

**CADD ASSIGNMENT -2**  
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**2022205**

**PART 1: Literature Review and Drug Identification:**

NUMBER	DRUG NAME	TYPE AND STATUS	MECHANISM	REFERENCE
1	Statins (e.g., atorvastatin, simvastatin)	Approved (HMG-CoA reductase inhibitors)	Statins (e.g., atorvastatin) reduce CD40 expression and CD40L-induced inflammation in human vascular cells, via dose-dependent mechanisms involving NOS and PPAR pathways. This effect is partially reversible by mevalonate, supporting their use as immunomodulators in atherosclerosis and transplantation.	<a href="https://academic.oup.com/cardiovasc/article/59/3/755/348297#google_vignette">https://academic.oup.com/cardiovasc/article/59/3/755/348297#google_vignette</a>
2	DRI-C21045	Experimental small-molecule inhibitor	DRI-C21045 is a small-molecule inhibitor that blocks the CD40-CD40L interaction with low micromolar IC50. It shows specificity for CD40L, lacks cytotoxicity, and has been confirmed in various assays and an in vivo murine transplant model.	<a href="https://pubs.acs.org/doi/full/10.1021/acs.jmedchem.7b01154">https://pubs.acs.org/doi/full/10.1021/acs.jmedchem.7b01154</a>
3	DRI-C21041	Experimental small-molecule inhibitor	Structurally related to DRI-C21045, DRI-C21041 also targets CD40L and inhibits CD40-CD40L interaction. Demonstrated activity in in vitro assays; considered for immune modulation and transplant models.	<a href="https://pubs.acs.org/doi/full/10.1021/acs.jmedchem.7b01154">https://pubs.acs.org/doi/full/10.1021/acs.jmedchem.7b01154</a>
4	Compound 6877002	Experimental small-molecule inhibitor	Inhibits CD40-TRAF6 interaction, blocking downstream signaling; suppresses CD40-induced IL-1β/IL-6 expression; shows potential in autoimmune and inflammatory conditions.	<a href="https://pmc.ncbi.nlm.nih.gov/articles/PMC5427621/?utm_">https://pmc.ncbi.nlm.nih.gov/articles/PMC5427621/?utm_</a>
5	TAS-119	Experimental small-molecule (Aurora A inhibitor)	Though not a direct CD40 inhibitor, TAS-119 modulates immune response by affecting Aurora A kinase pathways, which indirectly influence CD40/CD40L signaling. Investigated for immunomodulatory and anticancer effects.	<a href="https://pubchem.ncbi.nlm.nih.gov/compound/86663491">https://pubchem.ncbi.nlm.nih.gov/compound/86663491</a>

## 2. Preparation of Ligands and Proteins for Docking

- Drug molecules in sdf format (Downloaded)

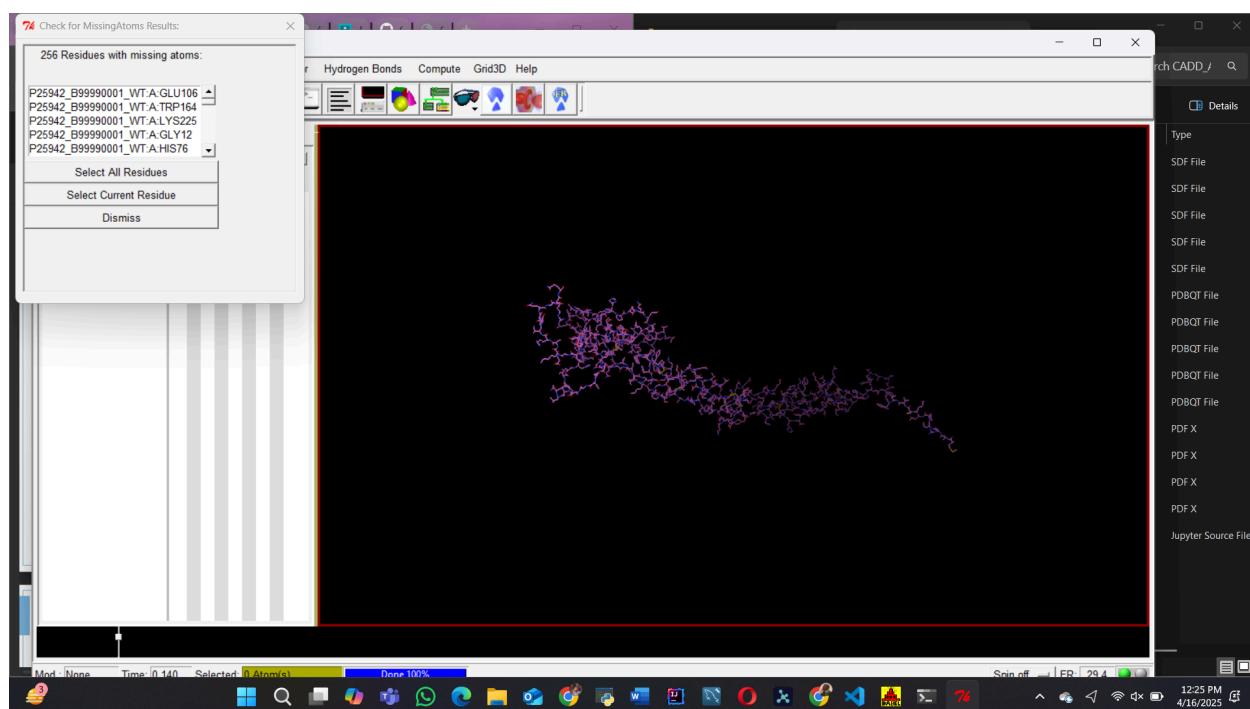
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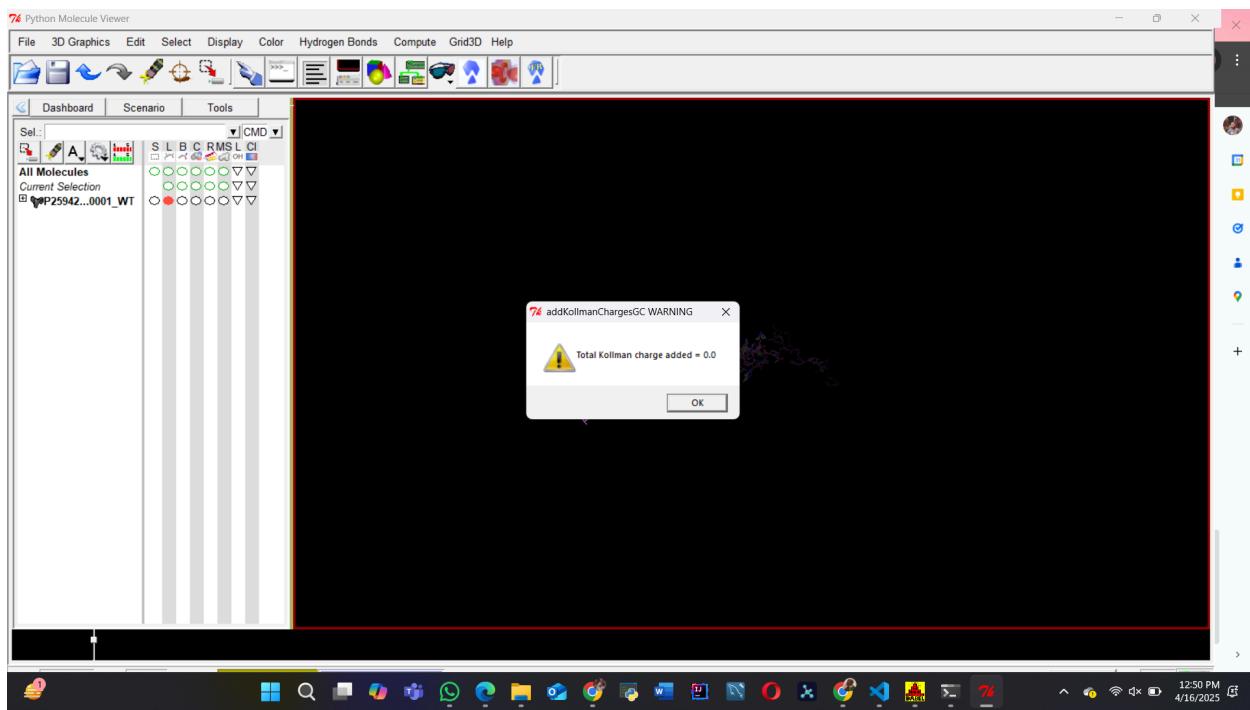
- Ligand preparation for Docking

