COMBINE workshop

25.11.2015, Singapore

SYCAMORE Tutorial

Build a kinetic model of the first two steps of glycolysis in human hepatocytes.

To set up the model, you will select respective reactions in SABIO-RK and check if kinetic data for these are available in the database. Then you will slightly adapt the model and run the simulation.

Modeling procedure:

1. Go to the Sycamore website, http://sycamore.eml.org/, and click the button “Enter SYCAMORE”
2. Use the second option by clicking on “build SABIORK model”
3. Enter the following search command into the search field :

   d-glucose 6-phosphate AND Organism:("homo sapiens" OR "rattus norvegicus") AND Tissue:"liver" AND PubMedID:("1435758" OR "14988235")

4. You get a list of all reactions stored in SABIORK that match your search restrictions. You can press the blue arrow on the left to get more details.
5. We are interested in the two following reactions:

   D-Glucose + ATP <=> ADP + D-Glucose 6-phosphate (Entry ID: 2580)
   D-Glucose 6-phosphate <=> D-Fructose 6-phosphate (Entry ID: 2616)

   You can find the Entry ID at the top of each reaction entry (click on the blue arrow). Select both entries (“Add to export cart”) and then click on the paper symbol to see the list of entries to be exported.
6. Click on the button “Send Data to SYCAMORE” and see which information was imported from SABIORK. Go to the bottom of the page and click 'add data to model'.
7. You should now see an overview of your model (including e.g. Reactions, Compounds Kinetic Parameters etc.). Here you can edit and change the information, which is sometimes necessary for consistency. Change the name of

   'reaction_0' to 'Glucokinase'
   and 'reaction_1' to 'Isomerase'

8. As a final step in setting up your draft model, you will likely want to make the system open. For this purpose you have to define an influx for Glucose and an efflux for Fructose-6-phosphate. This can be achieved by pressing the “add reaction” button on the bottom of the reaction list and defining two new reactions.

   1. For the influx reaction fill in the following values and add the reactions to the model:
      1. Reaction name: 'Influx'
      2. Select compound 'D-Glucose' from the selection list 'add existing compound'
      3. Specify the role of D-Glucose as 'product'
      4. Set the initial concentration to '1'

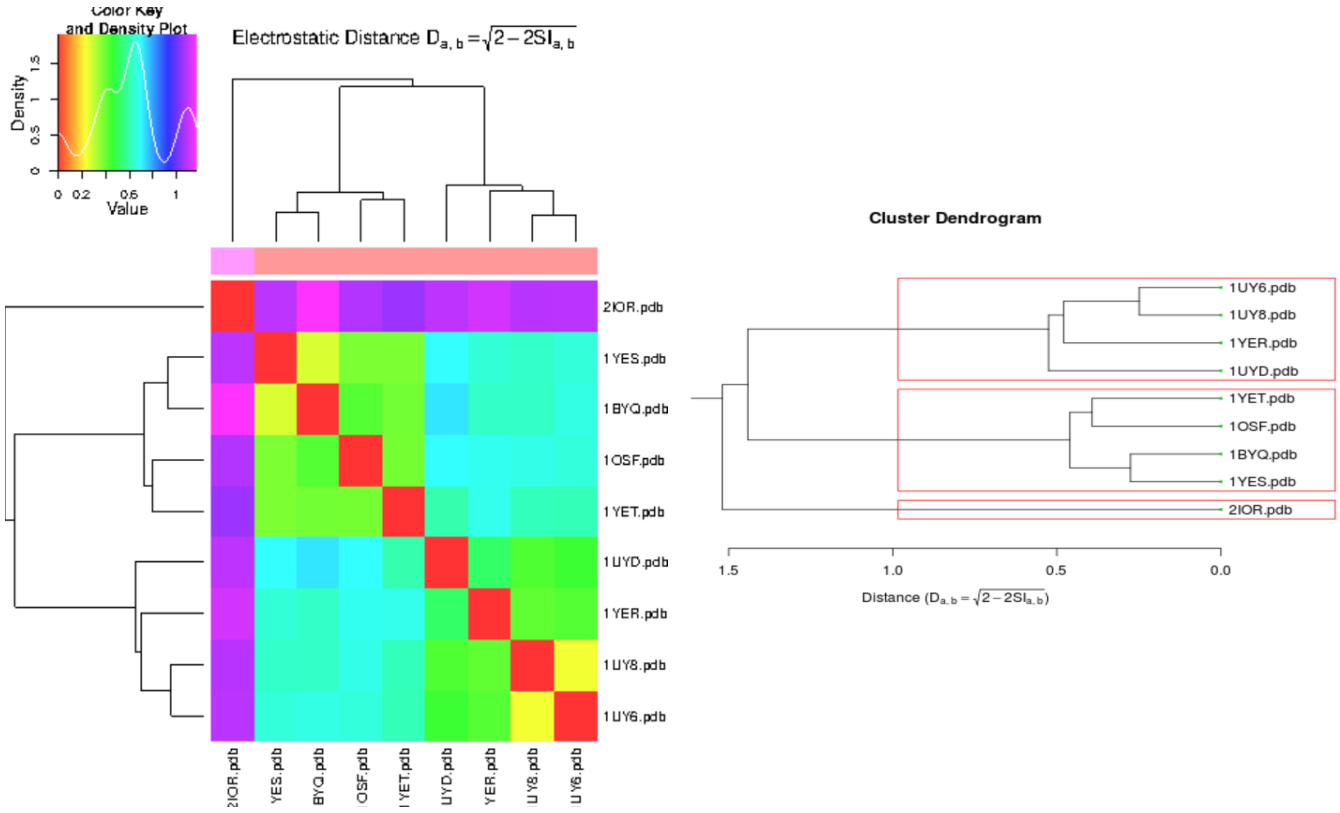
5. Define a parameter 'k' for this reaction, set the value to '0.1'
6. Enter 'k' as the formula for the kinetic law
7. Press the button 'add reaction to model and enter next reaction'

