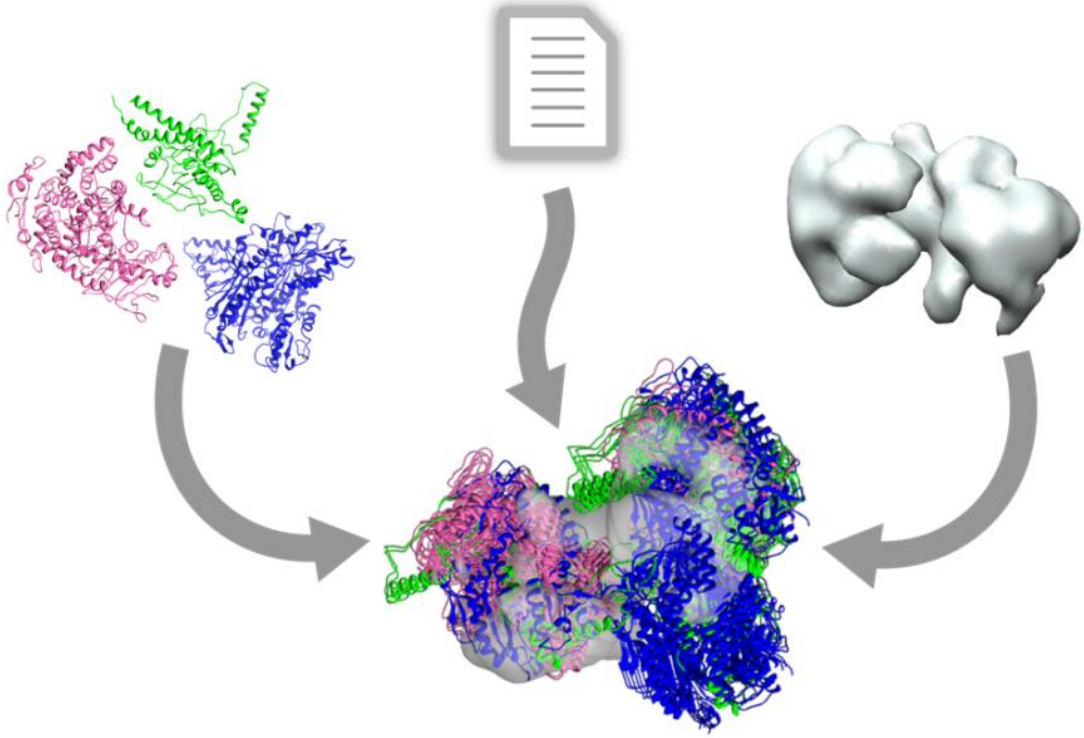
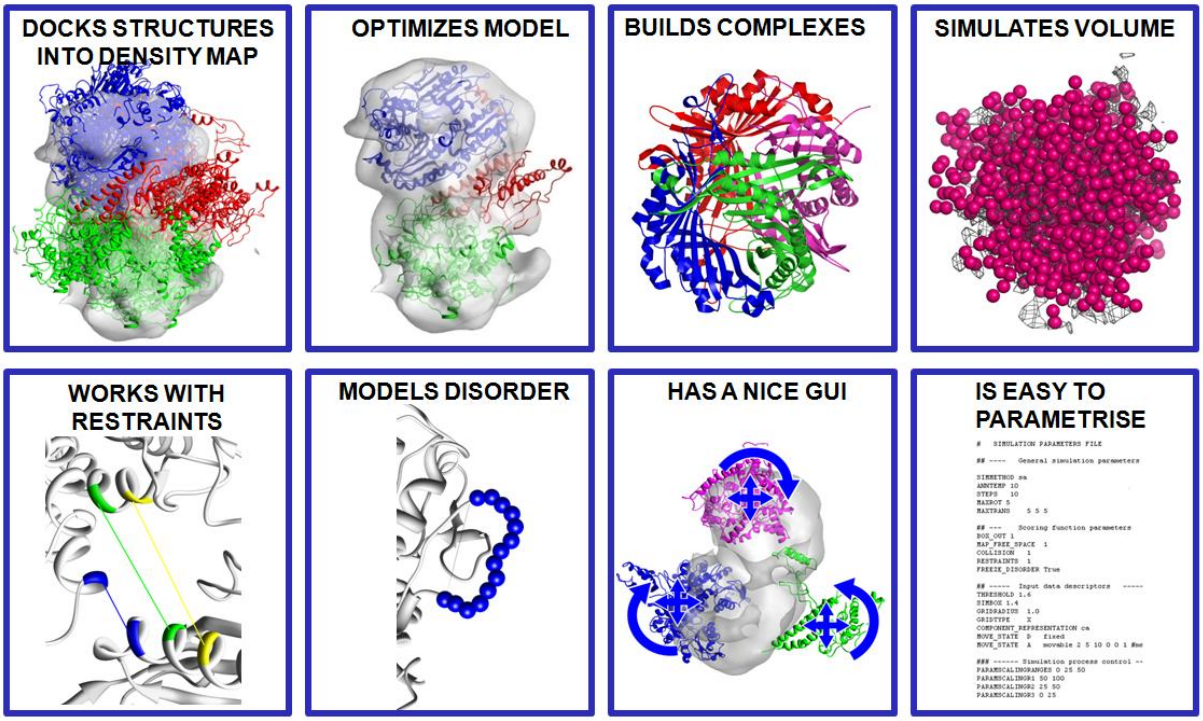
MODELING OF STRUCTURES FOR LARGE MACROMOLECULAR COMPLEXES