(Done using OpenBabel Application)

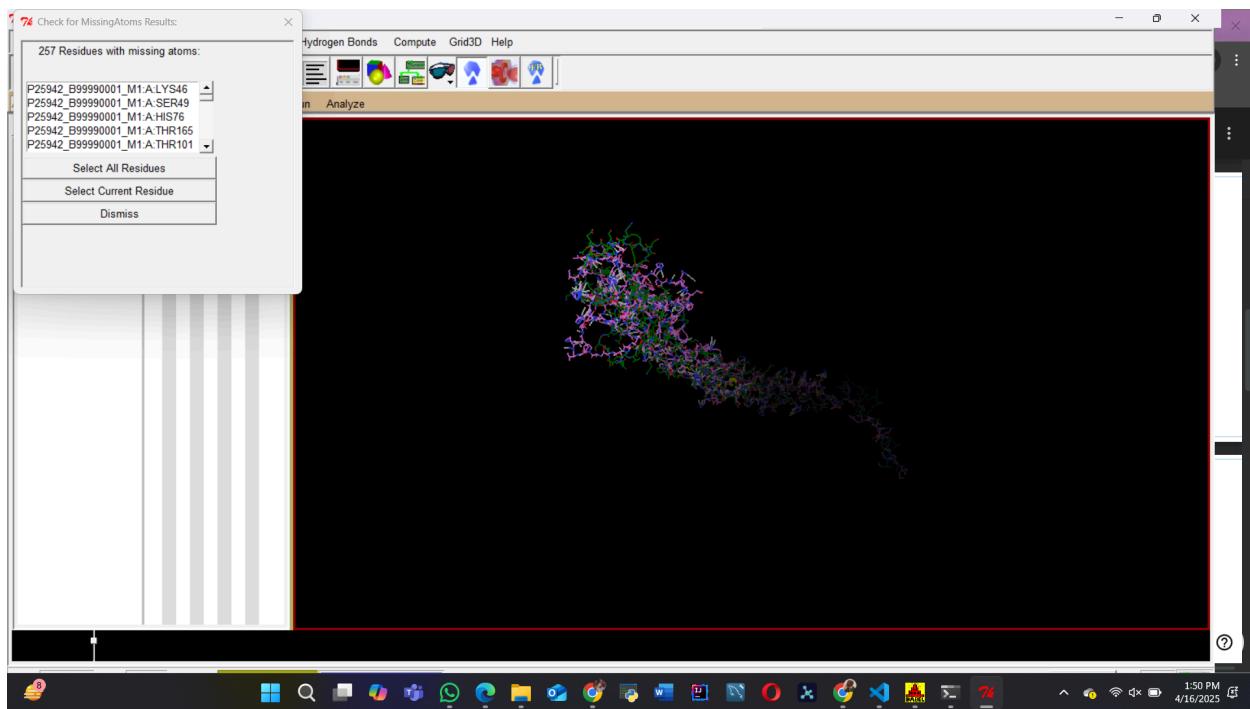
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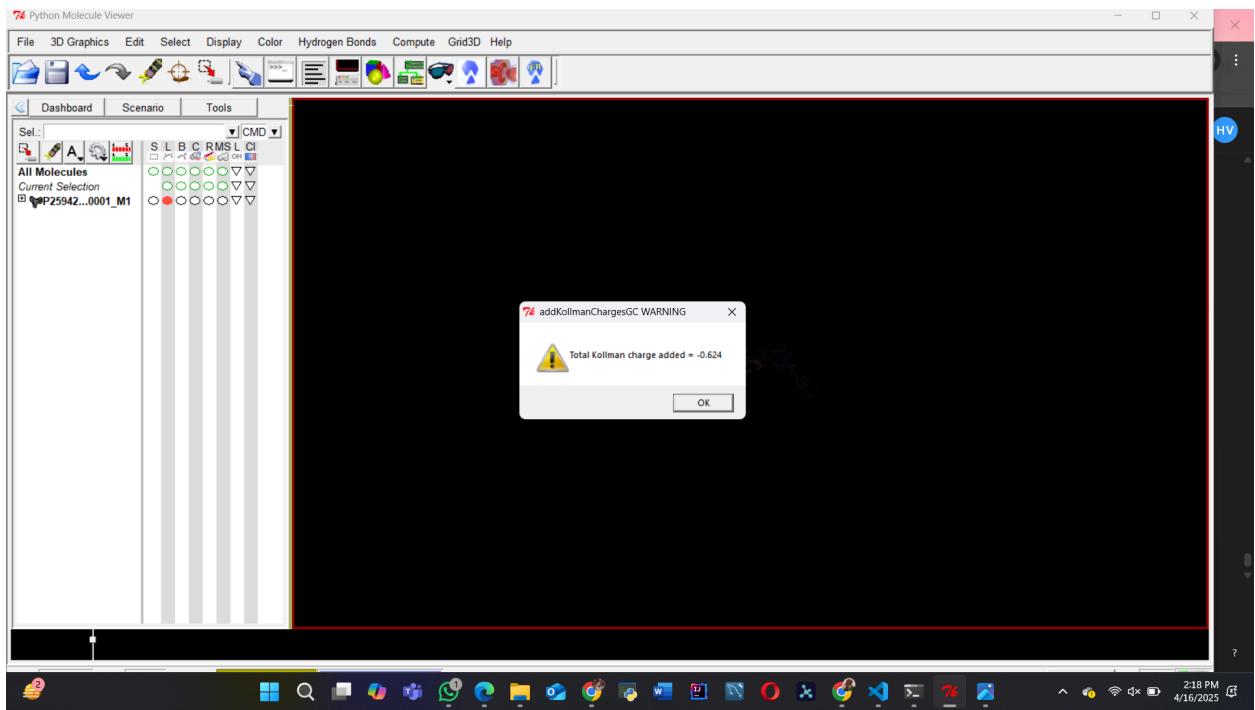
### WILD TYPE



