Graphical analysis of the ligand-receptor interaction

1. Introduction

The visualization and analysis of the ligand-receptor interactions is a fundamental aspect of the computational chemistry. This analysis can be developed in different ways using different programs. We will carry out this analysis using a graphical approach by means of Chimera program.

2. Getting started

In this tutorial we will analyse the interactions between an inhibitor (PP1) and the RET tyrosine kinase. The PDB code of this structure is 2IVV.
We first have to download the pdb file from the RCSB Protein Data Bank.

- Open Google Chrome (from "Applications" menu) and go to the PDB homepage (https://www.rcsb.org/)
- In the search panel, digit the code 2IVV and press the Search button. The page related to the desired X-ray structure will be loaded.
- Click on "Download Files" button and select "Legacy PDB format" from the drop-down menu.
- The file should be automatically saved in the "Downloads" folder of your home directory. Otherwise, select "Save File" in the popup window and click "OK".

Now we have to open the file inside Chimera

- Open Chimera program
- Click on File/Open
- Select the file 2ivv.pdb from the "Open File in Chimera" window, and click on "Open".

Now we need to:

a) remove water molecules
b) remove the formic acid

For removing the water molecules:

- Click on Select/Residue/HOH
- Click on Action/Atoms/Bonds/delete

For removing the formic acid:

- Click on Select/Residue/FMT
- Click on Action/Atoms/Bonds/delete

For focusing the structure:

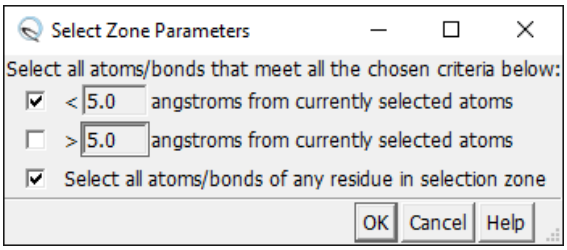
- Click on Select/Clear Selection (if something is selected)
- Click on Action/Focus

Colour by atoms the system:

- Click on Actions/Color/by element

Highlight the inhibitor and the binding site:

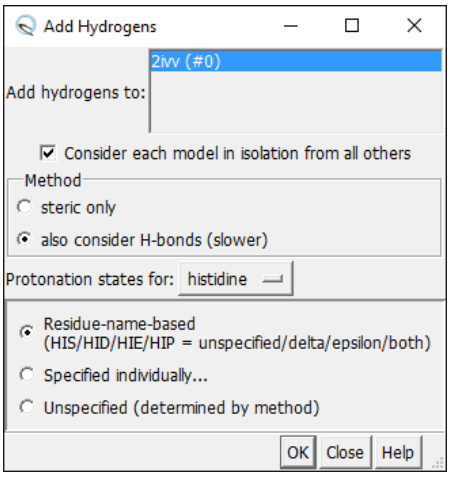
- Click on Select/Residue/PP1
- Click on Actions/Color/cyan
- Click on Actions/Color/by heteroatom



- Click on Select/Zone and change the parameters like the Figure; in this way the program will selected all residues within a radius of 5 Å from the ligands.
- Press the OK button

- Click on Actions/Atoms/Bonds/show only
- Unselect (Click on Select/Clear Selection)
- Click on Action/Ribbon/hide

Add the hydrogens to the structure:



- Click on Tools/Structure Editing/AddH
- Press OK leaving all the parameters as their default.
- Press Close in the warning panel (you can have a message about some atoms protonation followed by “Hydrogens added")

