Molecular Docking with PyMol

1. Firstly, the installation of the TeachOpenCADD software was carried out by following the steps outlined in Figure 1. The tutorial files provided contain detailed explanations of the procedures. The T015 protein-ligand docking tutorial will be followed based on these tutorials. Therefore, sample trial steps were applied, and the progression of the process was observed. It is crucial to interpret the obtained smina results, and as a result, the significance of each outcome has been understood.

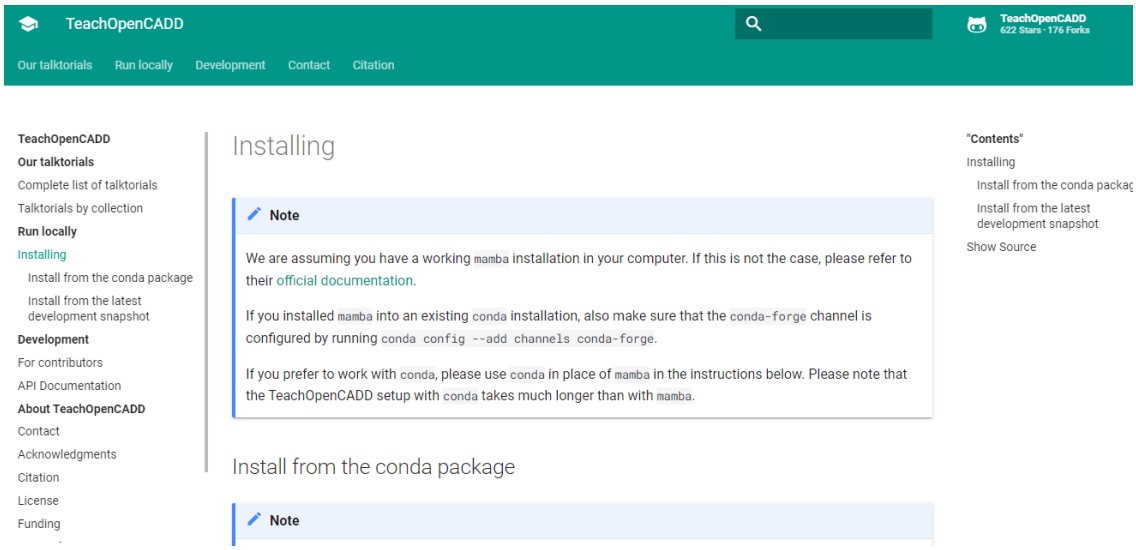

Figure 1. Installed the TeachOpenCADD.

2. The protein selected from the Protein Data Bank (PDB) is 2J6M, representing the crystal structure of the EGFR kinase domain in complex with the AEE788 protein. The respective protein is associated with the AEE ligand.

The visual representation of the 2J6M protein in PyMol is depicted in Figure 2.

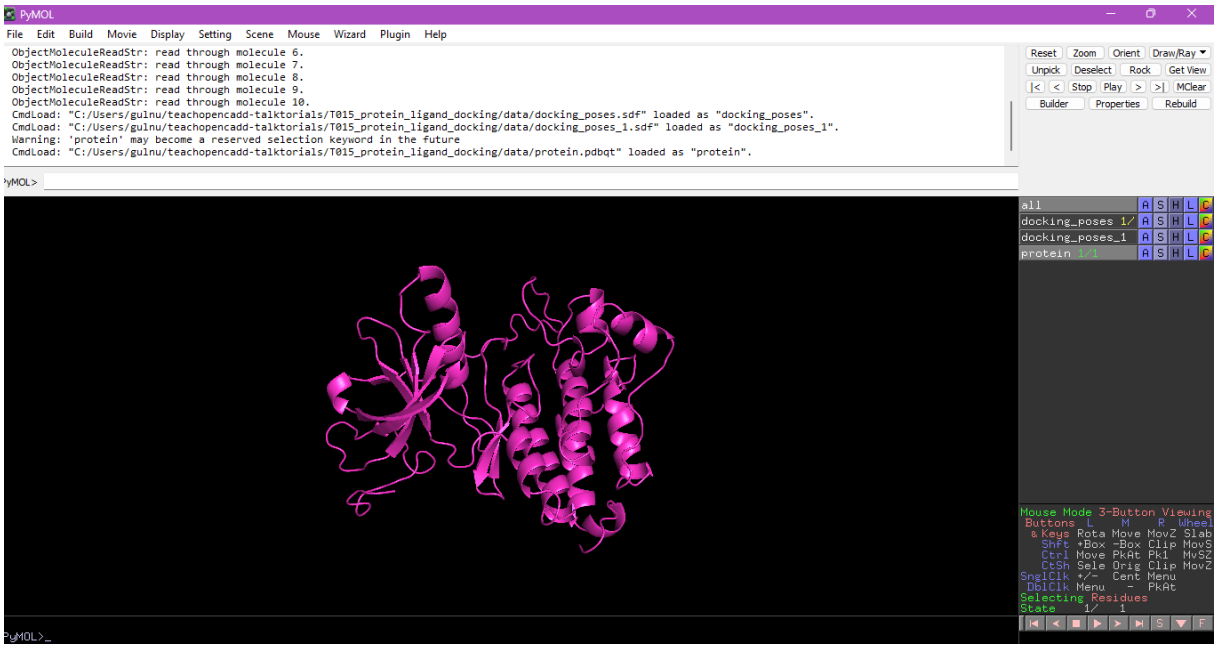

Figure 2. Visualization of 2JM6 on PyMol.

3. Abstract for the protein and inhibitor

2J6M-Crystal structure of EGFR kinase domain in complex with AEE788 (ligand: AEE)

EGFR kinase mutations contribute to the development of non-small-cell lung cancer. In our investigation to comprehend their activation mechanisms and impacts on drug binding, we explored the kinetics of the L858R and G719S mutants and elucidated their crystal structures in the presence of inhibitors such as gefitinib, AEE788, and staurosporine. Our findings indicate that these mutations activate the kinase by disrupting autoinhibitory interactions, resulting in a remarkable up to 50-fold acceleration of catalysis in vitro. The structures of inhibitors bound to both wild-type and mutant kinases revealed similar binding modes for gefitinib and AEE788. However, there was a notable rotation of staurosporine in the G719S mutant. Notably, direct binding measurements demonstrated that gefitinib binds 20-fold more tightly to the L858R mutant compared to the wild-type enzyme.

AEE-788 is a drug identified by the DB12558 code, classified under the "investigational" group. This drug has been utilized in trials for the treatment of Cancer, Glioblastoma Multiforme, and Brain and Central Nervous System Tumors. AEE-788 is also categorized under "Heterocyclic Compounds, Fused-Ring" and "Receptors, Vascular Endothelial Growth Factor, antagonists & inhibitors." This classification provides essential insights into the chemical structure and mode of action of the drug. The CAS number, serving as a unique identifier for its chemical structure, is 497839-62-0 (Figure 3).

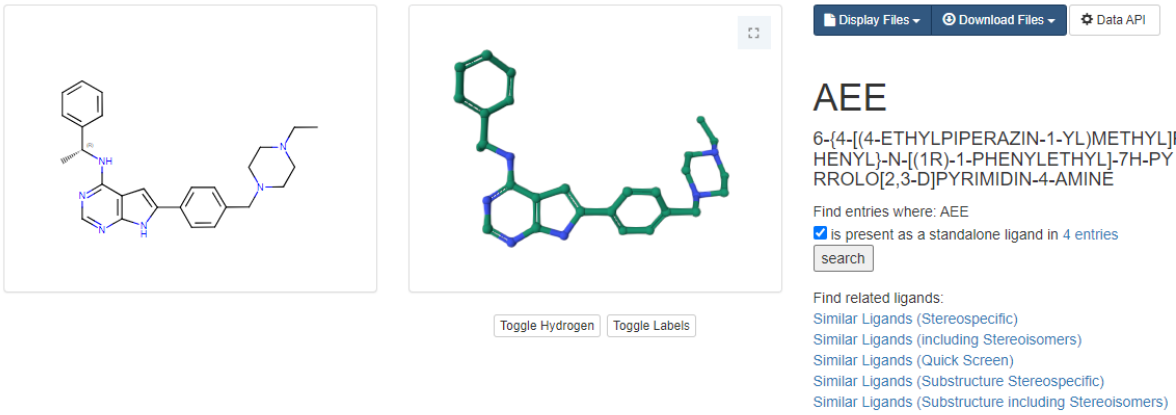

Figure 3. AEE Inhibitor.

4. For the redocking simulation, the talktorial template was adhered to, and smina was executed to obtain relevant results. For each exhaustiveness level, the individually predicted and average binding affinity scores by smina, along with the position of the ligand in the active region of the protein, were determined. Visualization was accomplished using PyMol. In addition to, 8 redocking simulations were performed for each exhaustiveness value. Given the requirement of at least 5 redocking simulations for each exhaustiveness value, 8 redocking simulations were conducted for every exhaustiveness value.

Exhaustiveness 8:

```
smina is based off AutoDock Vina. Please cite appropriately.

Weights      Terms
-0.035579    gauss(o=0,_w=0.5,_c=8)
-0.005156    gauss(o=3,_w=2,_c=8)
0.840245     repulsion(o=0,_c=8)
-0.035069    hydrophobic(g=0.5,_b=1.5,_c=8)
-0.587439    non_dir_h_bond(g=-0.7,_b=0,_c=8)
1.923        num_tors_div

Using random seed: -894080384

0%   10   20   30   40   50   60   70   80   90   100%
|----|----|----|----|----|----|----|----|----|----|
**************************************************

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
1       -9.6       0.000      0.000
2       -9.5       0.633      1.634
3       -9.3       1.144      2.717
4       -9.2       1.799      3.283
5       -8.9       1.815      2.826
6       -8.6       2.516      3.622
7       -8.4       1.952      3.588
8       -8.3       2.201      3.332
9       -8.2       2.499      3.391
10      -8.1       2.371      3.508
Refine time 54.977
Loop time 55.310
```

Figure 4. Report of the 8th redocking.

The affinity value of 9.6 implies a strong interaction between the two molecules. As this value decreases, it indicates a gradual reduction in the strength of the interaction.

The Root Mean Square Deviation (RMSD) value illustrates the structural similarity between docking results. An RMSD value of zero signifies almost no structural difference between the predicted structure and the reference structure. In this case, starting with an RMSD of 0, the value gradually increases, indicating a decreasing overlap between the docking results and the reference structure. This pattern is observed consistently across all exhaustiveness values.

