

Supplementary Information

Docking Simulations with the Software Rosetta ¹

Protocol for the simulations between FhuA and Ib-M6 (EWGRRMMGWGRGRRMMRRWW-NH2) peptide models

Steps:

1. The crystallographic structure of the FhuA protein was obtained from the Protein Data Bank database with PDBid 1QFG.
2. Create initial FhuA-IbM6 complexes using Pymol and the different models of the Ib-M6 peptide.
3. Use the Rosetta python script: cleanPDB to remove unknown atoms, water molecules and to renumber the atoms of the complexes.
4. Run the protocol for protein-protein docking for an initial global docking process between protein FhuA and the peptide:

docking_protocol.linuxgccrelease @**flag_global_docking**

- Details of **flag_global_docking** file:

```
-in:file:s input_files/complex.pdb
-unboundrot input_files/complex.pdb

-nstruct 500

-partners A_B
-dock_pert 3 8
-spin
-randomize1
-randomize2

-ex1
-ex2aro

-out:path:all output_files
-out:suffix _Model_
```

5. Analyze the best models in terms of I_{sc} and location of the peptide in the iron recognition domain.
6. Perform the refinement protocol using FlexPepDocking protocols
 - Perform a prepack step with the selected model, using the FlexPepDocking protocol with the file: **prepack_flags**

- Details of the **prepack_flags**
 - s input_files/best_model.pdb
 - ex1
 - ex2aro
 - use_input_sc
 - unboundrot input_files/best_model.pdb
 - flexpep_prepack
 - nstruct 1
- Rename the obtained file as best_model_ppk.pdb
- Run the FlexPepDocking protocol with the file **flagsNoLow**
 - Details of the file **flagsNoLow**
 - s input_files/best_model_ppk.pdb
 - out:pdb
 - out:path:all output_files
 - scorefile score.sc
 - nstruct 200
 - flexPepDocking:flexpep_score_only
 - flexPepDocking:pep_refine
 - ex1
 - ex2aro
 - unboundrot input_files/best_model.pdb
 - use_input_sc
 - mute protocols.moves.RigidBodyMover
 - mute core.chemical
 - mute core.scoring.etable
 - mute protocols.evaluation
 - mute core.pack.rotamer_trials
 - mute protocols.abinitio.FragmentMover
 - mute core.fragment
 - mute protocols.jd2.PDBJobInputter

7. Analyze the results using Chimera and VMD.

Protocol for the simulations between LPS and Ib-M6 peptide models

1. The crystallographic structure of the FhuA protein was obtained from the Protein Data Bank database with PDBid 1QFG.
2. Create initial FhuA-Ib-M6 complexes using Pymol and the different models of the Ib-M6 peptide.
3. Use the Rosetta python script: clean_pdb_keep_ligand.py to remove water molecules and to renumber the atoms of the complexes.

4. Run the protocol for protein-protein docking for an initial global docking process between LPS and the peptide:

docking_protocol.linuxgccrelease @**flag_global_docking**

- Details of **flag_global_docking** file:

```
-in:file:s input_files/complex.pdb
-unboundrot input_files/complex.pdb

-nstruct 500

-partners A_B
-dock_pert 1 1
-spin
-randomize2

-ex1
-ex2aro

-out:path:all output_files
-out:suffix _Model_
```

8. Analyze the best models in terms of I_{sc} and the proximity to the LPS.
9. Perform the refinement protocol using FlexPepDocking protocols

- Run the FlexPepDocking protocol with the file **flagsNoLow**

- Details of the file **flagsNoLow**
 - s input_files/best_model.pdb
 - out:pdb
 - out:path:all output_files
 - scorefile score.sc
 - nstruct 200
 - flexPepDocking:flexpep_score_only
 - flexPepDocking:pep_refine
 - ex1
 - ex2aro
 - unboundrot input_files/best_model.pdb
 - use_input_sc
 - mute protocols.moves.RigidBodyMover
 - mute core.chemical
 - mute core.scoring.etable
 - mute protocols.evaluation
 - mute core.pack.rotamer_trials
 - mute protocols.abinitio.FragmentMover
 - mute core.fragment