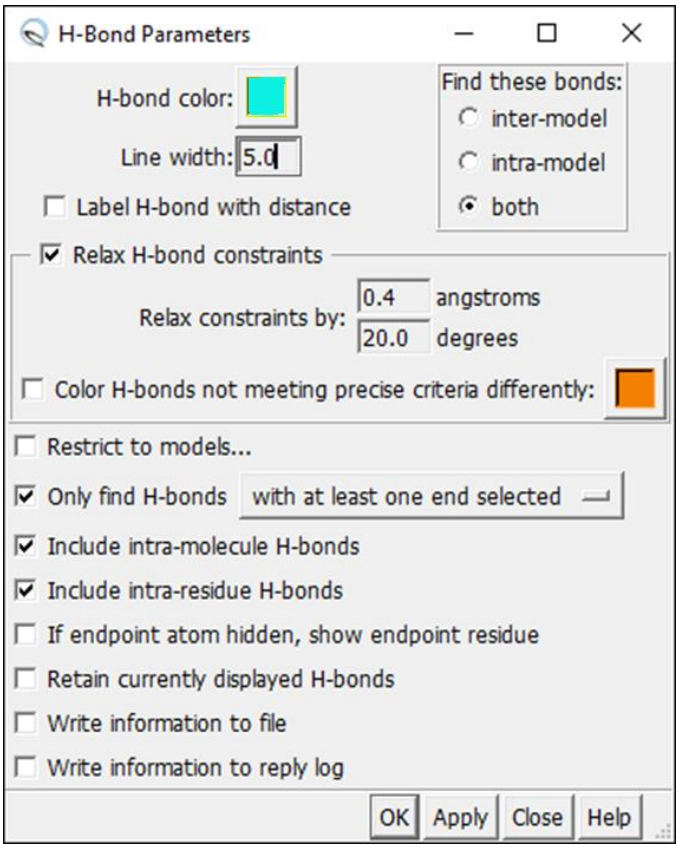
In order to hide the non-polar hydrogens (i.e. the hydrogens connected with carbon atoms)

- Click on Select/Chemistry/IDATM type/HC
- Click on Action/Atoms/Bonds/hide
- Unselect (Click on Select/Clear Selection)

3. H-bond analysis

Highlight the H-bond interactions between the ligand and the protein:

- Click on Select/Residue/PP1
- Click on Tools/Structure Analysis/FindHBond and modify the parameters as reported in Figure. Press OK



FindHBond uses atom types and geometric criteria to identify possible hydrogen bonds (H-bonds). "OK" starts the calculation and dismisses the panel, while "Apply" starts the calculation without dismissing the panel. "Close" dismisses the panel without performing any calculation. "Help" brings up this manual page in a browser window.

The scope of the calculation is controlled under "Find these bonds":

inter-model - between models only

intra-model - within models only

both (default)

The calculation can be further restricted with the options:

"Restrict to models..." shows a list of molecule models from which one or more can be chosen. Individual models or blocks of models can be chosen with the left mouse button. Ctrl-click toggles the

status of an individual model. To choose a block of models without dragging, click on the first (or last) and then Shift-click on the last (or first) in the desired block.

Only find H-bonds with [at least one end selected / exactly one end selected / both ends selected / between selection and atom spec…] reports only H-bonds with donor and/or acceptor heavy atoms within the current selection.

"Relax H-bond constraints" indicates which tolerances to Relax constraints should be applied to the precise geometric criteria. H-bonds within the tolerances but not meeting the precise criteria can be colored differently than the H-bonds that meet the precise criteria.

Additional options:

"If endpoint atom hidden, show endpoint residue" turns on the display of residues containing an H-bonding atom if that atom is not displayed initially (By default, if the atom on either end of a pseudobond representing an H-bond is not shown, the pseudobond itself is not shown, although it still exists; displaying the atom allows the H-bond to be shown.)

"Retain currently displayed H-bonds" retains a previously determined set of H-bonds through a subsequent round of H-bond detection

"Write information to file" writes H-bond information (atom specifications and distances) to a file.

<u>You should always check the results obtained with the Hbond panel because the program could add some improper interaction or some H-bonds could be missing.</u>

