BioNSi Cytoscape app – User Instructions

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Installing BioNSi

- In Cytoscape menu bar: Apps > App manager
- Go to the "Currently Installed" tab. Make sure a previous version of BioNSi is not installed. **If an older version is installed:**
    - o Choose the old BioNSi version and click Uninstall
    - o Close Cytoscape and then open it again.
    - o Open the App Manager again
- Under "Install Apps" tab, click "Install from File".
- You should see a new menu, a graph button, and a replay button. All BioNSi features are controlled from this menu.
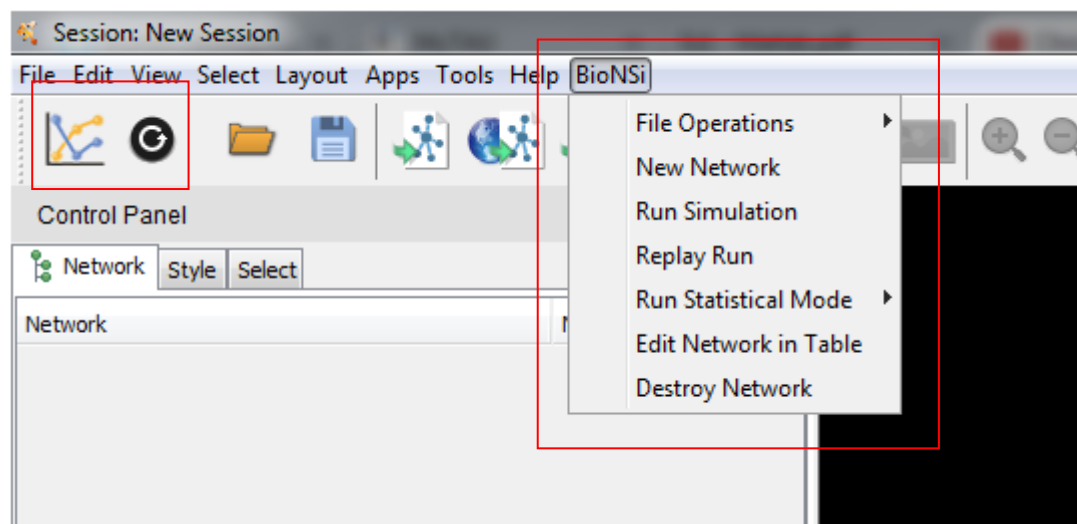
Creating a New BioNSi Network

- BioNSi >New Network
- There can only be one BioNSi network open at all times.

Opening a Previously Saved BioNSi Network

- BioNSi >New Network
- File > Open
- Choose your file (extension .cys)

Saving a BioNSi Network

- The same as saving any project in Cytoscape: File>save

Building and Editing Your BioNSi Network

Add new node

- Right click on the canvas > Add >Node. A "new Node" dialog box is opened.
- Set node's properties and press Ok.
- The new node will appear on the canvas.

- Node names should be unique in the network. If a name identical to a node that already exists in the network is inserted a number will automatically be added to the end of the new nodes' name.
- The color you will choose for your node will also be used to represent this node in the chart showing network step-by-step simulations. We recommend choosing different colors so you will be able to easily understand the results chart.
- Node shape is only a visual property - you can choose it as you wish.
- **Regular nodes** have 2 main properties**:**
    a. initial state – can be set between 0 and 9.
    b. maximum state limit - can be set between 0 and 9. (This is independent of the initial state and can be set higher, lower or equal to it)

- **Timed node:** Has 3 properties you can set. Unlike regular nodes, timed nodes states are not influenced by the changes in the network during a simulation run. (Usage example: pulse of light, day and night, injection).
    a. **Time table -** list of timed events. Each row describes a state and the amount of time that the node will be set to this state.
    b. **Lag -** step in simulation the timed node will enter and start following its pre-set time table.
    c. **Cyclic -** determine if the events in the time table will occur once or repeatedly. If left unmarked – the state after this will be 0.

