Model analysis using PyRy3D Chimera Extension

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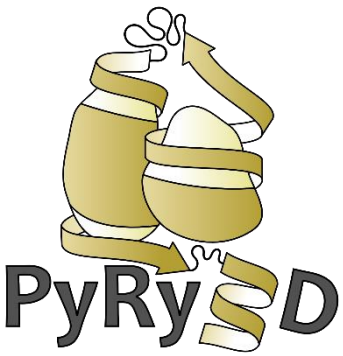
Coarse graining of biomolecules and beyond, Warsaw, 2017



In order to complete this workshop, you have to download and install UCSF Chimera and PyRy3D Chimera Extension. **PyRy3D Chimera Extension currently only works on Linux systems**.

You can get Chimera here:

https://www.cgl.ucsf.edu/chimera/download.html

You can get PyRy3D Chimera Extension here:

ftp://ftp.genesilico.pl/pub/software/pyry3d/PyRy3D_Chimera_Extension.zip

In order to install PyRy3D Chimera Extension, please follow the advice listed here:

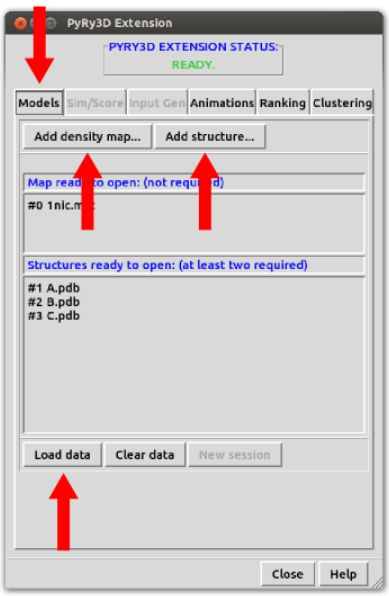
http://genesilico.pl/pyry3d/installing#gui-installation

In this tutorial, you'll have a chance to analyze a model of a protein complex using a single-model evaluation feature offered by PyRy3D Chimera Extension.

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 the 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.

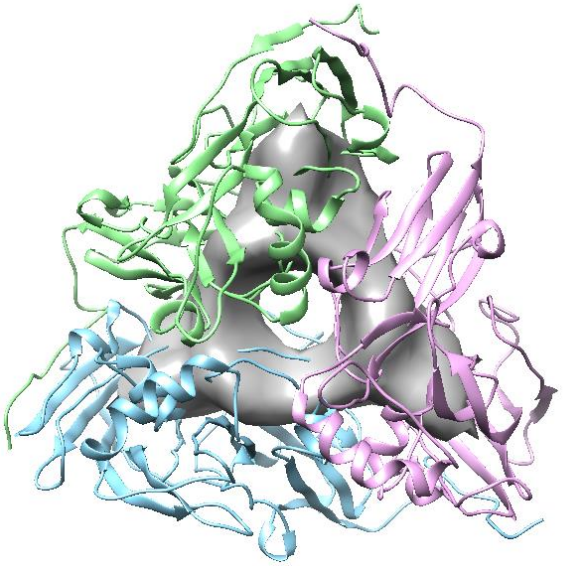


3. You can reset the lists by clicking **Clear data** button.

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

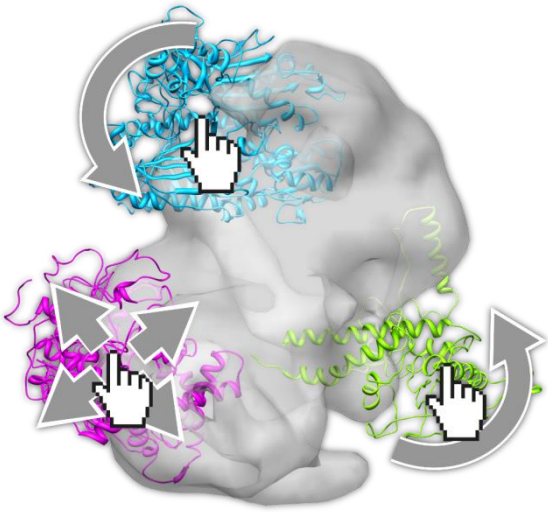
ATTENTION! Please keep in mind that all input files (map and structures) have to be loaded at this step. Later it will not be possible to delete or add any components into the viewer via Extension.

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

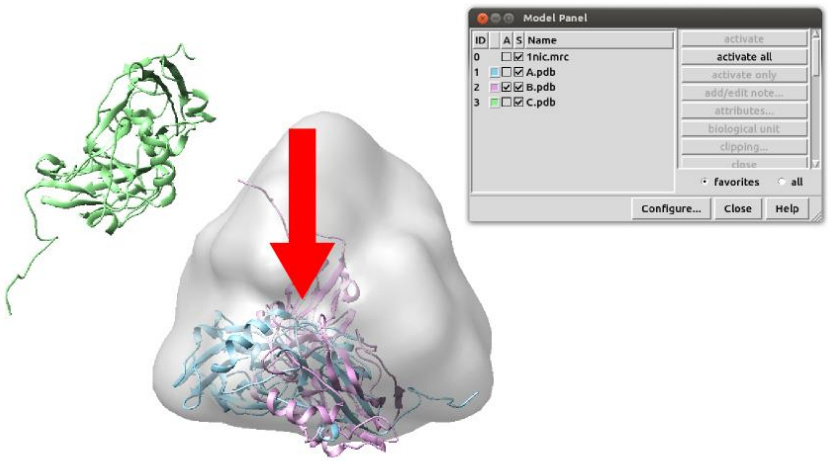


PyRy3D UCSF Chimera Extension can be used to evaluate particular arrangement of components, that a user set interactively using in UCSF Chimera window.

