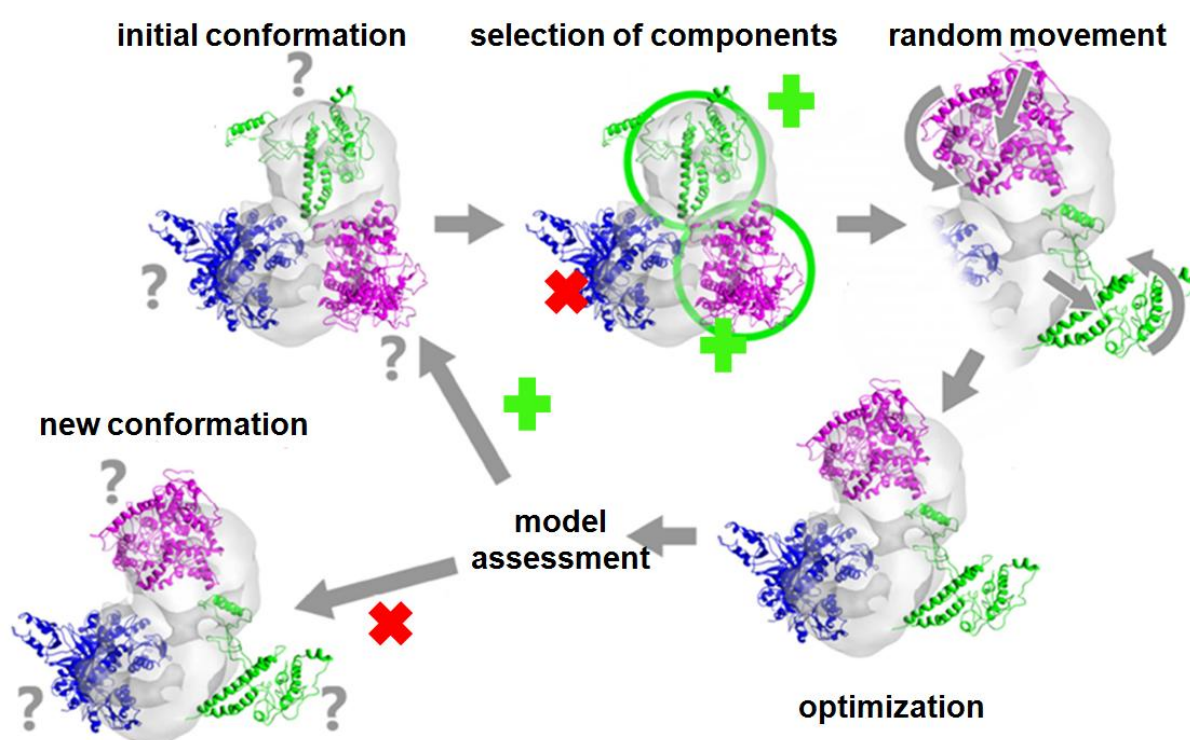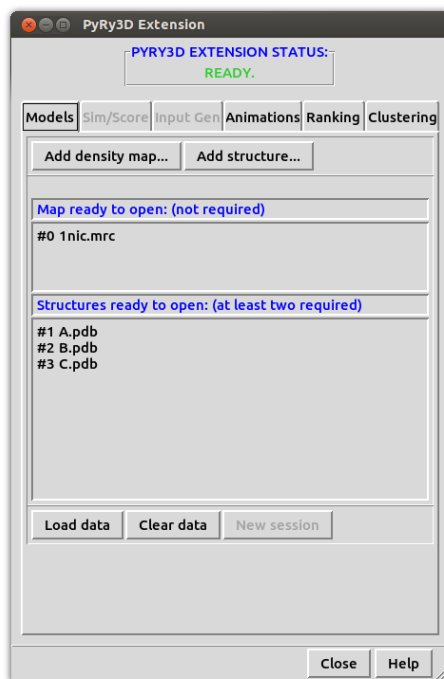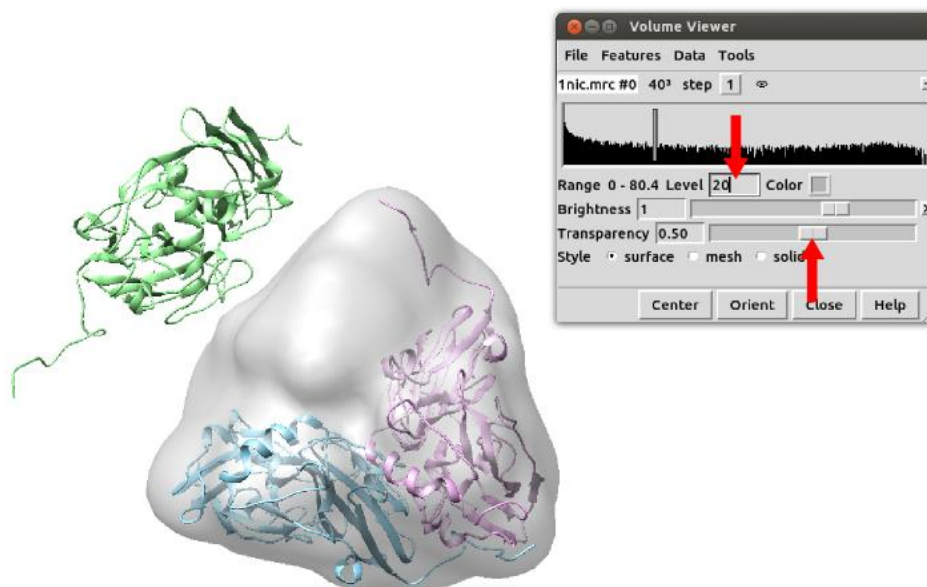## STEP 7 RUNNING PYRY3D SIMMULATION

