Assignment JWS online

JWS Online is a Systems Biology tool for simulation of kinetic models from a curated model database. This document serves to introduce JWS Online.

1 How to use JWS Online

1.1 The JWS Online home page
JWS Online is hosted on a number of mirror sites, each of them allow to select and run kinetic models in a browser. All of these models have been curated to reproduce the model behavior as published by the model constructors.
For the course you can run JWS Online from the VU server at: (http://jjj.bio.vu.nl). The home page has a number of links to various pages giving information on the JWS Online and Silicon Cell projects. For this tutorial we will mostly be using the Model database link, although at any point during a tutorial one might need to use the Help link as well.

1.2 Model selection
Clicking the Model database link, a window will load displaying three different methods to make a selection for the models in the database: The simplest method is to select all the models in the database by clicking on the select all button. As the name already implies the method is not very selective and it might be hard to find a specific model. The second method allows to make a search on any of the following fields: Author, Title, Journal, Organism, Category, Subcategory, Model type. Select one of these fields from the drop down menu, type the keyword in the text box and click the submit button. For the simple models with which we will start this tutorial a good selection method would be: select Model type from the drop down list, and type demo as keyword. Clicking submit, will display a list with all the demonstration models. The last selection method is the most advanced and allows for a quick selection of a model on the basis of a mouse over menu with organism and category as the selection criteria. Move your mouse over the menus and try, it is very easy to use. Importantly, to make a final selection you need to click the word on the button, just clicking the button won’t work. The older Internet Explorer versions do not support this method, use Firefox instead.

1.3 The Query result page
After you have made your selection a query result page will be loaded with the following information: Organism, Category/sub-category, Model, Manuscript details, Download model and a run link. Clicking the run link will load the JWS Online model interface to actually start running simulations with the model.

1.4 Running a simulation
The JWS Online model interface consists of two parts: 1) a Java applet, build up of several tables in which the user can change parameter values and make selections for the specific simulation, 2) a scheme for the system being simulated, with a mouse over facility to show the rate equations used for the specific reactions.
In the left hand table of the applet, the parameter values can be changed by clicking on the respective cell, changing the value and pressing the return (enter) key or clicking on another cell in the table. In the same table one can also change the values of the external (i.e. clamped) metabolites and the values for the initial concentrations of the variables. 2
1.4.1 time simulation
Below the Evaluate Model button there are three tabs Sim, State and MCA, that can be used to chose between time simulation, steady state analysis and metabolic control analysis. The Sim tab is selected by default (as indicated by its blue color). The user can enter the start and end time for the time simulation, select whether to plot reaction rates or metabolite concentrations (using the radio buttons, metabolites are selected by default) and in the table specific rates or metabolites can be selected. Clicking the Evaluate model button will send your request to the server and a plot of the selected variables against time will be loaded in a new window. Your browser must allow pop-ups from the site to see the new window.

1.4.2 steady state analysis
Selecting the State tab makes it possible to choose between different steady state analyses: Steady State, N matrix, K matrix, L matrix, Jacobian and Eigenvalues, which result in a table with steady state metabolite concentrations and flux values, a stoichiometric matrix for the system, a matrix with dependent and independent fluxes and metabolites, and simple stability analyses respectively upon clicking the Evaluate Model button.

1.4.3 scan
The third tab, Scan makes it possible to analyze the steady state systems behavior over a range of parameter values. The parameter for which the scan will be made can be selected by checking its box in the table on the left side of the applet. By default the first parameter is selected, and the name of the parameter is indicated in the scan panel. The user can select the range over which the parameter will be scanned by indicating the lower (MinVal) and upper value (MaxVal) and the number of steps (Nsteps) that needs to be taken. Finally the user needs to select whether metabolites or rates will be plotted (and which of these, if not all), similar as was done for the time simulation.

1.4.4 metabolic control analysis
The last tab, MCA gives access to metabolic control analysis for the system. This has not been explained yet, in the course, or at least only very briefly. Forget it for now.

2 Tutorial 1: Working with demonstration models
This is a tutorial for first time users of JWS Online, or people having very limited experience in working with kinetic models.

