Tutorial

Metabolic Network Analysis with *CellNetAnalyzer*

CellNetAnalyzer (CNA) – general information:

- *CellNetAnalyzer* (CNA) is a MATLAB toolbox with (optional) graphical user interface and various computational methods and algorithms for exploring structural and functional properties of metabolic, signaling, and regulatory networks.
- The focus of this tutorial is on CNA's functionalities for analysing metabolic networks based on stoichiometric and constraint-based modeling techniques, including flux balance analysis (FBA), metabolic flux analysis, elementary-modes analysis, minimal cut set analysis, and many more.
- You should also read the manual of CNA (on the CNA website or in the directory 'CellNetAnalyzer/doc'), in particular chapters 0 (quick start) and 2 and 3 (general information on mass-flow / metabolic networks in CNA)

Contents:

Example model: *E. coli* core metabolism

In the following we will use the compressed *E. coli* core metabolism model **EColiCore2-compressed (ECC2comp)** and provided along with *CellNetAnalyzer*. This model was published in [*Hädicke O, Klamt S (2017) EColiCore2: a reference model of the central metabolism of Escherichia coli and the relationships to its genome-scale parent model. Scientific Reports 7:39647*] and was received by reducing a genome-scale model *E. coli* model (iJO1366). It comprises the reactions of the central metabolism, four substrate uptake reactions (glucose, succinate, acetate, glycerol; here the focus will be on glucose), several standard fermentation products (ethanol, acetate, succinate, formate, lactate, hydrogen). Biomass synthesis is represented by one compressed growth reaction. Note that there are also other (partially related) *E. coli* network projects in the standard CNA contribution:

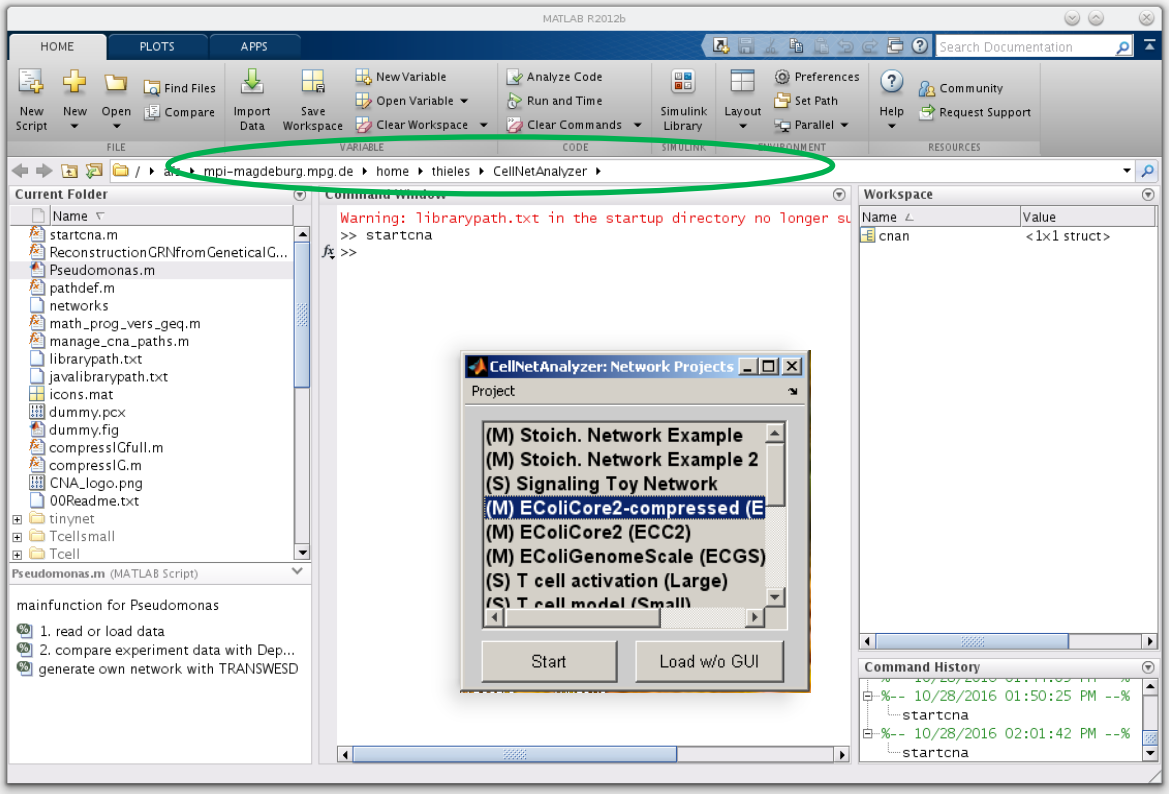
- **EColiCore2-compressed (Small map)**: the same as ECC2compressed with a smaller map (for monitors/beamers with smaller resolution)
- **EColiCore2 (ECC2)**: *E. coli* core with uncompressed biomass synthesis routes;
- **EColiGenomeScale (ECGS)**: the genome-scale model (slightly modified iJO1366) from which ECC2 and ECC2compressed were derived
- **Escherichia coli (small model)**: another smaller core model of *E. coli*'s central metabolism illustrating the use of biomass components and assembly routes in CNA

Use case scenario S1: Flux analysis

Test feasibility and perform metabolic flux analysis and flux variability analysis in the central metabolism of *Escherichia coli*.

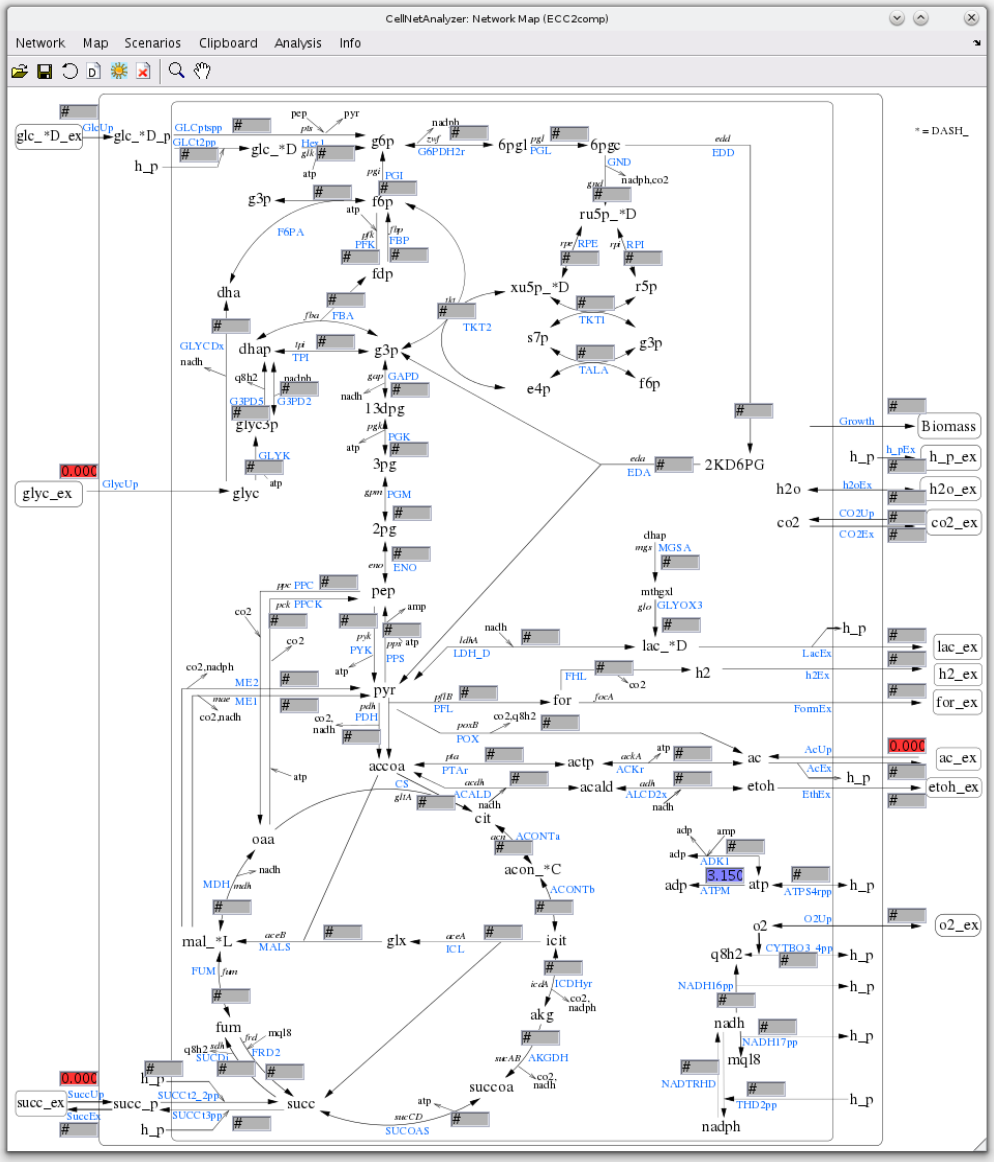
Starting *CellNetAnalyzer*:

- When starting *CellNetAnalyzer* the first time you might need to set some path variables in “startcna.m” and “javalibrarypath.txt ” (see manual sections 0.2 and 0.5)
- Start MATLAB; ideally you should choose the *CellNetAnalyzer* main directory as startup directory (important for correct setting of the paths for the JAVA CPLEX interface; only relevant for a few API functions; see also section 0.2 in the manual). **Alternatively** you can change the working directory to CNA’s main directory after loading MATLAB but then few CPLEX related functions will not work in this case).
- Start CNA by entering “startcna” into the command window
- The project manager window appears → you can now select and start a project:
  → EColiCore2-compressed (ECC2comp)



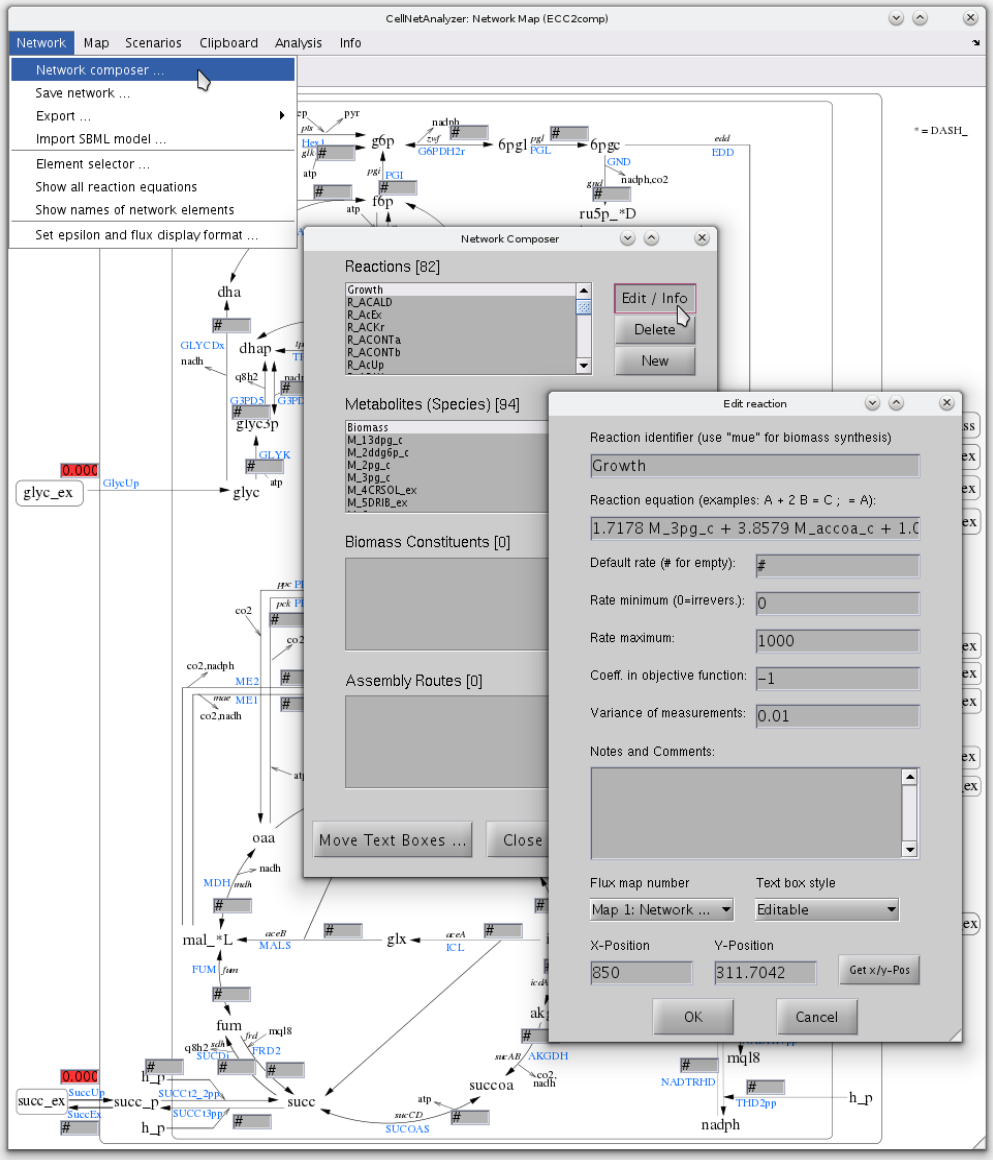
- NOTE: this project consists of only one interactive network map; a network project can have arbitrary many maps (see e.g. E. coli (small))

- In the map, each text box corresponds to one reaction (rate). The text boxes show initially the predefined default values of reaction rates (for most reactions the default value is unknown (‘#’)). Here, the default values can be used to model growth on glucose (glycerol, acetate, succinate cannot be taken up).



