

# BiNoM: Biological Network Manager

## Version 2.0 Manual

February 13, 2012

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# 1 Introduction

BiNoM (BIological NetWOrk Manager) is a Cytoscape plugin, developed to facilitate the manipulation of biological networks represented in standard systems biology formats and to carry out studies on the network structure. BiNoM provides the user with a complete interface for the analysis of biological networks in Cytoscape environment.

In an effort to exchange and curate pathway database knowledge, several standard formats have been developed (SBML, BioPAX [7] and others). Many softwares, which are centered on the description and representation of biological pathways, adopted these standards. CellDesigner[4] and Cytoscape[6], for instance, allow the visualization and manipulation of networks but meet some limitations. BiNoM was designed to facilitate the use of systems biology standards, the extraction and organization of information from pathway databases through BioPAX interface.

BiNoM concentrates on the following aspects: the import and export of BioPAX and (CellDesigner) SBML files and the conversion between them; the structural analysis of biological networks including decomposition of networks into modules, path analysis, etc.; the BioPAX query engine which provides the extraction of information from huge BioPAX files such as whole pathway databases; and various operations on graphs not offered by Cytoscape such as clipboard operations and comparison of networks.

This manual describes only the functions of plugin-in BiNoM. Cytoscape proposes some functions close to those of BiNoM (import, export, set operations ...) which are explained in Cytoscape manual (<http://www.cytoscape.org>).

BiNoM plugin with documentation, API and source code is available for download at: <http://bioinfo.curie.fr/projects/biynom>

## 2 BiNoM I/O

### 2.1 Import BioPAX 3 document

BioPAX level 3 information is fully supported (reaction network, interaction network, pathway structure, annotations).

#### Plugins⇒BiNoM I/O⇒Import BioPAX 3 Document from file

The model M-Phase.owl[5] is uploaded. A dialog window proposes to create three different interfaces to the BioPAX file: reaction network (RN), pathway structure (PS) and protein interaction (PP).

- Reaction network: M-Phase RN is a representation of the reaction network (figure 1).
- Pathway structure: M-Phase PS represents the pathway hierarchical structure. For this example, we choose to show a more detailed and complete pathway, the apoptosis sub-network extracted from Reactome database (figure 2).
- Protein interaction: M-Phase PP shows which proteins interact with each other.

For more details on BioPAX, its interfaces, etc, go to section 8.4.

In the case of creating the pathway structure interface, several choices are offered:

- Make Root Pathway Node: adds an extra node to which all pathways are connected. This feature can be useful for organizing the graph and joining separate and disjoint pathways.
- Include Next Links: shows the order of the reactions. From a node, an arrow indicates which node is the next step. This feature provides a timeline of the events in a pathway and could emphasize, for example, the linearity of a cascade.
- Include Pathways: includes green nodes (figure 2) which correspond to the names of the different pathways of the network.
- Include interactions: shows explicitly the reactions involved in the pathway (lower grey nodes in figure 2).

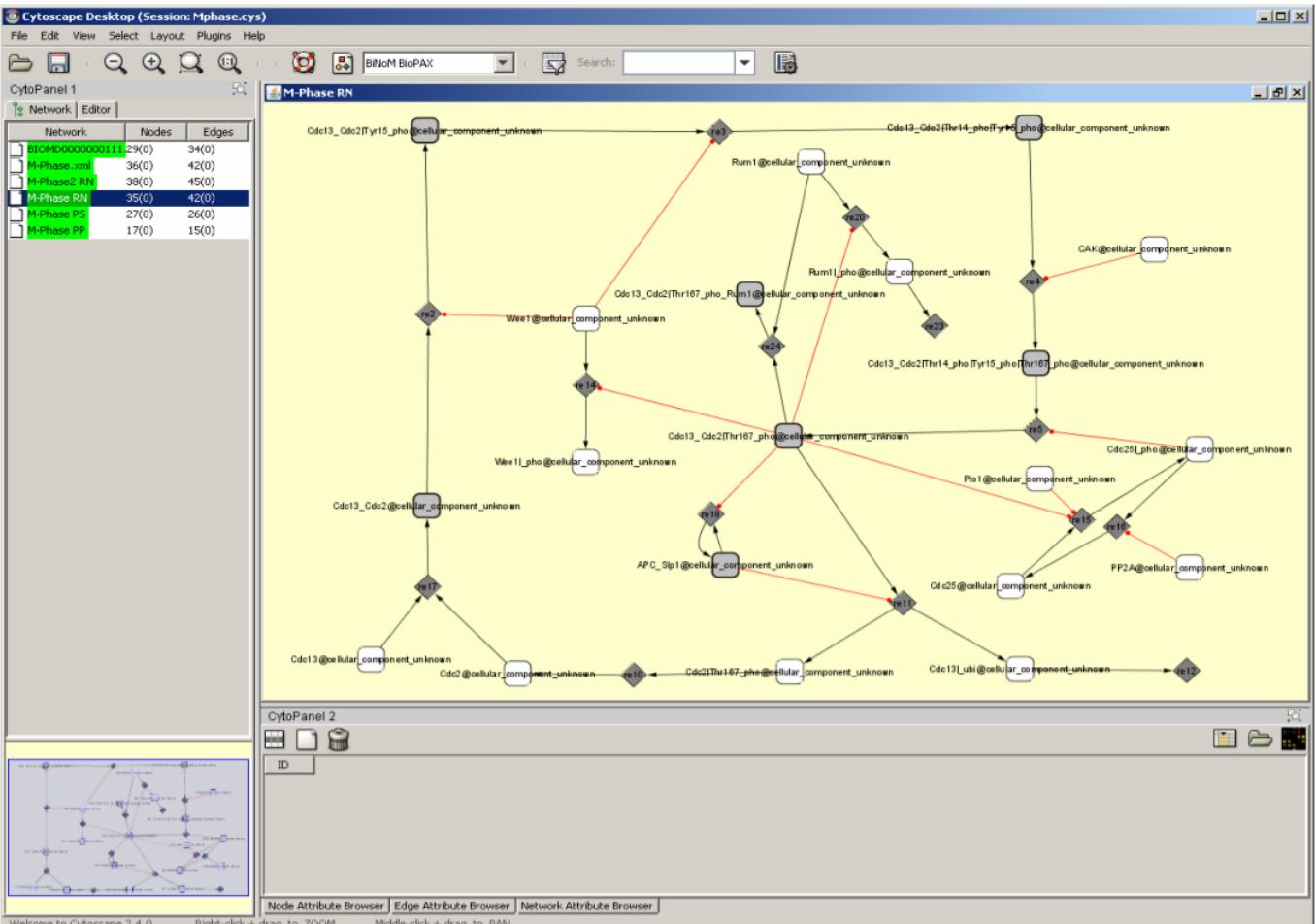


Figure 1: BioPAX view of Novak et al. model.

### Plugins⇒BiNoM I/O⇒Import BioPAX 3 Document from URL

A BioPAX 3 document can also be imported directly from a URL. The web address must be typed in the dialog window.

## 2.2 Import CellDesigner document

### Plugins⇒BiNoM I/O⇒Import CellDesigner Document from file

The model can be drawn or downloaded[5] in CellDesigner (figure 3) and saved as M-Phase.xml.

The Import CellDesigner Document from file function imports a model from CellDesigner to Cytoscape. A dialog window opens and M-Phase.xml needs to be selected and imported (figure 4).

Figure 3 and 4 show the same model viewed by CellDesigner and Cytoscape respectively. The layout information from CellDesigner is imported automatically into Cytoscape.

In specie notes in CellDesigner Attribute name:Value as HUGO:E2F1 (without blank) is converted in Cytoscape as the attribute HUGO with the value E2F1 for the specie.

### Plugins⇒BiNoM I/O⇒Import CellDesigner Document from URL

A CellDesigner document can also be imported directly from a URL. The web address must be typed in the dialog window.

## 2.3 Import CSML document

### Plugins⇒BiNoM I/O⇒Import CSML document

BiNoM imports a CSML (Cell System Markup Language, csml.org)

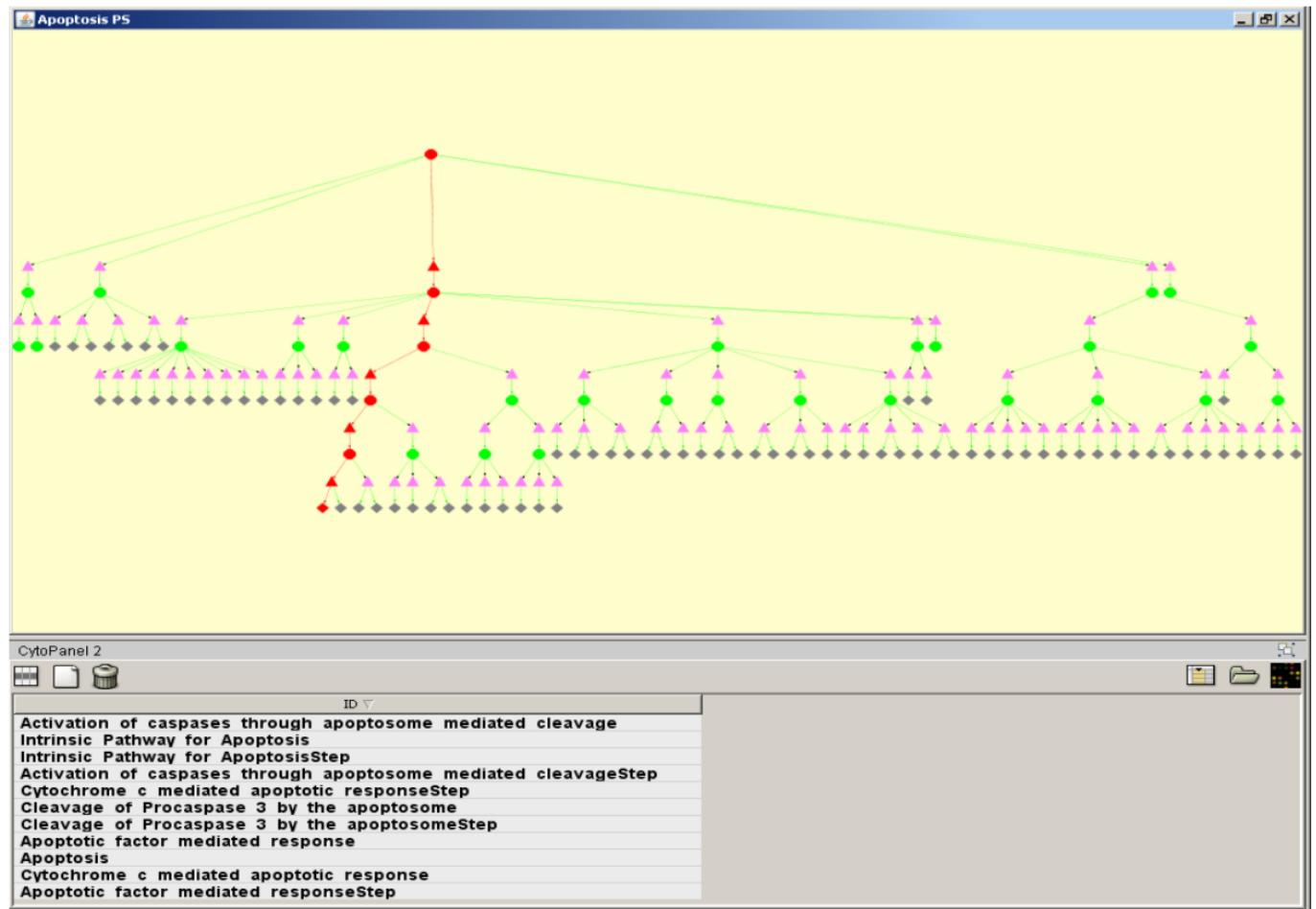


Figure 2: Apoptosis pathway hierarchical structure. Green nodes represent pathways, pink triangular nodes represent steps, and grey nodes represent reactions. From the apoptosis node (top node in red), the cell can choose through 5 different paths. The red-colored path shows one of them, the activation of apoptosis via the intrinsic pathway, leading to the cleavage of caspases 3.

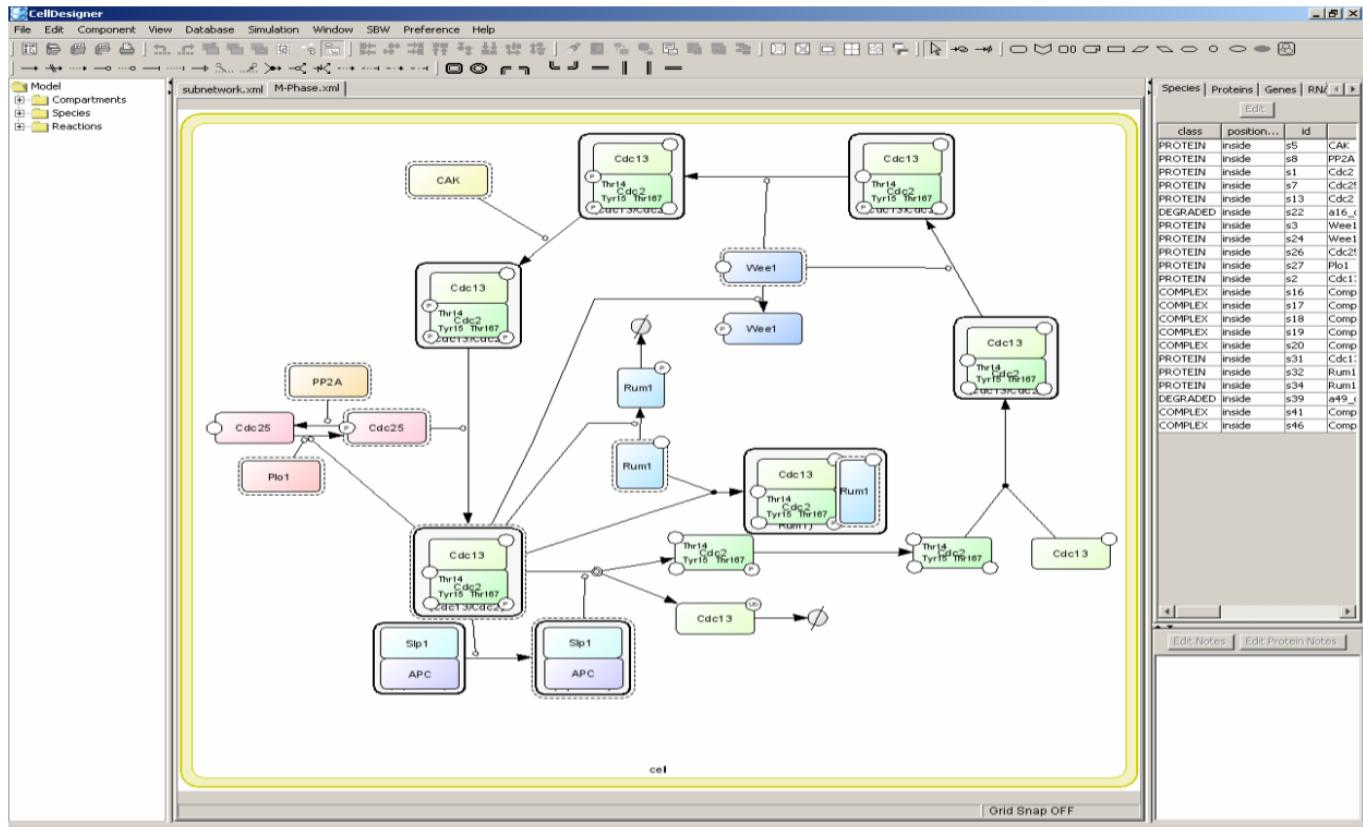


Figure 3: CellDesigner view of the cell division cycle model of fission yeast[5]

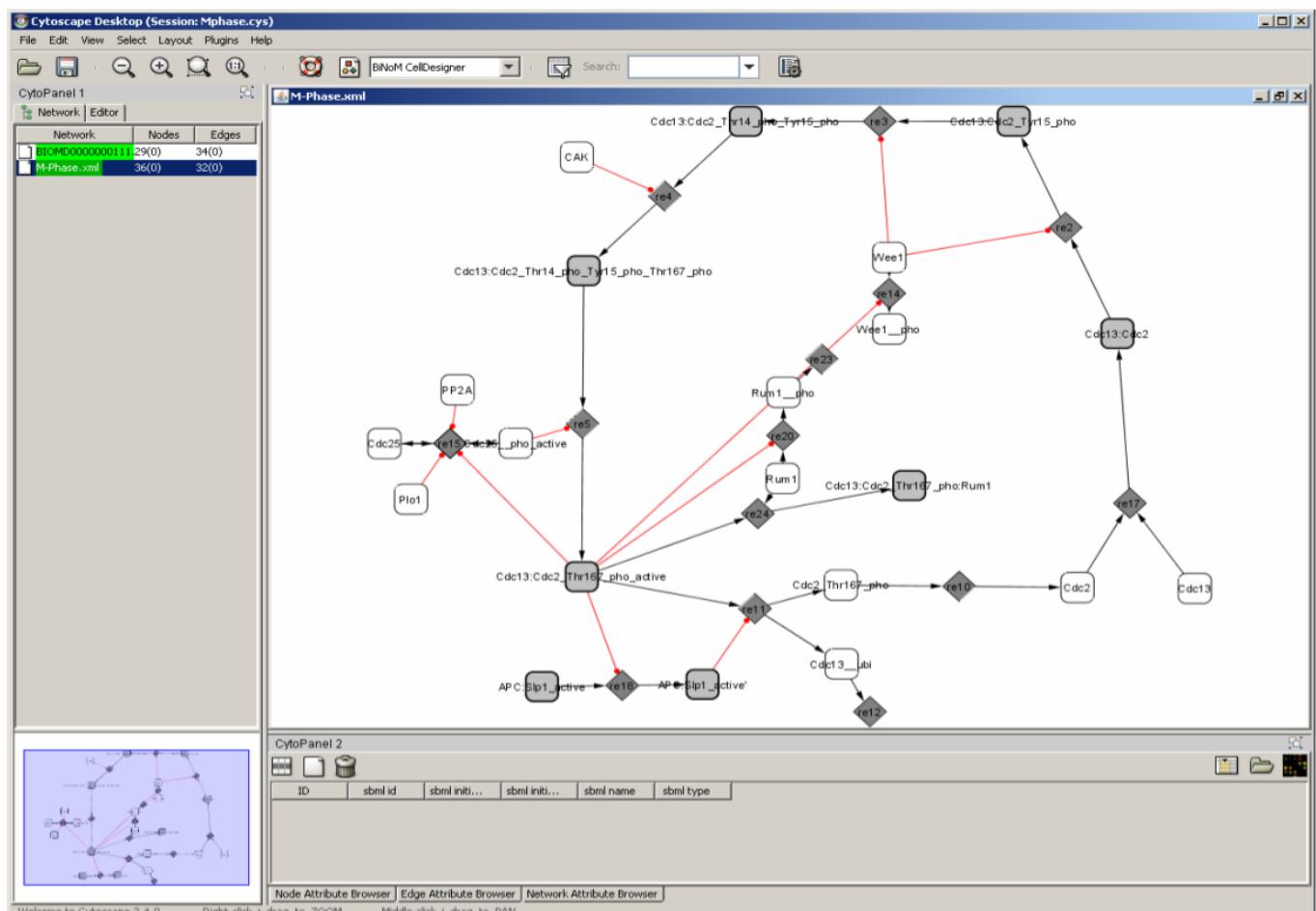


Figure 4: Cytoscape view of the cell division cycle model of fission yeast from a CellDesigner document

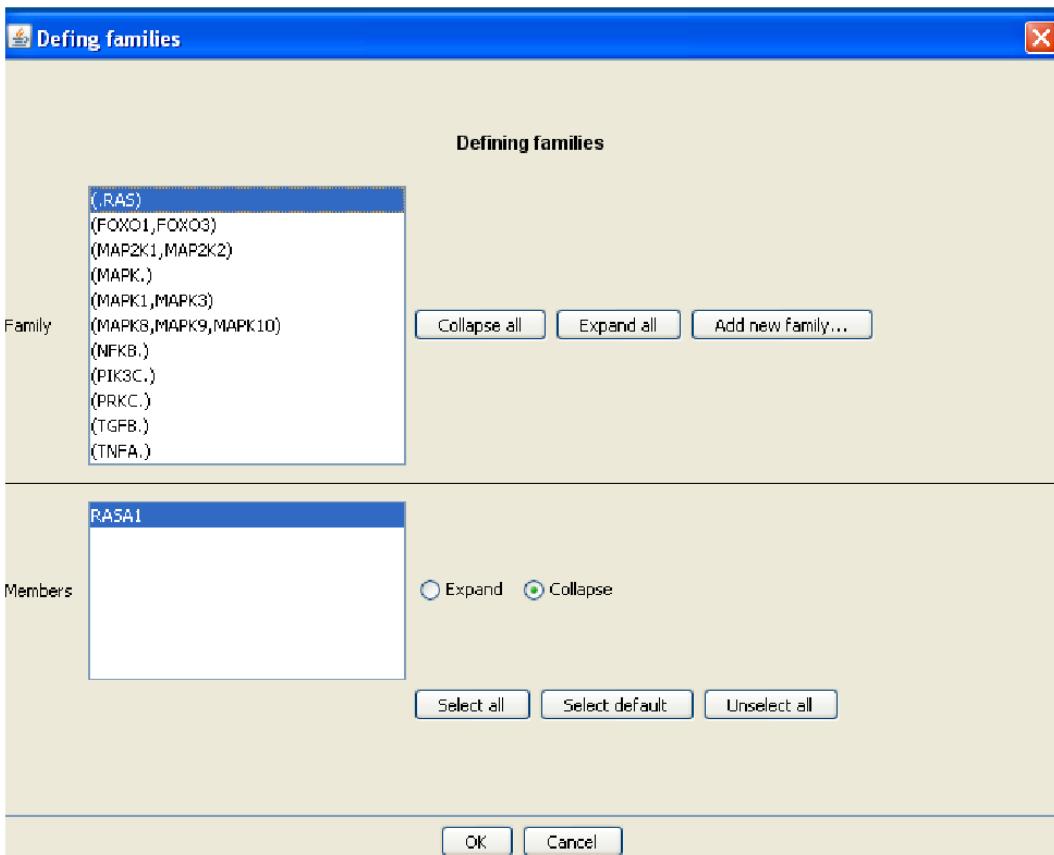


Figure 5: Dialog window for families management when importing apoptosis influence file. BiNoM recognizes AIN format for families and proposes to either collapse or expand them.

## 2.4 Import influence network from AIN file

The precise format of the influence file (AIN) is described in the appendices (section 8.5). It is an annotated list of links between genes, in a simple ASCII format.

### Plugins⇒BiNoM I/O⇒Import AIN file

The AIN file of the apoptosis model ExamplApop.txt is imported. First, the user is asked to manage the families (groups of genes or proteins, see the appendices for a precise description): they can be expanded (replacing the family by all its members) or collapsed (replacing all family members by the name of the family). See figure 5.

Then a dialog window proposes to add constitutive reactions: influences that link proteins (or families) to their complexes and proteins (or families) to their phosphorylated state. See figure 6.

The imported network is synchronized with BioPAX format that includes the annotations of the AIN file. All this information can be accessed via BioPAX 3 property editor (see BioPAX Utils, section 5.1).

## 2.5 Export current network to BioPAX 3, Export current network to CellDesigner

The Cytoscape networks can be exported in BioPAX and CellDesigner by:

- Plugins⇒BiNoM I/O⇒Export current network to BioPAX 3
- Plugins⇒BiNoM I/O⇒Export current network to CellDesigner

⚠ provided that they are associated to existing CellDesigner or BioPAX by:

- Associate BioPAX Source, see section 2.7.
- Associate CellDesigner Source, see 2.9.

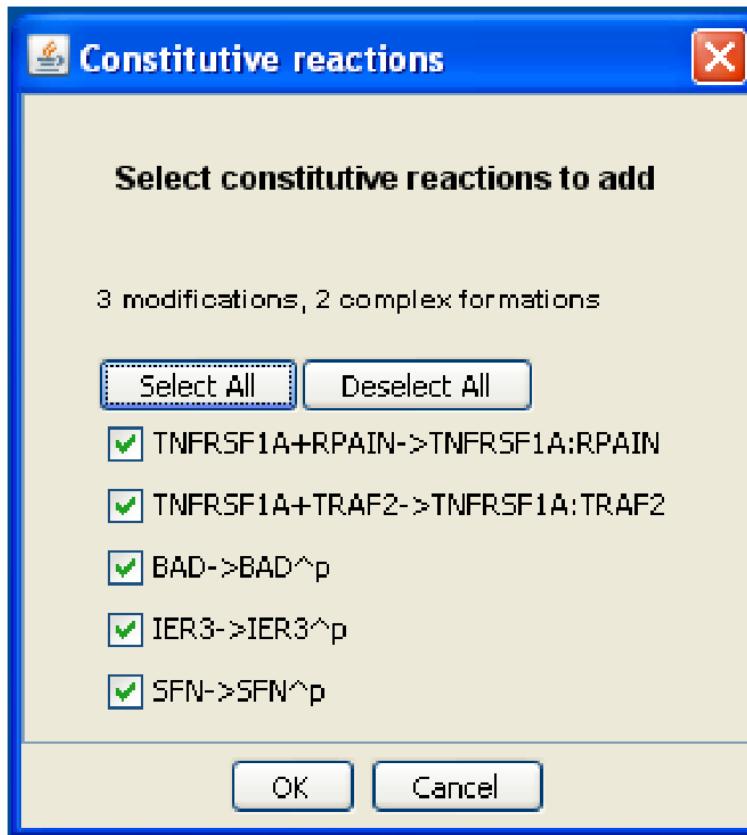


Figure 6: Dialog window for constitutive reactions when importing apoptosis influence file. BiNoM detects all possible constitutive reactions and proposes to add them.

More precisely, BiNoM is able to convert CellDesigner to BioPAX, and BioPAX reaction network interface to pure SBML. BiNoM is also able to export only a part of CellDesigner and BioPAX file, visible in the current Cytoscape network (interface). During the export operation, BiNoM is also able to merge a part of associated BioPAX file with already saved another part. BiNoM can modify the content of a BioPAX file.