Add new edge
- Right click on the <u>source</u> node > Add >Edge
- Draw the edge to the desired target node.
- Set edge properties in the opened "New Edge" dialog box and press Ok.
    a. **Weight** - the relative strength of the effect of the source node on the target node
    b. **Delay** - the number of simulation steps that the effect of the source node on target node will be delayed. If set to 0 (the default), the effect will take place right in the next simulation step.
    c. **Dependencies -** a set of rules conditioning when the edge is activated. If the conditions do not hold in a specific step in the simulation, the edge will be inactive in that step (as if it were temporarily deleted). The rules describe the effect of other nodes on the edge being edited. The conditions are of the form "node X is in state more than Y" or "node X is in state less than Y". In addition, one needs to set how many of the conditions should hold together for the current edge to be active.
- The new edge will appear on canvas.

Edges Graphical Representation

- Edge with a positive weight:
- Edge with a negative weight:
- Edge with weight higher (lower) than 1 (-1), the weight will annotate the edge in the graphical view.

- Edge with delay will be dashed:

- Dependencies of the edge: the figure shows an example case, where the activation of A by B depends on both C and D.
- Dependency edges are grey (edges from C and D)
- The shape of a dependency node (created
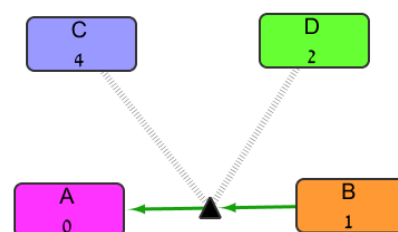
automatically when you create an edge with dependencies) is determined by the sufficient number of rules (that hold) in order to activate the edge:

   a. All of the rules hold - triangle
   b. At least 1 of the rules holds - arrow
   c. At least k of the rules hold (k is determined by you) - square

Edit node

- Right click on node > BioNSi> Edit node

Edit edge

- Right click on edge > BioNSi> Edit edge

Delete node/edge

- Save the project (recommended)
- Click on the object you wish to delete
- Press Delete button on your keyboard

There are some known issues when deleting an object in Cytoscape. If you follow these instructions, you'll probably be able to delete an object successfully:

- If nothing happens after pressing "Delete"
   a. Close and open Cytoscape again
   b. BioNSi >Build BioNSi network
   c. File>open
   d. Choose your saved project
- If a deletion was made: verify that the deletion went OK :
   a. **Deleting a node** incoming and outgoing edges from that node should have been deleted too.
   b. **Deleting a dependant node or deleting a dependant edge (full line)** all edges connected to it should have been deleted, and a normal edge should have been created instead (in the figure above all edges will be deleted and an edge between B and A will be created).
   c. **Deleting a depend edge (grey line) when there aren't any other grey line (representing other dependencies on the edge) connected to that depend node** depend node should have been deleted.
- If you have found any abnormality, restart Cytoscape, load your saved project and try to delete again.

Easy editing of BioNSi network properties in a table

Another way to edit properties of objects in your network is via the tables in the "Edit Network" dialog box, which allows easy editing of multiple objects in the same place.

