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Tutorial: Structural Analysis of a Protein-Protein Complex

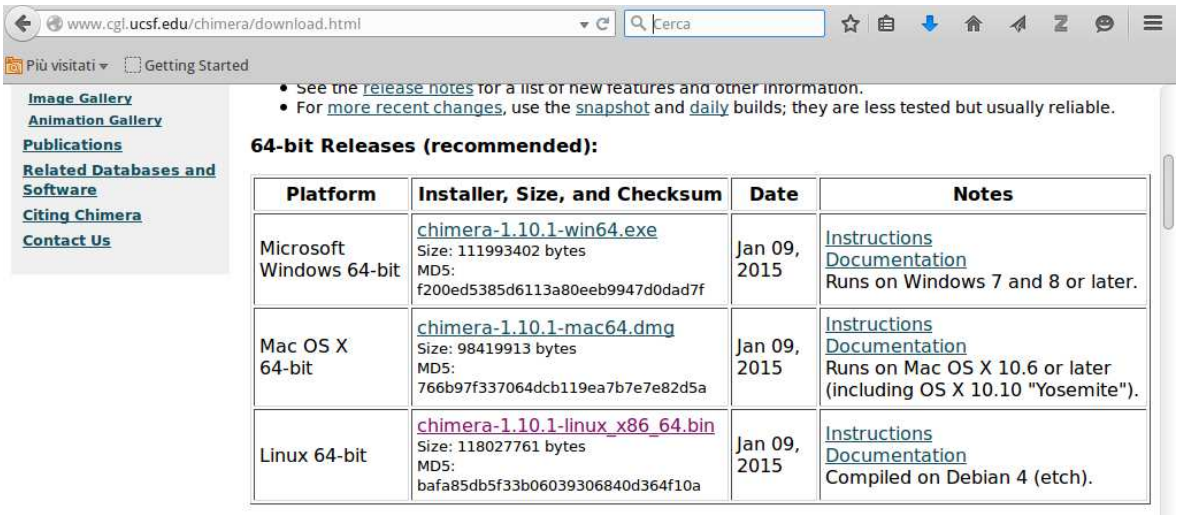
Goal: give the instruments to analyze a protein-protein complex. In particular, the tutorial will be focused to the identification of the protein-protein interactions and the evaluation of the complementarity between them. This will involve the study of the molecular surface and the projection on it of different properties using Chimera UCSF.

Brief Outline:

- How to retrieve a pdb structure file
- How to get information from the Protein Data Bank and from the .pdb file
- Visualize the complex on Chimera
- Analyze the molecular interaction and complementarity

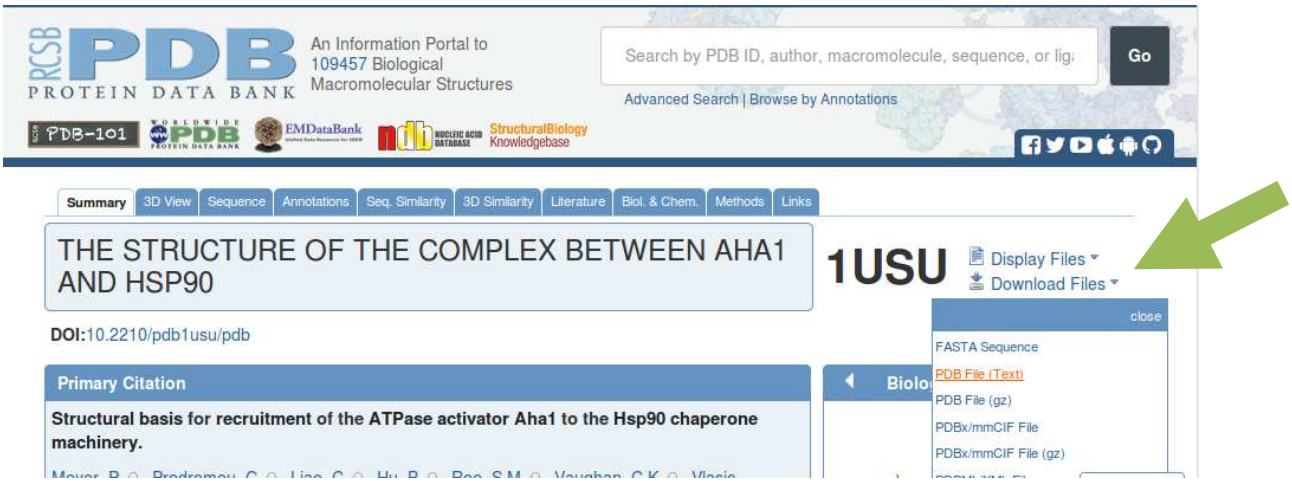
Prerequisite: Software Download and Installation