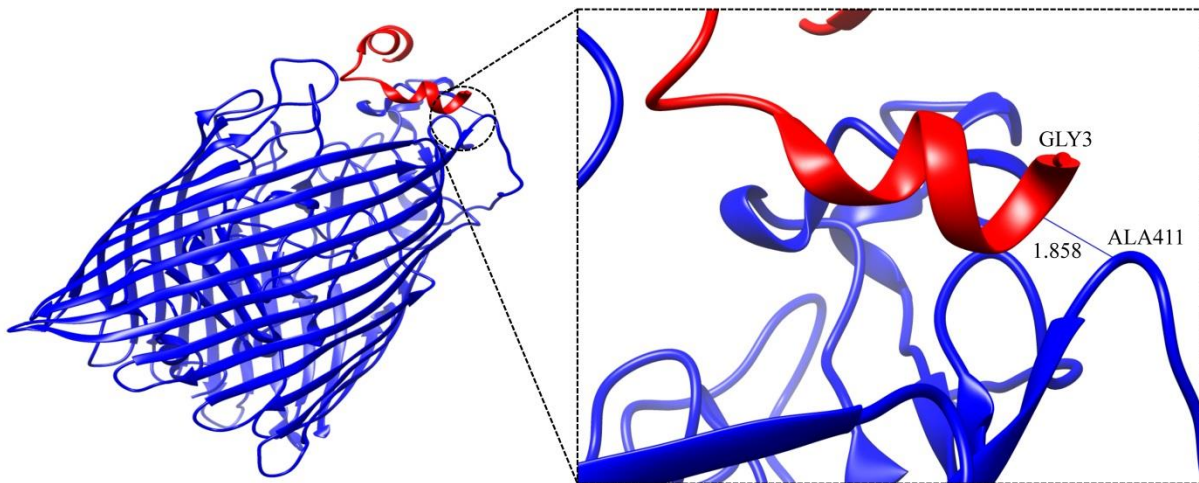
-mute protocols.jd2.PDBJobInputter

10. Analyze the results using Chimera and VMD.

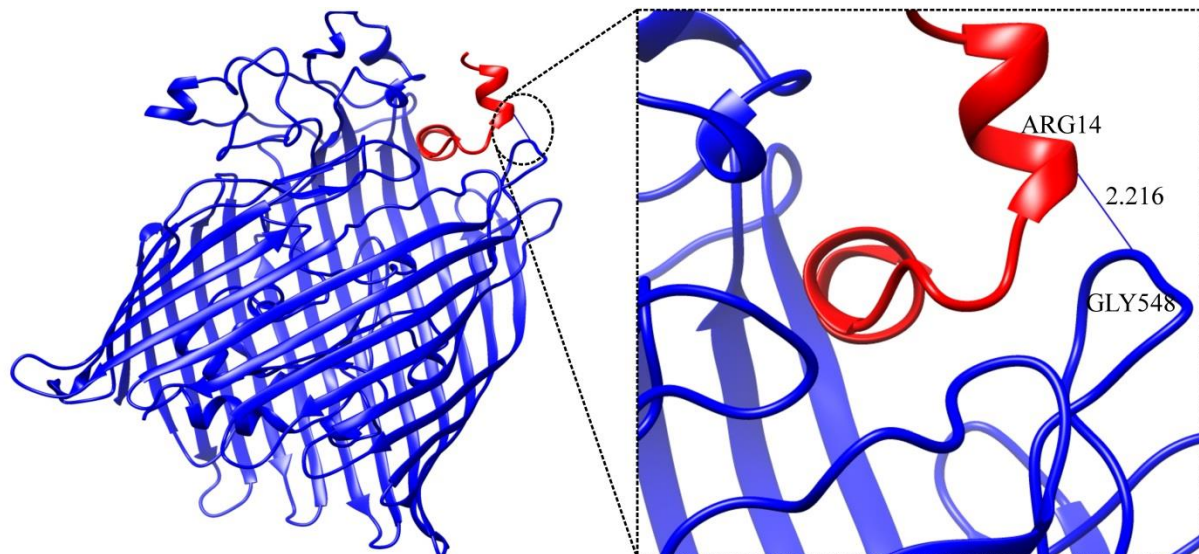
Images of the resulting complexes between FhuA and Ib-M6 peptide models

