STEP 5 AUTOMATIC COMPLEX SCORING

PyRy3D UCSF Chimera Extension can be used to evaluate particular arrangement of components, that a user set interactively using in UCSF Chimera window.
This feature lets to use **knowledge and/or intuition** to manually arrange the components inside an electron density map and then check how this particular arrangement will be scored by PyRy3D. The scoring can be performed for different sets of PyRy3D parameters.

We hope that a possibility to play around with structures and program parameters manually will help users to learn how the program assigns punctuations for particular complex features (such us fulfillment of distance restraints, collisions, density map filling etc) and to knowingly select values of configuration parameters to run successful modeling with our tool ☺

IMPORTANT! The most important aspect of complex structure characterization is to accurately describe all available information about a particular system. This is done by expressing available data as a scoring function which global minimum corresponds to native assembly structure.

In order to validate quality of generated structures in PyRy3D a scoring function was implemented which is defined as follows:

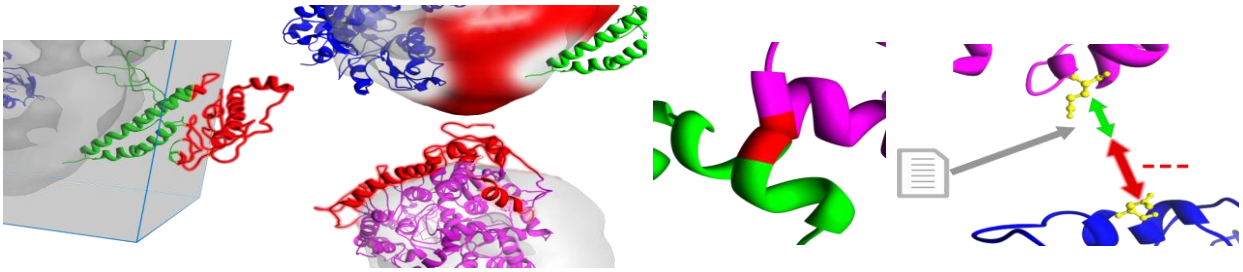
$$S = w_{\text{clashes}} * S_{\text{clashes}} + w_{\text{map\_filling}} * S_{\text{map\_filling}} + w_{\text{grid\_filling}} * S_{\text{grid\_filling}} + w_{\text{restraints}} * S_{\text{restraints}}$$

The scoring functions takes into account the following features of analyzed complexes:

- clashes between main chains atoms (S_{clashes}),

- occurrence of atoms outside the simulation area ($S_{grid\_filling}$),
- an electron density map filling ($S_{map\_filling}$),
- fulfillment of distance restraints defined by the user ($S_{restraints}$).

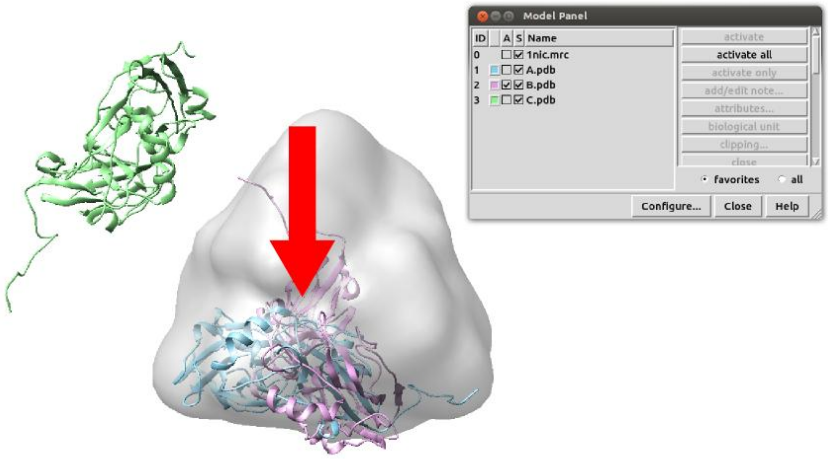
For each of these elements a user provides weights which corresponds to level of accuracy/reliability/importance for a particular feature.