- Download "UCSF Chimera" software from http://www.cgl.ucsf.edu/chimera/download.html. In the website you can find versions suitable for Windows and Mac, with related instructions for download.



1. Pdb file Download

- Go to the website http://www.rcsb.org/pdb/home/home.do , and search your protein by PDB ID (for example 1USU).
- Click on ***Download Files***, select ***PDB File (Text)*** from the menu and download the file.



NB: remember that in a PDB file you can find interesting informations beyond coordinates of atoms, so open the file with a text editor (such as WordPad, libreoffice etc.) and have a look at it!!

2. Get Informations From The PDB File

The PDB file is a textual file and as a consequence can be open with a text editor (such as WordPad, LibreOffice, Word, Gedit, Vim). It contains several sections and the information varies according the methodologies used to obtain the structural data.

Tip: Depending on release year of the PDB structure the information some information may be present or not. Take a look at the webpage corresponding the PDB ID, several tabs are available (i.e. Sequence, Biol. & Chem., Method, ...)

3. Visualize the Protein-Protein Complex.

- Start Chimera and load your PDB file clicking on:
  File → ***Open...*** → select your file → ***Open***

o 3.1 Basic Functions Of Chimera:

Here some basic functions of the software are explained, in particular the use of the menus:

- ***Select*** menu: using this menu you can make selection of part of the structures; the most useful options are
  - ✓ ***Chain***: to select different protein chains
  - ✓ ***Residue***: to select amino acids by residue type
  - ✓ ***Structure***: for example clicking on protein you can select all protein chains (the whole protein complex)
- ***Actions*** menu: from this menu you can choose how to render the structures (entire structures if nothing is selected, otherwise the rendering will affect only the selection)
  - ✓ ***Atom/Bonds***: to show/hide atoms and bonds and to choose their rendering
  - ✓ ***Ribbond***: to show/hide ribbon and to choose its rendering
  - ✓ ***Surface***: to show/hide surface and to choose its rendering
  - ✓ ***Color***: to select the color for the rendering

4. Analyze the molecular interaction and complementarity

o 4.1 Sequence Analysis

- Open the amino acid sequence of the protein clicking on:

Tools → Sequence → Sequence

In the "Show Model Sequence" window select one of the chains and click on ***Show***, in this way a window reporting the sequence of that protein will appear.

Placing the cursor over a residue in the sequence the name and number of the residue will appear at the base of the panel.

Portions of sequence highlighted in yellow corresponds to alpha-helix and green to beta-strand, while amino acids in a red rectangle are no solved in the structure.

- Open the amino acid sequence corresponding to the gene (UniProt):

Tools → Sequence → PDB/UniProt Info

In the "PDB/UniProt Info" window select with a double click one of the chains: in this way two windows will appear.

The first window shows the sequence corresponding to the protein codified by the gene (UniProt sequence). Portions of sequence absent from the structure are surrounded by a pink box.

The second window shows some interesting and useful information, such as missing residues on the structures and sequence mismatching (PDB vs UNIPROTKB).

