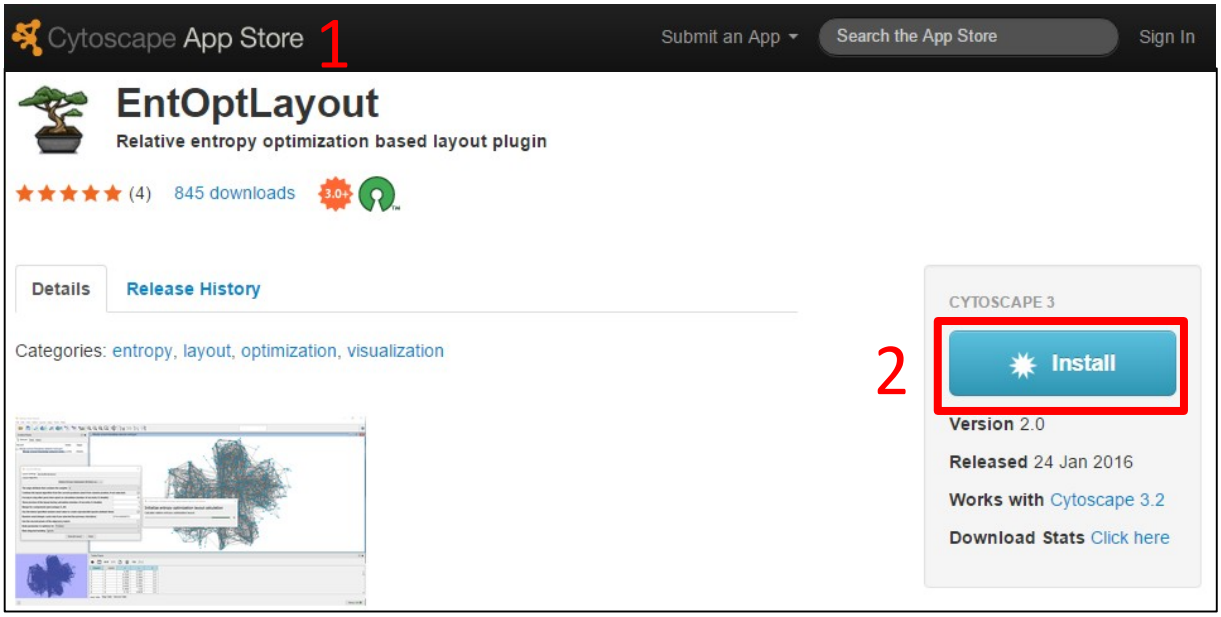
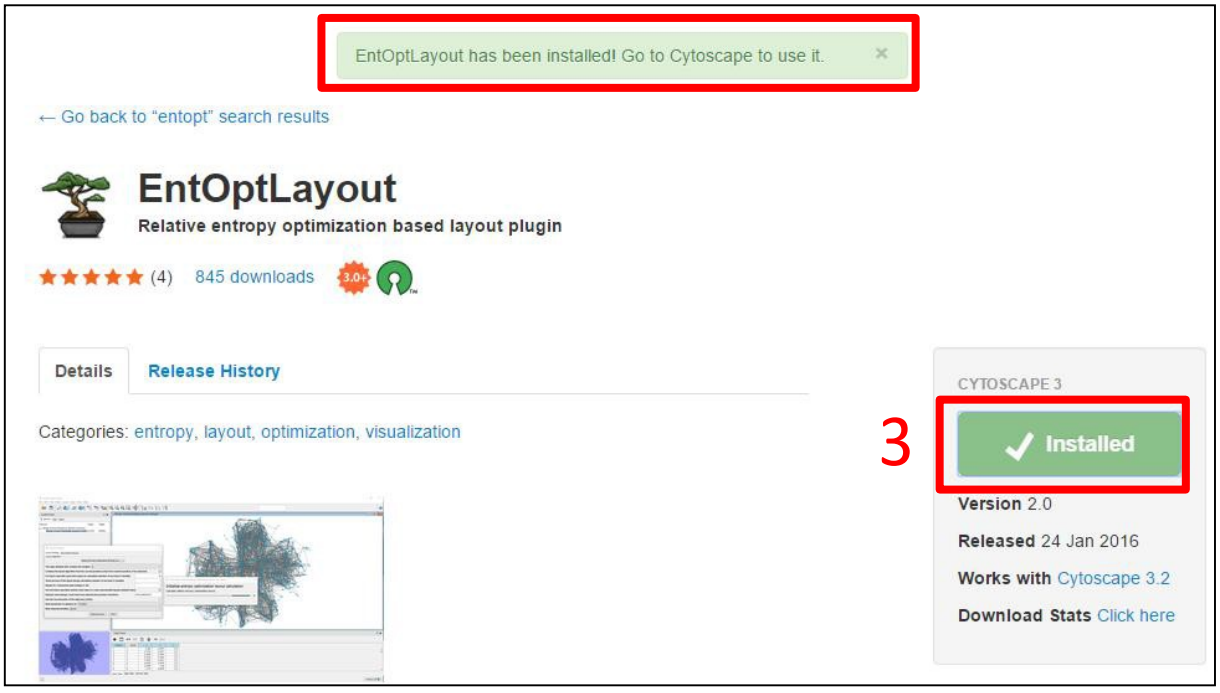
Tutorial