- BioNSi > Edit network in table
- Press Ok in order to apply the changes to the network.
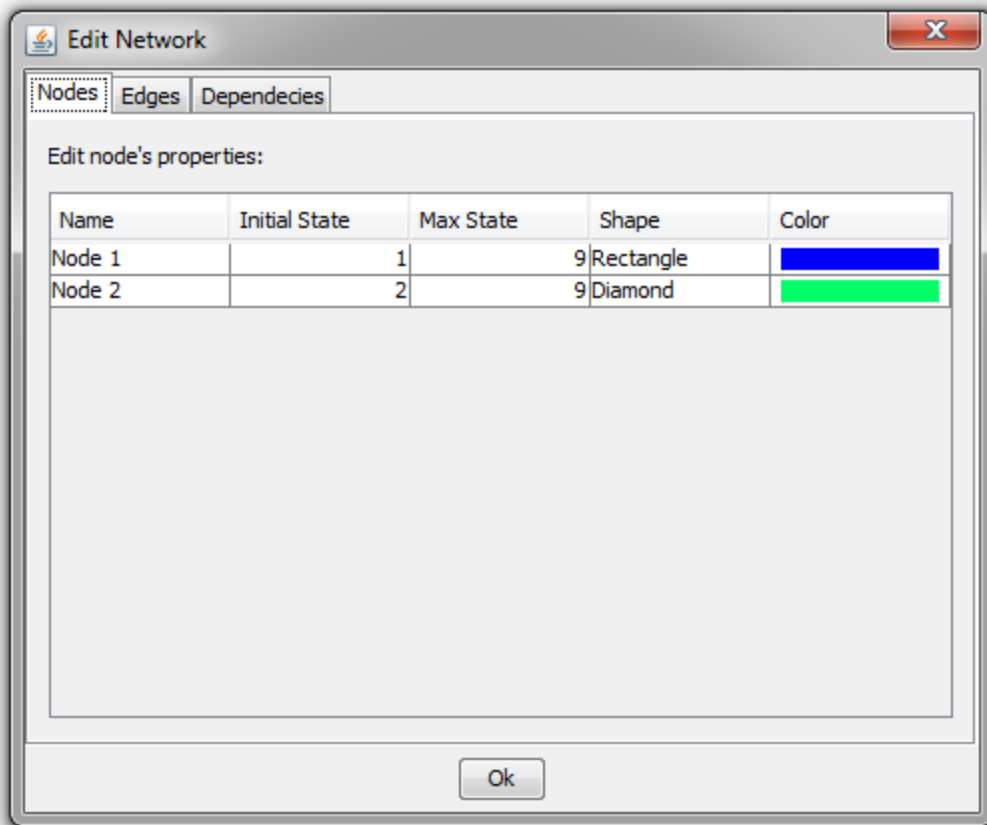
- Properties of nodes, edges and dependencies can be edited within the table by clicking on the relevant cell and entering the new value.
- Objects' names are not editable from this dialog (only from the "edit node" option).
- Timed nodes do not appear in these tables. You can only edit their properties individually (see page 5, edit node).
  ** In the edges table the delay value will only be set after pressing "Enter"**