NB: 3D-Structural and Sequence windows are synchronized, so you can select amino acids from the sequence window by left button clicking+dragging over the amino acids and localized them on the 3D-structural window.

o 4.2 Protein Preparation

First of all it's necessary to prepare the protein structure in terms of reconstruction of missing residues, protonation and calculation of partial charges, which are essential for the following calculation of electrostatic potential.

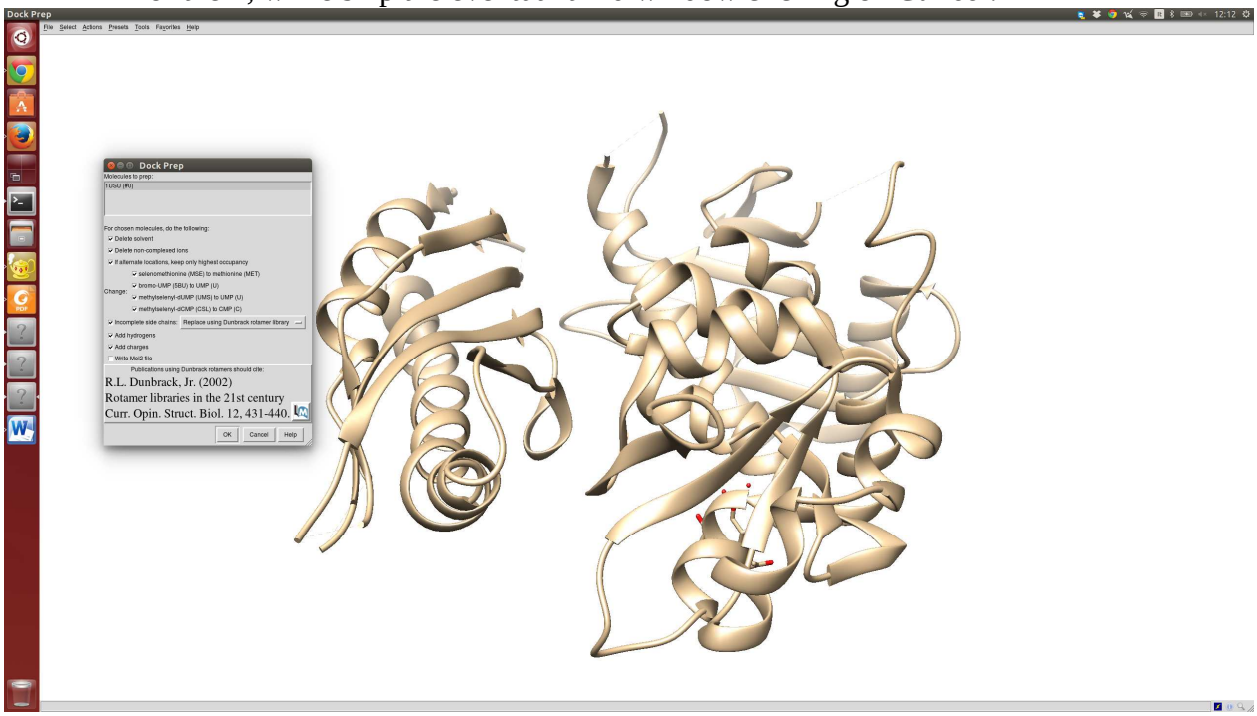
- Click on:

Tools → Structure Editing → Dock Prep

In the window that will appear flag:

- ✓ ***Delete solvent*** : this will remove the molecules of water
- ✓ ***Delete non-complexed ions*** : this will remove ions not in complex with the protein
- ✓ ***If alternate locations, keep only highest occupancy*** : in this way multiple conformations for the same atoms will be removed
- ✓ ***Incomplete side chains*** and leave the default option : this will generate complete side chains for incomplete residues
- ✓ ***Add hydrogens***: this tool aims to generate protonation states reasonable at physiological pH
- ✓ ***Add charges*** : this tool will assign partial charges to atoms

- Click ***OK***, leave the default options in the following two windows and click ***OK*** in both of them, while skip the eventual third window clicking on ***Cancel***.



o 4.3 Protein-Protein Intermolecular Interactions

H-BONDS:

In this section we will detect hydrogen bonds at the interface between the two proteins of the complex.

