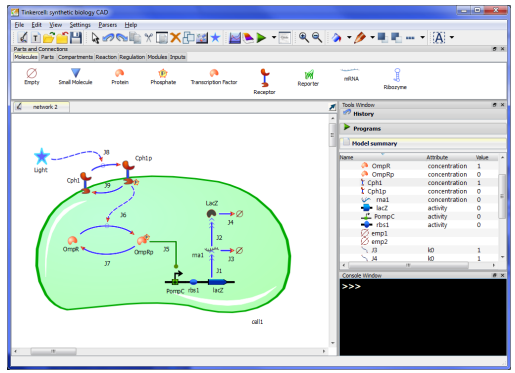
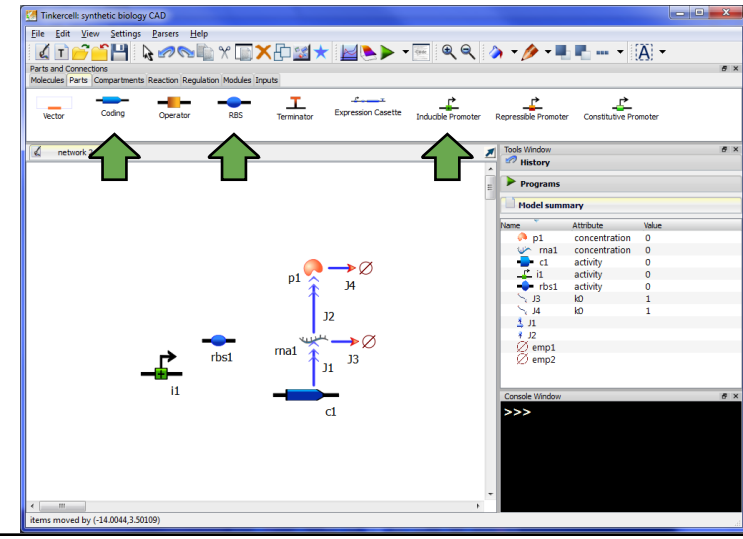
Tutorial #3

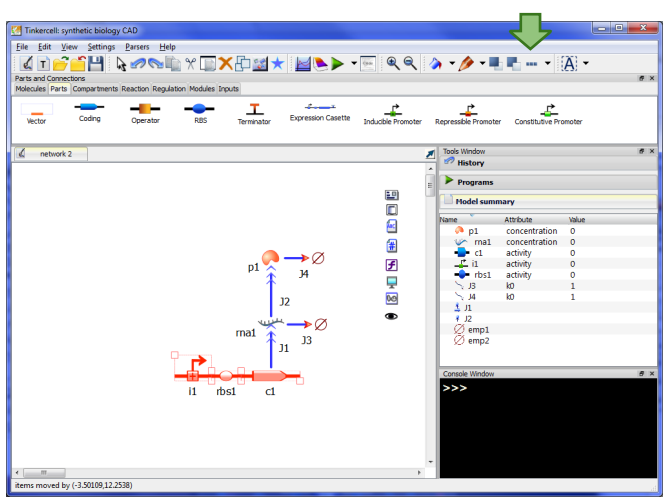
Light detecting system from [2006 Univ. Texas iGEM]



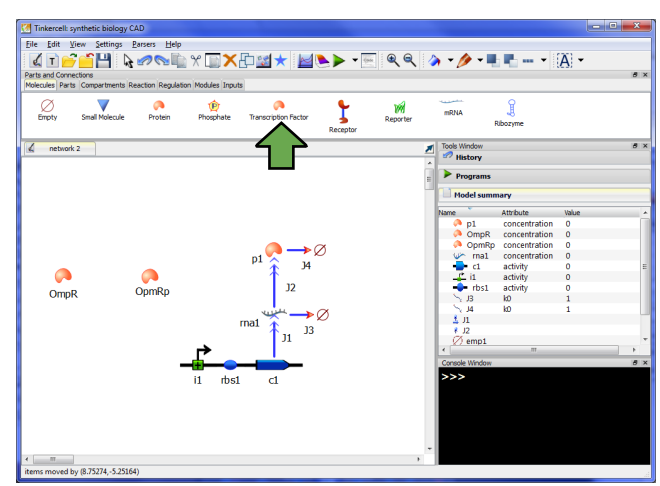
Insert promoter, RBS, and protein coding parts from the catalog of parts. The mRNA and protein will automatically appear from the protein coding part.