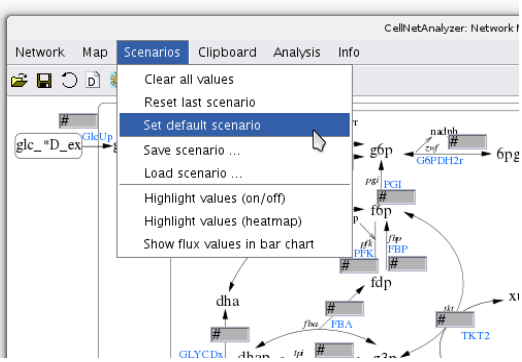
- To receive information about reactions and metabolites of the network open the network composer from the CNA menu (Network → Network Composer → Edit/Info); for more information see also use case scenario S5

- Properties of a reaction can also directly be seen and edited by a right click of the corresponding text box

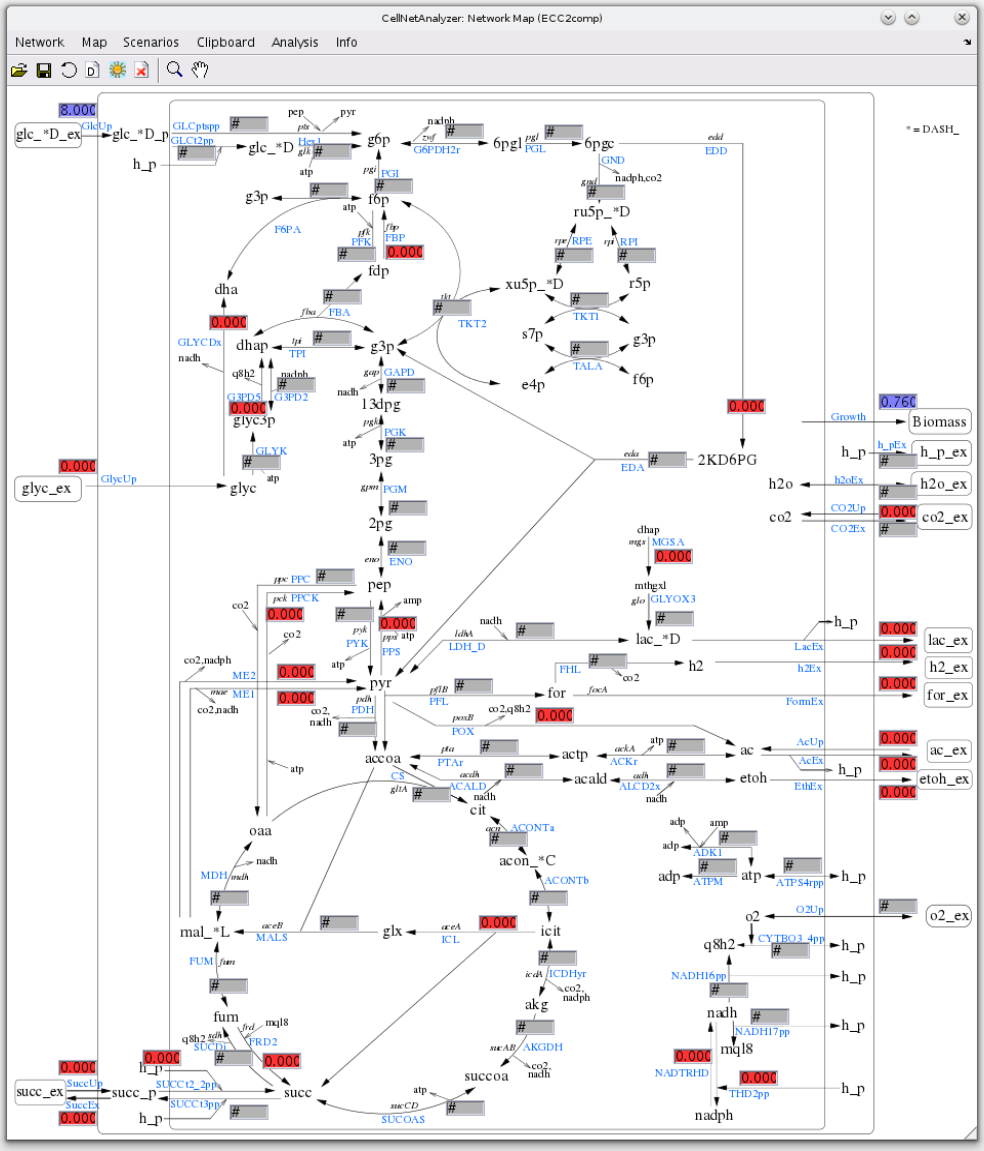


Select default scenario values:

- As mentioned, after opening a project the default scenario is displayed in the boxes.
- Manual selection (also at a later time point) is possible on two ways:
  - press the D-button in the toolbar
  - select: Scenario → Set default scenario



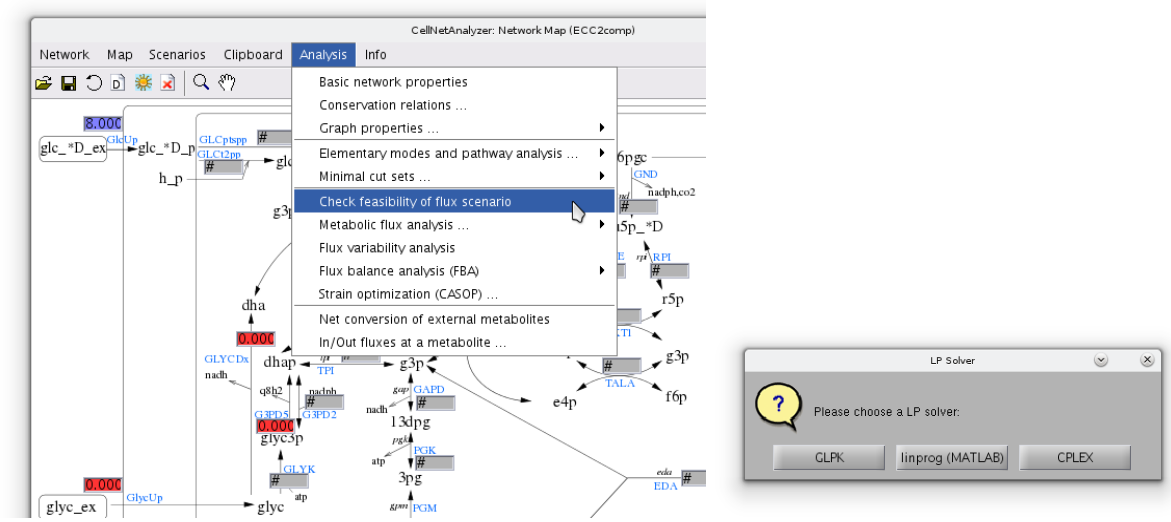
- In the same submenu you can also save and load scenarios. Here we load a scenario “example_flux_analysis.val” serving as an example for a flux analysis scenario for growth on glucose. The scenario file is located in the project directory which is shown in the dialog box appearing after clicking on “Load scenario”.



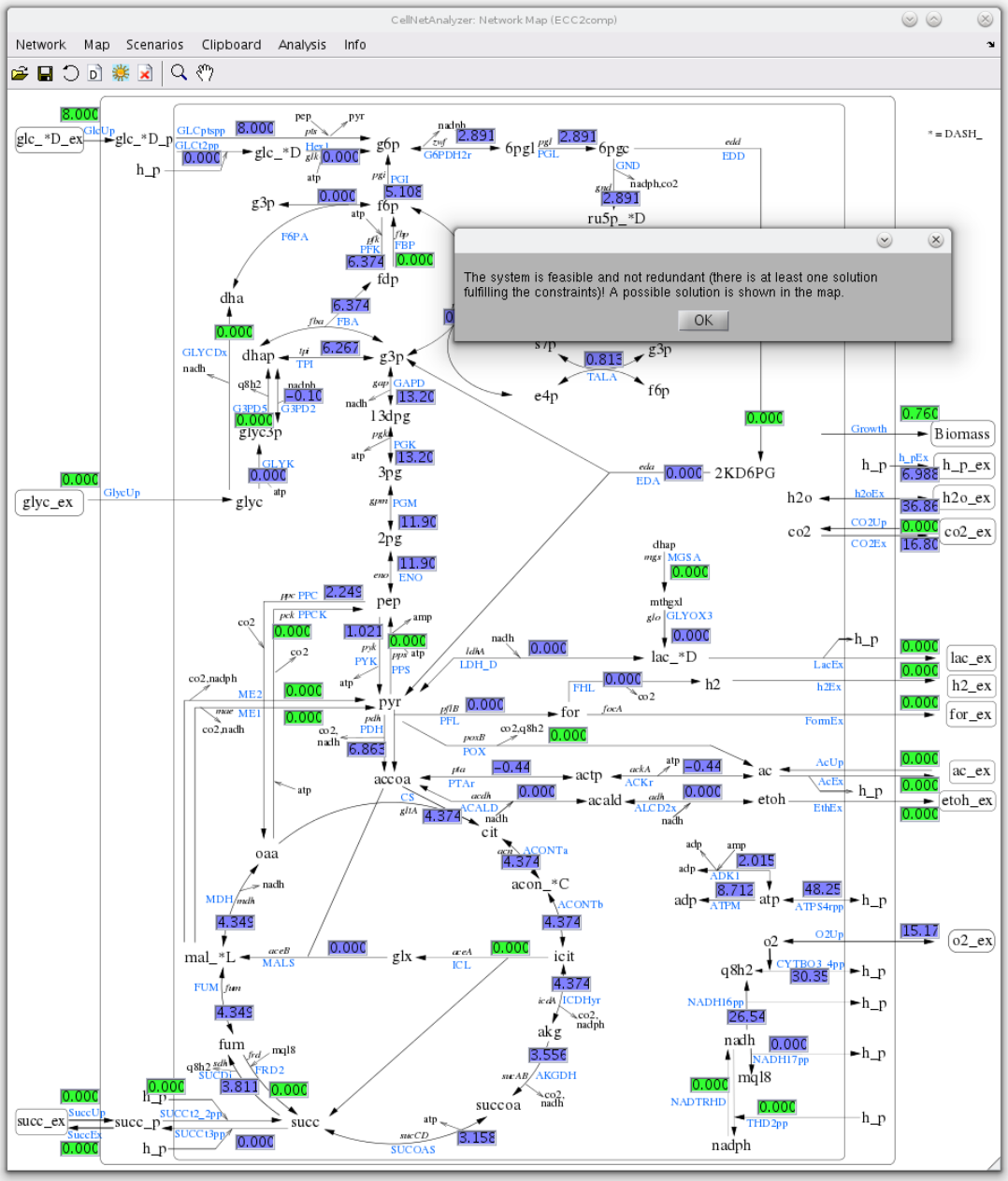
- After loading, the scenario is displayed in the maps and given values are highlighted.
- Legend: # ≙ reactions with undefined value (grey text box)
  red box ≙ disabled reaction (rate = 0)
  blue box ≙ given reaction rate ≠ 0

Check feasibility:

- We check the feasibility of the loaded scenario
- Press: Analysis → Check feasibility of flux scenario → choose “GLPK” as LP solver (you may also select a different solver if available (CNA supports GLPK, MATLAB linprog, CPLEX; see manual for installation)



- “Check feasibility of flux scenario” tests whether there exists at least one feasible flux distribution in the network consistent with the defined rates and flux capacity constraints (min/max reaction rates that can be specified in the property editor for each reaction; see also below) → one possible solution (if it exists) is shown in the map

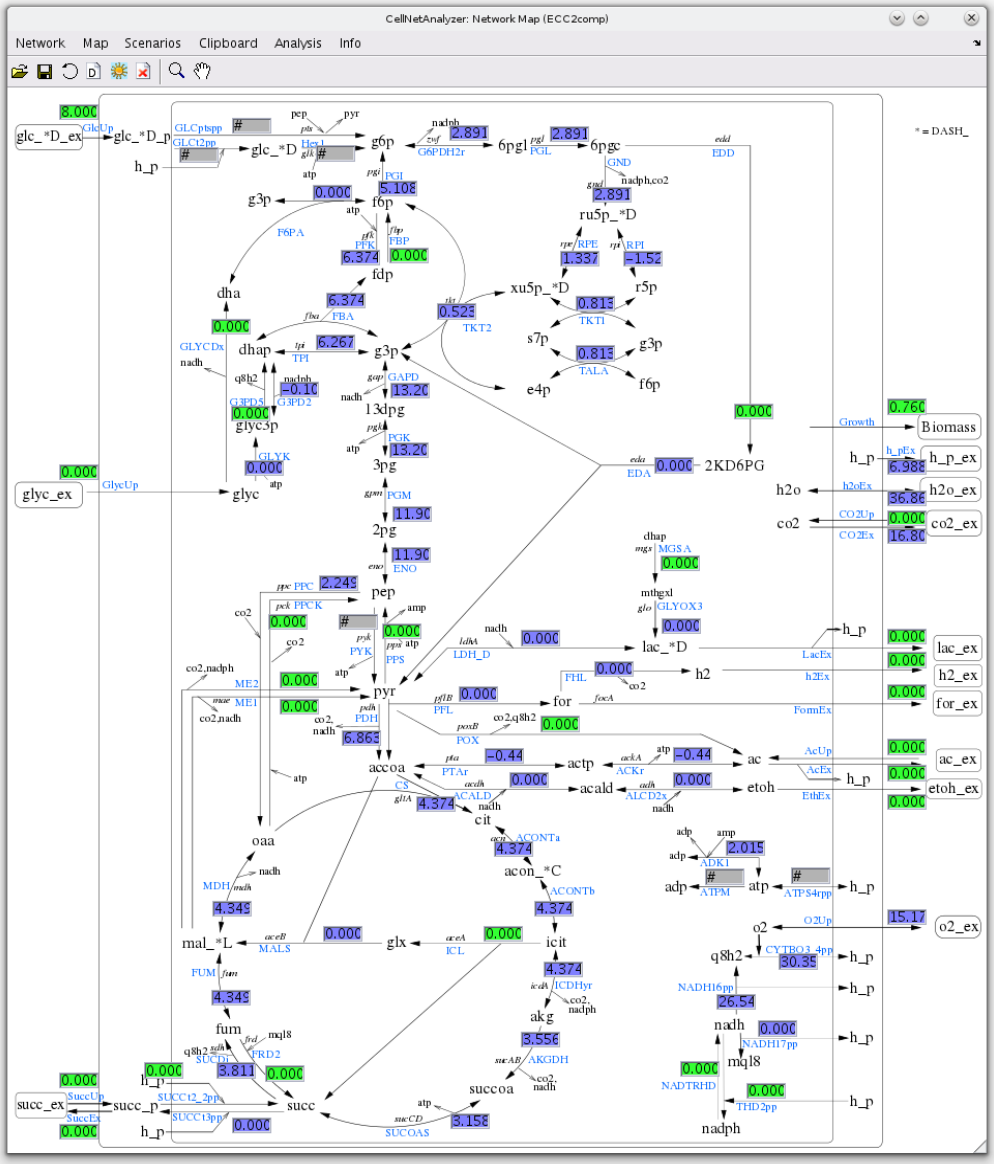


- green boxes ≙ predefined values; blue boxes ≙ calculated values

Flux analysis:

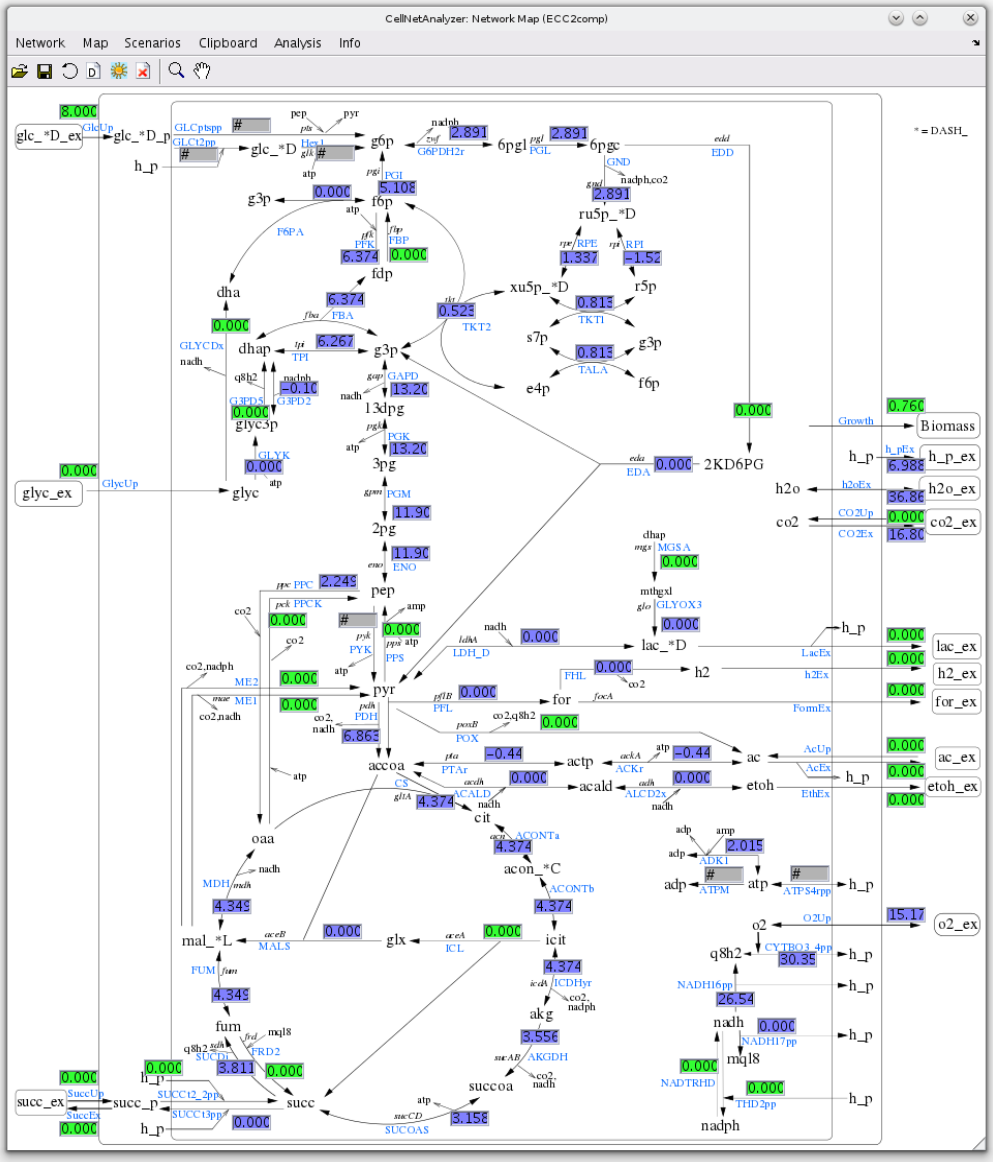
- Reload the latest scenario by clicking on in the toolbar → this was the flux example scenario we had loaded before.
- Press: Analysis → Metabolic Flux Analysis → Flux analysis
- The predefined, uniquely calculable and non-calculable (if the system is not fully determined) rates are displayed/indicated in the maps
- Legend: green boxes ≙ predefined values; blue boxes ≙ calculable values
  grey boxes ≙ non-calculable rates (here: 6 rates are undetermined due to unresolved fluxes in the glucose uptake pathway (PTS vs. hexokinase)

- In redundant (“overdetermined”) systems, a method for dealing with redundant systems must be chosen and balanceable rates will also be indicated (see manual)
- NOTE: flux min/max and reversibility constraints are not considered in this type of flux analysis, but violations of these constraints would be indicated in the MATALB command window



Flux variability analysis (FVA):

- FVA can also be used as a metabolic flux analysis tool. It accounts for the min/max (including reversibility) flux constraints and displays also feasible flux ranges for undetermined reaction rates, but it cannot deal with redundant systems. We apply FVA again to our flux scenario.
- **Load again the “example_flux_analysis.val” scenario.**
- Analysis → Flux variability analysis → choose e.g. GLPK as LP solver
- Results are displayed in the network maps in the blue and green boxes …



… and in the command window (here is an extract):

Results of Flux Variability Analysis:

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Growth: MinFlux: 0.76 , MaxFlux: 0.76

R_ACALD: MinFlux: 0 , MaxFlux: 0

R_AcEx: MinFlux: 0 , MaxFlux: 0

R_ACKr: MinFlux: -0.44276 , MaxFlux: -0.44276

…

R_ATPM: MinFlux: 6.7124 , MaxFlux: 8.7124

R_ATPS4rpp: MinFlux: 46.2522 , MaxFlux: 48.2522

R_CO2Ex: MinFlux: 16.8018 , MaxFlux: 16.8018

…

R_GLCptspp: MinFlux: 0 , MaxFlux: 8

R_GLCt2pp: MinFlux: 0 , MaxFlux: 8

R_GlcUp: MinFlux: 8 , MaxFlux: 8

…

R_PTAr: MinFlux: -0.44276 , MaxFlux: -0.44276
R_PYK: MinFlux: 1.0212 , MaxFlux: 9.0212
R_RPE: MinFlux: 1.3376 , MaxFlux: 1.3376
…

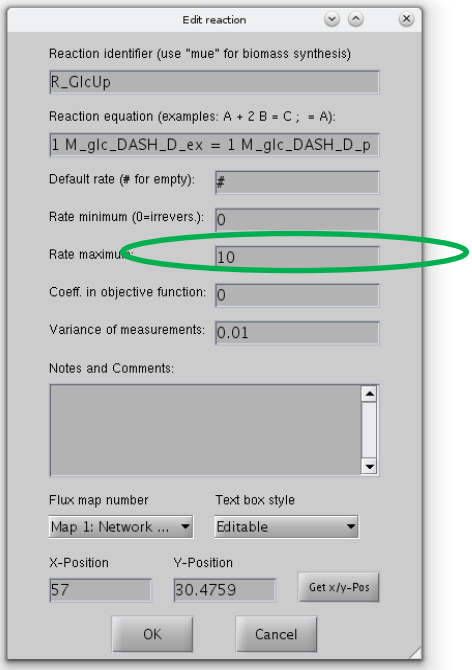
6 fluxes are undetermined	→ grey boxes
27 fluxes fixed.	→ green boxes
49 fluxes are determined by the given constraints.	→ blue boxes; anabolism included

- NOTE: only fixed and uniquely determined fluxes (min. rate = max. rate) are shown in the network maps; the range of non-unique rates is displayed in the command window

Use case scenario S2: Flux optimization

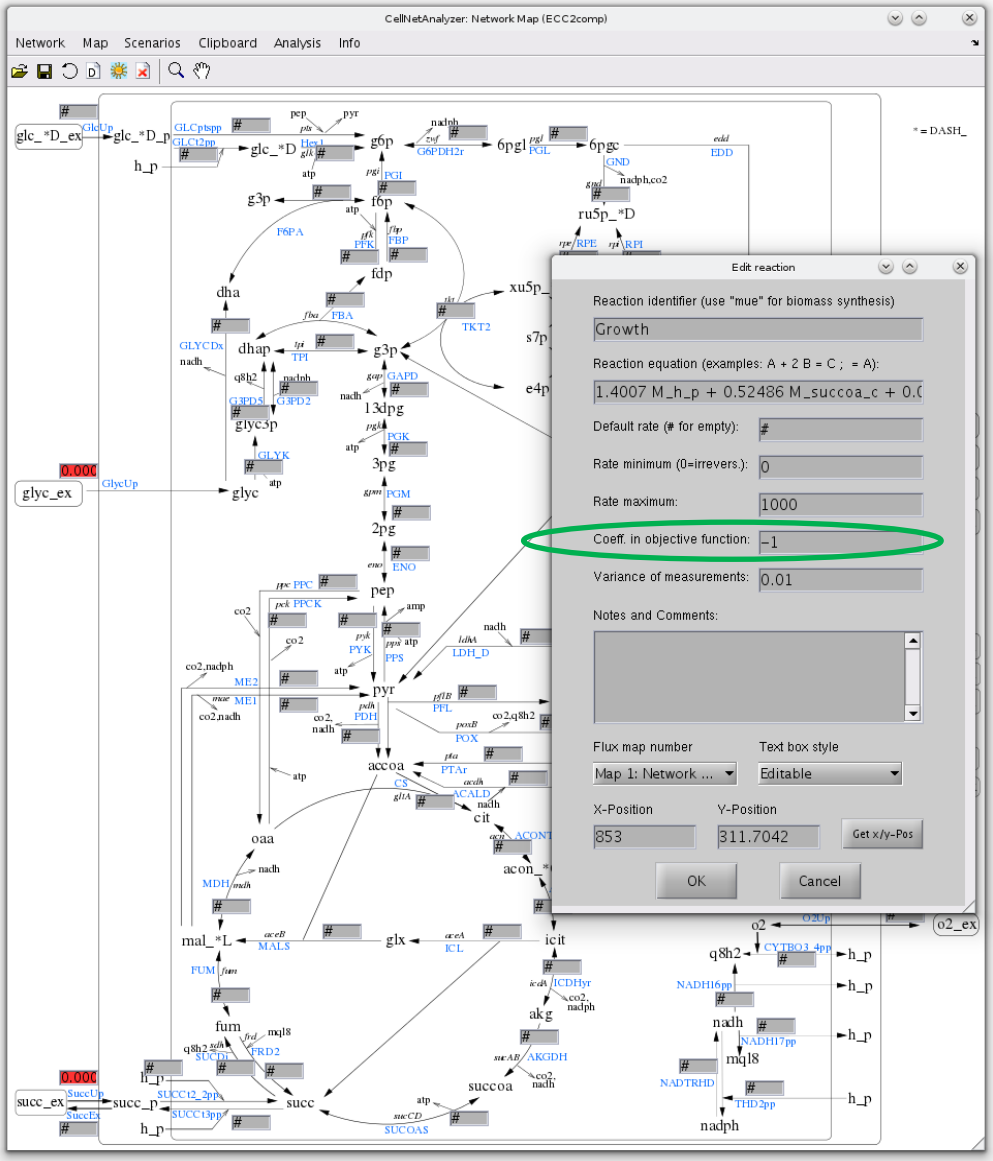
Find the optimal flux distribution maximizing cell growth on glucose as the only substrate. Subsequently, find the optimal flux distribution for ethanol production with some minimal growth.

- Press the -button in the window to set the default scenario (which is “growth on “glucose”)
- Verify that the glucose uptake reaction is set to a maximal rate of 10 in the field “Rate maximum” (Network→ Network Composer → “Edit/Info” –Button next to the “Reactions”-part in the window after selecting “R_GlcUp” -reaction). Alternatively click with the right mouse button at the reaction text box of “R_GlcUP”.

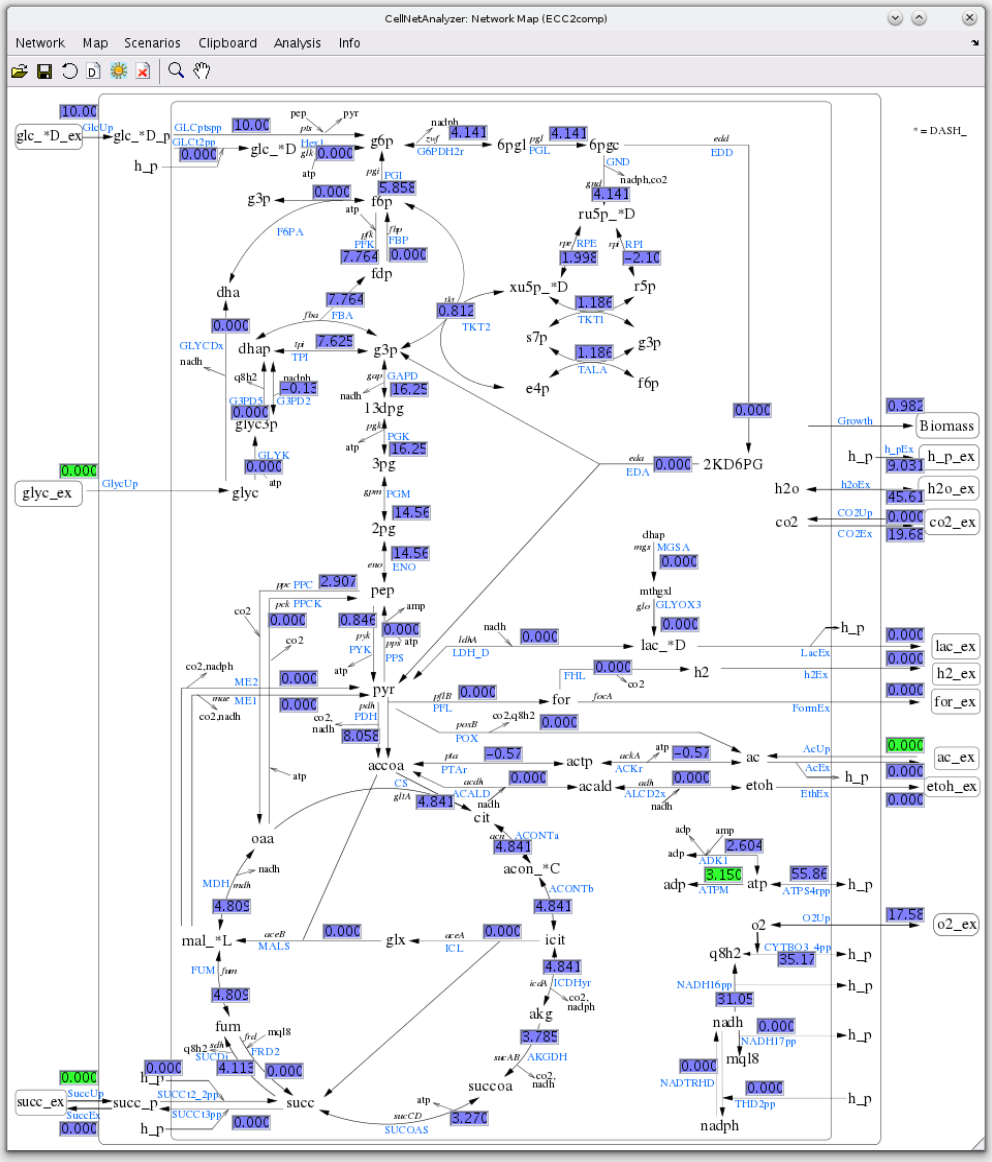


- Specify the right coefficient for the ‘growth’ reaction in the objective function as displayed below. Note that CNA **will minimize the** objective function, hence, for minimizing a rate you need to enter “+1” in the field “coeff. in objective function”; for maximizing enter “-1”; for no optimisation leave “0”. You may check the current objective function via

“Analysis→Flux balance analysis→Show objective function”



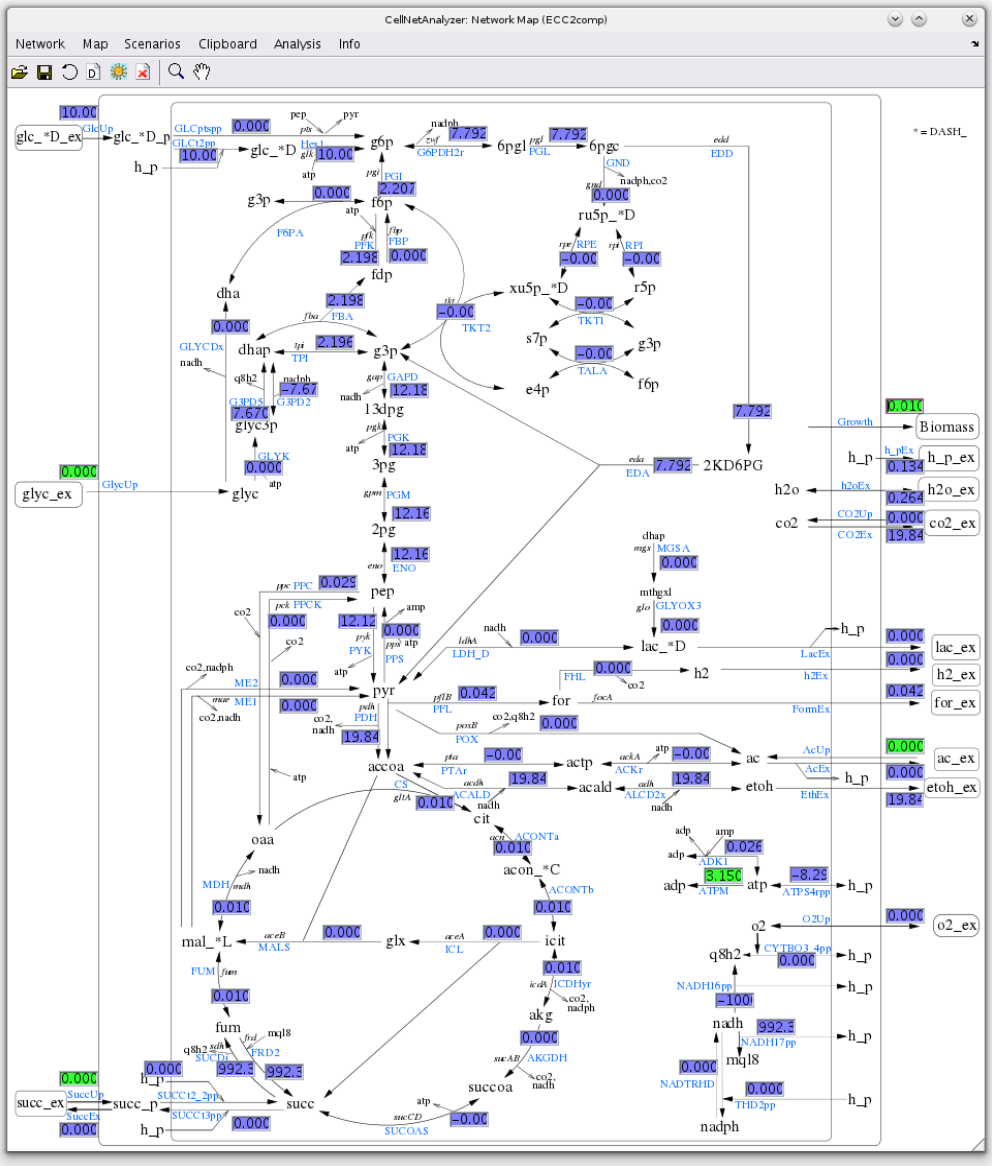
- For starting the flux optimization press: Analysis → Flux Balance Analysis (FBA) → Flux optimization
- Choose e.g. GLPK as LP solver.
- The resulting optimal flux distribution for the maximal biomass production is shown in the network maps.



