NetworkMaker Editor User's Manual v 1.0

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0. General function of this software:

The Gene network modifier tool has been developed to provide a user-friendly interface to construct, visualize and modify the gene network used for simulations with EmbryoMaker. The NetworkMaker files are constantly being updated and future versions may contain additional features that are not presented in version 1.0.

NetworkMaker is a software to edit networks and model parameters. The model itself is run through the EmbryoMaker software. For the model to run, some initial conditions (an initial spatial distribution of cells, cell nodes and concentration of different molecules in those) need to be provided to EmbryoMaker. In EmbryoMaker both initial conditions and model parameters are stored into single files. These files can be fed as input to EmbryoMaker (to run the gene network, model parameters from specific initial conditions). To view the respective network and model parameters, the same input files can be fed to NetworkMaker. By means of NetworkMaker, one can then modify (or design from scratch) those network and model parameters and store them into a new file which can later be simulated by EmbryoMaker. Note that the initial conditions as such are not modified by NetworkMaker. To manually edit or modify initial conditions, or any intermediate developmental stage, the embryo editor in EmbryoMaker should be used.

1. Download Software:

The NetworkMaker network editor software can be downloaded from

http://www.biocenter.helsinki.fi/salazar/software.html

2. Installation:

2.1 Linux

NetworkMaker has been made and compiled on 12.04 LTS, 64 bit Ubuntu, so it should compile

easily on any ubuntu version 12.04 or more recent.

2.1.1 Compilation:

2.1.2 Software requirements:

-gtk2.0+ libraries:

-*http://www.gtk.org*
-In Ubuntu this can be usually installed by running “sudo apt-get install gtk2.0+”

-gtk-2-fortran

- *https://github.com/jerryd/gtk-fortran/wiki*

or directly: *https://aur.archlinux.org/packages/gtk-2-fortran-git/*

-We provide the necessary library within the package, which should be sufficient for most users' requirements, so manual download may not be needed.

-gfortran compiler:

-*https://gcc.gnu.org/wiki/GFortran*
-In Ubuntu this can be installed by running “apt-get install gfortran”

2.1.3 Building:

Download the .tar file from the website and save it in the place you want to install the software. Uncompress the .tar file. You may do that by opening a terminal, going to the directory where the .tar file is stored and typing the following command:

tar -xzvf <NetworkMaker_directory>.tar.gz

Where <NetworkMaker_directory> stands for whatever name the downloaded .tar file has at that moment.

To compile go to the NetworkMaker folder and type:

./compile_NetworkMaker.sh

This compilation command assumes that you are using the gtk-2-fortran.pc from inside the same folder. If you are using it from a different location, adapt this command line or create a symbolic link to your current location.

2.2 Mac

We are currently trying to provide a version of NetworkMaker available for Mac. This is still in process. Check out source files and installation hints:
http://www.biocenter.helsinki.fi/salazar/software.html

2.3 Windows

We are currently trying to provide a version of NetworkMaker available for Windows. This is still in process. Check out source files and installation hints:
http://www.biocenter.helsinki.fi/salazar/software.html

2.4 Licences

As previously mentioned, this software package includes code downloaded as open-source from the internet. This applies to the gtk-2-library as well as to all source files that stem from the gtk-fortran project (h*ttps://github.com/jerryd/gtk-fortran/wiki*). They are available under the GNU General Public License version 3 (*http://www.gnu.org/copyleft/gpl.html*).

3. Command-line execution options:

This section provides a brief description of how to run the program executable and about its several command-line options:

-Simplest option. Open a terminal and go to the directory where the simulator has been installed. Then type:

./NetworkMaker-launcher

And press return. A small array of buttons will be prompted with the following options:

1. Choose input file. Pressing the upper button will open the system of files in the user's computer, thereby allowing to choose an input file. The choice is made by either double-clicking on the file name or pressing the “OPEN” button.
2. Create a new GRN. This will run the interface with a new network (without pre-existing genes)
3. Press the START button. A simple default network and model parameters will be used. If the user decides to save it in its original form or after modification, some default initial conditions will be written with it in the output file (which, again, can later be edited from EmbryoMaker).
4. Quit. This simply closes the program.

Alternatively, in order to run it with a defined input file in the first place, run:

./NetworkMaker-launcher <name of file>

Where <*name of file*> is an input file. Any output files that arise from EmbryoMaker can be used as an input file to NetworkMaker. This way the network and parameters used in a simulation in EmbryoMaker can be visualized. For example, in order to run a file named '010115_input.dat' inside an 'output' folder, simply type in the terminal:

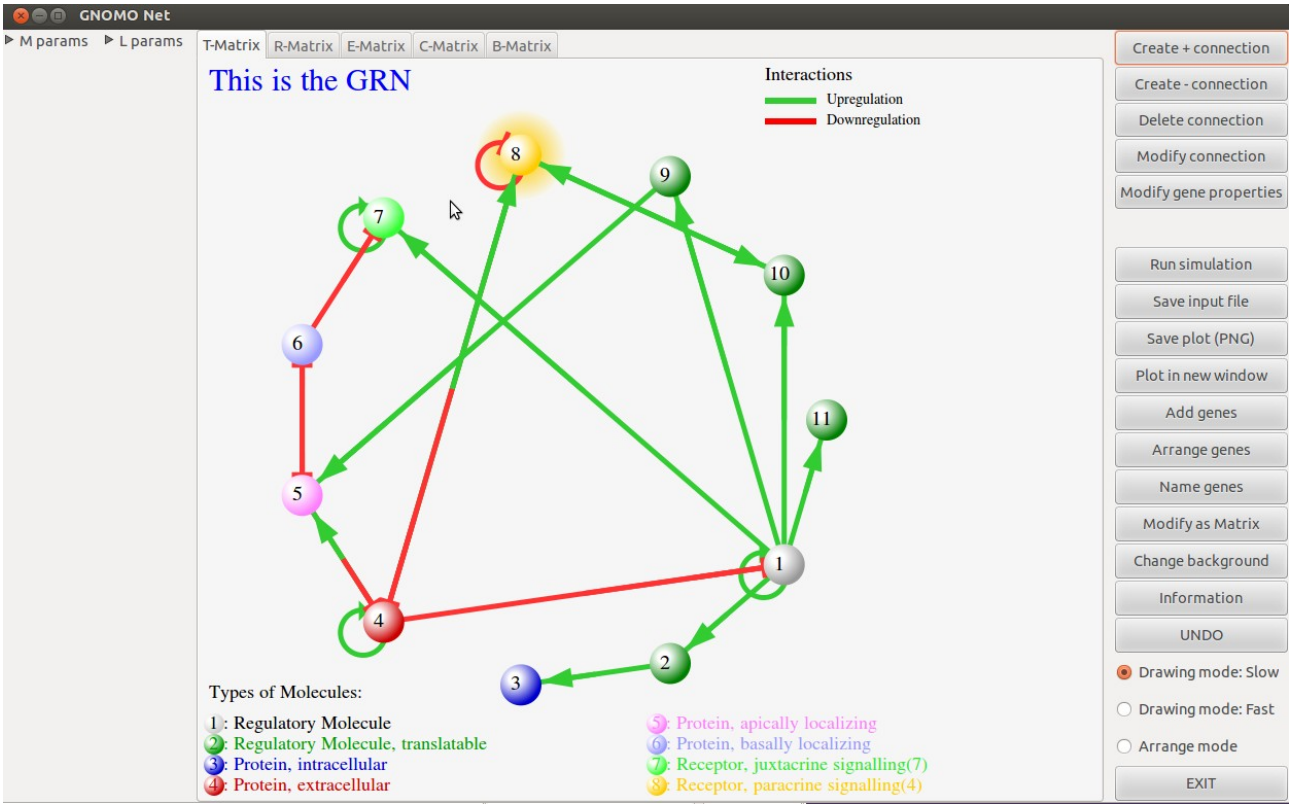
./NetworkMaker-launcher output/010115_input.dat

In this case, the file system will not be accessible and the visual interface is launched immediately. Note that too long file names (more than 140 characters) might lead to problems, since the length of the input string, consisting of the file name plus.

4. Running NetworkMaker with its visual interface:

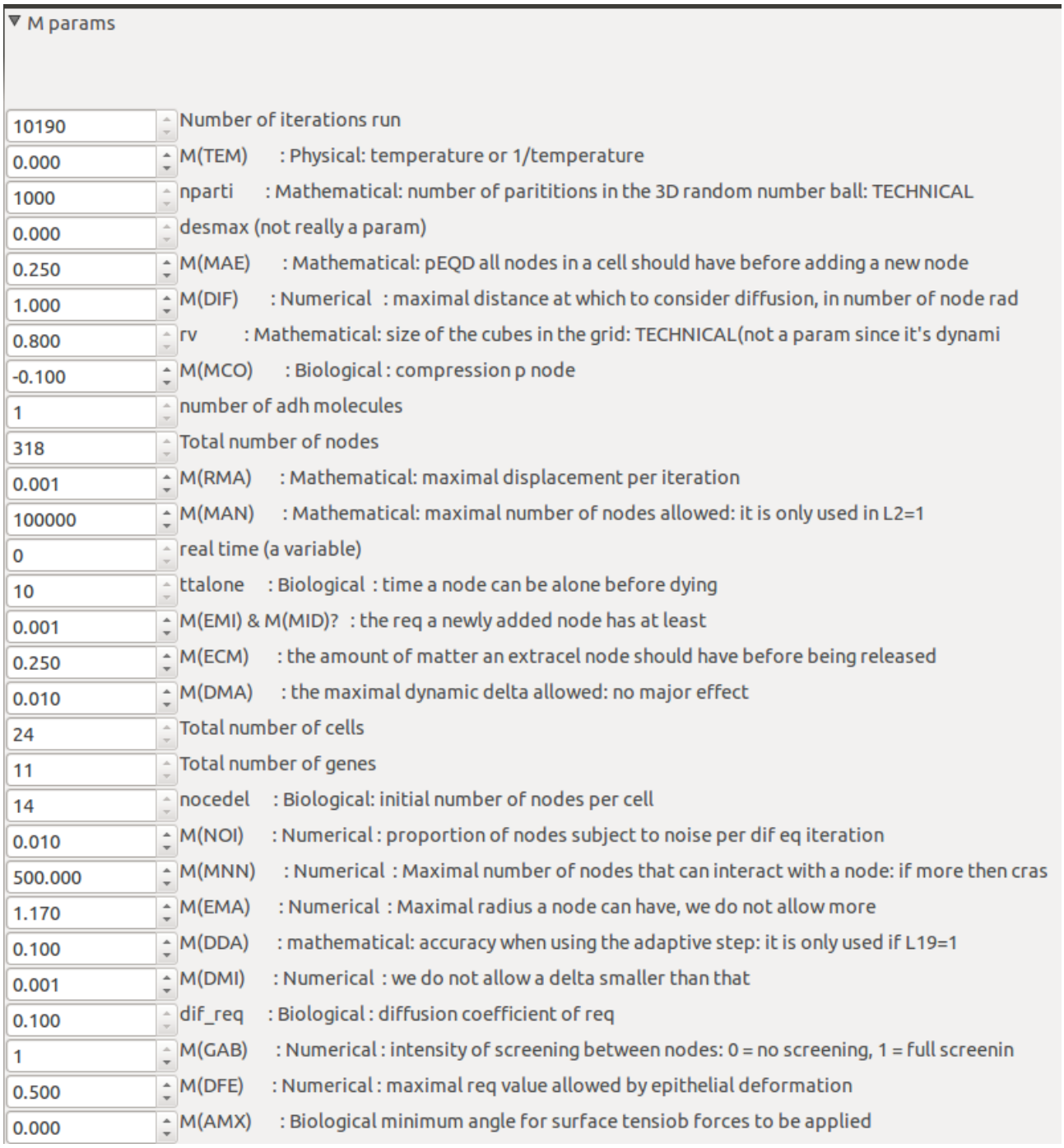
When the program is executed, a large panel with a number of boxes, menus and buttons will

appear. We will know describe the function of each of them starting from the left part of the panel.