**⚠** BiNoM is NOT able to create CellDesigner file with all graphical notations from BioPAX or from scratch, and it is not able to modify the content of a CellDesigner file.

Here are the typical scenarios when BiNoM export operations can help.

1. User imports a big BioPAX file as reaction network and using Cytoscape creates a new subnetwork from the global reaction graph. After he can export this subnetwork into a separate self-containing BioPAX file.
2. User imports the pathway structure of a big BioPAX file and selects only a few pathway or pathwayStep nodes he is interested in. After he can export a part of the BioPAX file necessary to define these pathways.
3. User imports a BioPAX file as reaction network, selects a subnetwork and exports it as pure SBML to be used for creation of a computational model of this subpart later.
4. User imports CellDesigner file, selects a subnetwork and exports it as a CellDesigner file: it can be useful for creating a CellDesigner image of a network module of a big reaction network.
5. User imports CellDesigner file, selects a subnetwork and exports it as a BioPAX file (some SBML-specific information such as parameters values will be lost).

The networks created as a result of the import operation are already associated to the corresponding BioPAX or CellDesigner files. However, if the XGMML file is saved and used in another Cytoscape session, or if a new network is created from the initial network with Cytoscape New menu then this association is lost.

To perform export operation, the network should be Re-associated to the corresponding file (from which

it is originated) through Plugins⇒BiNoM I/O⇒Associate... operation. For huge BioPAX files the association might take some time for the first association, but once the file is loaded into memory cache, the following associations are almost instantaneous.

To understand better what BiNoM can do or can not, read the sections 8.1, 8.3 and 8.4 about the BiNoM data model.

## 2.6 Export current network to SBML

**Plugins⇒BiNoM I/O⇒Export current network to SBML**

Export the current network to pure SBML level 2.

## 2.7 Associate BioPAX 3 Source

**Plugins⇒BiNoM I/O⇒Associate BioPAX 3 Source**

Associate a BioPAX 3 Source to allow exportation in BioPAX 3 as explained in section 2.5

## 2.8 Save whole associate BioPAX 3 as

When the content of the BioPAX file is modified (through BioPAX property editor, see section 5.1), it can be saved as a whole (not only visible part) by

**Plugins⇒BiNoM I/O⇒Save whole associated BioPAX 3 as**

Otherwise, all modifications made in the different interfaces are lost. Changes are visible but only recorded permanently when the document is save.

## 2.9 Associate CellDesigner Source

**Plugins⇒BiNoM I/O⇒Associate BioPAX 3 Source**

Associate a CellDesigner Source to allow exportation in CellDesigner as explained in section 2.5

## 2.10 List all reactions

**Plugins⇒BiNoM I/O⇒List all reactions**

Display list of reactions, can be copied by control+A then control+C.

## 2.11 List all nodes

**Plugins⇒BiNoM I/O⇒List all nodes**

Display list of nodes, can be copied by control+A then control+C.

## 2.12 Color CellDesigner proteins

**Plugins⇒BiNoM I/O⇒Color CellDesigner proteins**

Cytoscape allows coloring nodes according to values of attributes (for example expression data) by the powerful possibilities of VizMapper. The export to CellDesigner keeps the colors. This process can be used to color species in CellDesigner. The function Color CellDesigner proteins allows to color proteins in CellDesigner which describe the components of complexes.

The gene expression file is based on Hugo names, data in columns (first line title and tabulation as column separator):

Hugo names<Tab>expression level 1<Tab>expression level 2...

Open dialog box Color CellDesigner proteins, input CellDesigner file name and gene expression file, click on ok. BiNoM generate a file \*.conv where Hugo names are converted in protein names (links by annotation in CellDesigner, check if correct) and a CellDesigner file by column. When there are several Hugo name the highest is kept.

Figure 7 shows the aspect of colored proteins inside complexes.

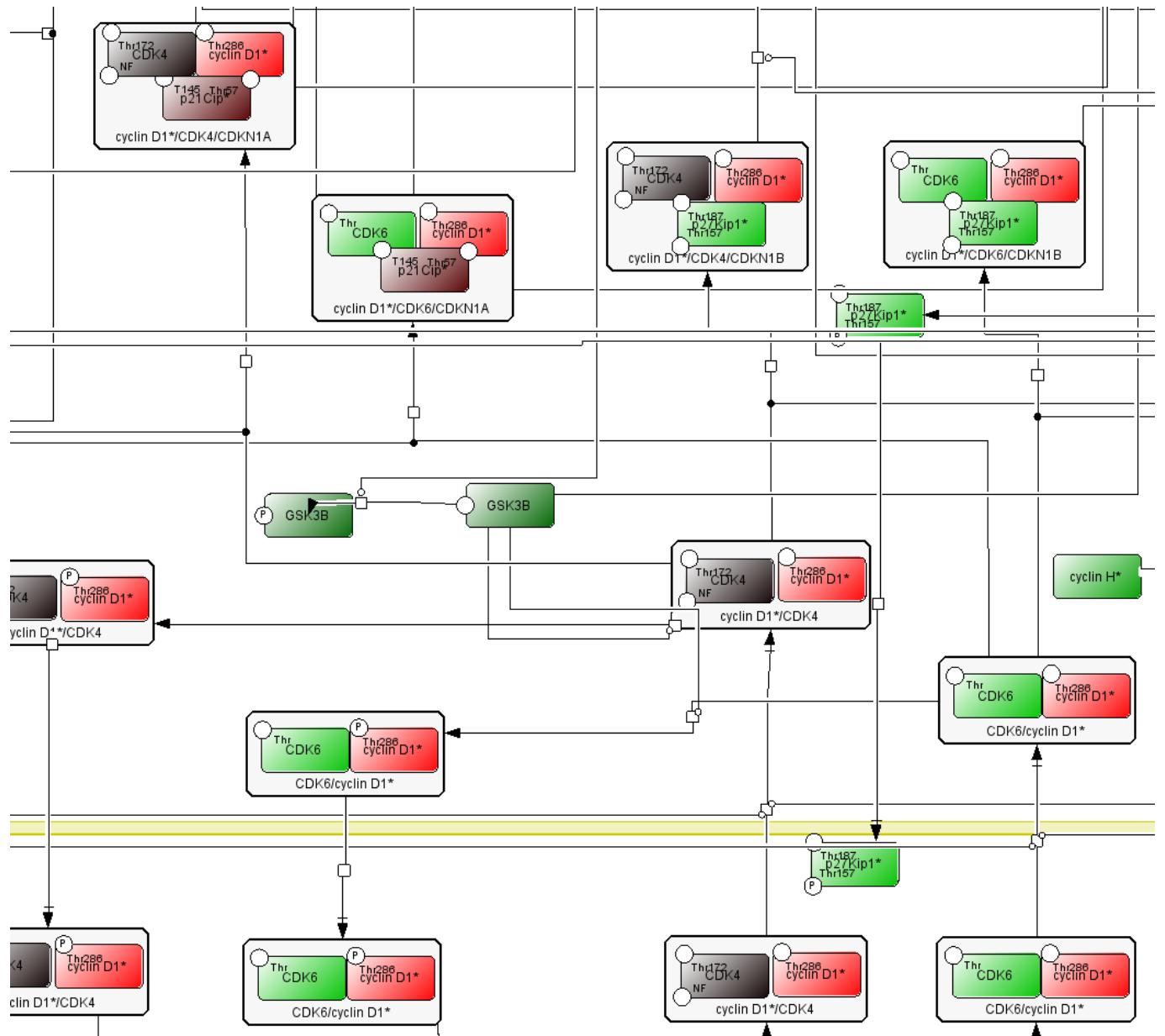


Figure 7: CellDesigner view of an extract from Rb-E2F[2] pathway colored by fictitious expression data

## **2.13 Modify CellDesigner notes**

**Plugins⇒BiNoM I/O⇒Color CellDesigner proteins**

Modify in Cytoscape the notes of CellDesigner file when exporting.

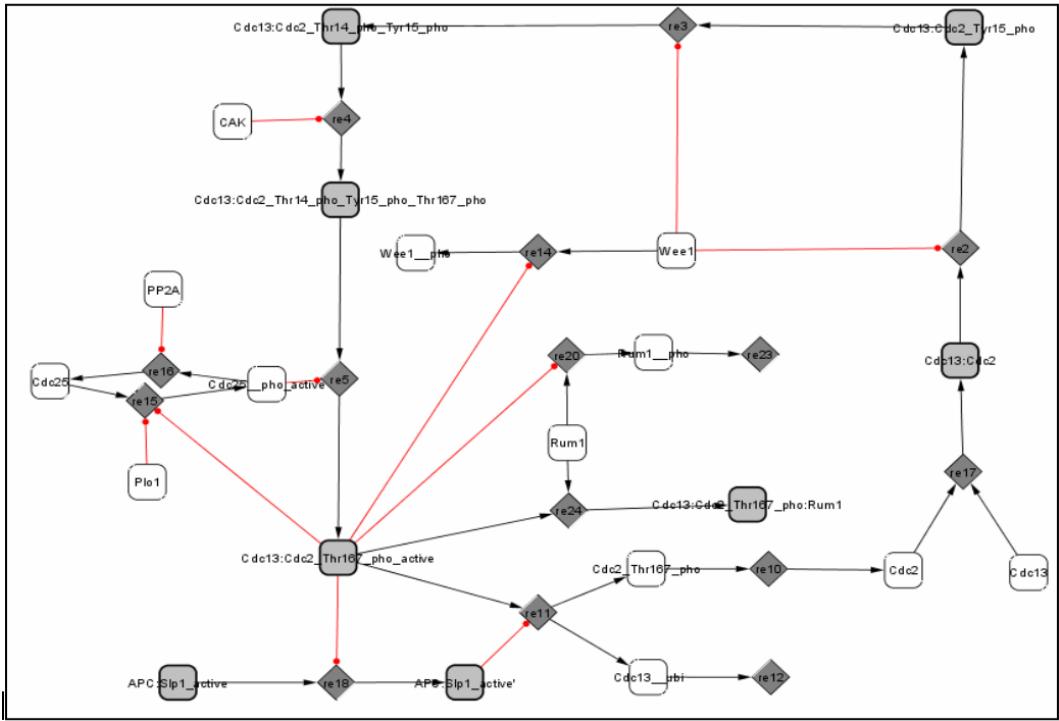


Figure 8: Cytoscape view of the M-Phase network

### 3 BiNoM Analysis

We illustrate, here, the different functions of BiNoM related to the structural analysis, using the modified version of the Novak et al. model, M-Phase.xml as an example (figure 8).

From the menu Plugins⇒ BiNoM analysis, we review all the functions one by one.

#### 3.1 Get connected components

Plugins⇒BiNoM analysis⇒Get connected components

This command dissociates the unconnected subparts of the network. In our case, since the network is already completely connected, the one obtained when choosing this function is the same as the initial one (called M-Phase.xml\_cc1).

#### 3.2 Get strongly connected components

Plugins⇒BiNoM analysis⇒Get strongly connected components

Based on Tarjans algorithm[8], the strongly connected components are isolated. In simple words, the obtained network, M-Phase.xml\_scc1(figure 9), insures that there exists a path from one node to another and deletes the components which do not respond to this requirement.

#### 3.3 Prune Graph

Plugins⇒BiNoM analysis⇒Prune graph

Pruning the graph is equivalent to separating the network into three parts(figure 10: what comes in (M-Phase.xml\_in), what goes out (M-Phase.xml\_out) and the central cyclic part (M-Phase.xml\_scc)).

This decomposition corresponds to the idea of the bow-tie structure developed by Broder and colleagues[1]. In our example, the central cyclic part is the same as figure 9, the strongly connected component. In other cases, it can be composed from several strongly connected components, connected or disconnected.

The Prune graph operation decomposes the current network into three parts: IN, OUT and SCC (the later can contain several strongly connected components).

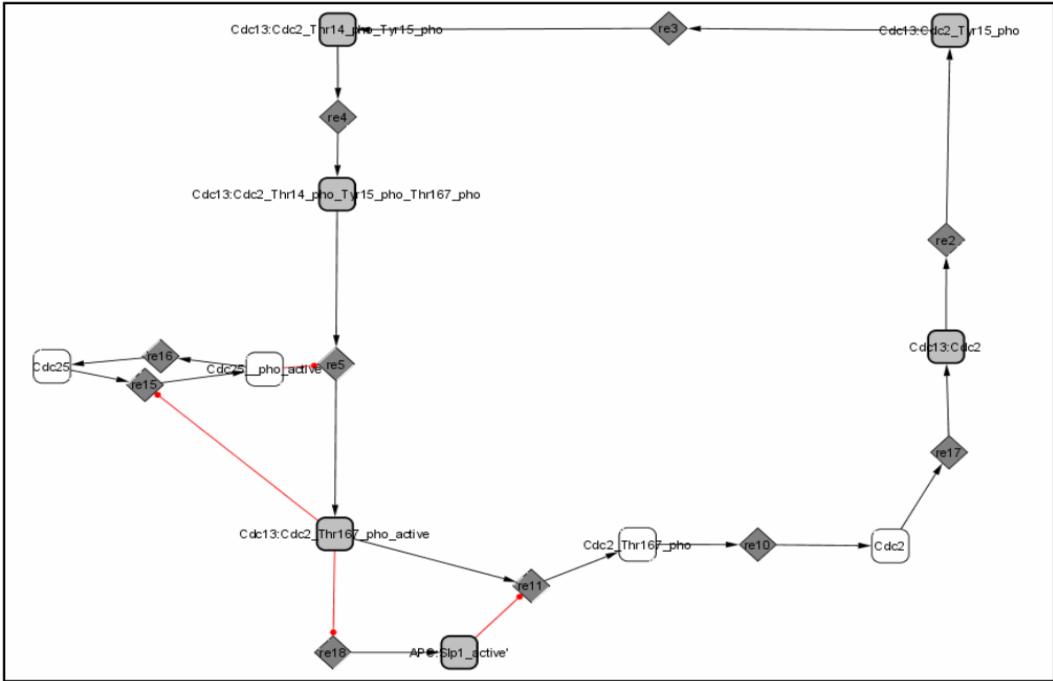


Figure 9: Strongly Connected Component of M-Phase network

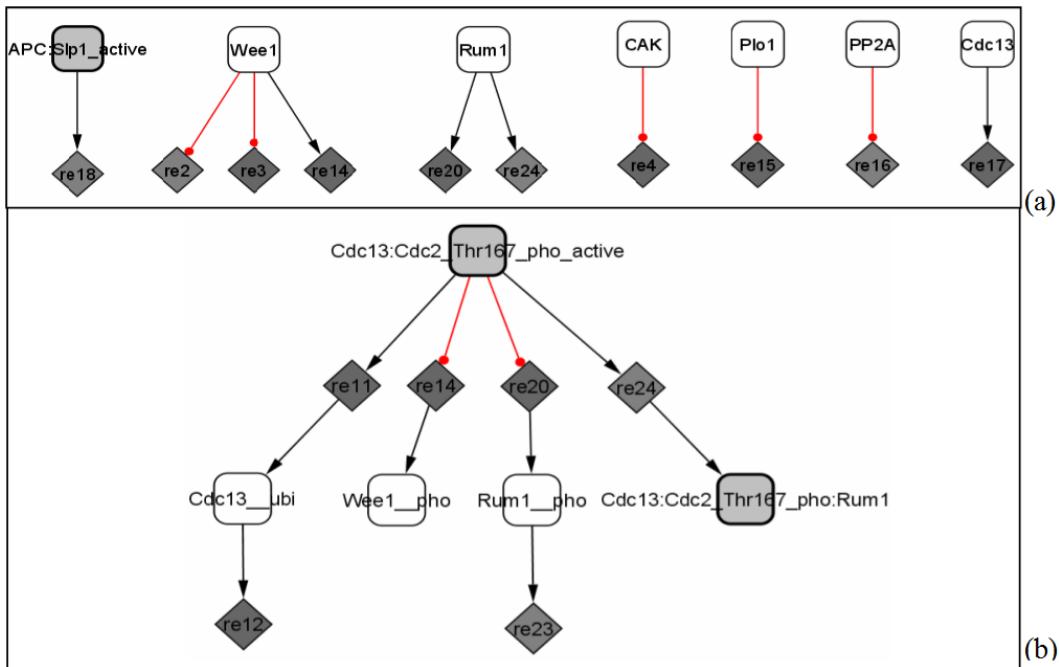


Figure 10: Prune the graph. (a) Incoming flux: molecules involved in the IN part of the network, and (b) Outgoing flux: molecules involved in the OUT part of the network.

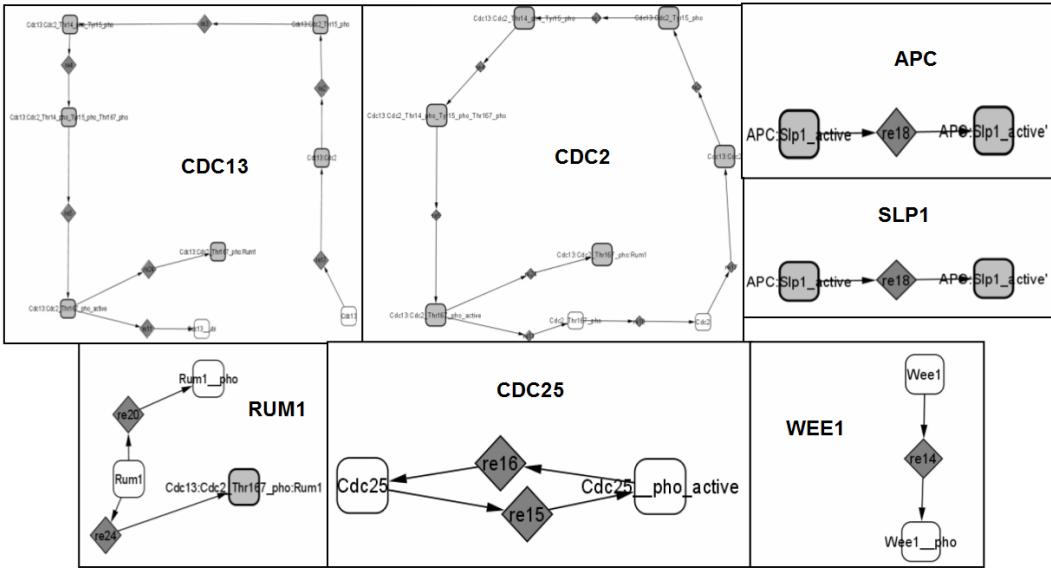


Figure 11: Material Components

### 3.4 Get Material Components

Plugins⇒BiNoM analysis⇒Get material components

This function uses node name semantics to isolate sub-networks in which each protein takes part. In our example(figure 11), seven sub-networks are created: M-Phase.xml\_Cdc13, M-Phase.xml\_Cdc2, M-Phase.xml\_Rum1, M-Phase.xml\_APC, M-Phase.xml\_Slp1, M-Phase.xml\_Cdc25 and M-Phase.xml\_Wee1. Some major overlaps between sub-networks are expected, as it is the case for Cdc2 and Cdc13 which form a complex.

### 3.5 Get Cycle Decomposition

Plugins⇒BiNoM analysis⇒Get cycle decomposition

This command decomposes the network into relevant directed cycles[3], using a modification of the Vismaras algorithm[9]. Often, this feature gives information about the life cycle of a protein or a complex, about the feedbacks of the studied network, etc(figure 12). Note that the union of all the cycles corresponds to the strongly connected component figure 9.

⚠ This operation can produce enormous number of cycles! Therefore it is rather suitable for analysis of small to moderate size networks. For a big network, one can start to understand the cyclic network structure by eliminating first the network hubs, which are contained in many network cycles. After that, the local, relatively short, cycles can be represented as meta-nodes (modules) and the analysis for cycles can be repeated.

### 3.6 Path Analysis

Plugins⇒BiNoM analysis⇒Path analysis

In a network, it can become handy to find out if there exists a path (or paths) from one species to another, or to verify that a protein or a protein complex is reachable from a starting molecule(figure 14). Provided (an) initial source and target protein(s) that are selected first on the graph then in the dialog window, the command Path analysis can find: the shortest paths, the optimal and suboptimal shortest paths, or all the non-intersecting paths (does not include inner loops), using a finite number of intermediary nodes (use finite breadth search radius), for either directed or undirected paths (figure 13).

⚠ In big networks the number of paths can be exponential! It is recommended to find the shortest path first, take its length and increment gradually the breadth search radius starting from this value to find the second shortest, third shortest, etc., paths.

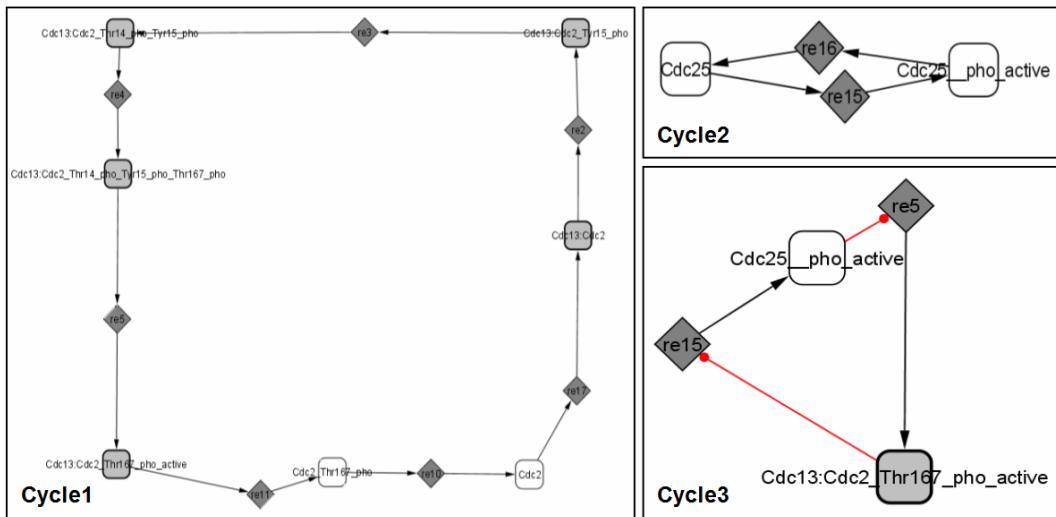


Figure 12: Minimal cycle decomposition of the M-Phase network. Cycle 1 includes CDC2 and CDC13 proteins, Cycle 2 CDC25 and Cycle 3 shows the feedback existing between CDC13/CDC2 and CDC25.

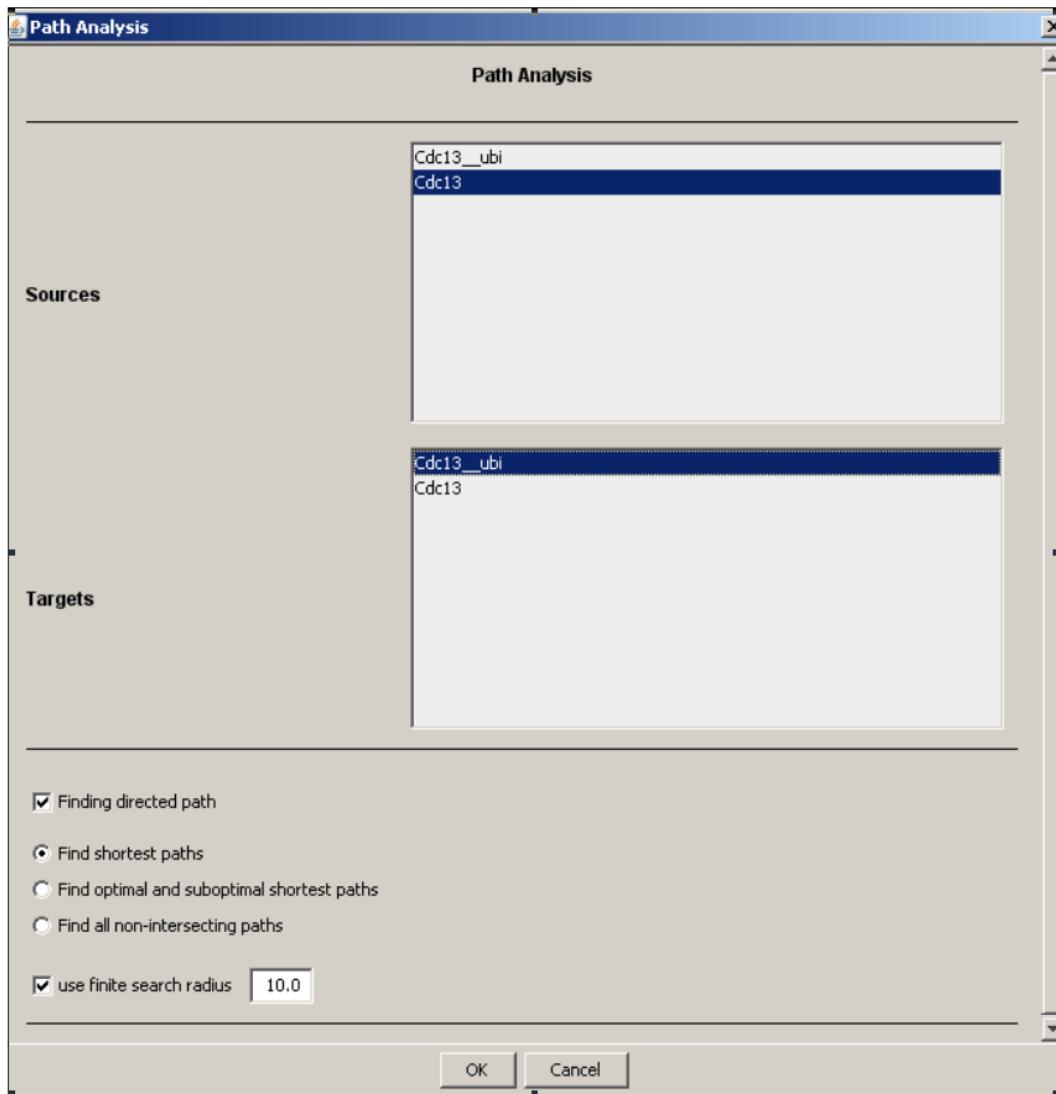


Figure 13: BiNoM Path Analysis: Pop-up window in which the source(s) and the target(s) need to be specified along with the type of paths (shortest, optimal shortest or all paths).