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 is scored by PyRy3D. The scoring can be performed for different sets of PyRy3D parameters.

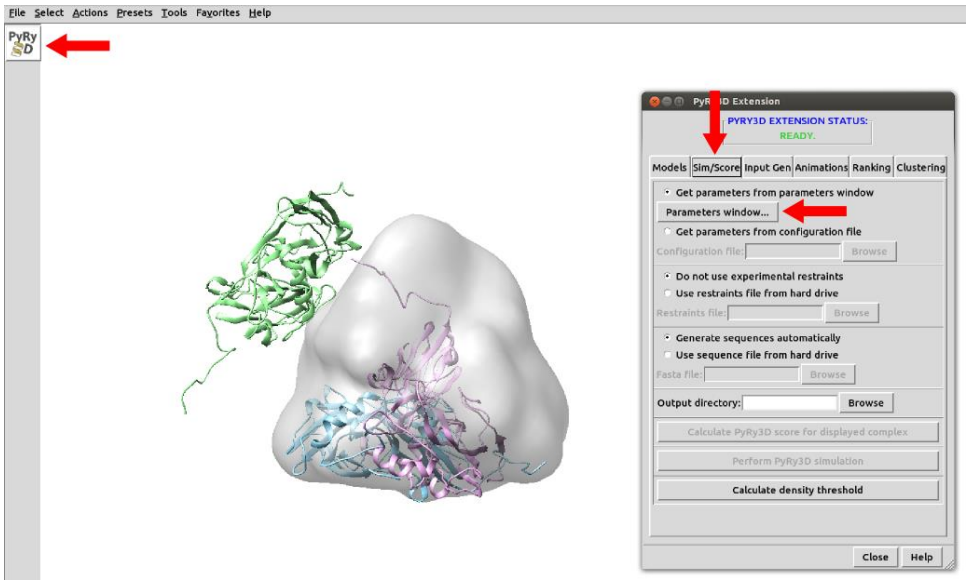


1. Use Chimera's manipulation tools to change the arrangement of components inside the density map. By default, it includes rotating active structures using the left mouse button, translating them using the scroll wheel, and zooming in/out using the right button (to select active / inactive models use "**Model Panel**" available from "Favorites" menu). 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 make at least two of the components collide. It means that some parts of two structures will overlap (check components pink and blue in the picture below).



2. 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.

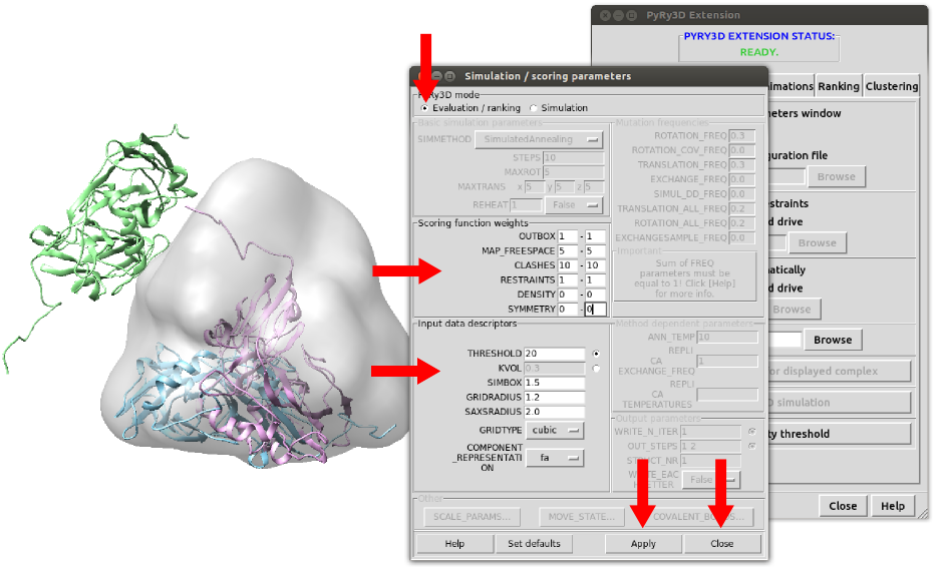


Our suggestions for the beginning are the following **Scoring function weights** values:

- **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 the complex volume),
- **SIMBOX** to 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**

3. 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).