5. Principal window options:

5.1 Global model parameters:

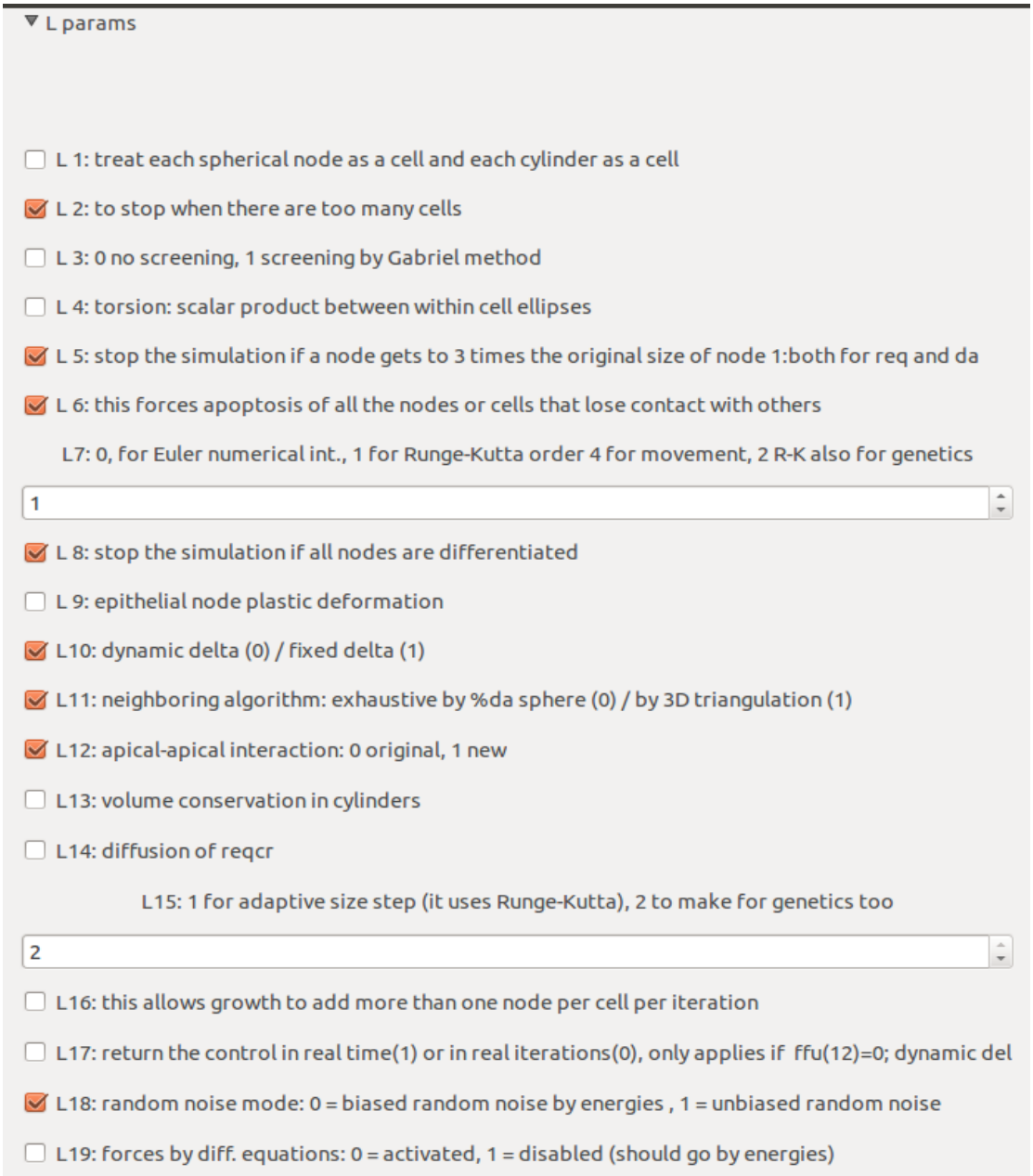


By clicking the label ”Mparams” in the left upper corner the list of the global model parameters will arise. The current values of each modifiable parameter are shown inside the entry fields and can be modified either manually or by using the tiny arrowheads. Values that cannot be changed within NetworkMaker by the user will appear without modification option (these are variables that are modified fro EmbryoMaker such as the number of elements or the simulation time). To hide these

parameters out of view, click again in the “Mparams” label in the upper left border. For a description of the global model parameters check the original article.