The ranking of the top 10 docking poses is as follows:

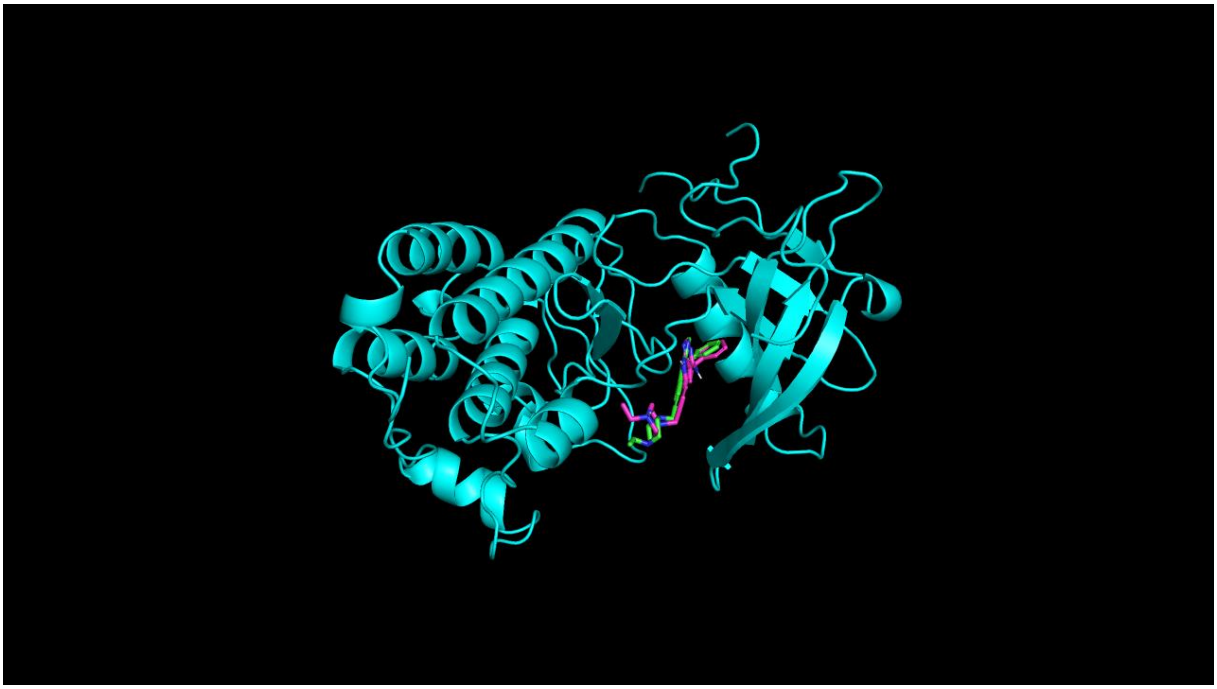

Figure 5. Best Docking Pose

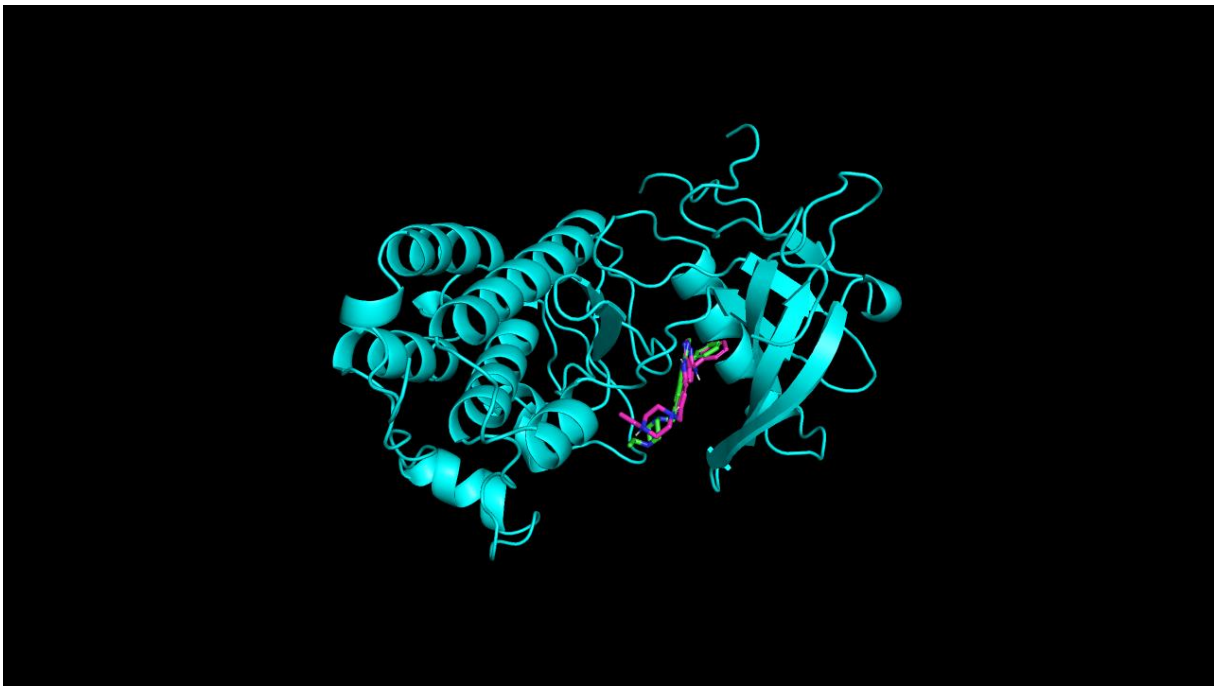

Figure 6. Second Best Docking Pose

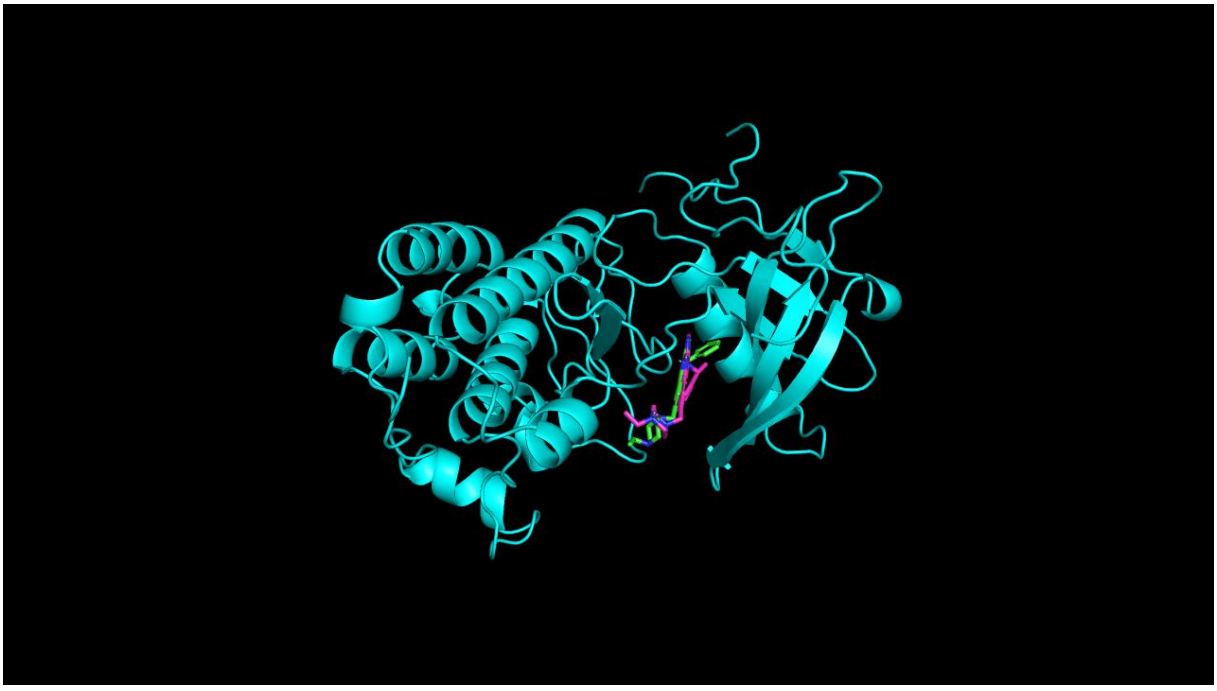

Figure 7. Third Best Docking Pose

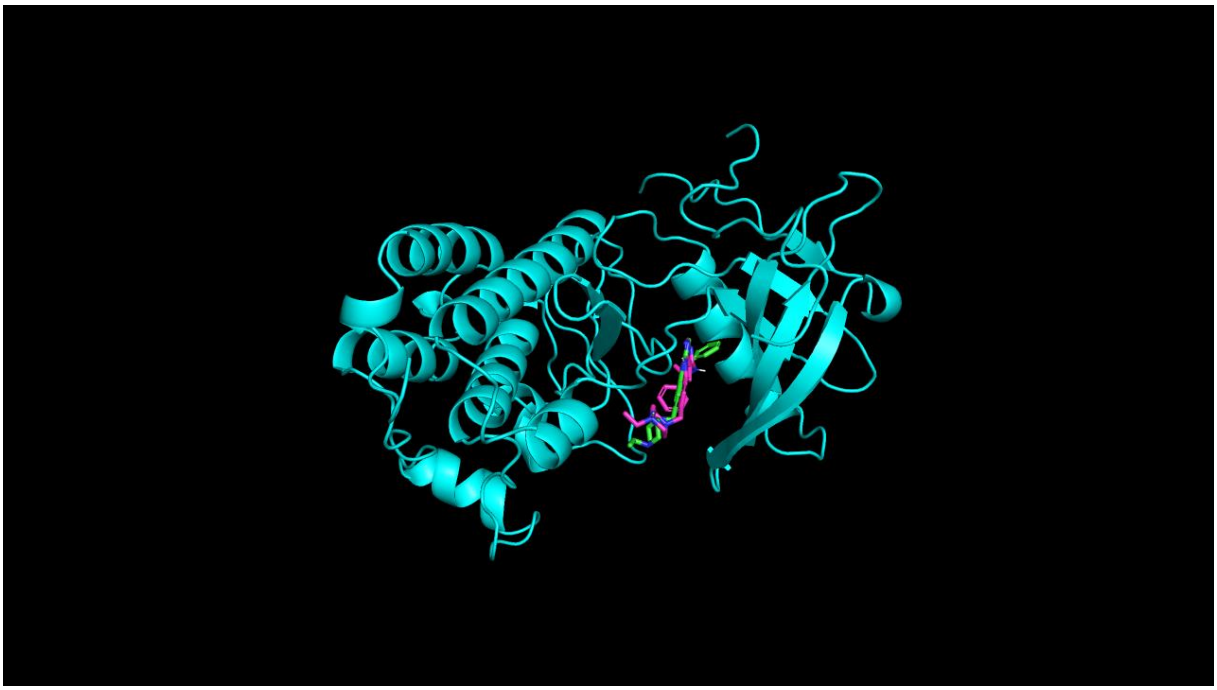

Figure 8. Fourth Best Docking Pose

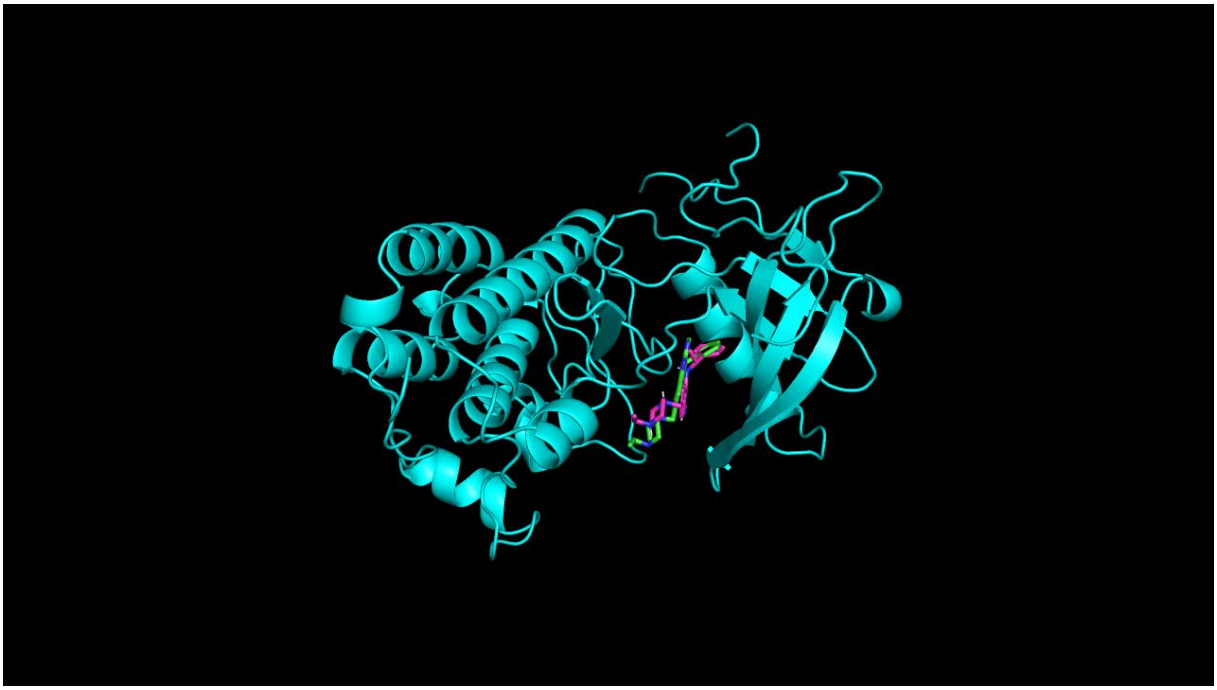

Figure 9. Fifth Best Docking Pose

Figure 10. Sixth Best Docking Pose

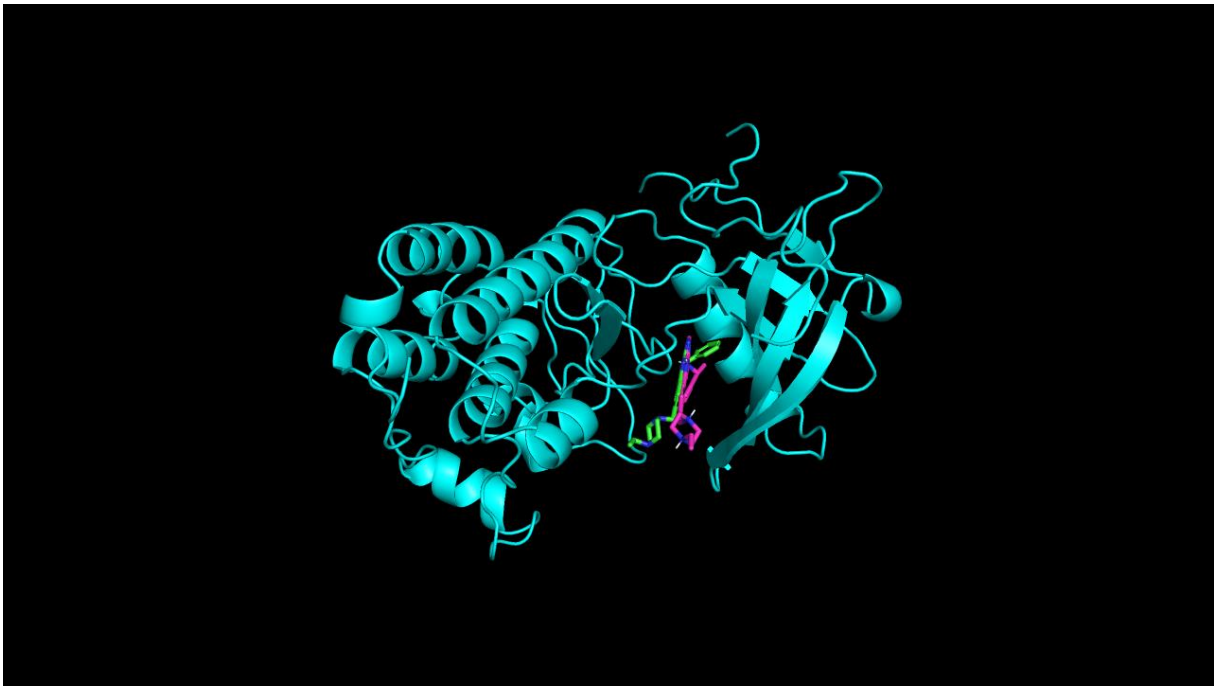

Figure 11. Seventh Best Docking Pose

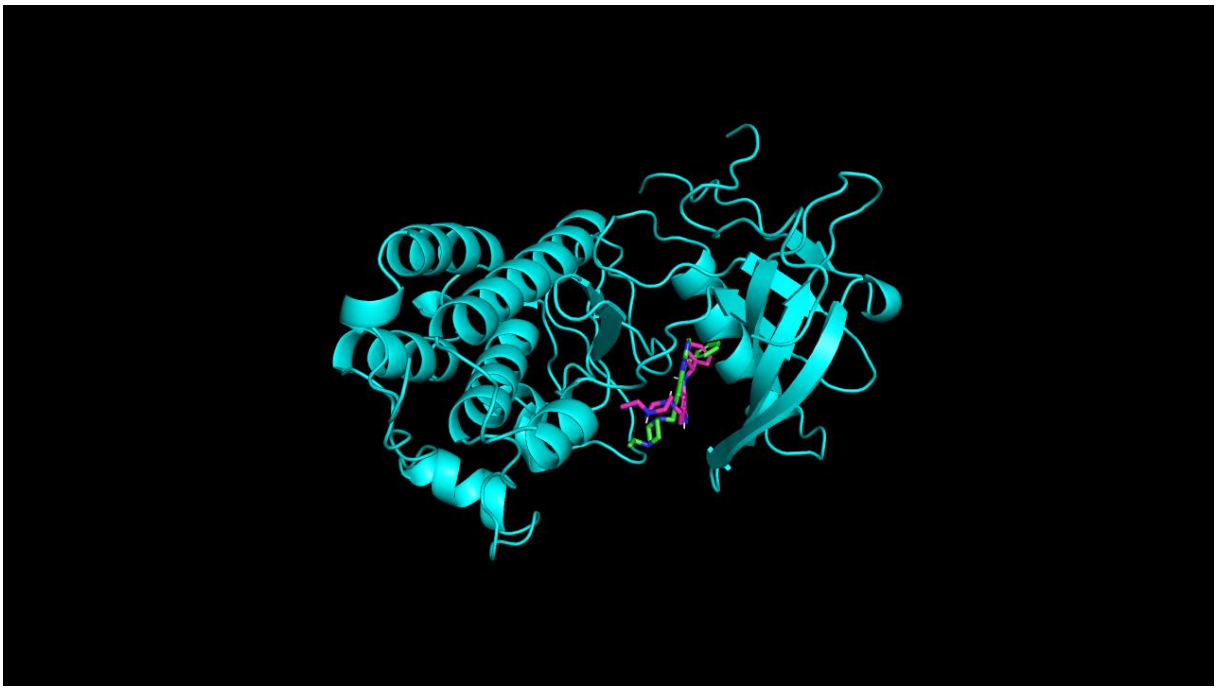

Figure 12. Eighth Best Docking Pose

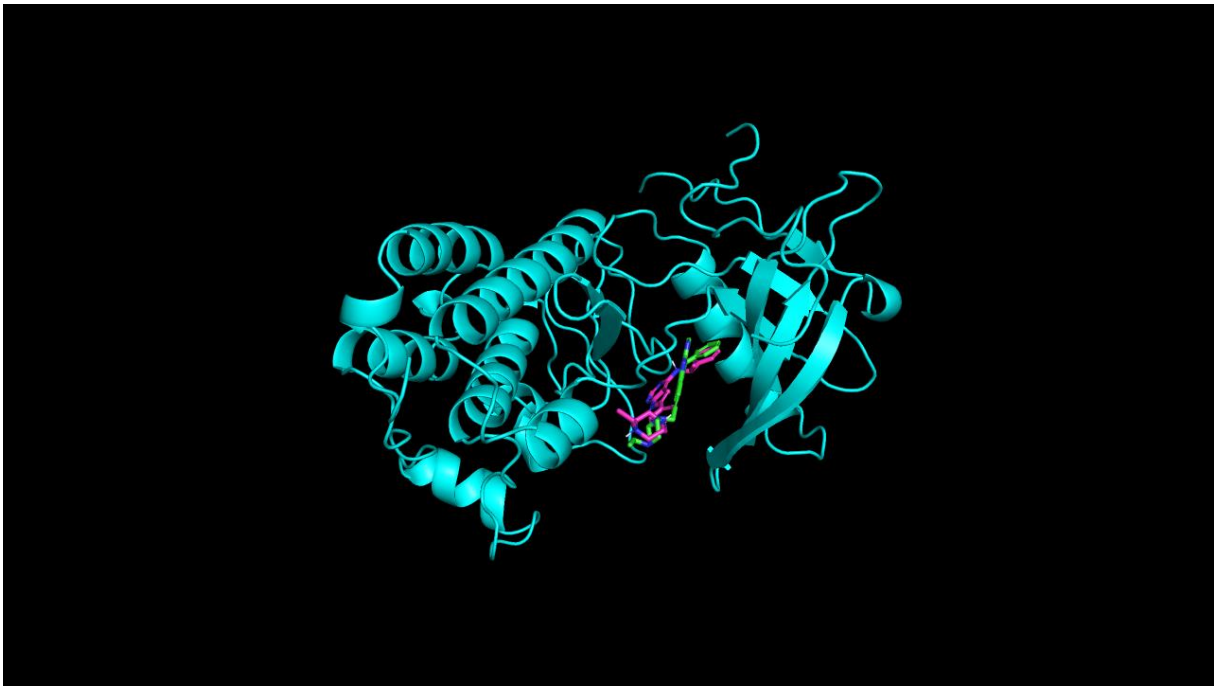

Figure 13. Ninth Best Docking Pose

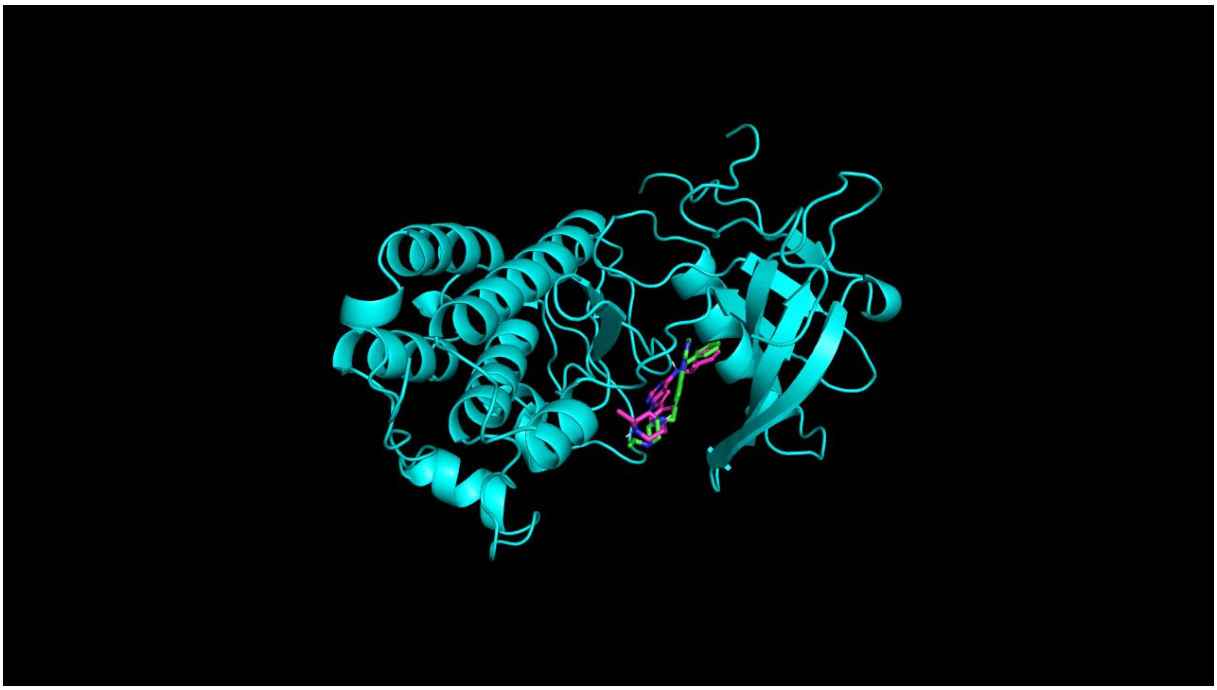

Figure 14. Tenth Best Docking Pose

Exhaustiveness 16:

```
smina is based off AutoDock Vina. Please cite appropriately.

Weights      Terms
-0.035579    gauss(o=0,_w=0.5,_c=8)
-0.005156    gauss(o=3,_w=2,_c=8)
0.840245     repulsion(o=0,_c=8)
-0.035069    hydrophobic(g=0.5,_b=1.5,_c=8)
-0.587439    non_dir_h_bond(g=-0.7,_b=0,_c=8)
1.923        num_tors_div

Using random seed: 957376272

0%   10   20   30   40   50   60   70   80   90   100%
|----|----|----|----|----|----|----|----|----|----|
**************************************************

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
1       -9.7       0.000      0.000
2       -9.3       1.807      3.133
3       -8.9       1.819      3.047
4       -8.8       1.695      2.289
5       -8.8       2.152      3.599
6       -8.8       2.282      3.431
7       -8.7       1.893      2.737
8       -8.5       2.208      3.800
9       -8.4       2.335      3.618
10      -8.4       2.336      4.082
Refine time 111.567
Loop time 112.236
```