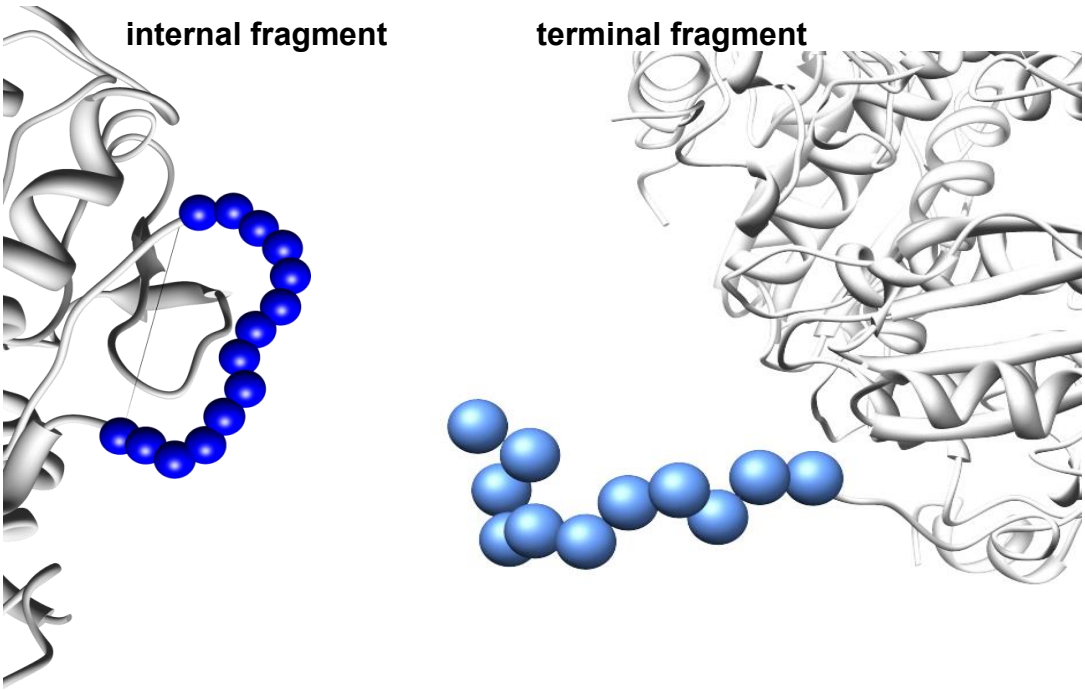
```
// 1NIC
dist (
                    (212) "A" -(212) "B" (<=11.0)
                    (212) "A"-(212) "C" (<=11.0)
                    (212) "B"-(212) "C" (<=11.0)

)
```

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

4. 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)

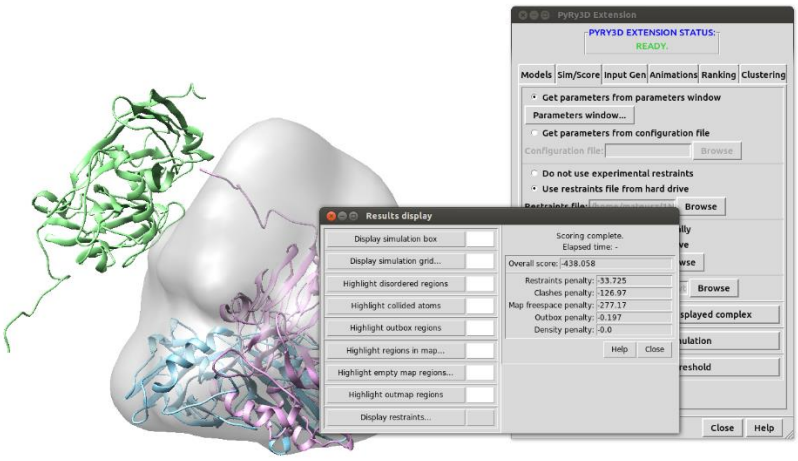


PyRy3D recognizes disordered/missing fragments by comparison of sequences from .PDB and .FASTA file for a particular component. Open **1nic_dd.fasta** where we added extra **'hhhhh'** at the beginning and the end of the "A" sequence to encode disordered residues with missing 3D coordinates. Save changes and use "**Use sequences from the hard drive**" to upload data into the Extension.

5. 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/**.

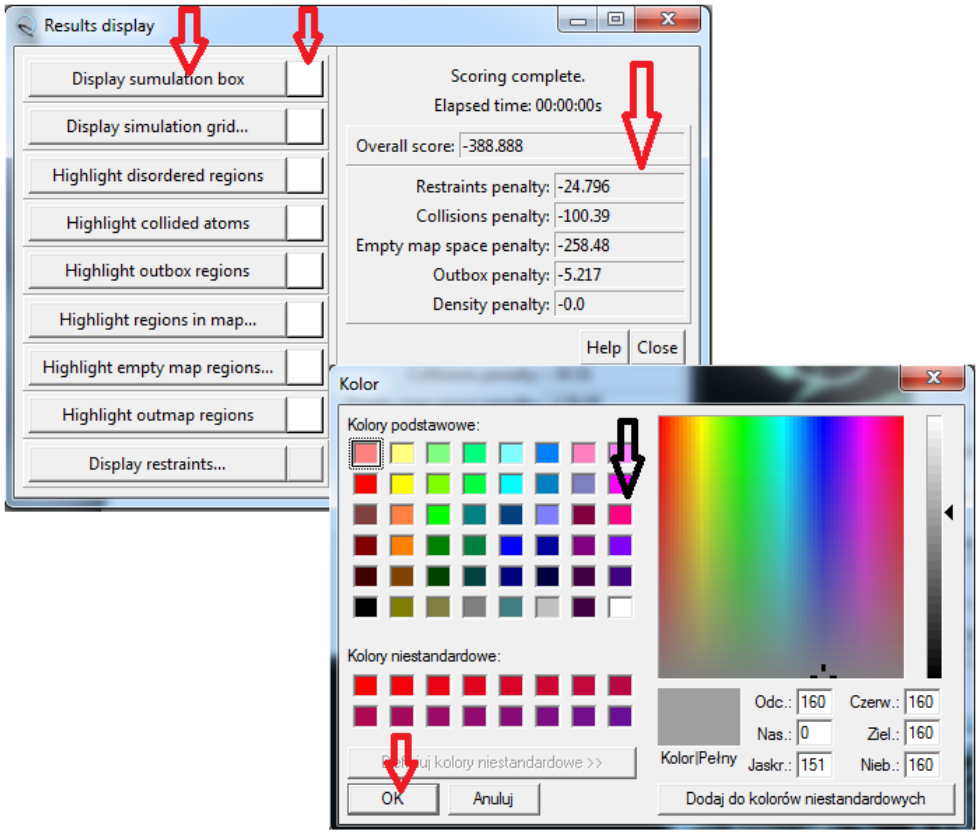
6. To start the evaluation, click **Calculate PyRy3D score for displayed complex**. The evaluation might take up to several minutes (depends on your computer's power). When it's finished, a window containing the overall score and it's elements will be displayed.