Model FhuA-M6_A

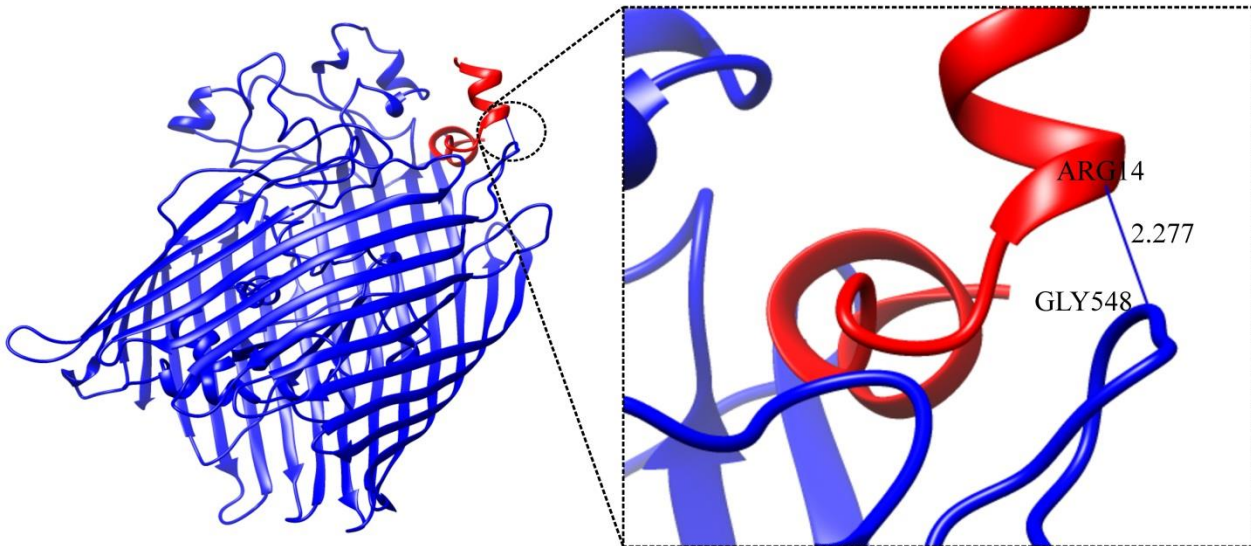
- Model FhuA-M6_A_1



- Model FhuA-M6_A_2

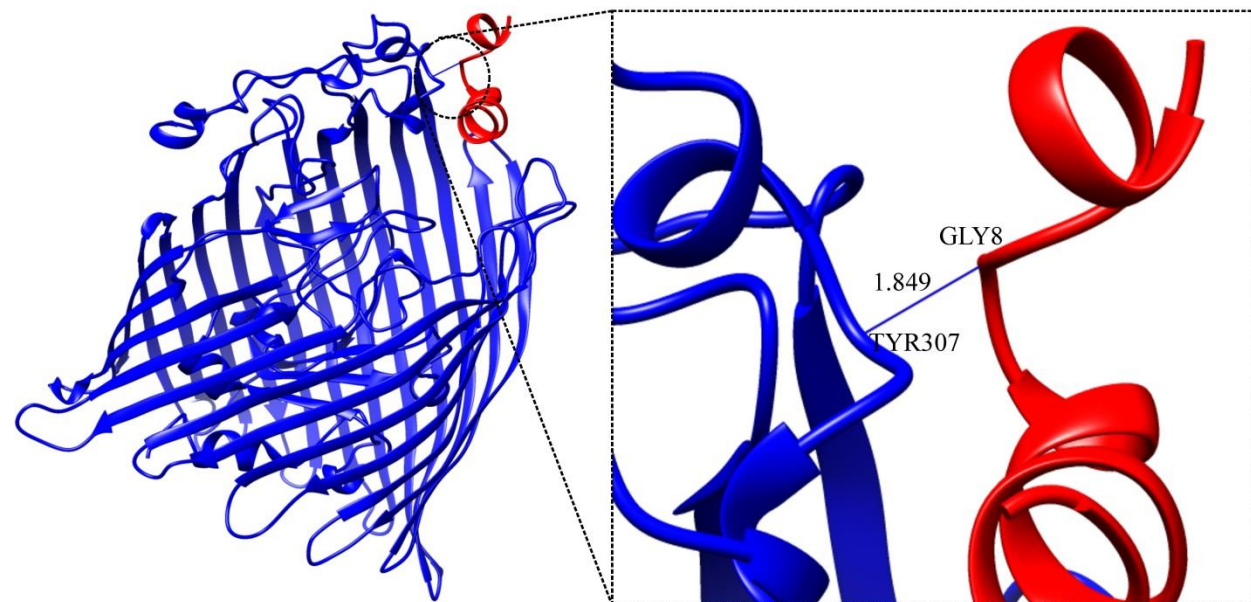


- Model FhuA-M6_A_3

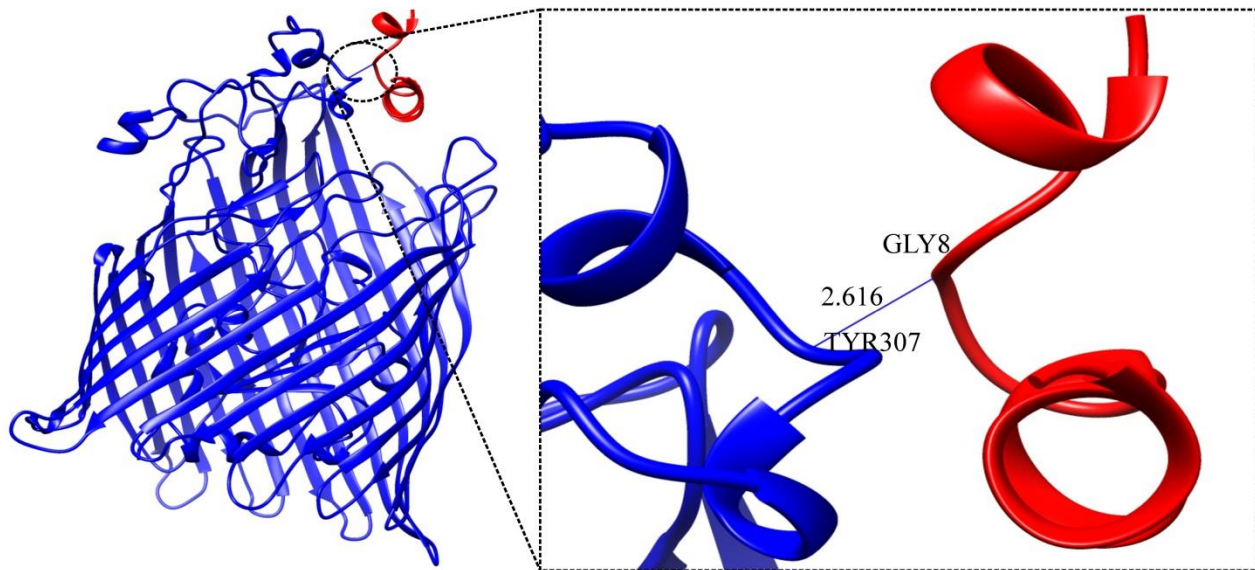


Model FhuA-M6_B

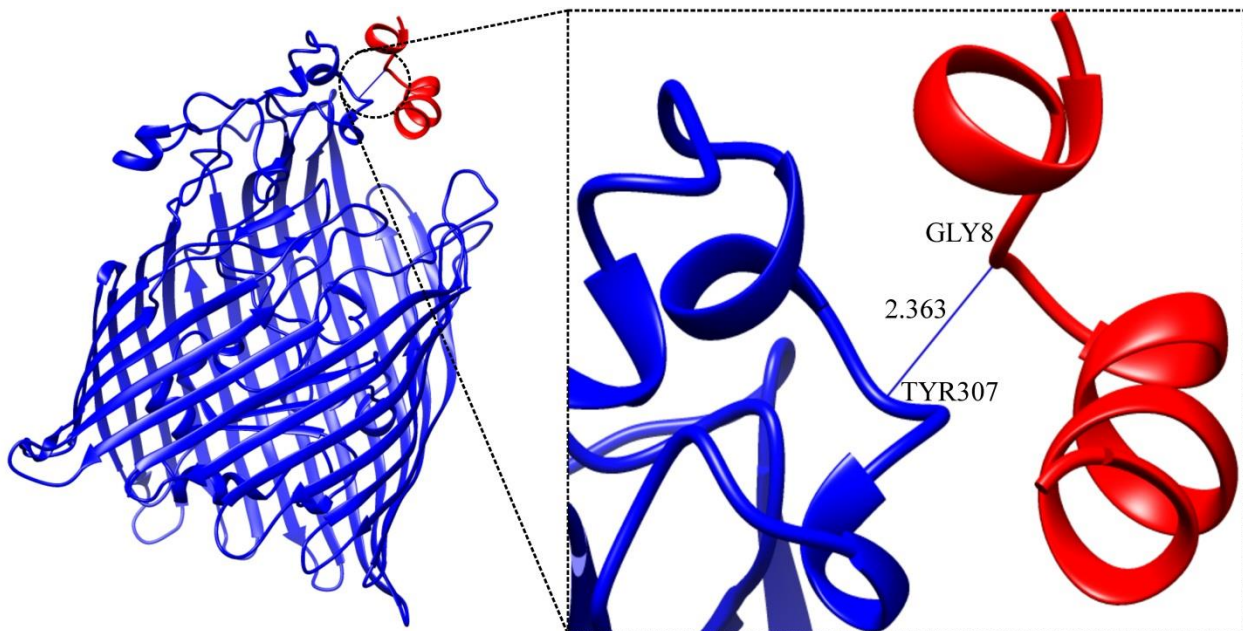
- Model FhuA-M6_B_1