EntOptLayout - Cytoscape 3.7.1 plugin

I. How to install the plugin?

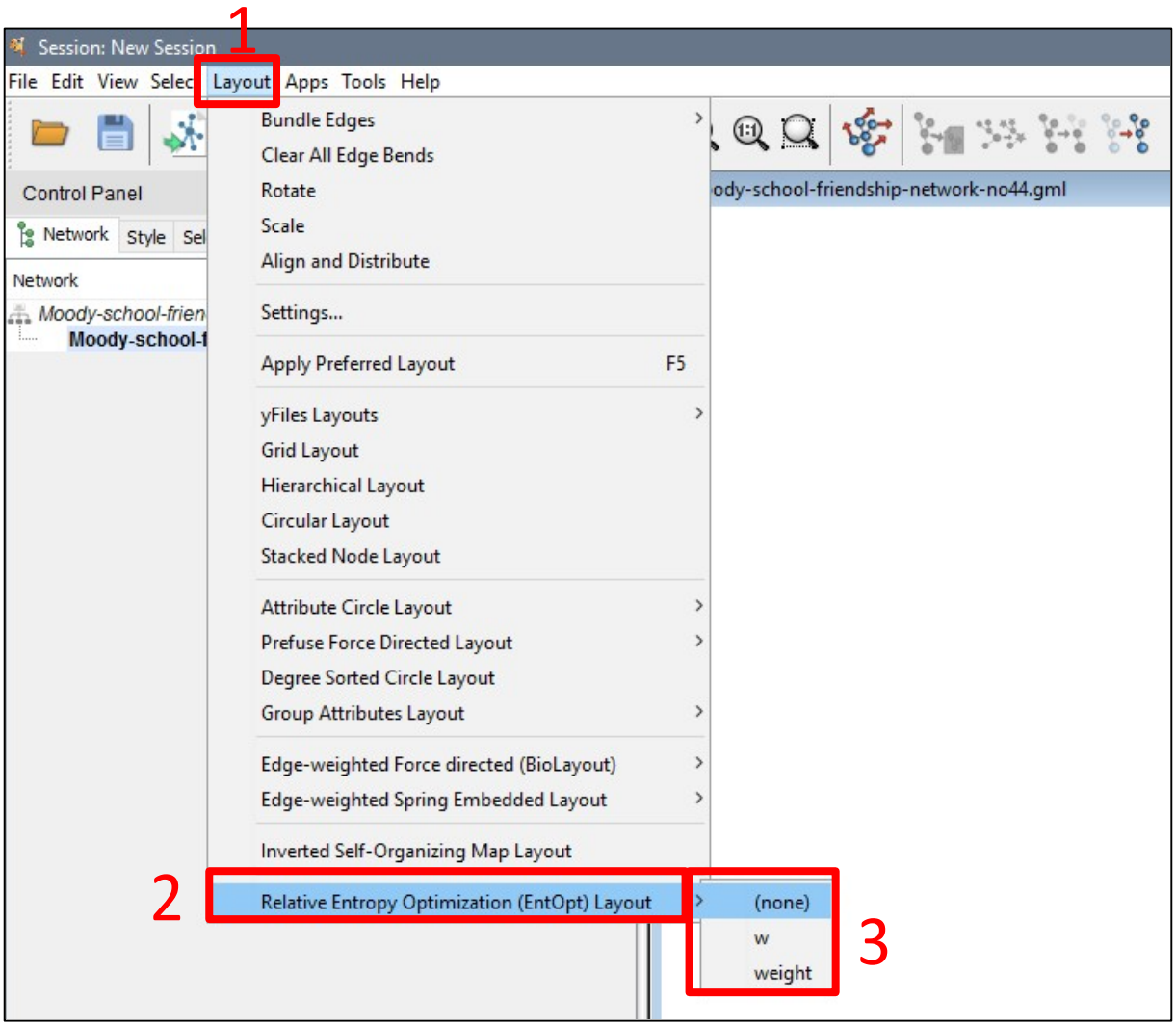
1. Visit the **Cytoscape App Store** at http://apps.cytoscape.org/apps/entoptlayout.
2. Install the EntOptLayout plugin by clicking on the **Install** button.
3. If the **Installed** button appears the installation was successful.





