TP: modeling project

Terms of the practical courses

- The students will be divided into six groups for this modeling project.
- Each group will write a short report with the results and their discussion.
- Theses reports will be submitted on the Virtual University (VU); the students will be informed about the deadlines to submit the reports.

Tasks to perform

For this practical course, you will need to install on your computer the *Modeller* and the *Pymol* programs. *Modeller* is available here: https://salilab.org/modeller/download_installation.html. Register first for a free license: https://salilab.org/modeller/registration.html.

Pymol is available on the Virtual University (VU).

You will work on the 3D structure of house dust mite allergens. These allergens are classified into different families. Allergens from group 5 have been isolated from several species: Der p 5 from *Dermatophagoides pteronyssinus*, Blo t 5 from *Blomia tropicalis,* Der f 5 from *Dermatophagoides farinae*, ... The 3D structures of Der p 5 and of Blo t 5 have been experimentally resolved, and their PDB code are 3MQ1 and 2JMH, respectively. These structures are described in the papers of Mueller *et al.* (2010) and Naik *et al.* (2008); these papers are available on the VU.

The PDB structure of Blo t 5 is a monomer composed of three alpha-helices, whereas Der p 5 is a dimer.

Task 1. Analysis of the proteins

A] Dowload and save the sequences of Der p 5 (Uniprot code P14004), Blo t 5 (Uniprot code O96870) and Der f 5 (Uniprot code Q3LHF8).

B] Analyze the quality of the 6 chains that are in the PDB file 3MQ1. Use for that purpose the PDB website (http://www.pdb.org) and the Electron Density Server (http://eds.bmc.uu.se/eds/ ; use the "Significant regions" and "Ramachandran" sections of the website). Which chain has the best quality?

C] Use the *PDBeFold* server (http://www.ebi.ac.uk/msd-srv/ssm/) to superimpose the 3D structure of 2JMH and chain A of 3MQ1. Visualize the structure superimposition in *Pymol* and analyze the result. Which structural feature of 3MQ1 allows the formation of the dimer?

D] Read the paper of Mueller *et al*. Which residues are considered as important for the dimerization of the protein? Use the sequence alignment provided on the VU to analyze the sequence conservation at these positions.

You will study *in silico* the propensity of Blo t 5 and Der f 5 to form a dimer and the importance of the residues identified in Task 1.D. for the stability of the dimers. For that purpose, we will (1) model the structure of Blo t 5 and Der f 5, (2) model the possible structure of their dimer by protein-protein docking.

Task 2. Modeling of the structure of Blo t 5 and Der p 5

A] Read the tutorial to build a model with *Modeller*: section 4 of the "Basic example" of the tutorial (http://salilab.org/modeller/tutorial/basic.html). The files used in this basic example are downloadable.

B] You will create a population of 10 models for Blo t 5 and for Der f 5 by using several templates. The pairwise global sequence alignment can be performed with Stretcher (available on http://mobyle.pasteur.fr/, section alignment/pairwise/global; ask for an output fasta format in the advanced options). Use the normalized DOPE score to rank the 10 models.

B1. Create a population of 10 models for Der f 5 by using 2JMH as template.

B2. Create a population of 10 models for Der f 5 by using chain A of 3MQ1 as template.

B3. Create a population of 10 models for Blo t 5 by using chain A of 3MQ1 as template.

Groups 1 and 2 will perform task B1; groups 3 and 4 will perform task B2 and groups 5 and 6 will perform task B3. The 6 groups will share the 3D models that they obtained and the normalized DOPE scores obtained for all the models.

C] Analyze the obtained models. Compare the scores obtained for the Der f 5 models with the 2 templates. Select the best model for tasks B1, B2 and B3, according to the normalized DOPE score.

D] Use *Pymol* to create a PDB file for a mutated Der p 5 structure. You will mutate the Val at positions 85 and 88 into Ala to test the assumption that they provide stability to the dimer (see paper of Mueller *et al*.). For that purpose:

- open 3MQ1 in *Pymol*;
- create an object that contains only chain A: "create 3MQ1A, chain A and 3MQ1";

- select residues 85 and 88 of chain A: "select res85-88, resi 85+88 and 3MQ1A";
- show these residues in sticks;
- mutate residues 85 and 88 into Ala: menu Wizard – mutagenesis, select "Mutate to ALA", click the first residue to mutate and then "Apply", click the second residue to mutate and then "Apply"; click "Done";
- save the mutated PDB: File – Save Molecule, and select 3MQ1A.

Task 3. Modeling of the structure of the dimers

You will use the *ClusPro* protein-protein docking server (https://cluspro.bu.edu/).

A] Which kind of energy function is used by *ClusPro*? Which criteria are used to rank the models?

B] You will first test the performances of the *ClusPro* server. For that purpose, submit the structure of chain A of 3MQ1 to *ClusPro*. Compare, with *PDBEFold*, the solutions obtained with *ClusPro* to the experimental structure of the dimer (chains A and B of 3MQ1). If you want to superimpose dimers with *PDBeFold*, unclick "match individual chains" on the submission form. Analyze the results. Which cluster contains the closest structure to 3MQ1 dimer (chains A and B): the most populated cluster or the cluster with the lowest energy structure?

C] You will generate a dimer structure for Der f 5, Blo t 5 and mutated Der p 5.

C1. Submit to *ClusPro* the best model of Der f 5 obtained with chain A of 3MQ1 as template (Tasks 2.B2 and 2.C).

C2. Submit to *ClusPro* the best model of Blo t 5 obtained with chain A of 3MQ1 as template (Tasks 2.B3 and 2.C).

C3. Submit to *ClusPro* the mutated structure of chain A of Der p 5 (Task 2.D).

Groups 1 and 2 will perform task C1; groups 3 and 4 will perform task C2 and groups 5 and 6 will perform task C3. The 6 groups will share the 3D models that they obtained (most populated cluster and cluster with the lowest energy structure).

D] Analyze these results. Are Der f 5 and Blo t 5 able to form a dimer that is structurally similar to Der p 5? Compare the energies of the dimers. Do the mutations Val 85 Ala and Val 88 Ala induce a change in the computed stability of the dimer and in the predicted structure of the dimer (compare with the dimer obtained in Task 3.B)?