- Model FhuA-M6_B_2

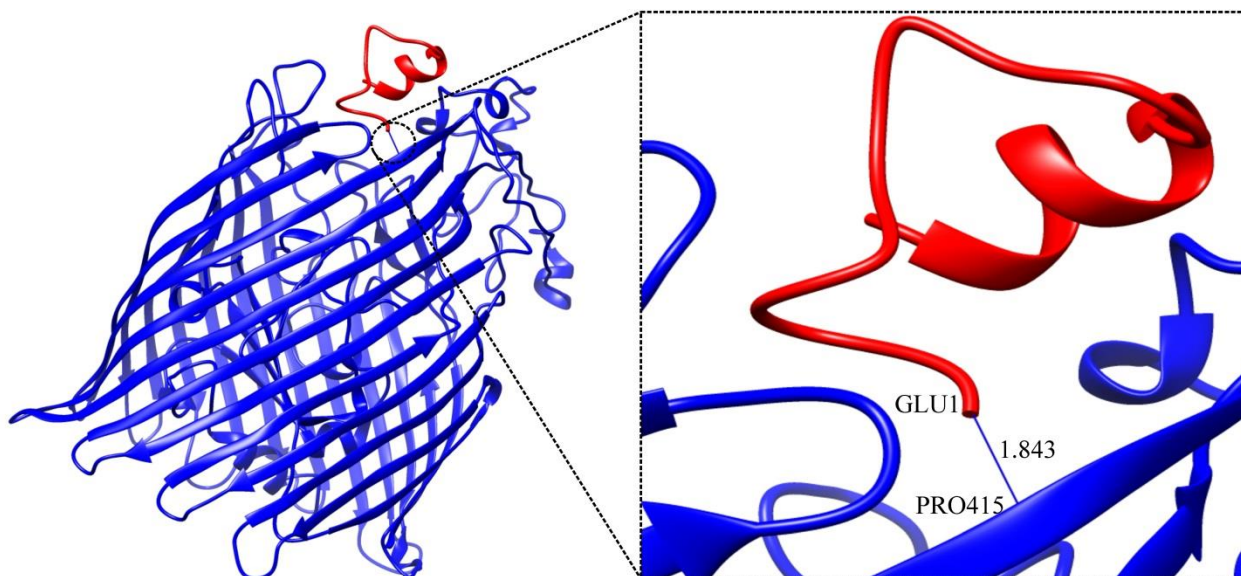


- Model FhuA-M6_B_3



Model FhuA-M6_D

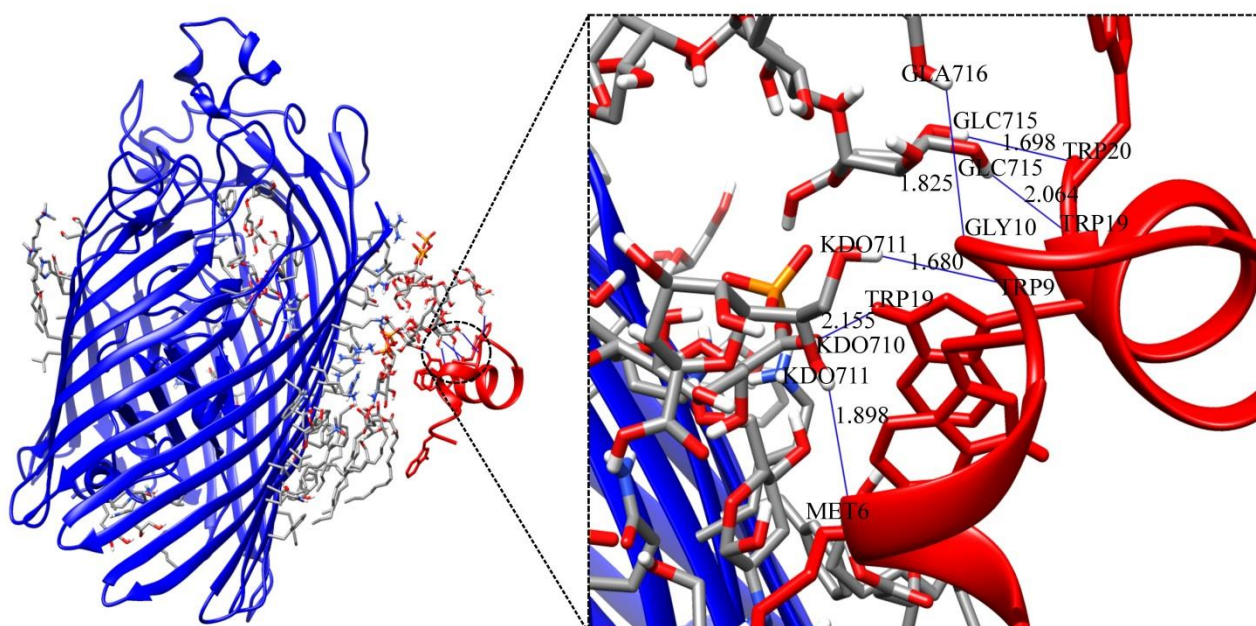
- Model FhuA-M6_D_1



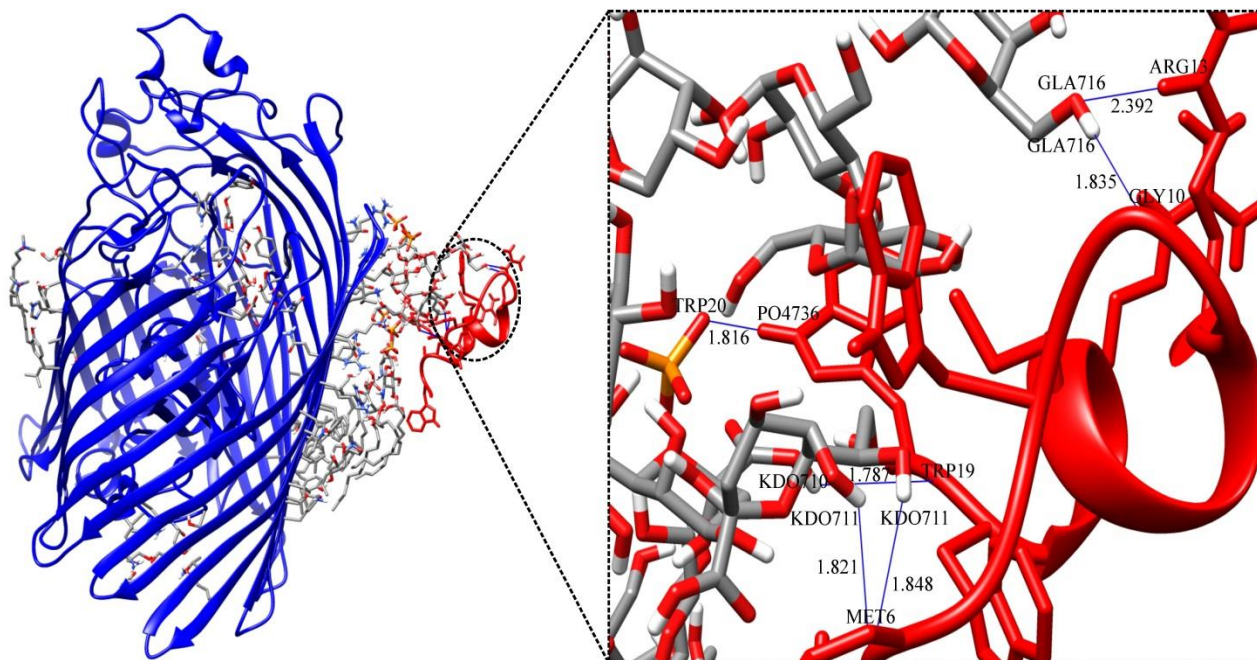
Images of the resulting complexes between LPS and Ib-M6 peptide models