The main purpose of PyRy3D software is to build models based on data provided by the user such as structures, electron density maps or restraints. To perform this task a program applies **Monte Carlo** simulations in order to find such mutual arrangements of components inside a density map that fulfill restraints. The simulation require to sample large number of solution and find one that fits best to data provided by the user.

1. Please close the Chimera and load a density map (**1nic.mrc**) and structures (**A.pdb, B.pdb, C.pdb**) of components again via PyRy3D Extension.
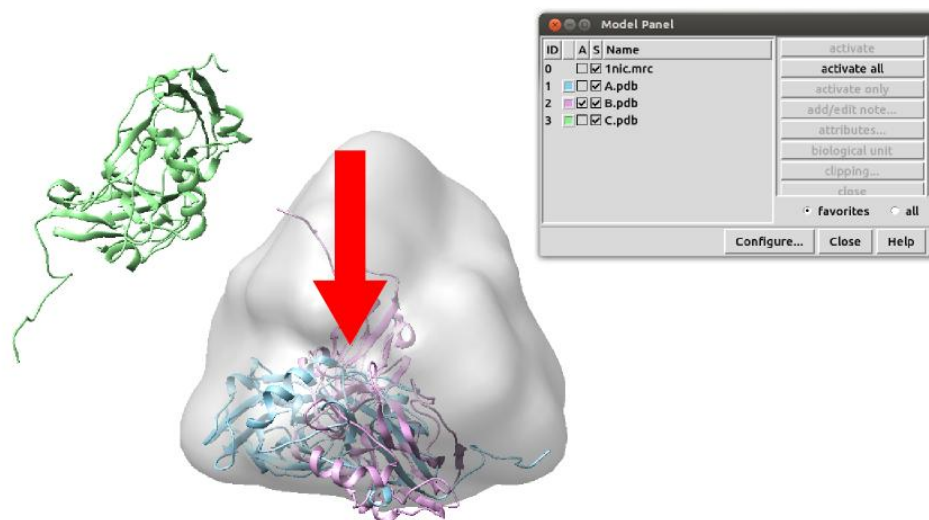
2. Choose values of a density map: **Level** 20, **Brightness** 10 and **Transparency** 0.50 in **Volume Viewer** Window.
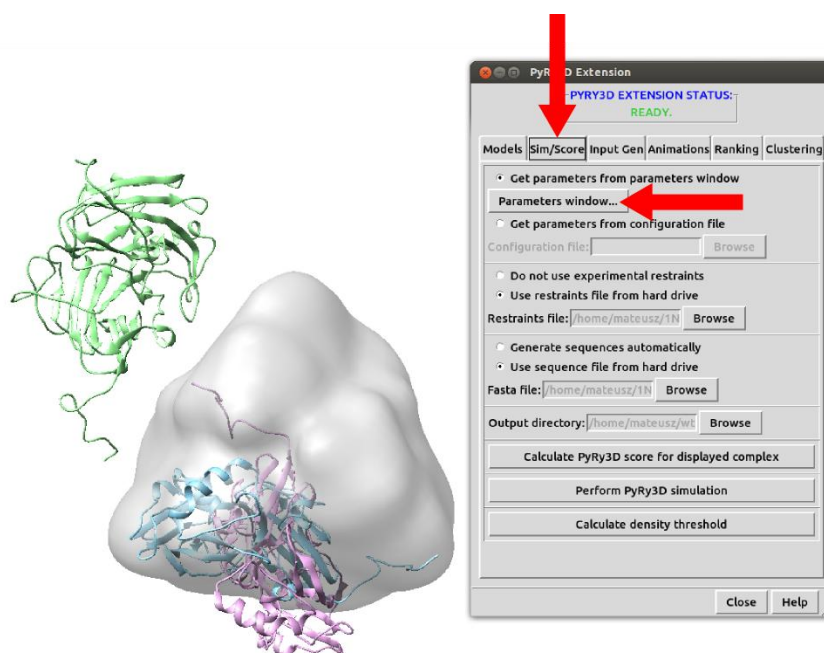