5.2 Logical model parameters

By



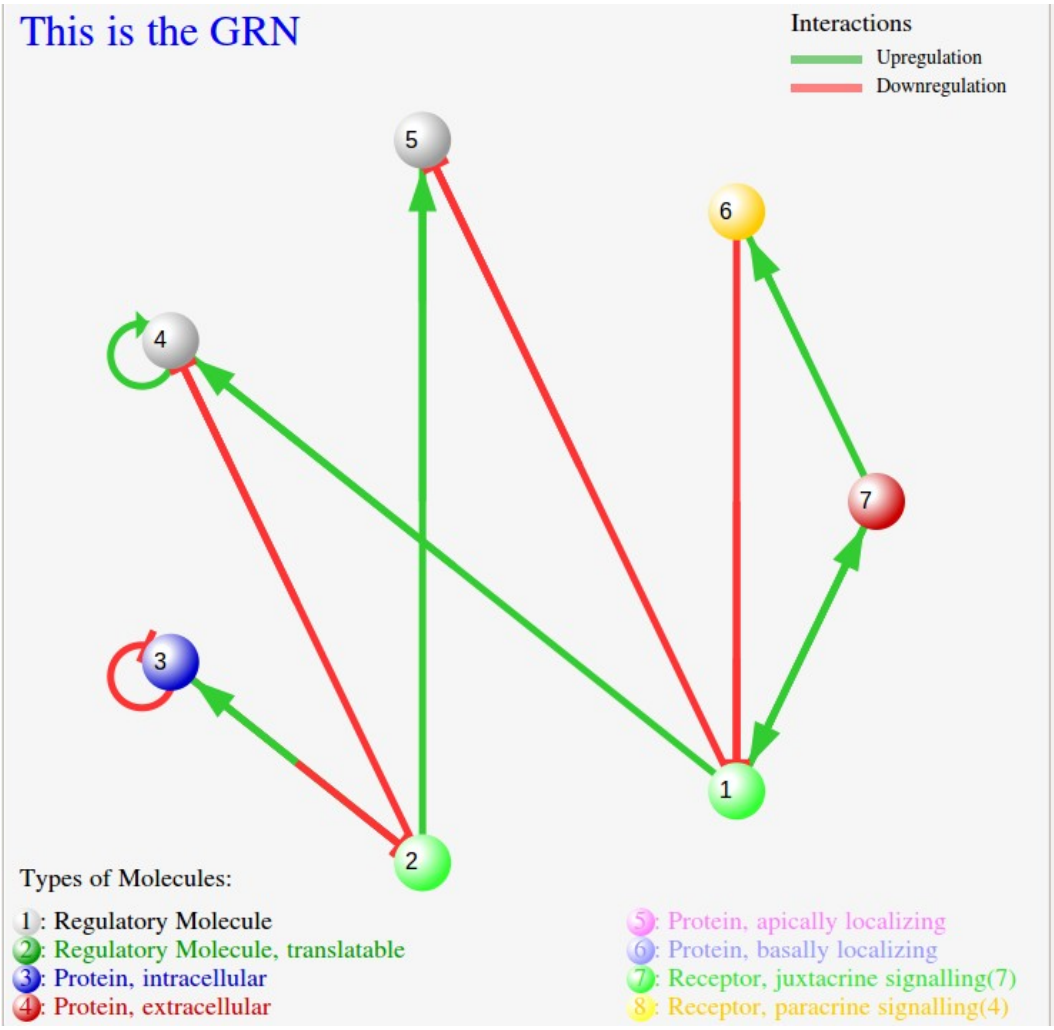
clicking the label ”Lparams” the list of the logical model parameters will arise. They can individually be toggled in order to start a simulation with different conditions. Non-binary decisions are made by a choice between numbers. For a description of the logical model parameters check the original article.

5.3 Main Window View

By default, the main window contains a drawing area, on which the network of regulatory molecules is depicted. By clicking on the different tabs in the upper part of the window different aspects of the gene network can be visualized and modified by the user.