- Click on:

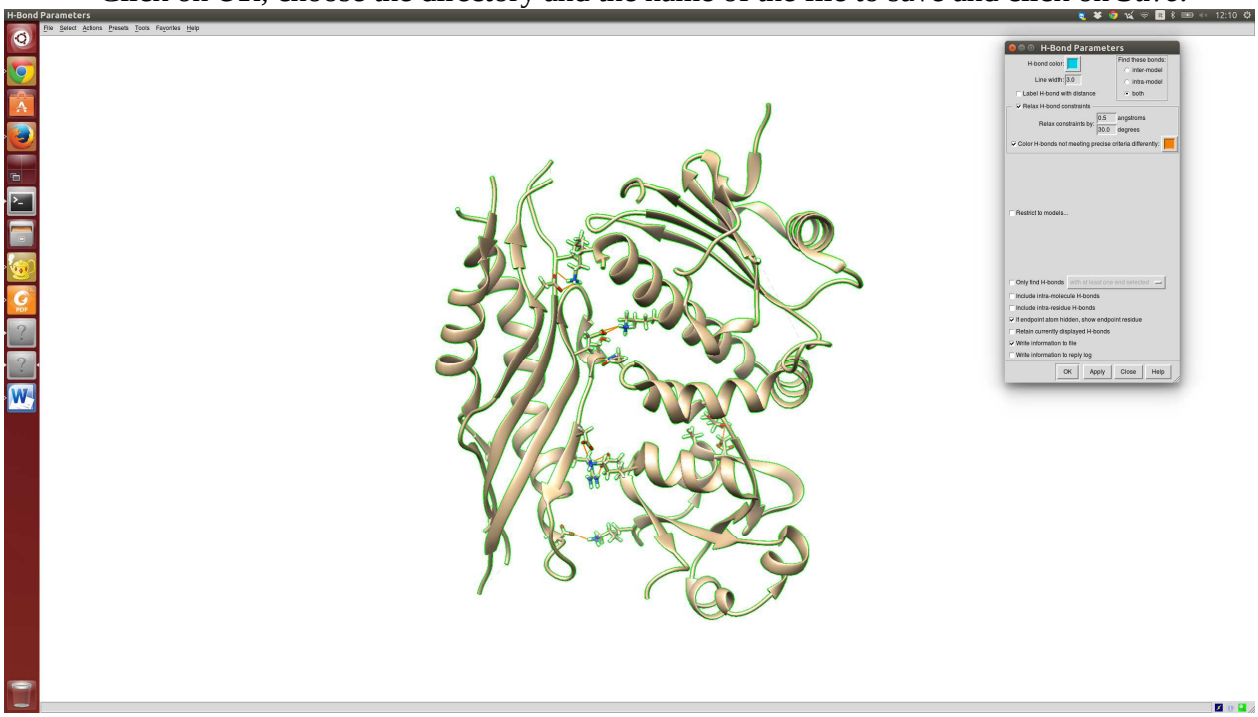
Tools → Surface/Binding Analysis → FindHBond

- In the "H-Bond Parameters" window that will appear flag:
  - ✓ ***both*** in the ***Find these bonds*** box
  - ✓ ***Relax H-bond constraints*** and set the values ***0.5 angstroms*** and ***30.0 degree*** : in this way also non-ideal H-bonds, falling in this range of deviation from ideality,

will be computed

- ✓ ***Color H-bonds not meeting precise criteria differently***: non-ideal H-bonds of the selected color will be drawn in the structure
- ✓ ***If endpoint atom hidden, show endpoint residue***: residues involved in H-bonds will be drawn in the structure
- ✓ ***Write information to file***: a list of the interacting residue will be saved

- Click on ***OK***, choose the directory and the name of the file to save and click on ***Save***.