Model LPS-M6_A

- Model LPS-M6_A_1

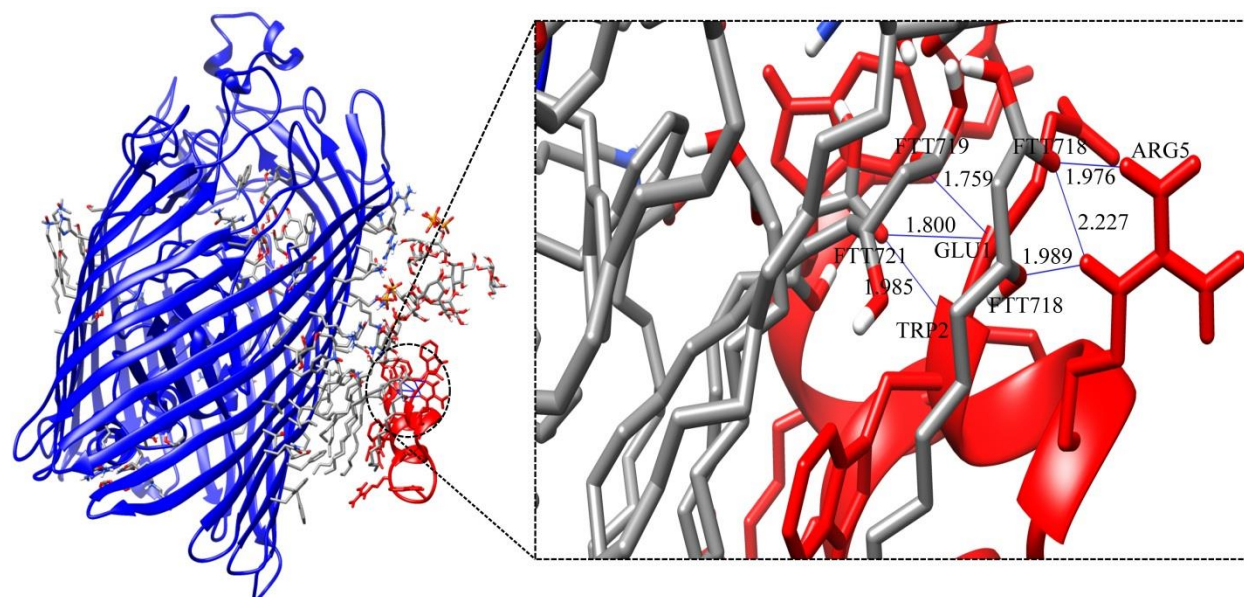


- Model LPS-M6_A_2

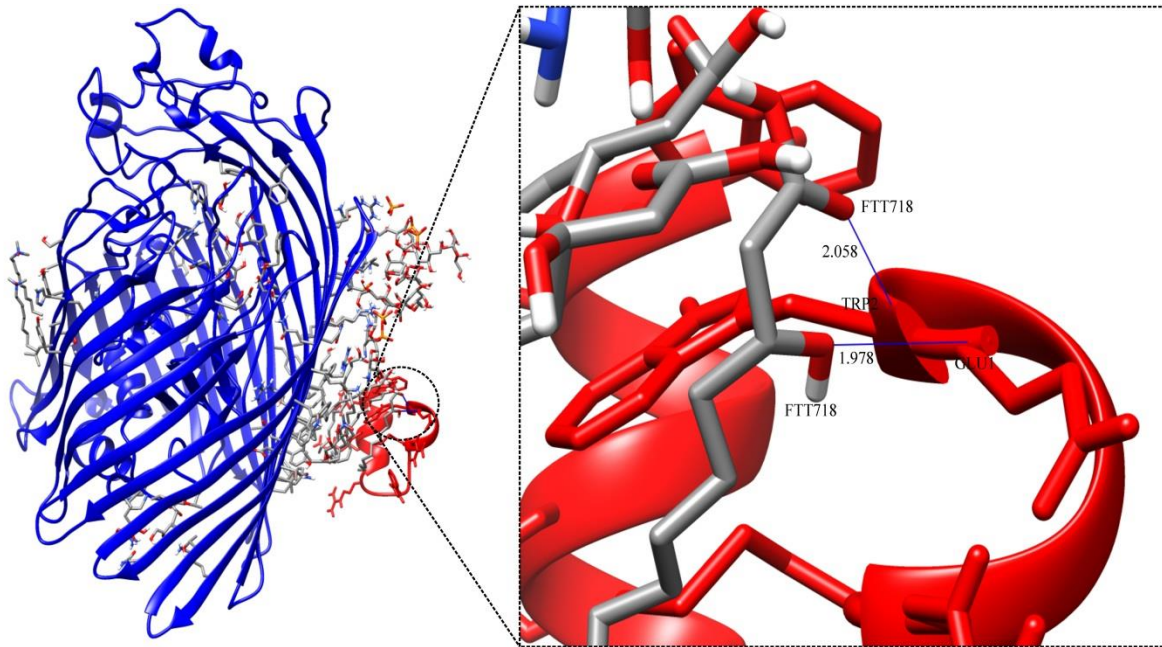


Model LPS-M6_B

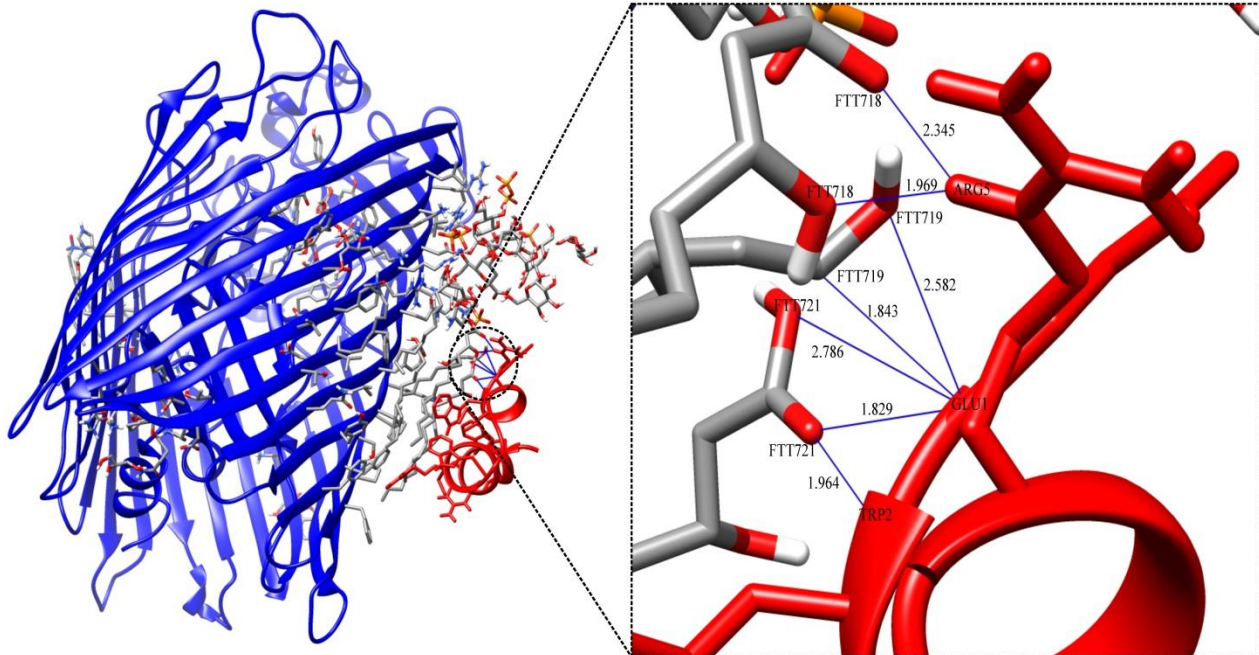
- Model LPS-M6_B_1



- Model LPS-M6_B_2

