

# Molecular docking tutorial

## Sulfonamide-type D-Glu inhibitor docked into the MurD active site using ArgusLab

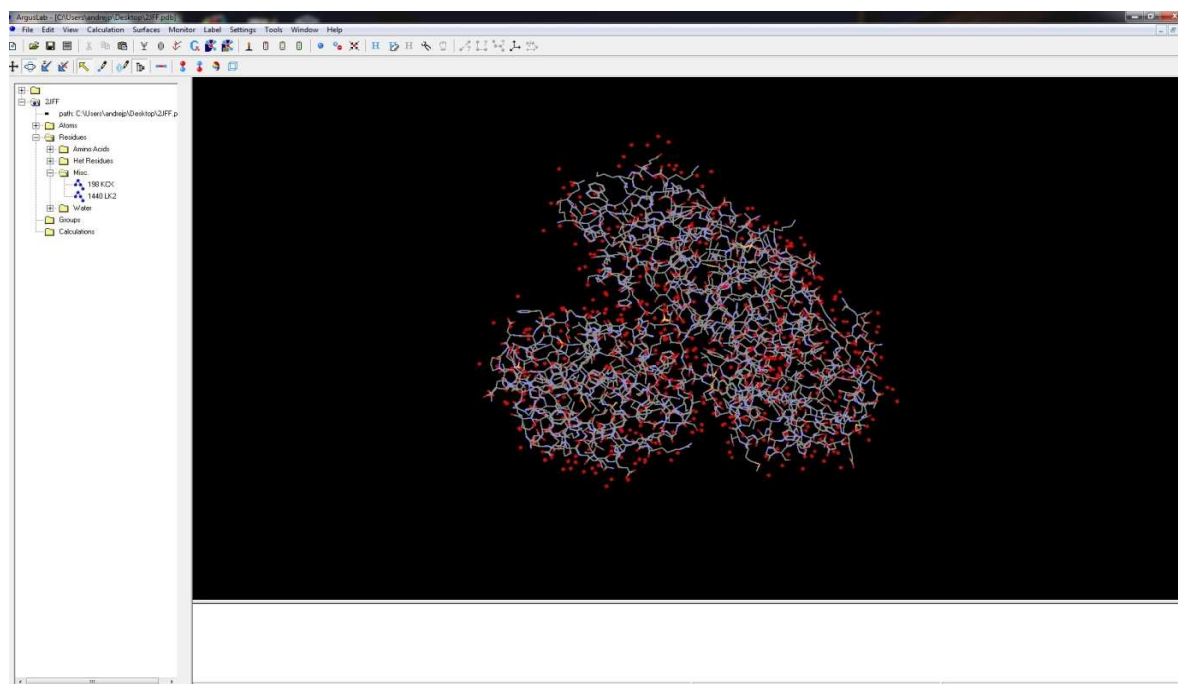
In this tutorial [1] you will learn how to prepare and run molecular docking calculations using ArgusLab software. You will first extract an experimentally determined binding conformation of a sulfonamide-type D-Glu inhibitor of the bacterial enzyme MurD and subsequently dock it into its back into its binding site. Finally, you will compare the docked structure with the x-ray structure to see how well they match. This type of calculations is often used when validating docking programs before performing large-scale virtual screening experiments. Before you can dock a molecule, you first need to define the atoms that make up the **Ligand** (drug, inhibitor, etc.) and the **Binding Site on the protein** where the drug binds. The crystal structure of a protein used in this exercise (PDB: code 2JFF) can be freely accessed and downloaded from the Brookhaven Protein Databank (PDB) and represents an important result of a European collaborative antibacterial research project where several Slovenian researchers were involved [2].

### 1. Download and open the PDB structure 2JFF.

Open the web browser and type in the **Brookhaven Protein Databank** ([www.rcsb.org](http://www.rcsb.org)) web address and in the search field type 2JFF. Download the displayed structure as PDB file (Text) preferably to Desktop. Open the 2JFF.pdb located in the folder you designated during download in ArgusLab program (Arguslab icon is located on your desktop). This is a **PDB file** so make sure you select the correct file type in the file open dialog (pdb type).