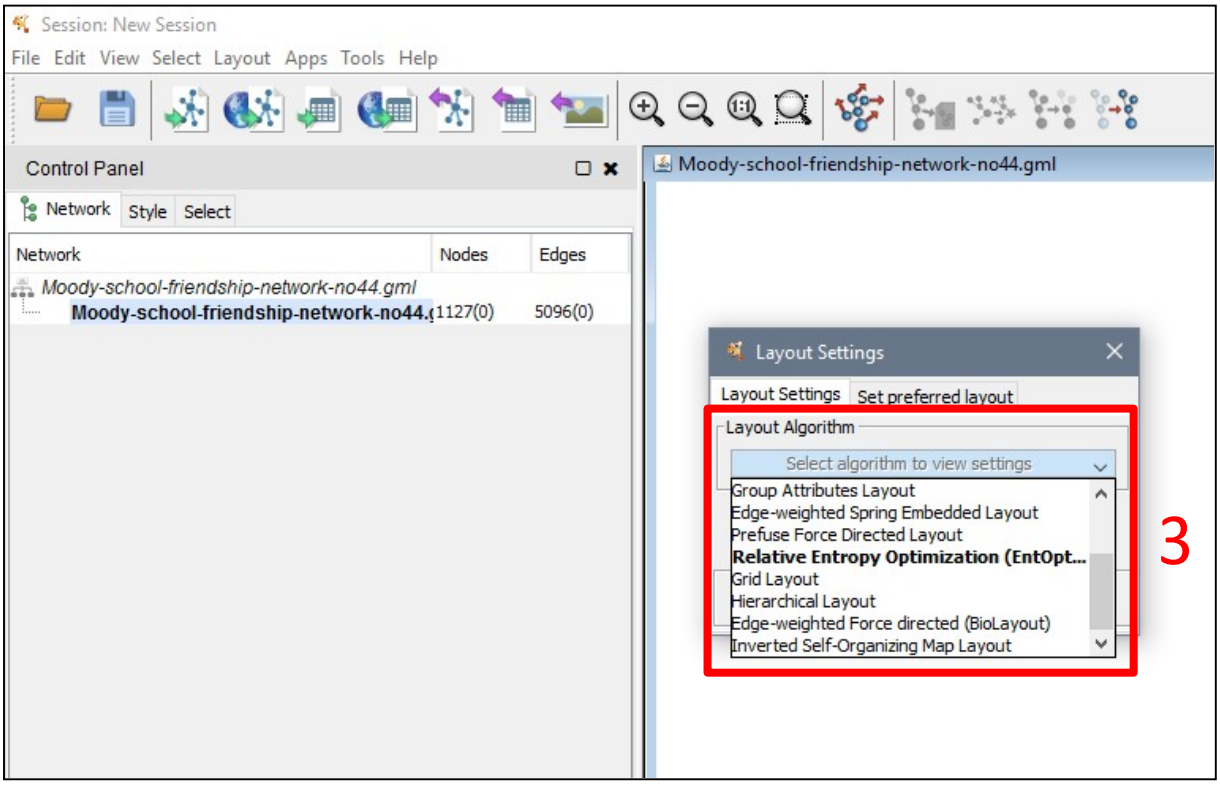
II. How to perform a quick EntOpt layout with default settings?

1. Click on the **Layout** button in the **Menu** bar.
2. Choose the **Relative Entropy Optimization (EntOpt) Layout** option.
3. If you would like to visualize a **weighted network** you can choose from the listed options which edge attribute to use as the weight during the layout. The **(none)** option means edge weights are not taken into account during the layout.