- Result: maximal cell growth (without ATP maintenance demand) is at μ=0.98 h^{-1} with no by-product formation except CO_2

- For finding the optimal flux distribution for growth on glucose with a maximal ethanol production and a defined minimal growth rate (μ=0.01 h^{-1}): repeat the flux optimization as follows:
  - Press the  -Button (“Reset values”) and all values predefined from the previous calculation will be shown again
  - Set the coefficient in objective function of the growth reaction to “0”
  - Restrict the reaction rate of growth (μ) to 0.01 h^{-1} by typing the value directly into the reaction text box of the map
  - Set the coefficient for the objective function for reaction “R_EthEx” as demonstrated previously for the growth reaction (enter “-1” in the field “coeff. in objective function”). You may check the correct setting of the objective function via “Analysis→Flux balance analysis→Show objective function”
  - Start flux optimisation via: Analysis → Flux Balance Analysis (FBA) → Flux optimization

- The resulting optimal flux distribution for maximal ethanol production under some minimal growth is shown in the flux maps (catabolic map is shown below) and we can see that the maximum ethanol production rate is 19.84 mmol/gDW/h.



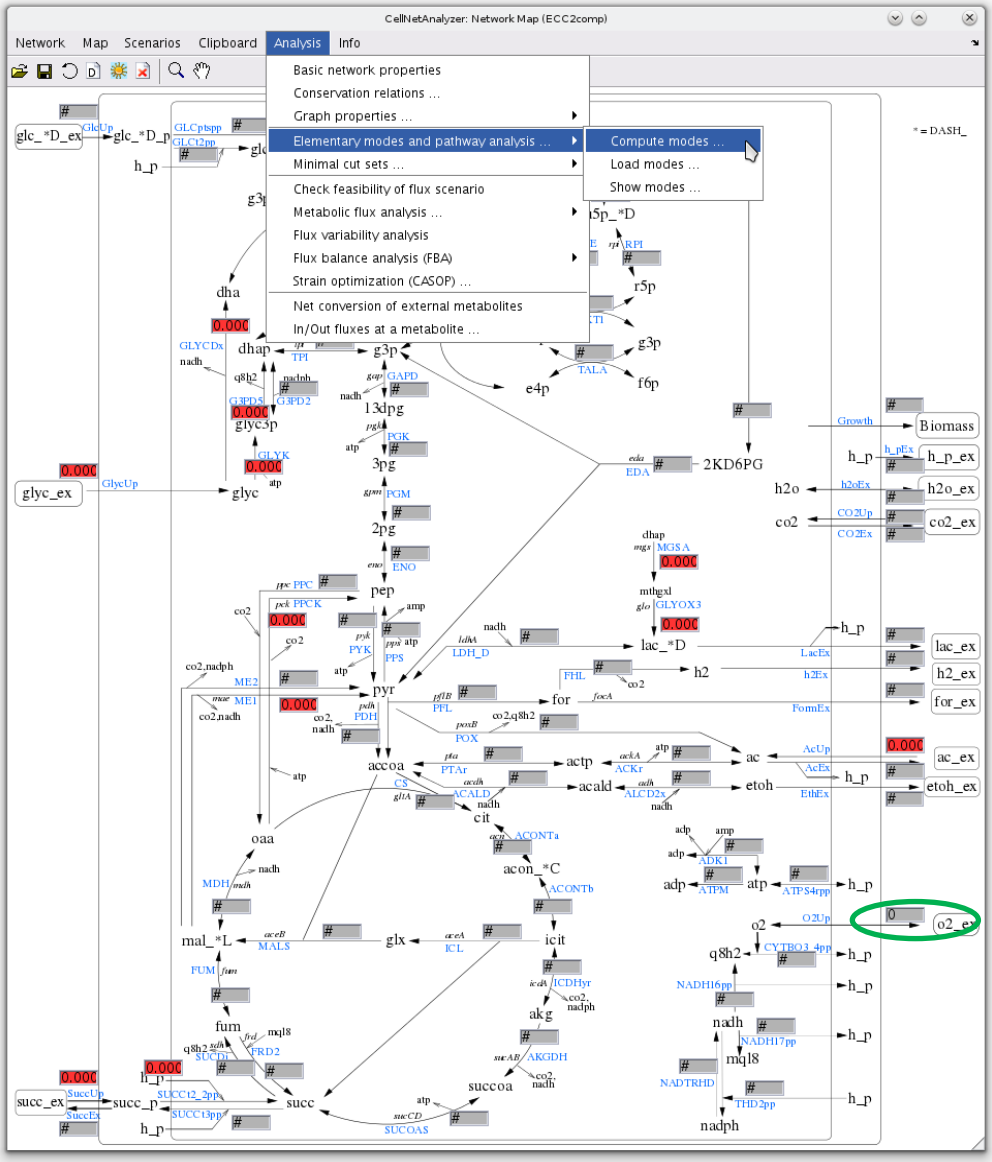
- Result: maximal ethanol production under these conditions is 19.84 mmol / (gDW h)

Use case scenario S3: Elementary modes (EMs) and Minimal Cut Sets (MCSs)

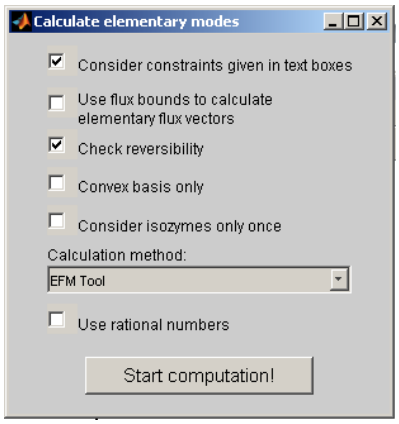
Compute elementary modes (EMs) for anaerobic growth on glucose in the central metabolism of *E. coli.* Visualize the 2D-yield plot for growth versus ethanol yield. How many modes exist, that contain in total only 20 reactions? Calculate the minimal cut sets (MCSs) deleting all EMs for anaerobic growth on glucose.

Calculating the EMs:

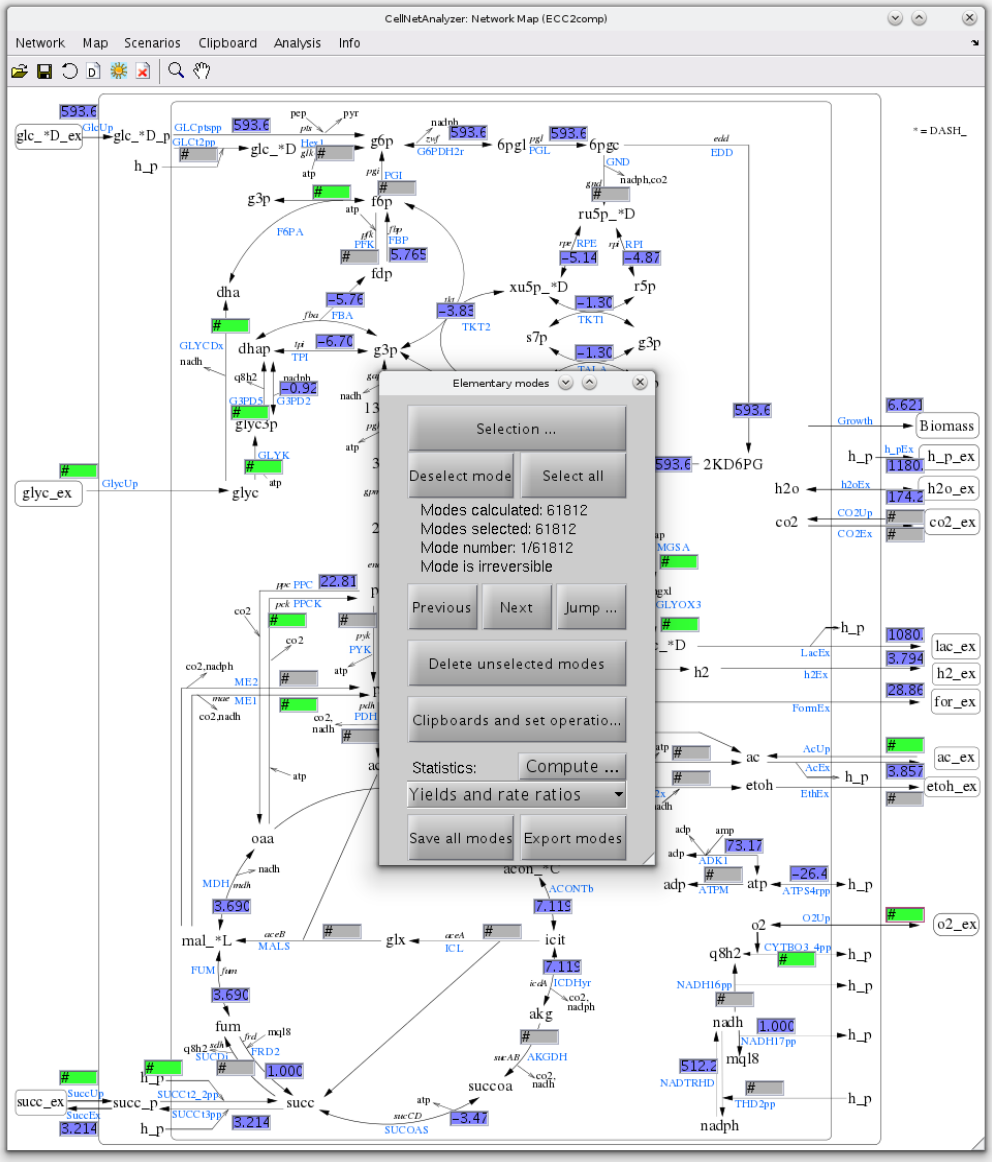
- Here we want to calculate the elementary modes for anaerobic growth on glucose. There is a predefined scenario file which fixes certain reaction rates to zero to simulate growth on glucose (this switches off substrate uptake reactions (succinate, acetate, glycerol) and some other reactions which are unlikely to be active under standard growth on glucose). Load the scenario file: Scenario → Load scenario and select the file "glucose_standard.val"
- **To ensure anaerobic conditions enter "0" in the box of reaction "R_O2up"**
- When CNA calculates the EMs, all reactions with a given zero rate will be excluded (hence, all computed EMs will not use these reactions).



- Calculate the EMs (Analysis → Elementary modes and pathway analysis → Compute modes); leave the options in the panel as they are (with efmtool as algorithm)

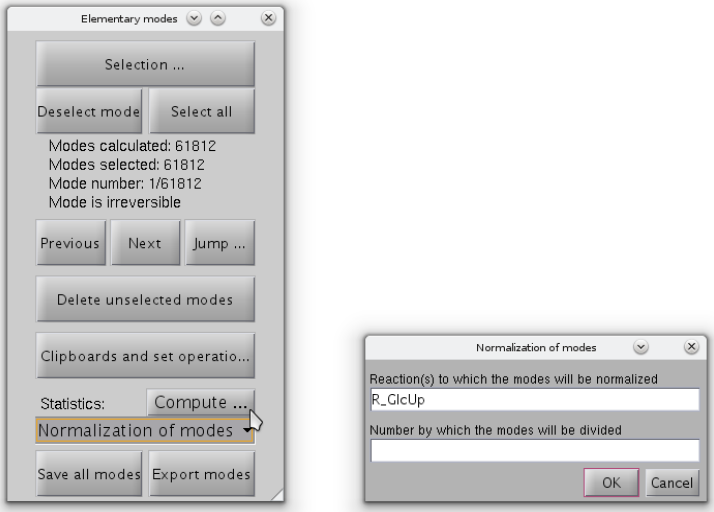


- Start computation, wait for the results: 61812 EMs are found
- The first EM is automatically displayed in the map and a control panel for EM analysis comes up. You may go through the set of EMs by clicking on “Next” or “Previous”



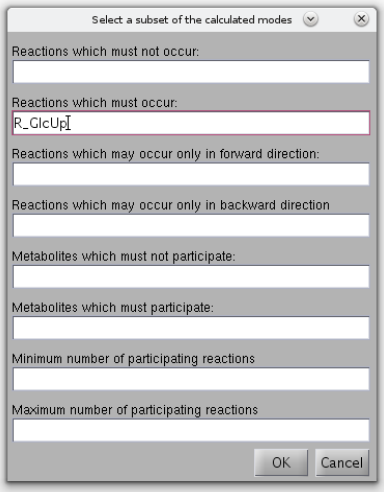
Select and normalize EMs with Glucose uptake:

- We should normalize the modes with respect to the substrate (glucose) uptake rate. Choose “Normalization of modes” in the “Statistics” pull-down menu and click on “Compute”. Write in the first line “R_GlcUp” to normalize all EMs with respect to their glucose uptake rate.

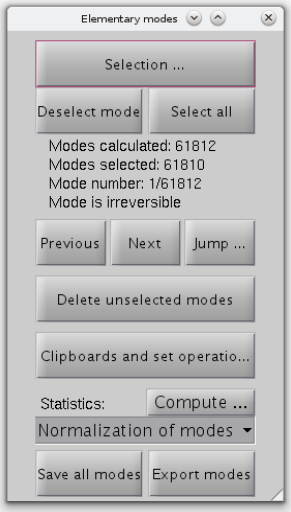


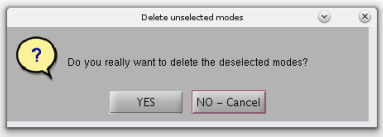
- Next we want to check whether there are (artificial) EMs that do not involve glucose uptake. Press the button “Selection…” in the “Elementary modes”-window and enter “R_GlcUp” as a reaction, that must NOT occur in the set of EM, and press “OK”. → There are two such EMs and in the next step we will delete them from the set of EMs.