2. For the efflux reaction fill in the following values:
    1. Reaction name: 'Efflux'
    2. Select compound 'D-Fructose 6-phosphate' from the selection list 'add existing compound'
    3. Specify the role of D-Fructose 6-phosphate as 'substrate'
    4. Set the initial concentration to '1'
    5. Define a parameter 'k' for this reaction, set the value to '0.1'
    6. Enter 'k * D-Fructose 6-phosphate' as the formula for the kinetic law
    7. Press the button 'add reaction to model'

9. Check if your model is complete by pressing the 'Model completeness' button at the bottom of the page. No errors should be detected.
10. Click on 'Model simulation', select all compounds and start the simulation. Have a look on the results. You can also start the simulation with, for example, different initial concentrations and see how it influences the overall result.

webPIPSA Tutorial
Use of PIPSA server to compare different interaction fields (electrostatics) for different proteins.

In this example we will focus on the chaperone HSP90 molecule. It stabilizes proteins including those that are required for tumor growth and that is why there is an interest to find HSP90 inhibitors. This protein has a binding site that is surrounded by helices. Some of these helices can be distorted. Here, we will compare the electrostatics surrounding the binding sites of nine different HSP90 structures and check if there are differences.
We have already performed a PIPSA analysis with these nine structures and grouped them into three clusters. You can see the cluster results below:



Question: Do you think the number of clusters is reasonable? What could be the reason for the bigger distance between some HSP90 proteins?

This previous analysis was performed on the whole (global) superimposed HSP90 structures. Now we want to perform a local analysis around the binding site with the distorted helix as center and see how the result changes.

1. Go to the PIPSA webserver http://pipsa.h-its.org/
2. Start a PIPSA analysis with PDB structures as input. Select and import the nine local PDB files from http://co.mbine.org/system/files/webPIPSA_PDBs.zip. These pdb files contain the already superimposed structures from the previous analysis. Give your email and use APBS (this goes faster). You do not need to superimpose the structures again.

To set the center type a name (e.g. center) and the following coordinates:
x: 16.305 y: 7.275 z: 24.916
As radius you can use 10 and/or 15 Å (you can define a center two times with a different radius)

3. When the analysis is complete (this can take several minutes), note the different clusters of structures. Use the tabs at the top to switch between the different analysis.
   Question: Which differences can you observe?
4. Look at the structures using the integrated applet. Focus on the distorted helices. What is your conclusion?
   Question: What is the difference between the global and the local comparison runs?

LigDig Tutorial

The LigDig webserver provides seven different modules. Here you will test two of them to get an impression how to use LigDig and for which purposes it might help you.

1. Go to the LigDig website: http://mcm.h-its.org/ligdig/ and click on 'Example Cases' in the right panel.
2. Select one example case which is most interesting for you.
3. Go through the example and test the respective module(s) in LigDig.