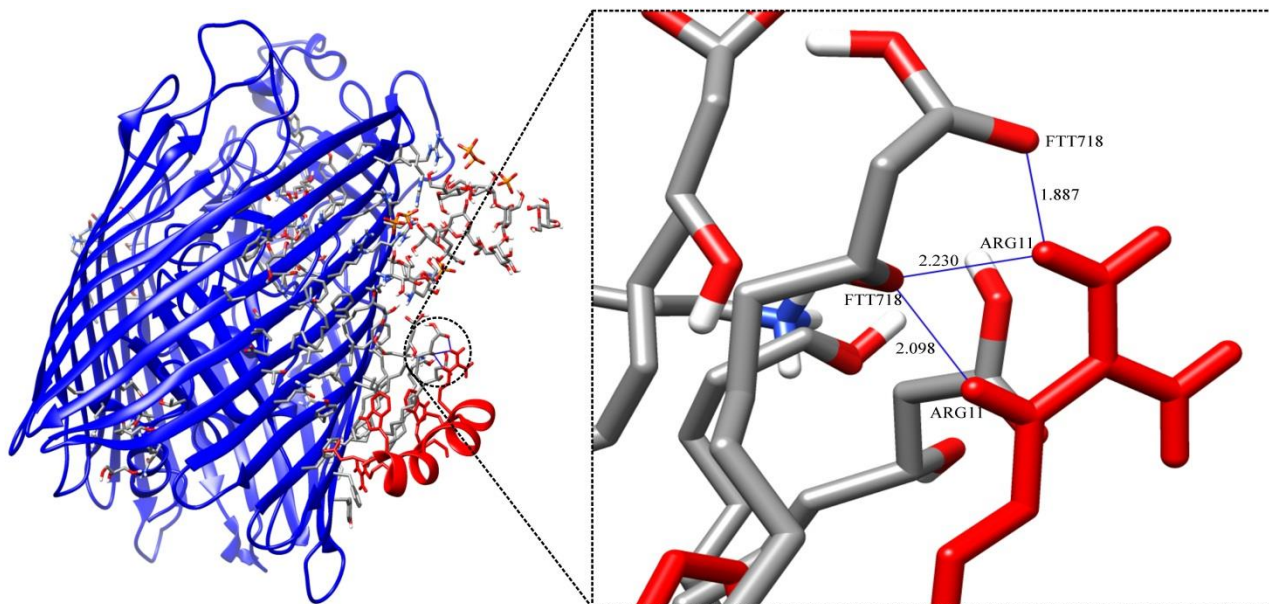


- Model LPS-M6_B_3

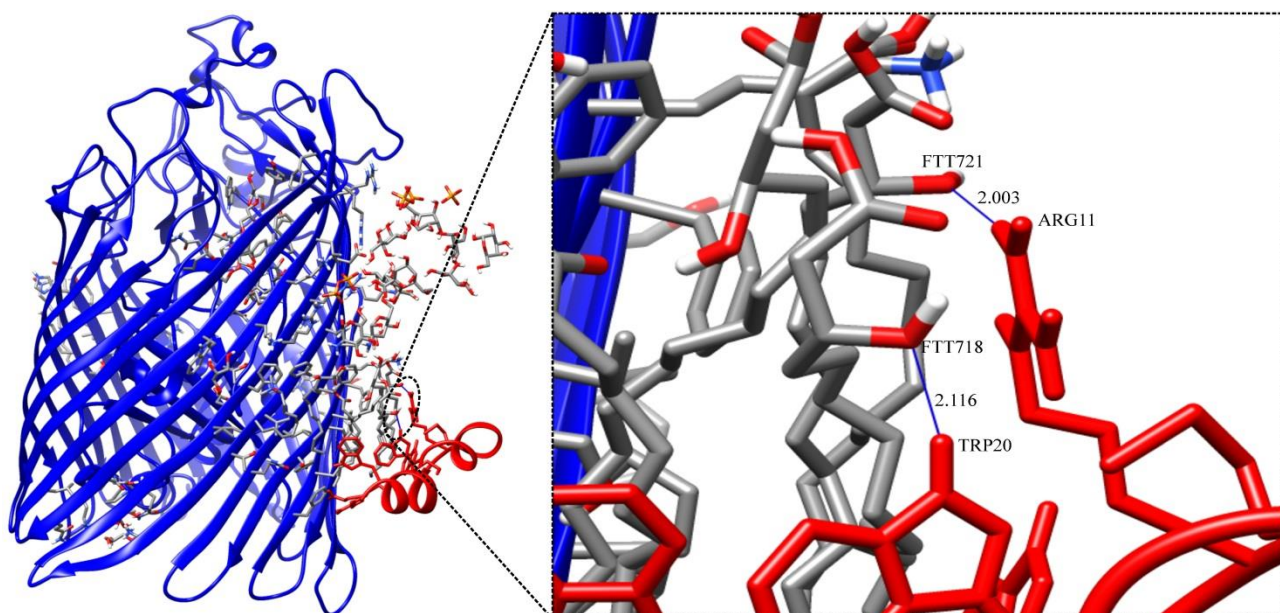


Model LPS-M6_C

- Model LPS_M6_C_2

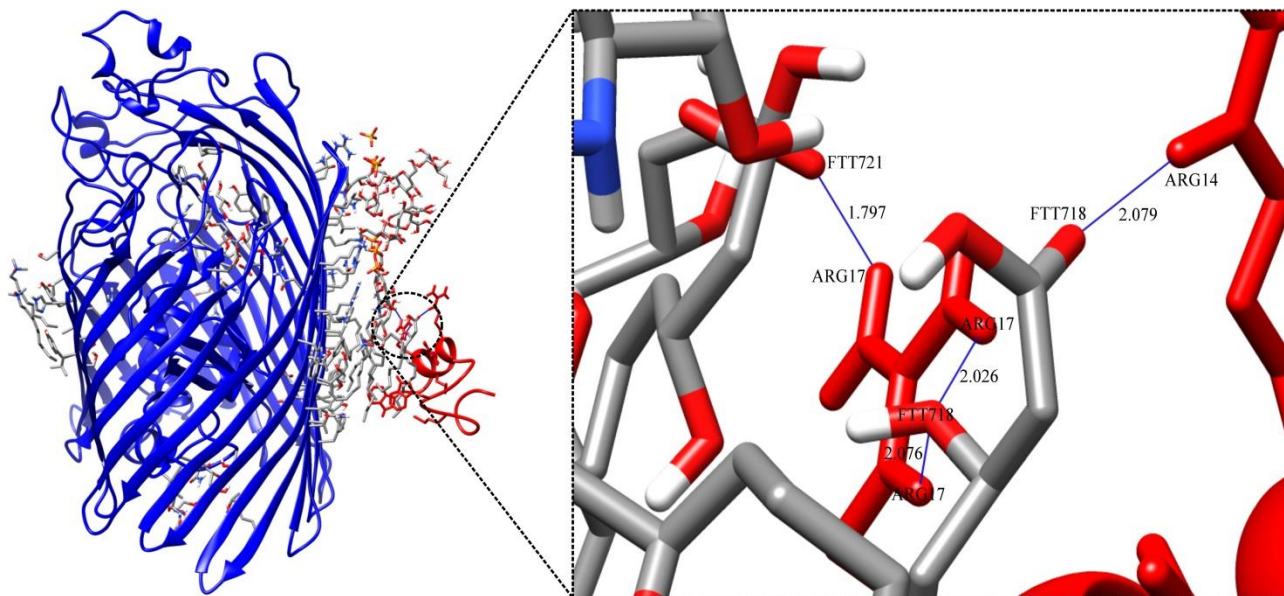


- Model LPS_M6_C_3

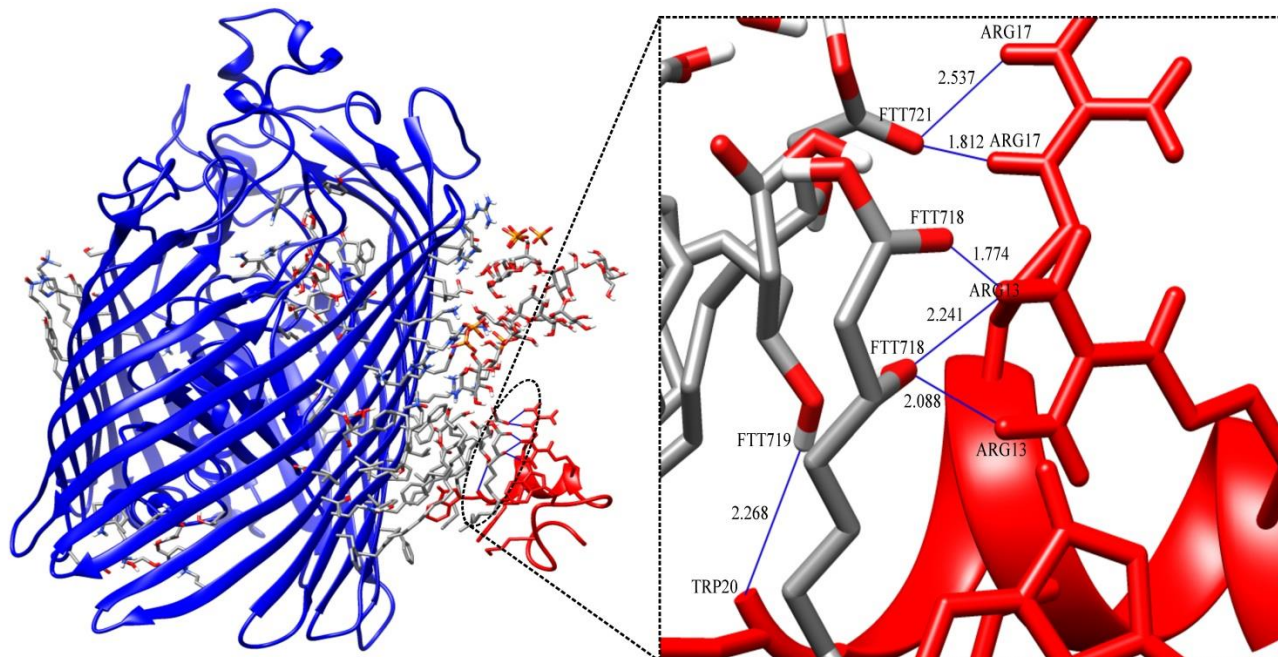