- Press “Select all” in the “Elementary modes” window. Use then “Selection…” in the “Elementary modes”-window and enter “R_GlcUp” as a reaction, that must occur in the set of EM, and press “OK”. (61810 EMs are selected).



- Delete the two (artificial) unselected EMS via “Delete unselected modes”-button in the “Elementary modes”-window



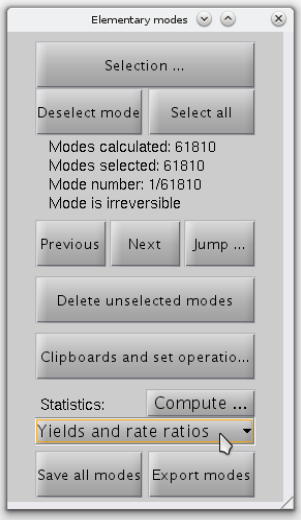


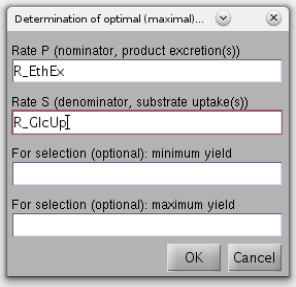
➔ Result: 61810 EMs are selected, all containing the glucose uptake reaction

➔ Before continuing you may save these EMs for later analysis via “Save all modes” (binary MATLAB file → this one can be reloaded by CNA) or via “Export modes” (as text file).

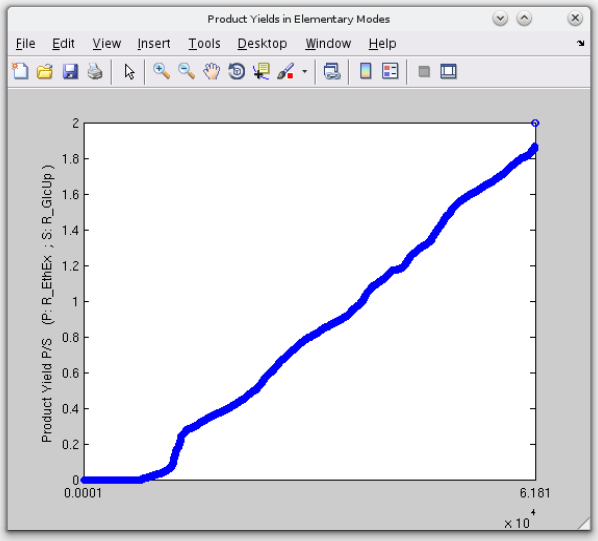
Analyzing product yields in EMs:

- We are now interested in ethanol as a potentially relevant product.
- Select “Yields and rate ratios” in the “Statistics” menu of the “Elementary modes” window and press the “Compute”-button
- Enter the names of product excretion reaction and the substrate uptake reaction (here: R_EthEx/ R_GlcUp)





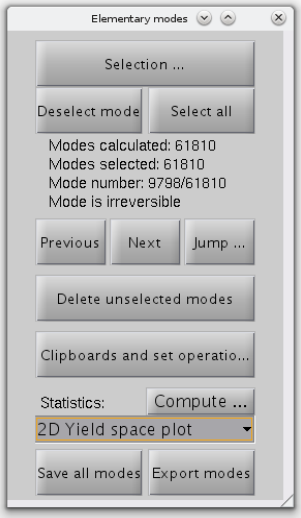
- The yield distribution within the EMs is shown in the graphic below. And more information is also given in the command window.

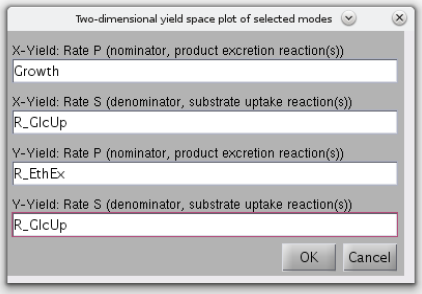


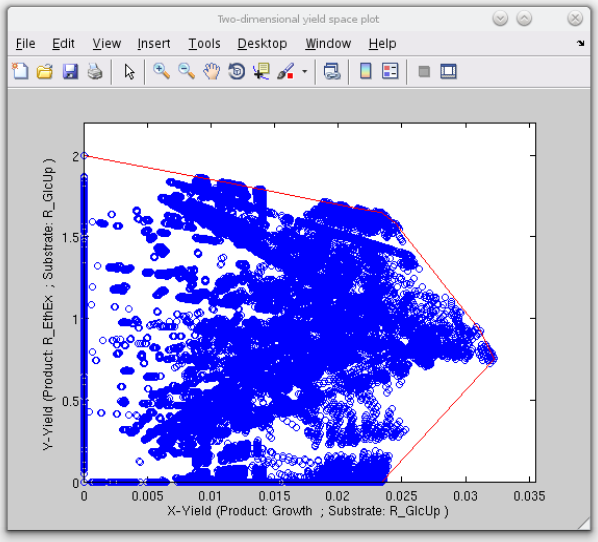
- You want to see the EMs with maximal ethanol yield of mmol/(mmol glucose)? Repeat the last step but write in the third row (Minimum yield) a 2. → 33 optimal EMs are selected. You can go through this set of EMs and study the different optimal pathways. You may compute Statistics (e.g. reaction participation) via the "Statistics" pull-down menu for these EMs.
- Click on "Select all" to select all EMs again.

Visualize the EMs in a 2D-Yield space plot: biomass yield vs. ethanol yield

- Ensure that all EMs are selected again (press "Select all").
- Select "2D Yield space plot" in the statistics part of the "Elementary modes" window and press the "Compute"-button
- Specify the yields to be displayed by giving the corresponding reaction names: first for the yield to be displayed at x-axis (product rate P: 'Growth' = biomass synthesis; substrate uptake rate S: 'R_GlcUp' = glucose uptake') and then for the yield to be displayed at the y-axis (product rate P: 'R_EthEx' = ethanol excretion; substrate uptake rate S: 'R_GlcUp' = glucose uptake)



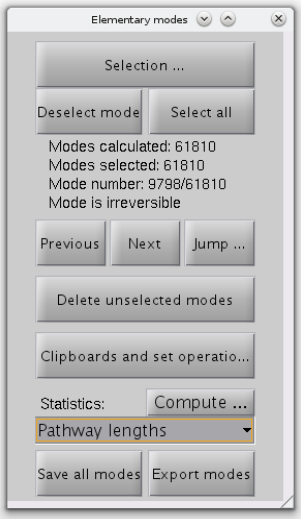


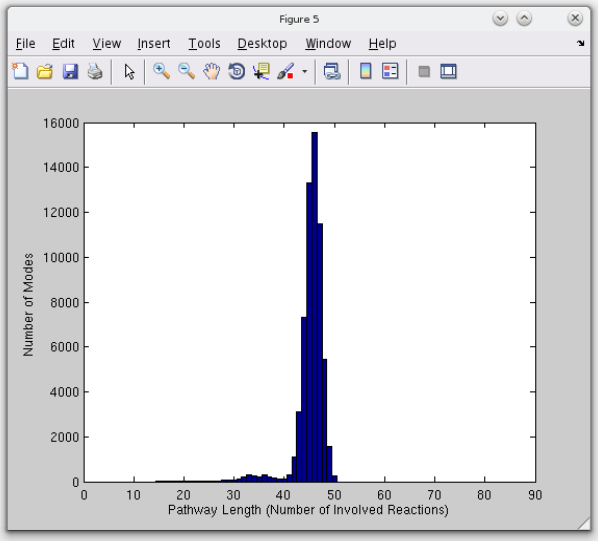


➔ Result: two-dimensional distribution of the elementary modes in the yield space with biomass yield on the x-axis and acetate yield on the y-axis. You can also see the maximum growth yield and maximum product (ethanol) yield again.

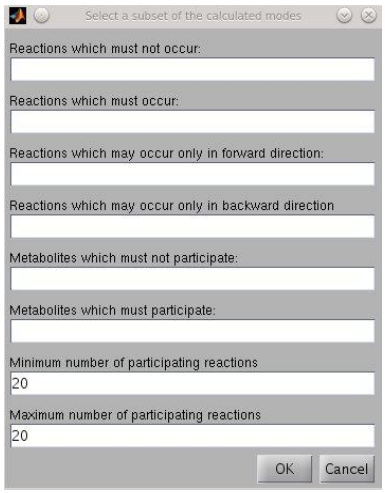
Analyze pathway lengths of EMs

- Select “Pathway lengths” in the statistics part of the “Elementary modes” window and press the “Compute”-button above



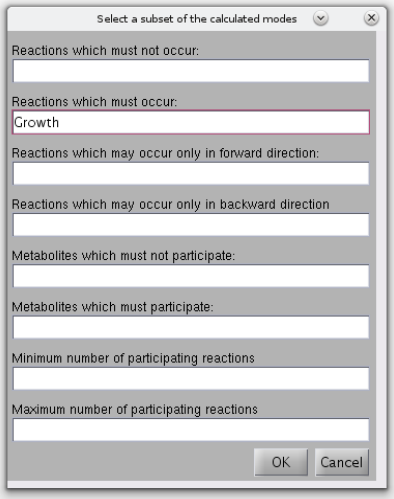


- You want to see the shortest EMs, e.g. using only 20 reactions or less? →
- Use the “Selection”-button in the “Elementary modes” window and enter “20” in the field “Maximum number of participating reactions”). → 42 EMs are selected

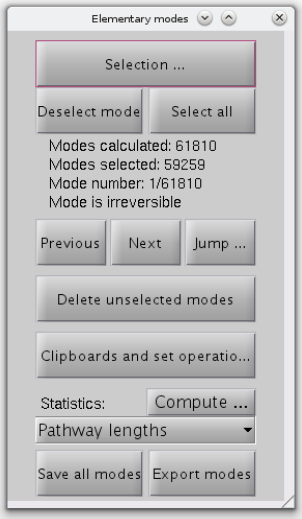


Select EMs with biomass synthesis:

- Press the “Select all”-button in the “Elementary modes”-window to select all EMs again.
- Press the button “Selection…” in the “Elementary modes”-window and enter “Growth” as a reaction, that must occur in the set of EM, and press “OK”



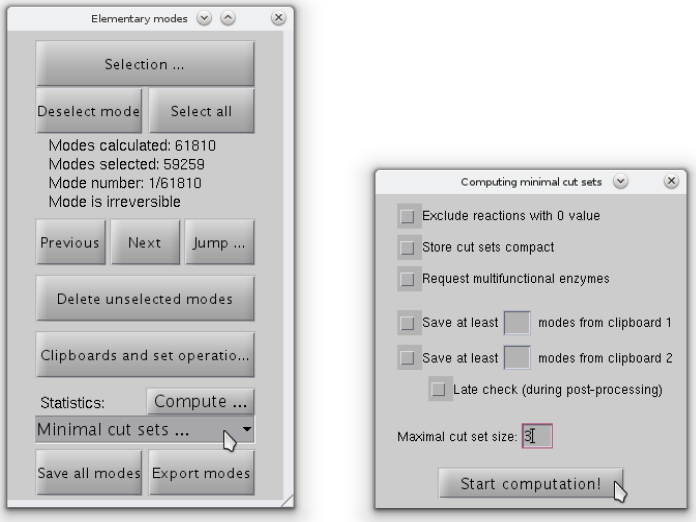
- The EMs with a growth rate >0 are selected, the first is displayed in the network map and the total number of selected EMs is shown in the “Elementary modes”-window



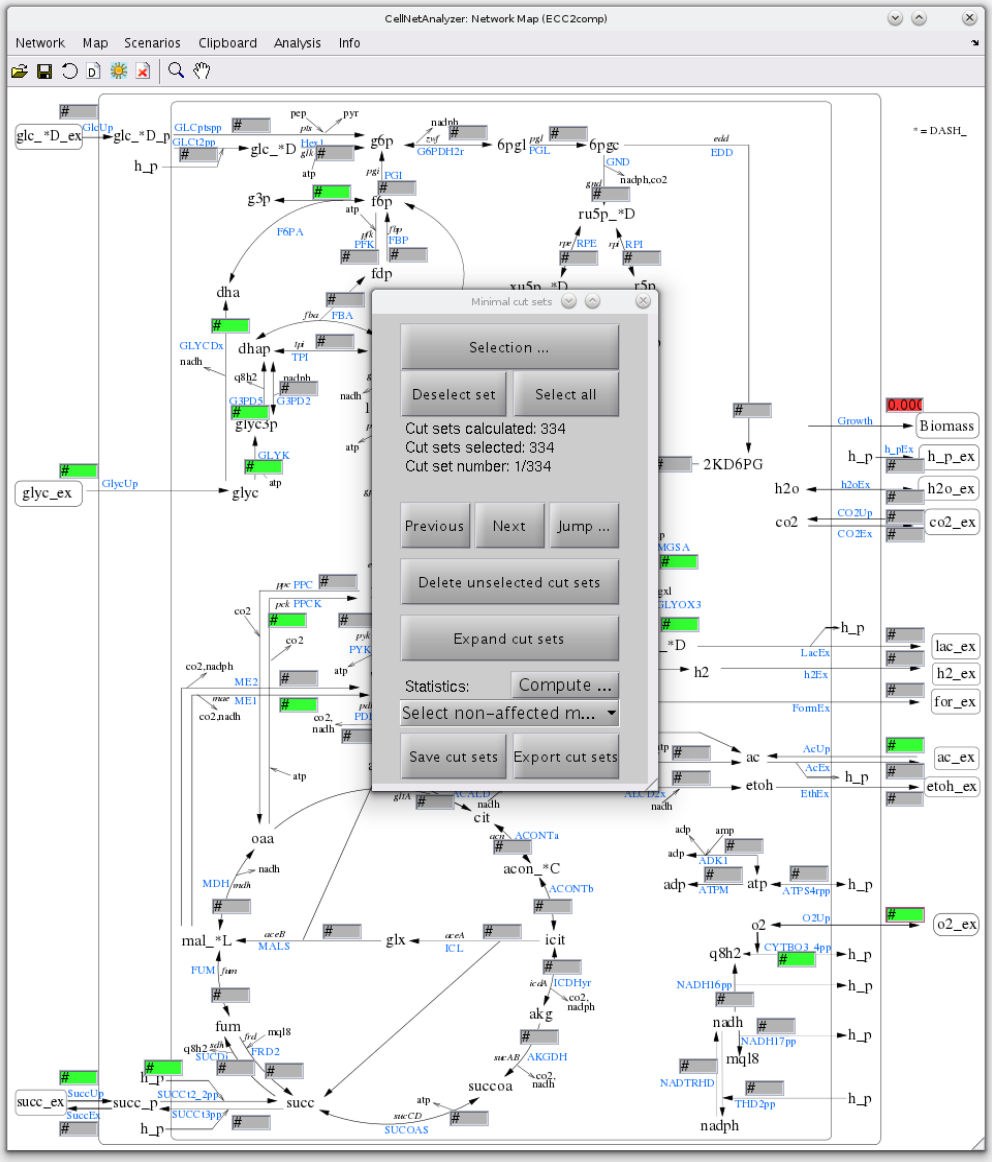
➔ Result: there are 59259 EMs allowing growth under anaerobic conditions with glucose as substrate.