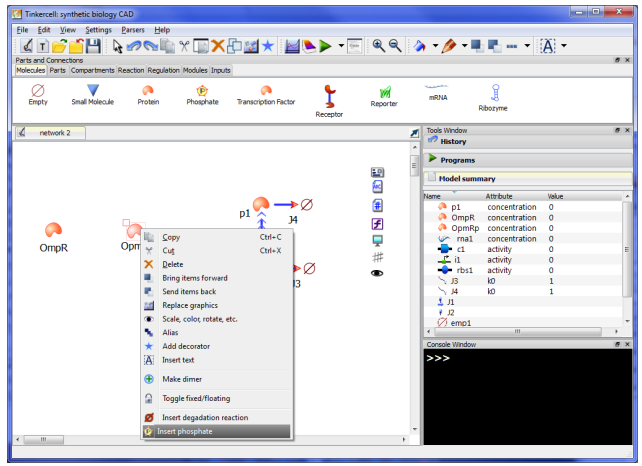
Drag the promoter, RBS, and coding next to each other. They should automatically align. *Alternatively*, you can use the "align compact" (see green arrow) option after selecting the three components.



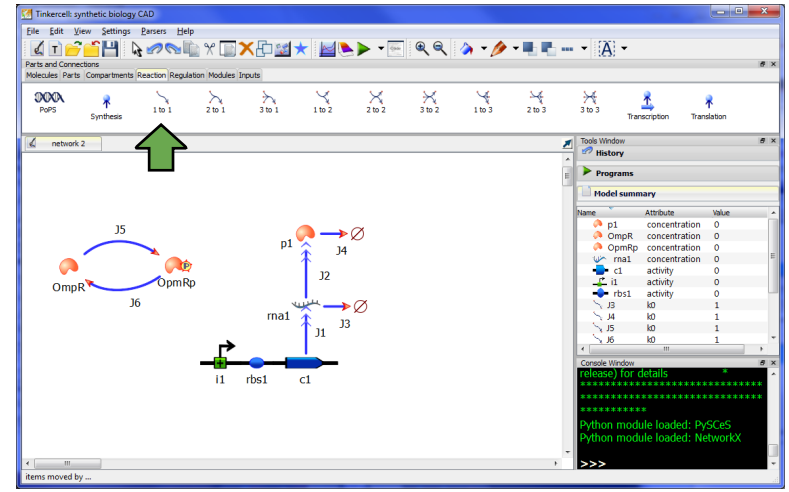
Select the "Transcription factor" component and click twice on the canvas to insert two transcription factors. Click on the name and rename them to OmpR and OmpRp. OmpRp represents the phosphorylated version of OmpR.



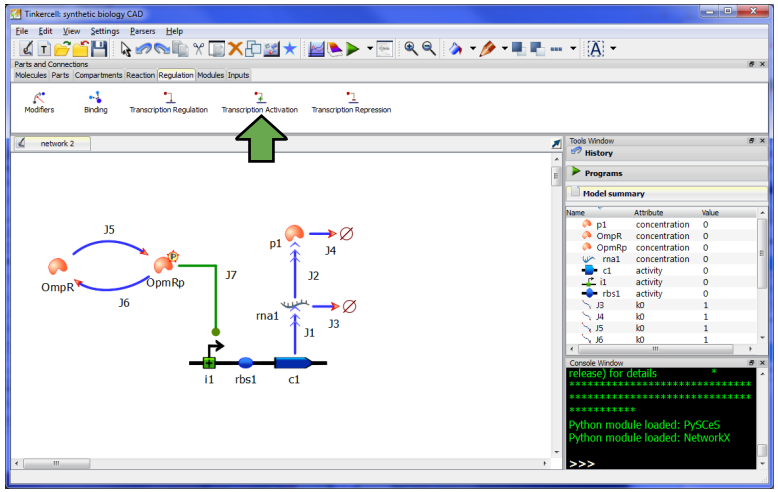
This step is optional. It is purely for visual appeal.
Right-click (or option+click on a Mac) on OmpRp and select the "Insert phosphate" option. You may move reposition the phosphate icon by holding the CTRL key and moving it with the mouse.