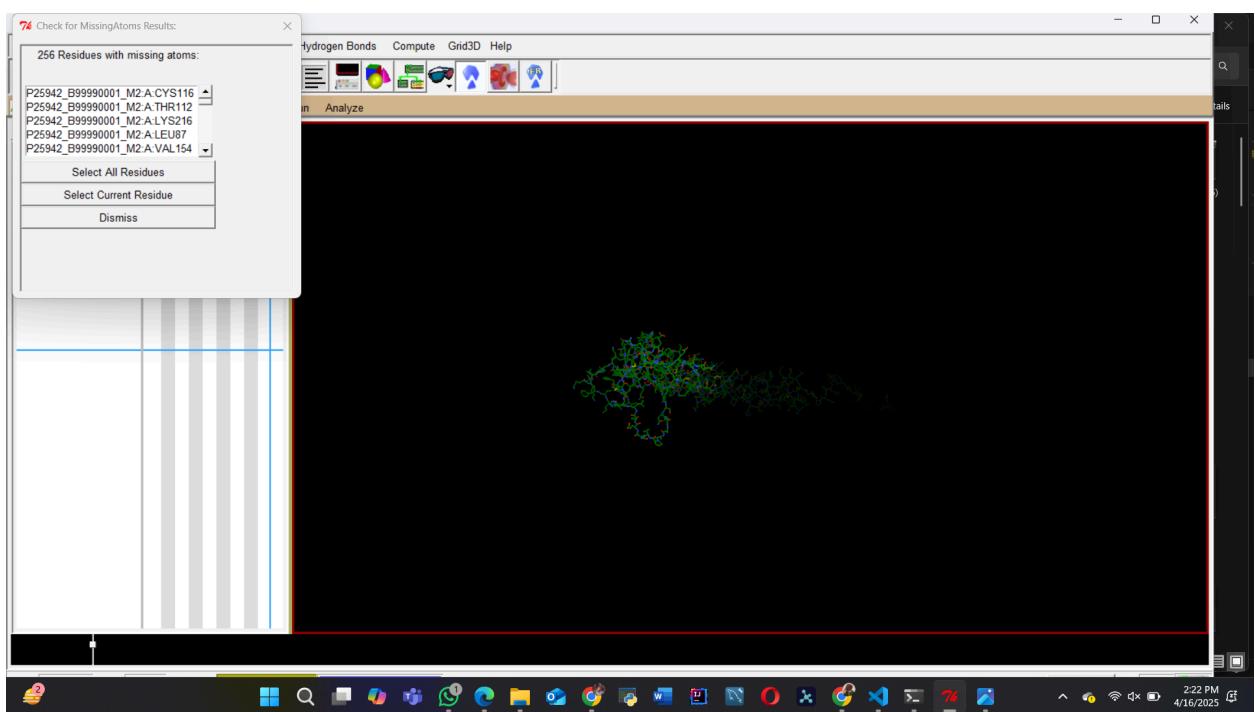


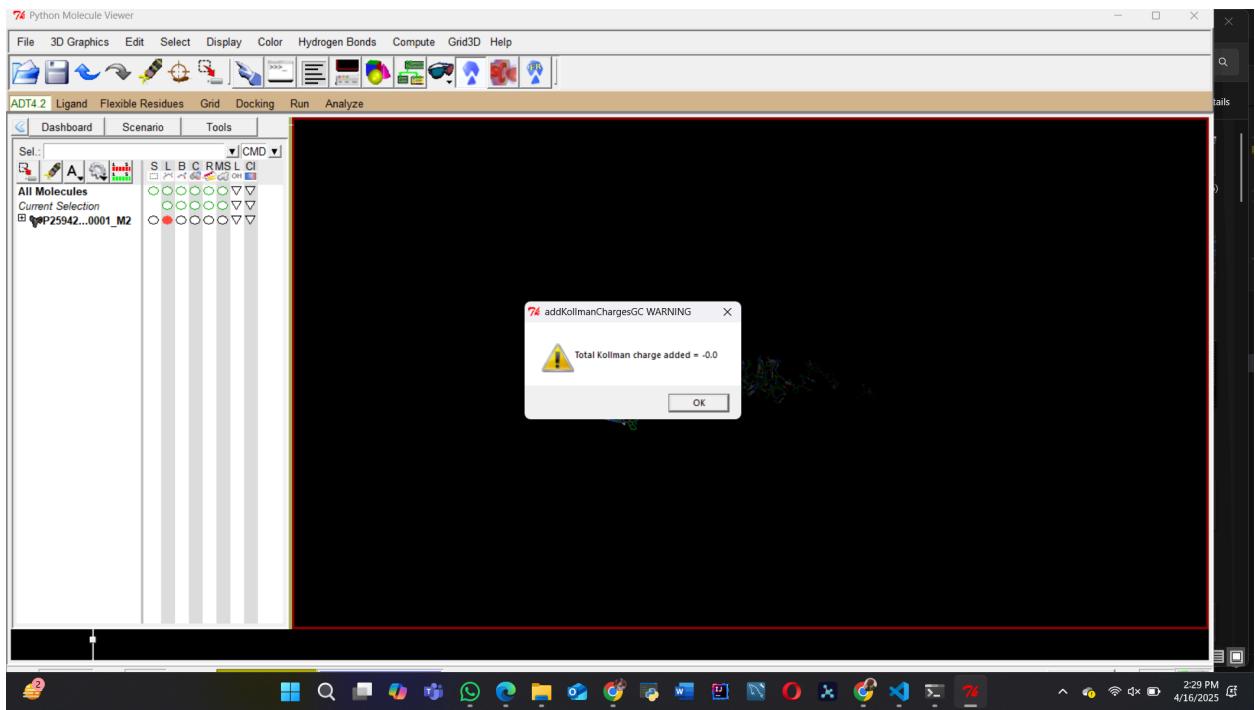
## Mutation1



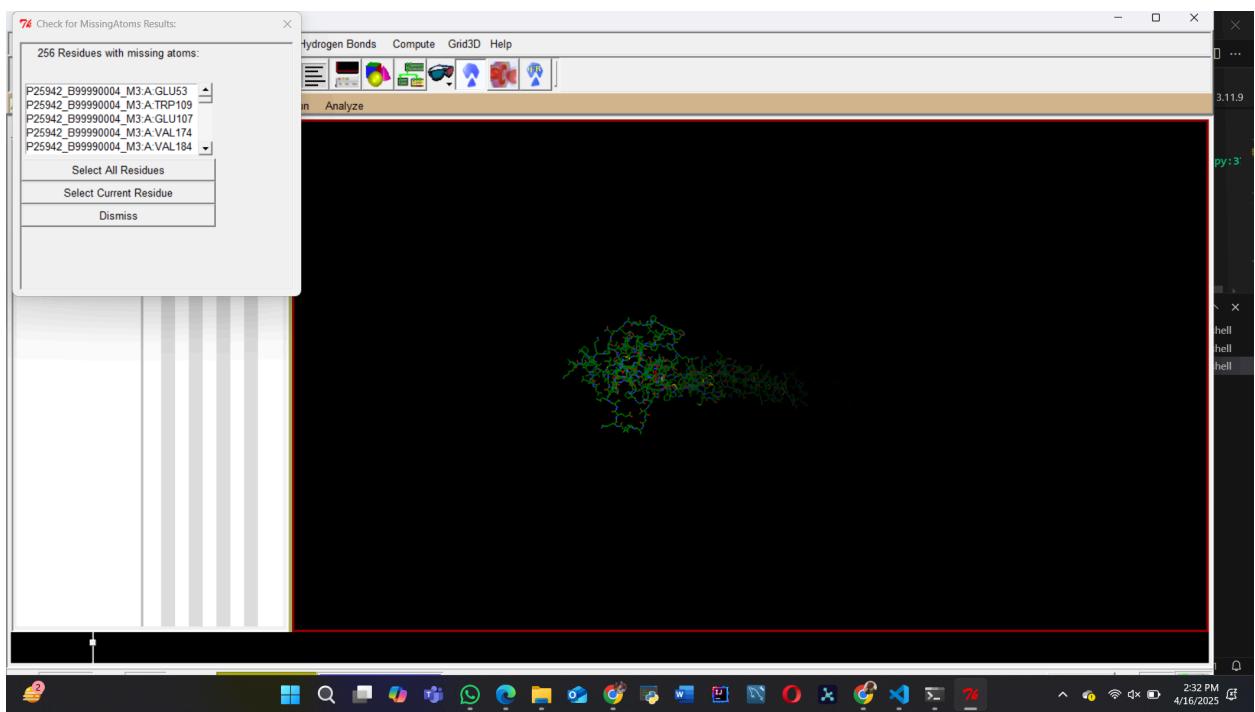