Expand the **Molecule Tree View tool** of 2JFF (located on the left side on the screen) and open up the *Residues/Misc* folder to show the sulfonamide inhibitor (**LK2**).


You should see something like this:




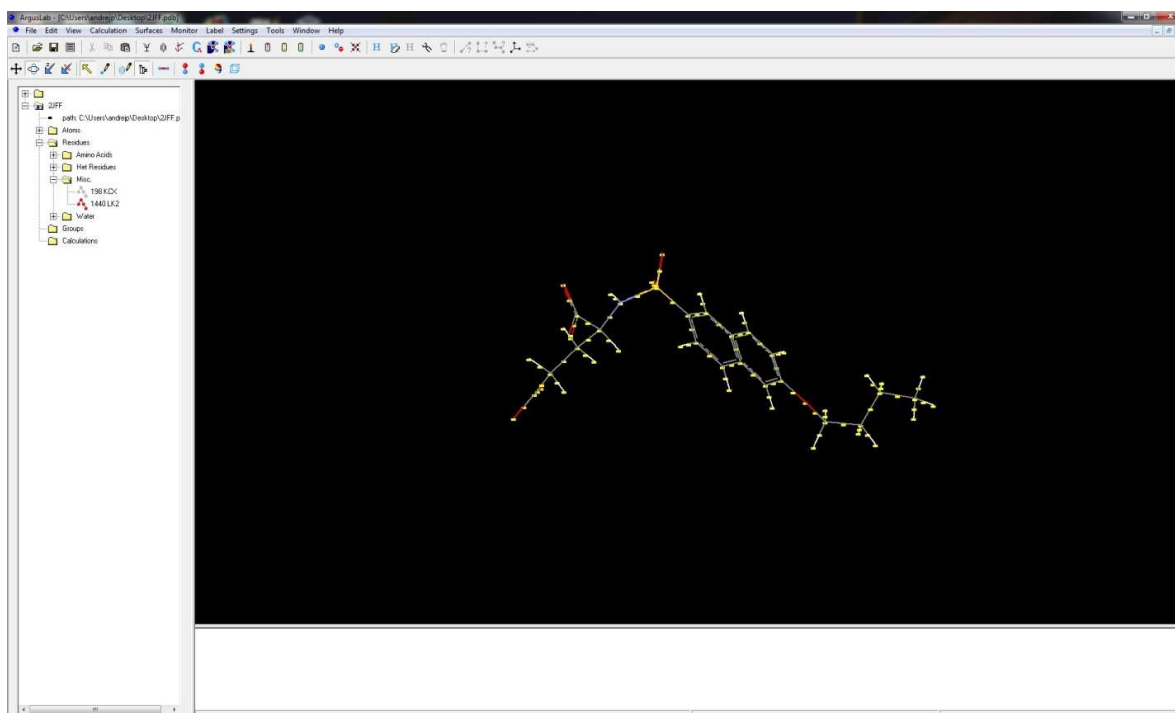
## 2. Create "Ligand and Binding Site groups".

Left-click on "1440 LK2" in the **Tree View** to select the sulfonamide-type D-Glu inhibitor. It should appear yellow.

Select the *Edit/Hide Unselected menu* option to hide all atoms that are not selected. The only atoms showing on the screen should be the D-Glu sulfonamide inhibitor.

Center the sulfonamide inhibitor in the window by selecting the *View/Center Molecule* in Window menu or pressing the  button on the toolbar.

While the inhibitor is selected, add hydrogens by pressing the  button on the toolbar. You should see something like this:



Right-click on "1440 LK2" in the **Tree View** and select the **"Make a Ligand Group from this Residue"** option. ArgusLab will construct a group underneath the **Groups** folder with the same name "1 LK2" that is of type = Ligand.