Run Network Simulation

- BioNSi > Run Simulation, or use the button on the left side of the tool-bar:
- Choose the running mode (constant\logarithmic), and when to stop running the simulation:
    a. after the simulator identified a loop or a steady state,
        - If a network has timed nodes, the simulation will not search for a loop/steady-state before the last (maximal) lag time of all timed nodes came into play
    b. after a pre-defined number of steps.
- You can also fill in a title for the simulation ("Tab Name").
- Press Ok.
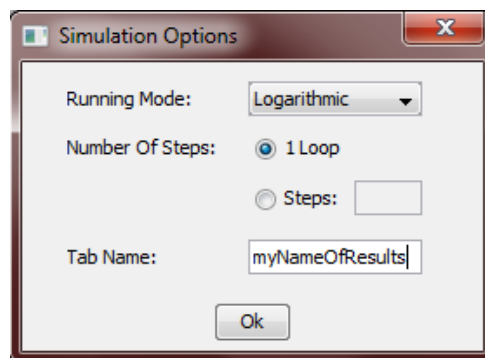
- A new tab in the Results Panel will be opened (if you accidentally close the results panel, use View>Show Results Panel)
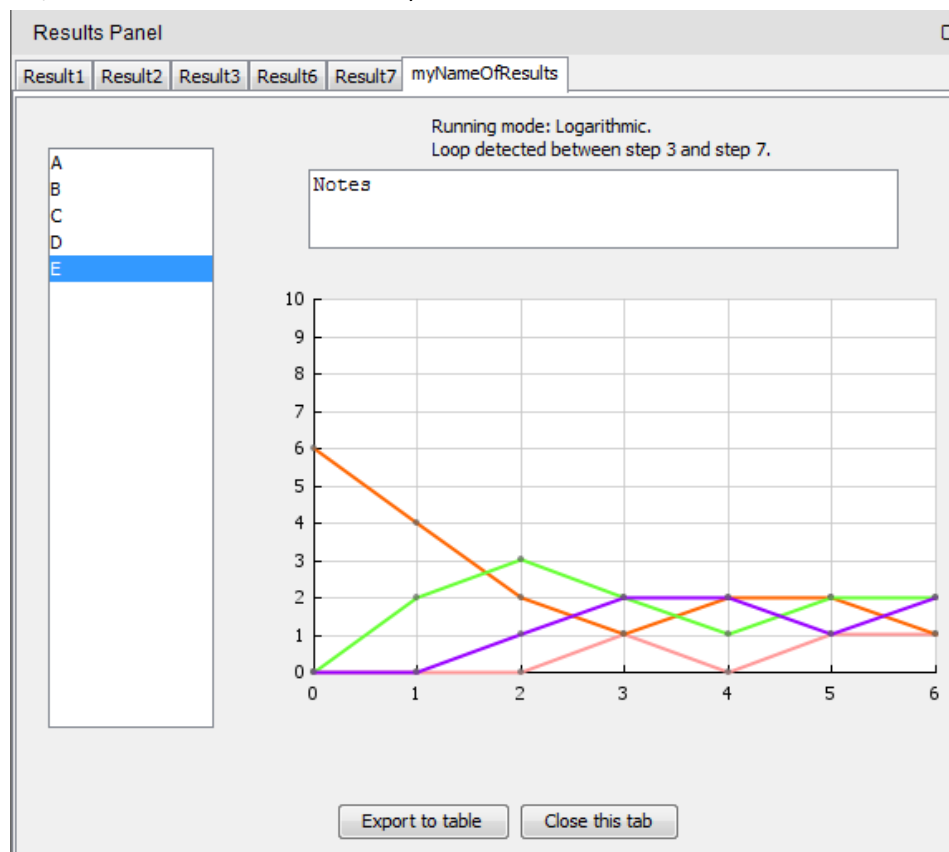
- **Top message:** There are 3 options for the message that appears on top:
    a. "System has reached steady-state after # steps"
    b. "Loop detected between step # and step #"
    c. When setting the number of steps manually, the message will be "Model ran for # steps", followed by the detected loop or steady-state steps, if the system has reached a loop or steady state within the chosen number of steps.
- **The graph:** x-axis shows the states, y-axis shows the steps. Each node is represented by a line with the same color as the node in the network.
- **Nodes list:** Click on a node name (the list on the left) in order to hide it from the graph. You can click and drag your mouse over the list in order to hide more than one node. Click again on a hidden node to make it visible again in the graph.
- **Export to table:** Used to create a .csv file (opens for example in Excel). The file includes the simulation results in a table (nodes states per step) and summary of the network properties on that run. This network summary is in the same format as the file created from "Export Network" action (see below).
- **Notes:** You can use the "Notes" text box for some quick self-reference (for example, document the parameters of the specific run). The notes are not saved after the tab is closed, or after Cytoscape is closed.

Replay Network Simulation

The parameters inserted when running a simulation (running mode, number of steps, tab name) are saved and "Replay Run" can be used to run simulations without needing to insert them again.

- BioNSi > Replay Run or use the button on the left side of the tool-bar:

Statistical Mode

The statistical mode allows you to run a large amount of simulations and gather statistical information about your network.

- BioNSi > Run Statistical Mode > Regular Nodes

- Fill in the running mode of all the simulations (logarithmic/constant).
- Choose an indicator node. The analysis will focus on the indicator node's states.
- Choose the nodes for which you want to run the simulations with a range of initial states. (We later relate to these nodes as "statistical nodes"). Notice that the number of simulation that will run is updated on the right.
- When pressing ok, the program will run a simulation for every possible combination of node states (that were chosen in the table). The other nodes' initial states will remain as they were originally set in the network.
- We do not recommend running more than one million runs, because running time is long.