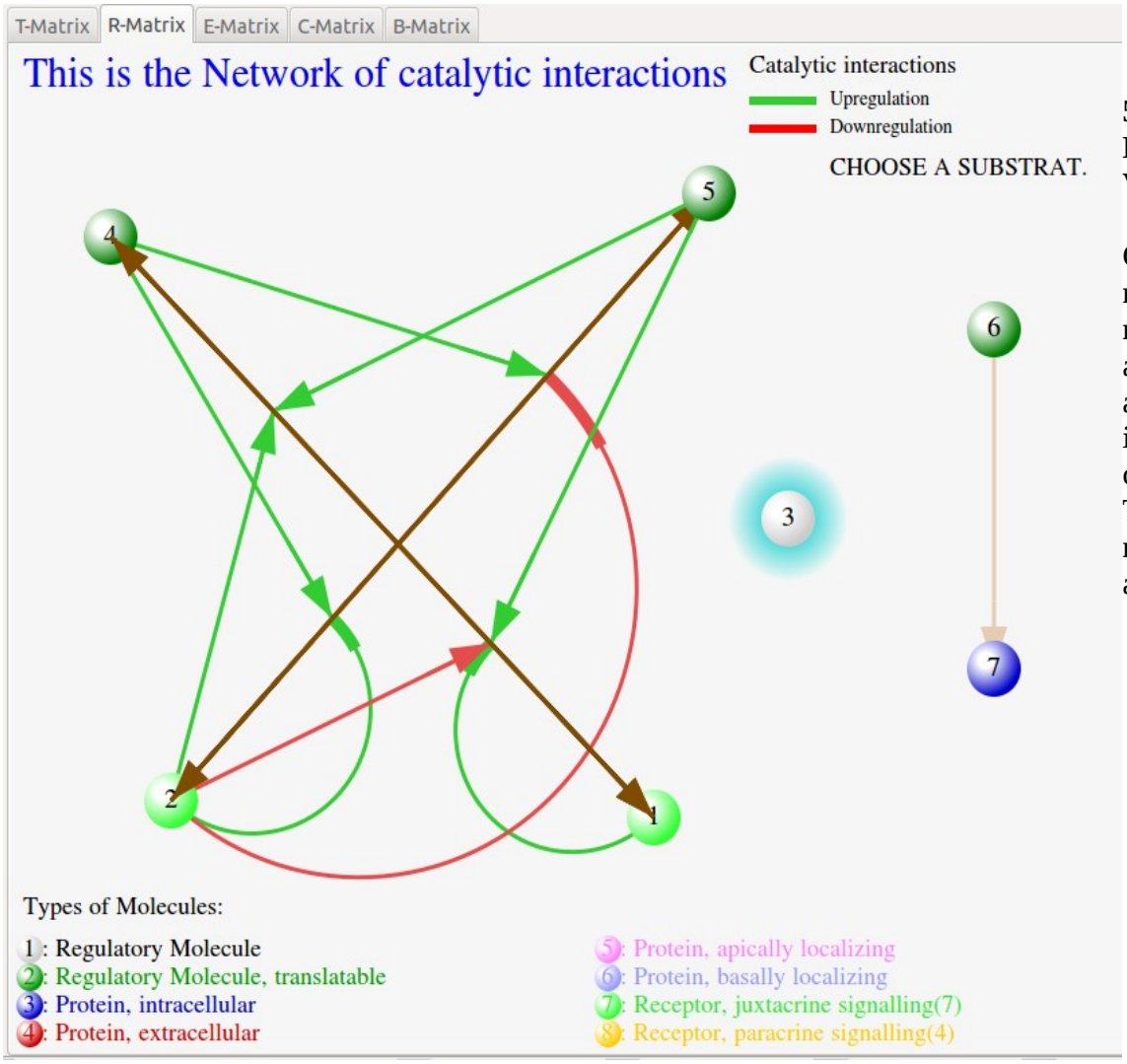
5.4 T-Matrix View

Regulatory molecules, such as genes and proteins, are symbolized by spherical nodes. These are the same as in the case of the R-matrix, E-matrix and C-matrix. By default, all regulatory molecules in the network used are arranged in a circle. Different colors represent some of their particular properties (which define, e.g., their localization within and between cells). A legend is always shown beneath the network. The T-matrix contains the interactions that make up the transcriptional network used in a particular EmbryoMaker simulation (see the original article for a description). Lines denote interaction between genes. Arrowheads point from the gene that acts as the transcriptional factor to the gene whose transcription is being regulated. Green lines denote positive transcriptional regulation, whereas red ones denote negative regulation. Autoactivation is drawn as a smaller circle around the respective node.



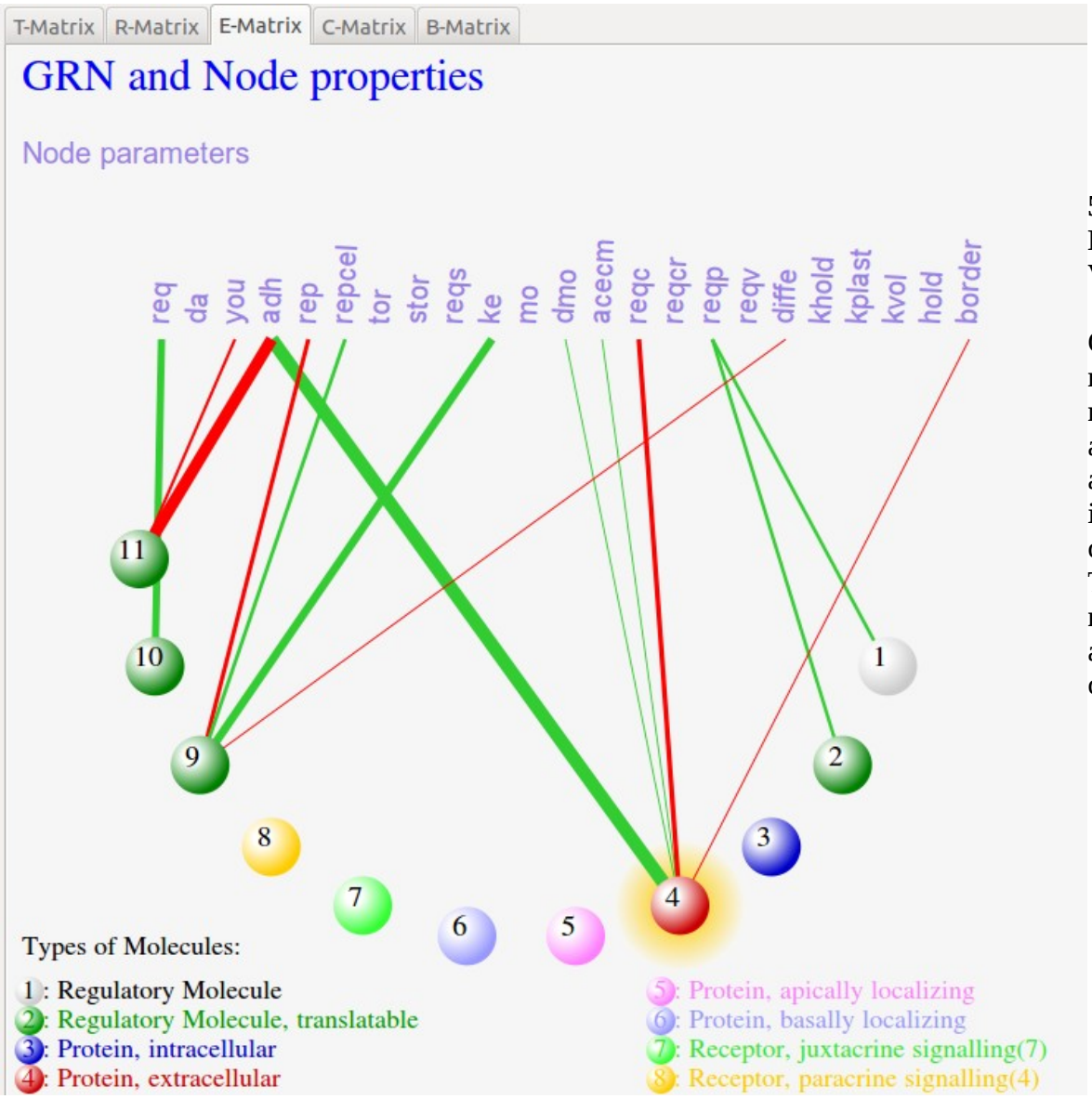
5.5 R-Matrix View

This is the matrix that specifies which regulatory molecules catalyze which reactions. Each node, spheres, corresponds to a different regulatory molecule (which may be a gene product or not, cf. Section 5.4.). Dark arrows between two nodes indicate that one molecule (the one from which the arrow starts) gives rise to the other one (the one pointed towards by the arrow). The regulatory molecules regulating (catalyzing) such a reaction are indicated by an arrow leading from the catalyst molecule to the reaction arrow (close to the product molecule).Green color indicates a positive regulation and red color a negative one. Since three molecules are needed to define a catalytic reaction, different background colours around the gene clicked last as well as an explicit comment indicate if substrate, product or catalyst are to be selected next.