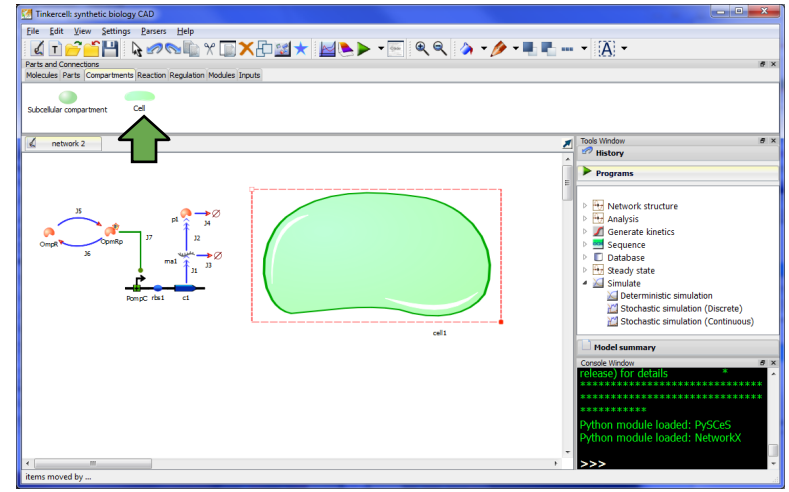
Select the "1 to 1" Reaction and click on OmpR and then OmpRp to create a reaction that converts OmpR to OmpRp. Similarly, create a reaction in the reverse direction.



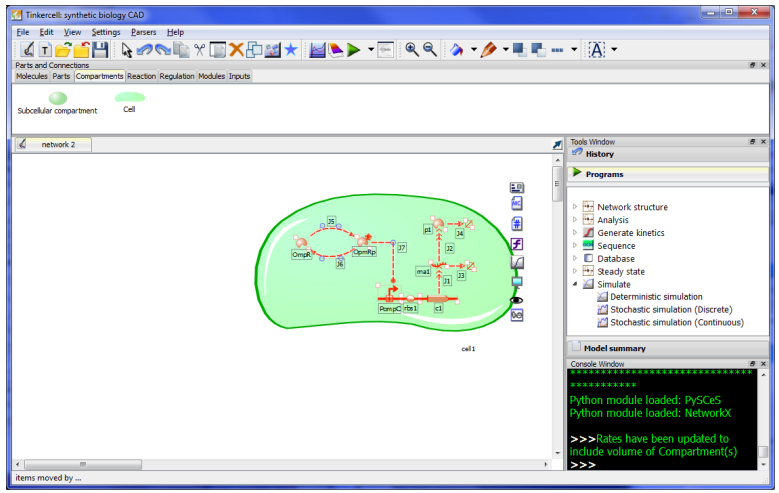
Select the "Transcription activation" Regulation and click on OmpRp and then the promoter. Additionally, rename the promoter to PompC



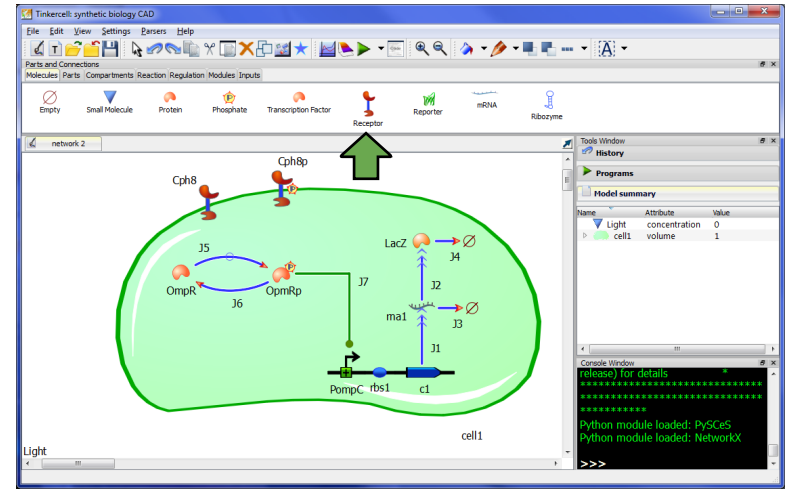
Insert a "Cell" on the canvas. Resize the cell so that it is big enough to hold the network we have just created.