These operations will show the amino acids involved in intermolecular hydrogen bonds and will write a file with a list of residues involved in these interactions. For each couple of residues H-bond donor, acceptor, hydrogen atom, donor - - acceptor distance and donor-H - - acceptor distance are reported in the file.

CONTACTS:

In this section we will identify all kind of interactions (polar and non-polar, favorable and unfavorable (clashes)) between the two proteins.

- Select both protein chains clicking on:

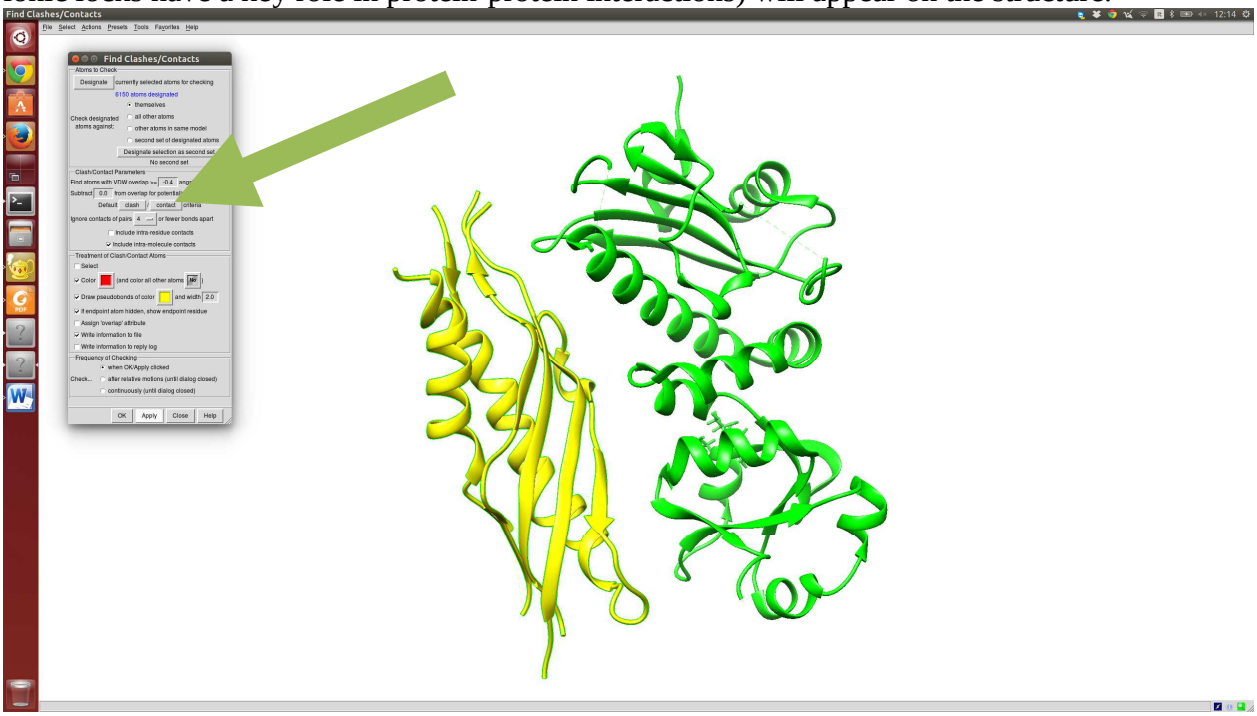
Select → Structure → protein

- Click on:

Tools → Surface/Binding Analysis → Find Clashes/Contacts

- In the "Find Clashes/Contacts":
  - ✓ click on ***Designate*** and flag ***themselves*** : in this way only contacts between the two proteins will be checked
  - ✓ click on ***contact*** in ***Default clash/contact criteria*** , in order to choose the contact instead of clash option
  - ✓ flag the ***Color*** option
  - ✓ flag the ***Draw pseudobonds of color*** option
  - ✓ flag ***If endpoint atom hidden, show endpoint residue*** , to draw residues involved in contacts
  - ✓ ***Write information to file***: a list of the interacting residue will be saved

At the end amino acids involved in intermolecular interactions of every kind (for example ionic locks have a key role in protein-protein interactions) will appear on the structure.