Figure 15. Report of the 8th redocking.

The affinity value of 9.7 implies a strong interaction between the two molecules. As this value decreases, it indicates a gradual reduction in the strength of the interaction.

The Root Mean Square Deviation (RMSD) value illustrates the structural similarity between docking results. An RMSD value of zero signifies almost no structural difference between the predicted structure and the reference structure. In this case, starting with an RMSD of 0, the value gradually increases, indicating a decreasing overlap between the docking results and the reference structure. This pattern is observed consistently across all exhaustiveness values.

The ranking of the top 10 docking poses is as follows:

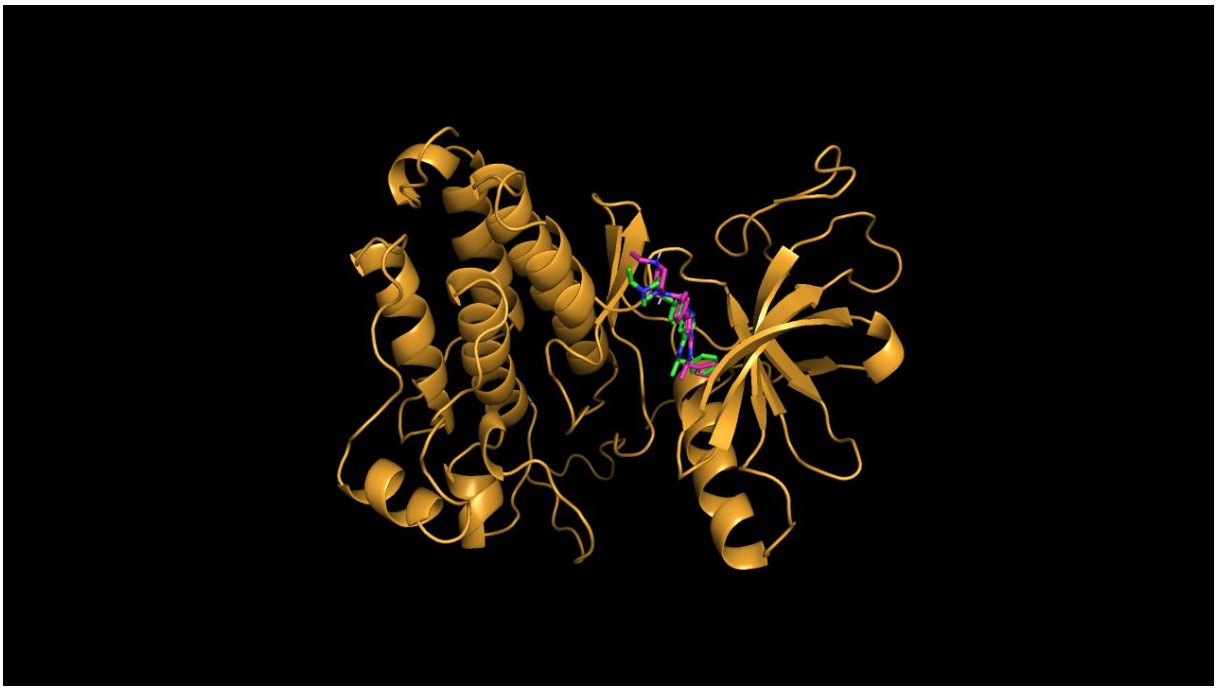

Figure 16. Best Docking Pose

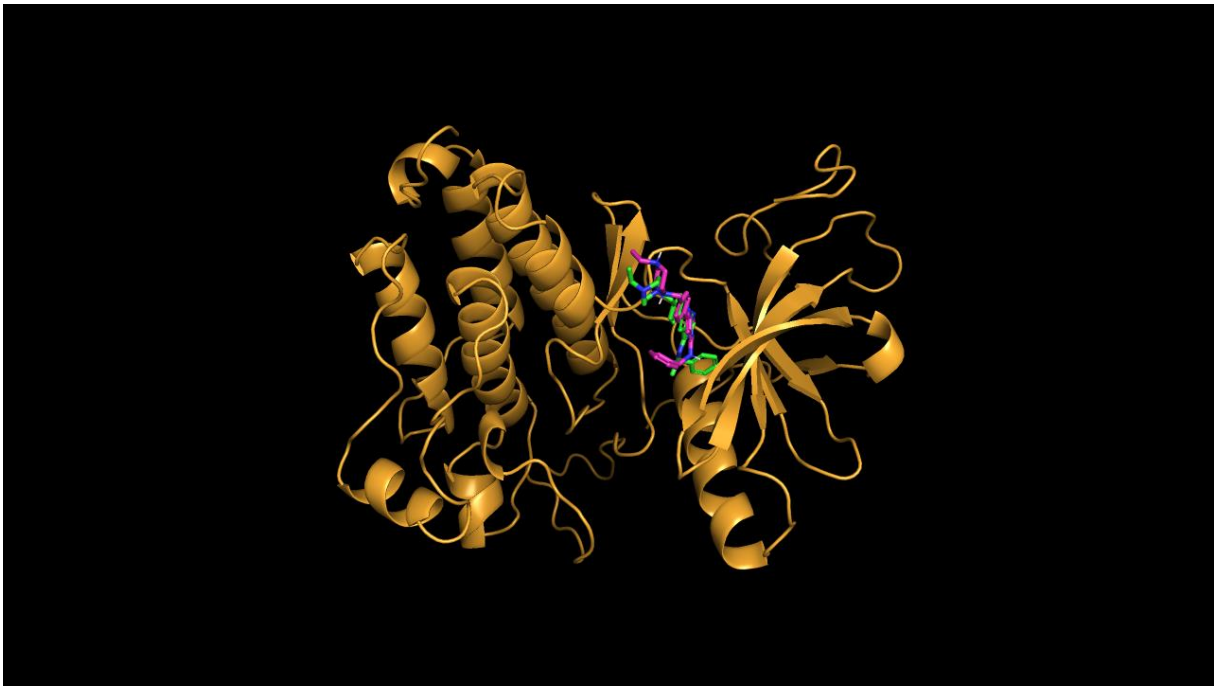

Figure 17. Second Best Docking Pose

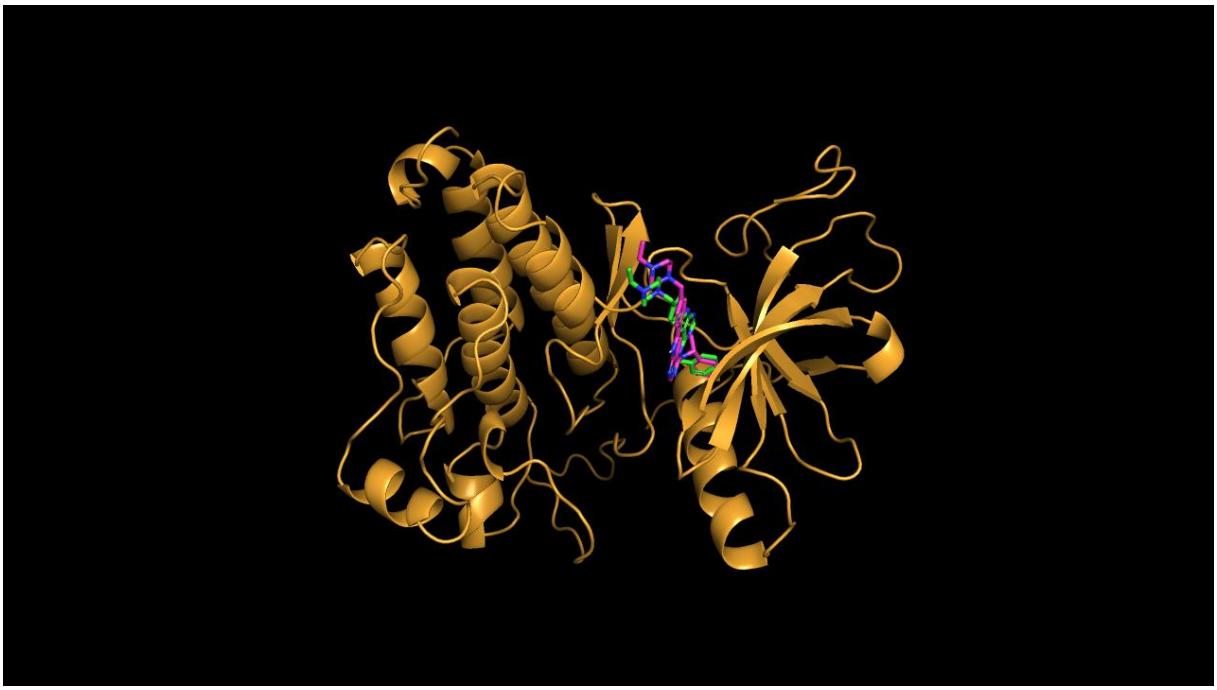

Figure 18. Third Best Docking Pose

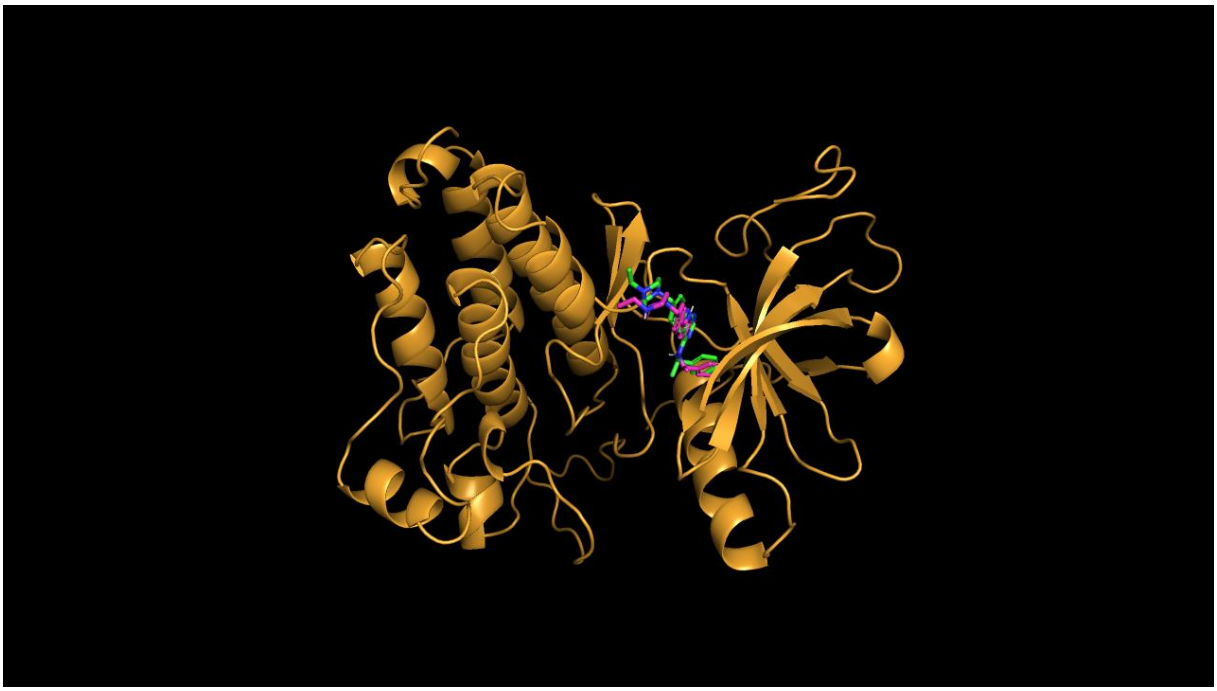

Figure 19. Fourth Best Docking Pose

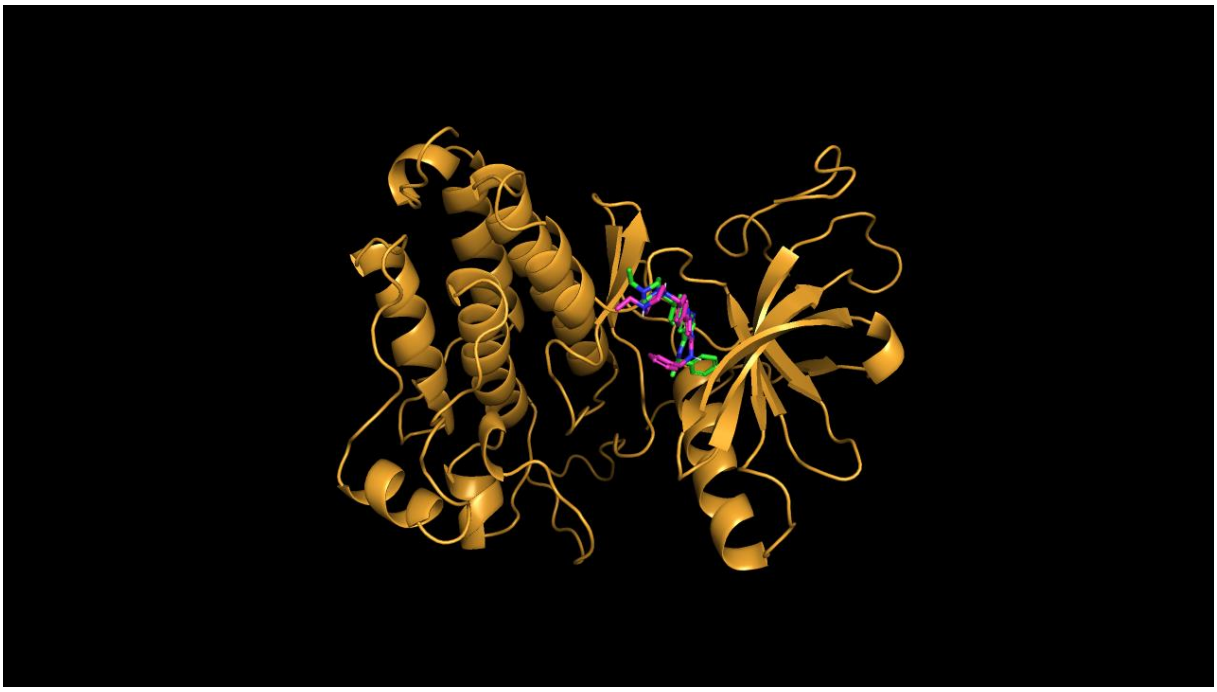