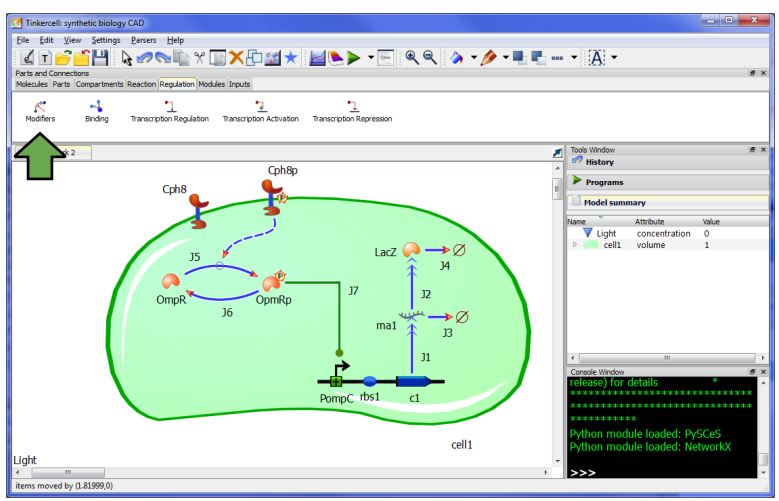
Select the entire network and drag it into the cell.



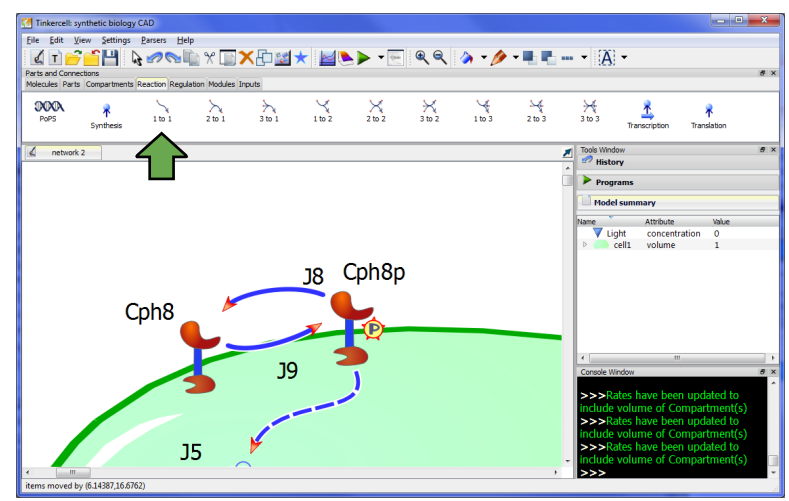
Select the "Receptor" component and insert two receptors on the canvas, inside the cell as shown. Rename them as Cph8 and Cph8p, the phosphorylated version of Cph8



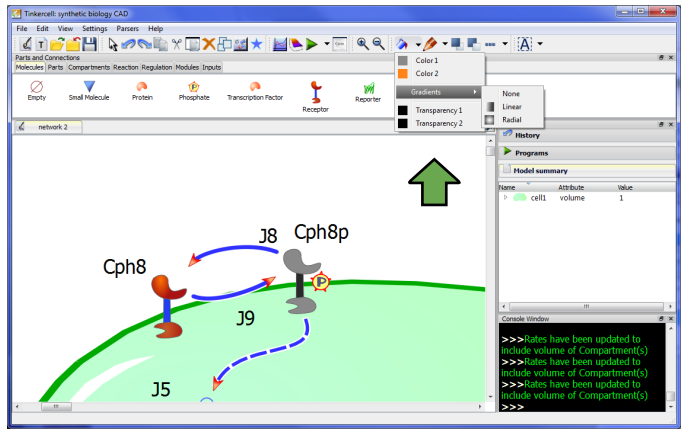
Select the "Modifier" regulation. This represents any reaction where one molecule modifies another. Click on Cph8p first and then click the **reaction arc** going from OmpR to OmpRp.