3. Now please arrange components in such a way that the fit of components into a cryo-EM map is bad e.g. put one component outside of the density map and place two other in the center of the map, heavily colliding with each other (Use **Model Panel** →

Action to manipulate with mobility of components). When you put all components in desired orientation go back to **Model Panel** and select all objects as **Shown** and **Active.**

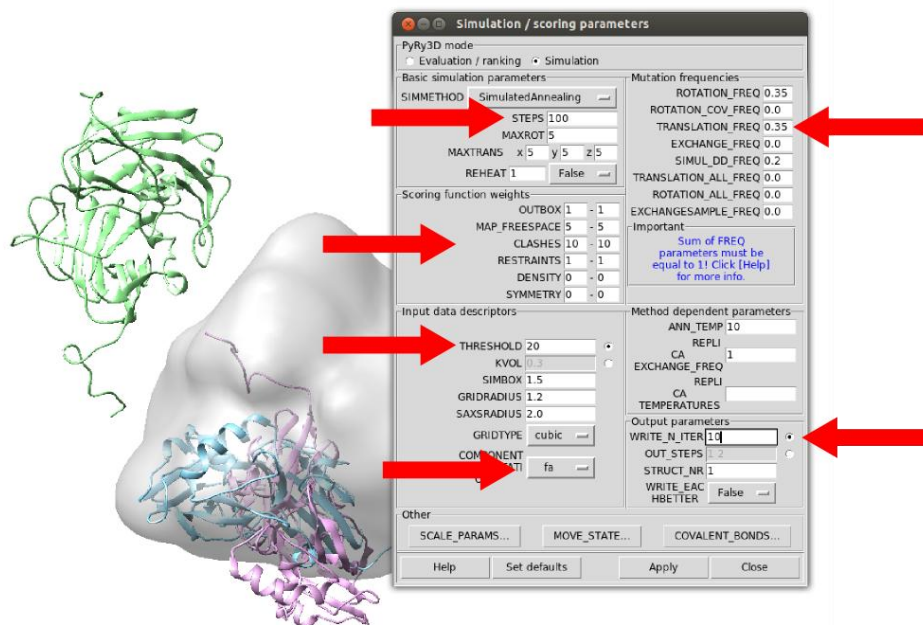!!! The point of this is step is to create many clashes (collisions between components), which will be hopefully eliminated during PyRy3D simulation.

4. Having STARTING ORIENTATION chosen and visualized in Chimera we can start selection of PyRy3D parameters for simulation (complex modeling process).

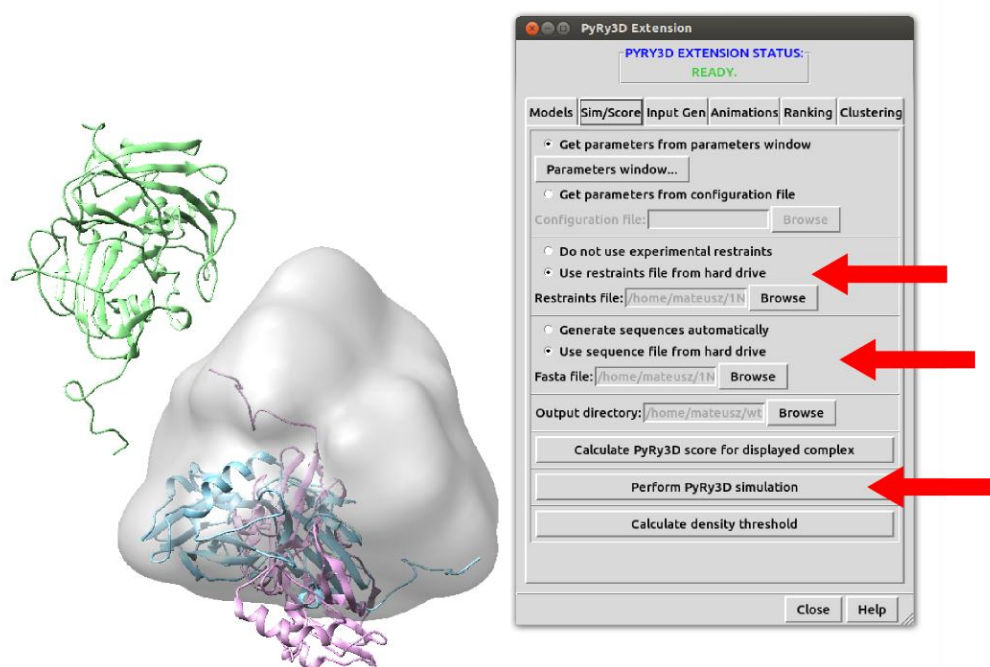   a. Go to **Sim/Score** Bookmark in **PyRy3D Extension** Window and choose **Parameters Window**