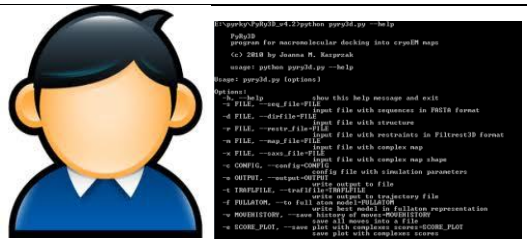
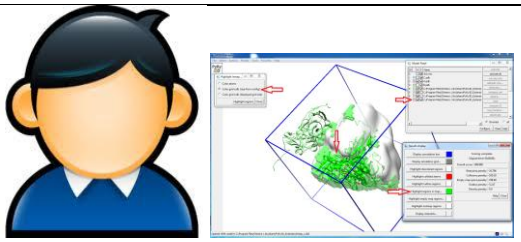


PyRy3D is a method for building low-resolution models of large macromolecular complexes. The components (proteins, nucleic acids and any other type of physical objects including e.g. solid surfaces) can be represented as rigid bodies (e.g. based on atomic coordinates of structures determined experimentally or modeled computationally) or as flexible shapes (e.g. for parts, whose structure is dynamic or unknown). The model building procedure applies a Monte Carlo approach to sample the space of solutions. Spatial restraints are used to define components interacting with each other, and a simple scoring function is applied to pack them tightly into contours of the entire complex (e.g. cryoEM density maps). This approach enables the construction of low-resolution models even for very large macromolecular complexes with components of unknown 3D structure.

Main features of PyRy3D program:



Aim of this tutorial is to show features of PyRy3D program and its UCSF Chimera Extension (which is a Graphical User Interface for the command-line PyRy3D application)

PyRy3D command-line (engine)	**PyRy3D Extension (GUI)**
	
Python command line	UCSF Chimera Viewer
Faster	Slower since it works inside the viewer
Scriptable	Limited to the features offered by GUI

Does not have any graphical features, performs modeling only	Allows to visualize and cluster results, prepare data, record movies

During the workshop you will have a possibility to build structural models for Cu-nitrite reductase from *Achromobacter Cycloclastes* complex (PDB id 1NIC)

Files for tutorial can be found:

- You can find all files for the tutorial on ftp: **ftp://ftp.genesilico.pl/pub/software/pyry3d/IIMCB_tutorial/**

STEP 0 FIRST RUN OF PyRy3D PROGRAM

To run simple docking of structures into a density map with a use of restraints dataset you will need:

- Structures of components stored in one common archive (**.tar**). Each component should be saved in a single PDB file (**1nic.tar**)
- Sequences of all complex components in Multi-FASTA file. Each sequence should be called the same as its chain identifier in the corresponding PDB file e.g. >A, (**1nic.fasta**)
- Electron density map – you can simply download it from the dedicated database: http://emdatabank.org/, (**1nic.mrc**)
- Restraints to define interactions between complex components (optional). Program takes files in filtrest3d format: http://filtrest3d.genesilico.pl/filtrest3d/help.html, (**1nic.restr.txt**)
- A configuration file – a simple text file where a user defines values for some program parameters (**config.txt**)

All results will be stored in "results" folder of your choice.

