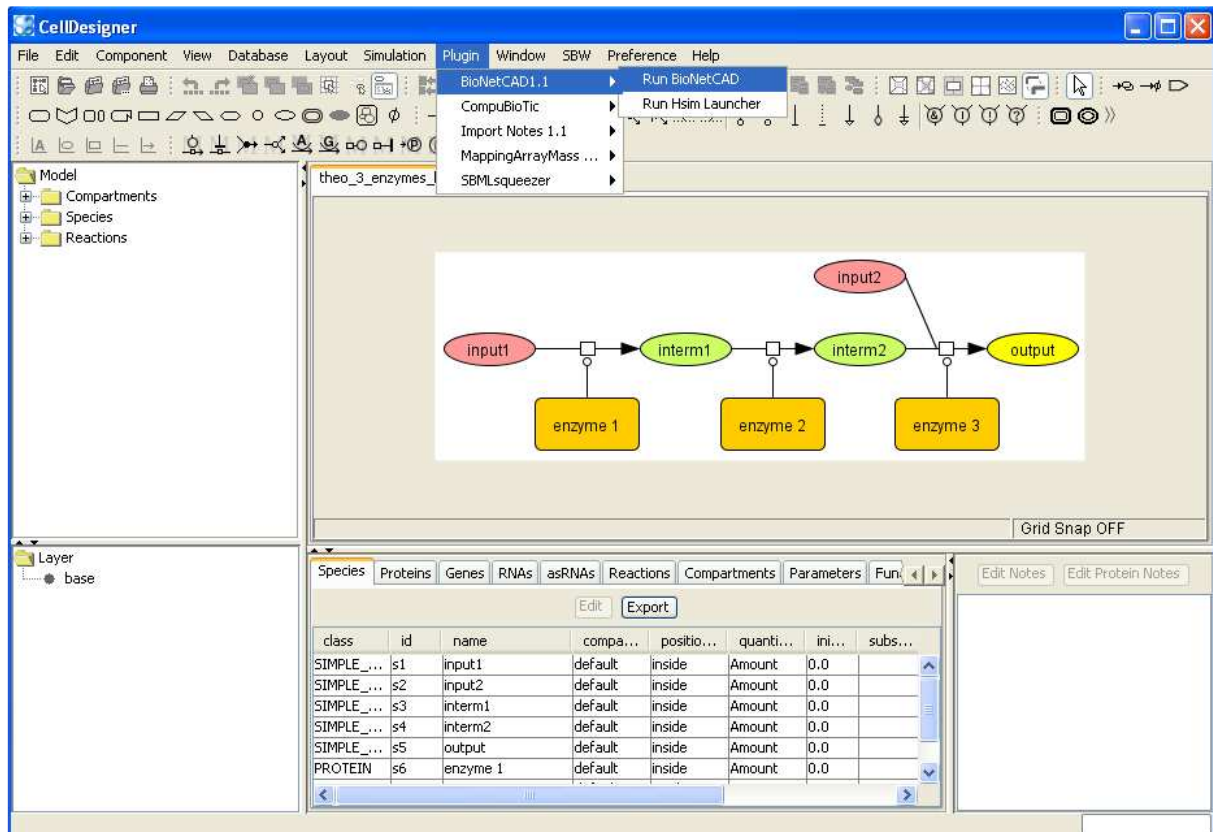


BioNetCAD1.1 tutorial

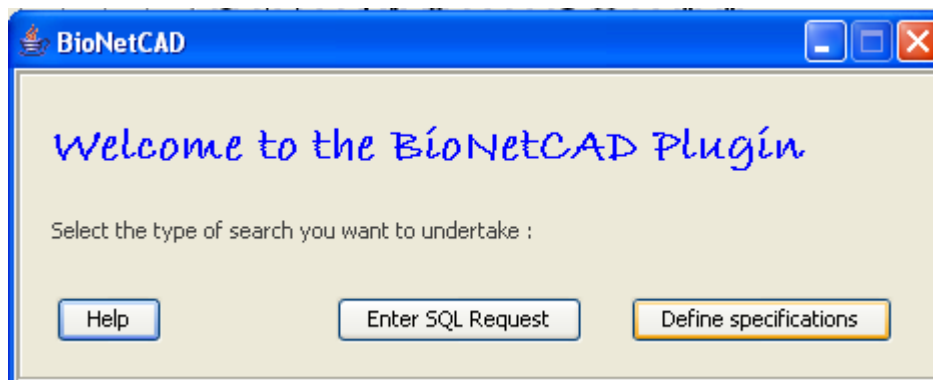
1. IMPLEMENTATION OF BIOCHEMICAL NETWORKS