For the beginning we suggest to change the following parameters mentioned in the table and shown in the following figure:

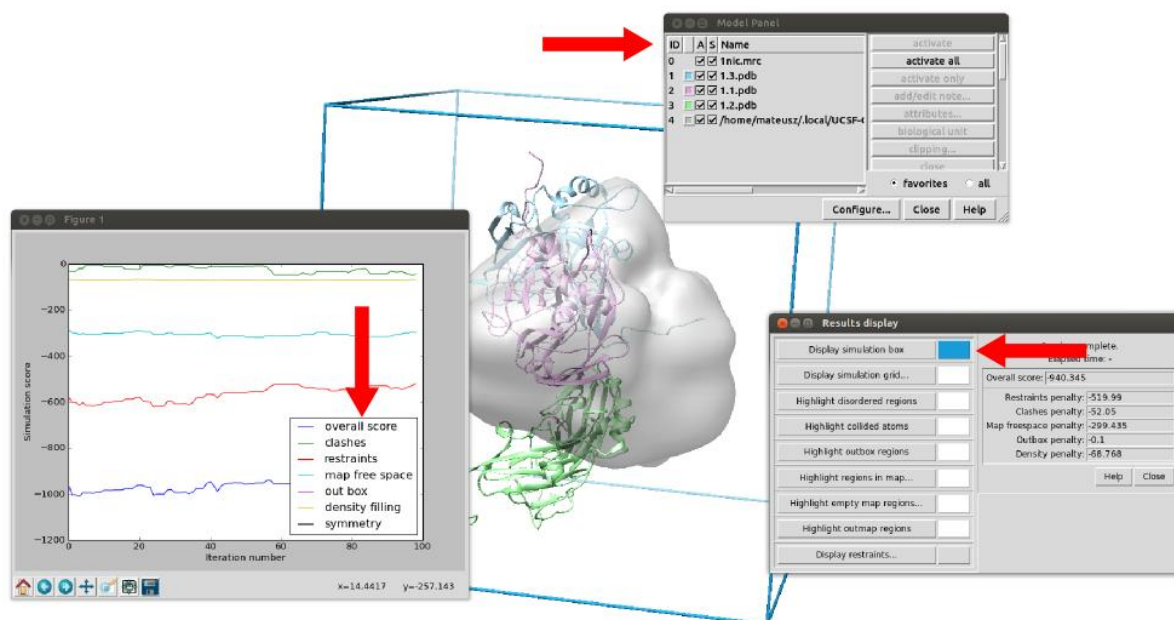| Parameter name	New value	Why we do this?
STEPS	**100**	To increase number of simulation step
MAP_FREESPACE	**5 5**	Increase weights for density map filling quality
CLASHES	**10 10**	Increase weights for clashes between components
DENSITY	**0 0**	Use 0, since we choose MAP_FREESPACE
THRESHOLD	**20**	Select 20, as this density threshold corresponds to complex volume; the same will you get by selecting KVOL 1.0
COMPONENT_REPRESENTATION	**fa**	Let's calculate a model in full atom representation (not reduced)
ROTATION_FREQ	**0.35**	Rotations will occur with 0.35 frequency
TRANSLATION_FREQ	**0.35**	Translations will occur with 0.35 frequency
SIMUL_DD_FREQ	**0.2**	Conformational change of disordered regions will occur with 0.1 frequency

5. After having all parameters values established click **Apply → Close**

6. Now please load files with restraints (**1nic.restr.txt**) and sequences (**1nic_dd.fasta**) again

7. Click the **Perform PyRy3D simulation** button.

ATTENTION! The simulation might take even a very long time (depending on your machine's computational power). **It is not recommended to perform long simulations (>1000 steps) using the PyRy3D UCSF Chimera Extension since it might slow down your station for long hours**. The command-line and server PyRy3D maximize the effectiveness of the simulations, so long simulations should be run via command line tool outside the Chimera viewer.