III. Where to change the settings of the plugin?

1. Click on the **Layout** button in the **Menu** bar.
2. Choose the **Settings…** option.
3. In the **Layout Settings** submenu select the **Relative Entropy Optimization (EntOpt) Layout** option.



IV. How to change the settings of the plugin?

1. **The edge attribute that contains the weights**
   If you would like to visualize a **weighted network** you can choose from the listed options which edge attribute to use as the weight during the layout. In this select box Cytoscape automatically lists all edge attributes available in the loaded network file that can be utilized as edge weight, i.e. edge attributes with an integer or floating point number type. Edge attribute names listed in this select box are also taken from the imported network file. For example in the EntOptLayout video tutorial "w" and "weight" edge attributes were available in this list, and as in this case both edge attribute contains the same weight values, the first "w" option was selected for the weighted network visualization. The **(none)** option means edge weights are not taken into account during the layout.

2. **Use the second power of the adjacency matrix**
   If checked, this advanced setting forces the layout algorithm to raise the adjacency matrix to the second power, and to use this squared matrix instead of the original adjacency matrix. This method helps to achieve a layout that emphasizes the features of the network related to mutual neighbors of the nodes.

3. **Initialize node positions with**
   With this option you can specify the way the EntOptLayout plugin determines the initial coordinates of nodes.
   - **EntOpt coordinates** – If selected, EntOptLayout initializes the positions with coordinates stored by the plugin internally. It is important to select this option, if you are planning to optimize for multiple node parameters in an alternating fashion, otherwise every optimization step would result in a scrambled layout because of the random initialization of node positions.
   - **Visible coordinates** – If selected, you can use EntOptLayout for fine-tuning of a network layout previously created by another algorithm, as this option allows for EntOptLayout to start the layout optimization with the visible node positions. **We suggest to start your network visualization with this option using the prefuse force-directed layout of Cytoscape as an initialization of the layout process, since some (up to 5-10%) nodes may become dissociated from their major network module, if random coordinates were used for initialization.** With this option you can also drag some nodes manually, and then use EntOptLayout to improve this manually modified layout. If you plan to perform multiple optimization steps for position, width or normalization, it is important to switch back to ***EntOpt coordinates*** (see above) after the first layout optimization step.
   - **Random coordinates** – This option forces EntOptLayout to start the layout optimization from random coordinates. So only select this option, if you would like to discard the calculated positions and start a new layout optimization process. If you plan to perform multiple optimization steps for position, width or normalization after the randomization of the coordinates, it is important to switch back to ***EntOpt coordinates*** (see above) after the first layout optimization step.