- BioNSi > Run Statistical Mode > Timed Nodes

- Fill in the running mode of all the simulations (logarithmic/constant).
- Choose an indicator node. The analysis will focus on the indicator node's states.
- Choose the timed node for which you want to run the simulations. The lag, initial state and duration fields are all text boxes that can hold multiple values separated by commas. Here are some examples to better understand the combination possibilities:
  a. Lag: 50,100,150

State	Duration
9	13
0	13

Will result in running 3 simulations with the same timetable and different lag times.

  b. Lag: 50

State	Duration

9,6	13
0,0	13

Will result in running 2 simulations, with two different timetables:

State	Duration
9	13
0	13

State	Duration
6	13
0	13

c. Lag: 50

State	Duration
9	13, 26
0	13,26

Will result in running 2 simulations as well with the following timetables:

State	Duration
9	13
0	13

State	Duration
9	26
0	26

d. Lag: 50

State	Duration
9,6	13,26
0,0	13,26

This time we will run 4 different combinations:

State	Duration
9	13
0	13

State	Duration
6	13
0	13

State	Duration
9	26
0	26

State	Duration
6	26
0	26

- Notice that all fields must have the same number of values so in examples b and d we insert "0,0" to match the number of states in the first row even though the actual value doesn't change.

Statistical analysis results

After running the simulations the results panel will contain the following tabs:

Stats Summary tab:

Results Panel

Name	Percent	Loop Size	Initial States ...	Average Run ...
Steady-State1	33.333	0	[00]	85.000
Loop1	33.333	26	[02]	150.000
Loop2	22.222	101	[04]	169.000
Loop3	11.111	78	[44]	78.000

- Table summarizing all the simulations results :
    a. Simulation results: steady-state or loop.
        ▪ Loop results are grouped together according to the loop states of the chosen indicator node.
        ▪ Steady-state results are grouped together according to the end states of all of the nodes in the network.
    b. The percentage of the simulations in which the system has reached the same result.
    c. Loop size (number of steps, 0 for steady state)
    d. Initial setting example – A "representative" for the initial states of the statistical nodes in which the system has reached this specific result.
    e. Average run length until reaching loop\steady-state, over all initial configurations that led to that loop\steady-state.
- Distribution of initial states:
    - Available only when running the statistical mode on regular nodes.
    - Opens a panel with different tabs for each result encountered during the simulations and two general tabs – "distribution over all loops" and "distribution over all steady-states"
    - Each tab shows a table that presents how many times the initial state of the nodes resulted in that loop/steady-state.
      For example, in the following table the simulations reached 3 different loops and 1 steady-state and you can see that "Loop1" occurred 3 times and only when the node "Clock" was in state 2

13

- View loop/steady-state start times:
    a. Available only when running the statistical mode on timed nodes.
    b. Here the tables show the distribution of the loops/steady-states start times for each result reached during the simulation runs.

Importing a BioNSi network

- BioNSi >File Operations>Import Network
- The imported file must be a CSV file (make sure it is saved that way in Excel):
  ** Note that to ensure that the information is imported properly, due to the CSV properties you can't use commas in any of the fields**
- Regular nodes table:
  a. Node – the name of the node, this field can't be left empty.
  b. Initial State - this field can't be left empty.
  c. Max State - this field can't be left empty.
  d. Shape – can be one of "Rectangle", "Diamond", "Ellipse", "Hexagon", "Triangle". If left empty the rectangle shape will be used as the default.
  e. Color – A hex string of the color, for example: #ff6666. If left empty #0000ff (blue) will be used as the default color
  f. Position(x;y) – the position of the node on the canvas. If left empty the nodes will be positioned in a grid. (Layout can then be changed with all of Cytoscapes' layout options in the "Layout" menu)
- Timed nodes table:
  a. Node,Shape,Color,Position(x;y) - same as the fields in the regular nodes table
  b. Entry Step – represents the "lag", this field can't be left empty
  c. Repeating – TRUE/FALSE field representing whether the node is cyclic or not. This field can't be left empty.
  d. Timed Expression (State:duration) – should match the time table in the "add node" panel. There can be more than one timed expression per timed node, In which case they should appear in the same row.
- Edges table:
  a. Source Node – this field can't be left empty and must contain the name of a node that appears in the regular or timed nodes tables.
  b. Target Node– this field can't be left empty and must contain the name of a node that appears in the regular or timed nodes tables.
  c. Weight – a positive or negative integer, this field can't be left empty.
  d. Delay – must be 0 or higher, this field can't be left empty.
  e. Sufficient number of rules – a number specifying when the condition holds. Can be between 0 and the number of the dependencies in that row.
  f. Dependencies –
     - conditions of the form "node X is in state more than Y" or "node X is in state less than Y"
     - There can be more than one dependency per edge, In which case they should appear in the same row

Exporting a BioNSi network

Exports all of the data in the current network to a given CSV file. This file can then be used to import the network.