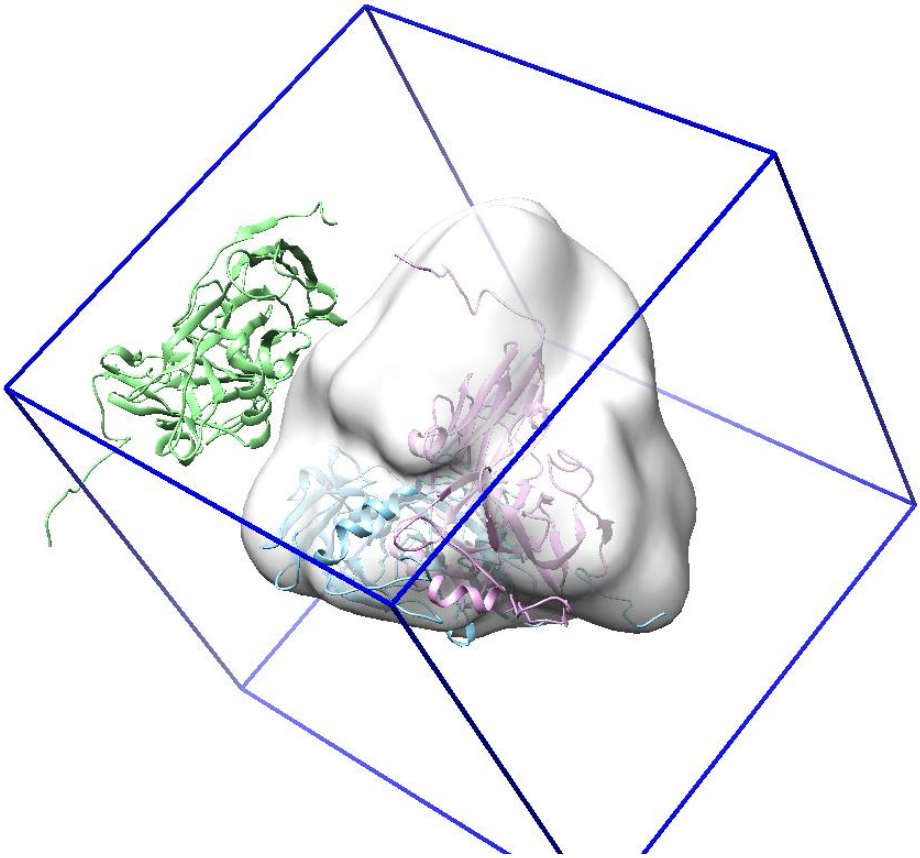
As authors of PyRy3D we wanted to make our software fully transparent for users. This means that we provided some functionalities to show how exactly the program works and how it interprets the structures. For example in the PyRy3D Chimera Extension we implemented Results Display tool that enables to visualize scores assigned to structures shown on the screen. Here one can check collided regions of structures, empty fragments of an electron density map or fulfillment of distance restraints.

Thanks to all that features a user does not have to use PyRy3D as a black box, but can do it with full understanding of the scoring process. We hope that such an approach will help to use our program and to create models of better quality.