CLASHES:

In this section we will identify unfavorable intermolecular interactions where atoms are too close together, that is clashes.

- If they are not already selected, select both protein chains clicking on:

Select → Structure → protein

- Click on:

Tools → Surface/Binding Analysis → Find Clashes/Contacts

- In the "Find Clashes/Contacts":
  - ✓ click on ***Designate*** and flag ***themselves*** : in this way only contacts between the two proteins will be checked
  - ✓ click on ***clashes*** in ***Default clash/contact criteria*** , in order to choose the contact instead of clash option
  - ✓ flag the ***Color*** option, and use a different color from the one used for atoms involved in contacts
  - ✓ flag the ***Draw pseudobonds of color*** option, and use a different color from the one used for contacts
  - ✓ flag ***If endpoint atom hidden, show endpoint residue*** , to draw residues involved in clashes
- De-select the proteins clicking on

Select → Clear Selection

At the end amino acids involved in clashes will appear on the structure.

o **4.4 Surface Analysis**

Protein-protein recognition depends on the complementarity between the surface of the two proteins, that is a complementarity in shape and in electrostatic and hydrophobic interactions.
Therefore it's necessary to calculate the surface of proteins in the complex.

SURFACE REPRESENTATION:

It's necessary to save two separate PDB for the two proteins before generating the molecular surface.

- Select chain A clicking on:

Select → Chain → A

- Delete Chain A clicking on:

Actions → Atoms/Bonds → delete

- Save the Chain B clicking on:

File → Save PDB → choose the directory and the name of the file to save **→ *Save***

- Close the session clicking on:

File → Close Session

- Open your original PDB and repeat the previous points deleting chain B and saving chain A
- Open the PDB of the chain B. Now you have loaded the structure of both chain A and chain B.
- Generate the molecular surface clicking on:

Actions → Surface → show

ELECTROSTATIC POTENTIAL

In this section we will color the surface according to the Coulombic potential.

- Click on:

Tools → Surface/Binding Analysis → Coulombic Surface Coloring

- Select both the surfaces in the "Coulombic Surface Coloring" window (left button+Ctrl)
- Use the default options and click on ***OK***

As a result the surfaces will be colored according to the Coulombic potential, going from red (negative potential) to blue (positive potential).

HYDROPHOBIC POTENTIAL

In this section we will generate a hydrophobicity surface coloring the molecular surface by amino acid hydrophobicity.

- Click on:

Tools → Depiction → Render by attribute

- In the window "Render/Select by attribute":
  - ✓ Select both chains (left button + Ctrl) and choose ***residues*** in the ***Attributes of*** section
  - ✓ Choose ***kdHydrophobicity*** in the ***Attribute:*** section
  - ✓ Choose Cyan-Maroon in the ***Palette:*** section
  - ✓ Use the remaining default options and click on ***OK***

As a result the surfaces will be colored according to amino acid hydrophobicity: more hydrophobic residues (larger positive values of hydrophobicity) are colored in maroon, while the hydrophilic residues (negative values of hydrophobicity) are colored in cyan.

STERIC COMPLEMETARITY

To observe shape complementarity, it's useful to move only one protein leaving the other fixed, in this way it will be possible to check if a pocket in one structure fits with a protrusion in the partner.
In the same way we can check if positive portions match negative ones, if hydrophobic portions match hydrophobic ones and if hydrophilic portions match hydrophilic ones.

- Click on:
  - ✓ ***Favorites → Model Panel***
  - ✓ Make one structure inactive by de-flagging the column A box in the line corresponding to that structure
  - ✓ move the structure using the central mouse button (avoid to rotate the structure, so don't use the left mouse button)

5. Conclusions

Thanks to the molecular surface and interaction analysis it's possible to understand some of the key features for protein-protein recognition, giving interesting hints about what happens in response to single point mutations.