Figure 20. Fifth Best Docking Pose

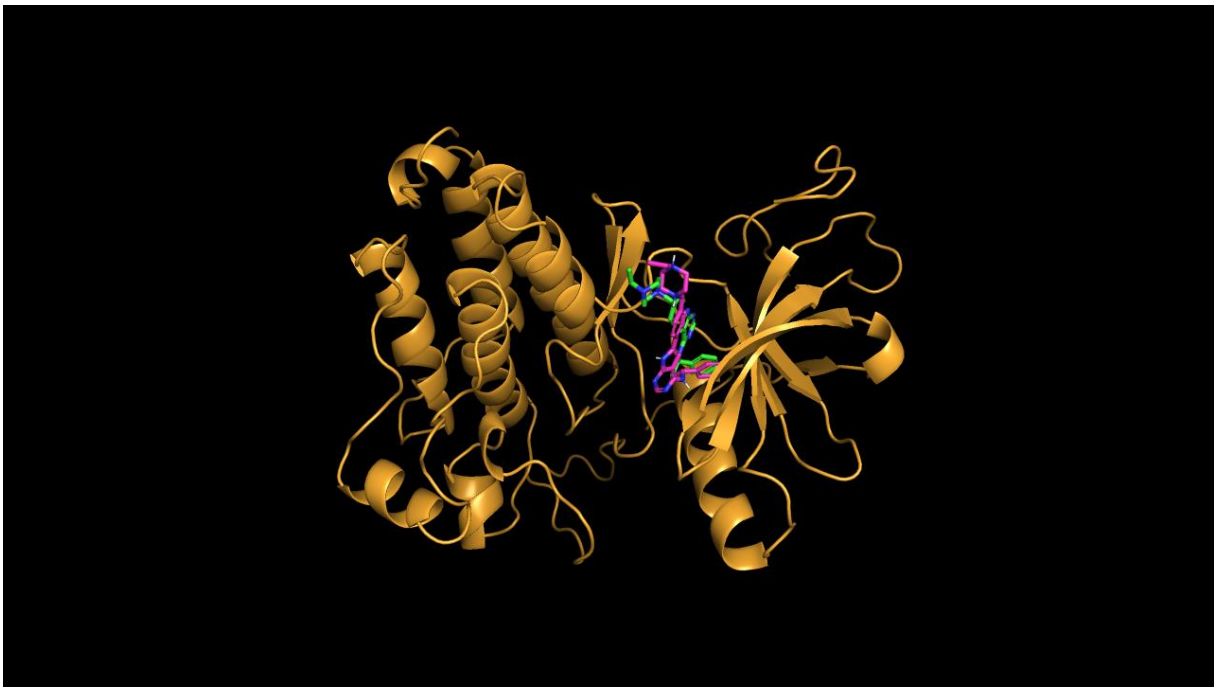

Figure 21. Sixth Best Docking Pose

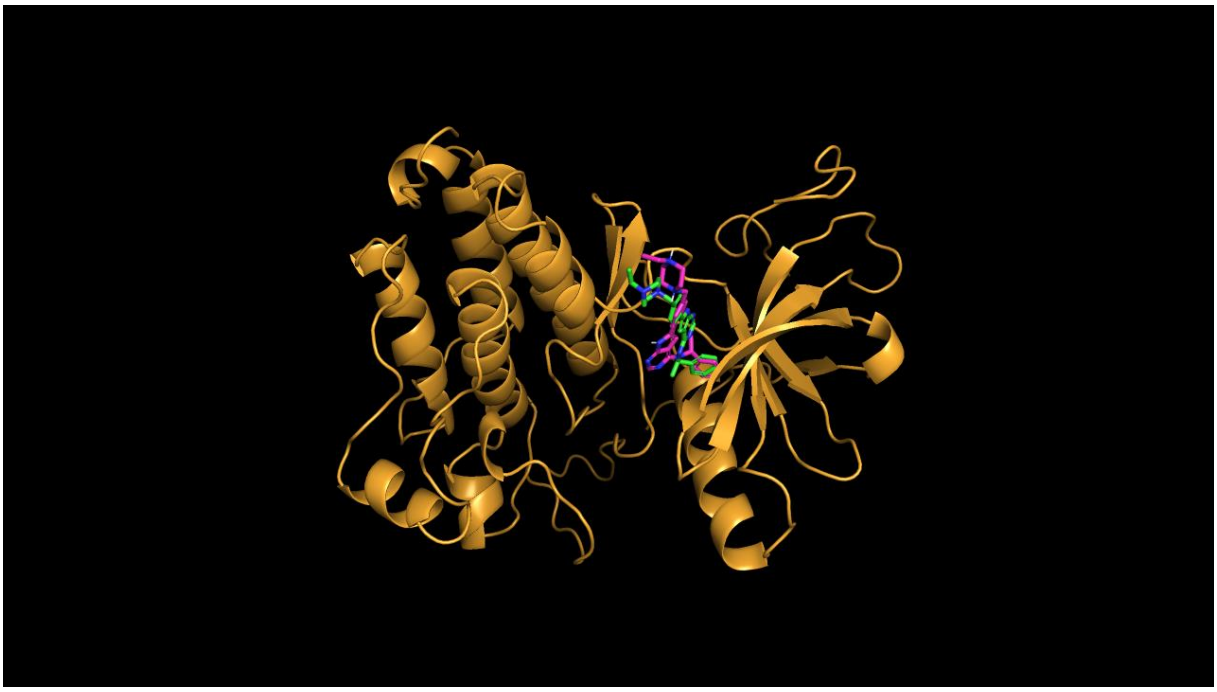

Figure 22. Seventh Best Docking Pose

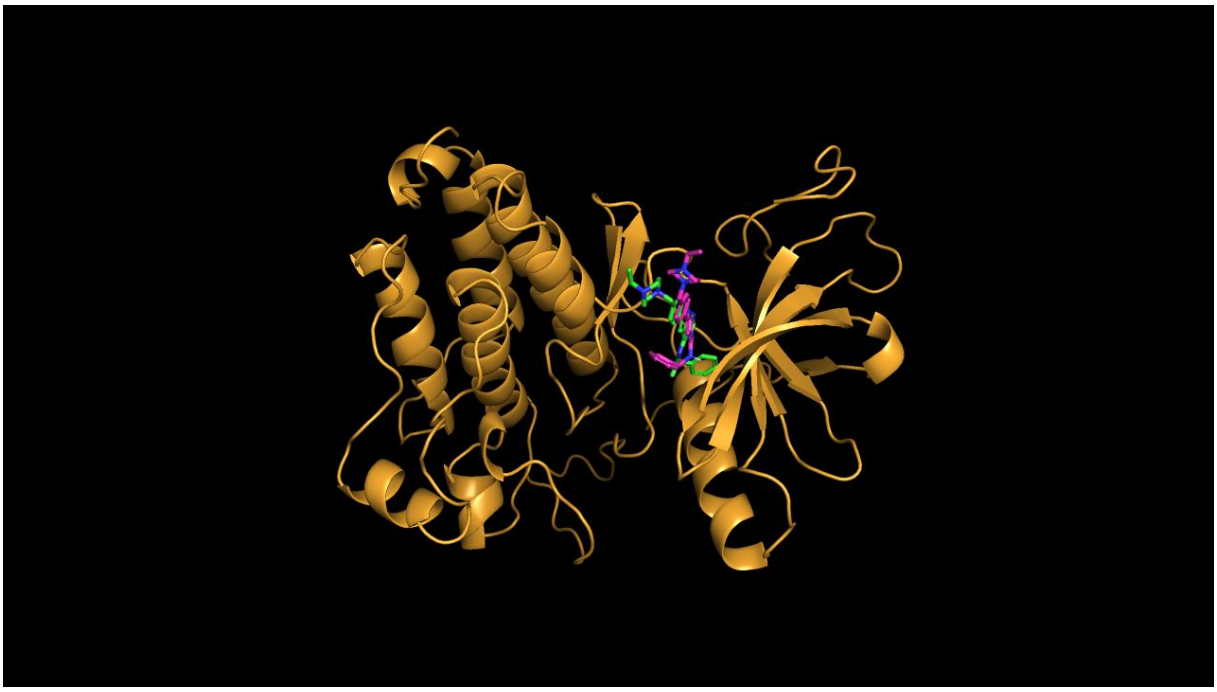

Figure 23. Eighth Best Docking Pose

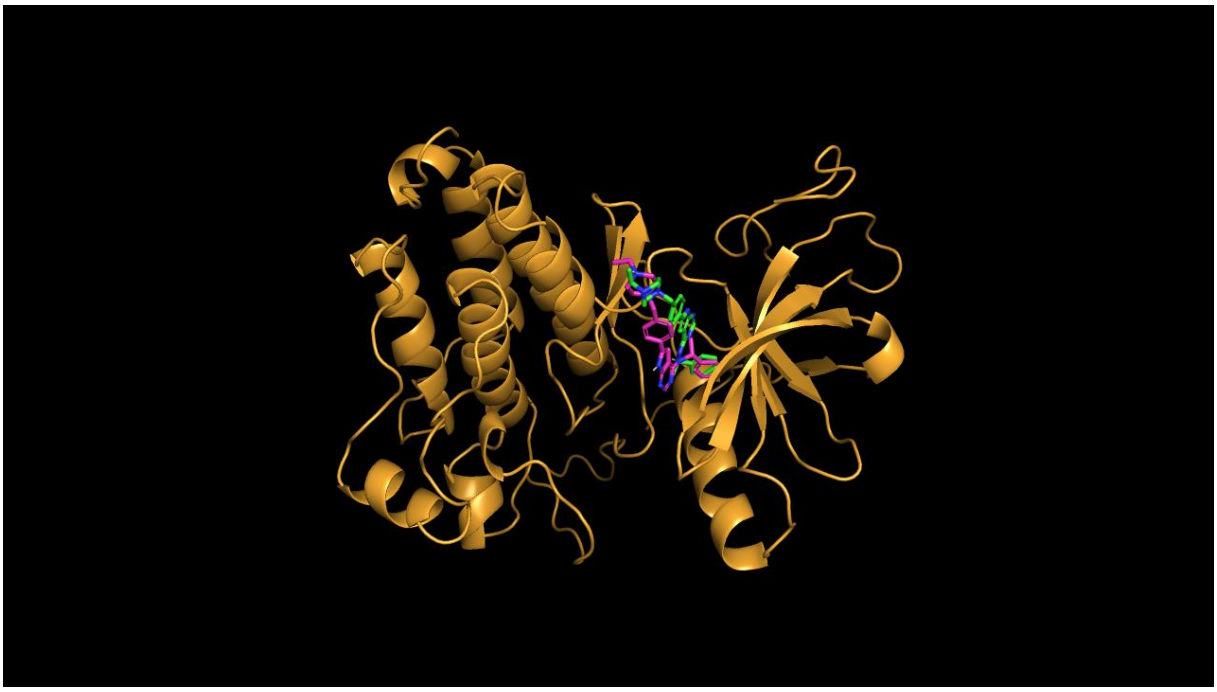

Figure 24. Ninth Best Docking Pose

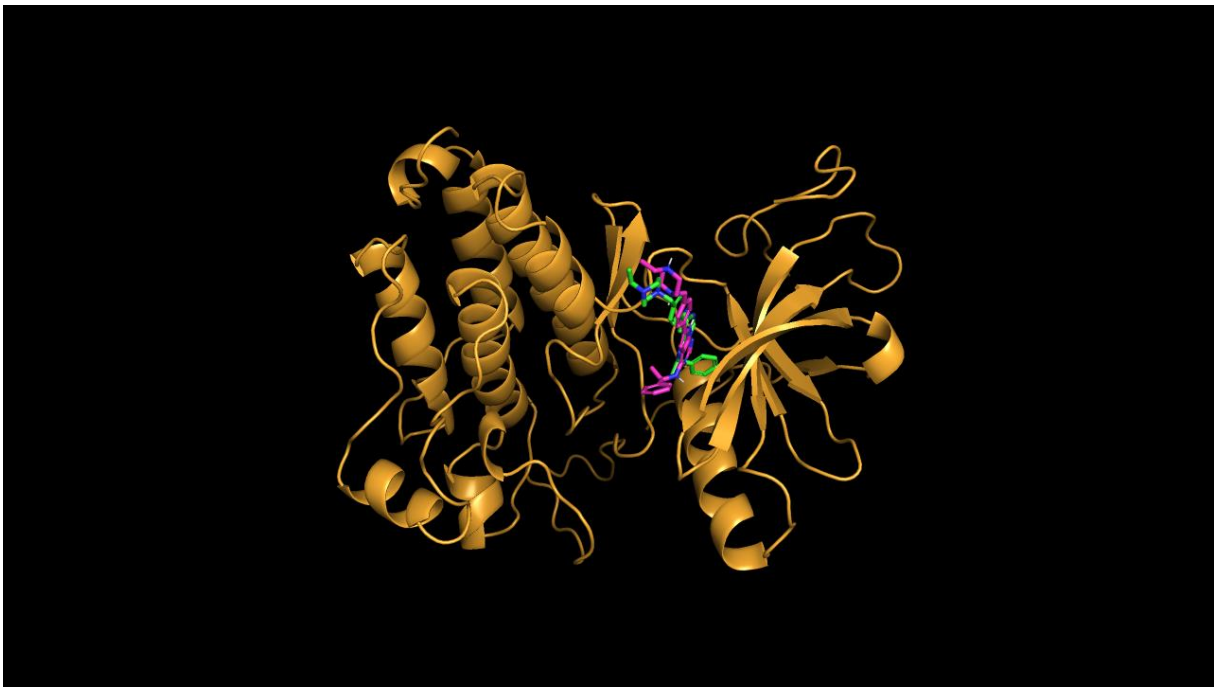

Figure 25. Tenth Best Docking Pose

Exhaustiveness 32:

```
smina is based off AutoDock Vina. Please cite appropriately.

Weights      Terms
-0.035579    gauss(o=0,_w=0.5,_c=8)
-0.005156    gauss(o=3,_w=2,_c=8)
0.840245     repulsion(o=0,_c=8)
-0.035069    hydrophobic(g=0.5,_b=1.5,_c=8)
-0.587439    non_dir_h_bond(g=-0.7,_b=0,_c=8)
1.923        num_tors_div

Using random seed: -1910113484

0%   10   20   30   40   50   60   70   80   90   100%
|----|----|----|----|----|----|----|----|----|----|
**************************************************

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
1       -9.7       0.000      0.000
2       -9.7       0.331      1.231
3       -9.3       1.145      2.728
4       -9.0       1.279      2.044
5       -8.9       1.805      2.830
6       -8.9       1.811      2.641
7       -8.8       1.812      3.448
8       -8.7       2.274      3.399
9       -8.6       2.014      3.212
10      -8.4       2.432      3.350
Refine time 227.764
Loop time 228.097
```

Figure 26. Report of the 8th redocking.

The affinity value of 9.7 implies a strong interaction between the two molecules. As this value decreases, it indicates a gradual reduction in the strength of the interaction.

The Root Mean Square Deviation (RMSD) value illustrates the structural similarity between docking results. An RMSD value of zero signifies almost no structural difference between the predicted structure and the reference structure. In this case, starting with an RMSD of 0, the value gradually increases, indicating a decreasing overlap between the docking results and the reference structure. This pattern is observed consistently across all exhaustiveness values.

The ranking of the top 10 docking poses is as follows:

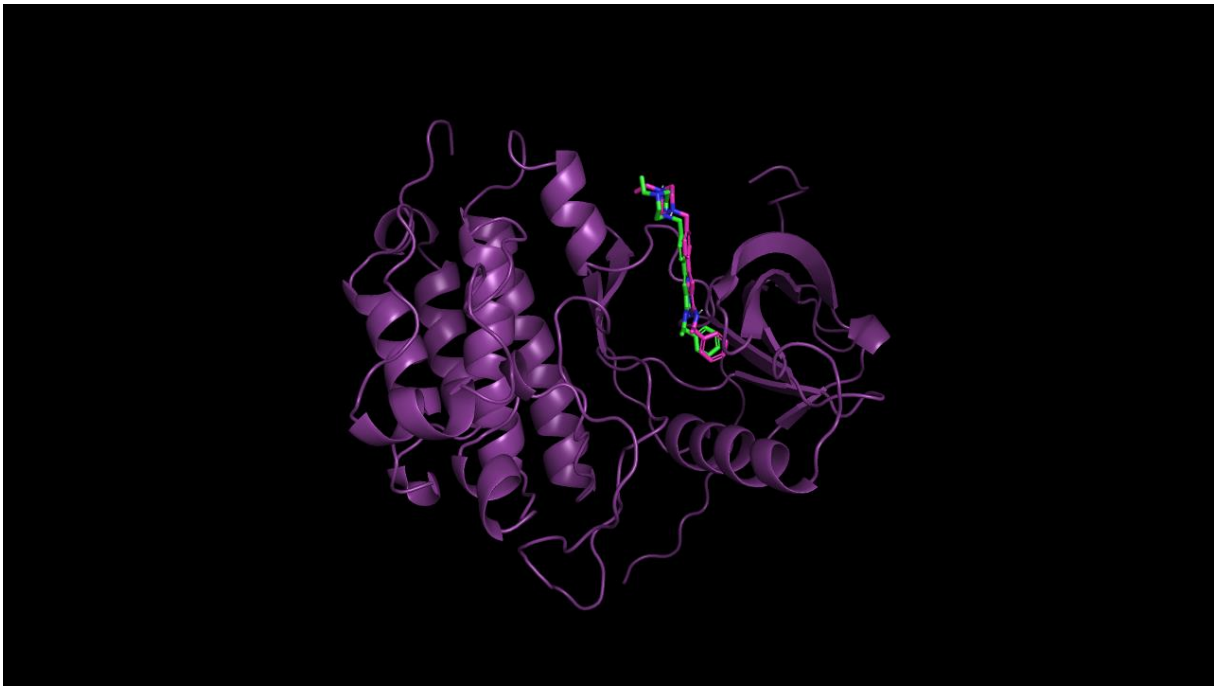

Figure 27. Best Docking Pose

Figure 28. Second Best Docking Pose

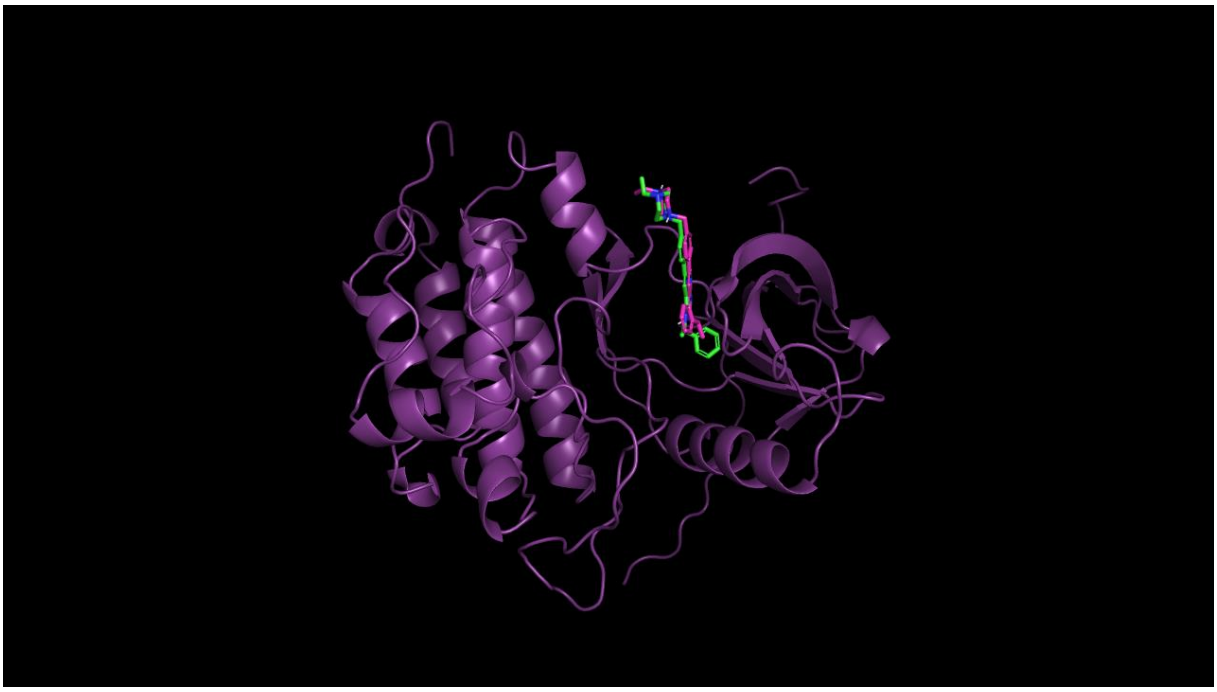

Figure 29. Third Best Docking Pose

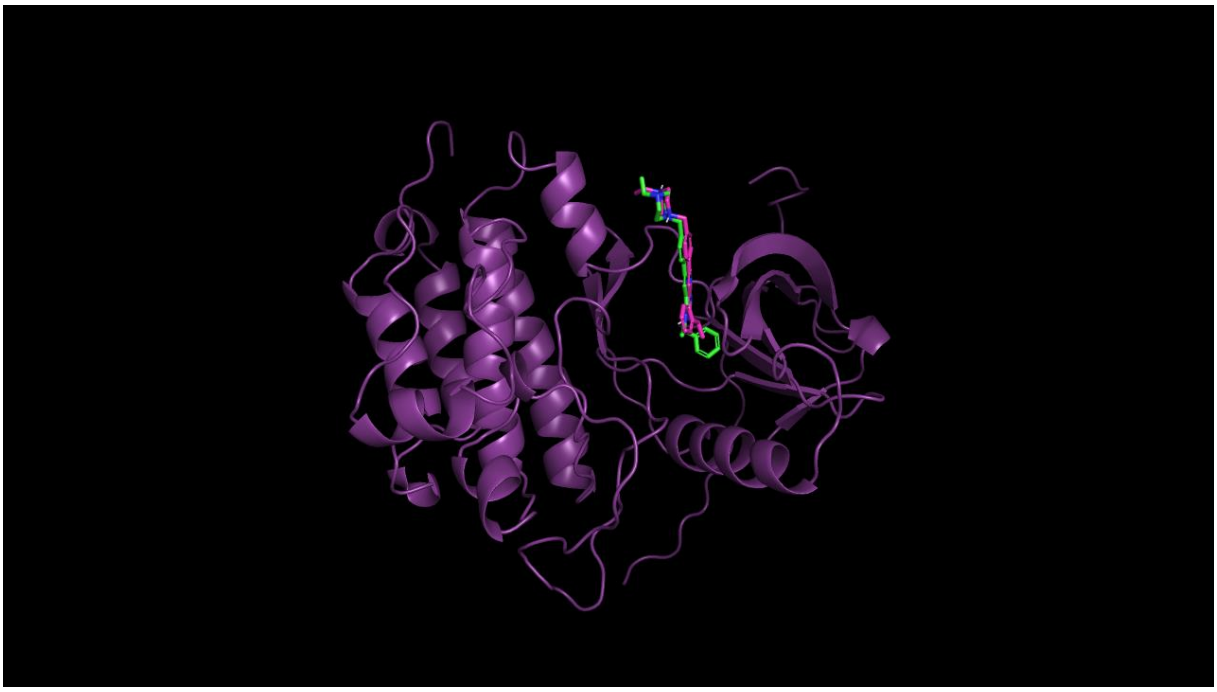

Figure 30. Fourth Best Docking Pose

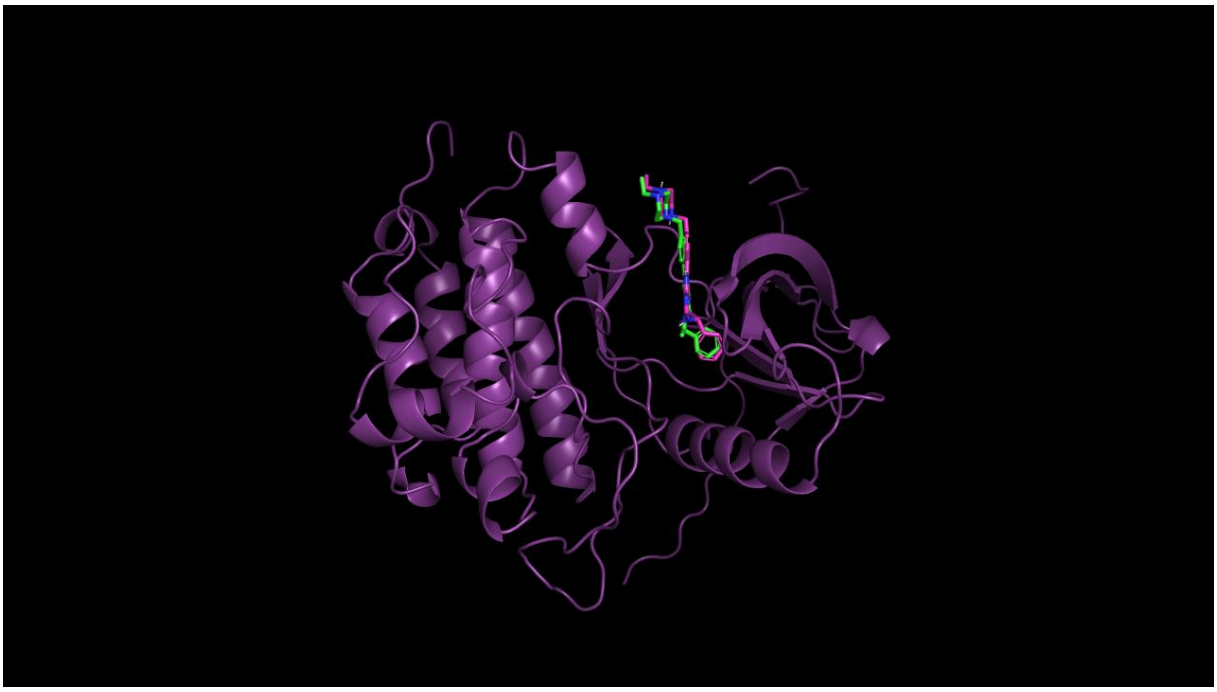

Figure 31. Fifth Best Docking Pose

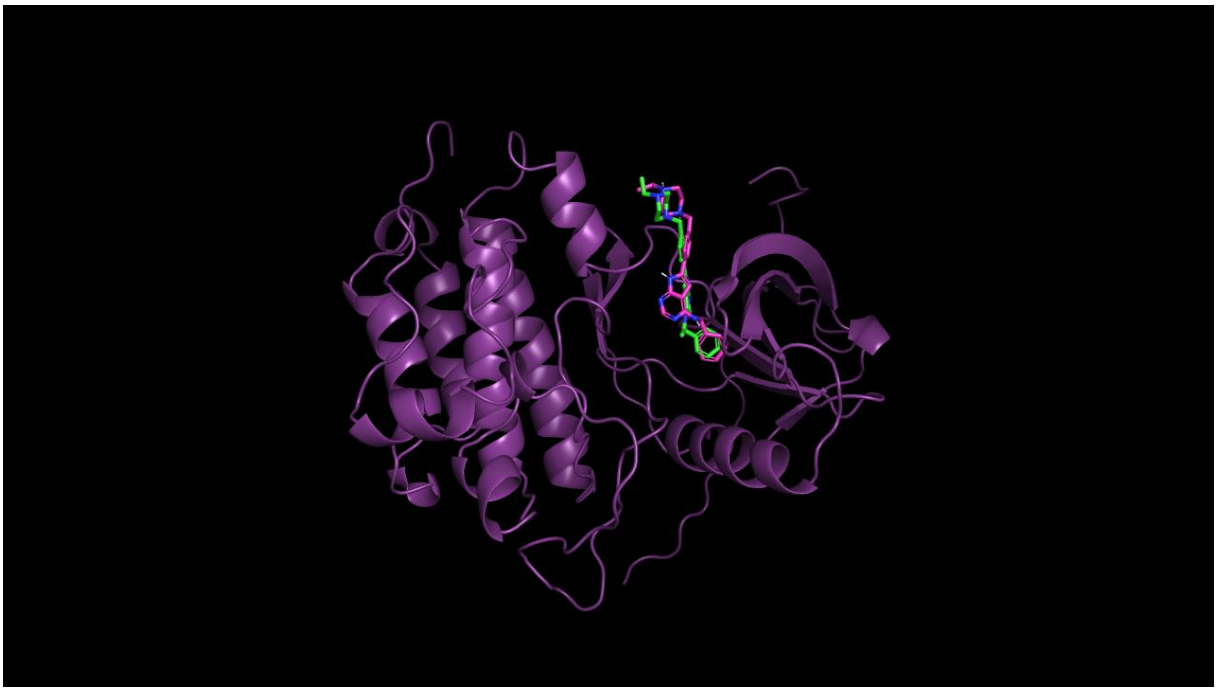

Figure 32. Sixth Best Docking Pose

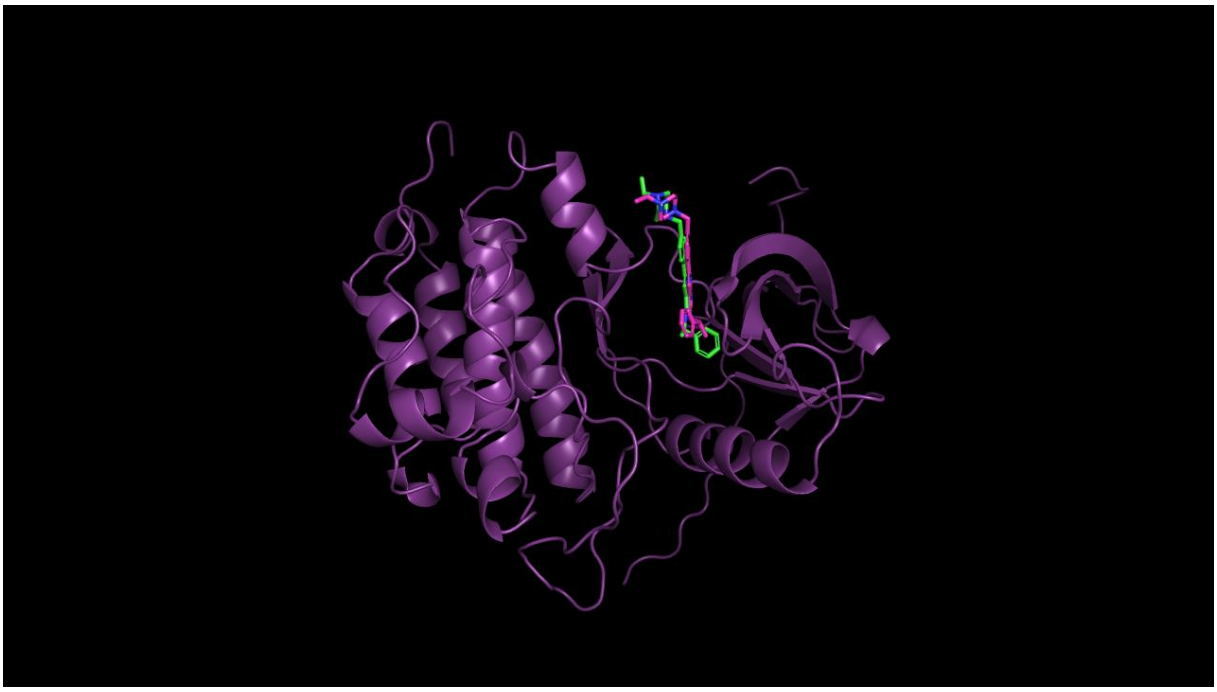

Figure 33. Seventh Best Docking Pose

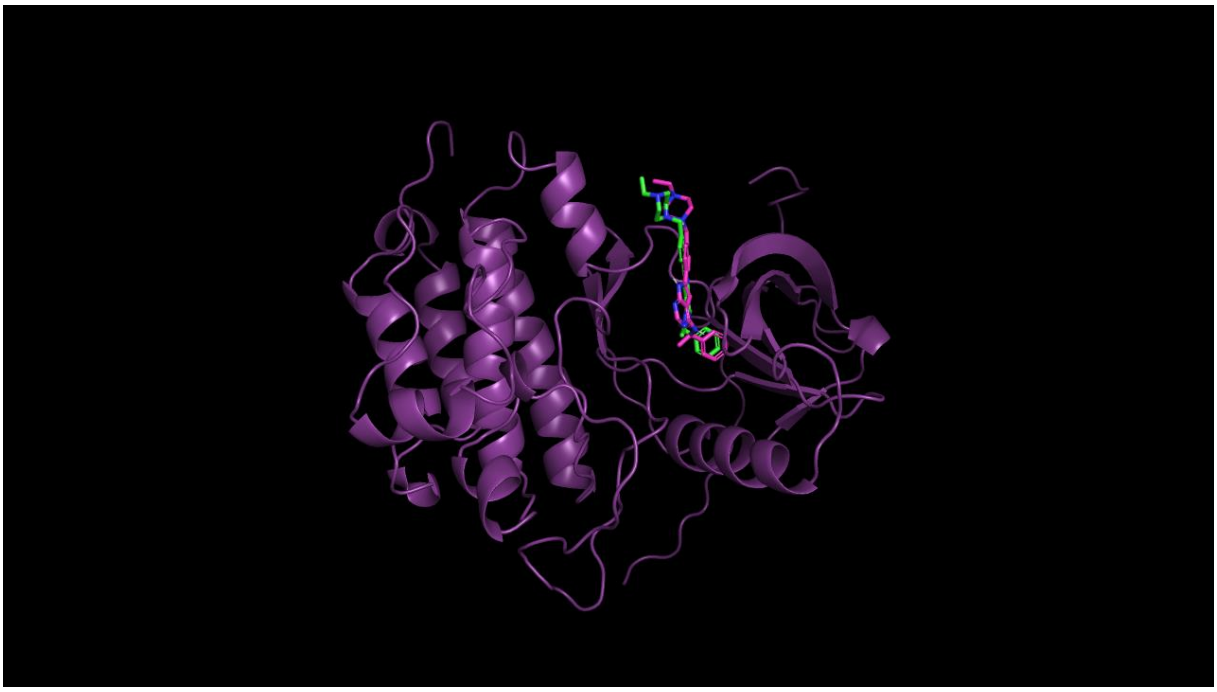

Figure 34. Eighth Best Docking Pose

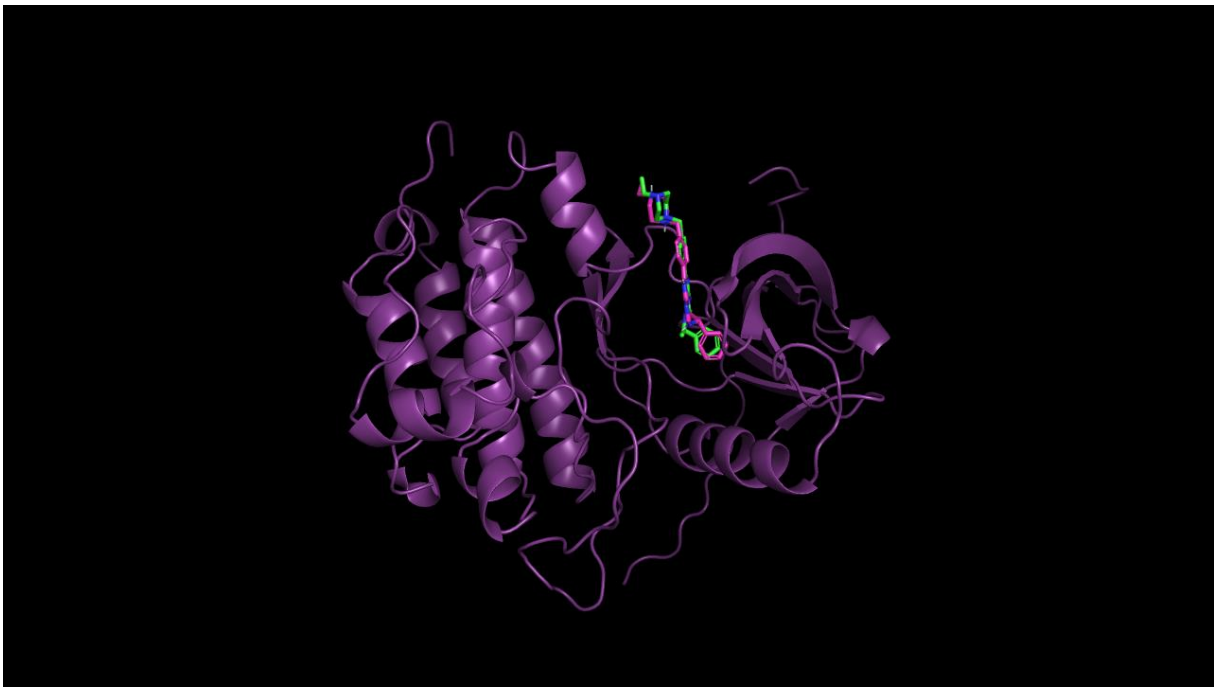

Figure 35. Ninth Best Docking Pose

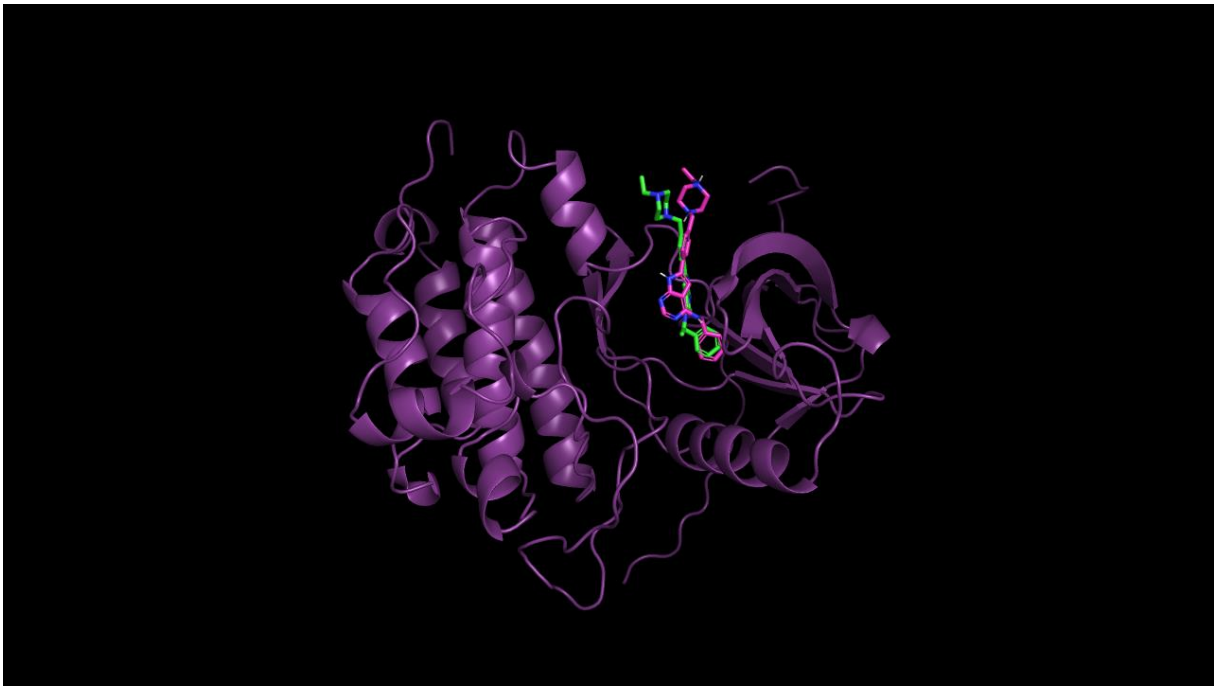

Figure 36. Tenth Best Docking Pose

The impact of exhaustiveness on the accuracy and consistency of molecular binding results:

Exhaustiveness is a parameter that determines how comprehensively docking programs will explore various conformations and binding modes of a ligand to the target protein. The higher the exhaustiveness value, the more conformations and binding modes are thoroughly examined.

Consistency of docking results refers to how similar the results are when repeated docking studies are conducted for the same ligand-target protein pair. It indicates the reliability of the program in consistently binding the same ligand to the same target protein under various conditions.

In our study, exhaustiveness values of 32, 16, and 8 were comparatively evaluated based on the obtained results.

A low exhaustiveness value (in our case, set to 8) may lead the program to explore a limited number of conformations and binding modes. Consequently, the program may produce different results under different conditions, potentially reducing its reliability.

A high exhaustiveness value (in our case, set to 32) allows the docking program to comprehensively understand various conformations and binding modes of the ligand. If the program is run with high exhaustiveness, and the results are reproducible, it can enhance our understanding of how the ligand binds to the target protein and contribute to obtaining reliable results.

However, exhaustiveness is just one factor, and there are other factors influencing the consistency of docking results. Factors such as the energy function used by the program, representation methods for the ligand and target protein, also affect the results. Therefore, while exhaustiveness is considered in docking studies, it may not be sufficient as a standalone indicator. Therefore, visualizing the conformations obtained for each different value is crucial. When examining the images obtained in PyMol for the conformations obtained at Exhaustiveness 32, it is noticeable that there is a significant overlap. Here, increasing the exhaustiveness value appears to have made the docking of the ligand to the protein more efficient and allowed for the acquisition of more useful conformations.