The file can be opened with excel and you can use all the Excel features on the tables (for example, sorting by a specific column).

- BioNSi >File Operations>Export Network
- Choose a directory and file name and press "Save". The file will be created it the selected directory.

Update initial states (New feature)

Update the whole network's initial states, by reading them from an expression file. An expression file is a simple 2-column csv file. The left column contains nodes' names, the right one contains initial expression levels. Expression values can be integers or decimal real numbers. For example:

symbol	expression level
HSPA6	5.167
PAX8	8.302
PLD1	6.009
DTX2P1	8.989
MAP4	9.111
CD24	13.224
…	…

- BioNSi > Edit network > Update initial states
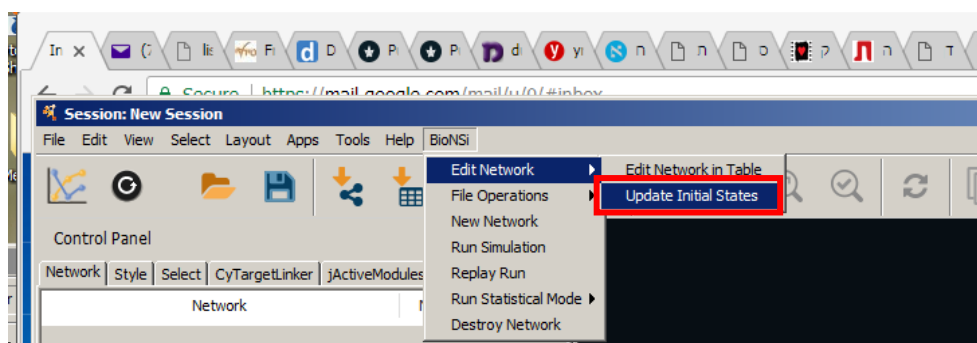
- Choose a directory with the required expression.csv file and click "Save".
- Note: A single csv file should be in the selected directory, named expression.csv (lowercase)
- The network will be updated with the new initial states.

See notes and more details in Box 1 below.

Box 1: Things you should know about the "Update initial states" feature

- Due to its simple format, generating an appropriate expression file is easy. You can either download a file from an existing database in this format (may require some modifications, depending on the format that the database supports), or, when the network is of moderate scale, prepare one on your own. An easy way to prepare such a file would be to copy and paste the node table in Cytoscape, and then specify the required initial state for each node.

- The expression file must be named expression.csv (lower case)

- BioNSi normalizes the expression levels to the range 0-9. This is done for consistency between sources that measure expression levels in different scales (such as logarithmic vs. linear).

- For convenience, after the update, nodes' fill colors will correspond to the initial states, and vary from white (0) to dark grey (9).

- Make sure the node names in the expression file are identical to those in the network (e.g. symbol, NCBI accession, etc). A node that does not appear in the expression file will get 0 for the initial state.

- If the same node appears more than once in the expression file, the last value will be taken, except for when a zero value is encountered after a non-zero value. Generally, it is advised to avoid multiple values for the same node, e.g. by averaging those.

- Some expression data may denote alternative names of e.g. genes in the format name1///name2///name3. We split this into the individual names "name1", "name2", "name3", so that each one of these will be recognized.

Merging KEGG pathway(s) from XML (New feature)

This new feature allows importing pathways from the KEGG database. Multiple pathways will be merged into a single BioNSi network.

Step 1 (generating KEGG pathway files): First go to the KEGG database (http://www.kegg.jp), navigate to "KEGG Pathway Maps", choose your required pathways and species and export each pathway as an xml format file by clicking "Download KGML" (some maps in KEGG may lack this option). Each pathway is represented by a single xml file. For example, suppose you want the ERBB signaling

pathway, denoted "hsa04012" in the KEGG database; go to http://www.genome.jp/kegg-bin/show_pathway?hsa04012 and click "Download KGML". Put all the xml files in a single folder.