After the program finishes it work in "results" directory you will get: log file with information about modeling process and scores for complex structures generated by the program. Also a set of pdb files will be saved on disc with models coordinates. Each .pdb file is named according to the following rule:

```
Outname_score_iteration_temperature.pdb
```

Where:

Outname – is given by the user, in our case "results"

Score – punctuation assigned by the program, **the closer to 0, the better** ☺

Iteration – in which simulation step this particular structure was created

Temperature – simulation annealing temperature

STEP 1 INTRODUCTION TO UCSF CHIMERA EXTENSION – PYRY3D GUI

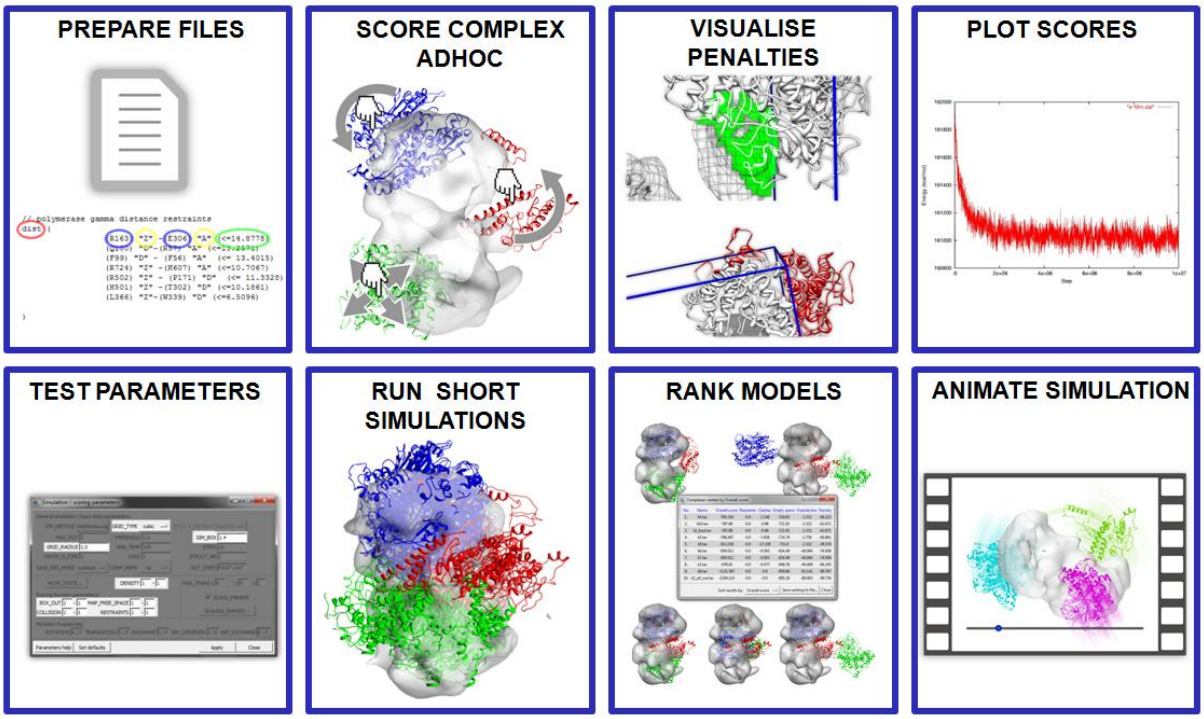
Since many researches do not like to use command-line applications, we developed a GUI available at http://genesilico.pl/pyry3d/pyryextension/ called **UCSF Chimera Extension** to make our method easy to use by a research community.

The GUI allows users to familiarize with main PyRy3D program (a command line application) by using it via UCSF Chimera (http://www.cgl.ucsf.edu/chimera/) - a popular viewer for interactive visualization and analysis of molecular structures.

The main goal of developing a **UCSF Chimera Extension** was to provide many features designed to make the **understanding and interpretation** of PyRy3D results for users easy and **INTUITIVE**.