Compute Minimal Cut Sets deleting all EMs for anaerobic growth on glucose:

- Work with the current EM-selection (all EMs with anaerobic growth on glucose) → these EMs will be deleted by the minimal cut sets to be calculated
- Select “Minimal cut sets ….” in the statistics part of the “Elementary modes”-window and press the “Compute”-button above
- Define the maximal cut set size (to accelerate the computation we choose here 3; when left empty all MCS are computed) and press the “Start computation!”-Button



- Note that CNA considers the SELECTED EMs as target to be deleted (here: all EMs for anaerobic growth on glucose)
- Wait for the results (you can check the progress in the command window): 334 MCSs up to size 3 are calculated for blocking anaerobic growth on glucose and the first is automatically displayed in the map
- Note that CNA also finds the trivial MCS of deleting the Growth reaction itself, which is shown as first MCS



- Explanation of the results:
  - The map of the catabolic network displays the comprised reactions of the calculated MCS in red in the corresponding reaction field
  - Using the “Next” or “Previous”-Buttons in the “Minimal cut set”-Window the user can jump between the calculated MCS
  - By using the “Jump”-Button in the “Minimal cut set”-Window the user can jump to a specific MCS by entering the number of the MCS

Use case scenario S4: constrained MCS for strain design

Calculate constrained MCSs (cMCSs) from the calculated EMs such that an ethanol yield of at least 1.5 mmol / mmol glucose is guaranteed while some minimum biomass yield (0.01 gDW/mmol glucose) is feasible. Restrict the MCS-size to 10. Show the histogram for all calculated cMCS-sizes. Verify one of the calculated cMCSs by flux optimization.

Procedure for calculation of constrained Minimal Cut Sets:

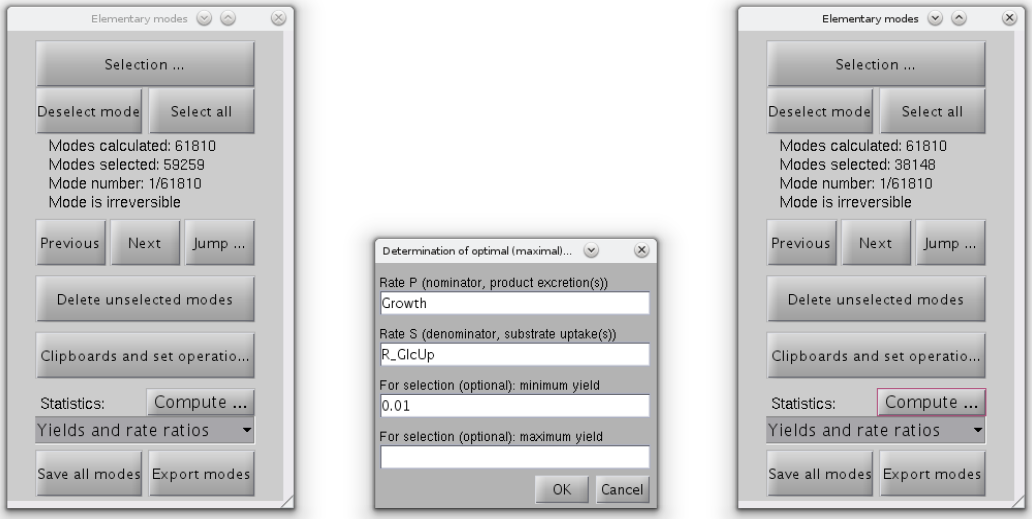
- define the set of desired EMs (DM), which will here be specified via 2 criteria:

1. select EMs with biomass yield $Y_{growth/glc} \geq 0.01$ (≙ TMP1)
2. select (on TMP1) EMs with ethanol yield $Y_{eth/glc} \geq 1.5$ (≙ DM1)

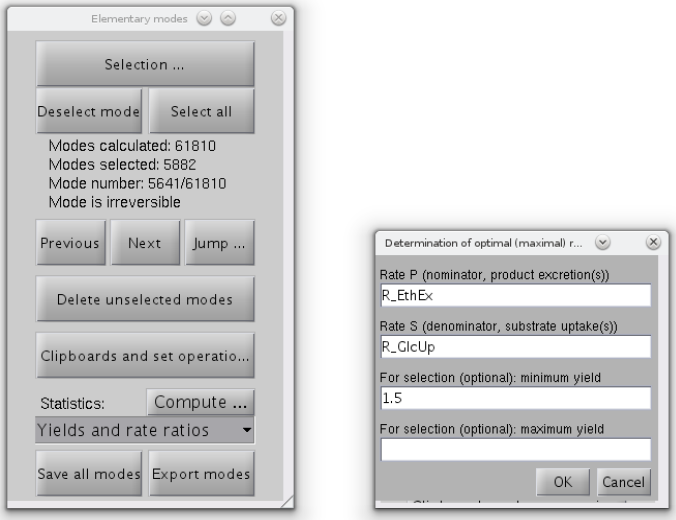
- define the set of target EMs (TM) to be deleted: EMs with $Y_{eth/glc} <= 1.5$
- calculate the cMCS; each cMCS will delete all target EMs (TM) and maintain a predefined number of desired EMs (DM).

Procedure in CNA:

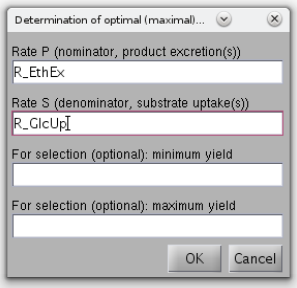
- We use again the EMs from the last scenario. Close the “Minimal cut sets”-window from the previous calculation.
- Select again all 61810 EMs.
- Select “Yields and rate ratios” in the “Statistics” pull-down menu and press the “Compute”-button
- Select all EMs with $Y_{growth/glc} \geq 0.01$ (→ set TMP1, cell growth is enabled). → Result: 38148 EMs are selected (the yield distribution over all EMs is also shown again).

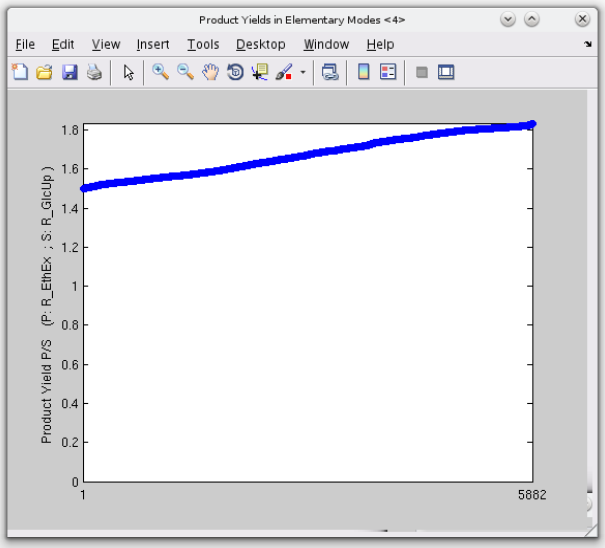


- from the current selection, select the subset of EMs that additionally fulfill $Y_{eth/glc} \geq 1.5$ (analogous as done before for the biomass yield).

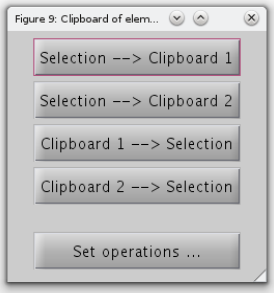


- Result: 5882 EMs selected → these are the desired EMs (set DM). You may check the current ethanol yield distribution from the current selection again via “Yield and rate ratios” (but this time without selecting for a min/max yield).

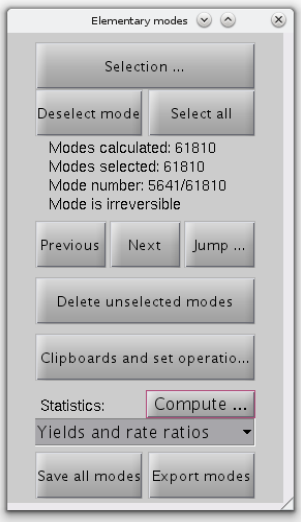


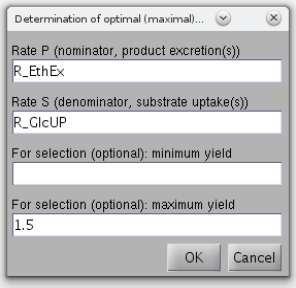


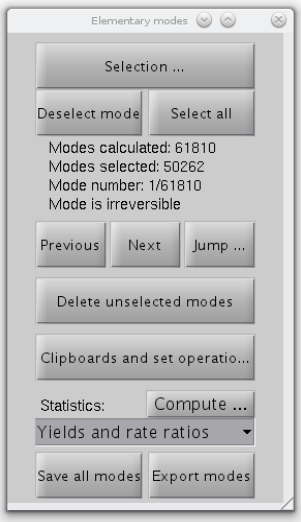
- We “save” the current selection of desired EMs on EM clipboard 1 by using “Clipboards and set operations”-button and then “Selection 1 → Clipboard 1”.



- For specifying the target modes (which need to be “hit” by the MCSs) select first the complete set of EMs again by pressing the “Select all”-button in the Elementary modes”-window and select then all EMs with ethanol yield below 1.5.

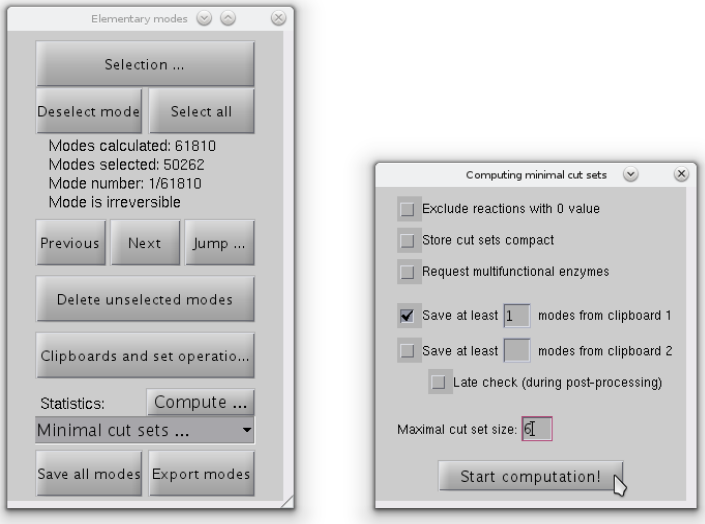




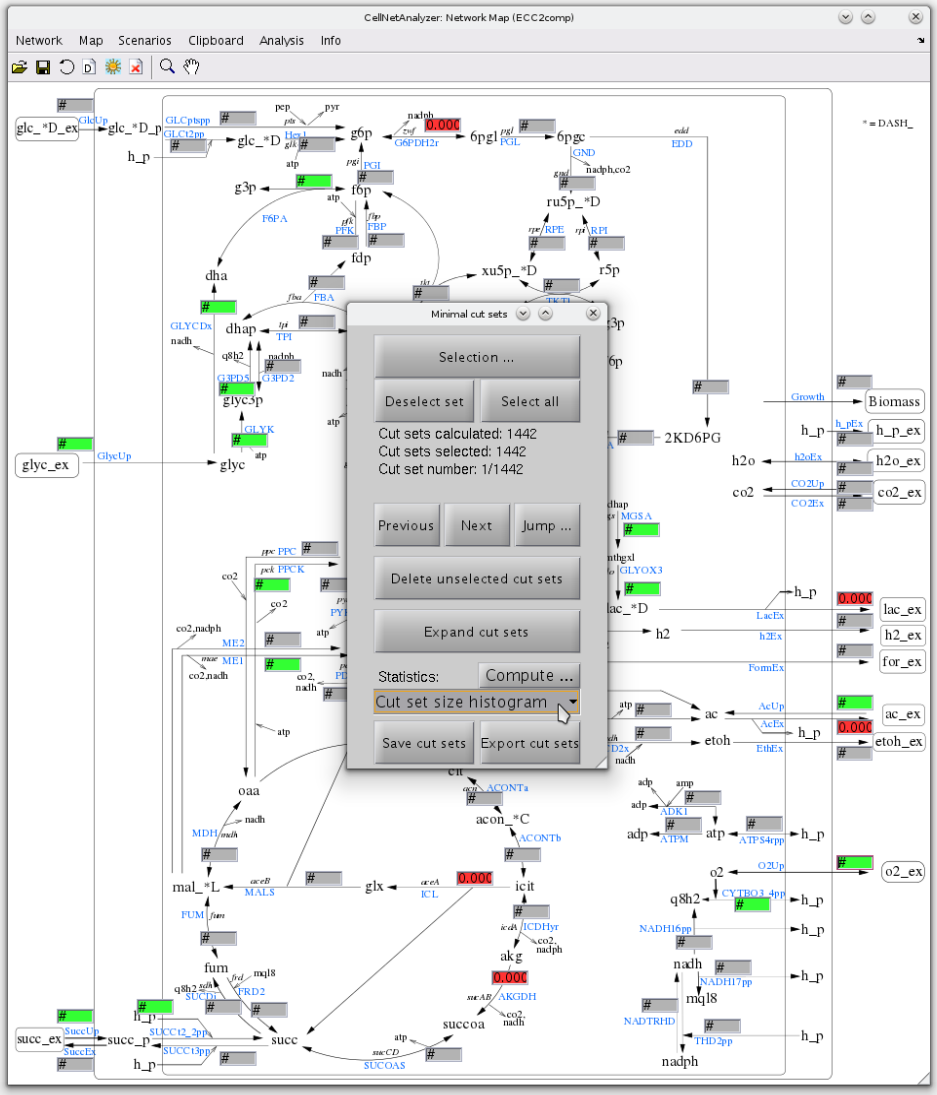


- Result: 50262 (target) EMs selected

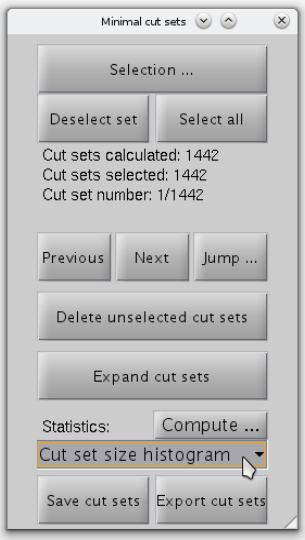
- Computation of constrained MCS:
  - Select “Minimal cut sets ….” in the statistics part of the “Elementary modes” window and press the “Compute”-button above
  - to maintain at least one desired EM (DM1) tick the selection “save at least…modes from clipboard1” and enter the number of modes that should be conserved (here: we choose 1)
  - define the maximal cut set size (here we choose 6) and press the “Start computation!”-button
  - Again: the current selection of EMs represents the set of target modes for the cut sets to be calculated!

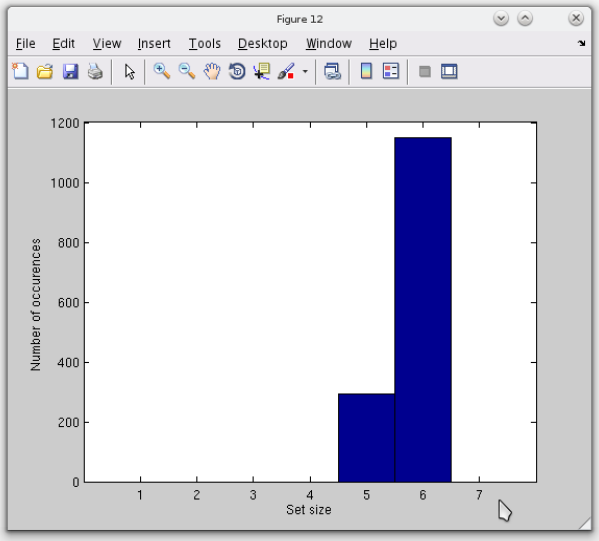


- Wait for the results (may take some minutes): 1442 cMCSs exists for the chosen constraints. The first cut set (comprising 5 KOs) is displayed. (for general explanations on how to handle and interpret the MCS see use case scenario S3)



- To show the MCS size distribution compute the histogram by selecting “Cut set size histogram” in the statistics part of the “Minimal cut sets”-window and press the “Compute”-button above. You can see that the minimum cut set size is 5 (anhd not larger than 6 since we set an upper bound of 6 for the cut set size).