ATTENTION. Please bear in mind that selection of values for simulation parameters (OUTBOX, MAP_FREESPACE/DENSITY, CLASHES, RESTRAINTS) is one of the most important steps in the modeling process. Choose values that describes your system most accurately → Playing with different values and testing how they work during complex scoring in PyRy3D Extension might be very helpful here ☺

1. Before evaluating the complex, you can quickly calculate the density threshold that corresponds to the volume of components loaded into Chimera's window. To do so, go to the **Sim/Score** tab in the Extension's window, and click **Calculate density threshold** button. After a brief moment, a small window will be displayed with the proposed threshold's value.

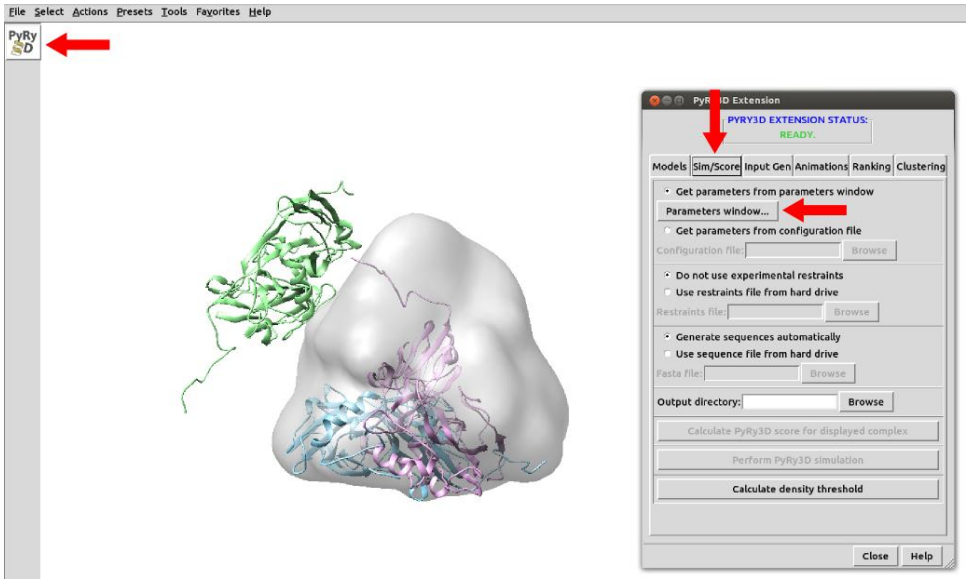
2. Use methods from **STEP 4 point 2.** to change the arrangement of components inside the density map. In order to test all the scoring function elements later, please try to place at least one of the components partially outside the density map, and at least two of the components collide (It means that some parts of two structures overlap - check components red and blue in the picture below). **ATTENTION!** After you position components make sure that all objects are **Active** and **Shown**.



An example of the components' arrangement, with blue and purple models colliding in the middle of the map, and the green model outside the map

3. When your arrangement is ready, you can choose values for PyRy3D program parameters. Open **PyRy3D Extension** Window → choose **Sim/Score** Bookmark (if it somehow disappeared just click with mouse on PyRy3D logo in the left panel of Chimera)

    - check the **Get parameters from parameters window** box
    - click **Parameters window...** button to open the parameters window.



You can learn the meaning of all the parameters here (http://genesilico.pl/pyry3d/index.php?option=com_content&view=article&id=52&Itemid=199#3.2).

Now select: **Evaluation/Ranking** mode on the top of the box.

!! In this particular task you will ***not*** use parameters describing simulation located in the following sections:

- Basic simulation parameters
- Mutations frequencies
- Output parameters
- Method dependent parameters

and also **Scale**, **Move_State**, **Cov_Bonds**. All these measures are specifically dedicated to Monte Carlo simulation, not simple scoring of a complex visualized on the screen. We will use them in further steps of the Tutorial.