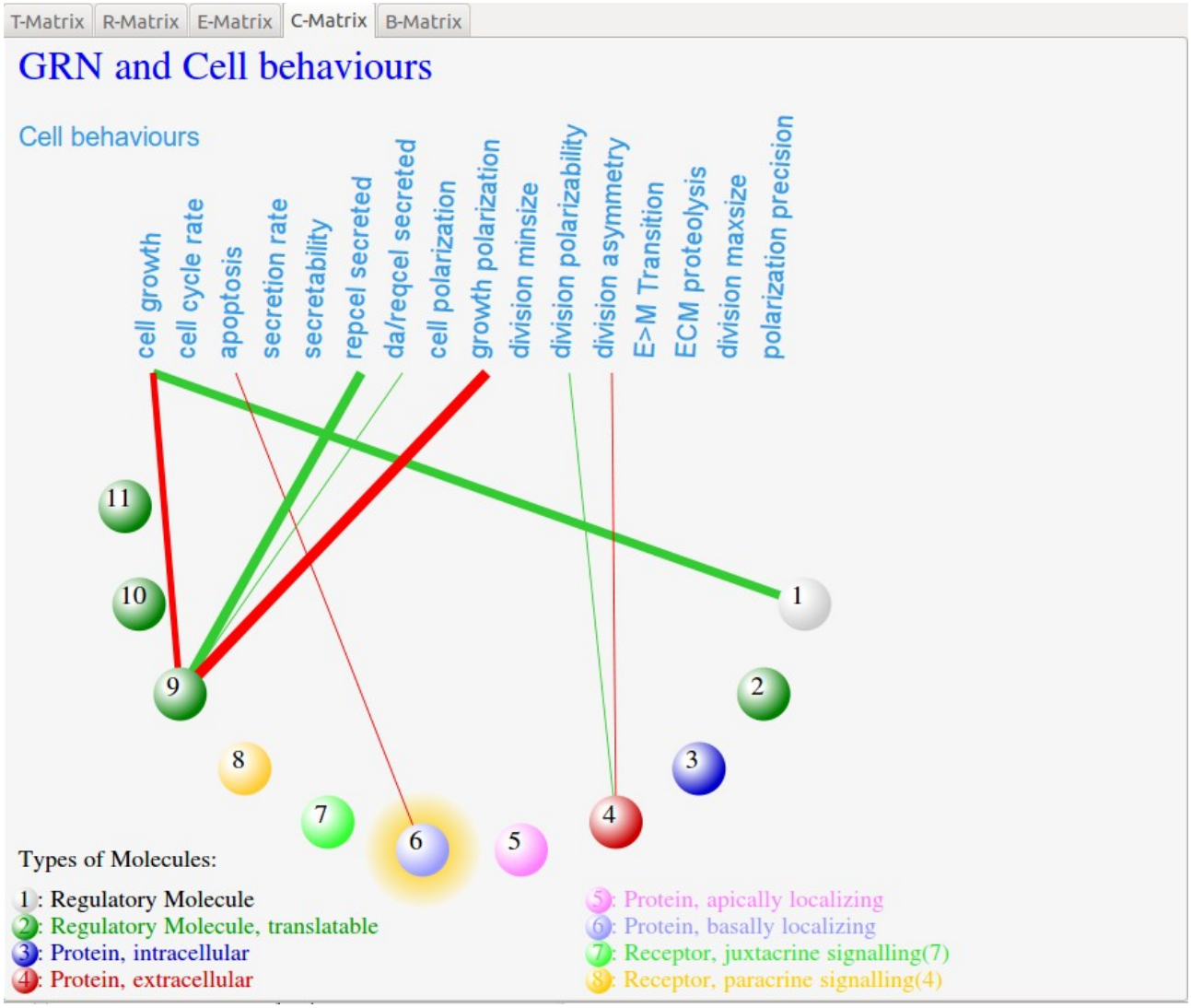
5.6 E-Matrix View

Gene regulation molecules are arranged in a semi-circle. The names of all the mechanical node properties are written in the upper part of the window. As before, positive regulation is depicted by a green line and negative regulation by red line, whose thickness represents the strength of regulation.