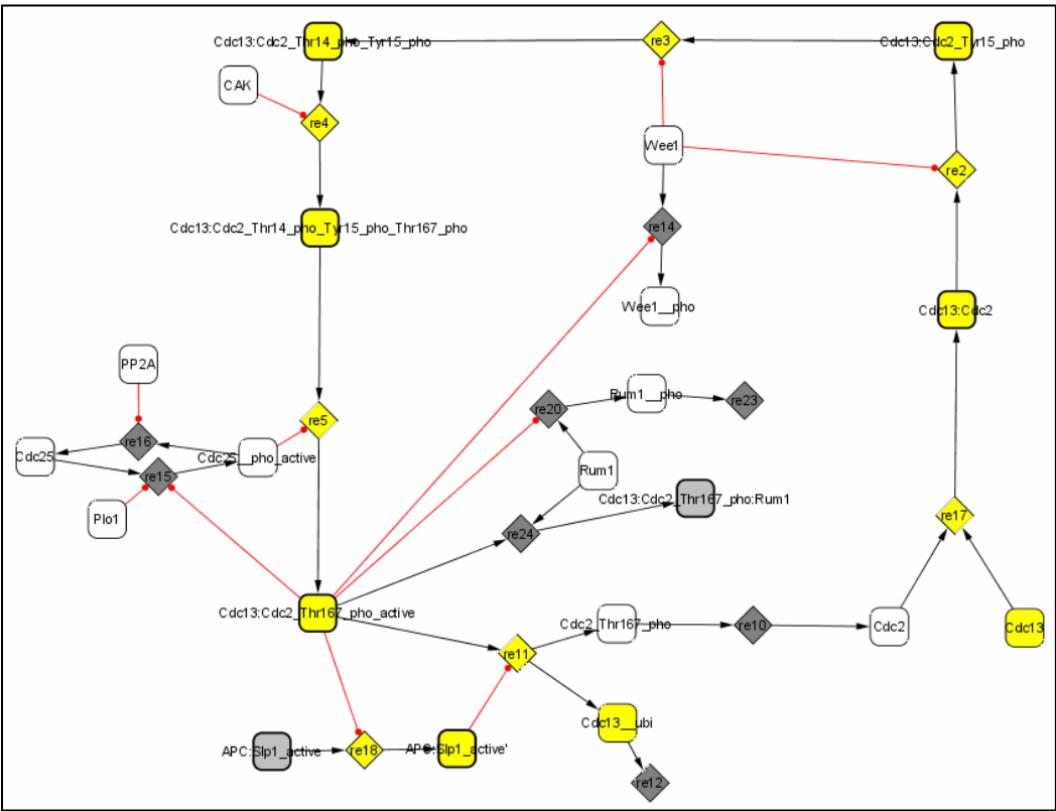


Figure 14: Path Analysis: All the paths leading from one molecular species (Cdc13) to another (Cdc13\_ubiquitinated form of Cdc13) are highlighted in yellow.

### 3.7 Extract subnetwork

**Plugins⇒BiNoM⇒analysis⇒Extract subnetwork**

Extract a subnetwork from selected nodes of a network with various options (figure 15).

### 3.8 Calc centrality, Inbetweenness undirected, Inbetweenness directed

**Plugins⇒BiNoM⇒analysis⇒Calc centrality⇒Inbetweenness undirected Plugins⇒BiNoM⇒analysis⇒Calc centrality⇒Inbetweenness directed** Display centrality of nodes in cases undirected and directed.

### 3.9 Generate Modular View

**Plugins⇒BiNoM⇒analysis⇒Generate modular view**

Given the initial diagram and some modules (which could be sub-networks of the initial network), it is possible to reconstruct a modular view of the network. For our example, we choose the initial network to be M-Phase.xml and the subparts or modules, the seven sub-networks corresponding to the material components described in (4). From these seven sub-networks only six are selected since two of them, Slp1 and APC, are exactly the same.

The sub-networks or modules need to be specified in the creating modular view window (figure 16).

There are different types of modular views. The modules are connected by: (1) the number of shared interactions (figure 17, upper panel); (2) the number of shared nodes (reactions + species) for which case the box Compact module intersection must be checked (figure 17, middle panel); and (3) the shared nodes and reactions showed explicitly (figure 17, lower panel).

### 3.10 Cluster Networks

**Plugins⇒BiNoM analysis⇒Cluster networks**

This command lumps together the modules that share a certain proportion of nodes. At a first glance, it can easily be concluded from Figure ?? (middle panel) that, for example, the modules M-Phase.xml\_Cdc13 and M-Phase.xml\_Cdc2 share a lot of proteins or protein complexes. Therefore, we can assume that these

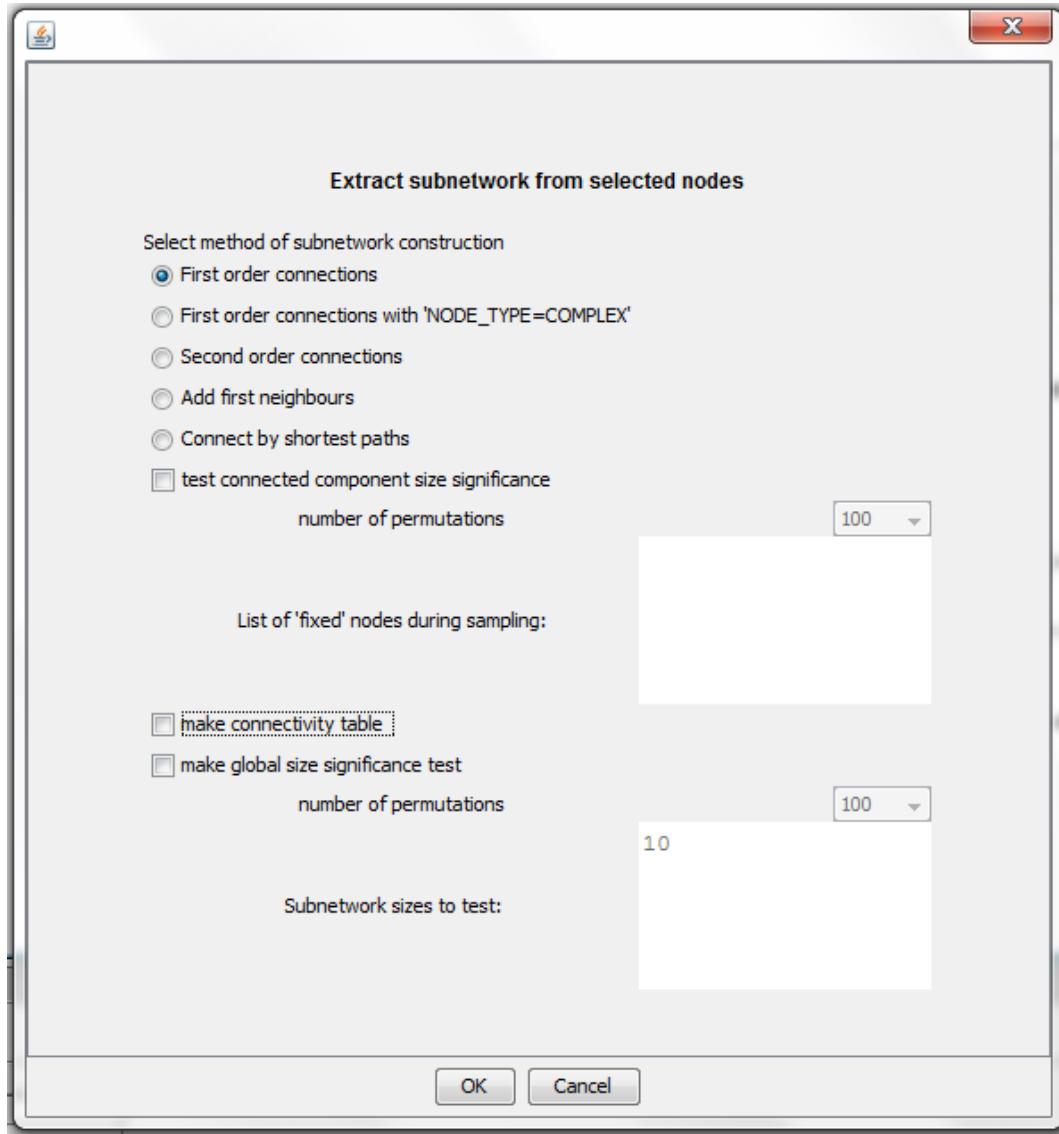


Figure 15: Dialog of extract subnetwork showing options.

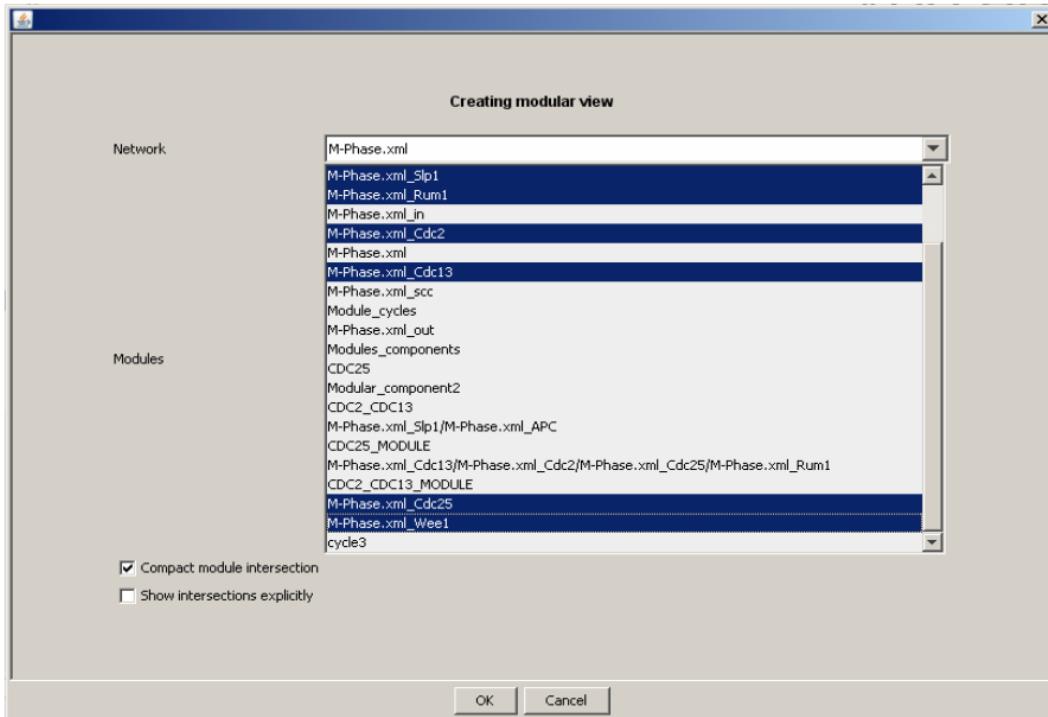


Figure 16: BiNoM modular view of the newtork: Pop-up window in which the initial graph and the modules are specified.

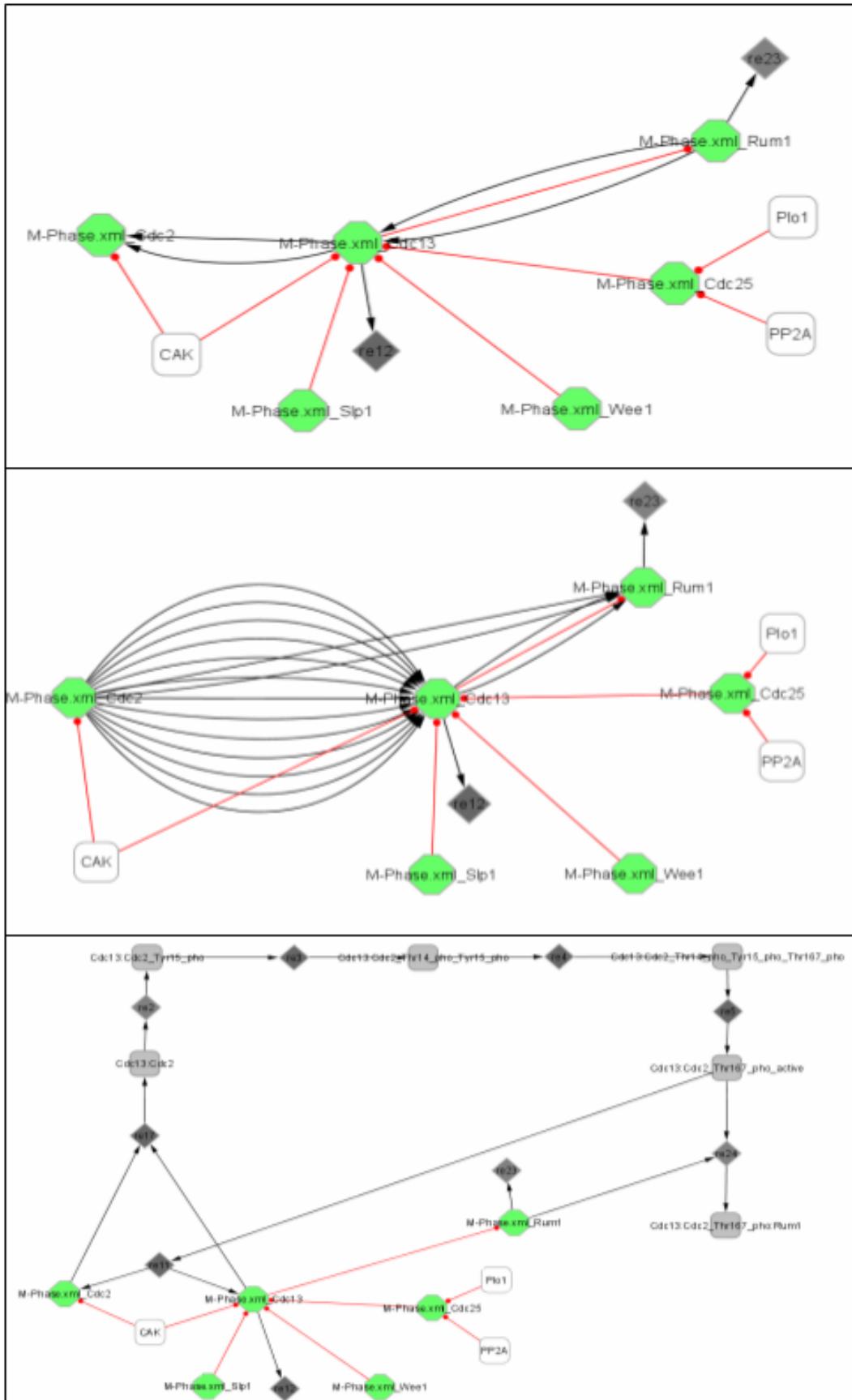


Figure 17: BiNoM modular view of the newtork: The resulting modular network (upper panel) with compact module intersections (middle panel) and with explicit intersections (lower panel).

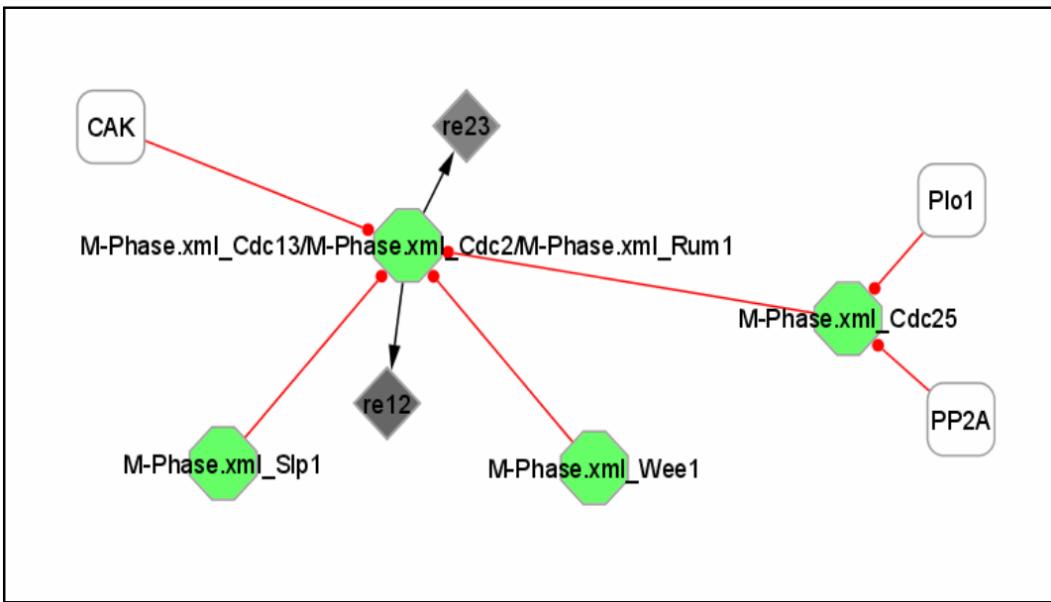


Figure 18: Clusters of modules. The obtained diagram is a compact modular view of the M-Phase network using the material decomposition and material components clustering

two modules will collapse into one big module. To determine the clusters, the intersection threshold can be set (from 0 to 100% intersecting components). For a 30% intersection threshold, Figure 18 is obtained. Four clusters of modules were proposed and linked.

An alternative modular view has been obtained using the cycle decomposition instead of the material decomposition. The cycles are presented in Figure 12. They are obtained by clustering the three cycles into two (cycle 1 + cycle2/cycle3) and organized into a modular view (Figure 19).

### 3.11 Mono-molecular react.to edges

#### Plugins⇒BiNoM analysis⇒Mono-molecular react. to edges

This command transforms monomolecular (with one reactant and one product) reaction nodes into influence edges. Thus, monomolecular (linear) reactions are represented as edges and the reaction graph is not bi-partite anymore. When the reaction nodes have the type of influence specified (through the EFFECT attribute), the graph is transformed automatically into an influence graph (see Figure 20: upper panel: BioPAX network, lower panel, the equivalent influence network). Non-linear non-monomolecular reactions (such as complex assemblies) are not transformed and remain to be represented as network nodes.

### 3.12 Linearize network

#### Plugins⇒BiNoM analysis⇒Linearize network

Remove reactions and reconnect edges according to a supposed influence (figure 21)

**⚠** The got network is not an influence network in the biological sense. But, it can be used to build an influence network.

### 3.13 Exclude intermediate nodes

#### Plugins⇒BiNoM analysis⇒Exclude intermediate nodes

This function opens a dialog where nodes to be excluded can be selected (figure 22). It creates a network without the selected nodes and reconnects edges.

### 3.14 Extract Reaction Network

#### Plugins⇒BiNoM analysis⇒Extract reaction networks

This function cleans up the diagram to only keep the reaction network. Only nodes with XXXX\_REACTION and XXXX\_SPECIES attributes (where XXXX stands for any word) are kept as a result of this operation.

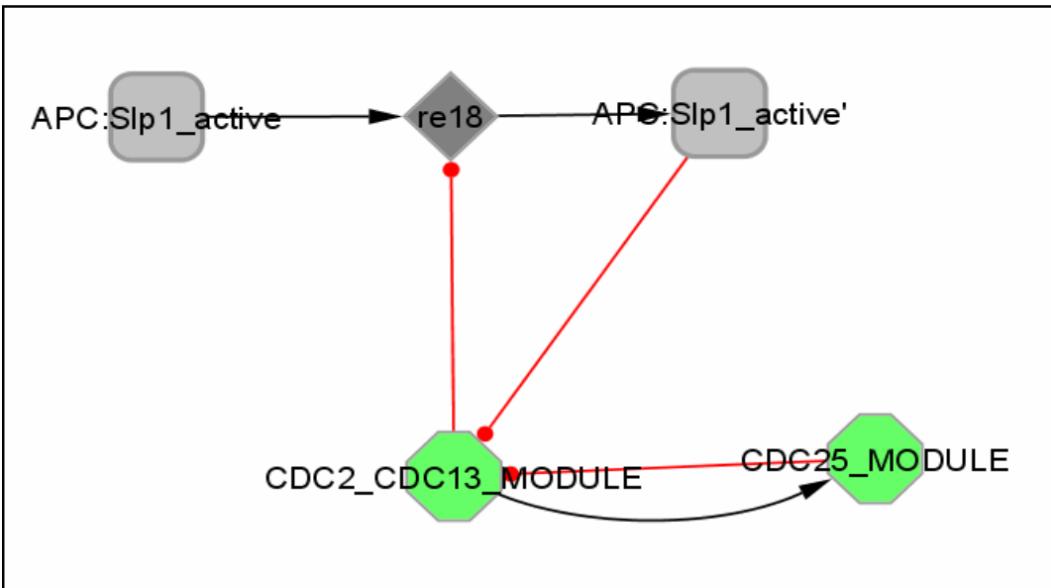


Figure 19: Clusters of modules. The obtained diagram is a compact modular view of the M-Phase network using the relevant cycle decomposition and cycle clustering

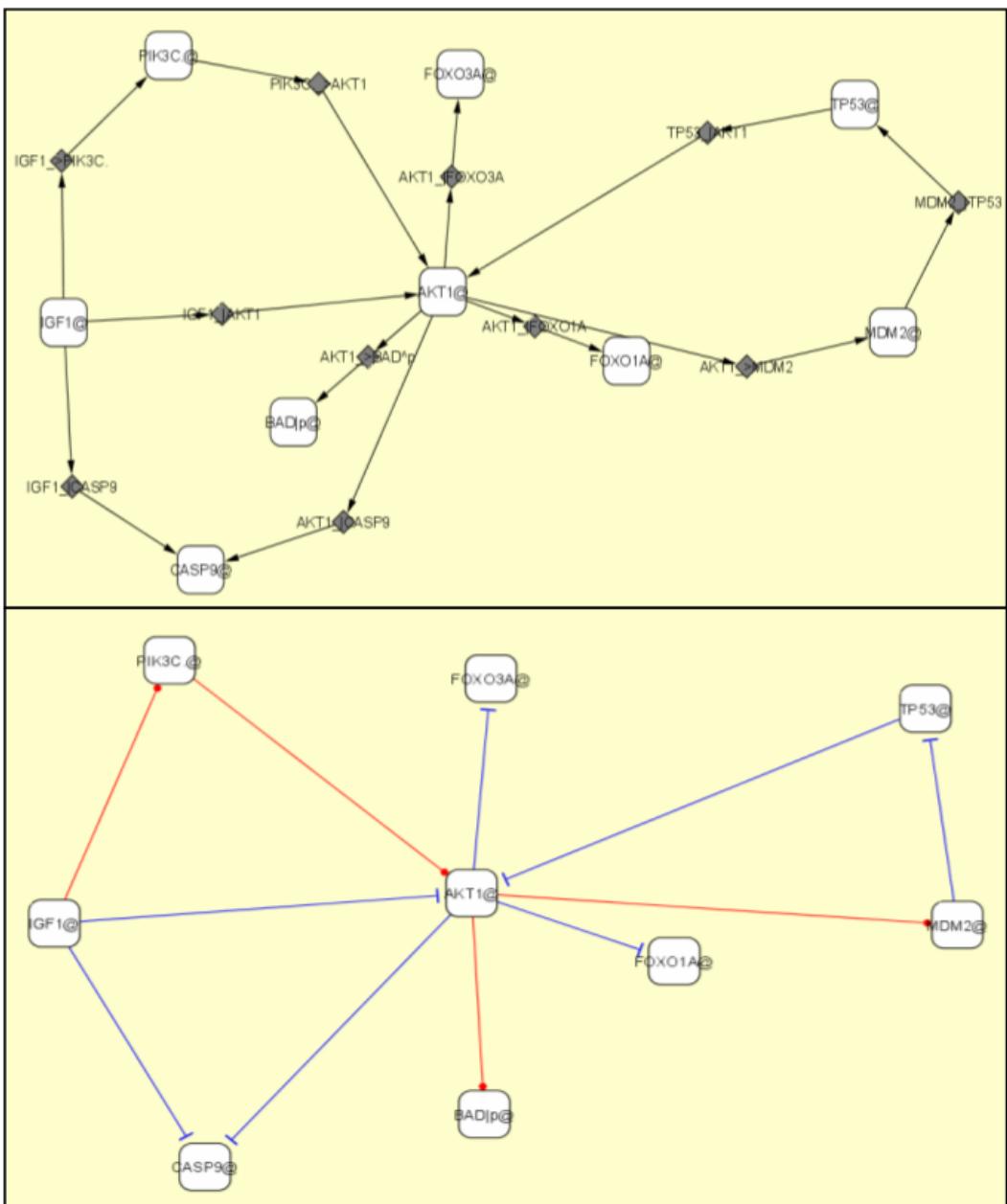


Figure 20: From a BioPAX network (upper panel) to an influence graph (lower panel).

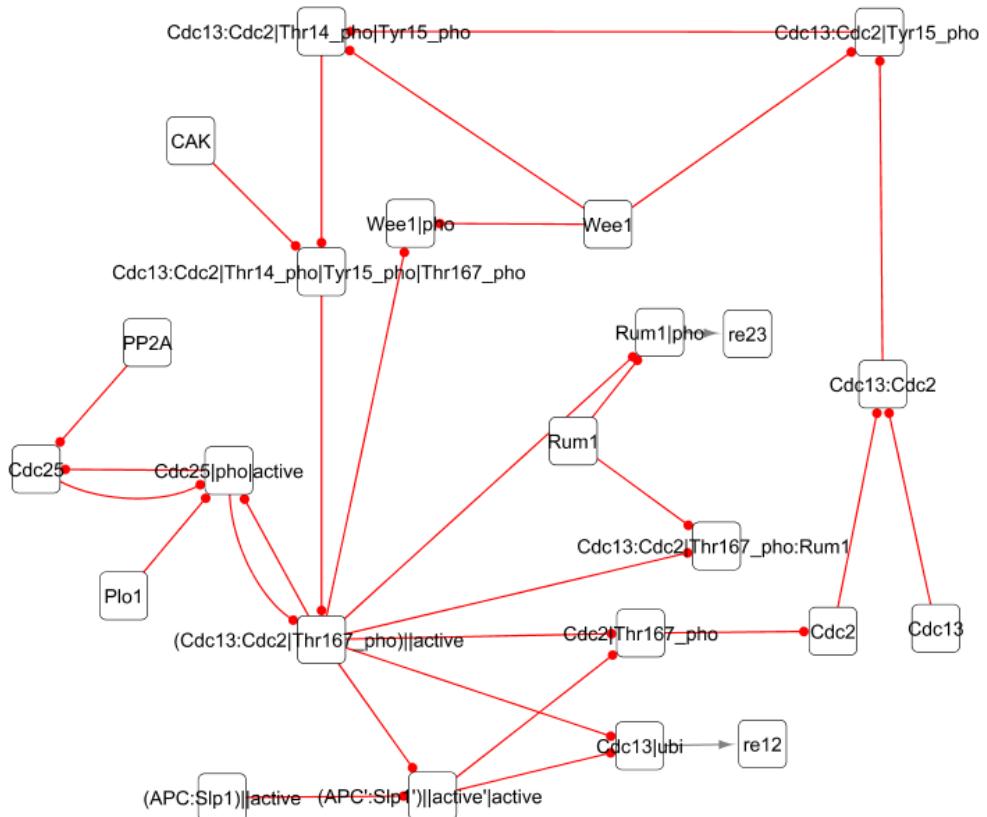


Figure 21: Result of applying "Linearize network" to M-Phase.