However the sections:

- Scoring function weights
- Input data descriptors

should be changed according to your intuition ☺

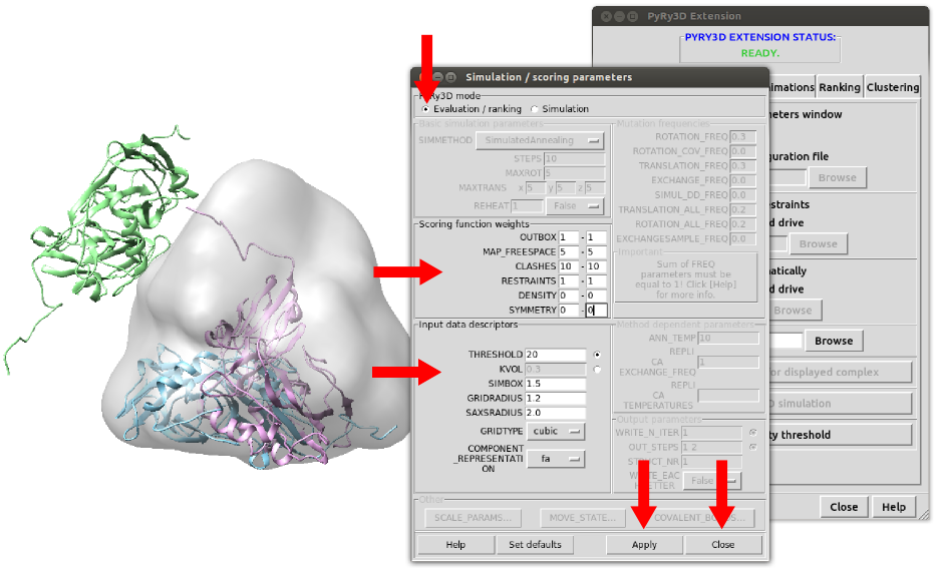
You can switch between sets of parameters used in single complex evaluation and simulations, by clicking radiobuttons on top of the configuration parameters window.

Our suggestions for the beginning are the following **Scoring function weights** values:

- **OUTBOX** 1 1,
- **MAP_FREESPACE** 5 5 (to put emphasis on docking components tight into a density map without leaving empty spaces)
- **CLASHES** 10 10 (to penalize clashes between atoms significantly)
- **RESTRAINTS** 1 1,
- **DENSITY** 0 0 (MAP_FREESPACE will score filling),
- **SYMMETRY** 0 0

And from the **Input data descriptors section**:

- **THRESHOLD** parameter to **20** (this value corresponds to a complex volume),
- **SIMBOX** into 1.2 (to build simulation area not much bigger than a density map)
- **GRIDRADIUS** to 1.6 [Å] (to allow accurate scoring)
- **COMPONENT_REPRESENTATION** to fa (full atom, all complex atoms will be considered).



After selection of your values please press **Apply → Close**

** You can also prepare configuration text file with parameters outside of Chimera. Just load it by **Get parameters from configuration file** option button and then click **Browse** to find the file on your disk.

4. Check the **Use restraints file from hard drive** option and click **Browse** to choose the file containing restraints (**1nic.restr.txt**). This particular file is used to provide data

about interactions between complex components e.g. from chemical cross-linking or two-hybrid system experiments. In the picture below we put an example of restraints where for each pair of chains a distance between residues 212 is smaller or equal to 11 Å (symmetry).

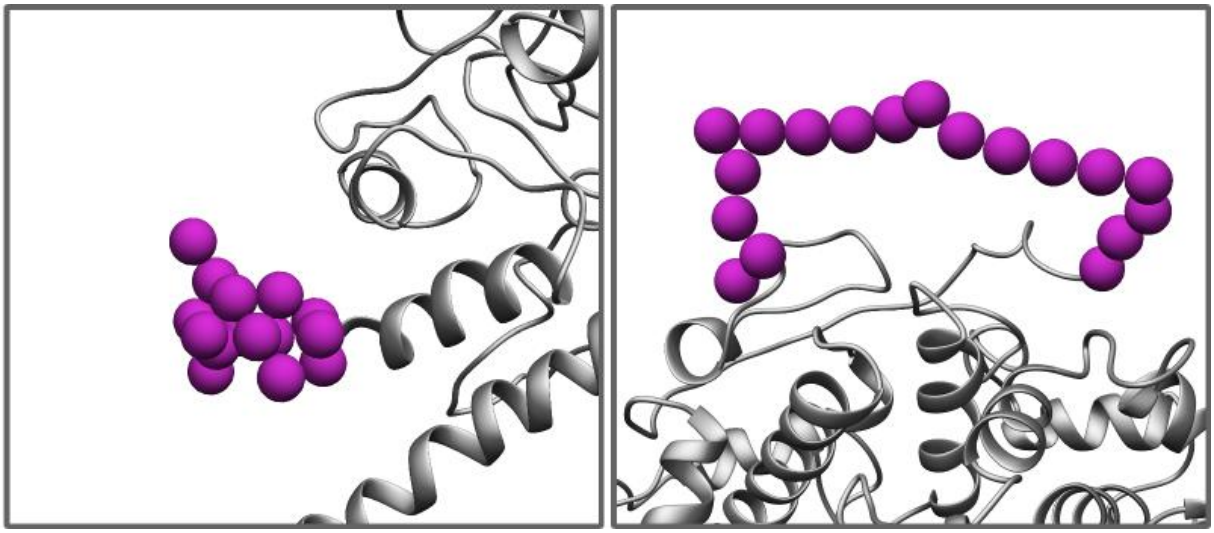
```
// 1NIC
dist (
                    (212) "A" -(212) "B" (<=11.0)
                    (212) "A"-(212) "C" (<=11.0)
                    (212) "B"-(212) "C" (<=11.0)

)
```