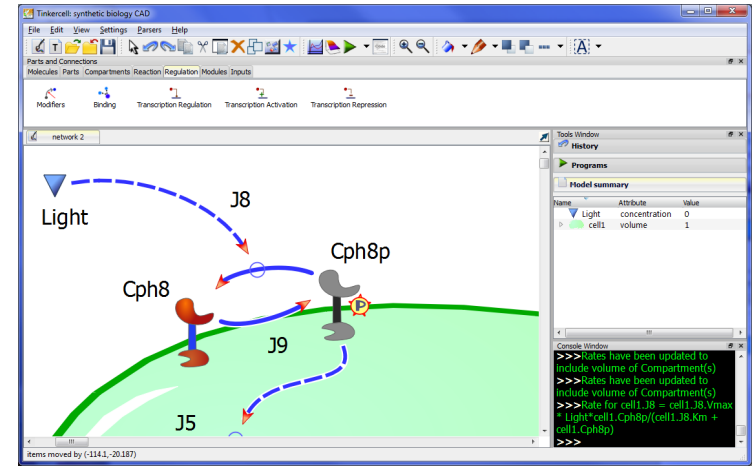
Insert a "1 to 1" reaction from Cph8p to Cph8 and also in the reverse direction, as shown. If you have trouble selecting components, use CTRL+ and CTRL- to zoom in and out, resp.



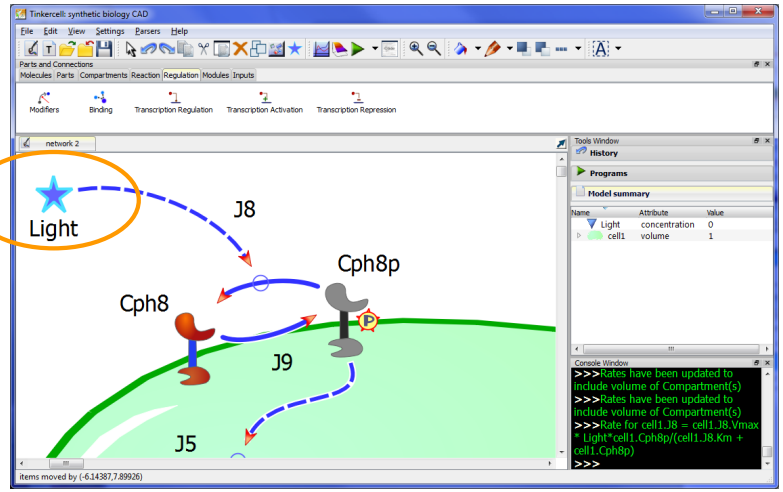
This step is optional. It is purely for visual appeal.
Select the paint bucket icon at the top. Use the menu within the button to select colors and gradient options. Click on the Receptor that you want to color. You may color individual sub-sections of the receptor as well.



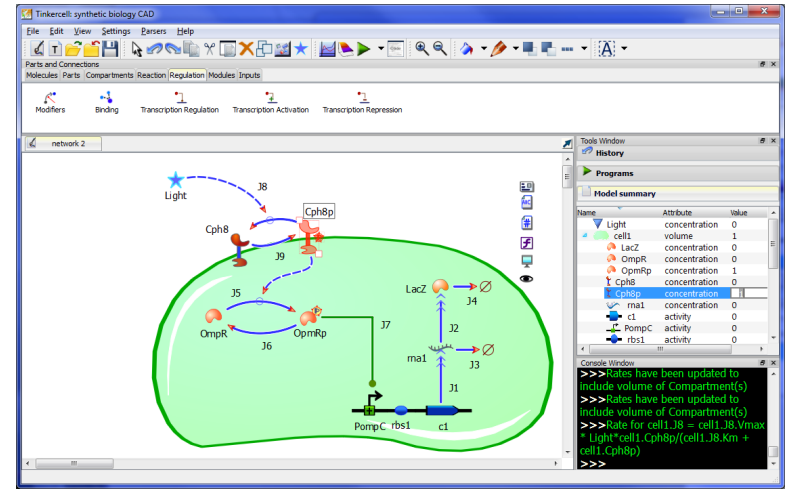
Insert a "Small molecule" on the canvas. Rename it to "Light". Then connect a "Modifier" connection from Light to the reaction converting Cph8p to Cph8. Note that we are substituting light with a small molecule, just for convenience.