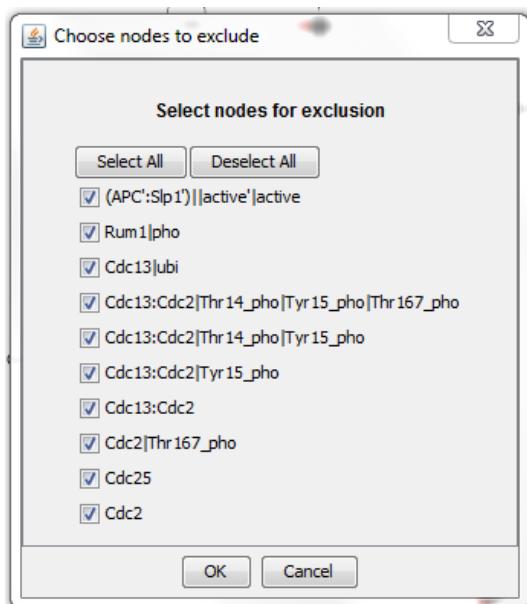


Figure 22: Dialog to select nodes to be excluded in the created network.

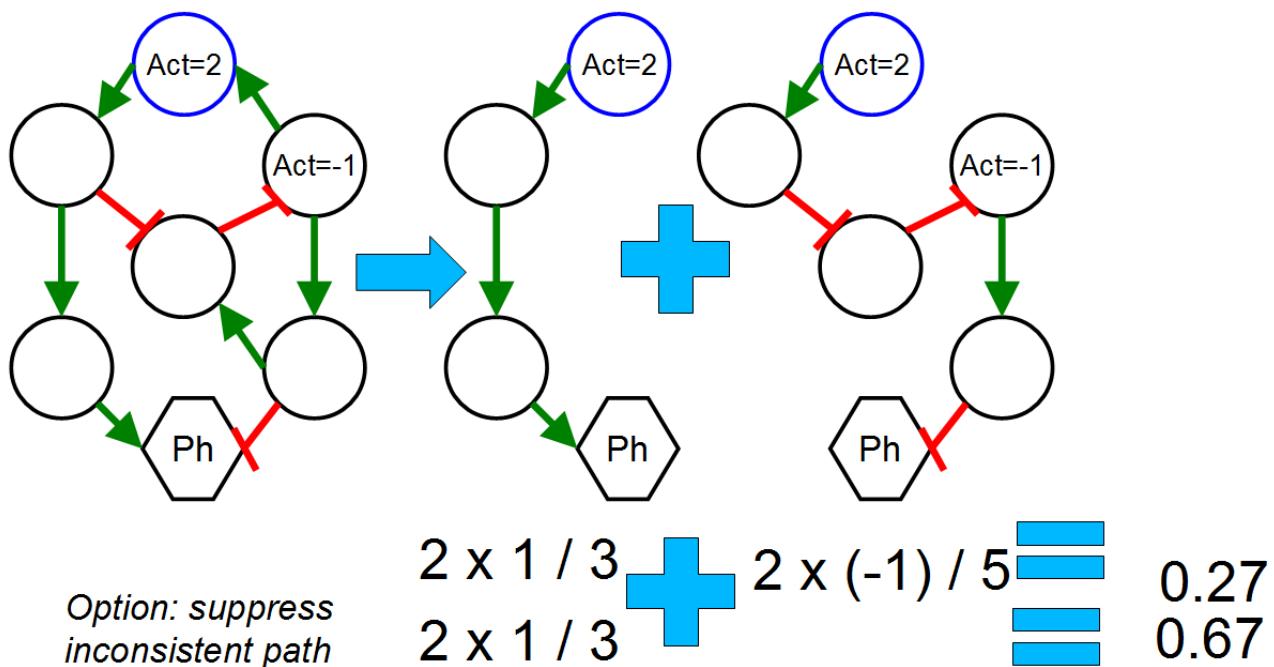


Figure 23: Computing influence in a simple example.

For example, it helps to clean the reaction network interface from the result of querying BioPAX index (which contains many other node types such as entities and publications).

### 3.15 Path consistency analysis

#### Plugins⇒BiNoM analysis⇒Path consistency analysis

This function is based on an algorithm of Path Influence Quantification (PIQuant). Shortly, for every annotated nodes, the influence is computed by summing (optimal and sub-optimal) paths contribution ( $1/\text{length of the path}$ ), multiplied by the nodes annotation that represent biological data. For global influence computation, influences are summed on all over the annotated nodes. The figure 23 shows the computing in a simple example.

⚠ The edge attribute which signs influence is "EFFECT" which can take 2 values EFFECT:activation and EFFECT:inhibition. An AIN file updates this attribute (section 2.4) or it can be imported as an edge attribute by Cytoscape.

For global influences, a p-value can be computed, by shuffling the annotation. Among the possible options accessible in BiNoM, the possibility of removing inconsistent path (paths that have an intermediate node with inconsistent annotation) was sometimes used.

The dialog has 2 steps: input parameters and options, select path and display results.

Step 1 see figure 24:

- Select activity attribute name and update list, only active nodes are displayed in box.
- Targets are phenotypes, any node can be selected.
- Choose options of paths, see glossary in section 8.7.
- Click OK.

Step 2 see figure 25:

- Click on a target node, paths and features of path evaluated according to PIQuant are displayed.

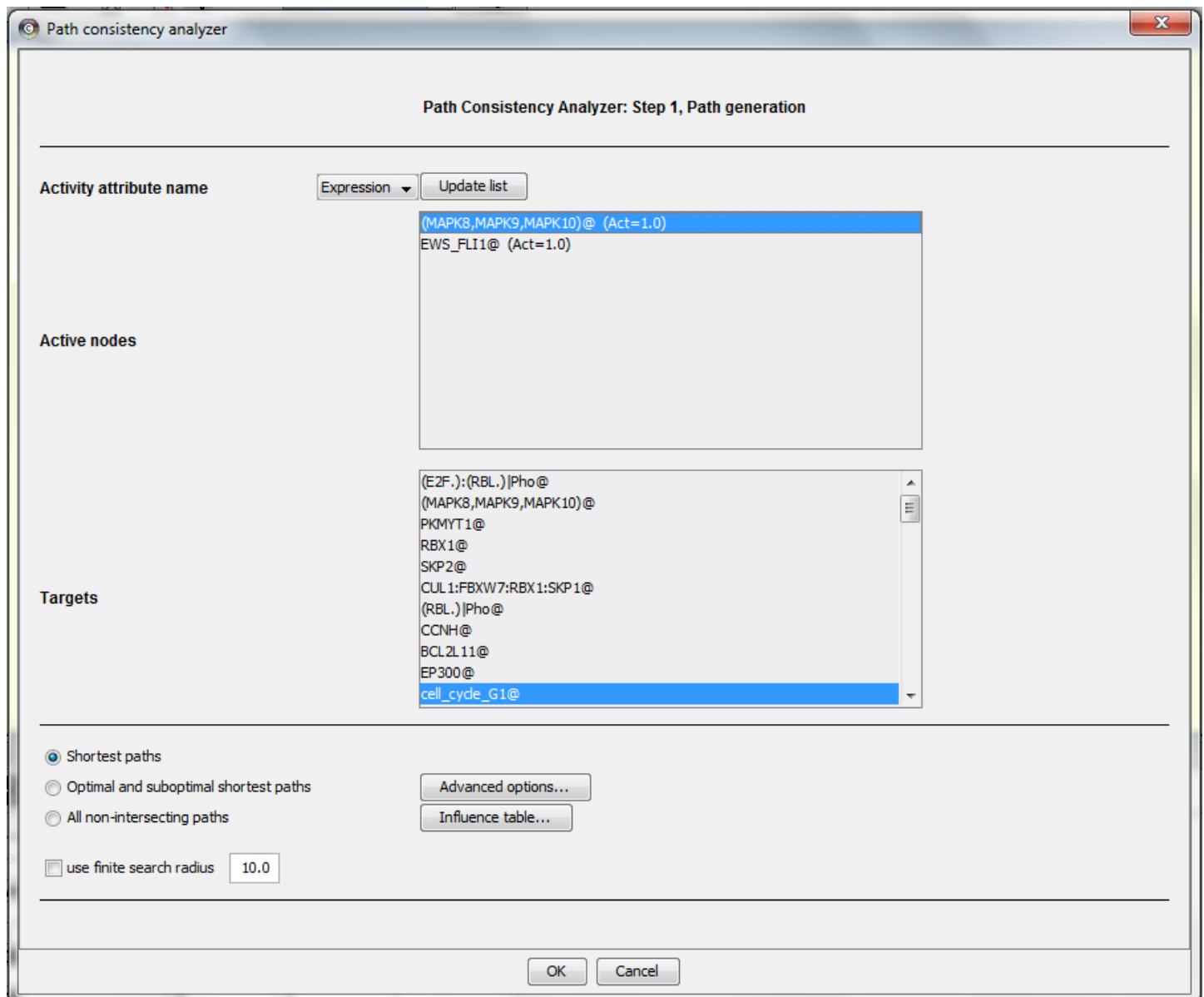


Figure 24: Path consistency analysis: dialog of step 1, select attribute, sources, targets and options.

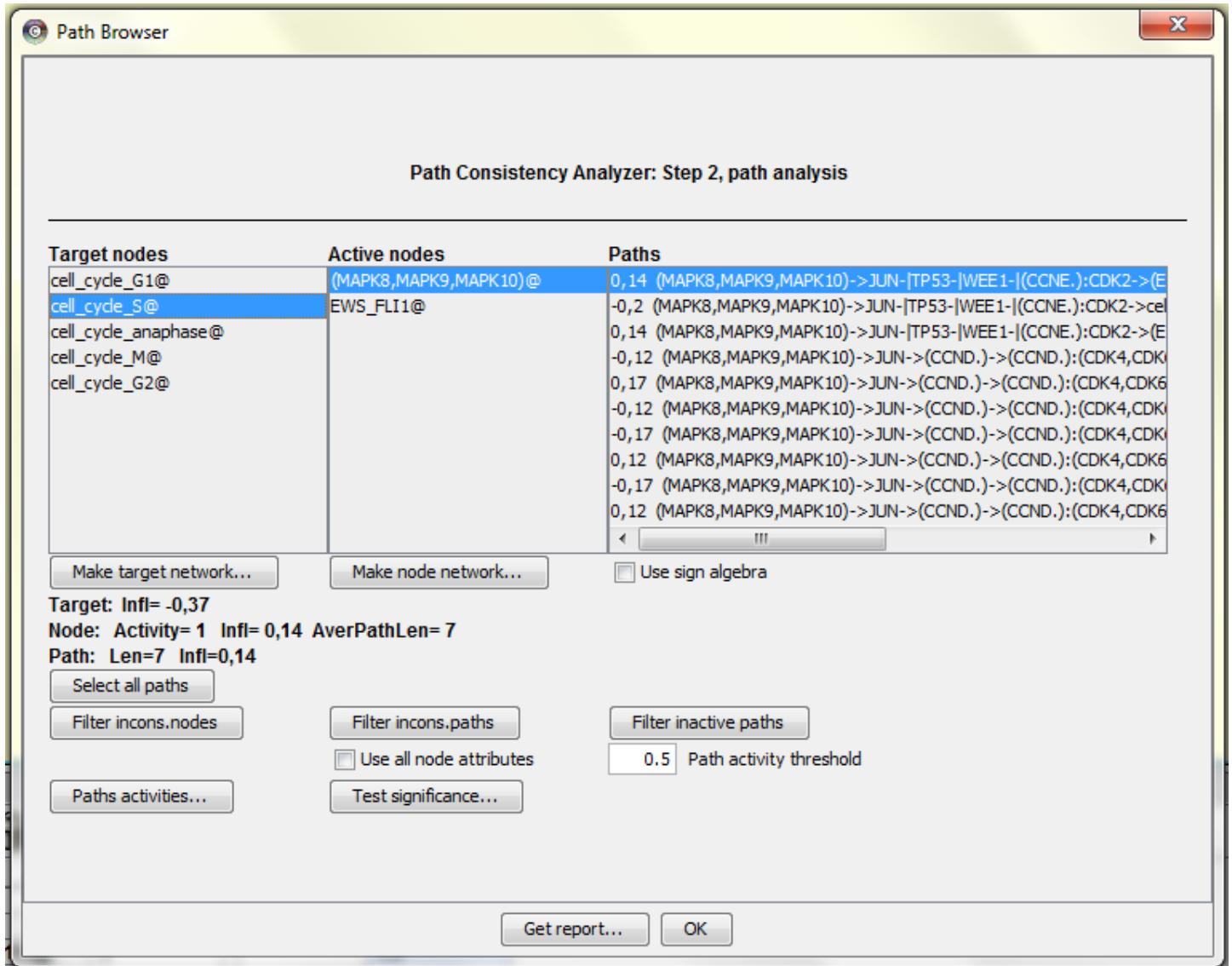


Figure 25: Path consistency analysis: dialog of step 2, display paths and activities, get all results

- A text report about optimal cut set can be get by "path activities".
- Possibility to filter nodes and paths.
- A significance test by p-value allows to insure got activities are significant.

### 3.16 OCSANA analysis

Plugins⇒BiNoM analysis⇒OCSANA analysis



### 3.17 Create neighborhood sets file

Plugins⇒BiNoM analysis⇒Create neighborhood sets file

This function creates a file \*.gmt (a text file where nodes are separated by <Tab>) containing the neighbors of selected nodes according to option of the dialog(figure 26)

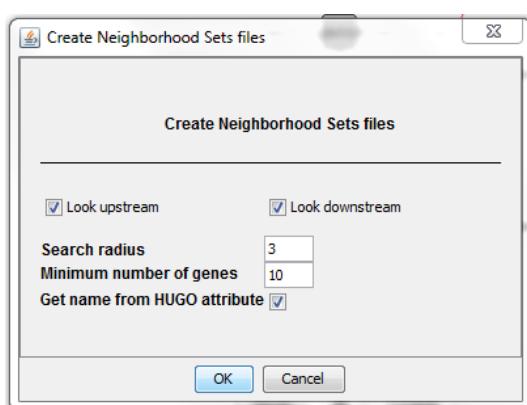


Figure 26: Dialog for options of creating a neighborhood sets file.

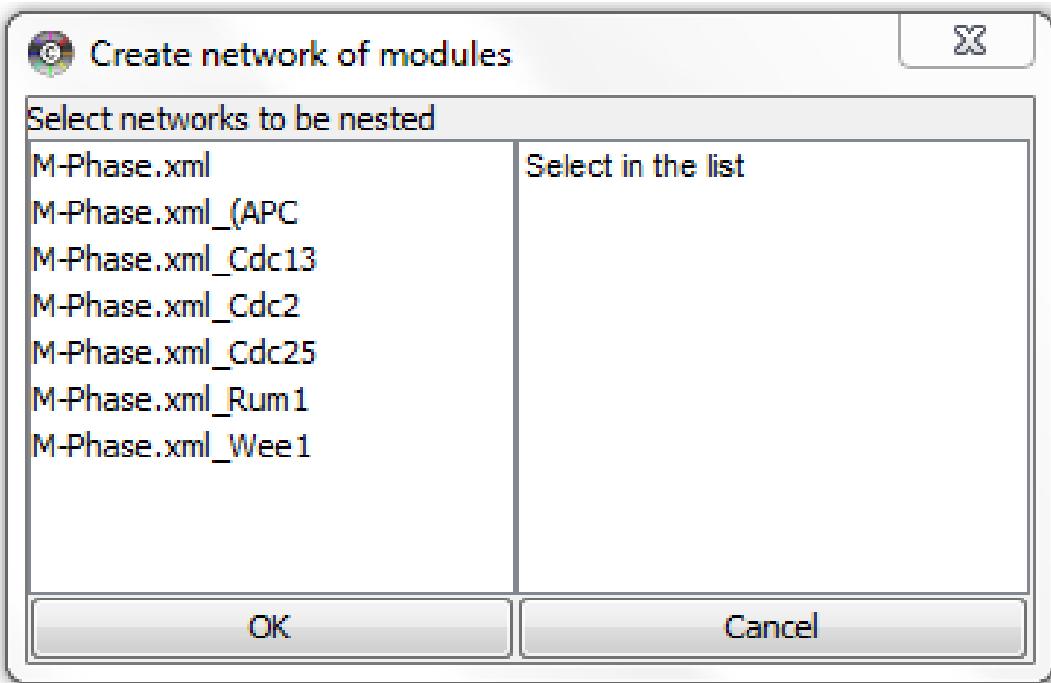


Figure 27: Dialog for select networks to become modules in modular0

## 4 BiNoM Module Manager

Module manager is useful for creating modular view of large networks without loosing details of modules (using nest, object of Cytoscape v7 and after).

### 4.1 Create network of modules

**Plugins⇒BiNoM module manager⇒Create network of modules**

Create a new network from a list of sub-networks (sub-networks are selected in the network list see figure 27). Nodes=modules, no edge. Visual style created in VizMapper for module network . The got network is as 28 without edge and with nodes on grid.

⚠ Module names and node names must be different, all network names too.

To go from module to sub-network:

select node⇒CRight click⇒CNested Network⇒Go to Nested Network.

### 4.2 Create connections between modules

**Plugins⇒BiNoM module manager⇒Create connections between modules**

Create edges linking modules from all edges of the selected network.

Links are simplified, no distinction between left and right (molecule flow), no duplication if same interaction. Warning message if duplicated or absent nodes (may disturb links).

### 4.3 Create modules from networks

**Plugins⇒BiNoM module manager⇒Create modules from networks**

Create modules in the active network from a list of sub-networks (sub-networks are selected in the network list)

All edges are kept. See edge attribute PREVIOUS\_ID for their origin.

The attribute BIOPAX\_NODE\_TYPE is set to pathway (see visual style BiNoM BioPAX).

⚠ All nodes of sub-networks must be found once in the active network (no intersection between sub-networks).

### 4.4 Agglomerate the nearest nodes in modules

**Plugins⇒BiNoM module manager⇒Agglomerate the nearest nodes in modules**

Create modules and a modular view by agglomerating the nearest nodes in the active network (see algorithm in

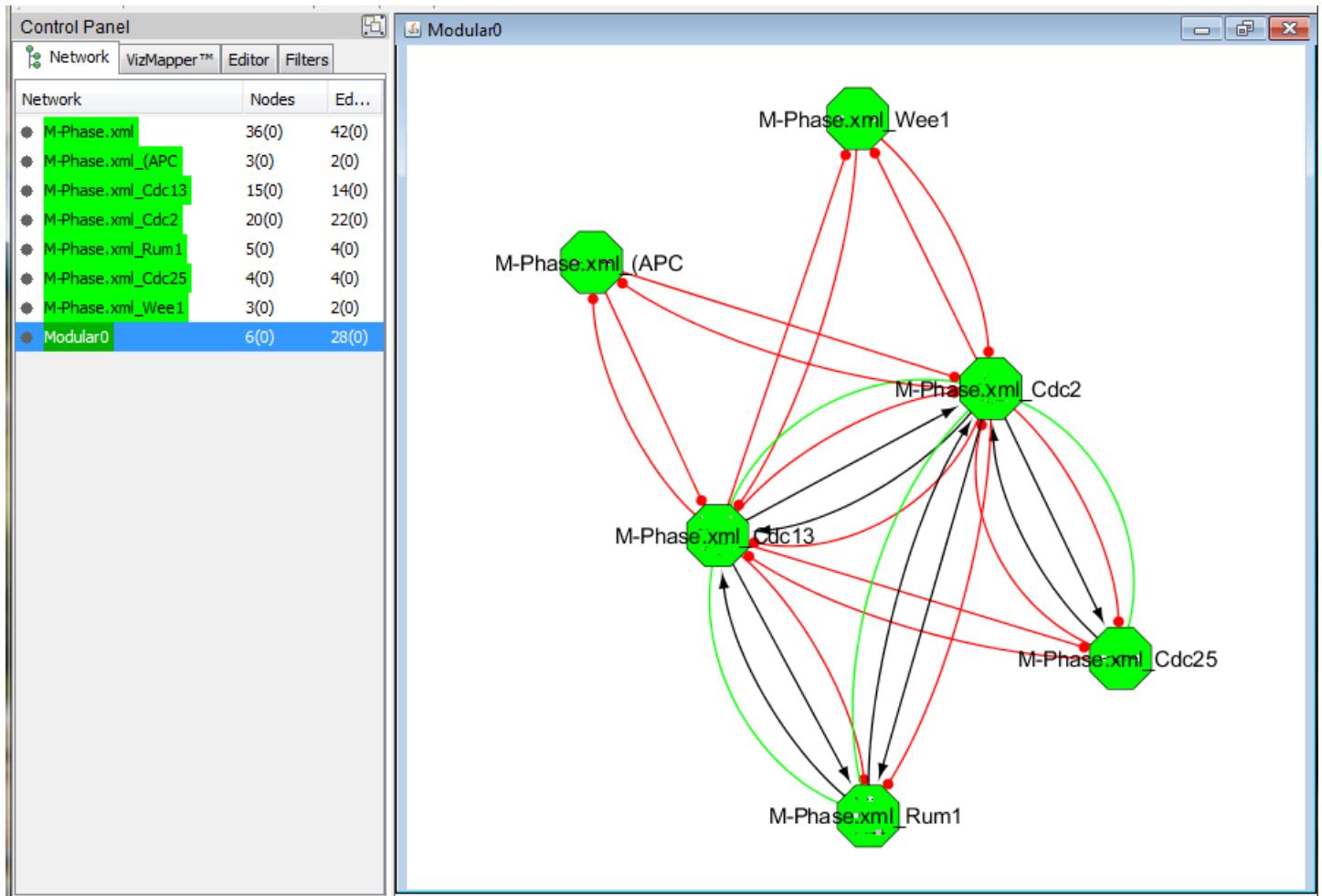


Figure 28: M-Phase is divided into modules by get material component. The modular view is got by creating network of modules with organic layout. The function "Create connections between modules" links modules according to the reference network. The function "Find common nodes in modules" creates intersection edges.

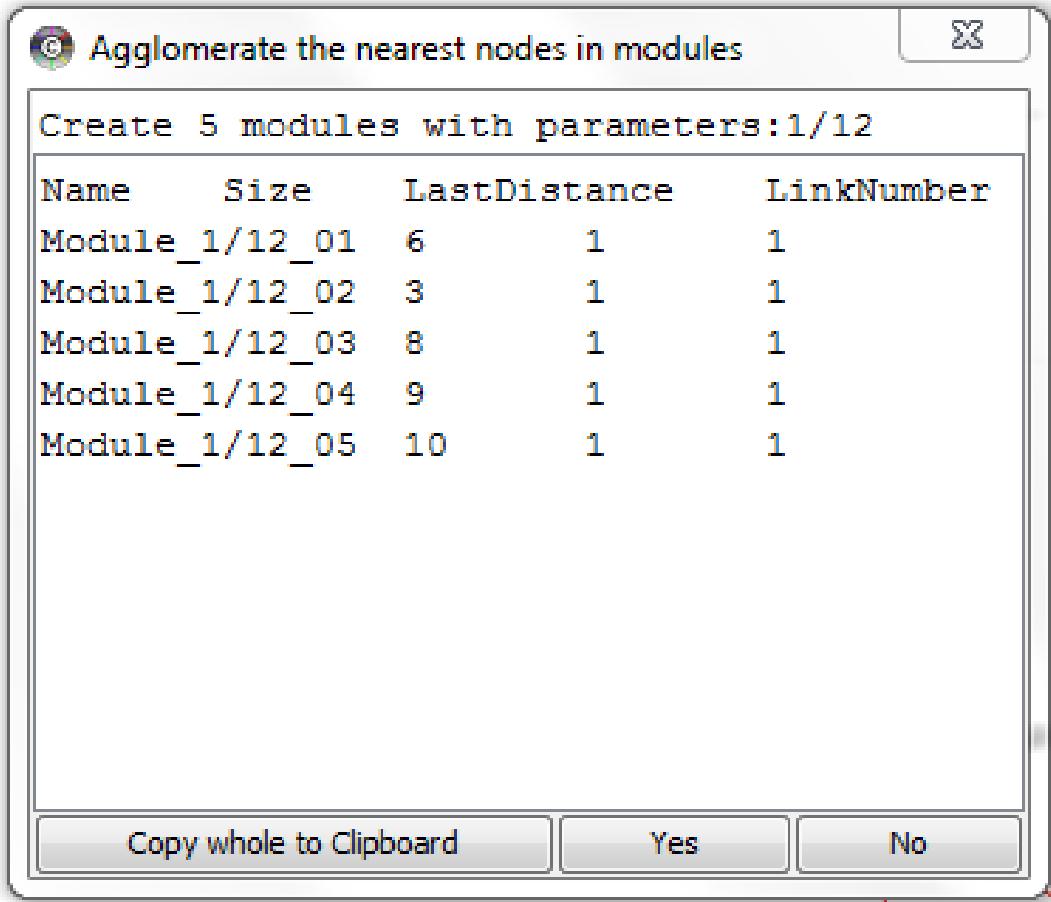


Figure 29: This window displays modules, number of node, last distance and number of links of agglomerating process. Yes lauches the process of agglomerating.

section 8.6.

Input 2 parameters to get not too big sub-networks containing not too far nodes:

- Maximal distance between nodes or modules in number of edges,
- Maximal number of nodes in modules.

