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".

😸 CellDesigner					
File Edit Component View Database	Layout Simulation Plugin	Window SBW Prefe	rence Help		
	■ 53 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	VetCAD1.1	Run BioNetCAD Run Hsim Launcher	. i † 9 † @	(∅ ∅ ∅ 0 0 (∅ ∅ ∅ 0 (∅ ∅ ∅ 0 (∅ 0 (
Model	theo_3_enzymes_I SBM	opingArrayMass 🕨			
				(input2)	
	(int	out1	interm1	interm2	
		Ļ	Ļ		
		enzyme 1	enzyme	2 enzy	me 3
	1				Grid Snap OFF
Laver	.			14-11 AV	
base	Species Proteins Genes	RNAs asRNAs React	ions Compartments P	arameters Fun 📢 🖡	Edit Notes Edit Protein Notes
1720-1. (M-2010, M-2019)		[Edit] (Ex	port		
	class id name	e compa	positio quanti	ini subs	
	SIMPLE s1 input1	default	inside Amount	0.0	
	SIMPLE s2 input2	default	inside Amount	0.0	
	SIMPLE s3 interm	1 default	inside Amount	0.0	
	SIMPLE s4 interm:	2 default	inside Amount	0.0	
	SIMPLE s5 output	: default	inside Amount	0.0	
	PROTEIN s6 enzym	e 1 default	inside Amount	0.0	
	<	1111		>	

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

👙 BioNetCAD						
Welcome to the BioNetCAD Plugin						
Select the type of search you want to undertake :						
Help Enter SQL Request 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".

👙 BioNetCAD	
Step 1 : select a molecule in the network and Validate	ις 9 Τ 7 4 9 4 ΦΛΦΔ Π α
Selected molecule : output	
Help Previous Next Cancel	input2
input1 interm1	interm2 output
enzyme 1	enzyme 2 enzyme 3

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".

🖆 BioNetCAD	
Step 2: Selected Molecule Specificat	tions
Selected Molecule Type	
Selected molecule : output	
Type or molecule : SIMPLE_MOLECULE	
Selected Molecule Constraints	
Localization of the molecule	Diffusion accross membrane
 Nondescript 	
🔿 Membrane	
🔿 Transmembrane	Nondescript
Soluble Inside	○ No
🔾 Outside	Ves Active
Detection method	Via transporters
🔿 Nondescript	By cargos
 Colorimetry 	
Other	
Is not detectable by these methods	
Help	Previous Next Cancel

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".

👙 BioNetCAD							
Step 4: Close Surrounding Network Specifications The selected molecule is implicated in 1 reaction(s).							
inte	erm2 substrate(re3)	Specify constraints					
inp	out2 substrate(re3)	Specify constraints					
en:	zyme 3 modifier	Specify constraints					
C	Help Previous	Next Cancel					

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".

molecule(s) has l	been found in CompuBioTic DataBas	e corresponding to the constra	ints you specified for outp	iut and il	ts close r	network	2	
SELECT * FF	ROM real_molecule, sma	ll_molecule WHERE :	idMOL=RM_idMOL8	AND (rmDet	ectio	on	
Molecule ID Molecule Name Close Network								
SM00016	O-dianisidine (oxidised)	re3						
		Reaction 3 : Donor + H	(2)O(2) = oxidized dono	or + 2 H	(2)0			
		interm2	input2		new product		enzyme 3	1
		Hydrogen peroxide	O-dianisidine (redu	iced)	water		Peroxidase	0
		O-dianisidine (reduced	l) Hydrogen peroxide		water		Peroxidase	0
SM00050	2,2-Azino-bis(3-	re3						
	ethylbenzthiazoline- 6-sulfonic acid, ABTS oxidised	Reaction 53 : ABTS + H(2)O(2) = oxidized ABTS+ 2 H(2)O						
		interm2	input2		new product		enzyme 3	ļi.
		2,2-Azino-bis(3- ethylbenzthiazoline- 6-sulfonic acid, ABTS	Hydrogen peroxide		/ater		Peroxidase	0
		Hydrogen peroxide	2,2-Azino-bis(3- ethylbenzthiazoline- 6-sulfonic acid, ABTS	2,2-Azino-bis(3- water ethylbenzthiazoline- 6-sulfonic acid, ABTS			Peroxidase	۲
SM00064	nitrophenol	re3						
		Reaction 5 : A phosphate monoester + H(2)O = an alcohol + phosphate						
		interm2	interm2 input2		new product enzyn		e 3	
		nitrophenylphosphate	water	Phosphate Alkali		ie phosphatas	e C	
		water	nitrophenylphosphate		Phosphate Alkalir		ie phosphatas	e C