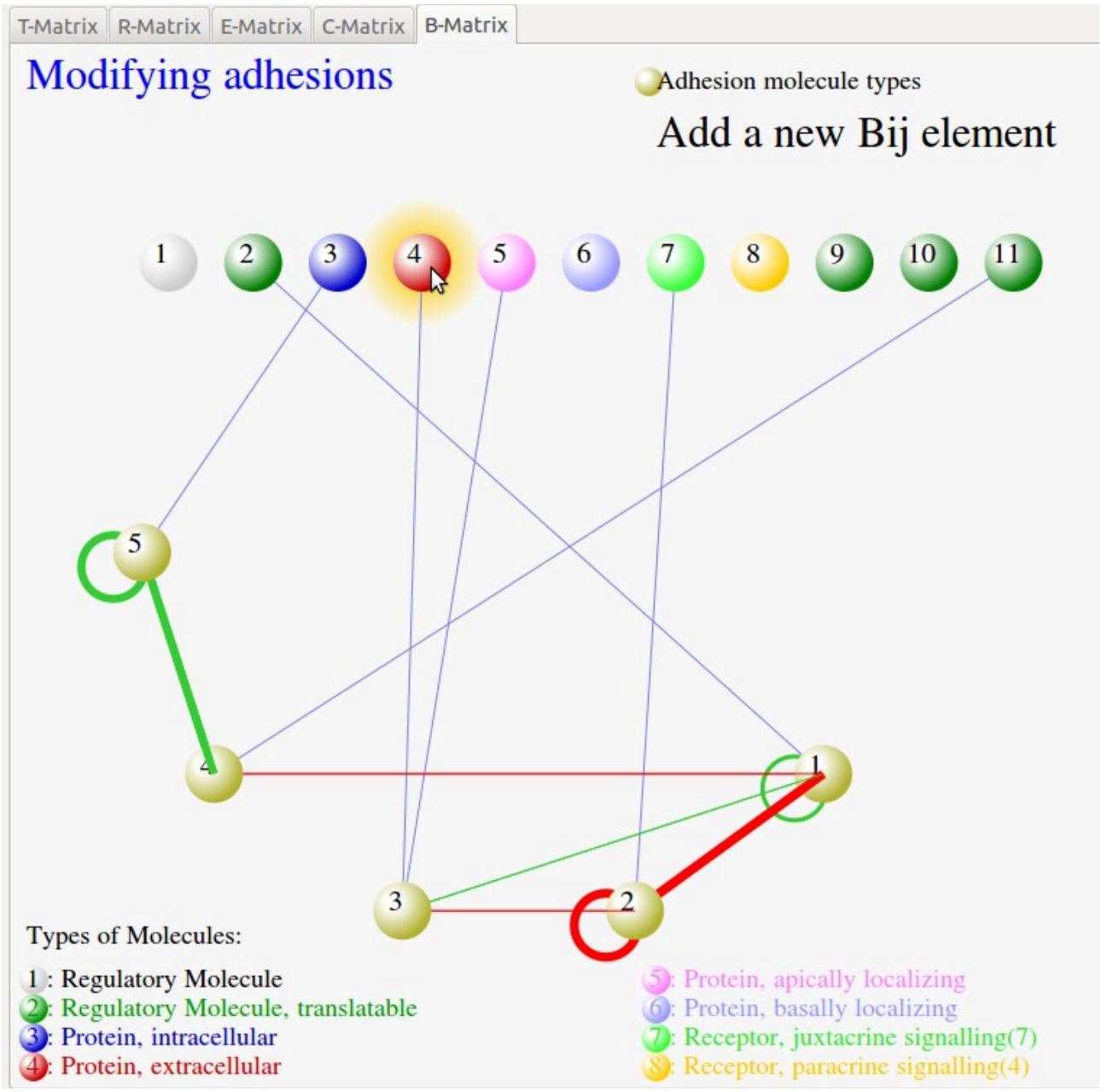
5.7. C-Matrix View

Gene regulation molecules are arranged in a semi-circle. The names of all the cell behaviours included in GNOMO are written in the upper part of the window. As before, positive regulation is depicted by a green line and negative regulation by red line, whose thickness represents the strength of regulation.



5.8 B-Matrix View

This sheet shows the specific adhesive or repulsive interactions mediated by the regulatory molecules of the network. Within the model, these interactions are stored in the B matrix. This view shows the specific adhesive interactions mediated by the regulatory molecules of the network. Green lines drawn between nodes mean that the respective molecules mediate adhesion (usually by surface protein interaction) between the cells in which they are expressed, red lines that they mediate repulsion between cells. Spheres that iconize those adhesions are colored golden and by default arranged in a semi-circle, while spheres representing genes (or other regulatory molecules) are colored as usual and lined up in the upper part of the sheet. Click on two nodes to see and modify the adhesion between them. A sub-menu window will pop up to allow modification.



6. Designing gene network

In the right most panel there are four buttons that allow to modify the network. The actions performed by these buttons apply only to the matrix (T, R, E, C or B) that is currently active (by the tabs in the upper part of the window).

6.1 Create + connection

Click on this button to activate the addition of connections between regulatory molecules or between a regulatory molecule and a property (for the E and C matrices). The interaction strength will be positive, with a default value of 0.1.

6.1.1 T-matrix: By clicking on a regulatory molecule and then on another regulatory molecule, the latter becomes positively transcriptionally regulated by the first. The interaction strength of the added connection is 0.1. This strength and other aspects of this interaction can be modified subsequently by clicking on the "Modify connection" button.

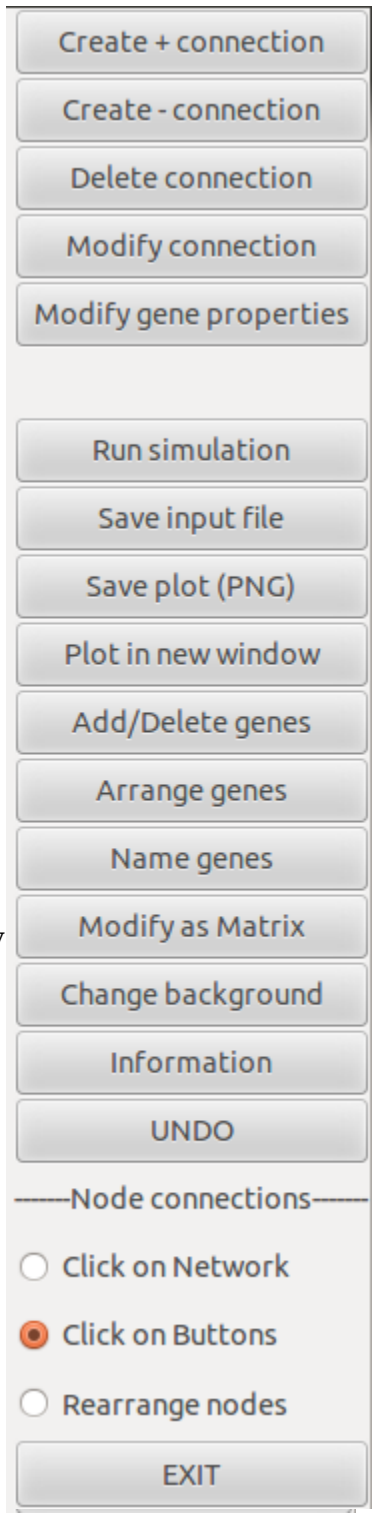
After clicking this button an interaction will be added between two regulatory molecules if the user clicks any two successive (and draw in the display) choose with the mouse button two genes with.

6.1.2 R-matrix: Two regulatory molecules A and B can be connected by a new arrow by sequentially clicking on them with the left mouse button, which means that the first molecule gives rise to the second one. Clicking on a third molecule C with the right button will choose a positive catalyst of the reaction from A to B, i.e. C becomes the catalyst of the reaction leading from A to B. The interaction strength of the added connection is 0.1. This strength and other aspects of this interaction can then be modified by clicking on the "Modify connection" button.

6.1.3 E-matrix: A new positive regulation of a node property by a regulatory molecules is added by simply clicking on a regulatory molecule first and then on one of the node properties. The interaction strength of the added connection is 0.1. This strength and other aspects of this interaction can then be modified by clicking on the "Modify connection" button.