Confirm creation if agree with displayed result (see dialog 29).

Sub-networks are created and gathered in a packed network as the function "Create modules from networks" (see figure 30).

#### 4.5 List nodes of modules and network

**Plugins⇒BiNoM module manager⇒List nodes of modules and network**

List nodes of network and nodes included in modules.

Result in text box can be simply copied in a spreadsheet through clipboard.

#### 4.6 Find common nodes in modules

**Plugins⇒BiNoM module manager⇒Find common nodes in modules**

Display in text box the belonging matrix of nodes (modules in columns, nodes in rows, size of modules in last row, frequency in modules in last column); result more easily usable after copying in a spreadsheet (see 31).

Create intersection edges with number of common nodes as attribute (COMMON\_NODES), green edges in figure 28.

Create node attribute containing the node numbers of modules (NODE\_NUMBER).

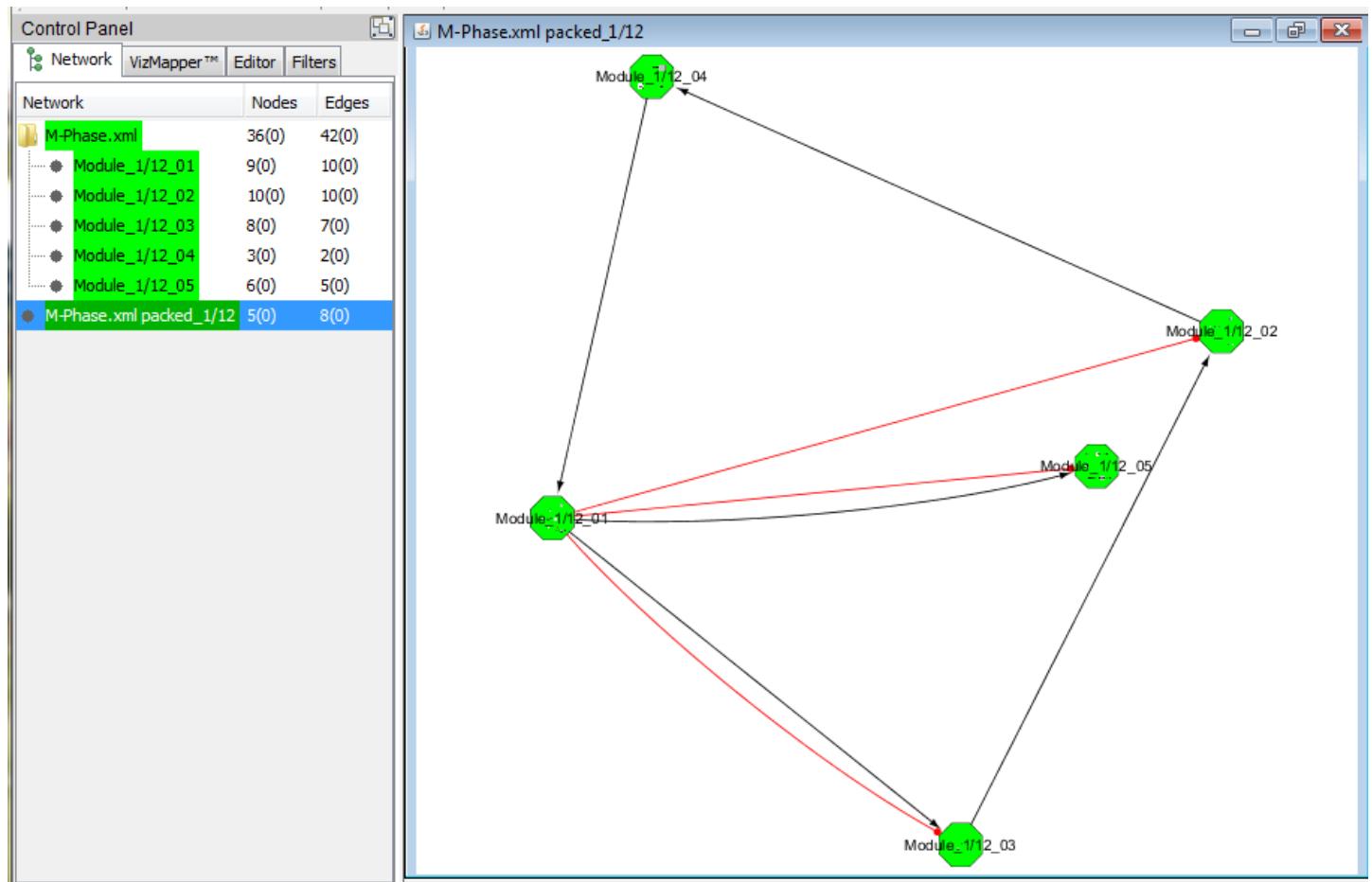


Figure 30: M-Phase is got by creating network from modules, modules created by agglomerating the nearest nodes (maximal distance=1, maximal size=12 nodes).

	M-Phase.xml _Wee1	M-Phase.xml _Rum1	M-Phase.xml _Cdc25	M-Phase.xml _Cdc2	M-Phase.xml _Cdc13	M-Phase.xml _(APC)	Frequency
(APC:Slp1)  active	0	0	...	0	0	0	1
Cdc13:Cdc2 Thr14_ph0 Tyr15_ph0 Thr167_ph0	0	0	...	0	1	1	0
Cdc13:Cdc2 Tyr15_ph0	0	0	...	0	1	1	0
Wee1	1	0	...	0	0	0	1
Cdc13:Cdc2	0	0	...	0	1	1	0
Wee1 ph0	1	0	...	0	0	0	1
Cdc13:Cdc2 Thr167_ph0:Rum1	0	1	...	0	1	1	0
...	...	...	...	...	...	...	...
Cdc25 ph0 active	0	0	...	1	1	0	0
re3	0	0	...	0	1	1	0
(APC':Slp1')  active'active	0	0	...	0	0	0	1
Cdc13	0	0	...	0	0	1	0
re15	0	0	...	1	1	0	0
re2	0	0	...	0	1	1	0
re20	0	1	...	0	0	0	0
<b>Size</b>	3	5	...	4	20	15	3

Figure 31: Matrix of nodes: modules in columns, nodes in rows, size of modules in last row, frequency in modules in last column.

Module Visual StyleCan be adapted to the wished visual aspect by hands in VizMapper, for example:

- To visualize NODE\_NUMBER: double click Node Size, select NODE\_NUMBER, continuous mapping, adjust width by graphical view.
- To visualize COMMON\_NODES double click Edge Line Width, select COMMON\_NODES, continuous mapping, adjust width by graphical view.

#### 4.7 Assign module names to node attribute

Plugins⇒BiNoM module manager⇒Assign module names to node attribute

Create a node attribute (named as the modular network), containing module names. This attribute may be used to visualize modules in the reference network.

#### 4.8 List components of species in network and modules

Plugins⇒BiNoM module manager⇒List components of species in network and modules

List components of species (their names must respect BiNoM syntax). Useful to name modules.

#### 4.9 Create network from union of selected modules

Plugins⇒BiNoM module manager⇒Create network from union of selected modules

Create a network from union of selected modules and its corresponding module in the current network (named by module names separated by &).

#### 4.10 Create network from intersection of 2 selected modules

Plugins⇒BiNoM module manager⇒Create network from intersection of 2 selected modules

Create a network from intersection of 2 selected modules and its corresponding module (named by module names separated by |).

Confirm for deleting the common nodes in the selected modules.

#### 4.11 Recreate lost connections inside modules

Plugins⇒BiNoM module manager⇒Recreate lost connections inside modules

Recreate connections inside modules which may have been lost by modularizing operations.

#### **4.12 Destroy networks unused as module**

**Plugins⇒BiNoM module manager⇒Destroy networks unused as module**

Select networks to be deleted among a list of networks which are not used as modules in the current network (simplify cleaning session).

## 5 BiNoM BioPAX3 Utils

### 5.1 BioPAX 3 Property Editor

Plugins⇒BiNoM BioPAX 3 Utils⇒ BioPAX 3 Property Editor

All the information available in a BioPAX file can be easily retrieved using the BioPAX Property Editor function. A component on the diagram must be selected first (CDC2 in Figure 32) and a window appears with all available information concerning the molecule.

In the menu of the Property Editor, several options are offered:

- Display valid attributes / Display all attributes hides all the empty fields (for example, in Figure 33: Availability or Evidence have <empty object list> and would be hidden) / shows all the available fields, even the empty ones.
- << and >> correspond to back or forward buttons and follow the historical exploration of the Property Editor (similar to Back and Forward buttons of a network browser).
- Close current tab or Close all tabs closes the current page of the property editor or all the open pages.
- Display / Edit shows a simple display of the page editor where no change can be made (Figure 33) / allows changes in the fields by adding, removing or updating information (Figure 32). For the latter, click first on the Edit tab on the upper menu, then on update situated near the field to modify. In Figure 32, as an example, some comments were added manually: CDC2 is a kinase that binds to CDC13 to form a dimer. In the Apoptosis example (Figure 33), extensive information is already available concerning the pathway, references, etc.



Figure 32: BioPAX Property Editor: example of the properties concerning CDC2 component in M-Phase model

For more details on BioPAX description standard, visit the webpage: <http://www.biopax.org/>

### 5.2 BioPAX 3 Class Tree

Plugins⇒BiNoM BioPAX 3 Utils⇒BioPAX 3 Class Tree

All the statistics concerning the pathway are listed: the number of reactions, associations or catalyses, the number of proteins or complexes, etc (figure 34). More information can be accessed by selecting a specific object which, when clicked on, leads to the BioPAX 3 Property Editor window (see section 5.1).

To complete the network, the user can easily add new information or a new protein, protein complex, type of interaction, etc., by clicking on the New Instance tab.

### 5.3 Use Simplified URI Names

**Plugins⇒BiNoM BioPAX 3 Utils⇒Use Simplified URI Names**

In the BioPAX Class Tree, protein names can have either URI names (Uniform Resource Identifier used to give a unique identification to proteins) or BiNoM Naming Service names. For example, for the apoptosis pathway, the protein BAD is referred to as

*UniProt\_Q92934\_Bcl2\_antagonist\_of\_cell\_death\_BAD\_Bcl\_2\_binding\_component\_6\_Bcl\_XL\_Bcl\_2\_associated\_death\_promoter\_Bcl\_2\_like\_8\_protein*

in the URI case and just BAD in the BiNoM Naming Service case. For the rules of how BiNoM generates names see section 8.3.

### 5.4 Synchronize networks with BioPAX 3

**Plugins⇒BiNoM BioPAX 3 Utils⇒Synchronize networks with BioPAX 3**

This command updates all the interfaces according to the changes made to individual BioPAX objects.

**BioPAX Property Editor**

All attributes displayed    Display valid attributes    <<    >>    Close current tab    Close all tabs    Edit

**Apoptosis**

**Class pathway**  
**Object Apoptosis**

**AVAILABILITY**  
<empty string list>

Apoptosis is a distinct form of cell death that is functionally and morphologically different from necrosis. Nuclear chromatin condensation, cytoplasmic shrinking, dilated endoplasmic reticulum, and membrane blebbing characterize apoptosis in general. Mitochondria remain morphologically unchanged. In 1972 Kerr et al introduced the concept of apoptosis as a distinct form of "cell-death", and the mechanisms of various apoptotic pathways are still being revealed today. <BR>The two principal pathways of apoptosis are (1) the Bcl-2 inhibitable or intrinsic pathway induced by various forms of stress like intracellular damage, developmental cues, and external stimuli and (2) the caspase 8/10 dependent or extrinsic pathway initiated by the engagement of death receptors<BR> The caspase 8/10 dependent or extrinsic pathway is a death receptor mediated mechanism that results in the activation of caspase-8 and caspase-10. Activation of death receptors like Fas/CD95, TNFR1, and the TRAIL receptor is promoted by the TNF family of ligands including FASL (APO1L OR CD95L), TNF, LT-alpha, LT-beta, CD40L, LIGHT, RANKL, BLYS/BAFF, and APO2L/TRAIL. These ligands are released in response to microbial infection, or as part of the cellular, humoral immunity responses during the formation of lymphoid organs, activation of dendritic cells, stimulation or survival of T, B, and natural killer (NK) cells, cytotoxic response to viral infection or oncogenic transformation. <BR> The Bcl-2 inhibitable or intrinsic pathway of apoptosis is a stress-inducible process, and acts through the activation of caspase-9 via Apaf-1 and cytochrome c. The rupture of the mitochondrial membrane, a rapid process involving some of the Bcl-2 family proteins, releases these molecules into the cytoplasm. Examples of cellular processes that may induce the intrinsic pathway in response to various damage signals include: auto reactivity in lymphocytes, cytokine deprivation, calcium flux or cellular damage by cytotoxic drugs like taxol, deprivation of nutrients like glucose and growth factors like EGF, anoikis, transactivation of target genes by tumor suppressors including p53.<BR> In many non-immune cells, death signals initiated by the extrinsic pathway are amplified by connections to the intrinsic pathway. The connecting link appears to be the truncated BID (tBID) protein a proteolytic cleavage product mediated by caspase-8 or other enzymes.

**COMMENT**

**DATA\_DASH\_SOURCE**  
ReactomeDataSource

**EVIDENCE**  
<empty object list>

**NAME**  
Apoptosis

**ORGANISM**  
Homo\_sapiens

**PATHWAY\_DASH\_COMPONENTS**  
Activation\_of\_Effector\_CaspasesStep  
Extrinsic\_Pathway\_for\_ApoptosisStep  
Intrinsic\_Pathway\_for\_ApoptosisStep  
Activation\_myristylation\_of\_BID\_and\_translocation\_to\_mitochondriaStep  
Apoptotic\_execution\_phaseStep

**SHORT\_DASH\_NAME**  
Apoptosis

**SYNONYMS**  
<empty string list>

**XREF**  
15218528@PubMedWilliams, MacFarlane, JEMBO, Rev, 5, 674, R, 2004

click + drag to ZOOM    Middle-click + drag to PAN

Figure 33: BioPAX Property Editor: example of apoptosis pathway node properties

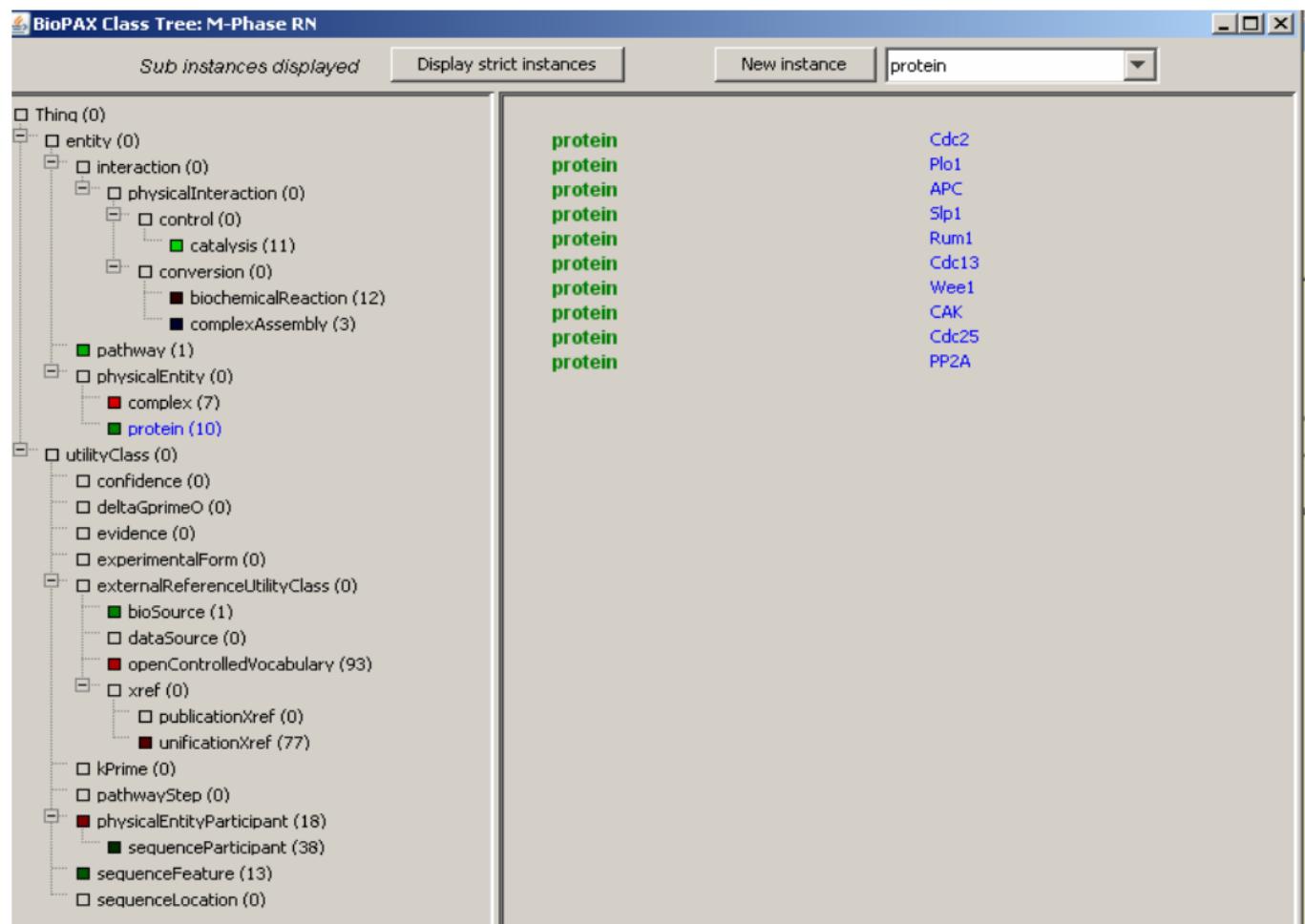


Figure 34: BioPAX Class tree. On the left frame, the model is described in terms of interactions, entities, etc. On the right frame, the proteins, selected in the left frame, are listed. The links are clickable and open a BioPAX Property Editor window.

## 6 BiNoM BioPAX3 Query

The purpose of the functions related to the query language is to work with huge BioPAX files and extract from the BioPAX documents only the information that is of interest. For this part, we will use the apoptosis example initially extracted from Reactome database: Apoptosis.owl. This set of functions can be used with big pathway databases already exported to BioPAX: Reactome, BioCyc, NetPath (see <http://www.biopax.org> for the complete list).

### 6.1 Generate Index

Plugins⇒BiNoM BioPAX 3 Query⇒Generate Index

Using this function BiNoM maps the content of BioPAX file onto a labeled graph (referred to as index). It creates an \*.xgmml file from an \*.owl one (figure 35). For the definition of BioPAX index, see section 8.4.

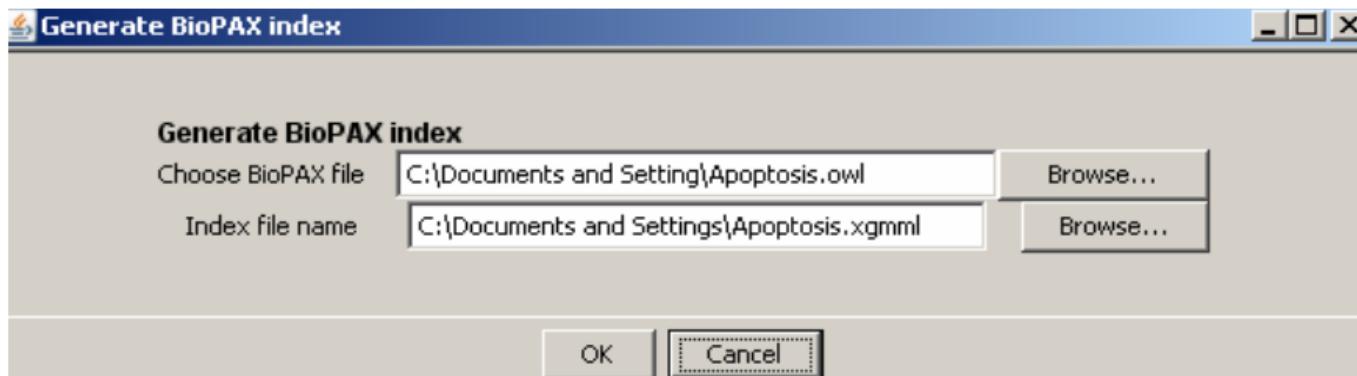


Figure 35: Generate BioPAX Index.

### 6.2 Load Index

Plugins⇒BiNoM BioPAX 3 Query⇒Load Index

Once the xgmml is created, it can be loaded into memory. The index is global object, i.e. only one index can be used at a time. Load Index loads the index file from xgmml format (figure 36).

Together with the index, you can also upload a tab-delimited accession number file which corresponds to

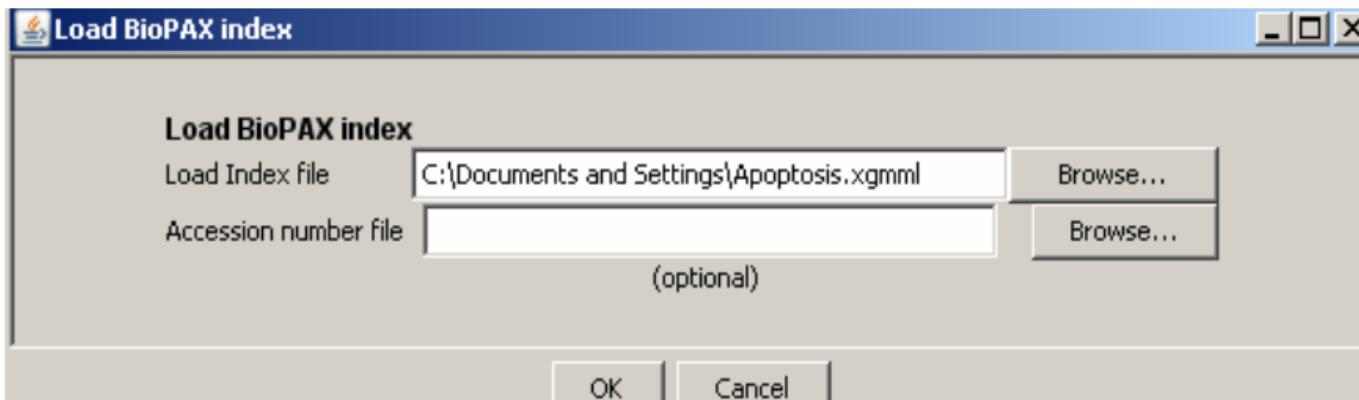


Figure 36: Load Index dialog.

a list of synonyms for the genes/proteins ids used in a network (see an example of the content of some accession number file at figure 37). An entity in the index can be identified by its id, by any XREF attribute (see section 8.4), by node name, or by any synonym from the accession table (if it is provided).

### 6.3 Display Index Info

Plugins⇒BiNoM BioPAX 3 Query⇒Display Index Info

This command opens a window indicating the name of the graph, the name of the file, the accession number

cAMP-dependent protein kinase	ID001
cAPK	ID001
InterPro:IPR000719	ID001
PKA catalytic subunit	ID001
PKAc	ID001
PKAc-beta	ID001
Protein kinase	ID001
protein kinase A	ID001
protein kinase A catalytic subunit	ID001
protein kinase A catalytic subunit	ID001
Protein kinase C-terminal domain	ID001
Serine/threonine protein kinase	ID001
Serine/threonine protein kinase, active site	ID001
Tyrosine protein kinase	ID001
ADP-ribosylation factor	ID002
ADP-ribosylation factor family	ID002
ARF	ID002
Rab	ID002
Ran family	ID002
Ras family	ID002
Rho family	ID002
Rho/Rac family	ID002
small G-proteins	ID002
small GTPases	ID002
small guanyl-nucleotide binding proteins	ID002
activator of G-protein signaling	ID003
AGS	ID003
InterPro:IPR001806	ID003
InterPro:IPR001806	ID003
InterPro:IPR003577	ID003
InterPro:IPR005225	ID003
InterPro:IPR005225	ID003
p21ras	ID003
Rad	ID003
Ral	ID003
Rap	ID003
Ras	ID003
Ras family	ID003
Ras GTPase superfamily	ID003
Ras GTPase superfamily	ID003
Ras homolog enriched in brain	ID003
Ras small GTPase, Ras type	ID003

Figure 37: Example of accession Number file. First column is a synonym (which can have structure <database> : <standard;d>), the second column is the id used inside the BioPAX file.

file, when available, the number of records, and the various statistics of the index: number of publications, proteins, physicalEntities, complexes, biochemical reactions, pathways, pathwaySteps, catalyses, and modulations (necessary proteins for catalyses). See figure 38.

## 6.4 Select Entities

### Plugins⇒BiNoM BioPAX 3 Query⇒Select Entities

The BioPAX document is often too big to find the protein or gene that needs to be studied. To access it easily and rapidly, it is possible to find the component directly with this command and build a specific network around that molecule.

For example, in Apoptosis.xgmml, we choose to find the caspases 8 and extend the network around it. When choosing Plugins =; BiNoM BioPAX Query =; Select entities from the index, a dialog window pops up and offers the possibility to find a protein or a gene by its name or id or XREF attribute or synonym, from the current network when a network is already opened, or from the list of identities associated with the BioPAX index (figure 39).

For our example, we choose the second option. To increase the probability to find the protein in the list, we propose, in figure 39, three different versions of the same name: CASP8, Caspase8 or caspases\_8, all separated by space (the separator can be also comma and semi-colon or line-break symbol). One of them (CASP8) corresponds to the name from the BioPAX list and a new network is created with only one protein, CASP8 (= MCH5 in the index), at the center of it. The other ones were not found (see output in figure ??). It is also possible to select more than one entity, in this case, the components all appear in the same window.