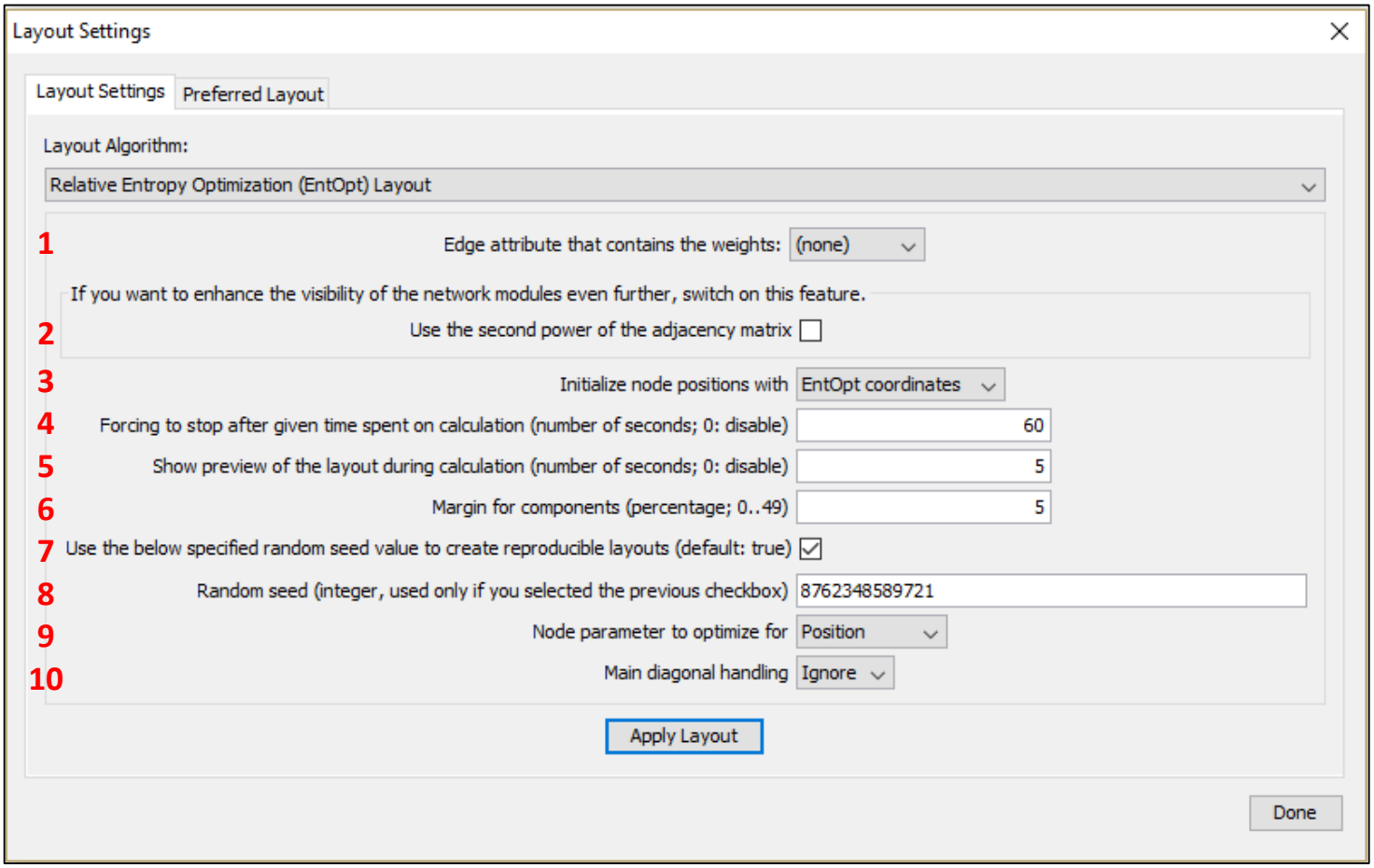
4. **Forcing to stop after given time spent on calculation (number of seconds; 0: disable)**
   This option allows you to run the network layout optimization for a limited amount of time. By default the plugin will only optimize for 60 seconds, but for bigger networks more time needed. If you would like to disable this limit simply write 0 to the box.

5. **Show preview of the layout during calculation (number of seconds)**
   The optimization is continuous, but the resulted layout can be showed only as a preview during the calculation before the final layout is ready, therefore it is suggested not to write 0 here, since it disables this function. Use this option to change the frequency of the preview update. By default the preview will be updated after 5 seconds.

6. **Margin of components (percentage; 0..49)**
   Using this option the dimensions of the space used for the layout can be optimized, which helps the better visibility adjusted to the size of the visualized network.

7. **Use the below specified random seed value to create reproducible layouts (default: true)**
   In order to create reproducible layouts you can define an integer in the **Random seed** box, where the optimization starts from. Using this option the layouts for the network will be the same after every independent run. By default this option is enabled, uncheck the box to disable.

8. **Random seed (integer, used only if you selected the previous checkbox)**
   Set an integer used as a random seed to perform reproducible layouts.