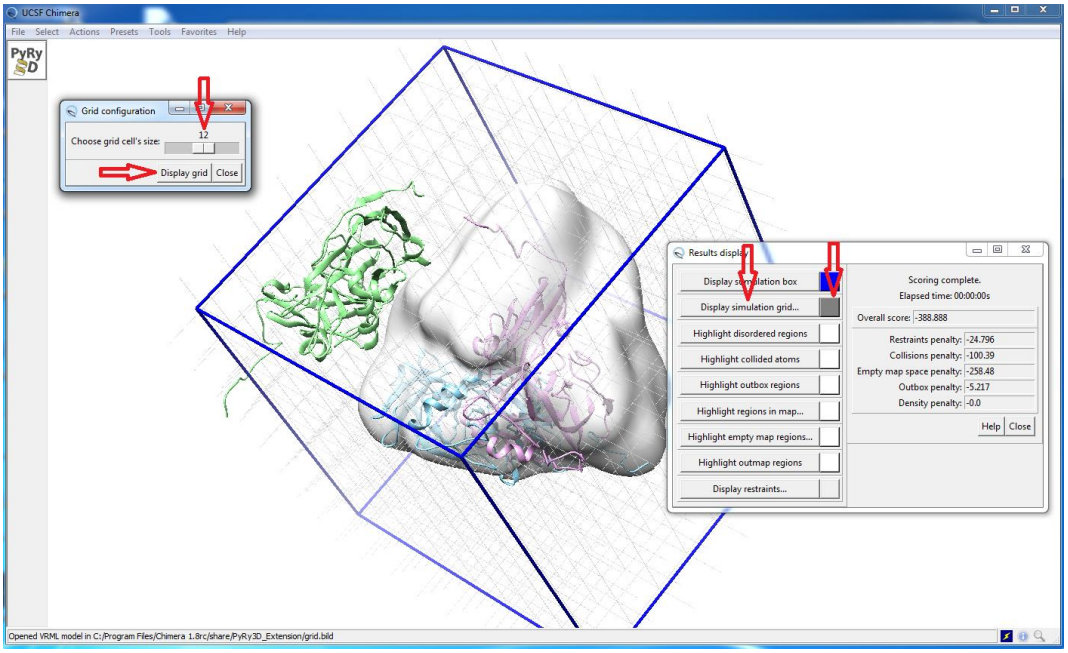
During the following steps, Chimera objects will be generated and added to the "model panel". Please, always have all models active while manipulating them, so that you don't change their coordinates respective to each other before you finish the analysis.



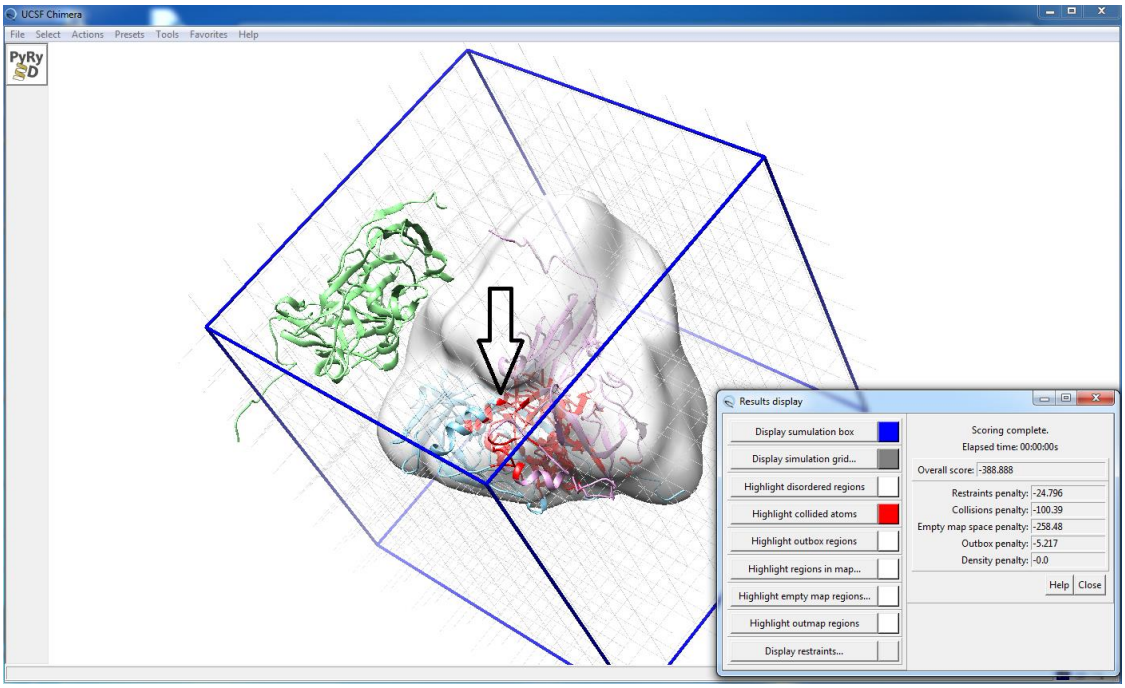
1. **Display simulation box** - displays the simulation box which limits the area of the simulation. Click the square button on the right, **choose blue color and draw the simulation box**. This is the area where structures can be moved during simulation process; all positions outside this box are penalized. The simulation box will appear as a new object in **Model Panel**. You can easily hide it be uncheck **Show**.