A Logic “AND” gate

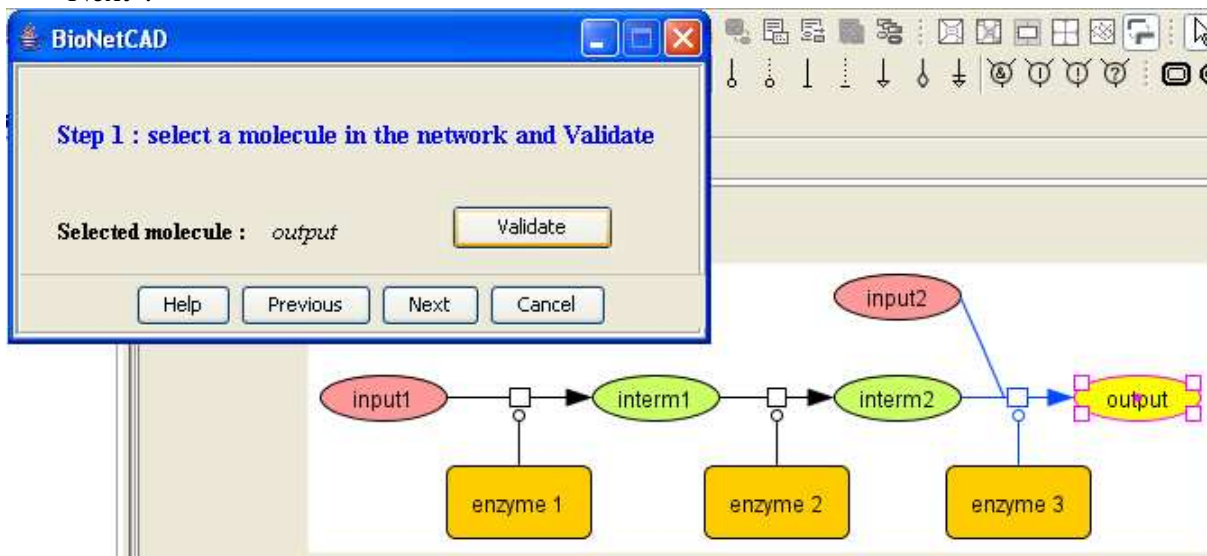
1. Open file in *CellDesigner*: theo_3_enzymes_logic_gate.xml
2. Once a model is opened in *CellDesigner*, go to menu Plugin and choose BioNetCAD1.1, and click on “Run BioNetCAD”.