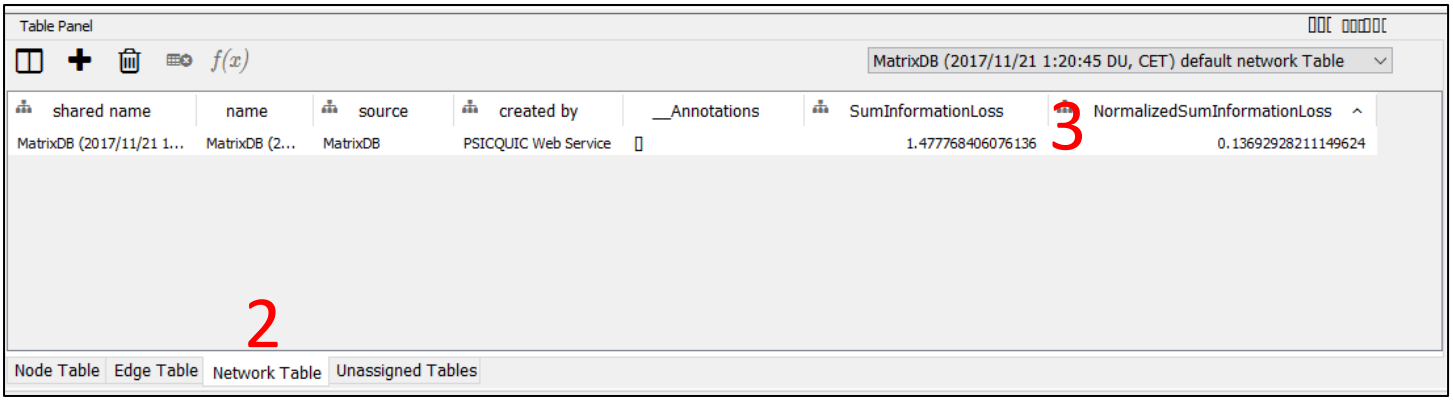
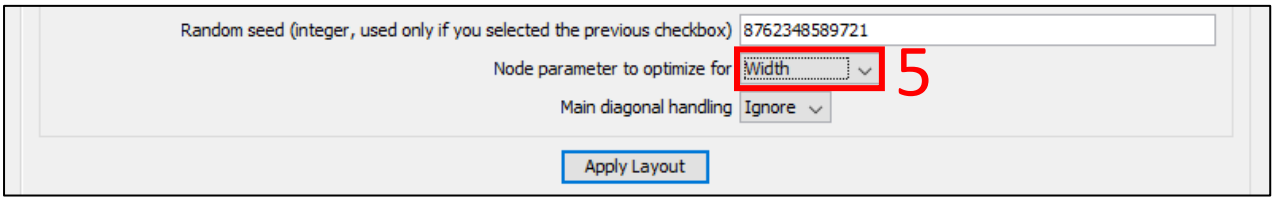


9. **Node parameter to optimize for**
   Alternating optimization for the listed node parameters, especially **position** and **width**, offers a network visualization with higher quality compared to the case when only optimization for position is applied. Although in some cases intermittent optimization for **normalization** may also improve the layout, it is generally not recommended.
   Optimization for **order** does not have an effect on the actual network layout, but it generates a table with nodes in the correct order, which appears under the unassigned tables tab marked by the name of the network. This table can be exported as a spreadsheet (e.g. CSV).

10. **Main diagonal handling**
    This setting determines the way entries in the main diagonal of the adjacency and overlap matrix are considered during calculations:
    - **Ignore** - Optimal if self-links are impossible or irrelevant in the network.
    - **Single** - Kept only for compatibility purposes.
    - **Double** - Optimal if self-links (being already doubled in the symmetric adjacency matrix, below and above the diagonal) are as relevant as the rest of the links in the network.

V. Suggested optimal use of EntOptLayout algorithm

Here we describe the optimal use of the EntOptLayout algorithm as suggested in the legend of Figure 1 of our paper Ágg et al.: "*The EntOptLayout Cytoscape plug-in for the efficient visualization of major protein complexes in protein-protein interaction and signalling networks*". First, the network is visualized by the Cytoscape prefuse force-directed layout option. The coordinates of this layout are used as an input to the EntOptLayout plug-in. For that purpose we first set the "Initialize node positions" option to "Visible coordinates". The square of the adjacency matrix function is switched on, the square of the diagonal function is ignored. Use the 5 percentage default margin for components value. (Similarly, leave the random seed at default.) With these settings consecutive optimization steps are performed for 10000 sec each for node position, node width, node position, node width and node position. Please pay attention to switch back the "Initialize node positions" option from the "Visible coordinates" value used at the very first node position optimization to "EntOpt coordinates" and keep this setting for all consecutive optimization steps. Such a procedure is usually enough to produce a relative entropy (D) measure which does not significantly decrease by using further optimization steps.

VI. How to check the quality of the network layout?

1. Perform a network layout with EntOptLayout as described above.
2. Click on the **Network** tab at the bottom of the Cytoscape window.
3. In the table that appears you can check the overall normalized relative entropy of the whole network (the so called D value) in the **NormalizedSumInformationLoss** column. This value measures the quality of the layout. The lower the relative entropy value the better the layout.
4. After performing further layout optimization steps with EntOptLayout you can follow the decrease in the value of **NormalizedSumInformationLoss**.
5. You may compare the information loss by EntOptLayout and other visualizations, if you open the Cytoscape file of your network containing the node coordinates determined by the other visualization method and you optimize it by EntOptLayout only for the node width.