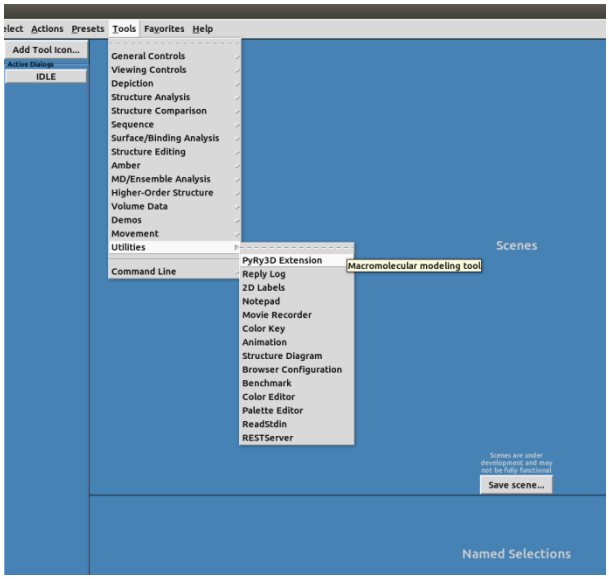
With PyRy3D GUI a user can:

- prepare input files ready for running command-line PyRy3D,
- visualize how the program assess a quality of complexes visualized on the screen,
- set up simulation parameters that accurately describe a particular complex,
- create a ranking of complexes according to PyRy3D scoring function,
- record movies from simulations,
- draw score plots,
- run short simulations from UCSF Chimera window with chosen simulation parameters and observe model building process,
- and much more!

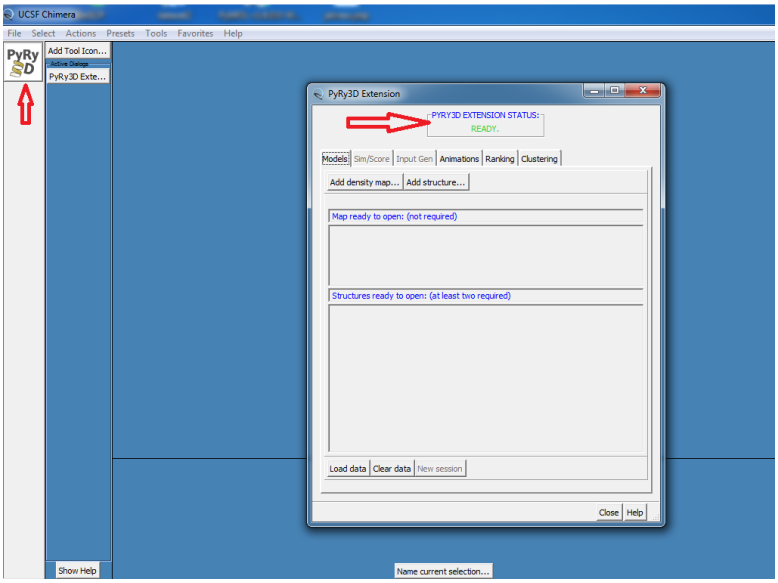


STEP 2 UCSF Chimera Extension RUNNING

1. Start UCSF Chimera. Go to the **Tools/Utilities** menu and click **PyRy3D Extension**.



2. Now you can start using PyRy3D UCSF Chimera Extension! PyRy3D logo will appear in the left panel and program window will pop up!

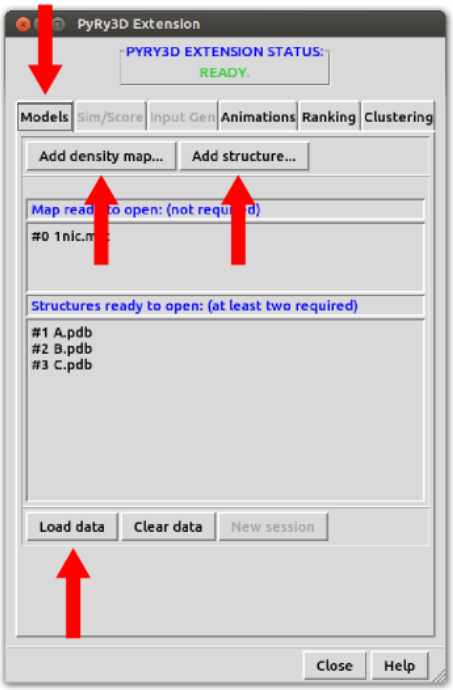


STEP 3 LOADING STRUCTURES AND ELECTRON DENSITY MAP INTO UCSF CHIMERA WITH GUI.

First step in working with PyRy3D Chimera Extension is to load complex data into UCSF Chimera. Bear in mind that loading all data for PyRy3D must be performed via **PyRy3D Extension** Window.