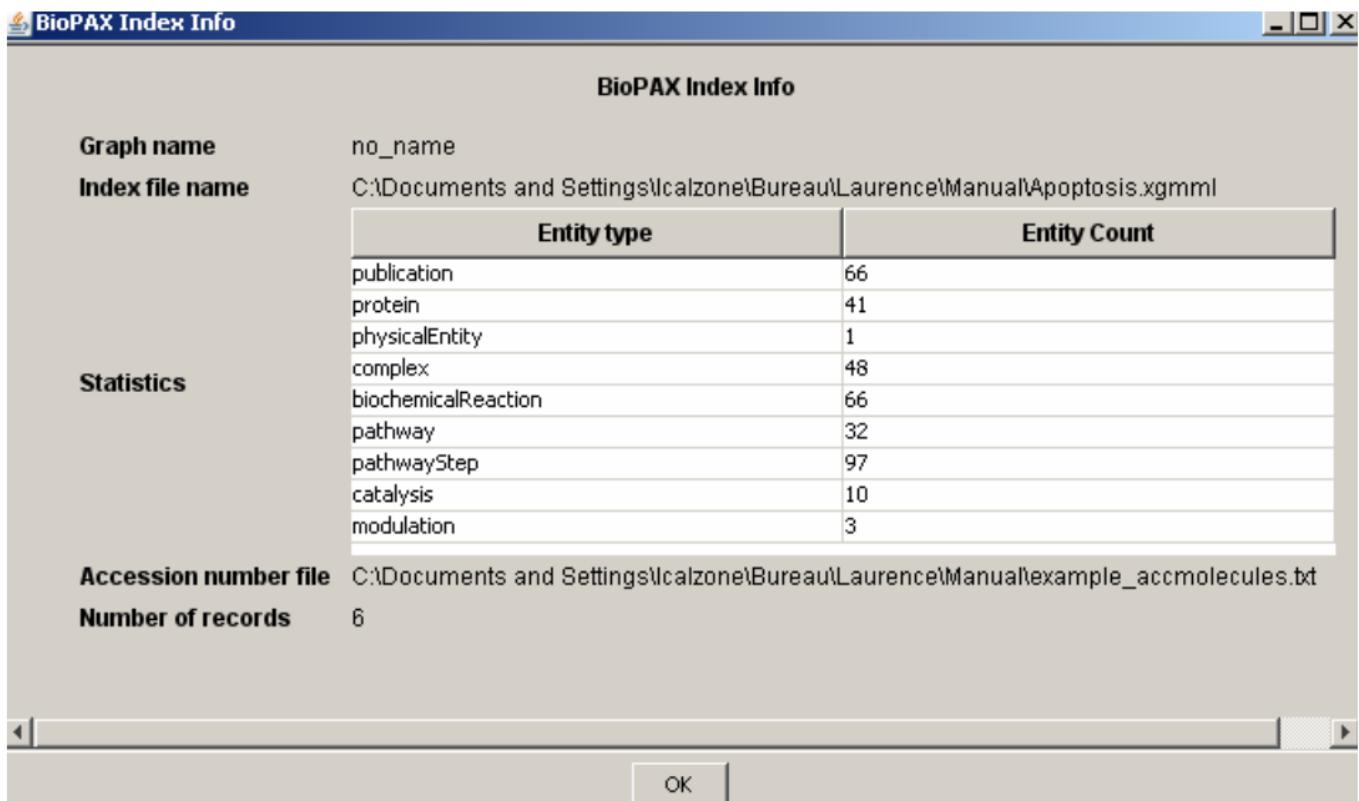


Figure 38: Display Index info.

The output is chosen to appear in a new network (selection is made at the bottom of the dialog window in figure 39) but it is also an option to view several genes or proteins in the same network by checking output in the current network.

A network is created with only one node, caspase 8, called MCH5 in the index. Note that for this part, it is advised to use the BiNoM BioPAX visual style to view the resulting network.

## 6.5 Standard Query

### Plugins⇒BiNoM BioPAX 3 Query⇒Standard Query

This command proceeds through a series of actions that will extend or make the studied network more specific to the users needs.

Lets start with diverse queries from the network created for the Caspase 8 entity. A dialog window opens as figure 40.

All the options proposed in the dialog window (figure 40) are listed here:

#### 6.5.1 In the input section

All nodes / Selected nodes: In the network, you can submit queries that concern all the nodes in the network or only the selected nodes (highlighted in yellow).

#### 6.5.2 Once you decide on which proteins you wish to work, you can:

- Add complexes
  - no expand adds only the homodimers of the molecule (figure 41a). If several proteins were queried, then all hetero-dimers in which all the proteins participate would appear.
  - expand adds all the complexes in which MCH5 is involved (figure 41b). The green arrow with a diamond ending represents the inclusion of one protein in a complex form.

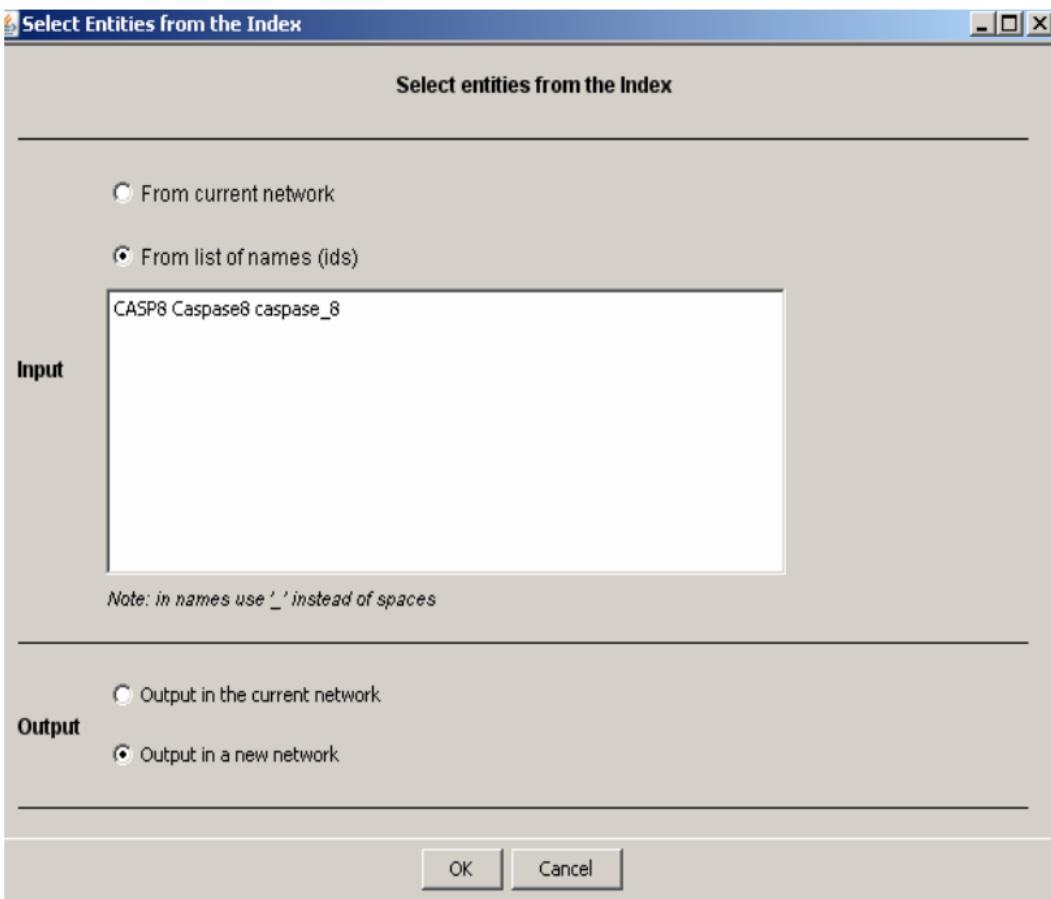


Figure 39: Select entities from the index.

- Add chemical species

This function adds, for each species, the cellular location and its specified modifications. It is linked to the protein with a grey edge (see figure 42).

- Add reactions
  - connecting reactions connects all present species that have common reactions (connected by a yellow node in figure 43).
  - all reactions includes all the reactions involving the chemical species (figure 44).
  - make reactions complete adds all the sources and targets of the reactions (figure 45) listed in the BioPAX index, including, for example, the pathway nodes and publications links.

- Add publications

When available, this function adds all the references associated with a reaction (see figure 46).

### 6.5.3 Output

The result of the queries can be seen either in the current network or in a new network.

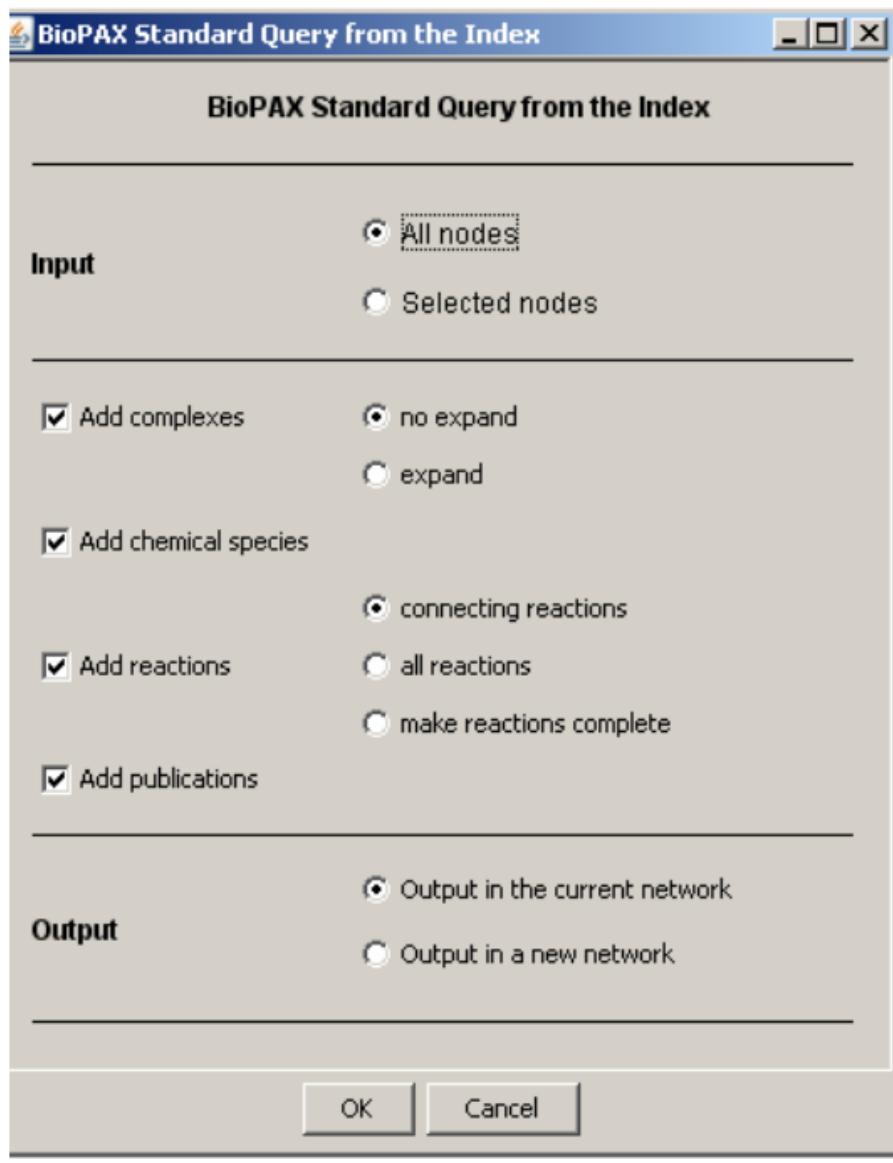


Figure 40: BioPAX Standard Query: dialog window.

## 6.6 Index Path Analysis

### Plugins⇒BiNoM BioPAX 3 Query⇒Index Path Analysis

This command finds the directed or non-directed, shortest, optimal or suboptimal, non intersecting paths with a pre-defined number of intermediaries in an index file. Note that the species need to be selected on a graph before this query.

This part of the query engine uses the same algorithms and options as Path analysis dialog (see section 3.6), however, with the network (index) kept completely in memory, without explicit visualization. Moreover, the network is slightly modified before this type of query: in particular, all non-directed edges (of CONTAINS, SPECIESOF and some other types) are represented as bi-directional, some nodes (publications and, optionally, smallMolecules) are removed.

For example, the following steps

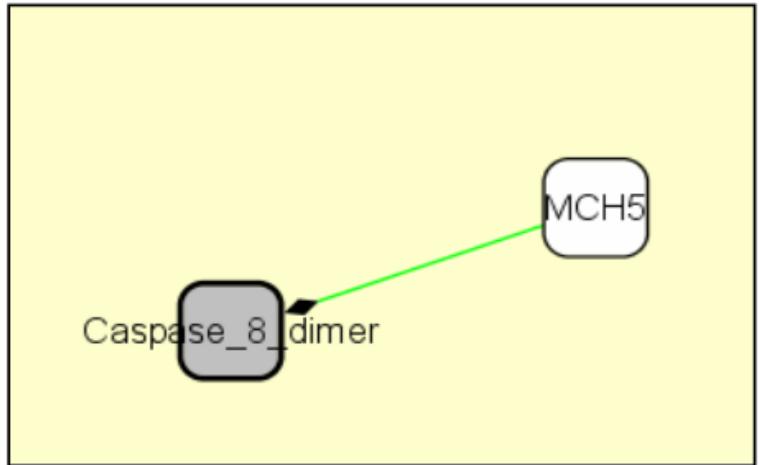
1. Select Entities: specify CASP8 and FAS proteins.
2. Select the two nodes.
3. Index Path analysis: Find all non-intersecting paths.
4. BiNoM Analysis: Extract Reaction Network.

produce the following network connecting CASP8 and FAS proteins (it also involves FADD because it complexes with FAS and CASP8 on the membrane). See figure 47.  
40

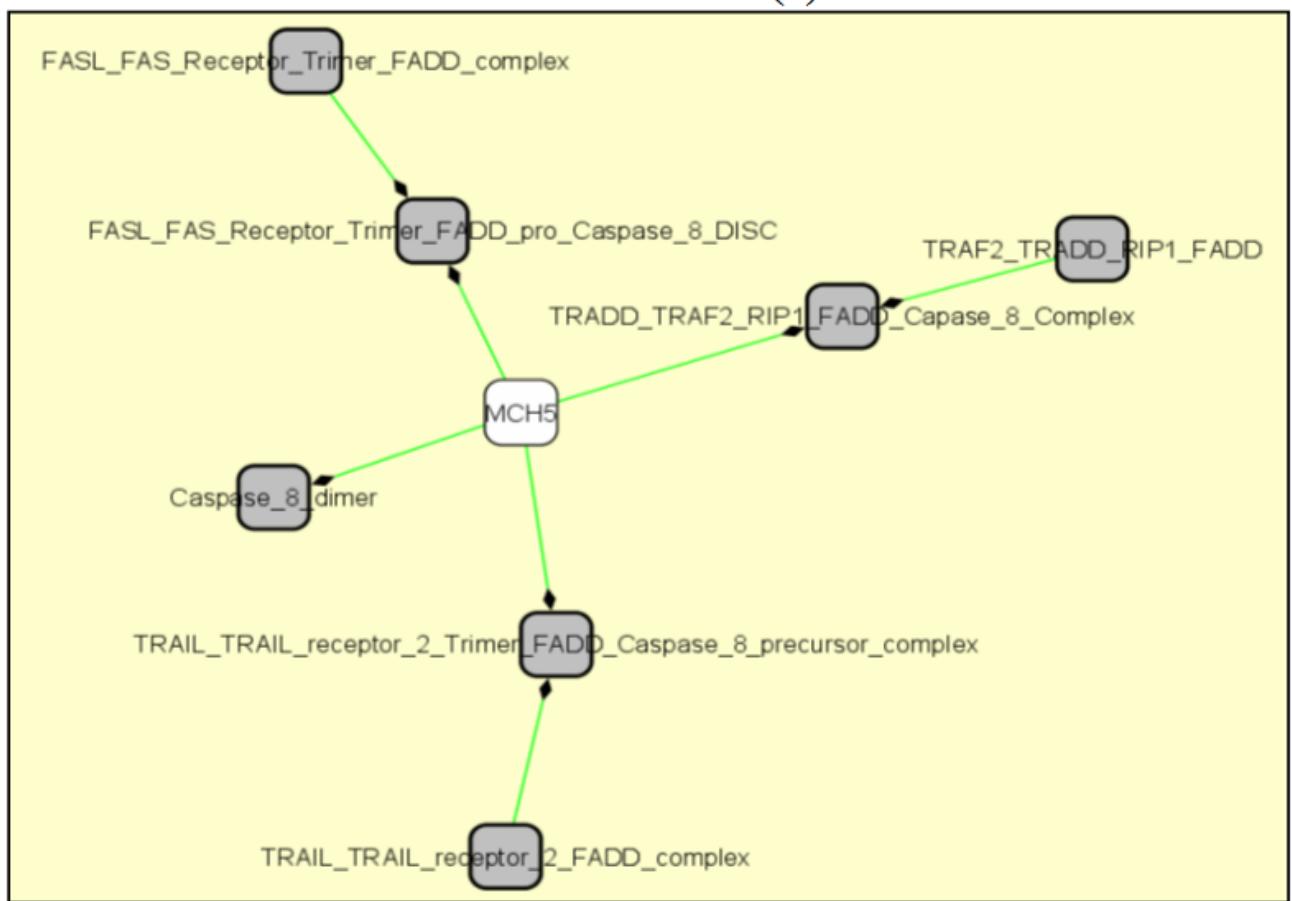
## 6.7 View Query Log

Plugins⇒BiNoM BioPAX 3 Query⇒

In this window (figure 48), are recapitulated all the queries done during the session.



(a)



(b)

Figure 41: BioPAX Standard Query: Add complexes with (a) the no expand or (b) expand option.

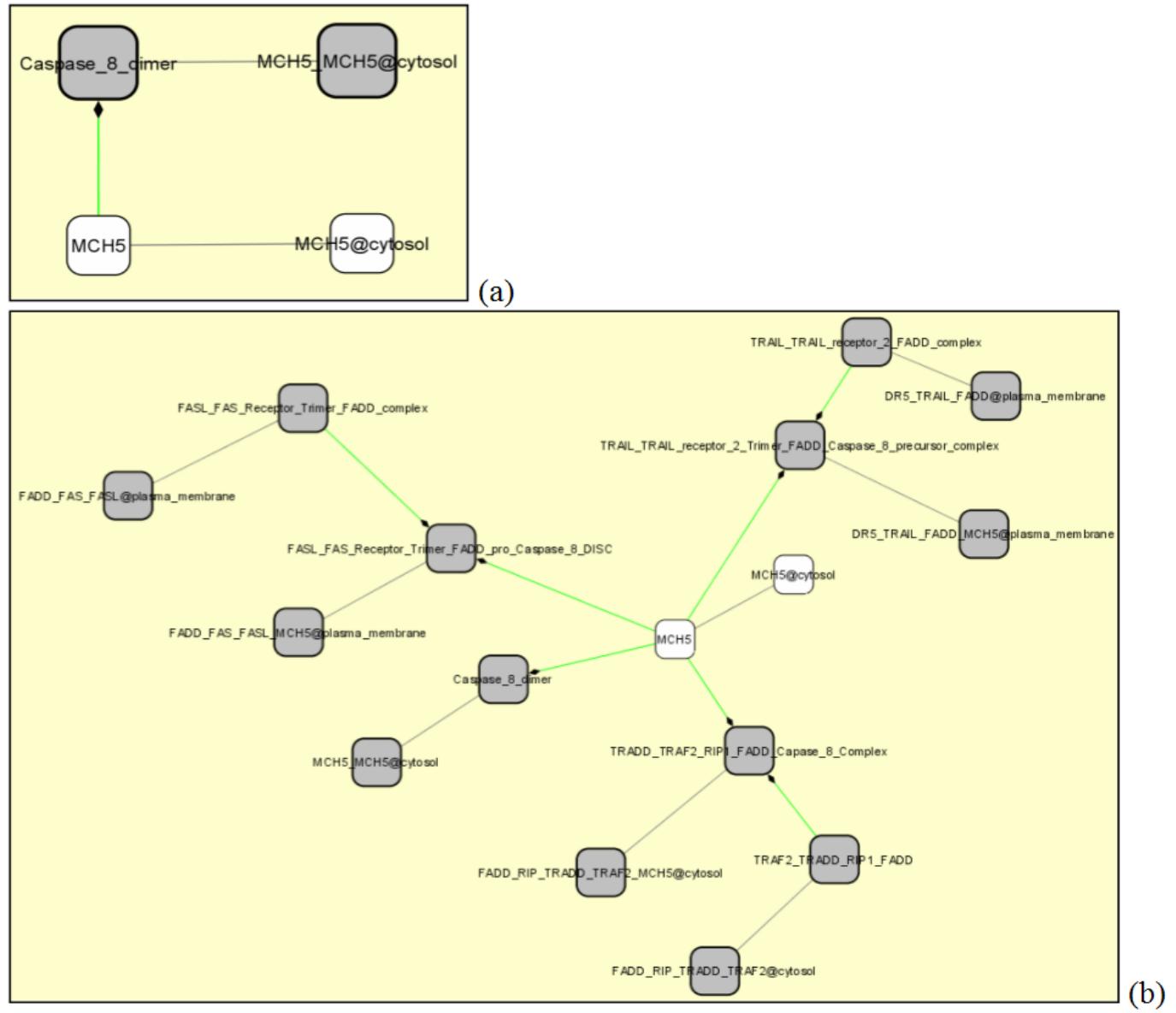


Figure 42: Chemical species. Cellular locations of all forms of MCH5.

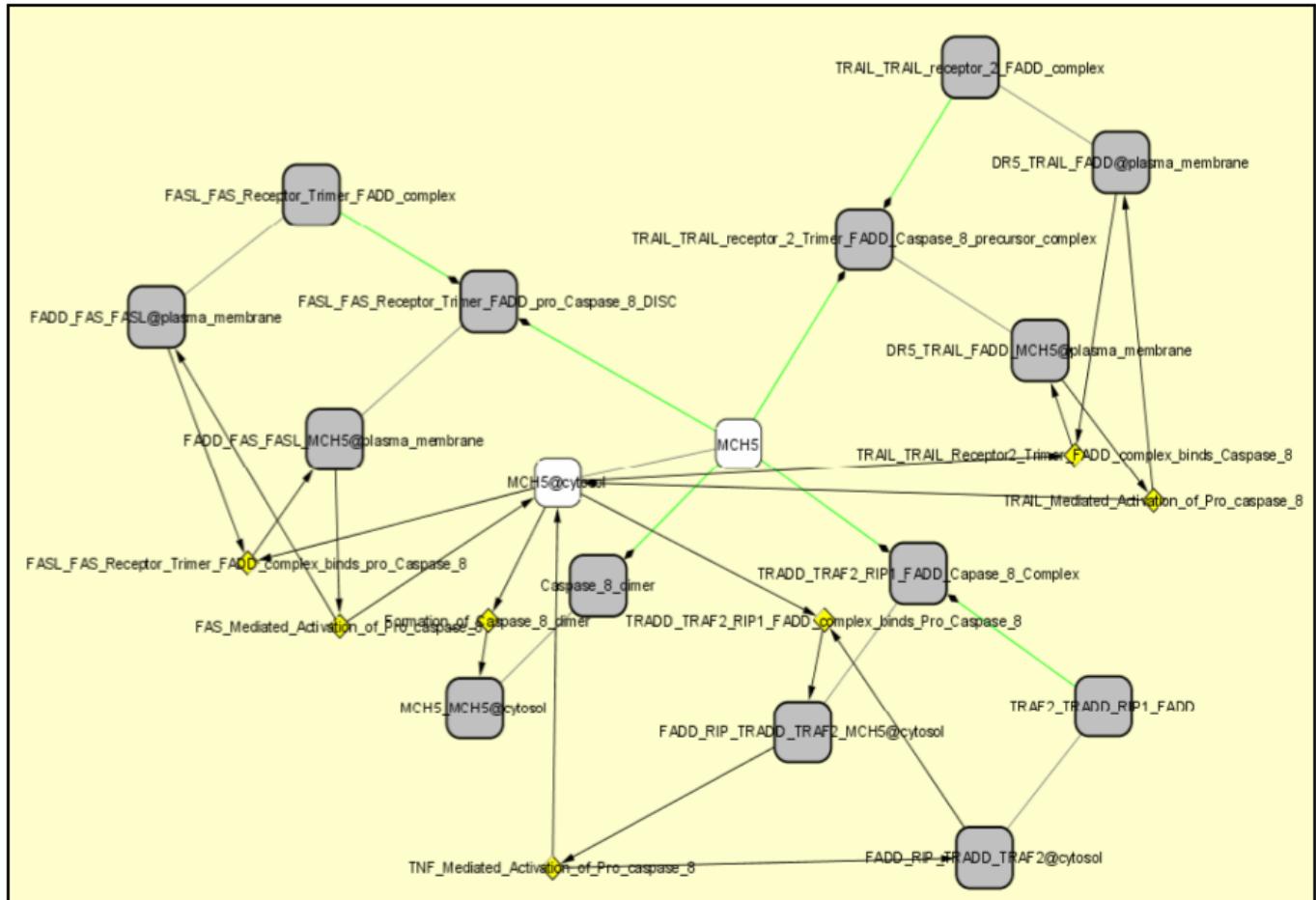


Figure 43: Adding reactions: example when all connecting reactions.