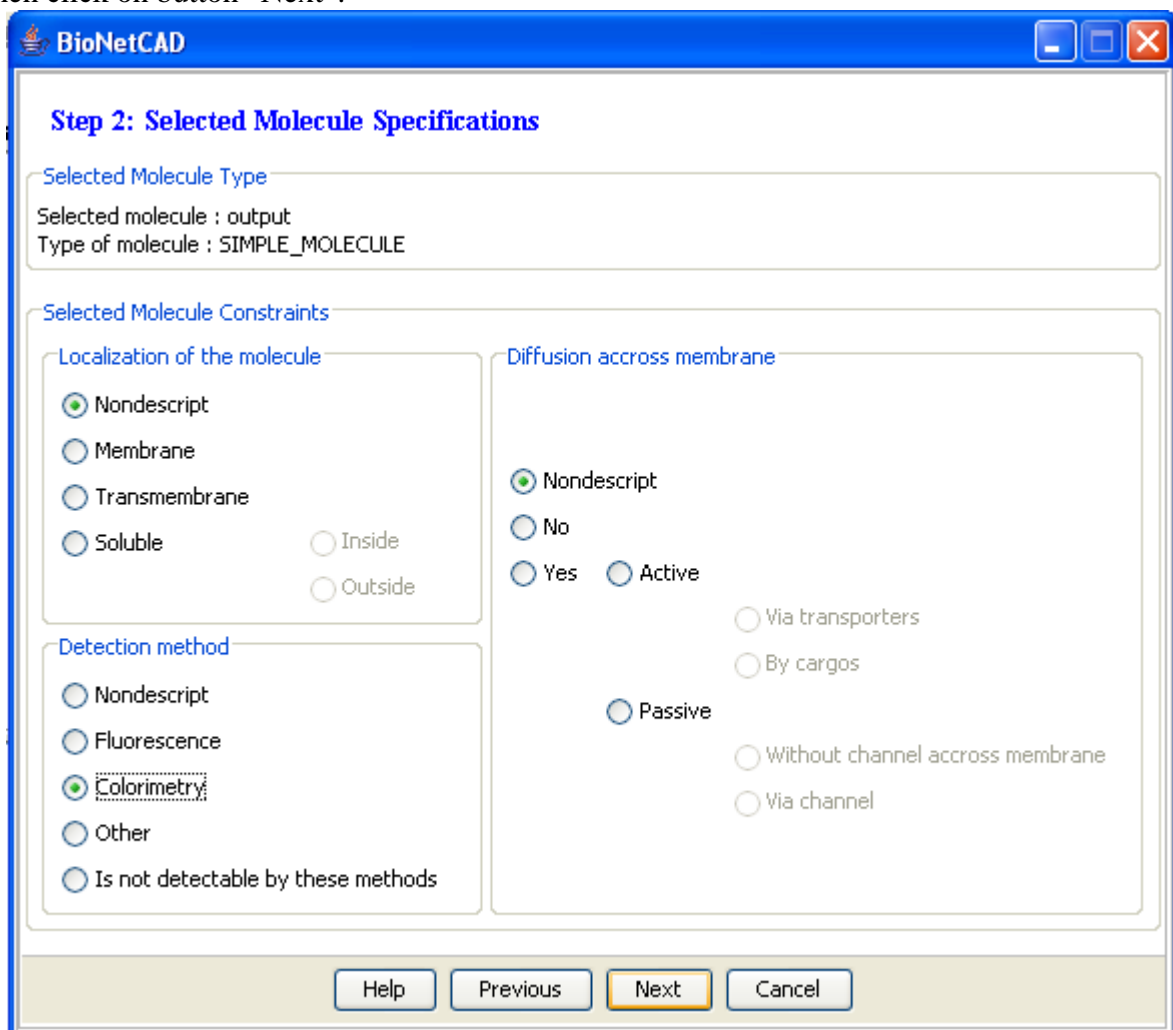
3. The BioNetCAD interface opens and asks you to choose between writing an SQL query and defining specifications about the network. Choose “Define specifications”.



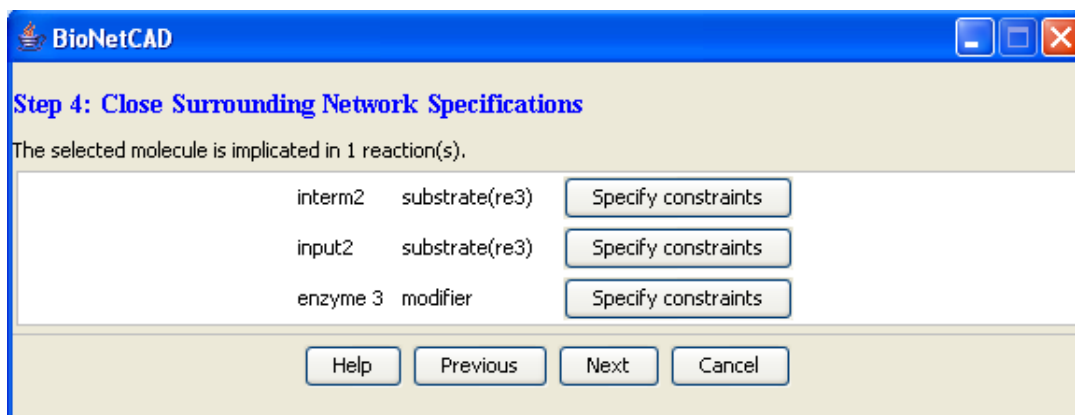
4. Click on the schema of “output” in the *CellDesigner* model in order to select it and then click on button “Validate” in the BioNetCAD interface to validate your choice. Then click on button “Next”.