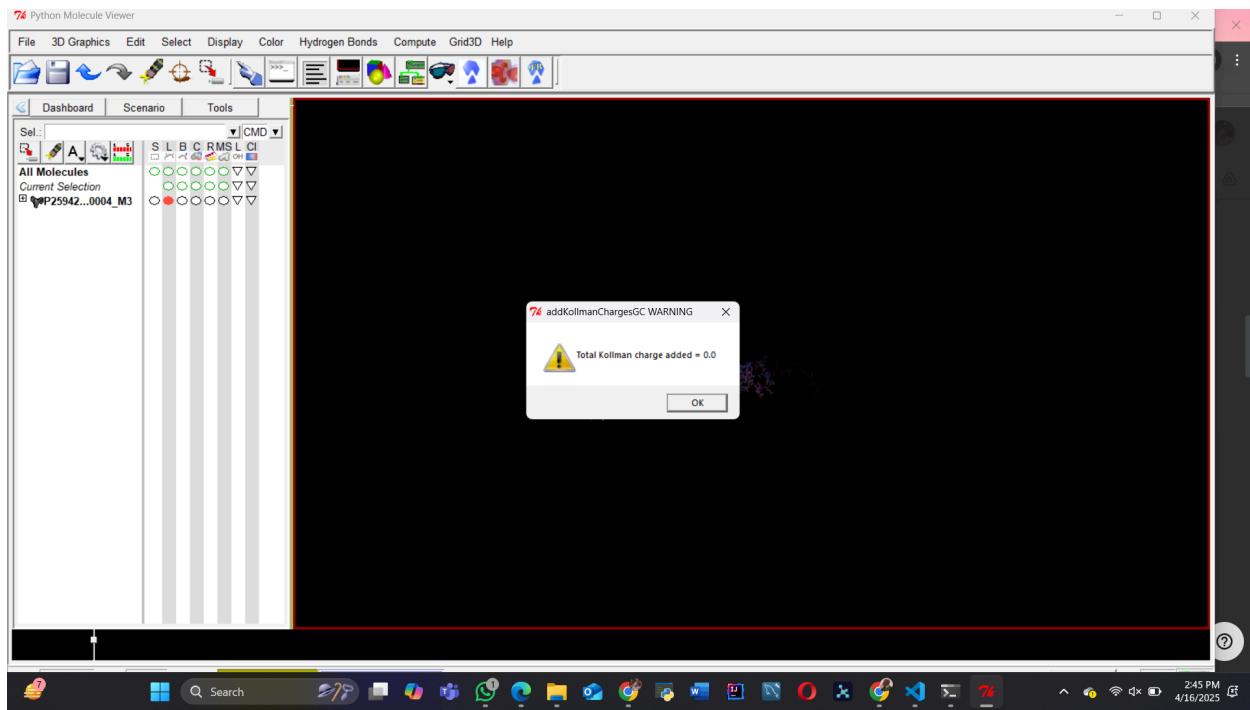
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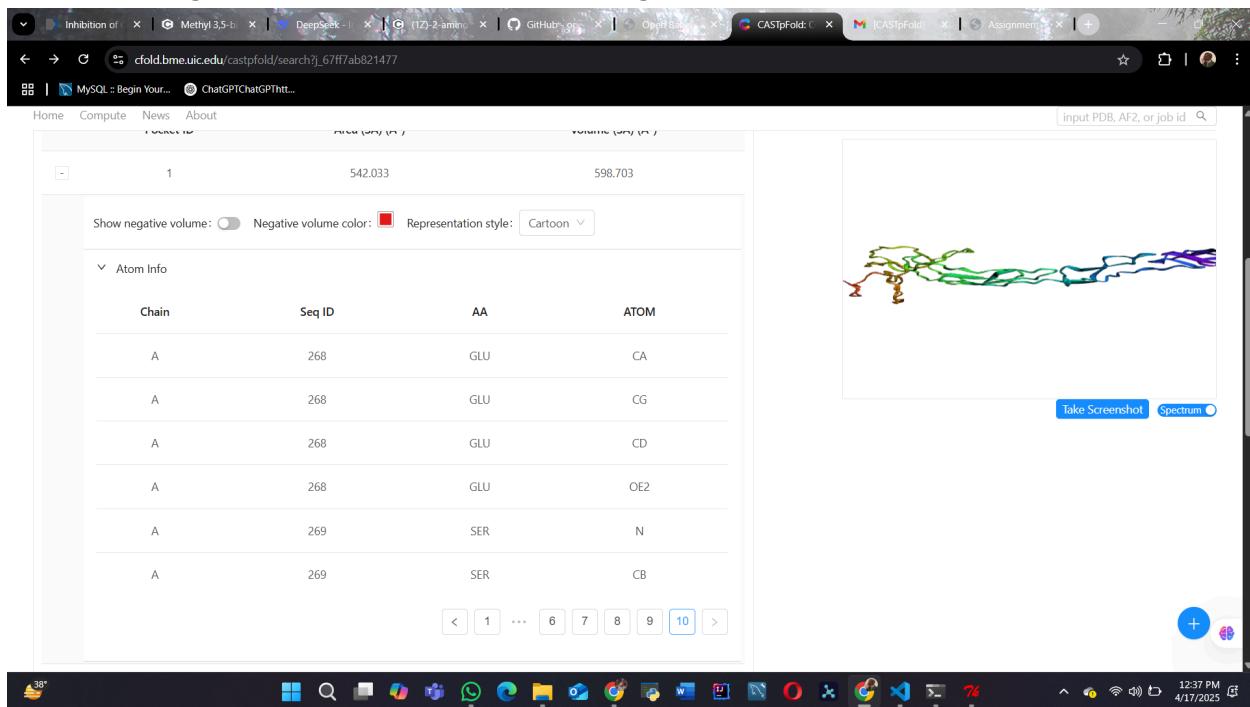