1. Load an electron density map (**1nic.mrc**) into the UCSF Chimera, by using **PyRy3D Extension** window and **Models** bookmark. Select **Add density map...** button and choose 1nic.mrc file.

2. To load the structures into Chimera, use the **Add structure…** button. You can select multiple files at once (with Shift button on **1nic/A.pdb, B.pdb and C.pdb**) and load them by clicking **Open**. Their names will be listed in the **Structures ready to open** field.

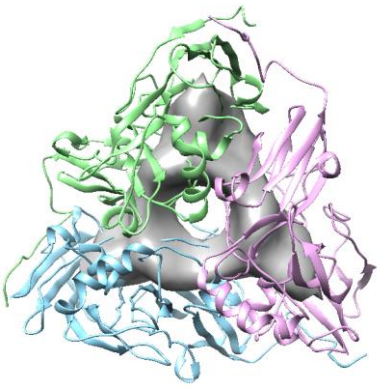


3. You can reset the lists by clicking **Clear data** button.

4. Make sure you have selected all the models you need and press **Load data** to load them into the viewer.

ATTENTION! Please bear in mind that all input files (map and structures) have to be loaded at this step. Later it will not be possible to delete or add any components into the viewer via Extension.

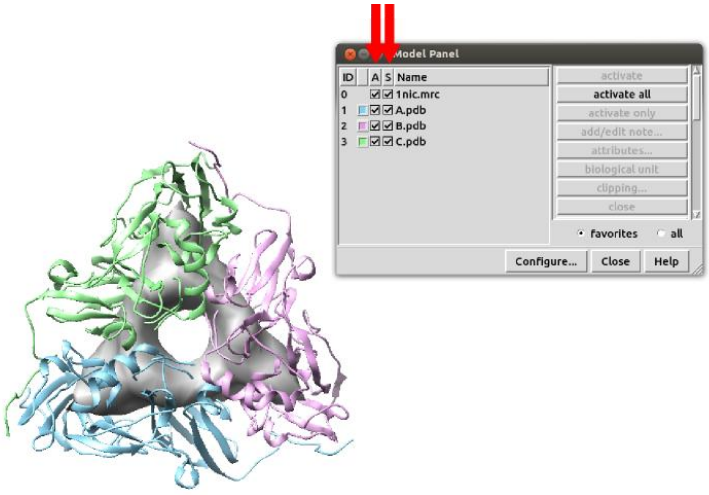
5. After **Loading data** into UCSF Chimera you should see a complex as the one in the following Figure:



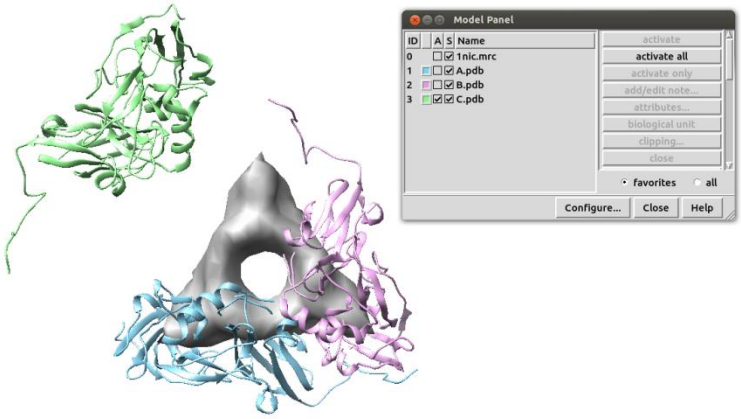
STEP 4 DISPLAY OPTIONS AVAILABLE IN UCSF CHIMERA

In this part of the Tutorial you will learn how to manipulate objects loaded into the UCSF Chimera viewer (like changing their location, selecting models, manipulating electron density map values). However we will focus only on features useful for working with PyRy3D Extension.
If you would like to learn more about UCSF Chimera, we encourage you to take a look at the official viewer tutorial http://www.cgl.ucsf.edu/chimera/current/docs/UsersGuide/frametut.html.