ATTENTION: PyRy3D uses Filtrest3D format to describe interactions between complex components. The file can be prepared automatically by the filtrest3D server: http://filtrest3d.genesilico.pl/filtrest3d/index.html

5. By default, a file containing sequences is generated automatically, based on PDB files (this means that sequences are identical as in structures).

   However if pdb structures miss some coordinates, PyRy3D can add them into structures and treat as flexible fragments with changeable conformation (check the image below)



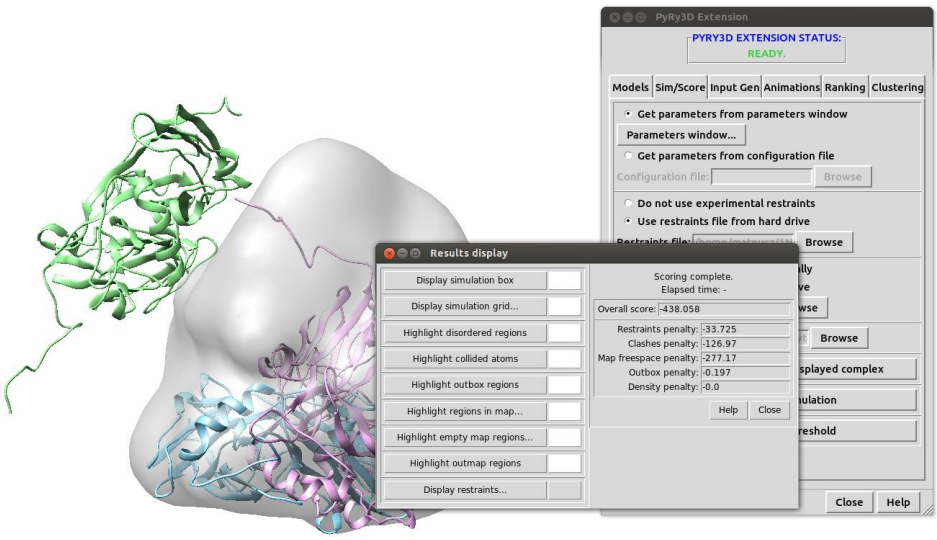
PyRy3D recognizes disordered/missing fragments by comparison of sequences from .PDB and .FASTA file for a particular component. Open **1nic_dd.fasta** where we added extra **'hhhhh'** at the beginning and the end of the "A" sequence to encode disordered residues with

missing 3D coordinates. Save changes and use "**Use sequences from the hard drive**" to upload data into the Extension.

6. To indicate where PyRy3D results will be stored on your hard drive please use **Output directory** field.

ATTENTION!! As an output directory please do not choose a folder where you store important data since all the files from this location will be deleted. We encourage to create a new **EMPTY** folder eg. **single_scoring/**.

7. To start the evaluation, click **Calculate PyRy3D score for displayed complex**. The evaluation might take up to several minutes (depends on your computer's power and the complexity of the system you're evaluating). When it's finished, a window containing the overall score and it's elements will be displayed. Learn more about PyRy3D score here (http://genesilico.pl/pyry3d/concepts/#complex-scoring-function/).



8. In **Output directory** you selected in **point 5** you will find a .pdb file with complex structure you have created by manipulations on the screen and a **pyry.log** file with a list of PyRy3D parameters used and their values.