- 9. 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".
- 10. 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".

elected Molecule Type elected molecule : enzyme 2 rpe of molecule : PROTEIN rpe of protein : GENERIC	
elected Molecule Constraints	
Localization of the molecule	Diffusion accross membrane
Nondescript	
	Nondescript
	🚫 No
	🔿 Yes 🔿 Active
Godiside	 Via transporters
Detection method	By cargos
Nondescript	O Passive
Fluorescence	○ Without channel accross membrane
O Colorimetry	◯ Via channel
Other	
\bigcirc Is not detectable by these methods	
Protein's function	EC Class
	 Nondescript
Enzymatic function	○ EC1 - Oxydoreductases - ○ EC4 - Lyases -
Binding function	C EC2 - Transferases -
	© EC3 - Hydrolases -

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	

SioNetCAD - Network component co	nstraints	X
Step 3: Linked Molecule Molecule Sp	ecifications	
Cinked Molecule Molecule Type		
Linked Molecule molecule : Hydrogen peroxide		
Type of molecule : SIMPLE_MOLECULE		
 Direct entry of the molecule (if you know it) 		
Enter one or more of the following :		
CheBI Identifier		
Linked Molecule Molecule name		Hydrogen peroxide
Formula		
O Specify constraints of the molecule		
Linked Molecule Molecule Constraints		
Localization of the molecule	Diffusion accross men	brane
 Nondescript 		
() Membrane		
○ Transmembrane	Nondescript	
◯ Soluble ◯ Inside		
Outside	O Yes O Active	Uis kussessakava
Detection method		
 Nondescript 	Passive	() by cargos
○ Fluorescence	01 035140	○ Without channel accross membrane
◯ Colorimetry		
Other		
◯ Is not detectable by these methods		
	<u>.</u>	
Help	Validate Clo	se

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.

SellDesigner	
File Edit Component View Database	Layout Simulation Plugin Window SBW Preference Help
	◙●▩०▯→┿┾┉┉┿┾┽ぺぺぷぇ╎╎╵╵╵Ѵ╹=๏๏» ▫◙▫®▯ױױ▯๚๚๚๚๚๚๚๚๚๚๚๚๚๚๚๚๚๚๚๚๚๚๚๚๚๚๚๚๚๚๚๚๚๚
	ዿዿ಼∞н+®®-∞ 含含
Model Compartments Species Reactions	theo_3_enzymes_logic_gate_implemented.xml
• *	

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
   Re<sub>2</sub>
          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".

4	BioNetCAD - Hsim lau	ncher				
		Welcome to th	ne BioNetCAD HsimLa	auncher Plugin		
	Geometry settings					
	cell height	200	cell width		200	
	re2					^
	kcat =			0.3		
	D-Glucose kM (mM) =			31.8		
	re1			31.0		
	koat =			60		
	KLOU -					
	lactose kM (mM) =			1.4		
	Peroxidase		0.3			
	ABTS	-	2.5			
	oxidized ABTS		0			
	Gluconic acid	-	0			
	Hydrogen peroxide	-	0			
	Glucose oxidase	-	0.09			
	Galactose	-	250			
	D-Glucose	-	0			
	Beta-galactosidase		0.011			
						Y
		Help	Cancel La	unch Hsim		