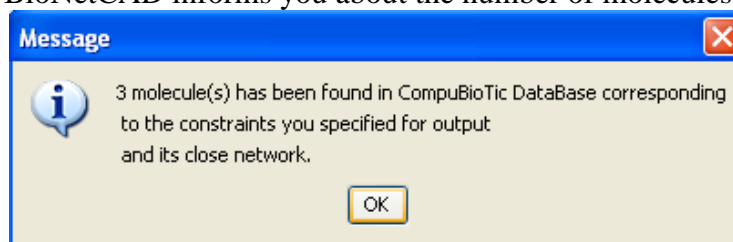
5. Next interface allows you to specify constraints about the molecule you have selected (“output” in this case).
Choose “Colorimetry” in the “Detection method” section.
Then click on button “Next”.



6. BioNetCAD proposes you to specify constraints about molecules directly linked to the selected molecule by reactions.
Here, no constraints will be specified. Thus, click on “Next”.



7. Now BioNetCAD performs a research on the database taking into account the constraints you specified for the selected molecule, the constraints about the network's molecules, and the topology of the network drawn under *CellDesigner*.
After the research, BioNetCAD informs you about the number of molecules found. Click “Ok”.



8. The results of the research on CompuBioTicDB are displayed. Choose one combination by *CellDesigner* reaction, by selecting a radio button: for instance, select SM00050 with hydrogen peroxide as implementation of “interm2”. Once your choice is made, click on “Update the network”.

BioNetCAD

Results

3 molecule(s) has been found in CompuBioTic DataBase corresponding to the constraints you specified for output and its close network.

```
SELECT * FROM real_molecule, small_molecule WHERE idMOL=RM_idMOL8 AND (rmDetection
```

Molecule ID	Molecule Name	Close Network															
SM00016	O-dianisidine (oxidised)	re3 Reaction 3 : Donor + H(2)O(2) = oxidized donor + 2 H(2)O <table border="1"> <thead> <tr> <th>interm2</th> <th>input2</th> <th>new product</th> <th>enzyme 3</th> <th></th> </tr> </thead> <tbody> <tr> <td>Hydrogen peroxide</td> <td>O-dianisidine (reduced)</td> <td>water</td> <td>Peroxidase</td> <td><input type="radio"/></td> </tr> <tr> <td>O-dianisidine (reduced)</td> <td>Hydrogen peroxide</td> <td>water</td> <td>Peroxidase</td> <td><input type="radio"/></td> </tr> </tbody> </table>	interm2	input2	new product	enzyme 3		Hydrogen peroxide	O-dianisidine (reduced)	water	Peroxidase	<input type="radio"/>	O-dianisidine (reduced)	Hydrogen peroxide	water	Peroxidase	<input type="radio"/>
interm2	input2	new product	enzyme 3														
Hydrogen peroxide	O-dianisidine (reduced)	water	Peroxidase	<input type="radio"/>													
O-dianisidine (reduced)	Hydrogen peroxide	water	Peroxidase	<input type="radio"/>													
SM00050	2,2-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid, ABTS oxidised)	re3 Reaction 53 : ABTS + H(2)O(2) = oxidized ABTS + 2 H(2)O <table border="1"> <thead> <tr> <th>interm2</th> <th>input2</th> <th>new product</th> <th>enzyme 3</th> <th></th> </tr> </thead> <tbody> <tr> <td>2,2-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid, ABTS)</td> <td>Hydrogen peroxide</td> <td>water</td> <td>Peroxidase</td> <td><input type="radio"/></td> </tr> <tr> <td>Hydrogen peroxide</td> <td>2,2-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid, ABTS)</td> <td>water</td> <td>Peroxidase</td> <td><input checked="" type="radio"/></td> </tr> </tbody> </table>	interm2	input2	new product	enzyme 3		2,2-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid, ABTS)	Hydrogen peroxide	water	Peroxidase	<input type="radio"/>	Hydrogen peroxide	2,2-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid, ABTS)	water	Peroxidase	<input checked="" type="radio"/>
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SM00064	nitrophenol	re3 Reaction 5 : A phosphate monoester + H(2)O = an alcohol + phosphate <table border="1"> <thead> <tr> <th>interm2</th> <th>input2</th> <th>new product</th> <th>enzyme 3</th> <th></th> </tr> </thead> <tbody> <tr> <td>nitrophenylphosphate</td> <td>water</td> <td>Phosphate</td> <td>Alkaline phosphatase</td> <td><input type="radio"/></td> </tr> <tr> <td>water</td> <td>nitrophenylphosphate</td> <td>Phosphate</td> <td>Alkaline phosphatase</td> <td><input type="radio"/></td> </tr> </tbody> </table>	interm2	input2	new product	enzyme 3		nitrophenylphosphate	water	Phosphate	Alkaline phosphatase	<input type="radio"/>	water	nitrophenylphosphate	Phosphate	Alkaline phosphatase	<input type="radio"/>
interm2	input2	new product	enzyme 3														
nitrophenylphosphate	water	Phosphate	Alkaline phosphatase	<input type="radio"/>													
water	nitrophenylphosphate	Phosphate	Alkaline phosphatase	<input type="radio"/>													