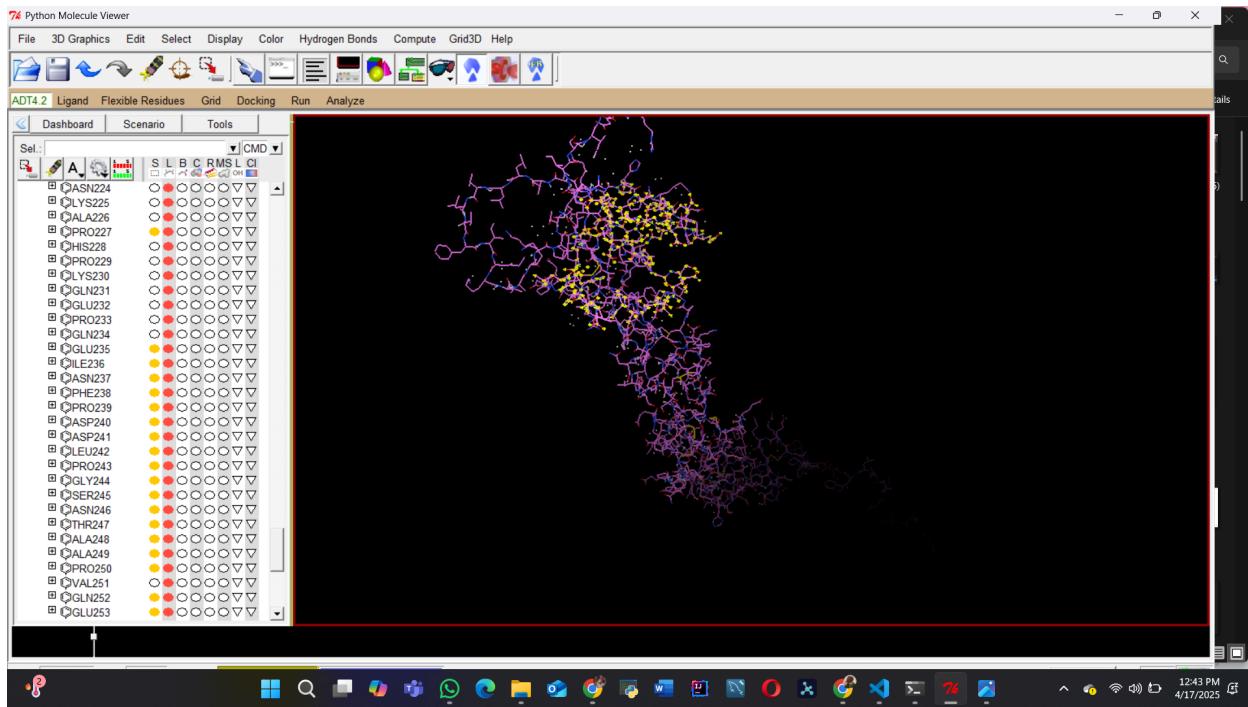
### Mutation 3





- **Finding active sites of the protein using Castp**



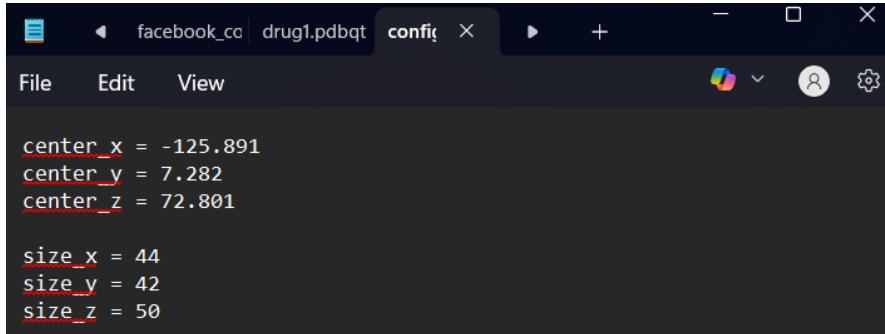


- All 4 protein models (1 wild-type + 3 mutated) preparation

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### 3. Molecular Docking: Perform docking of the five drug molecules with all four protein models (1 WT + 3 Mutated)

- Mention the parameters used (e.g. config file)



```
center_x = -125.891
center_y = 7.282
center_z = 72.801

size_x = 44
size_y = 42
size_z = 50
```

**center\_x = -125.891**

**center\_y = 7.282**

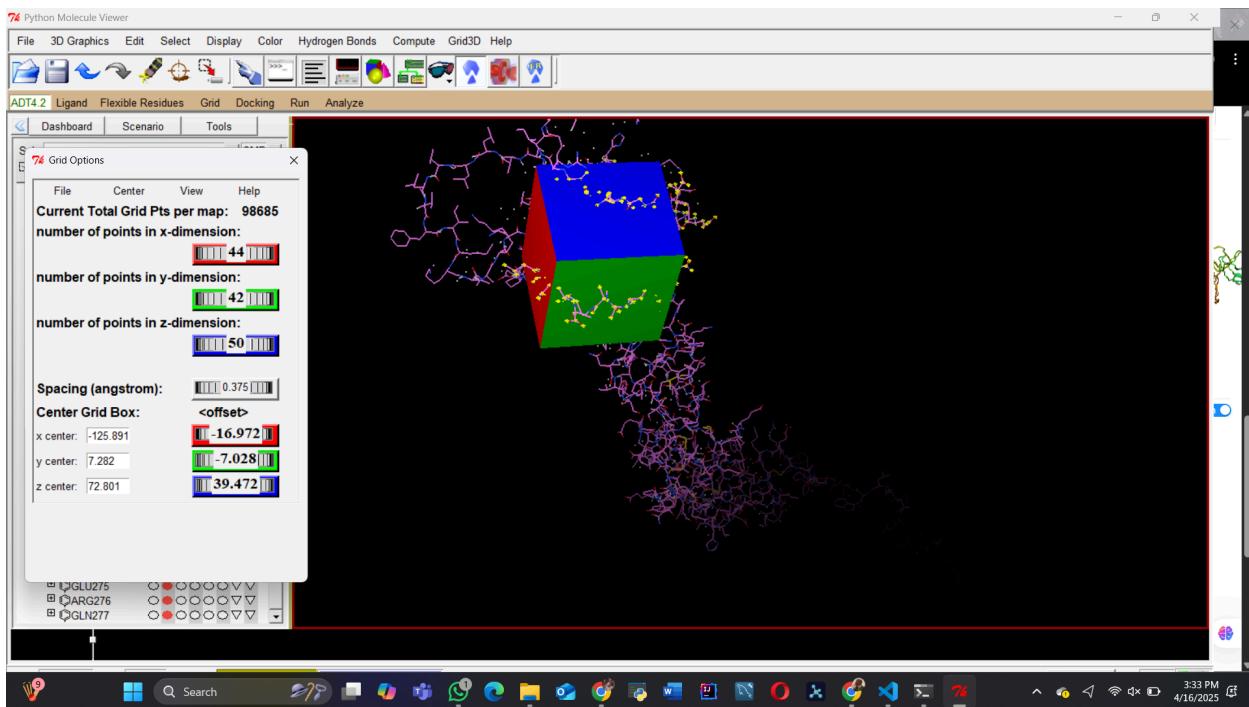
**center\_z = 72.801**

**size\_x = 44**

**size\_y = 42**

**size\_z = 50**

- Show the grid box taken WT



**4. Comparative Analysis of Docking Results:** Analyze the docking results and compare the binding affinities (binding energy scores) across the wild-type and mutated proteins for each drug