Left-click on either the "1440 LK2" residue in the *Residues/Misc folder*. This action will again select the atoms of the Ligand on the screen.

Copy and Paste the selected residue. Look in the *Residues/Misc* folder in the **Tree View tool** and you will see a new highlighted residue with a name "2480 LK2". (different number is also possible). This is an extracted conformation of a ligand that you will dock into its binding site.

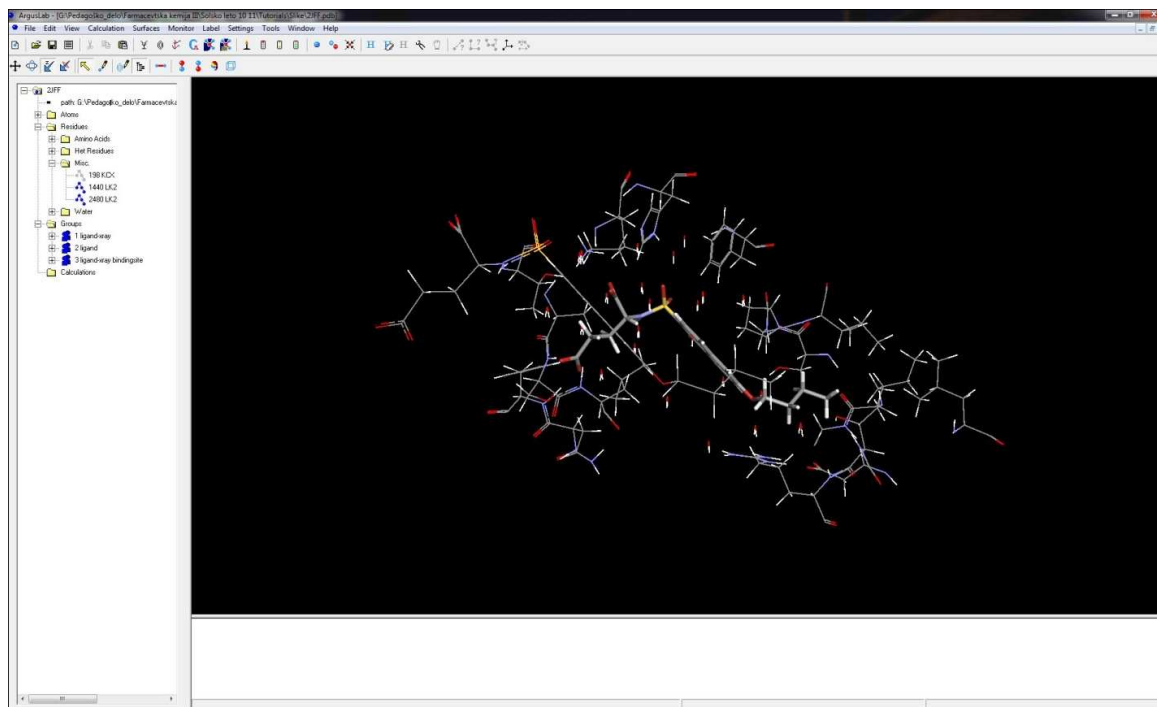
Make a Ligand group of this new residue in the same manner you did previously. Right-click on "2480 LK2" and select "**Make a Ligand Group from this Residue**". You should now have two Ligands in the **Groups** folder named "1 LK2" and "2 LK2".

Rename the two Ligand groups: Right-click on "1 LK2" in the **Groups** folder and select "*Modify Group...*" option. In the Modify Group Dialog box, type in a new name. Let's call it "**ligand-xray**". Make sure you don't change the Groups type. It should be a Ligand. Do the same for "2 LK2", but name it "**ligand**".

To more easily distinguish the two ligand in the graphics window, you can do a number of different things. One would be to change the rendering style of one of the ligands. Right-click on the **Group** named "**ligand-xray**" and select "*Set Render Mode*" and choose the "*Cylinder med*" option.