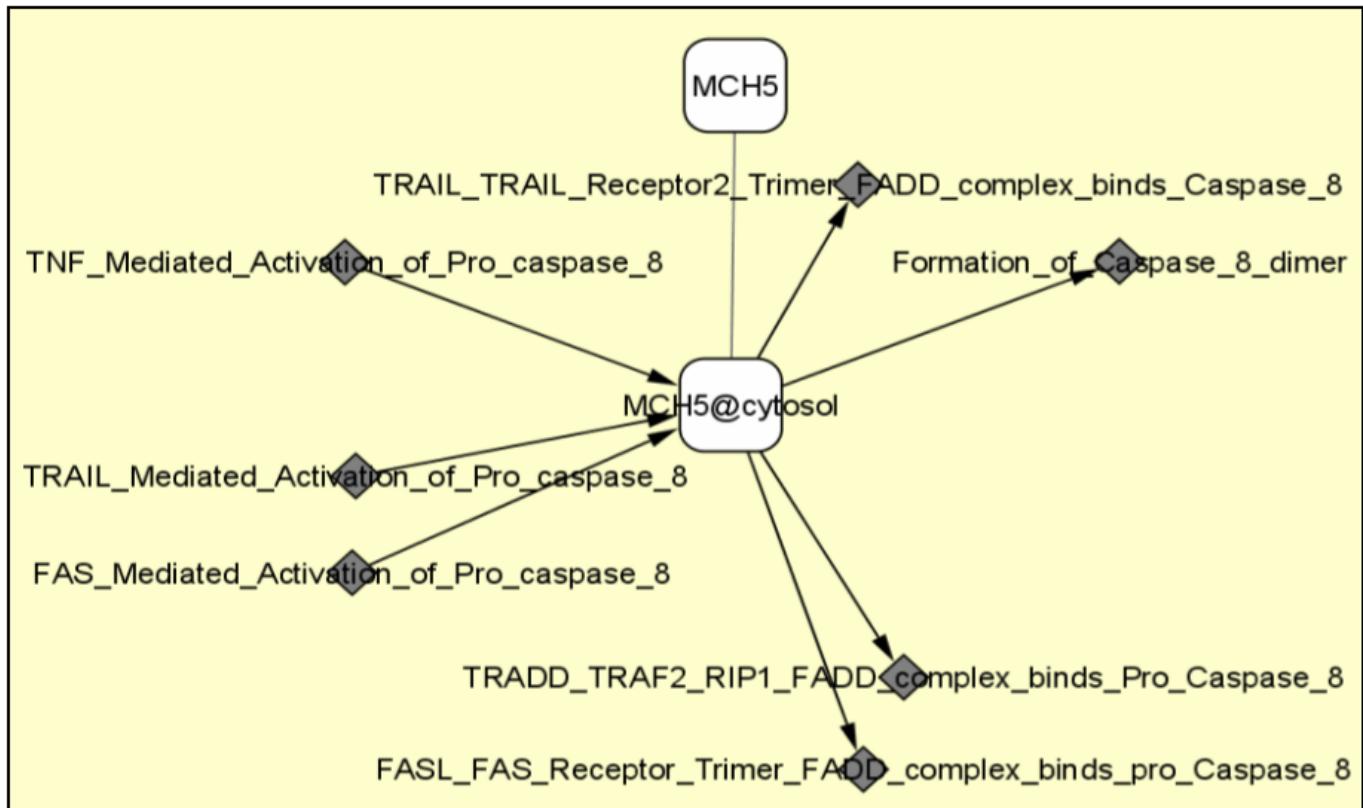


Figure 44: Adding reactions: example adding all reactions.

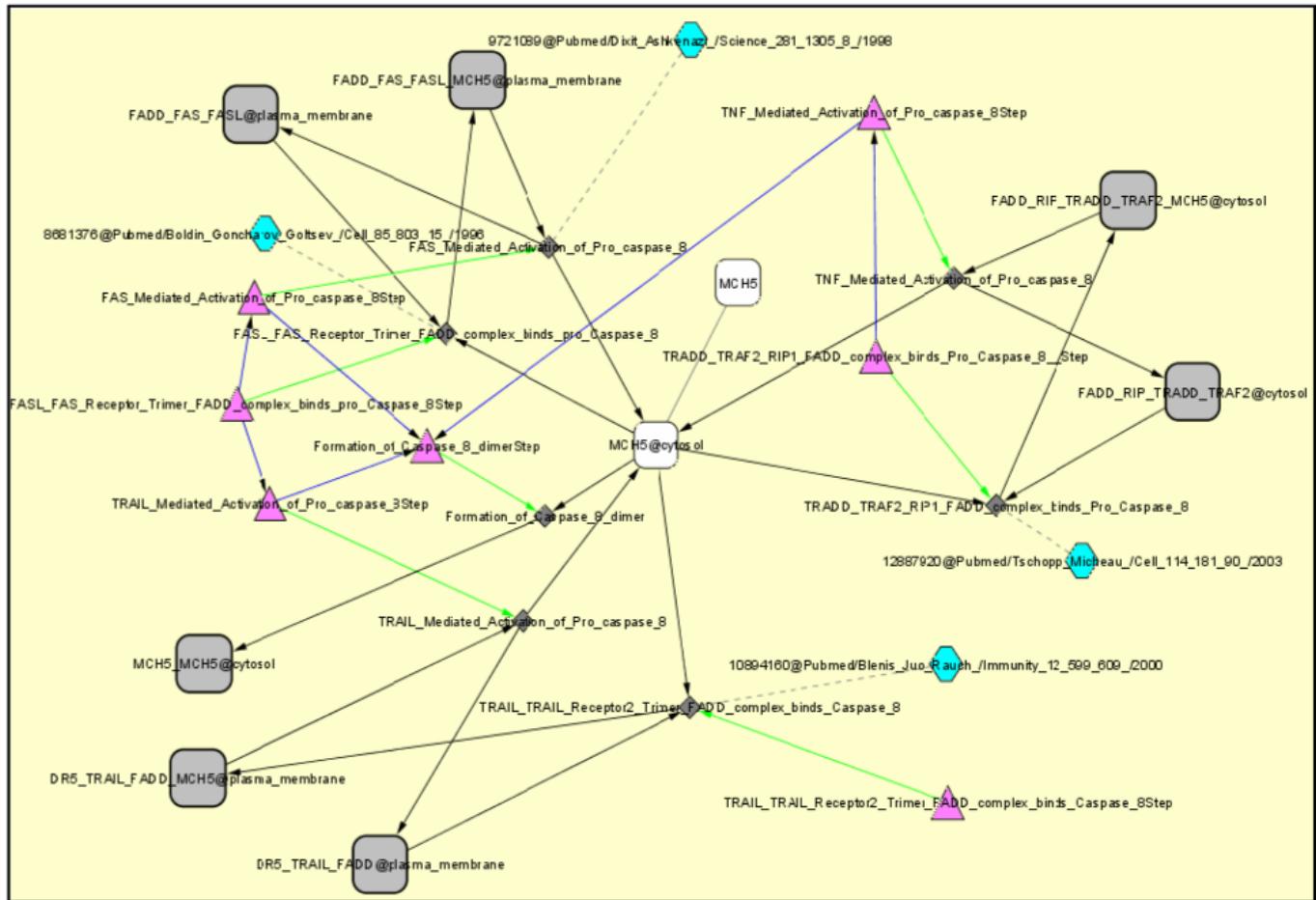


Figure 45: Adding reactions: example when making the reactions complete.

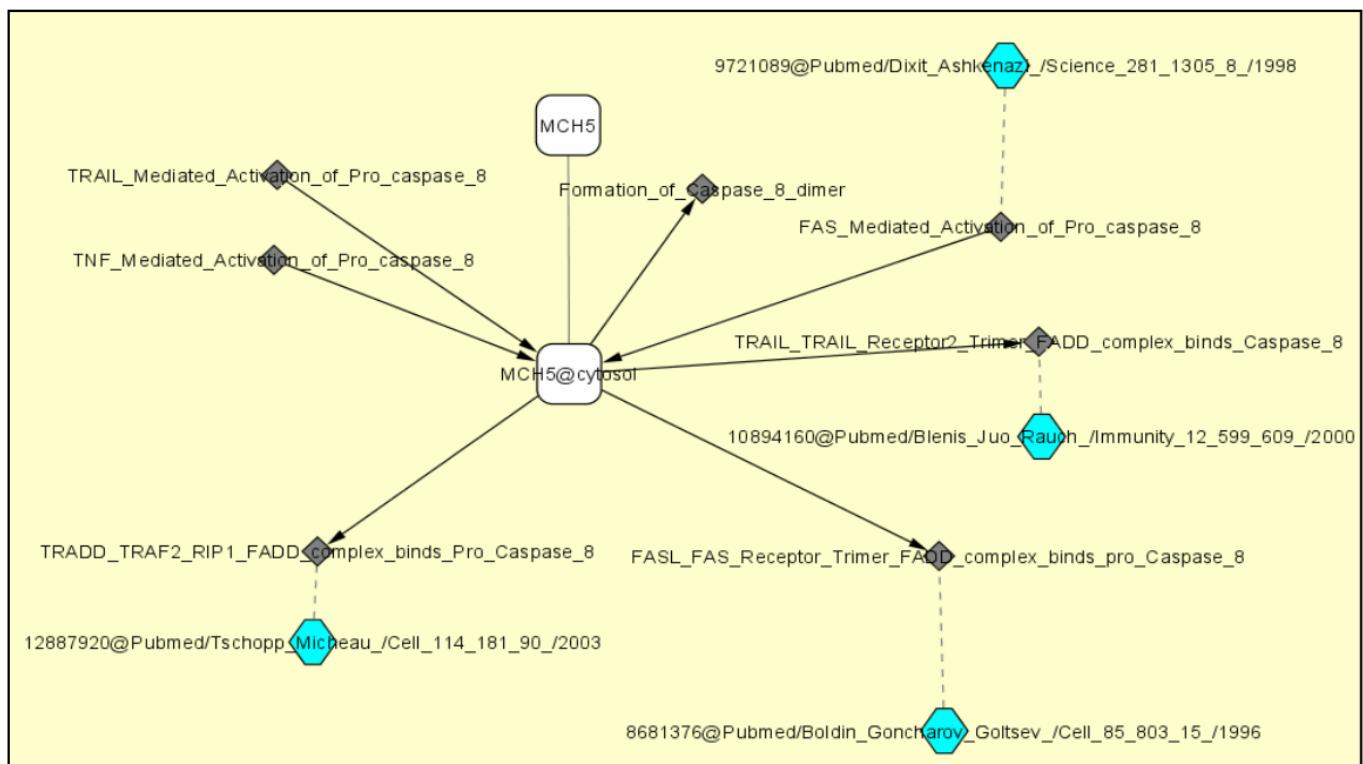


Figure 46: Adding publications.

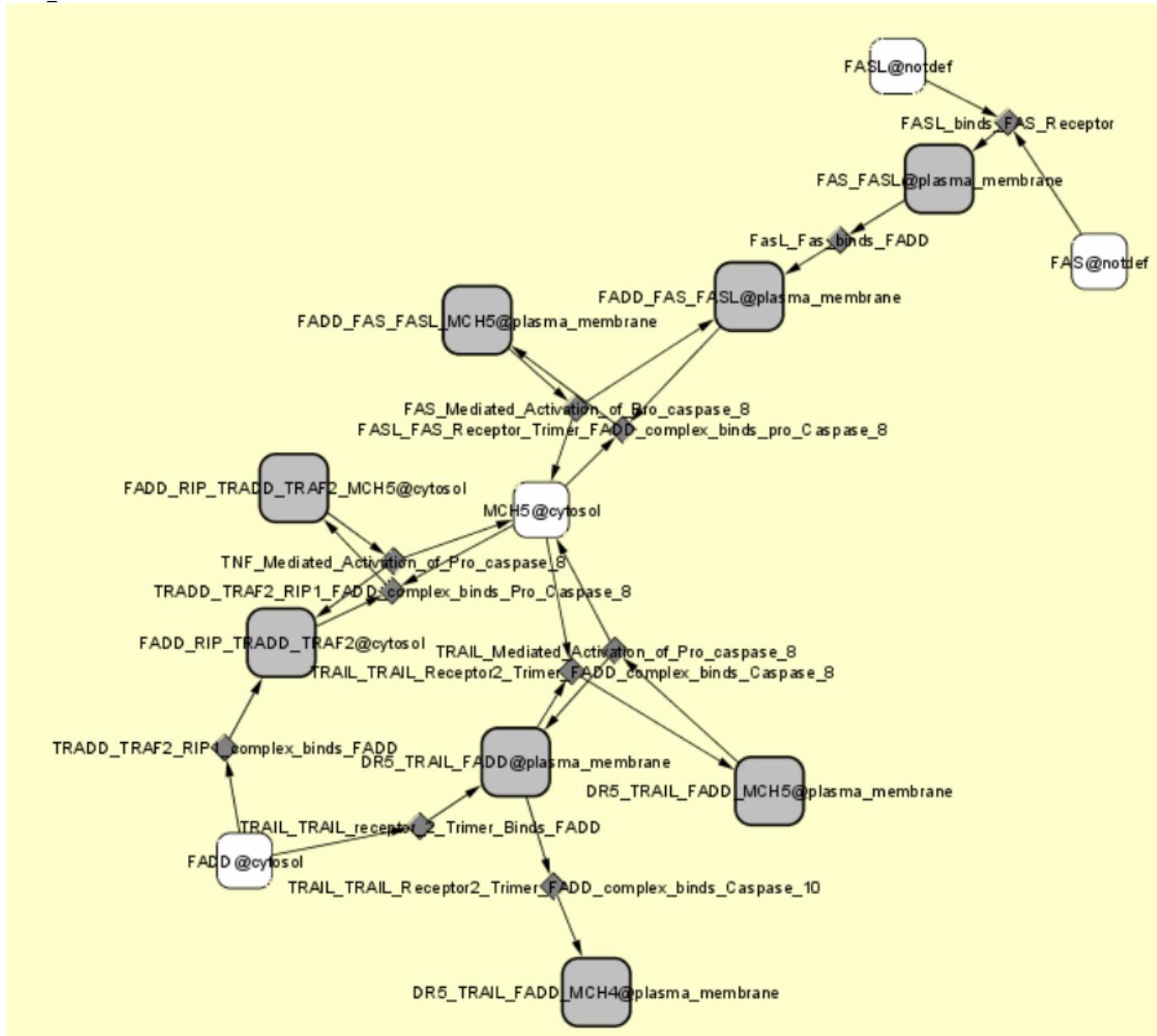


Figure 47: Index path Analysis with CASP8 and FAS.

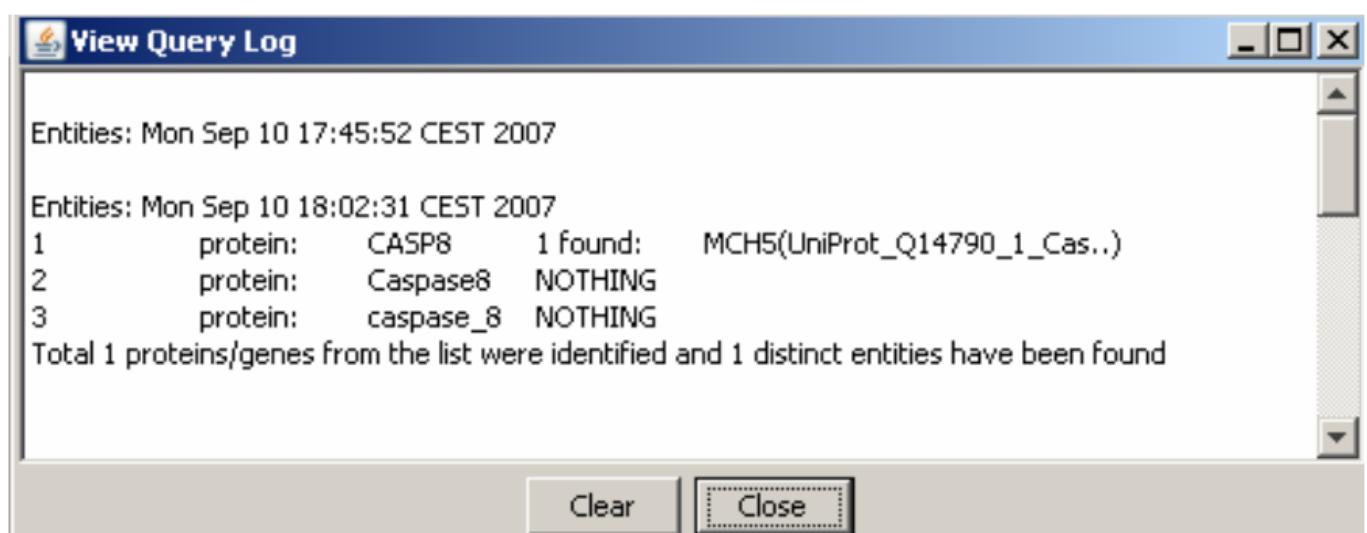


Figure 48: View Query Log Dialog

## 7 BiNoM Utilities

There are various functions that facilitate the manipulation of the networks, the copying and pasting of its subparts, the selection of some portions of it, etc. Those of them are missing in Cytoscape.

### 7.1 Select Edges between Selected Nodes

**Plugins⇒BiNoM Utilities⇒Select Edges between Selected Nodes** or f8

When some components are selected by their names (Select⇒Nodes⇒By Name) or simply with the mouse, the edges between the nodes are not selected. This function allows to remedy this problem.

This might be especially useful when the selection is copied and pasted in another network, although it is possible to paste the nodes with the edges connecting them, without selecting them, by choosing File⇒New⇒Network⇒From selected nodes, all edges when pasting them.

### 7.2 Select upstream neighbours

**Plugins⇒BiNoM Utilities⇒Select upstream neighbours** or ctrl+8

Select upstream neighbours of selected nodes. Whole upstream neighbours can be selected by repeating the command until no more node is selected.

### 7.3 Select downstream neighbours

**Plugins⇒BiNoM Utilities⇒Select downstream neighbours** or ctrl+9

Select downstream neighbours of selected nodes. Ditto downstream.

### 7.4 Double Network Differences

**Plugins⇒BiNoM Utilities⇒Double Network Differences**

A network is composed of nodes and edges. With this command, two networks A and B can be compared and the difference observed between the two is created in two new graphs: A-B for the differences of A compared to B ( $A - A \cap B$ ), and B-A for the differences of B compared to A ( $B - A \cap B$ ). Note that in the output networks, the layout of the first graph is conserved.

### 7.5 Update Networks

**Plugins⇒BiNoM Utilities⇒Update Networks**

When a network is modified, it is possible to update all the networks that are related to it, either because they are modules, sub-networks or older versions of it. That way, any changes, additions or deletions in a network can be propagated to the sub-parts that are derived from the initial version of that network. The user specifies which network is added, which one is deleted (when necessary) and which networks need to be updated in the proposed list.

Whatever is added or deleted in each sub-network is presented to the user in a separate window before any action is made. The user can agree or disagree by checking or unchecking the box in front of the network names. The previous version of the updated diagrams will not be deleted but saved under *network.name.xml.old*.

### 7.6 Update connections from other network

**Plugins⇒BiNoM Utilities⇒Update connections from other network**

The dialog (figure 49) propose to select:

- From network, the reference network where are the connections.
- Networks to update: networks in which connections are copied.

Some connections may be duplicated. So a Cytoscape command (version 8.0) may be used to delete them:

**Plugins⇒Network Modifications⇒Remove Duplicated Edges**

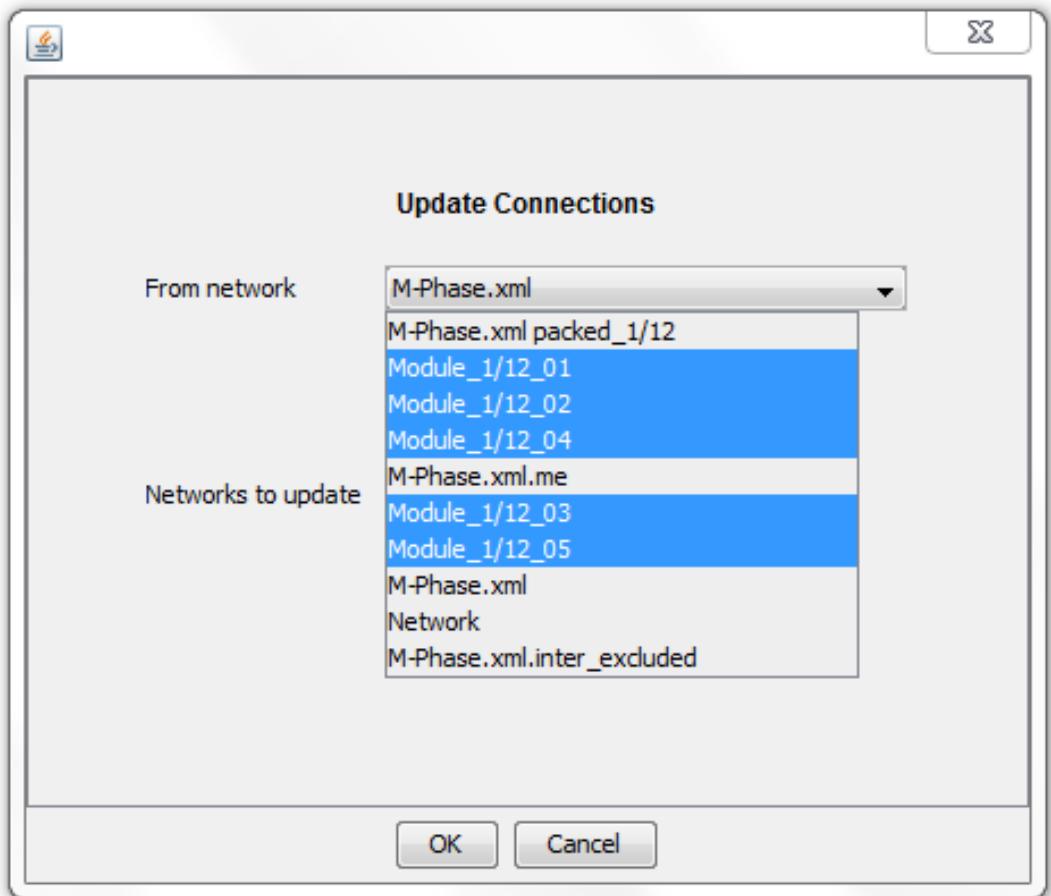


Figure 49: Update connections from other networks Dialog

## 7.7 Merge Networks and Filter by Frequency

**Plugins⇒BiNoM Utilities⇒Merge Networks and Filter by Frequency**

Create a network by merging the selected networks where the percentage of common nodes is greater than intersection threshold (see dialog figure 50).

## 7.8 Clipboard

The description of the commands, which work only inside a Cytoscape session, is self-explanatory.

**Plugins⇒BiNoM Utilities⇒Clipboard⇒Copy selected nodes and edges to clipboard**

**Plugins⇒BiNoM Utilities⇒Clipboard⇒Add selected nodes and edges to clipboard**

**Plugins⇒BiNoM Utilities⇒Clipboard⇒Paste nodes and edges from clipboard**

**Plugins⇒BiNoM Utilities⇒Clipboard⇒Show clipboard contents**

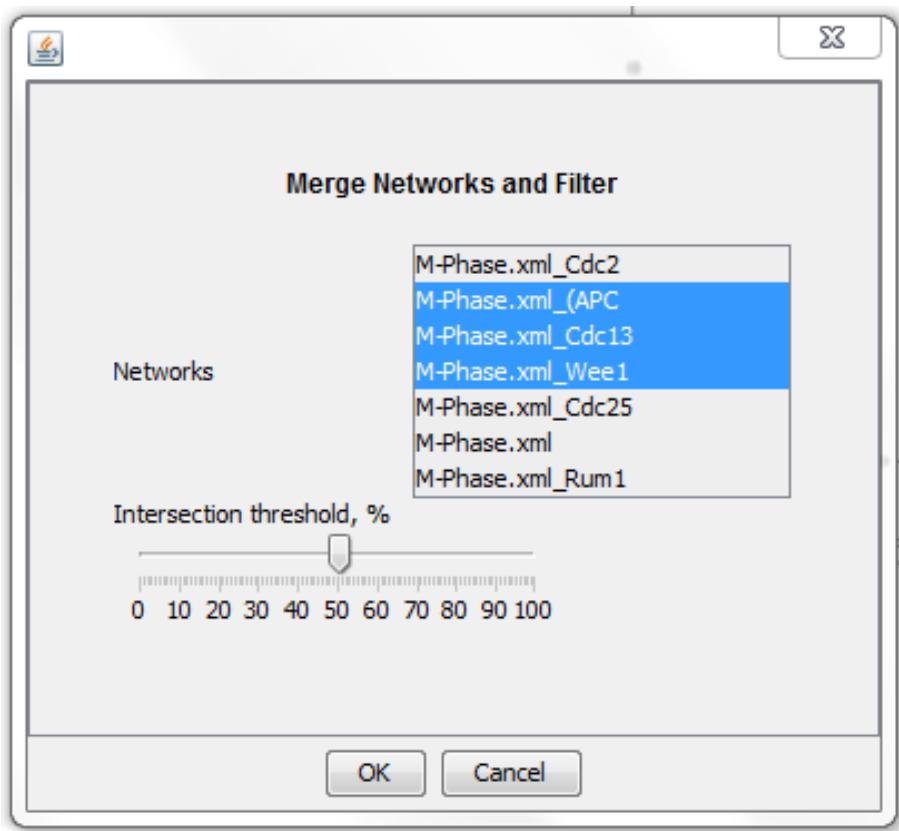


Figure 50: Merge Networks and Filter by Frequency Dialog

## 8 Appendices

### 8.1 Attributed graph model

BiNoM manipulates the information contained in the standard systems biology files by mapping it onto a labeled graph, called index. The index does not try to map the totality of all details; it rather serves as a connection map for the objects contained in other ontologies such as BioPAX. In other words, the index contains the minimum information needed to graphically represent objects and connections between them. Index elements (nodes and edges) are annotated by identifiers sufficient to find these objects in the original files and extract and edit the information related to them.

This approach has several advantages, in particular, with respect to synchronization issues. BiNoM index is a light-weight construction which can be easily regenerated, does not duplicate the information in existing files and serves only to facilitate the visualization and to access existing systems biology files.

Currently, BiNoM index is mostly developed to map BioPAX ontology files and CellDesigner object schema. In future versions, other mappings will be available, for instance, a mapping to SBML files annotated with Systems Biology Ontology (<http://www.ebi.ac.uk/sbo/>).

The table 51 lists all attributes used by the index.

### 8.2 BiNoM CellDesigner and BiNoM BioPAX visual mappers

BiNoM has two built-in visual mappers supporting the visualization of the whole index or of its parts. The legend for deciphering the different types of visualization is provided in figure 52.

### 8.3 BiNoM Naming Service

When importing pathway information, BiNoM tries to generate meaningful, unique and short names for index entities. This function of the plugin is performed via BiNoM Naming Service. For proteins and other entities, the shortest available synonym is used. For genes, a g symbol is added at the beginning of the name, and for RNAs, a r symbol is added in order to avoid mixing genes and mRNAs with their products. If this leads to an ambiguity, it is resolved by adding a suffix specifying a unique id of the entity.