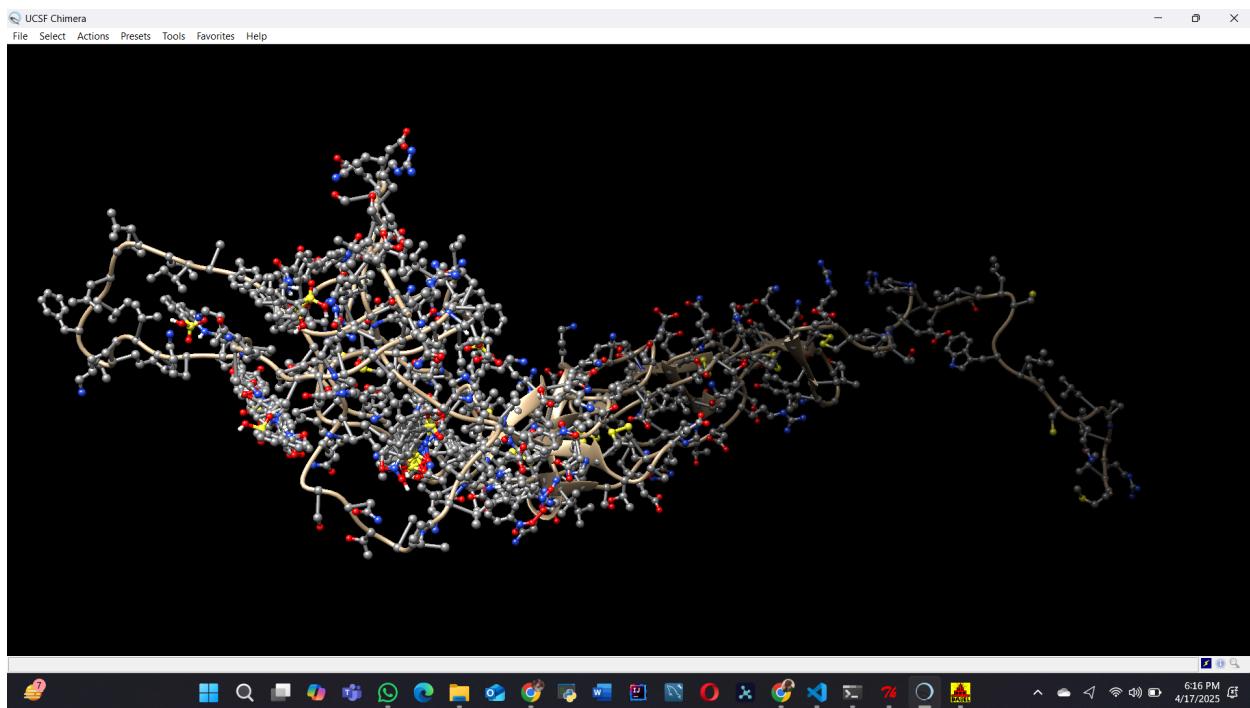
- Tabulated data of binding energies for each protein-ligand combination

COMBINATION	BEST AFFINITY SCORES
WT + DRUG 1	-6.354
WT + DRUG 2	-7.766
WT + DRUG 3	-8.6
WT + DRUG 4	-8.419
WT + DRUG 5	-6.902
M1 + DRUG 1	-6.756
M1 + DRUG 2	-7.858
M1 + DRUG 3	-9.044
M1 + DRUG 4	-8.773
M1 + DRUG 5	-7.896
M2 + DRUG 1	-7.856
M2 + DRUG 2	-8.346
M2 + DRUG 3	-8.757
M2 + DRUG 4	-8.432
M2 + DRUG 5	-6.787
M3 + DRUG 1	-7.505
M3 + DRUG 2	-8.865
M3 + DRUG 3	-8.695
M3 + DRUG 4	-8.529
M3 + DRUG 5	-7.355

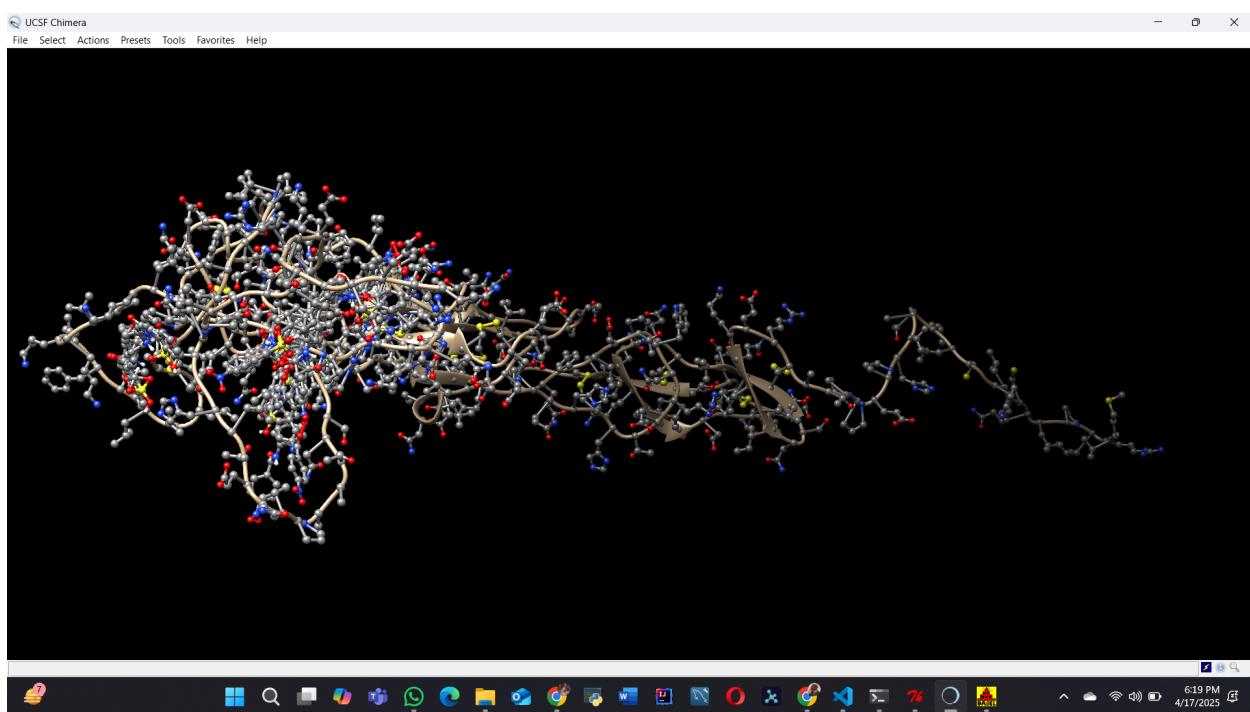
	<b>Protein</b>	<b>Mutation</b>	<b>Drug</b>	<b>Binding Energy</b>
0	M1	drug1	log.txt	-6.756
1	M1	drug2	log.txt	-7.858
2	M1	drug3	log.txt	-9.044
3	M1	drug4	log.txt	-8.773
4	M1	drug5	log.txt	-7.896
5	M2	drug1	log.txt	-7.856
6	M2	drug2	log.txt	-8.346
7	M2	drug3	log.txt	-8.757
8	M2	drug4	log.txt	-8.432
9	M2	drug5	log.txt	-6.787
10	M3	drug1	log.txt	-7.505
11	M3	drug2	log.txt	-8.865
12	M3	drug3	log.txt	-8.695
13	M3	drug4	log.txt	-8.529
14	M3	drug5	log.txt	-7.355
15	WT	drug1	log.txt	-6.354
16	WT	drug2	log.txt	-7.766
17	WT	drug3	log.txt	-8.600
18	WT	drug4	log.txt	-8.419
19	WT	drug5	log.txt	-6.902