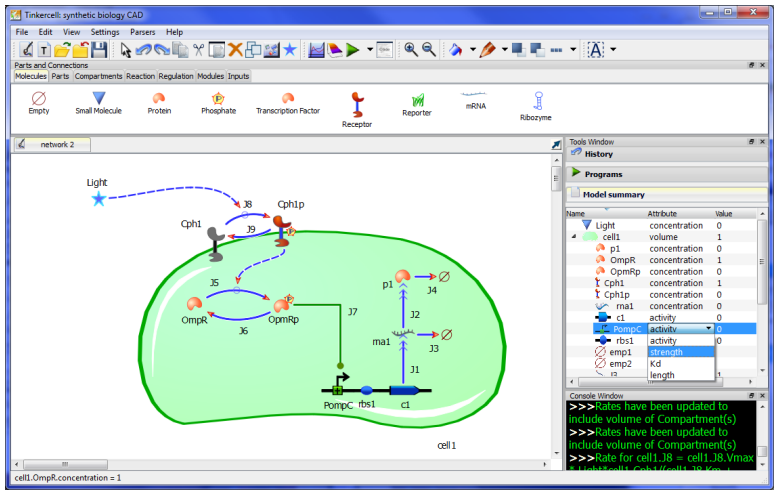
This step is optional. It is purely for visual appeal.
Right-click (or option+click on a Mac) on Light and select "Replace graphics". Then select the star icon under the "Decorations" tab.



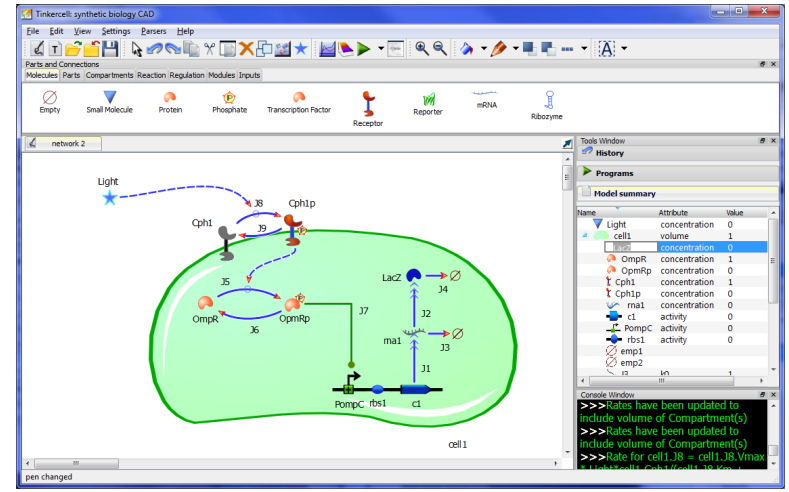
Now we are ready to simulate the system... lets set the initial concentrations first. Use the "Model summary" window on the right to set the concentrations of Cph8p to 1 and OmpR to 1. Set all the other concentrations to 0.



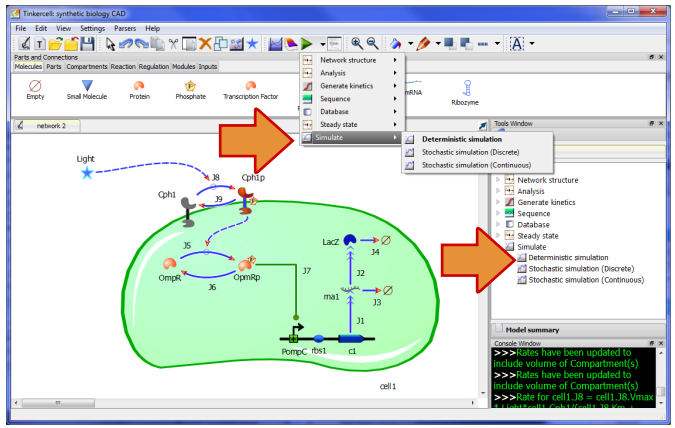
We can also set promoter strengths, RBS strengths, and other parameters using the Model Summary window, as shown in this screenshot.