- The network is implemented with the choice of molecules you have made. Click on “Make another research” to search implementations for the molecule “enzyme 2”.
- Repeat steps 3 and 4 of the tutorial with selection of “enzyme 2”. In the “Step 2: Selected Molecule Specifications” interface, select “Enzymatic function” in the “Protein’s function” section. Then click “Next”.

BioNetCAD

Step 2: Selected Molecule Specifications

Selected Molecule Type
 Selected molecule : enzyme 2
 Type of molecule : PROTEIN
 Type of protein : GENERIC

Selected Molecule Constraints

Localization of the molecule

Nondescript
 Membrane
 Transmembrane
 Soluble Inside
 Outside

Diffusion across membrane

Nondescript
 No
 Yes Active
 Via transporters
 By cargos
 Passive
 Without channel across membrane
 Via channel

Detection method

Nondescript
 Fluorescence
 Colorimetry
 Other
 Is not detectable by these methods

Protein's function

Enzymatic function
 Binding function

EC Class

Nondescript
 EC1 - Oxydoreductases - EC4 - Lyases -
 EC2 - Transferases - EC5 - Isomerases -
 EC3 - Hydrolases - EC6 - Ligases -

Help Previous Next Cancel

11. Click on “Specify constraints” for the molecule “Hydrogen peroxide”. Click “Validate” on the next interface.

BioNetCAD

Step 4: Close Surrounding Network Specifications

The selected molecule is implicated in 1 reaction(s).

interm1	substrate(re2)	Specify constraints
Hydrogen peroxide	product(re2)	Specify constraints

Help Previous Next Cancel

BioNetCAD - Network component constraints

Step 3: Linked Molecule Molecule Specifications

Linked Molecule Molecule Type
 Linked Molecule molecule : Hydrogen peroxide
 Type of molecule : SIMPLE_MOLECULE

Direct entry of the molecule (if you know it)

Direct entry
 Enter one or more of the following :
 CheBI Identifier
 Linked Molecule Molecule name
 Formula

Hydrogen peroxide

Specify constraints of the molecule

Linked Molecule Molecule Constraints

Localization of the molecule

Nondescript
 Membrane
 Transmembrane
 Soluble
 Inside
 Outside

Diffusion across membrane

Nondescript
 No
 Yes
 Active
 Via transporters
 By cargos
 Passive
 Without channel across membrane
 Via channel