Model LPS-M6_D

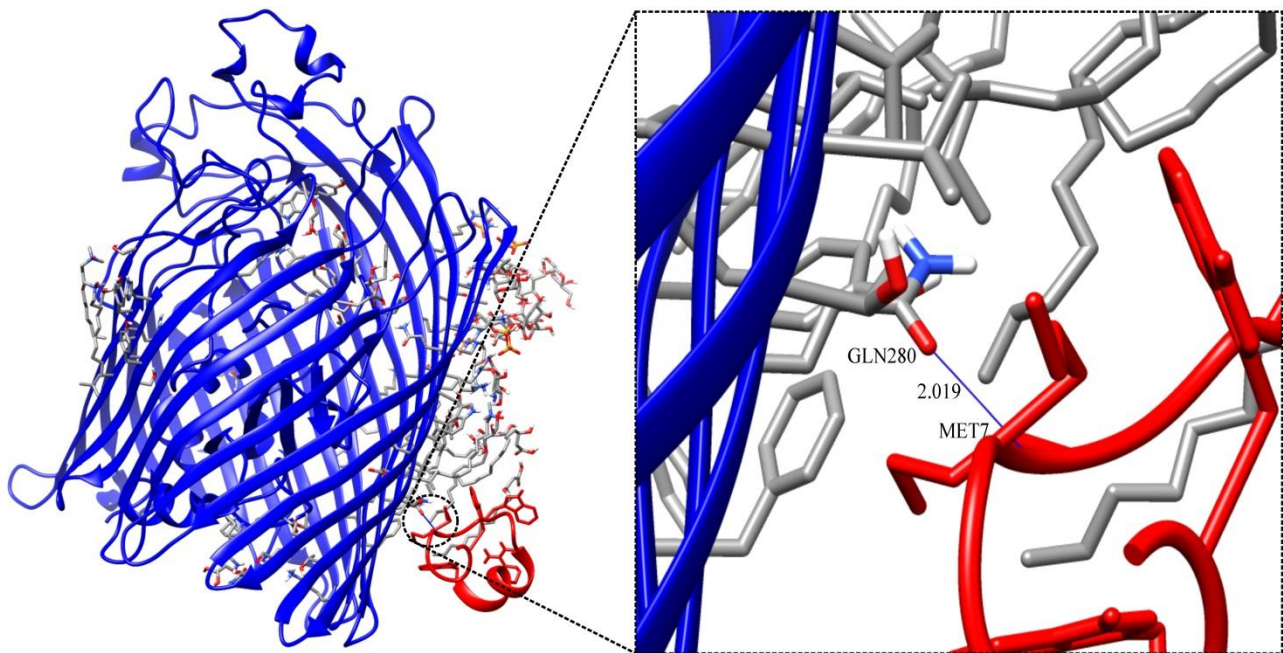
- Model LPS_M6_D_1



- Model LPS_M6_D_2

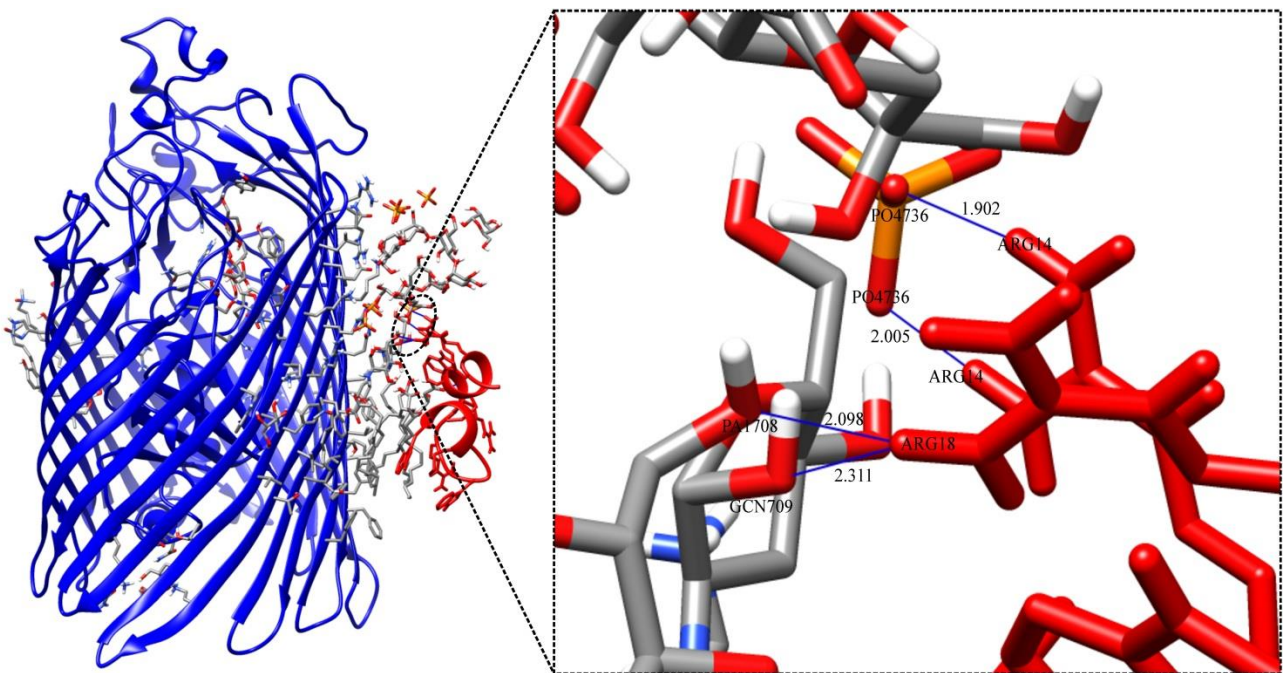


- Model LPS_M6_D_3

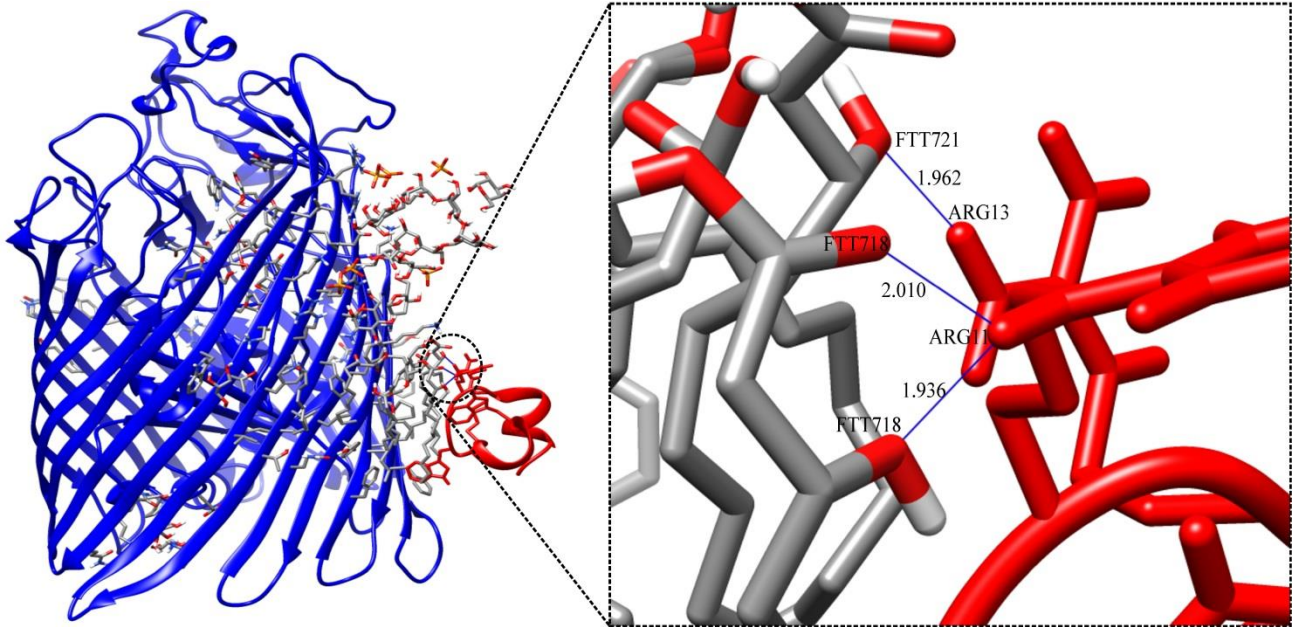


Model LPS-M6_E