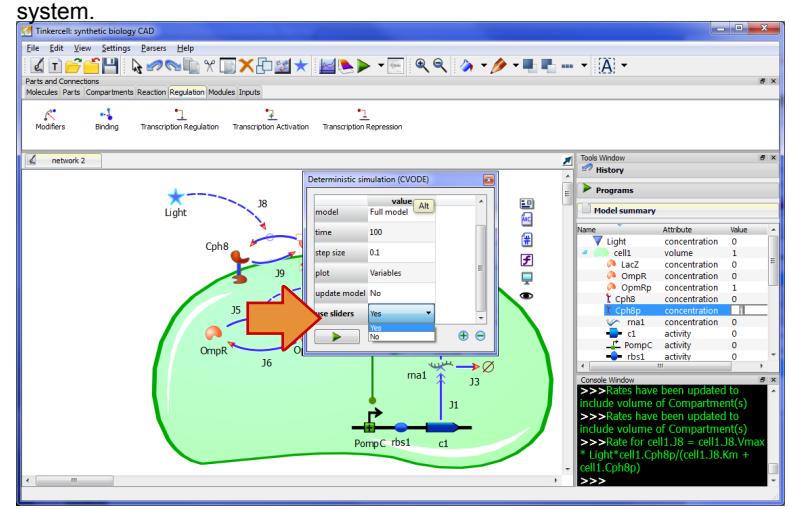
We can also rename components using the Model Summary window. Rename the protein, p1, to "LacZ".



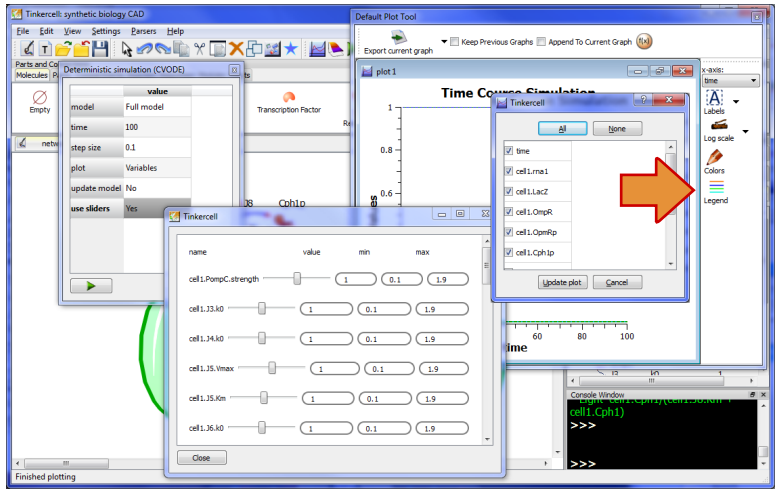
Select "Deterministic simulation" from programs menu. This menu can either be found at the top (a play button) or on the side.



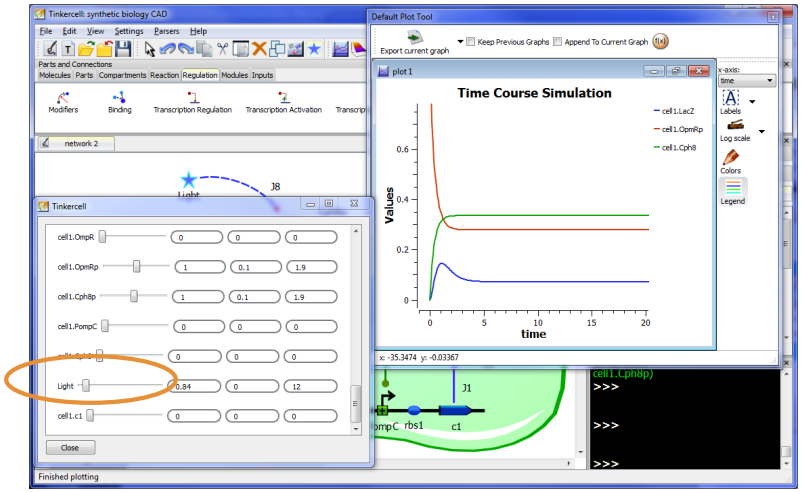
Select "Deterministic simulation" from programs menu. In the popup window, set "use sliders" to "yes". Click the play button to simulate the system.



Use the "Legend" button to select only the items we are interested in plotting: LacZ, OmpRp, and Cph8p (or other variables if you want)



Change the value of Light using the sliders to notice how the values of the molecules change. *Bug: you need to set the min/max values next to the sliders if they are all 0. If you see that a parameter is listed twice in the sliders list, use the second (bottom) one.*



You can also use the "steady state analysis" to see how the concentrations change as a function of Light. To do this, select "Light" as the free variable. For other analysis such as Stochastic simulations, it may be required to adjust the other parameters, otherwise the simulations will not show the same results due to high levels of noise.