Make the **binding site** for the **ligand-xray** group. Right-click on the ligand-xray group in the Groups folder and select the "**Make a BindingSite Group for this Group**" menu option. This will generate a BindingSite that consists of all residues that have at least one atom within 3.5 Angstroms from any atom in the ligand-xray group

You should see something like this:

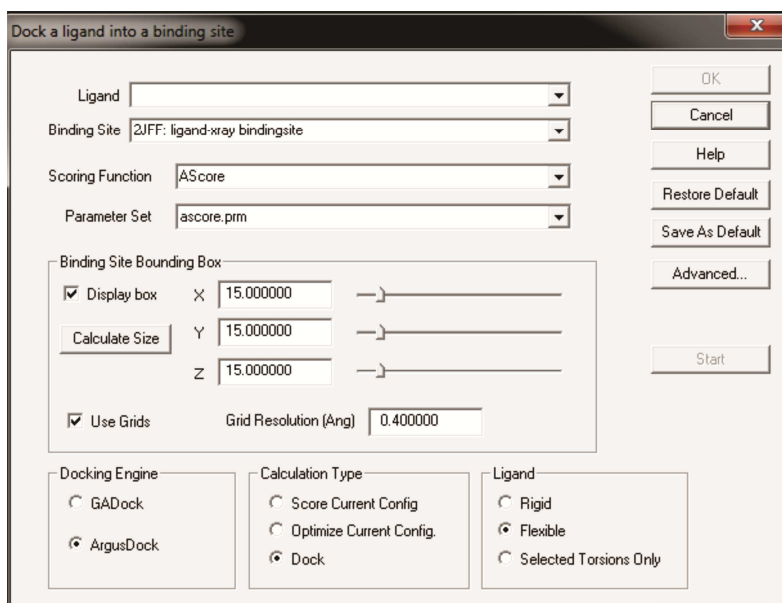


### 3. Dock the ligand into the defined binding site

Bring up the Dock Settings dialog box by selecting the *Calculation/Dock a Ligand* menu

option or clicking on the  button on the toolbar.

Here is what the Dock Settings dialog box looks like:



Select the ligand to dock in the "**Ligand**" drop-box. Make sure to select the group named "**ligand**" group and **NOT** the "**ligand-xray**" group.

Click on the "**Calculate Size**" button and a docking box tailored to the binding site will be made and shown on the screen.

Make sure "**ArgusDock**" is the selected docking engine, the **Calculation type = Dock**, and the Ligand is **Flexible**.