A chemical species in BiNoM is defined as a physical entity (such as protein) with some cellular localization and some (post-translational) modification (possibly none). The general template of the species label is the following:

Entity1\_name|Modification1|Modification2|: Entity2\_name|Modifications...[\_active|\_hmN]@compartment  
Here, the colon symbol : delimits the different components of a complex if the species has several components. Optional suffixes active or hm describe active state of the chemical species or N-homodimer state, respectively.

Several examples of naming chemical species are presented:

- Naming chemical species shown in Systems Biology Graphical Notation standard figure 53
- A conversion from CellDesigner figure 54.
- A conversion from BioPAX figure 55.

### 8.4 Standard BioPAX interfaces

BiNoM index serves as a visual connector to the content of a network file. However, with all types of relations, the index is a highly connected graph and not very insightful when represented entirely. A subgraph of the index can be extracted according to a specific purpose and used to understand a specific aspect of the pathway information. We will call interface such a subgraph of the entire index.

When importing a BioPAX file, BiNoM proposes to generate three standard BioPAX interfaces referred to as

Node attributes		Edge attributes	
Attribute name	Meaning and possible values	Attribute name	Meaning and possible values
<b>BioPAX attributes</b>			
BIOPAX_NODE_TYPE	Type of entity represented by the node: protein, dna, rna, smallMolecule, transport, biochemicalReaction	BIOPAX_EDGE_TYPE	Type of connection between nodes: LEFT, RIGHT, CATALYSIS, NEXT, REFERENCE
BIOPAX_SPECIES	Species unique name	BIOPAX_EDGE_ID	Unique edge id
BIOPAX_REACTION	Reaction id	BIOPAX_URI	Full URIs of the objects associated with the edge
BIOPAX_NODE_SYNONYM	All entity synonyms		
BIOPAX_NODE_XREF	All entity accession numbers		
BIOPAX_URI	Full URIs of the objects associated with the node		
<b>CellDesigner attributes</b>			
CELLDESIGNER_NODE_TYPE	Type of entity represented by the node: PROTEIN, GENE, STATE_TRANSITION...	CELL_DESIGNER_EDGE_TYPE	Type of connection between nodes: such as LEFT, RIGHT, CATALYSIS
CELLDESIGNER_SPECIES	For species nodes -- SBML species id		
CELLDESIGNER_REACTION	For reaction nodes -- SBML reaction id		
CELLDESIGNER_ALIAS	CellDesigner node alias		
<b>Common attributes</b>			
EFFECT	For reactions and influence edges -- effect of the influence. If something is known, such terms as 'activation', 'inhibition', 'catalysis' should be used		

Figure 51: All attributes of graph model used by the index

BiNoM·BioPAX·visual·mapper		BiNoM·CellDesigner·visual·mapper	
BIOPAX · NODE_TYPE <sup>a</sup>	BIOPAX_EDGE_TYPE <sup>a</sup>	CELLDESIGNER · NODE_TYPE <sup>a</sup>	CELLDESIGNER_EDGE_TYPE <sup>a</sup>
        	<p>protein      LEFT, RIGHT</p> <p>complex     CATALYSIS, ACTIVATION —→</p> <p>dna          CATALYSIS_UNKNOWN —→</p> <p>rna          MODULATION —→</p> <p>small molecule    INHIBITION —→</p> <p>publication    CONTAINS —→</p> <p>pathway      SPECIESOF —→</p> <p>pathwayStep    STEP —→</p> <p>conversion    NEXT —→</p> <p>reference    REFERENCE —→</p> <p>physicalInteraction —→</p>	             <p>PROTEIN      LEFT, RIGHT</p> <p>COMPLEX     CATALYSIS —→</p> <p>GENE          CATALYSIS_UNKNOWN —→</p> <p>RNA          INHIBITION —→</p> <p>ION          MODIFIES —→</p> <p>SIMPLE_MOLECULE    TRANSCRIPTIONAL_ACTIVATION —→</p> <p>PHENOTYPE    TRANSCRIPTIONAL_INHIBITION —→</p> <p>PATHWAY    TRANSLATIONAL_ACTIVATION —→</p> <p>STATE_TRANSITION    TRANSLATIONAL_INHIBITION —→</p> <p>DISSOCIATION    TRANSCRIPTIONAL_ACTIVATION —→</p> <p>HETERO_DIMER    TRANSCRIPTIONAL_INHIBITION —→</p> <p>ASSOCIATION    TRANSLATIONAL_INHIBITION —→</p> <p>TRANSPORT    TRANSCRIPTIONAL_ACTIVATION —→</p> <p>TRANSPORT    TRANSLATIONAL_ACTIVATION —→</p> <p>TRANSPORT    TRANSCRIPTIONAL_INHIBITION —→</p> <p>TRANSPORT    TRANSLATIONAL_INHIBITION —→</p>	

Figure 52: Types of visualization in BioPAX and CellDesigner

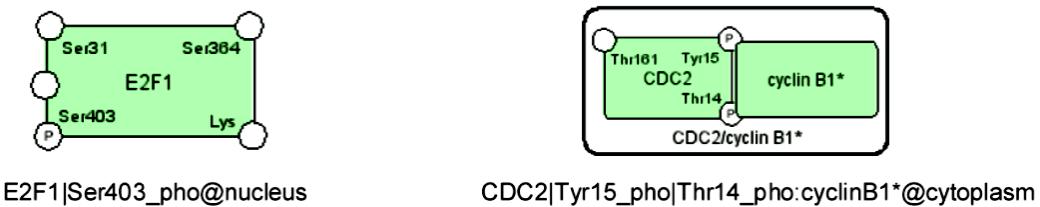


Figure 53: 2 examples of naming chemical species shown in Systems Biology Graphical Notation standard.

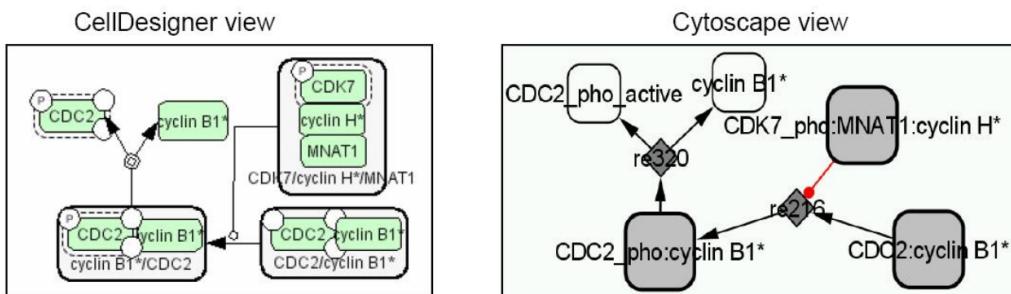


Figure 54: Conversion of a little network from CellDesigner Graphical Notation to BiNoM index representation

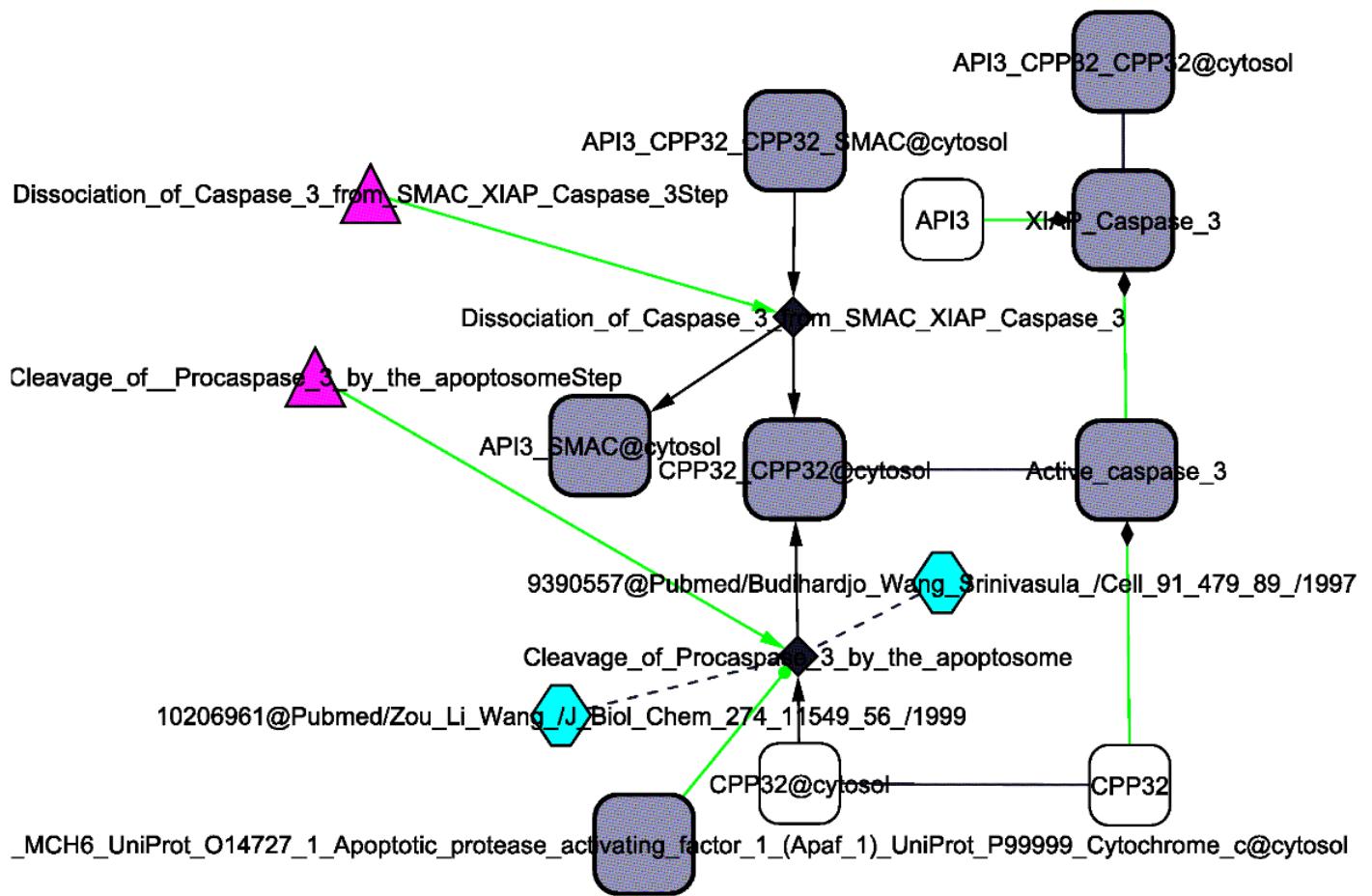


Figure 55: Small fragment of BioPAX index generated for Apoptosis pathway and extracted from Reactome database

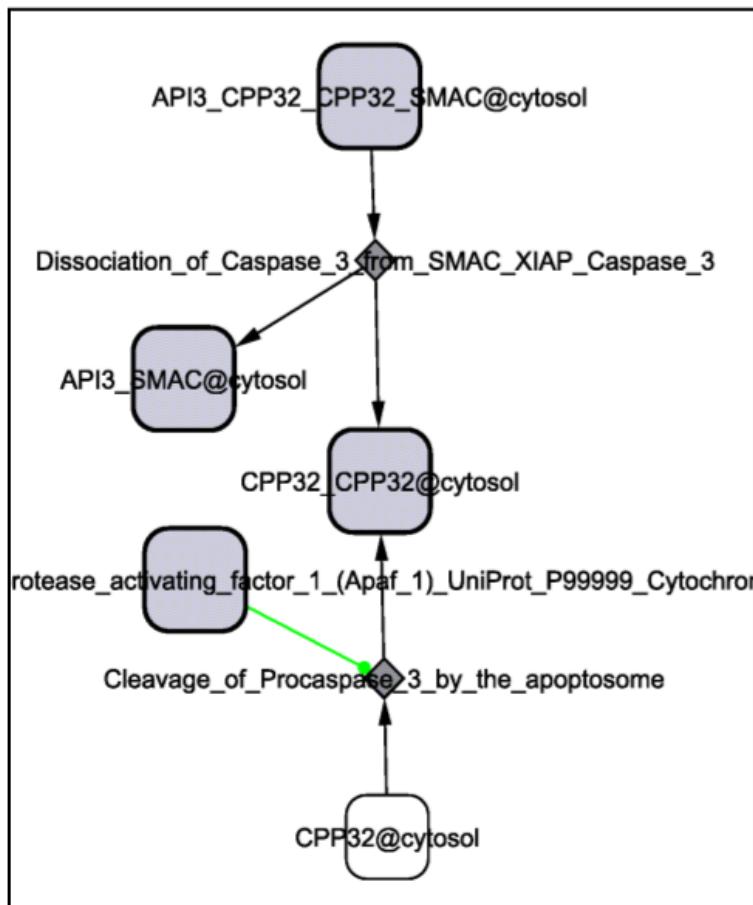


Figure 56: Fragment of Apoptosis from Reactome as Reaction Network.

- Reaction Network.
- Pathway Structure.
- Protein-Protein Interaction.

#### 8.4.1 BioPAX interface as Reaction Network

The Reaction Network interface is a bipartite graph which contains nodes of only two types: species and reactions. Reactants are connected to reactions through edges of type LEFT, products are connected through edges of type RIGHT. Modifier species are connected through CATALYSIS, MODULATION and other edges. See figure 56.

Some BioPAX objects (catalysis, for example) are represented by edges with the corresponding BIOPAX\_URI attribute. A chemical species node can correspond to several grouped physicalEntityParticipants, thus, it can have several BIOPAX\_URI attributes. When calling BioPAX editor, all of them will be opened.

Standard Reaction Network interface can be exported to pure SBML format (level 2) and serve as a draft for further computational modeling.

#### 8.4.2 BioPAX interface as Pathway Structure

Pathway Structure interface contains only nodes of pathway, pathwayStep and interaction types. The types of the edges connecting them are CONTAINS, STEP and NEXT. See figure 57.

#### 8.4.3 BioPAX interface as Protein-Protein Interaction

Protein-protein Interaction interface contains only entities (not chemical species) with edges of CONTAINS and physicalInteraction type. This interface allows to visualize the composition of complexes like the Caspase3

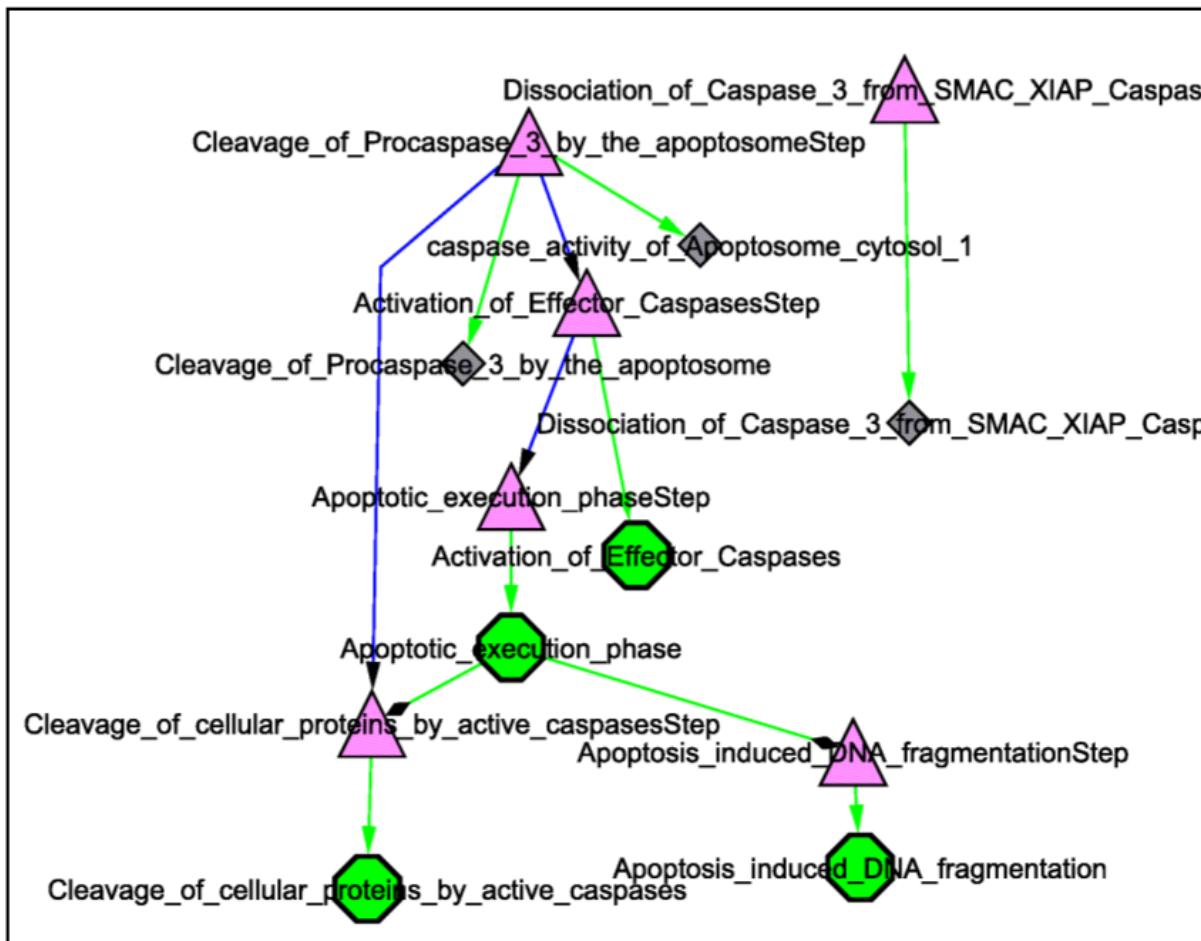


Figure 57: Fragment of Apoptosis from Reactome as Pathway Structure.

example of the Apoptosis pathway (left, first), or, explicit information about protein interaction with TGF $\beta$ 1 (left, second), as in the NetPath TGF-beta BioPAX file. See figure 58.

## 8.5 AIN file format

The AIN format describes a list of influences between genes, proteins, modified proteins or families. It is a table in ASCII, where the columns are separated by one tabulation (<Tab>).

The first line must start with the name of each column as follows (the titles are fixed):

ReviewRef ExperimentRef Link ChemType Delay Confidence Tissue Comment

(each space corresponds to a <Tab> on your keyboard).

- For the references (ReviewRef and ExperimentRef), if one wants to include a PUBMED number, it should have the form PMID:123456.
- The Link column describes a connection (activation or inhibition) between two entities, like A->B or A|B. The entities can be simply the name of a gene or a protein, but it can also be a complex ((C:D)), a phosphorylated protein ((C^p)) or a family. In the latter case, the family can be given explicitly by the list of all its members ((C1,C2,C3)) or implicitly, by an undefined name ((C.)), where the . can be replaced by any character..
- In the other columns, if the user wishes to add more than one word in each field, the sentences need to be inserted between .
- If a field cannot be filled, a simple dot should be inserted.
- A # in first column makes the line comment.

For an example of AIN format, one can open the file ExamplApop.txt in a simple text editor or in spreadsheet as EXCEL. All the information in this AIN file is translated in BioPAX format when the file is imported in Cytoscape via BiNoM.

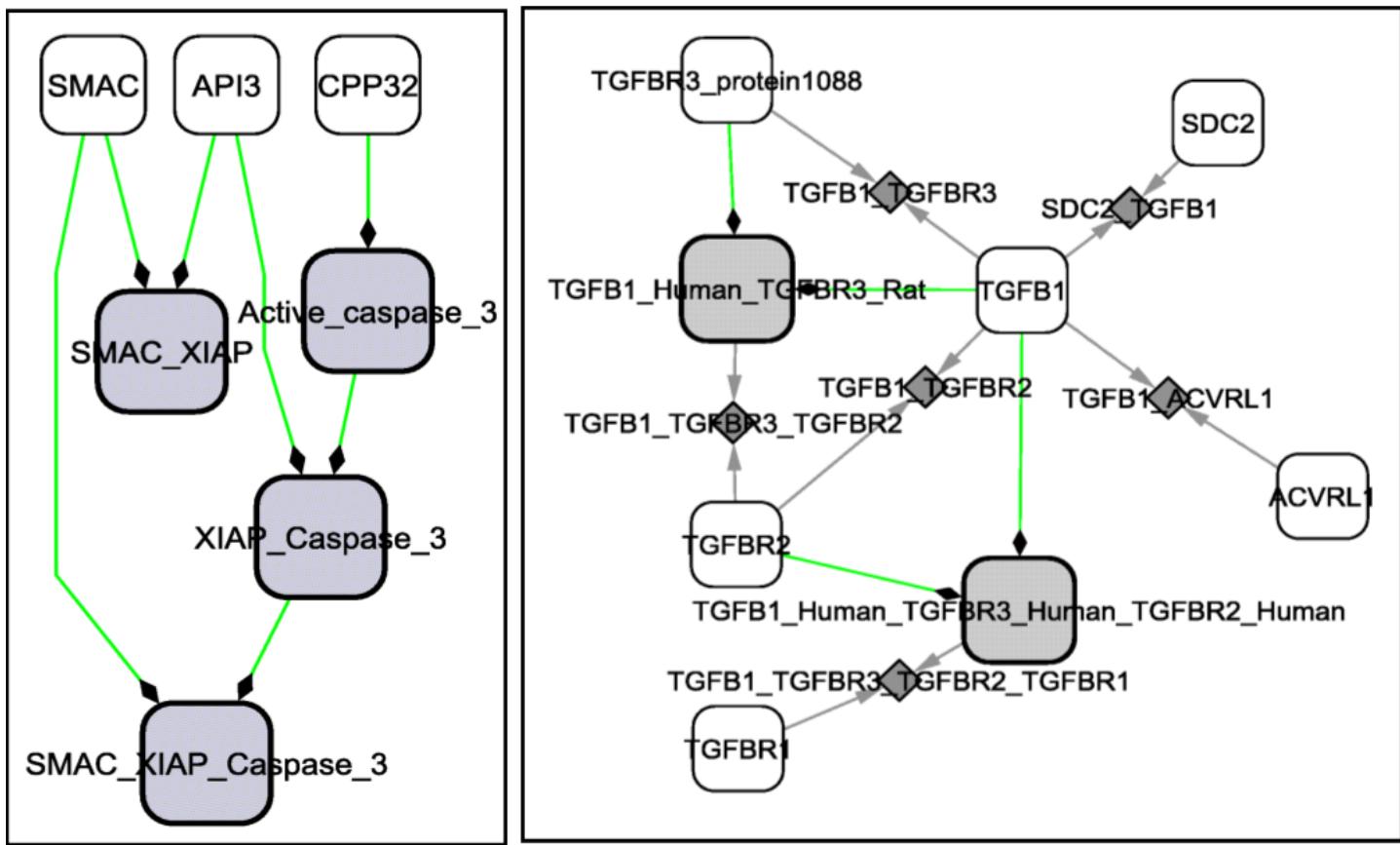


Figure 58: Fragment of Apoptosis from Reactome as Protein Protein Interaction.

## 8.6 Modularization by shortest path clustering

When only the structure of a network is known, the simplest method to agglomerate nodes in a network is to put the closest nodes together. And so modules may have the fewest links between them. This method can lead to an algorithm of modularization of an oriented network. The notion of closeness and the process of creating modules are to be clarified.

The distance between nodes is based on the length of the shortest paths and the number of occurrences if several paths are equal (the equality of the shortest path is frequent in a strongly connected network). The distance from node 1 to node 2 is generally different from distance from node 2 to node 1.

The used distance is the minimal linkage applied to the base distance, which is necessary to respect the triangular inequality. The distance between A and B is the minimum of distances from nodes in A to nodes in B and from nodes in B to nodes in A. And so, the agglomerative hierarchical clustering can be applied to build modules.

To avoid too speed increasing of clusters, they are ranked in a queue and the last created cluster is put at the end of the queue. For the same reason, nodes are sorted by in degree (sources in first). Despite of these precautions, the algorithm applied to strongly connected network gives unbalanced clusters (often a huge cluster and several tiny clusters). So, a ceiling number of nodes in a cluster must be fixed.

The agglomerative clustering gives 1 cluster at the end, which has no interest. Thats why; these 2 stop conditions are added:

- The length of the shortest path between 2 clusters reaches the maximal length.
- The number of clusters to be compared in the queue is less than 2.

The first stop condition make that too far clusters are not merged. When the last cluster to be created contains more than the maximal number of nodes, the largest cluster is excluded from the queue. Only the clusters remaining in the queue are to be compared by distance and they must be 2 or more.

The next page shows 3 examples (network inspired by toynet ). If the maximal length of the shortest paths is 1, nodes inside clusters are connected as a clique in a not oriented graph. But, if not, it may not be the case.

From a **practical point of view**, the input of ceiling number of nodes and maximal length of the shortest paths gives a set of not intersecting sub-networks. They are a partition of the network; their union is the whole network. This process is only useful for connected networks. Obviously, isolated nodes or sub-networks are not merged unless the maximal distance is infinity.

## 8.7 GLOSSARY

### 8.7.1 BioPAX

BioPAX is an OWL (Web Ontology Language) document designed to exchange biological pathways. BioPAX format provides separate layers of information: information about the reactions involved in the networks along with the participants, information about the structure of the pathway, and information about the protein-protein interactions.

### 8.7.2 CellDesigner

CellDesigner is a structured diagram editor for drawing gene-regulatory and biochemical networks. Networks are drawn based on the process diagram, with graphical notation system proposed by Kitano.

### 8.7.3 BiNoM Index

Directed labeled graph representing the objects in CellDesigner and BioPAX ontologies and their connections. Index maps only the information needed to display it and to identify the relevant information in the original CellDesigner or BioPAX files.

### 8.7.4 BiNoM interface

Part of the BiNoM index (subgraph) visually presented by Cytoscape network. There are standard interfaces (Reaction network, pathway structure, protein interaction) which can be combined to construct a user-defined interface.

### 8.7.5 Optimal / suboptimal shortest paths

Shortest paths in weighted directed graph paths in the graph between source and target nodes with minimal sum of weights of the edges making the path. Suboptimal path is constructed by removing all edges in all shortest paths one by one and one at a time and finding the shortest path.

### 8.7.6 Strongly Connected Components (SCC)

A subgraph in a directed graph, in which there is path from any node to any node

### 8.7.7 Relevant cycle

Any cyclic path in the graph which can not be decomposed further into simpler cycles

### 8.7.8 SBML

Systems Biology Markup Language (SBML) is a standard for representing models of biochemical and gene-regulatory networks.

## References

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