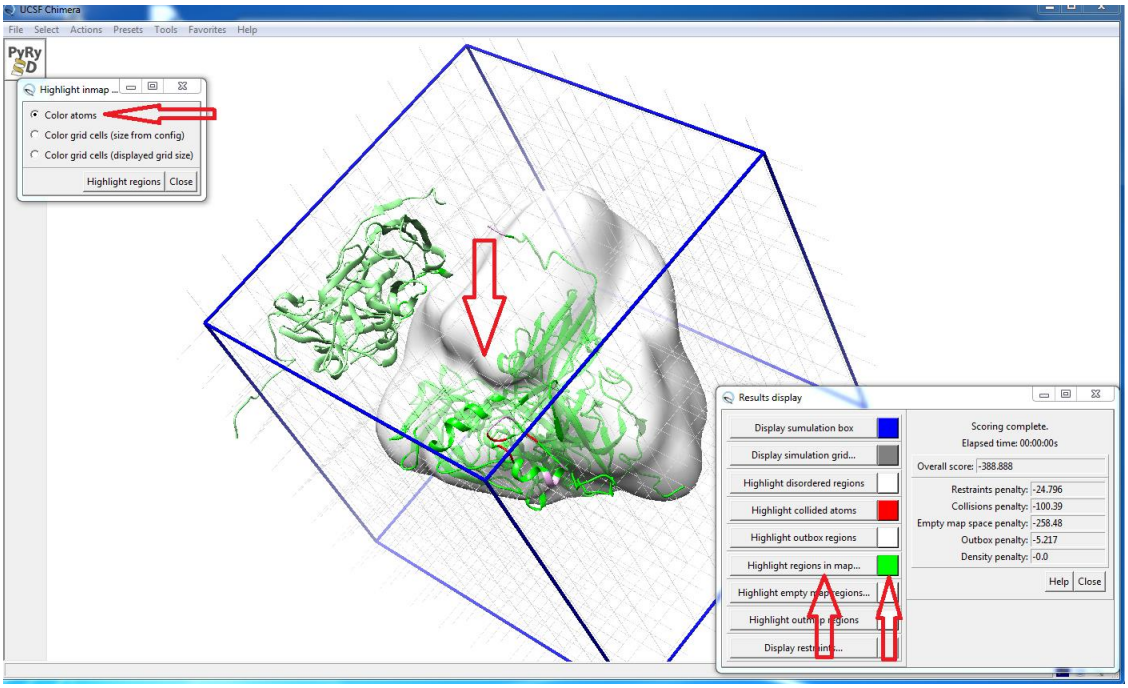
2. **Display simulation grid...** - displays the grid on which the evaluation was based. You can choose the size of the grid's cells. Because of the computational issues, very high-resolution grids cannot currently be displayed. Display a grey grid with cells' edges **12** Ångstrems long**. Please do not choose to small values (<6 Å) since this process might be very time consuming!**



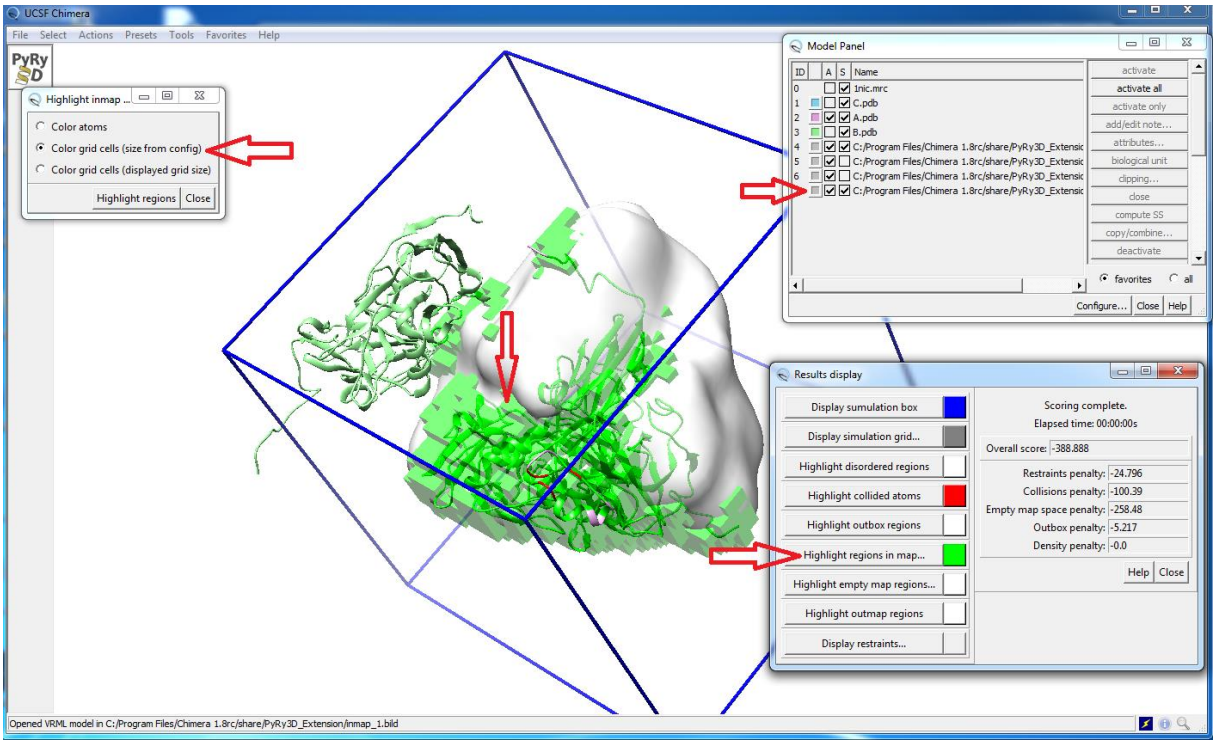
3. **Highlight collided atoms** - colors regions (atoms) where collisions between complexes' components occur. **Color them red**. This operation might be time consuming.