- **Step 2 (expression file):** In order to set the initial states of the nodes in the merged network, a single expression file has to be located in the same folder. Same as in the "update initial states" option, the expression file must be named expression.csv (lower case), and this file is a simple 2-column csv file. The left column contains nodes' names, the right one contains initial expression levels (see example above). **Note:** If you have no data on most or all initial states, use the expression.csv file from the website, which assigns 9 to every node, and change the nodes for which you do have an initial state.

Step 3:
- BioNSi >File Operations> Import KEGG pathway(s) from xml
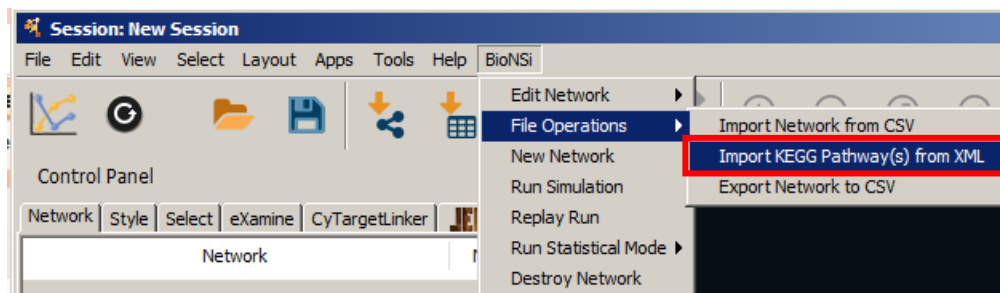
- Choose the folder with all the KEGG xml files and csv expression data.
- A window will pop up with edges' weights for the user to set, according to the KEGG type of the edge (see box 2). For convenience, this list contains default values (activation is +1, inhibition is -1, all the rest are 0).

Activation -->
2

Inhibition --|
-1

Indirect Effect ..>
0

State Change ...
0

Binding/Associa...
0

Dissociation -+-
0

Missing Interact...
0

Phosphorylatio...
2

Dephosphorylat...
-1

Glycosylation +g
0

Ubiquitination +u
0

Methylation +m
0

Self Inhibition
-1

OK

- The new network will be opened in BioNSi. For convenience, nodes' fill colors correspond to the initial states, and vary from white (0) to dark grey (9).

Box 2: Things you should know about "Merging KEGG pathways from xml" feature

<u>Files</u>

- All the xml files you downloaded from KEGG should be placed at the same directory, together with an expression file names expression.csv.
- A <u>singe</u> expression file named expression.csv (lowercase) must be provided. You can use the expression.csv file from the website, which assigns 9 to every node, and change the nodes for which you have an initial state (if any).

<u>Nodes</u>

- Only nodes of KEGG type "gene", "group" and "compound" are selected for the merging. All other types are typically irrelevant (e.g., "map") and thus ignored.

- Nodes default shapes:

Type	shape
Gene	rectangle
group	hexagon
compound	triangle

- Some node named contain "…", to denote the existence of alternative names. This is removed from the name. For example, "Ime1…" will become "Ime1".

- Nodes with no edges are filtered out

<u>Initial states</u> - same notes as in box 1

<u>Edges</u>

- KEGG has internal notations for edge types, which are listed below (see also http://www.kegg.jp/kegg/xml/docs/). We give these types default weights, which the user can override.

KEGG notation	Meaning	Default weight in BioNSi
-->	Activation	+1
--|	Inhibition	-1
..>	indirect effect	0
...	state change	0
---	binding/association	0
-+-	Dissociation	0
-/-	missing interaction	0
+p	phosphorylation	0
-p	dephosphorylation	0
+g	Glycosylation	0
+u	ubiquitination	0
+m	methylation	0

- Every node in the merged network gets a negative self-loop with weight -1, reflecting e.g. self-degradation of a protein. This default value can also be changed.

- When the same edge appears more than once, with different types, we prioritize --> over --\| over all the rest (among which the first occurrence is chosen