Detection method

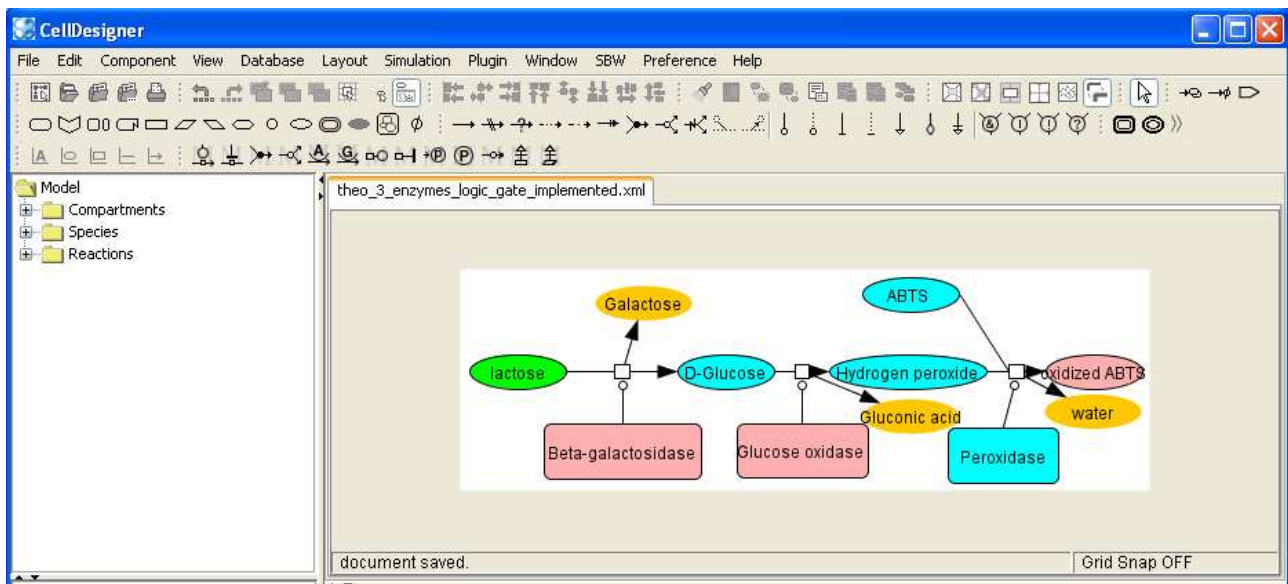
Nondescript
 Fluorescence
 Colorimetry
 Other
 Is not detectable by these methods

Help Validate Close

BioNetCAD will search for enzymes having hydrogen peroxide as product.

12. Again, results are displayed. Choose “glucose oxidase” with D-glucose as implementation of interm1. Repeat tutorial steps 9 to 11. For the last search, choose “enzyme 1” as firstly selected molecule and specify constraints about the D-glucose in the close network.

13. Finally, an implemented network is obtained.



2. HSIM SIMULATIONS

1. Open theo_3_enzymes_logic_gate_implemented.xml or theo_3_enzymes_logic_gate.xml after implementation in Section 1 of the present tutorial.
2. In the Plugin menu of CellDesigner and choose BioNetCAD1.1, and click on “Run Hsim launcher”.
3. Hsim launcher interface asks for several parameters needed for the Hsim configuration file.

As an example, enter the following parameters:

Geometry settings: cell height:200; cell width: 200

Reactions parameters settings:

Re3

kcat=736

Hydrogen peroxide kM=0.005

ABTS kM=0.18

Re2

Kcat=0.3

D-glucose kM=31.8

Re3

Kcat=60

Lactose kM=1.4

Initial quantities settings:

Water 0

Peroxidase 0.3

ABTS 2.5

Oxidized ABTS 0

Gluconic acid 0

Hydrogen peroxide 0

Glucose oxidase 0.09

Galactose 0

Lactose 250

D-glucose 0

Beta-galactosidase 0.011

When all the parameters are specified, click on “Launch Hsim”.

BioNetCAD - Hsim launcher

Welcome to the BioNetCAD HsimLauncher Plugin

Geometry settings

cell height: cell width:

re2

kcat =

D-Glucose kM (mM) =

re1

kcat =

lactose kM (mM) =

Peroxidase	<input type="text" value="0.3"/>
ABTS	<input type="text" value="2.5"/>
oxidized ABTS	<input type="text" value="0"/>
Gluconic acid	<input type="text" value="0"/>
Hydrogen peroxide	<input type="text" value="0"/>
Glucose oxidase	<input type="text" value="0.09"/>
Galactose	<input type="text" value="0"/>
lactose	<input type="text" value="250"/>
D-Glucose	<input type="text" value="0"/>
Beta-galactosidase	<input type="text" value="0.011"/>