8. After the simulation is finished, the complex with the best score is opened automatically (previous files are closed). New structures will load as new objects (see **Model Panel**).

9. Obtained model can be analyzed the same way as in **STEP6** with possibility do display complex features and scores assigned for particular features. Please repeat procedures from **STEP6** and check how scores and quality of fit changed.

10. Another window that pops up after simulation is ready is a **Plot** (Figure 1) where you can see how score (and its elements) change during complex formation.
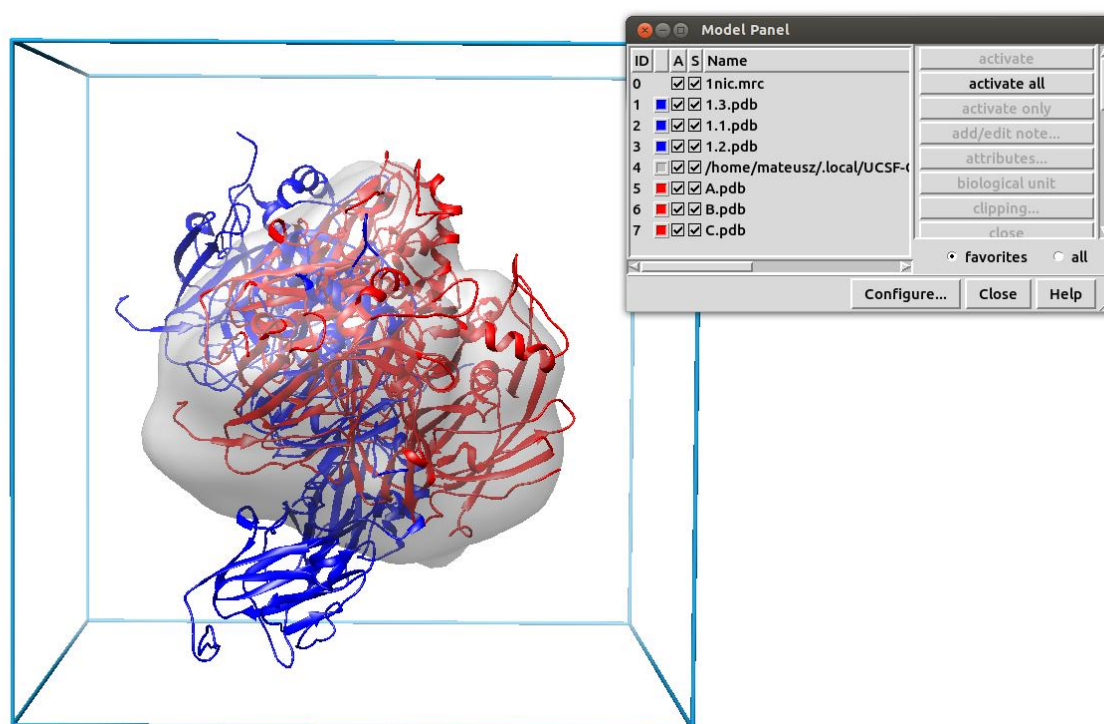

11. Go to the **Output Folder** (the one you selected to store results of simulation) to check how many files with structures have been created. Since we chose to write each 10th model there should be 10 files. Also to check details of simulation you can read a **pyry.log** file where all parameters are listed and also for saved structures their detailed scores are given.

STEP 8 COMPARISON OF OBTAINED MODEL AND CRYSTAL STRUCTURE AND EVALUETION OF THE MODEL

Crucial step in structure prediction is model evaluation, each model should be verified, without any evaluation it is unreliable and useless!!
In case of 1NIC complex a crystal structure is known and model obtained in **STEP 7** can be compared to native eg. by measuring RMSD. For regular modeling (were native structure is unknown) CCC value can be used to check a quality of fitness between a model and a density map (**Tools → Volume Data → Fit in map**)

1. To check how close our models were to the native structure, just open **1NIC.pdb** using Chimera's standard File / Open option.

2. As you can see, the obtained model is far from ideal, but we definitely managed to get rid of many collisions in just 100 simulation steps. Better results can be obtained by increasing the number of simulation steps (REMEMBER to use the command line version to run long simulations).