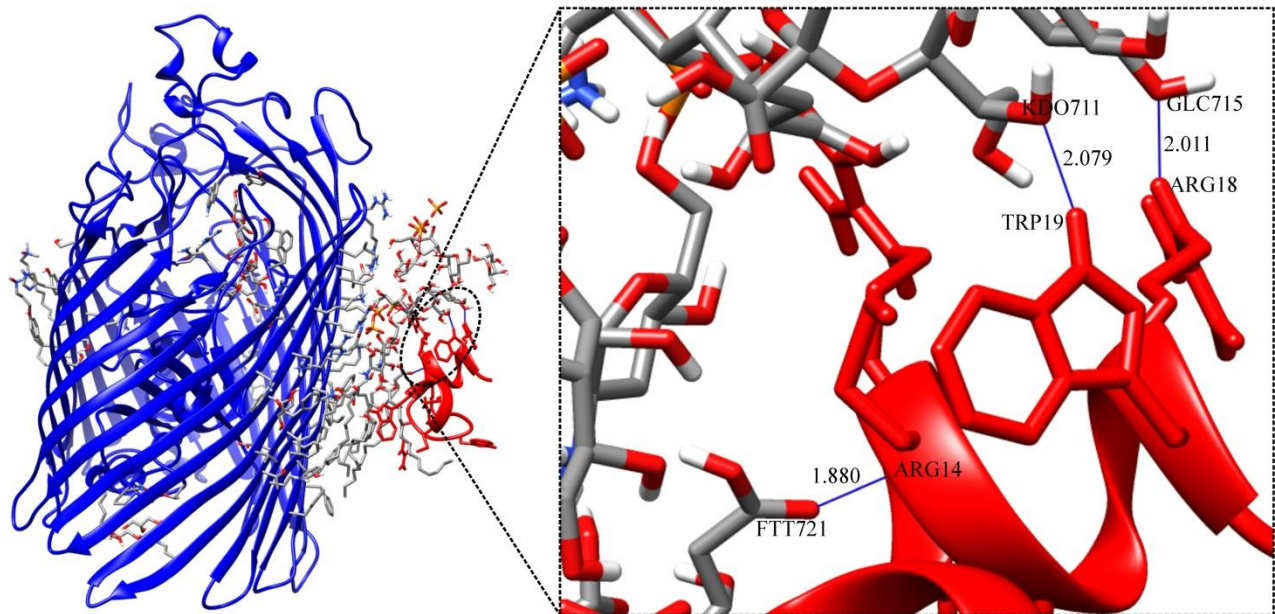
- Model LPS_M6_E_1



- Model LPS_M6_E_2



- Model LPS_M6_E_3



References

- 1 N. London, B. Raveh, E. Cohen, G. Fathi and O. Schueler-Furman, *Nucleic Acids Res.*, 2011, **39**, W249-53.