4. Binding disposition and lipophilic analysis

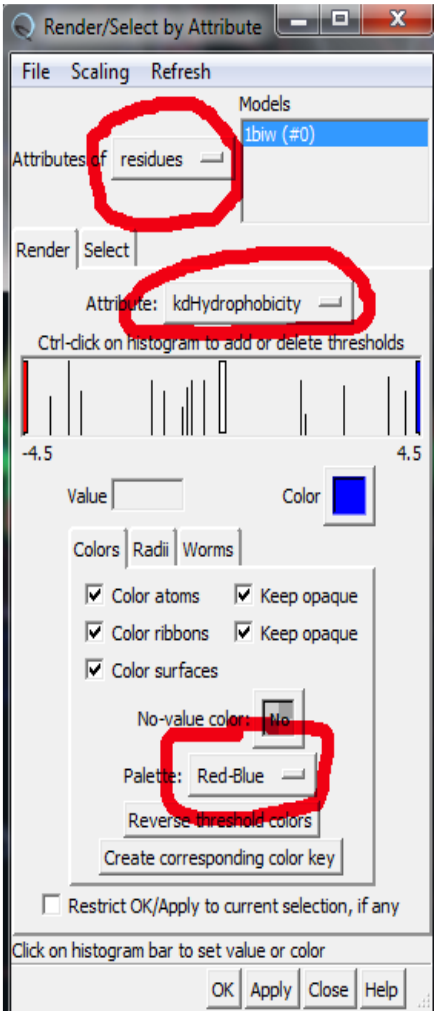
In order to analyse the binding disposition of the ligand we can show the protein surface

- Click on Select/Clear selection
- Click on Favorites/Model Panel
- In Model Panel click on "surface main"

In this way we can analyse the geometry of the binding site and the disposition of the ligand.

You can also colour all the lipophilic residues, in order to have an idea of the binding interactions of the ligand.

- Click on Tools/Structure Analysis/Render by Attribute
- Change the panel parameters as shown in Figure and click on Apply



In this way, amino acid residues will automatically receive an attribute named **kdHydrophobicity**, with values according to the hydrophobicity scale of Kyte and Doolittle.

Residue Type	**kdHydrophobicity**[a]
Ile	4.5
Val	4.2
Leu	3.8
Phe	2.8
Cys	2.5
Met	1.9
Ala	1.8
Gly	-0.4

Thr	-0.7
Ser	-0.8
Trp	-0.9
Tyr	-1.3
Pro	-1.6
His	-3.2
Glu	-3.5
Gln	-3.5
Asp	-3.5
Asn	-3.5
Lys	-3.9
Arg	-4.5

[a] A simple method for displaying the hydropathic character of a protein. Kyte J, Doolittle RF. *J Mol Biol.* 1982 May 5;157(1):105-32.

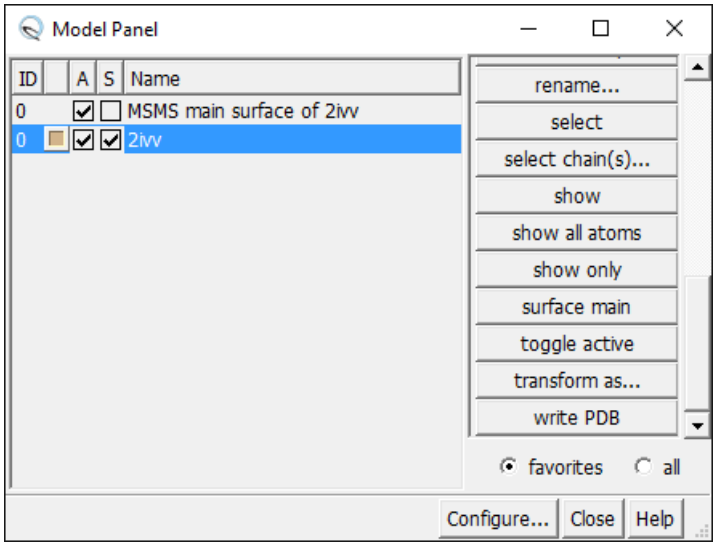
In order to better analyse the ligand interactions, it is important at this level to investigate which are the most important binding interactions of the ligand. For this step it is necessary to analyse the residues of the binding site.

- Click on Select/Residue/PP1
- Click on Tools/Surface/Binding analysis/Surface Zone, type a Radius of 4.0, press the Zone button and then press the Close button
- Click on Select/Clear selection