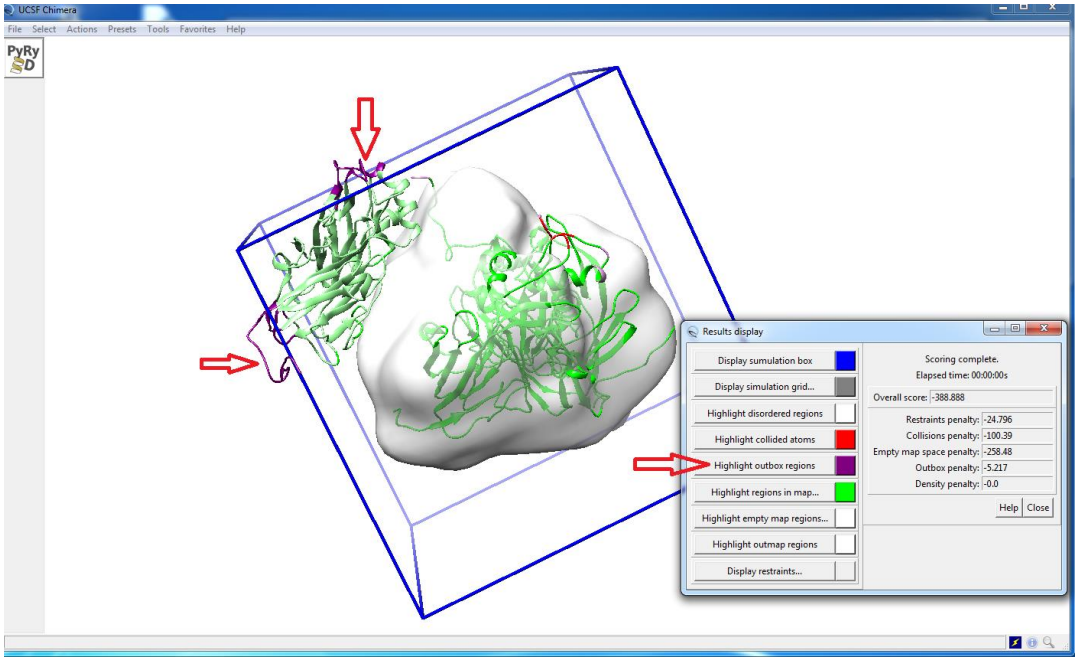
4. **Highlight regions in map** - colors regions (atoms or simulation grid cells) that correspond to atoms fitted inside the electron density map. **Color atoms** green. Coloring atoms might take some time.



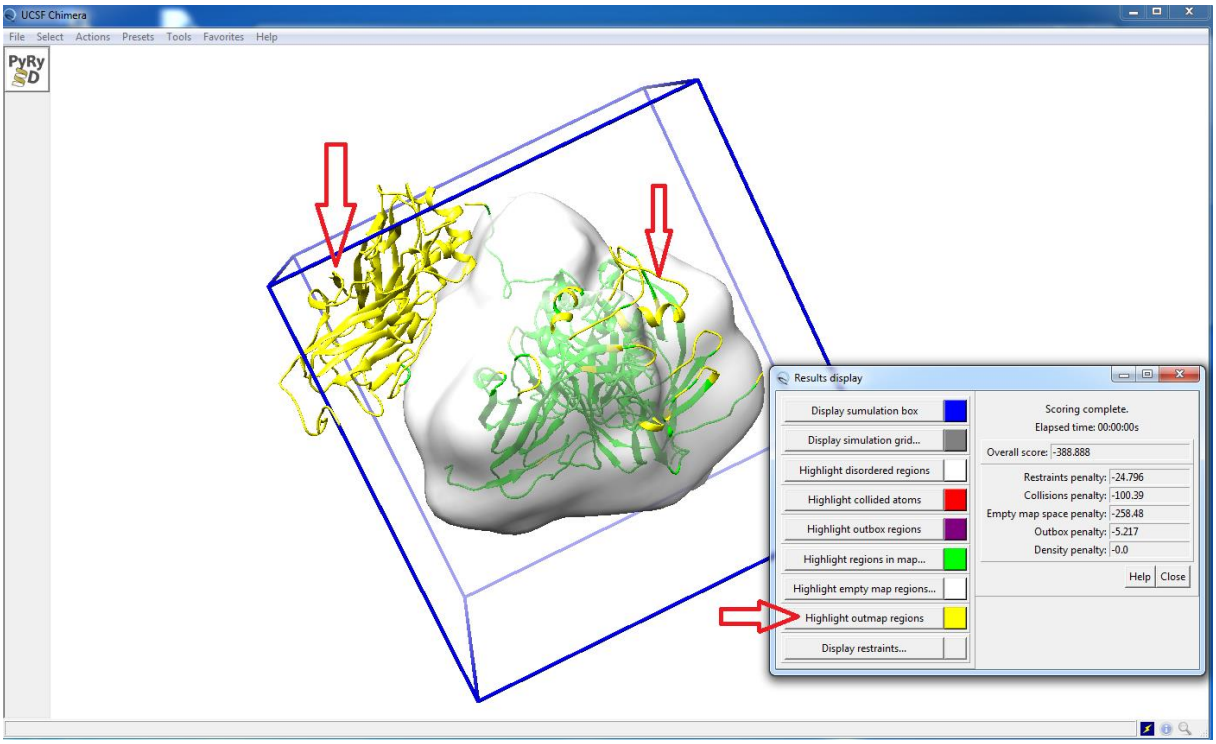
Color grid cells green:



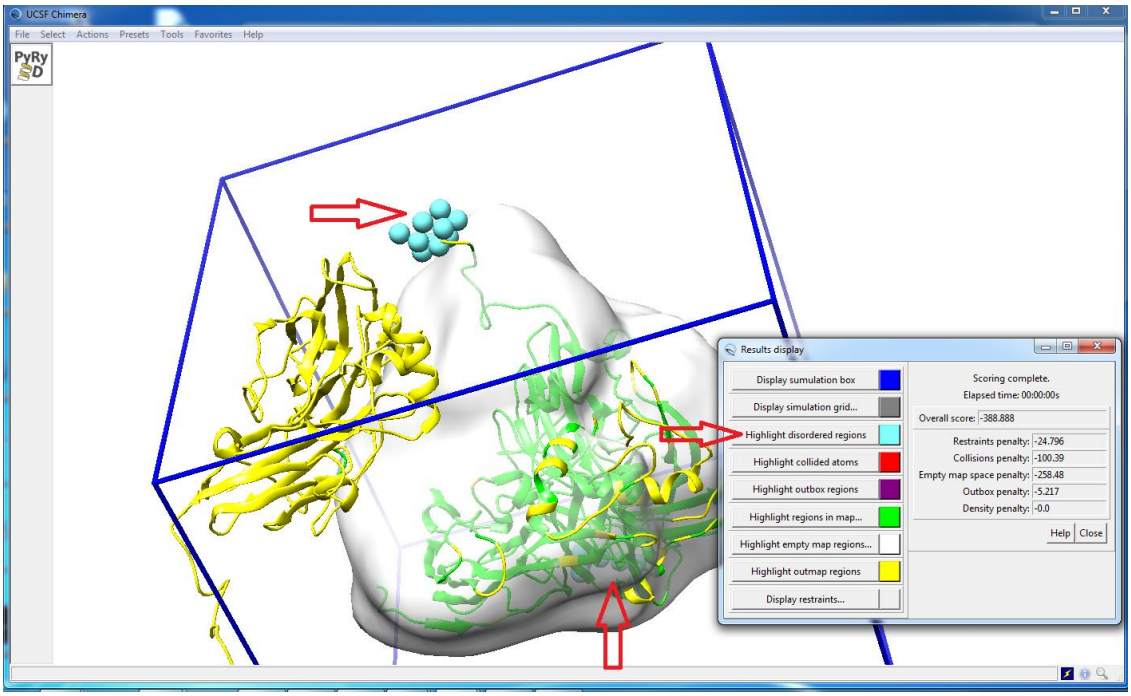
5. **Highlight outbox regions** - colors heavily punished atoms which are situated outside of the simulation box. **Color them purple**. This operation might be time consuming.



6. **Highlight outmap regions** - colors regions (atoms) that are situated outside the electron density map. **Color them yellow**. To create the following image the structures in Chimera where rotated to show corresponding regions.

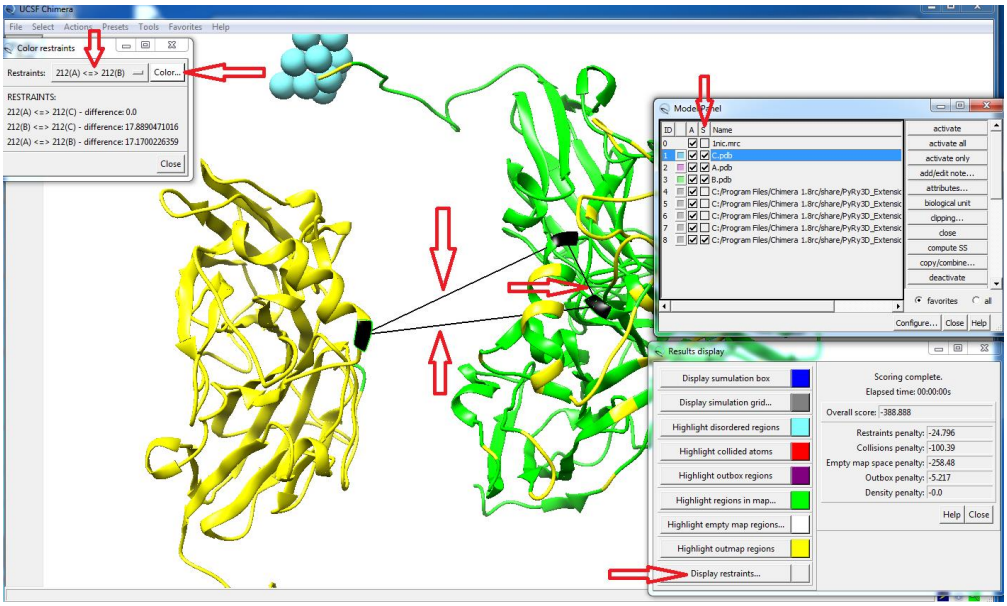


7. **Highlight disordered regions** - if there's a disordered region in the structure that's being evaluated, pseudoresidues are generated to fill the region without structural coordinates. The feature allows for visualizing the pseudoresidues as colored spheres. **Display the pseudoresidues by coloring them** cyan.



8. **Display restraints** - if you're using spatial restraints, you can see their complete list with differences between user defined values (as in **1nic.restr.txt** file) and values occurring in the evaluated complex. Select a restraint of interest in **Restraints** field and choose a **color** ->

program will draw a line between mentioned atoms. To prepare the following image we hidden a density map, simulation map object and reoriented a complex to make the interpretation easier.



MORE DETAILS, DOCUMENTATION, AND TUTORIALS AT:

genesilico.pl/pyry3d

WEBSERVER AT:

pyry3d.icm.edu.pl