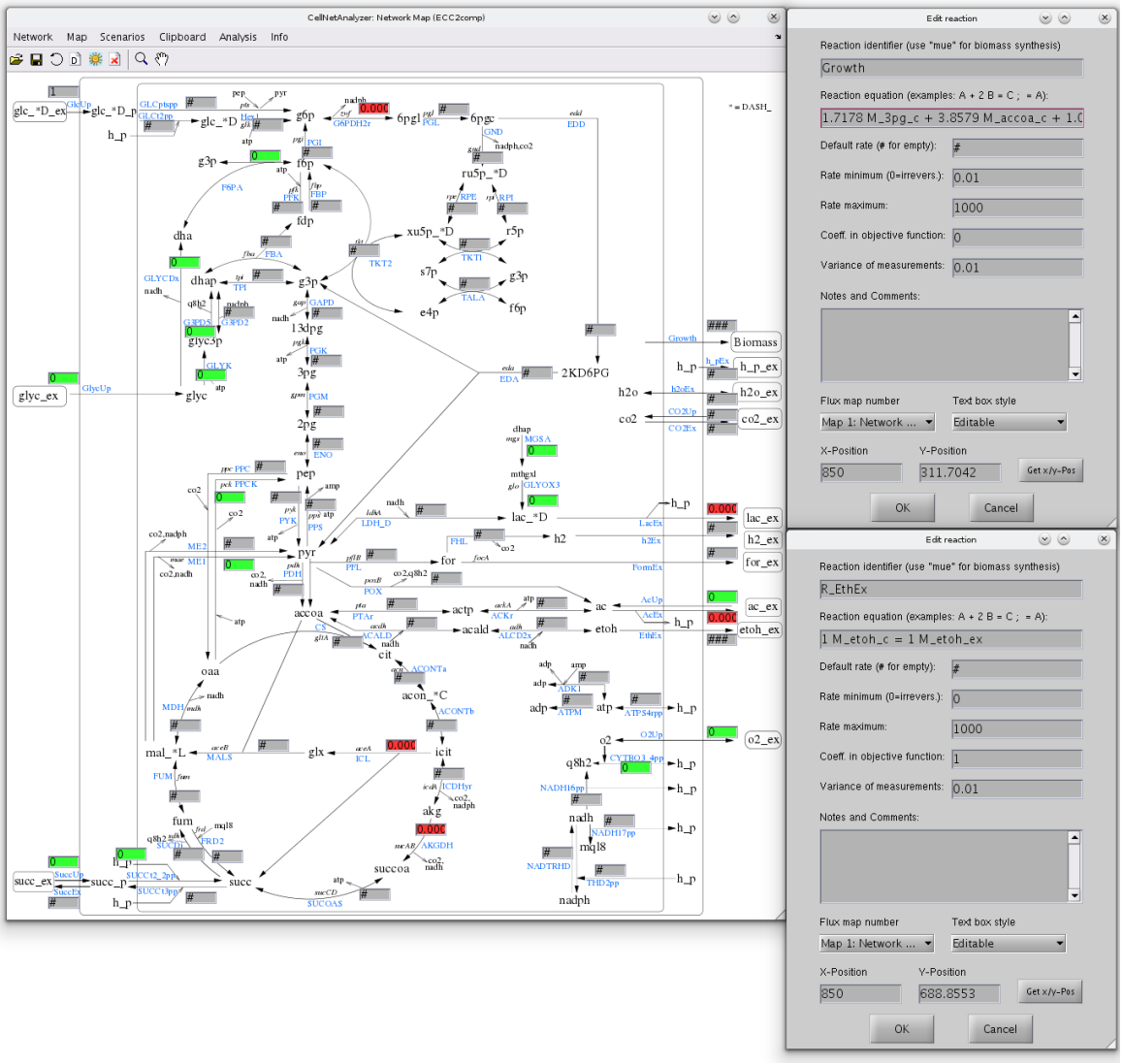




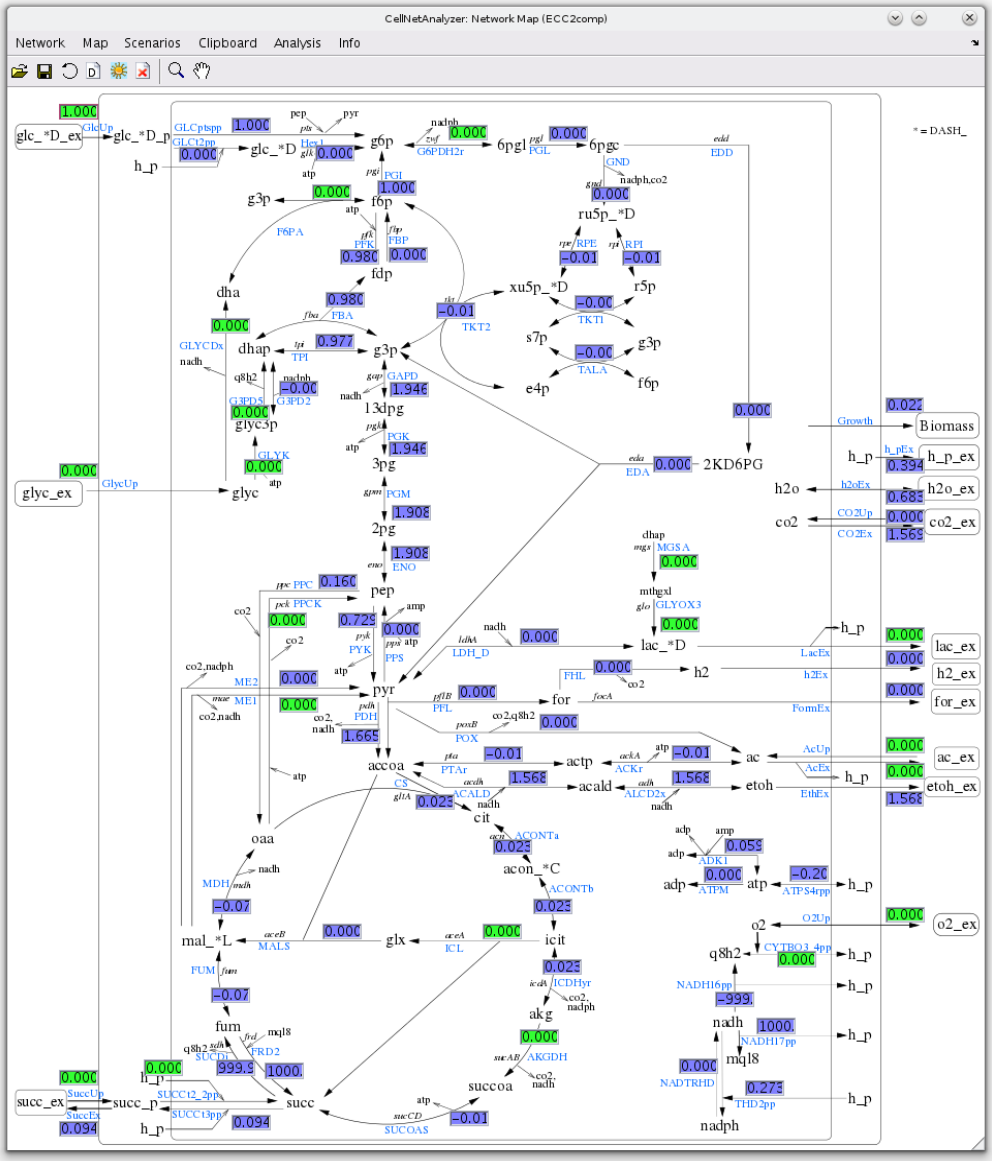
Verify correctness of the calculated cMCSs: flux optimization for minimal acetate yield

- We verify one of the cMCSs calculated above: if we “implement” an MCSs (with glucose as the only substrate, oxygen uptake is 0, and with a minimum biomass yield of 0.01) then the minimum ethanol yield must be 1.5.
- We take the first calculated cMCS which comprises five reaction knockouts (R_AcEx R_LacEx R_AKGDH R_ICL R_G6PDH2r) → these reactions are displayed with zero values in the flux map.

- In addition we need to set zeros for the “off-reactions” for anaerobic growth scenario on glucose. Luckily, these reactions are indicated in green in the flux maps. So set a zero value in all green text boxes. (Alternatively you might load the scenario file “glucose_standard.val” and then add zero rates for the five reaction knockouts of the cut set).
- Set a (normalized) uptake rate of glucose “1” in the associated text box of of glucose uptake rate.
- Modify the properties of reaction “Growth” such that the minimum rate is 0.01 and set the coefficient in the objective function for growth to 0 (you may click with the right button of the mouse into the text box of the “Growth” reaction).
- Modify the objective function value for reaction R_EthEx such that it is **minimized** (we therefore need to enter “+1” in the field “Coeff. in objective function”)
- Ensure that no other reaction in the network is participating in the objective function (you may check the current objective function via “Analysis → Flux balance analysis (FBA) → Show objective function”)



- Start flux optimisation (Analysis → Flux balance analysis (FBA) → Flux optimization) choose GLPK as LP solver)
- The resulting optimal flux distribution for the chosen cMCS with the minimal ethanol production is shown in the flux maps.



- Result: the minimum resulting ethanol yield for the chosen cMCS is ~1.56 and thus greater than 1.5 as demanded.

Use case scenario S5: Network adjustments

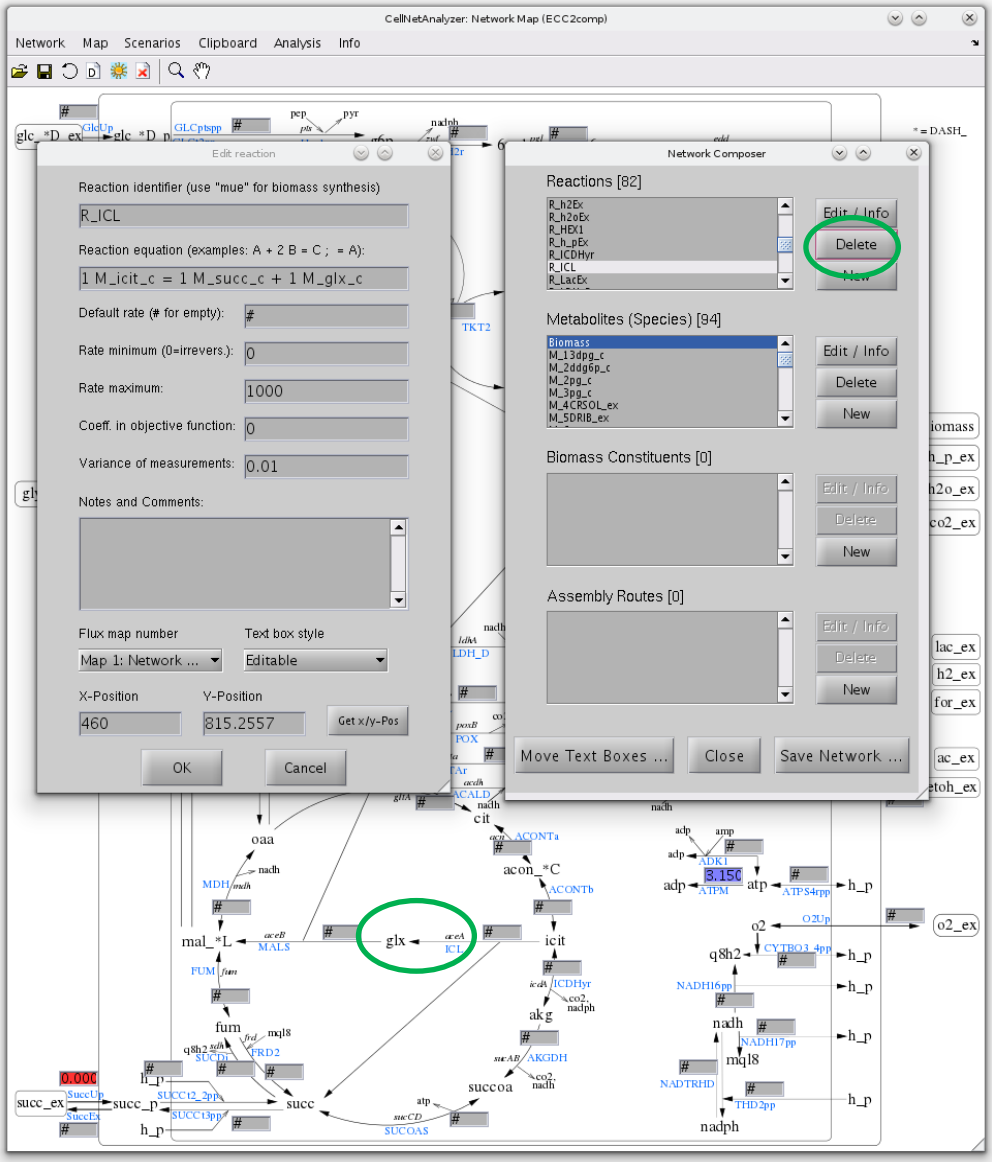
Modify the metabolic network by

(A) deleting the reaction of the glyoxylate pathway that converts isocitrate to glyoxylate and succinate

(B) adding an irreversible reaction for the uptake of pyruvate

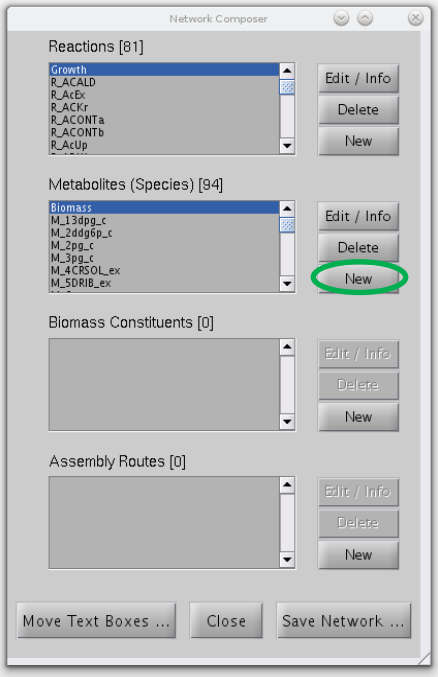
Task (A):

- to identify the reaction name click with the right cursor into the reaction field on the map, that needs to be deleted
- open the network composer (“Network → Network composer”)
- select the corresponding reaction “R_ICL” in the reaction list and press “Delete”