2.1 Three enzyme linear pathway: time simulation
From the home page select Model database, in the Keyword search, select Model Type from the drop down menu and type as keyword demo and click the Submit button (see 1.2). From the list of models, press the run link for the 3 enzymes linear path model. After the applet has been loaded, you can choose between the different types of analysis as explained in 1.4. As the name suggest the model describes a linear pathway that consists of three enzymes, converting the external metabolite s into p, via the intermediates x2[t] and x3[t].
Q1 Click the Evaluate Model button, and describe the resulting time course plot (if no result is obtained, make sure your browser allows popups from the server). The condition where the concentrations of x2[t] and x3[t] do not change anymore is called the steady state condition, this condition occurs when v[1] = v[2] = v[3]. Check this by extending the time period of the time simulation (see 1.4.1).
Note that you can click either on rates or metabolites. Looking at rates is easier to check that v[1]=v[2]=v[3].
Q2. Why is it likely that this steady state condition will be reached after a sufficiently long time period, with the concentrations of s and p clamped? In what direction is the flux running, by the way?

Q3 Note from the rate equations that these are all reversible reactions with an equilibrium constant. The overall equilibrium constant from s to p can be found by multiplying the individual Keq’s, and therefore Keq overall = 1. Estimate the concentration of p where the direction of the flux changes, and check that with simulation.

Q4 What condition would be reached if s and p would be free variables (not fixed, external metabolites), and what would be the concentrations of s and p in that condition?

Q5 Click the Reset button, such that the original values for the parameter are loaded. Change the Keq of reaction 1 to 0.05, and p to 0.1. What would be the direction of flux? Check by simulation (only after answering the previous question!).

So: external metabolites concentrations and the (product of) equilibrium constants are very important, as they set the direction of flow caused by a thermodynamic driving force known as the “chemical potential difference” (in analogy to ions moving in an electric field).

Q6 Change the initial conditions of x2[t] and x3[t] and run simulations. What happens to the steady state concentration of x2[t] and x3[t]? Do you understand this? Estimate the concentrations of x2[t] and x3[t] and the values of the reaction rates in the steady state condition.

2.2 Three enzyme linear pathway: steady state analysis
Q7 Select the State tab in the three enzyme linear pathway model applet (see also 1.4.1). Click the Evaluate Model button and compare the steady state concentrations for x2[t] and x3[t], and the rate values for v[1], v[2] and v[3] with those estimated from the time course. The rate values at steady state are called fluxes (symbol J), and in a linear path all enzymes have the same rate, i.e. there is one steady state flux value (check).

Q8 change the Vm of the first enzyme incrementally between 0 and 10 and study the effect on the steady-state flux, so either v[1], v[2] and/or v[3]: go to the scan tab and select v[1] in the parameter window on the left, put minimum value to 0 and maximum value to 10, with 20 steps, and select rates in the middle panel. Explain the observed pattern (i.e. why does the curve has a steep slope close to 0, and is almost flat close to Vm1 = 10?).

Q9 Change the Vm of all three enzymes to 10. Compare the steady state with the original one of Q7. Does it make sense to you?

2.3 Three enzyme linear pathway: oscillations
Let’s move to another model, also three enzymes but different kinetics and stoichiometry. Go back to the home page and select the model from Bier et al, via author search. Inspect the model. Note that the box with the 2 in it -above reaction 2- means that two T are being produced in this reaction. Change the time end value to 300 and run a time simulation... Nice he?
Q10. Now change the input rate of reaction 1 (k1); try lower values (e.g. 0.1), and higher ones (e.g. 1). What do you observe (note frequency and amplitude)?

Q11. If you increase k1 too much, an “explosion” will take place. Set end value of time to 10000 and run a simulation with k1 = 2.9 and k1 = 3.1. What do you observe? You can understand the difference if you inspect the rate equation of reaction 3, the reaction that consumes T (notably its maximal capacity).

So: dependent on the kinetic parameters (in this case k1), the system can behave qualitatively different, not only quantitatively! Sudden transitions in behaviour can happen (here from oscillation (k1 = 0.36) to damped oscillation finally reaching steady state (k1 = 1), to explosions (k1 >3)); these are called bifurcations. We will look at them again in the modeling course.