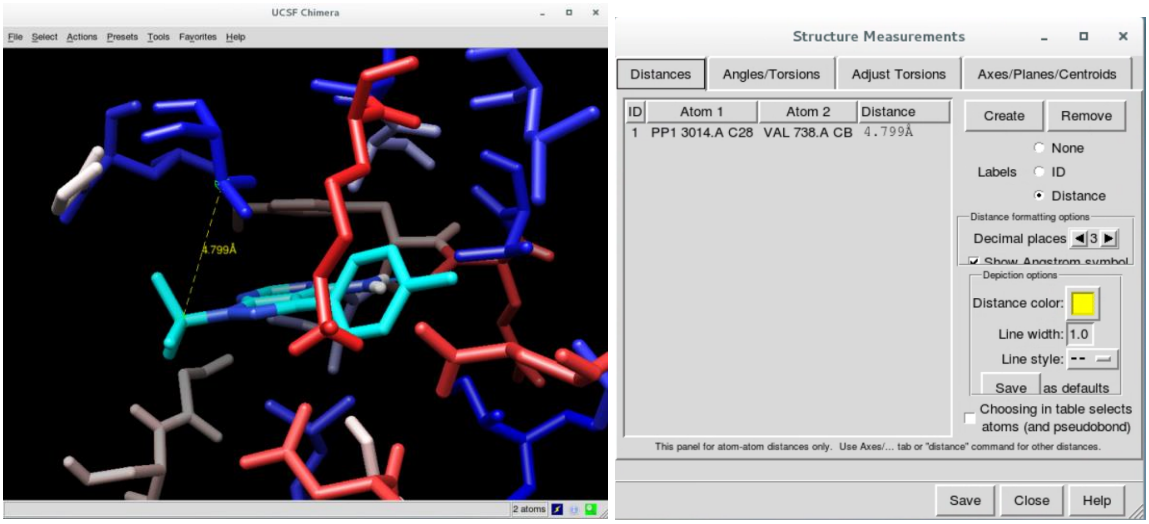
From this picture it is evident that the p-methylphenyl substituent shows interactions in the lipohilic (blue) cleft main delimited by Leu779, Ile788, Leu802, and Val804. Furthermore, the t-butyl group of the ligand shows lipophilic interactions with Leu730 and Val738.
It is also possible to evaluate the distance between different atoms. For example for measuring the distance between the t-butyl group of the ligand and Val738:

- Click on Favorites/Model Panel

- Deselect the 'show/hide square' of the surface as shown in the Figure



- Click on the central carbon of the t-butyl group (atom C28) with the CTRL-left mouse and click on the CH portion of Val738 (atom CB) with the CTRL-Shift-left mouse combination (rember: for selecting one atom press “Ctrl” and then click with the left button on one atom. For adding a selection hold down the “Ctrl” and “Shift” key and click with the left mouse button on the atom)
- Click on Tools/Structure Analysis/Distances and press create



You can do this measurement for the other lipophilic interactions and also for the H-bonds.

In the next page is reported a summary of typical interaction distances of noncovalent interactions.

5. Example

Starting from the PDB code 3RZB do the same analysis

Interaction type	Typical distance range [Å]		
Hydrogen bonds	OH	NH	
Carbonyl/sulfonyl O	2.7 - 3.0	2.8 - 3.1	
Heteroaromatic N	2.7 - 3.0	2.8 - 3.2	
Carboxylic acid O	2.6 - 2.8	2.7 - 3.0	
Halogen bonds	Cl	Br	I
Carbonyl O	3.0 - 3.4	3.0 - 3.5	2.9 - 3.5
Multipolar interactions			
F ··· carbonyl C	3.0 - 3.7		
Interactions with Aliphatic C			
F	3.3 - 3.9		
Cl	3.6 - 4.3		
Aliphatic C	3.7 - 4.4		
Sulfonyl O	3.3 - 3.9		
Divalent S	3.8 – 4.2		
Interactions with Aryl C	**In plane**	**Above plane**	
Aromatic C ··· divalent S	3.7 – 4.2		
Aromatic C ··· F	3.3 - 3.7		
Aromatic C ··· Cl	3.6 - 4.1	3.5 - 4.0	
Aromatic C ··· Br	3.7 - 4.2	3.5 - 4.1	
Aromatic C ··· $-CH_2-$	3.8 - 4.4	3.6 - 4.2	
Aromatic C ··· CH_3O-		3.4 - 4.0	
Aromatic C ··· CN+-		3.4 - 4.0	
Aromatic C ··· Amide-N		3.4 - 3.8	
	Parallel displaced	**Edge to face**	
Aromatic C··· aromatic-C	3.4 - 3.6	3.6 - 3.8	

A Medicinal Chemist's Guide to Molecular Interactions J Med Chem. 2010 53(14):5061-5084