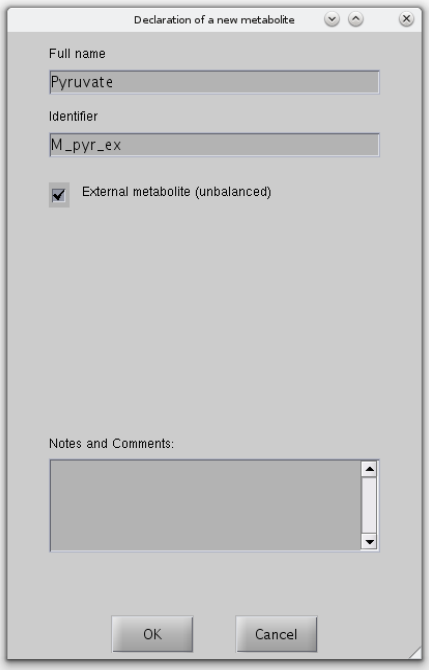


Task (B):

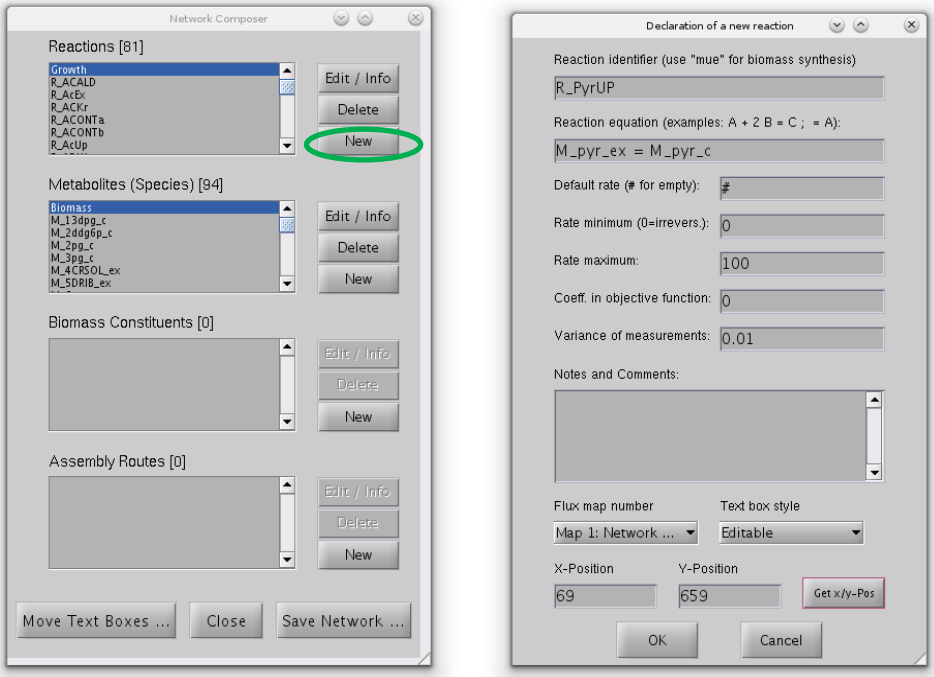
- open the network composer (Structure → Network composer) and press the “New”-Button next to the Metabolites list



- define the external Pyruvate as a new metabolite and set it as an external metabolite



- press the “New”- Button next to the reactions list and define a new reaction R_PyrUp (“Get x/y-Pos”-Button locates the reaction field within the catabolic map)



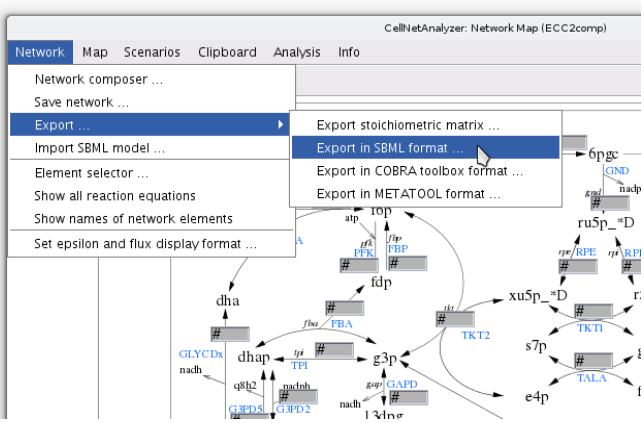
The textbox of the new reaction will appear. Note that (graphical) changes in the map need to done by the user outside of MATLAB, by using certain drawing programs (e.g. Inkscape)).

Note: once you have changed a network project and would like to save the made changes you can click on the “Save network …” button in the network composer window (but you should maybe not save this changed ECC2compressed example project).

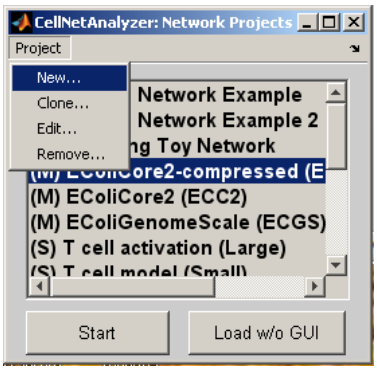
Use case scenario S6: Export and import of SBML models and creating new CNA projects

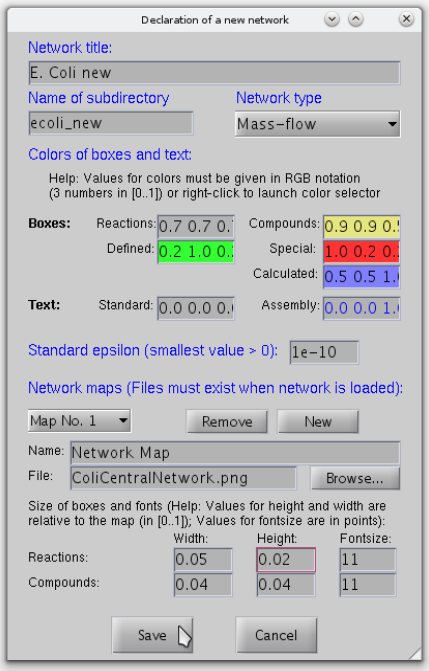
In this scenario we will first export the model in SBML format and then, to illustrate creation of a new CNA project and import of SBML models, build a new network project (which is then essentially a copy of the original model).

- To export the E. coli model as SBML file choose Network → Export → Export in SBML format from the CNA menu.

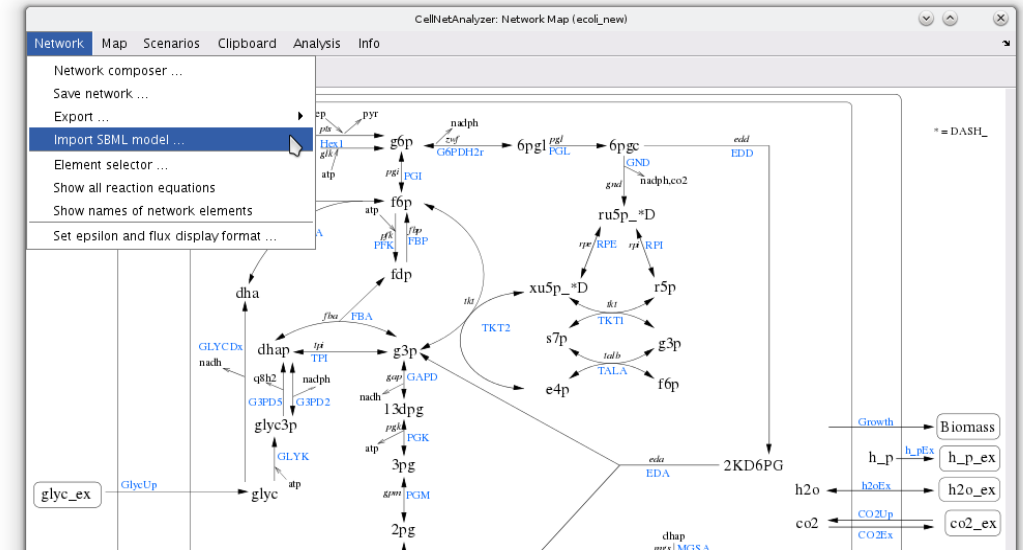


- Create directory ecoli_new and copy the map from ECC2comp/ColiCentralNetwork.png to ecoli_new/ColiCentralNetwork.png
- Initialize a new network project by choosing (Project → New) from the menu of the CNA Project manager.
- Name the project “E. Coli new”, the project directory “ecoli_new”, choose the copied E. Coli map as network graphics, adapt the text box sizes for reactions and then click the “Save” button.



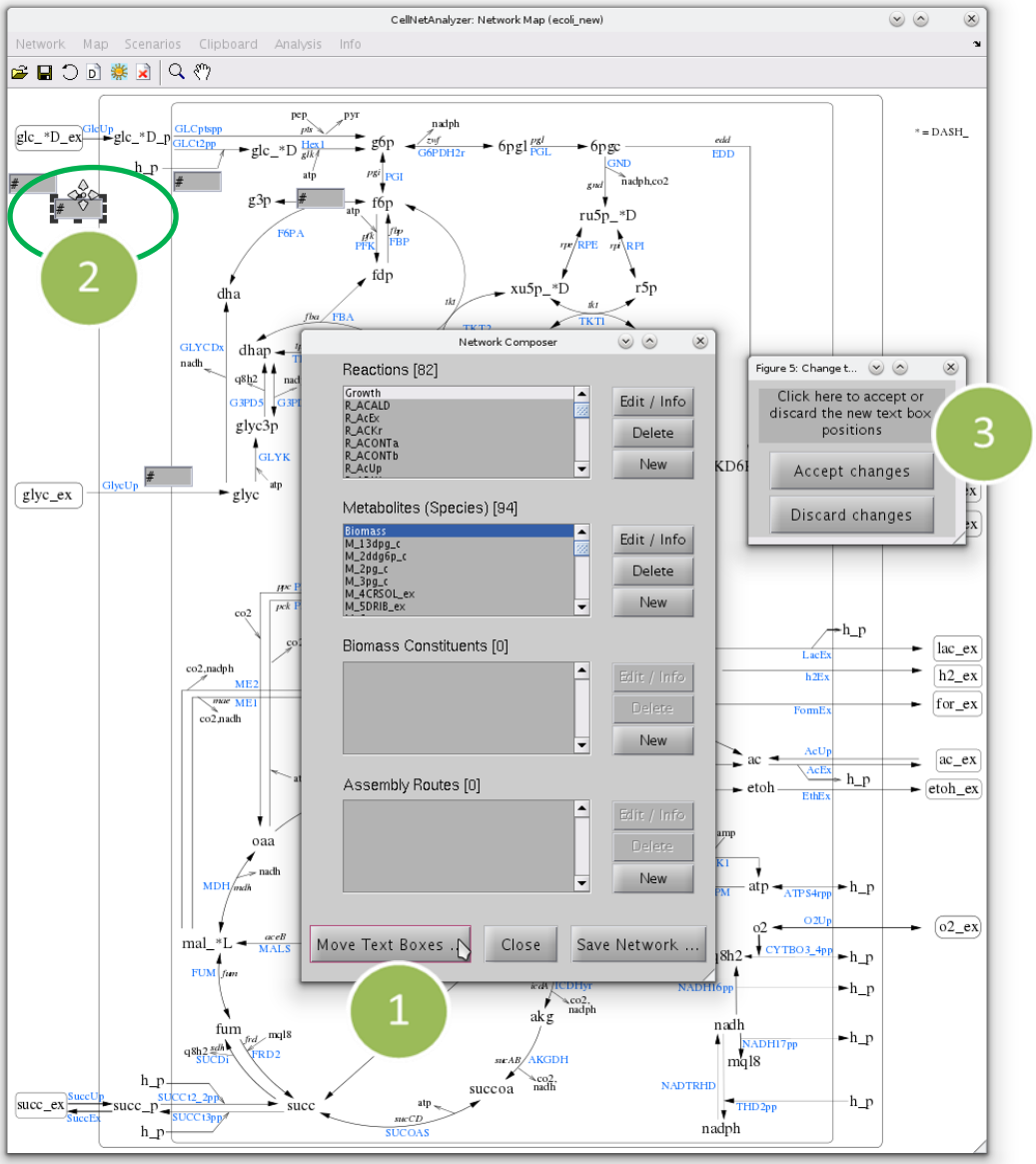


- Import the previously saved SBML file into the new project by choosing (Network → Import SBML model) from the CNA menu.



- Now, the model has been loaded, however, all text boxes are initially placed on the upper left corner on the map and you must now move the text boxes to the right position on the map. To identify which text box belongs to which reaction we click Network → “show

names of network elements". Then open the network composer from the CNA menu (Network → Network composer), click "Move Text Boxes" (1), drag the text boxes to their desired position on the network map (2), and finally accept the new text box positions (3).



Use case scenario S7: Application Programming Interface (API) of CNA

Many of the CNA-commands/calculations that can be started from the CNA GUI can also be started from MATLAB's command line by using CNA's Application Programming Interface (API). Moreover, the GUI of a CNA project can be accessed, e.g. to read/write values from/to the text boxes in the network maps. For general information regarding CNA's API and its application see manual chapter 7. The complete set of CNA's API functions can be found in

the directory *CellNetAnalyzer/code/api*. For information on a particular API function you may enter 'help <API function>' (note: CNA must have been loaded before). Most API function receive as input a CNA project variable.

Some examples of API functions:

- CNAcomputeEFM: Compute elementary modes of the network
- CNAoptimizeFlux: calculate optimal flux distribution.
- CNAfluxVariability: flux variability analysis
- CNAdeleteSpecies/ CNAdeleteReaction: Deletion of single metabolite or reactions from the network
- CNAreadMFNValues / CNAwriteMFNValues: reads values from / writes values to the text boxes within the GUI of a CNA project

Example 1: We want to recompute the elementary flux modes (EFMs) from scenario S3 (anaerobic growth on glucose) in the ECC2comp network, this time from command line:

- Redo the first steps of scenario S3:
- Load the scenario file which fixes certain reaction rates to zero to simulate growth on glucose (this switches off substrate uptake reactions (succinate, acetate, glycerol) and some other reactions which are unlikely to be active under standard growth on glucose). Load the scenario file: Scenario → Load scenario and select the file "glucose_standard.val"
- **To ensure anaerobic conditions enter "0" to the reaction field "R_O2up"**
- We now need to read the values from the text boxes (these fluxes need to be set to zero for EFM calculation). Therefore, enter in the MATLAB command window:

  [reacval]=CNAreadMFNvalues(ECC2comp);

  'ECC2comp' is the network project variable of the project. The returned vector "reacval" contains the values of the reaction rates currently set in the text boxes in the map (NaN for unknowns ('#')).
  - We now start the calculation of EFMs from command line by:

    efm = CNAcomputeEFM(ECC2comp,reacval);

    The "reacval" variable indicates the reactions that must be set to zero. The standard solver used for EFM calculation is *efmtool*.
  - The calculation (~ 5 min) yields 61812 EMs (the same number as found earlier in scenario S3) which are stored as rows in the matrix variable efms (the columns correspond to the reactions; the IDs of the reactions can be found in ECC2comp.reacID).

Example 2: Repeat use case scenario S5 - Task (A) (deletion of a reaction):

- Load the ECC2compressed project
- Enter in the MATLAB command window:

  [ECC2comp]=CNAdeleteReaction(ECC2comp,strmatch('R_ICL',ECC2comp.reacID));

  Remark: "strmatch('R_ICL',ECC2comp.reacID)" gives the reaction index for the reaction 'R_ICL' that is to be deleted.
- reaction "R_ICL" has now been deleted in the "ECC2comp" project (you will also see that the corresponding reaction box has disappeared at the flux map).

For further information see:

CNA web site (with manual): https://www2.mpi-magdeburg.mpg.de/projects/cna/cna.html

User forum: https://groups.google.com/forum/#!forum/cellnetanalyzer-user-forum

Contact: klamt@mpi-magdeburg.mpg.de