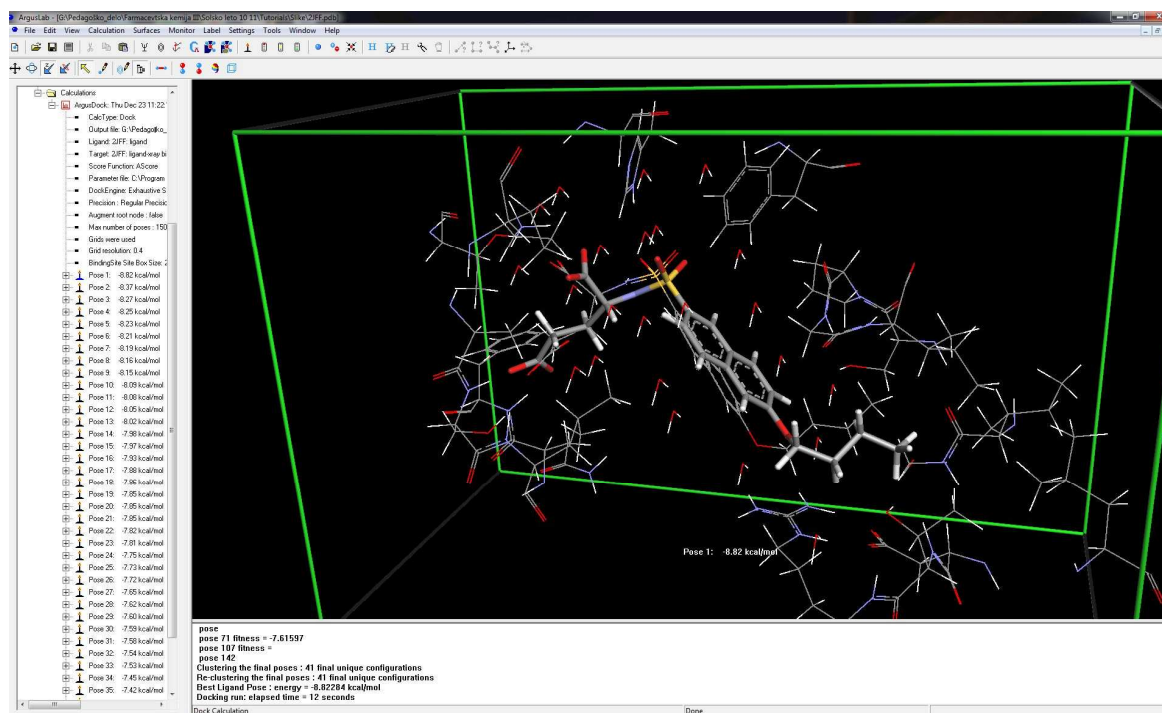
Click the "**Start**" button and the docking calculation will begin.

Notice the messages that appear in the status line at the bottom of the molecule window. You should see ArgusLab first generate the scoring grids used during the docking, then the various search phases will occur, and finally the candidate poses should be processed until the calculation is done. Only the coordinates of the group named "ligand" should have been modified.

#### 4. Analyze and save the results

There are two sources of information about the performed docking calculation: The separate log file and information located in the **Tree View tool**. Expand the ArgusDock calculation in the **Calculations folder** in the **Tree View**. You will see a listing of the docking settings and several "**poses**" starting with the most stable as **Pose 1** according to the calculated energy in kcal/mol. (A term "*pose*" is usually used to designate the specific set of coordinates of a docked structure). Right click on pose 2 and select the "Display" option. The coordinates of ligand will change to this pose and its score will be displayed on the screen.

The screen should look like this:



Now you can examine how close the docked structure is to the x-ray structure. You will calculate the RMSD difference between the x-ray and docked ligand - Pose 1 which you must select. Then in the **Tree View tool**, select both the **ligand** and **ligand-xray** groups by holding down the "Ctrl" key and left-clicking on both groups in the **Groups** folder. Both groups should be highlighted on the screen. Now, right-click on the **"Groups" folder tab** in the **Tree View** and select option *"Calc RMSD position between two similar Groups"*. An information dialog will appear on the screen giving the root mean square deviation (**RMSD**) difference in the coordinates of the two ligands. In order to be able to conclude that the experimental and calculated conformation are comparable the RMSD value should be less than approximately 2.5 Angstroms. Write the calculated RMSD value between the **Pose1** and experimental **ligand-xray structure** on your seminar answer sheet.

Finally, to save the work you have done, you will need to save this molecule to an **ArgusLab .agl file**. Select the *File/Save As...* menu option and choose the *ArgusLab* file type and save it. The file name should be: **Naloga4.agl**.

**Literature:**

1. Original Arguslab docking tutorial available on-line ([www.arguslab.com](http://www.arguslab.com)) and in the help section of the program.
2. Article describing the X-ray structure: Kotnik M, Humljan J, Contreras-Martel C, Oblak M, Kristan K, Hervé M, Blanot D, Urleb U, Gobec S, Dessen A, Solmajer T **Structural and functional characterization of enantiomeric glutamic acid derivatives as potential transition state analogue inhibitors of MurD ligase.** *J Mol Biol* **2007**, 370:107–115.