6.1.4 C-matrix: A new positive regulation of a cell property by a regulatory molecules is added by simply clicking on a regulatory molecule first and then on one of the cell properties. The interaction strength of the added connection is 0.1. This strength and other aspects of this interaction can then be modified by clicking on the "Modify connection" button.

6.1.5 B-matrix: New adhesion types (new Bij) elements can be generated by clicking on the "Create a new Bij element"line on top. Clicking on two different Bij elements creates a line between them to create a new heterotypic adhesive interaction. Analogously, by clicking twice on the same Bij element will define a homotypic adhesive interaction, displayed by a circle around the respective element. The default interaction strength is 0.1, but can then be modified by clicking on the "Modify connection" button. In order to connect Bij elements with the regulatory elements they are mediated by, click on a regulatory element sphere and then on a regulatory element (or vice versa). A blue line will be drawn to visualize this connection.



6.2 Create – connection

As above, 6.1, but a negative interaction is created instead of a positive interaction with a default value of -0.1. For the different matrices, the same options apply as explained for the positive interactions.

6.3 Delete connection

As above, 6.1. The regulatory molecules are chosen as above but instead an existing connection is deleted.

6.4 Modify the connection

As above, 6.3, an existing connection can be selected. Upon selecting it an additional window will pop up and the user will be able to modify the strength and other aspects of each interaction, depending on the matrix currently active.

6.5 Modify gene properties.

After selection of a regulatory molecule (by clicking a gene with the right mouse button), this button can be pressed in order to modify the properties of the chosen regulatory molecule. This can be done in an additional window which will pop out then. Modifiable properties of regulatory molecules include a gene diffusion rate (that would be intra-cellular if the gene is not a growth factor), degradation rate and the kind of gene (transcriptional factor, growth factor, etc...). See the original article of the model for a more detailed description of those properties. Pressing ”Accept” will accept all modifications. In addition, each gene may be given a name of 1-12 characters (instead of a number label) by typing it into the empty window and pressing ”Apply name”.

7. Other right panel buttons

7.1 Run simulation

Runs EmbryoMaker with the current networks and parameter settings edited by NetworkMaker and with the initial conditions in the original file (the one provided in the command-line when NetworkMaker was executed or opened through the menu) or some default initial conditions if that file was not provided. Notice that this option launches EmbryoMaker and that its behavior can not be affected by NetworkMaker once it is launched.

7.2 Save input file

Pressing this button will create a file with all the genetic and model parameters. The initial conditions will be the ones in the original file (the one written in the command-line when NetworkMaker [ORIGINAL FILENAME] was executed or opened through the menu) or some default initial conditions if that file was not provided. You will be prompted to name the file in a dialog window. This function allows you to save all changes you have made to the network in order to either modify them later or use them in EmbryoMaker.

7.3 Save plot (PNG)

This button allows to save the current view in the .png format. The name of the file has to be defined in a dialog window that opens once the button is pressed.

7.4 Plot in a new window

Clicking this button redraws the current model matrix (network) on display, displaying one interaction matrix, as a new window. This may be useful, if different matrices shall be shown or compared at the same time.

7.5 Add/Delete genes

This button allows the addition of new nodes. Writing 0 into the entry field of the prompted sub-menu window will create an empty new node (with basic properties and without interactive connections). Writing a number between 1 and the current number of genes will create the new gene as a copy of the selected gene. Press ”Add it now” in order to make the addition happen. The ”Delete a gene” button causes a chosen gene to disappear from the network.

7.6 Arrange genes

This button opens a sub-menu which allows you to rearrange the positions of the nodes.

7.6.1 Arrange circular

This will rearrange the nodes in the circular manner that is shown as a default case in the beginning.

7.6.2 Arrange manually

This option allows to shift the positions of the nodes. Click on a node to select it and click again to assert its new position. Note that, after pressing this button, no node connections can be made or modified. In order to do this, you have to return to the modification mode by pressing the ”Return to GRN modification” button.

7.6.3. Save arrangement

Saves the current positions of all nodes.

7.6.4 Retrieve arrangement

Retrieves and plots the node positions that have been saved before.

7.6.5 Return to GRN modification

Pressing this buttons allows to return to the default mode, in which gene properties and interactions can be modified.

7.7. Name genes

As a default option, games are numbered. However, you can also name them. Pressing this button will open a table with the list of currently used genes. Besides each gene, there is an empty field in which a name of a length of up to 12 characters can be created. Press the “Apply all” button to attribute all names to the genes. Unnamed genes will keep their numbers.

7.8. Modify as matrix

This option will display the current matrix as a table, where changes can be made, which may be useful if entire pre-defined GRNs shall be read in.

7.8.1. Accept Changes

Accepts the changes you have made. However, it requires that the cursor is not placed inside a single entry field before pressing this button.

7.8.2 Show original

Shows the ”original” Matrix, before any changes were introduced.

7.9 Change background

You can toggle between a white and a black background on which the networks are drawn by clicking here.

7.10. Information

Pressing this button displays information on how to use the interface and provides additional information on the matrices used. Using the command line, the user can switch between several topics.

7.11. UNDO

This button allows you to undo made decisions or even long chains of decisions. Note, however, that the number of deleted genes that can be restored is limited.

7.12. List of mode toggling buttons

Here, three modes between which can partly also be chosen by actions described above, are displayed here in order to yield a better accessibility and facilitate fast switching between them.

7.12.1 Node connections: Click on buttons

If this button is toggled, all changes, including drawing connections, require an ACCEPT' button (in the dialog appearing after pressing the 'Create + connection', 'Create – connection', 'Delete Connection' or 'Modify connection' button) to be clicked. This option allows to define interaction strengths precisely in the first place.

7.12.2. Node connections: Clock on network

If this button is toggled, lines and arrows will be drawn automatically by clicking on the respective spheres or strings.

7.13. EXIT

Click this button to leave the network modifier or change the file you are reading in. The main network view will disappear and a sub-menu with several options. Basically, this allows you to choose a new project or input file, or to close NetworkMaker altogether.

Enjoy.