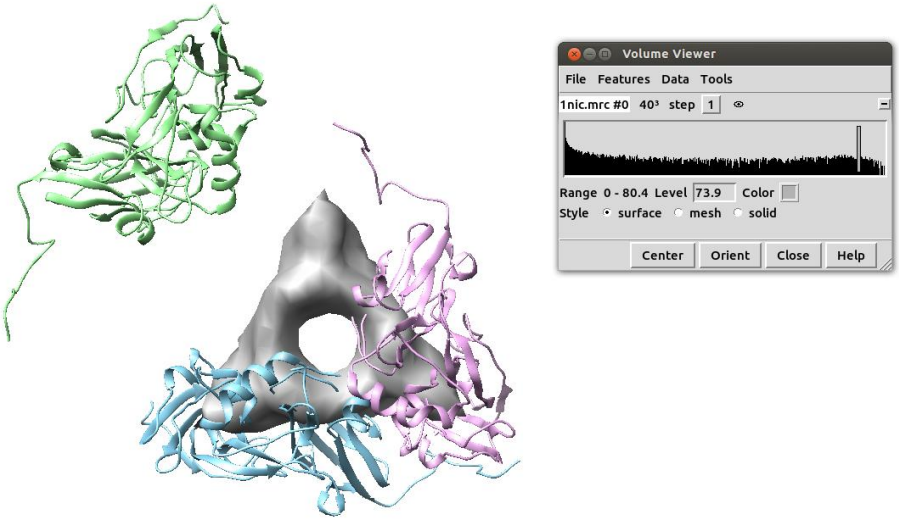
1. Open the **Model Panel** that is available in the **Favorites** menu. The table shows all the objects that are currently opened in Chimera. Un-ticking the field in the **Shown (S)** column, hides the particular object, but does not close it (you can always display it by ticking the field back). If an object is **Active (A)**, it means it will respond to the manipulations that you apply on the objects in the main window (in simple words, it means that you can rotate and move it around using your mouse). Deactivating an object freezes it until you activate it again.



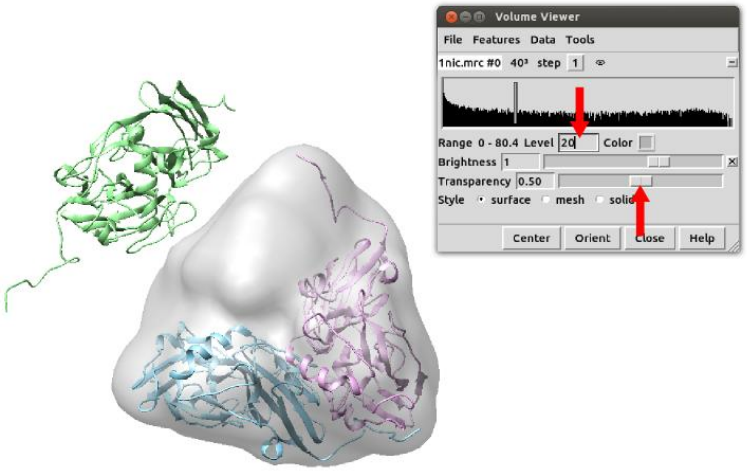
2. Now activate one of the structures (e.g. B.pdb as Active, deactivate A.pdb, C.pdb and 1nic.mrc) and move B.pdb around the main window. Holding down left mouse button and moving the mouse around rotates the model. Holding down right mouse button and moving it up and down, zooms the scene out and in. Holding down the middle button (mouse scroll) lets you move the structures along X and Y axis.



3. Now we can start manipulations with an electron density map. While loading files into Chimera a **Volume Window** should have popped up (If not please open it using **Tools →Volume Data → Volume Viewer**).



This tool enables users to change parameters for visualization of electron density maps. For example you can change map's volume by providing a density value in a **Level** field. To set accurate density value for this particular map please select **20**. Now a map on the screen should increase and become big enough to store all structures. Chimera also offers options to change density map brightness and transparency. Thanks to these features you can easily see what is located inside map object. Try to change the map by **Features → Brightness and Transparency** and use scroll bars to manipulate brightness and transparency values. We recommend to set **transparency** to **0.5**. You should now see the complex as the one in the following Figure:



IMPORTANT. The electron density threshold value that we provided in the **Load field** is the value suggested by the map's authors as the one that corresponds the best to the complex's volume (please check: http://www.ebi.ac.uk/pdbe/entry/EMD-1410/map). The threshold's value is one of the most important factors determining PyRy3D simulation's accuracy since it determines a shape and volume of the modeled complex. To high value will return a density map much larger than volume of the complex structure, with to low value it will not be possible to fit all components into the shape.