- Visualization of at least one docked complex for each mutation + wild-type

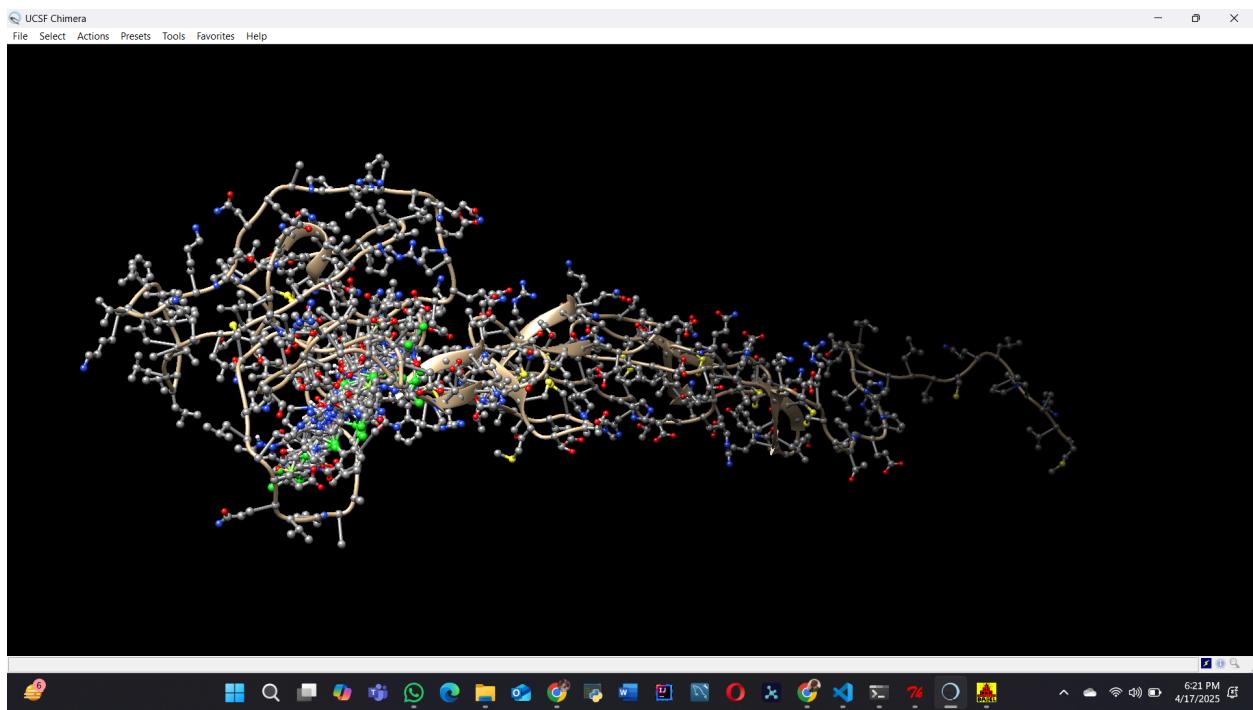
## Mutation 1



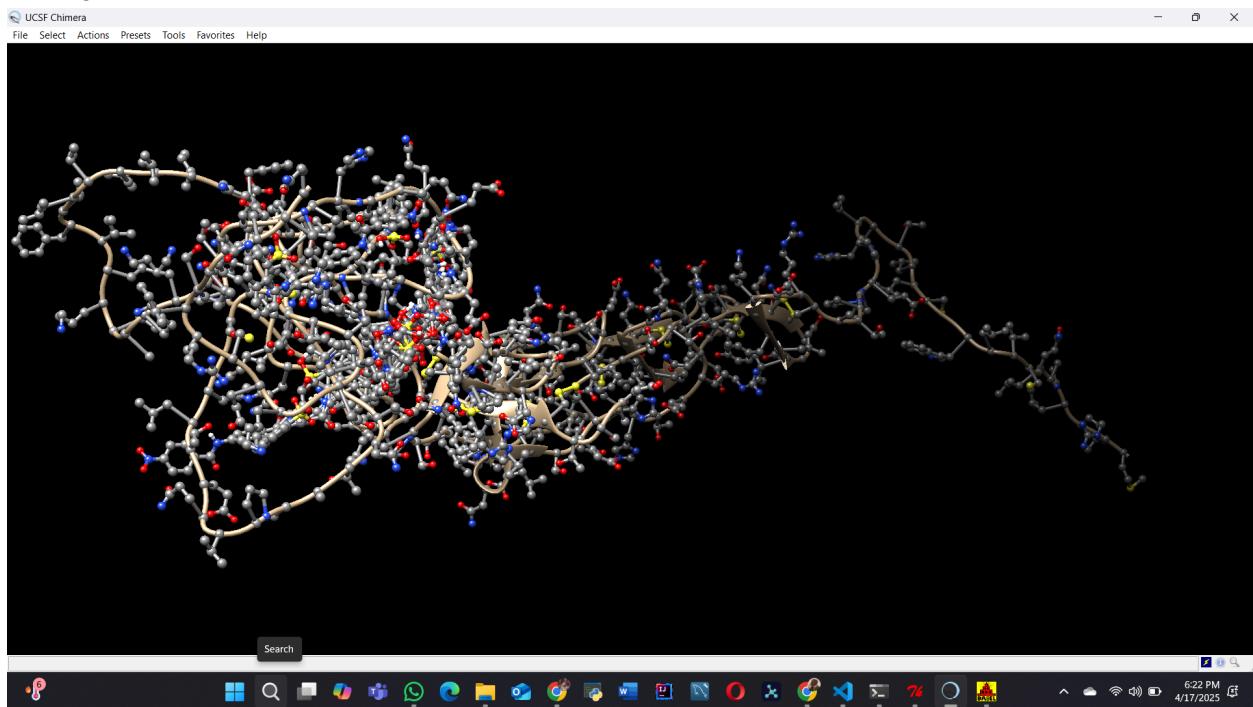
## Mutation 2



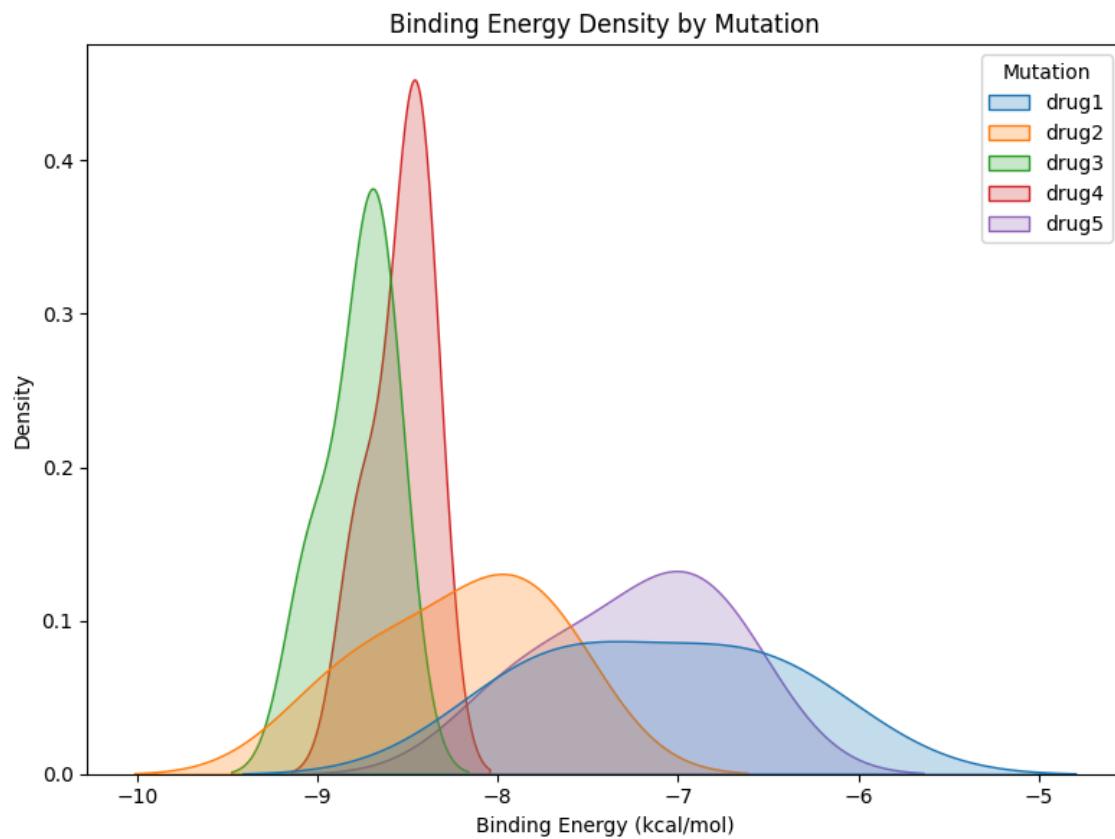
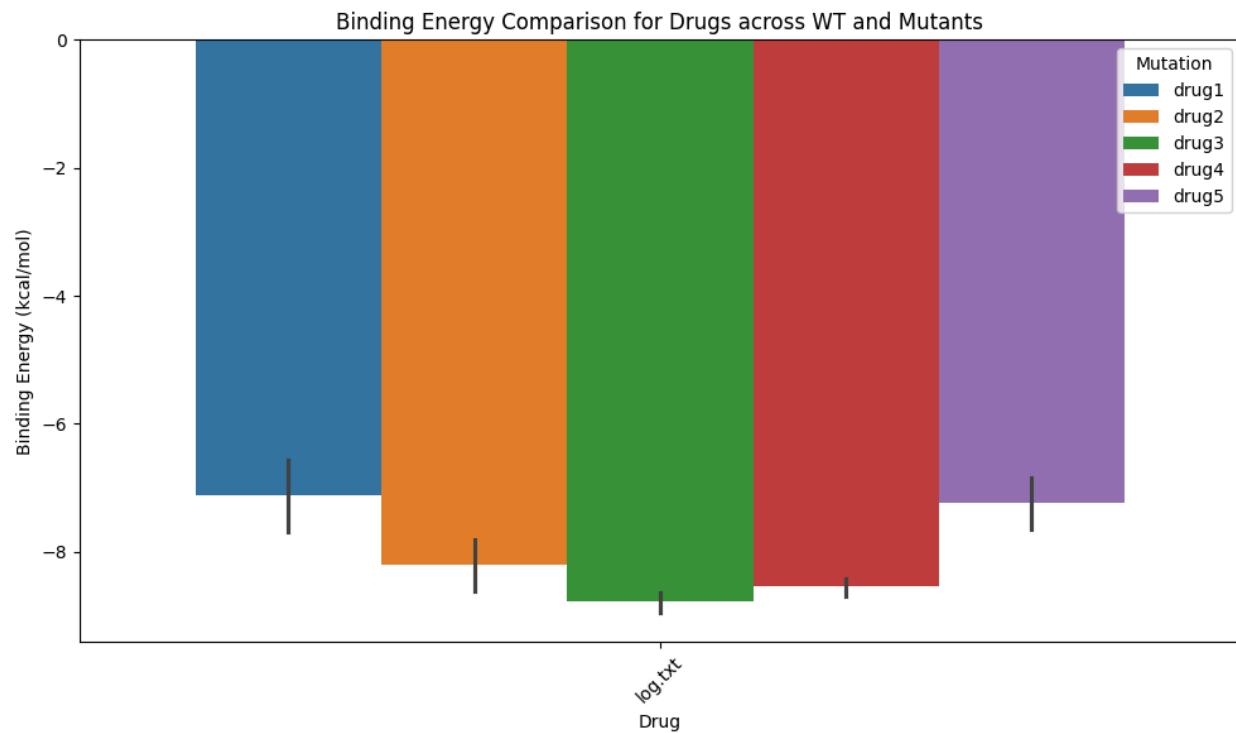
### Mutation 3



### Wild type



- Graphical representation of comparative binding affinities (e.g density distribution graph/bar graph showing binding energy distribution for all the ligands)



- **Highlight key differences observed in binding mode or affinity due to mutations**

1. Drug3 and Drug4 consistently show stronger binding affinities (more negative values) across all mutations compared to the wild-type.
2. M1 mutation enhances binding affinity especially for Drug3 (-9.044) and Drug4 (-8.773), which are both better than the wild-type for the same drugs (-8.600 and -8.419, respectively).
3. M2 and M3 mutations also improve binding for most drugs, particularly:
  - M3 + Drug2: -8.865 (better than WT -7.766)
  - M2 + Drug3: -8.757 (better than WT -8.600)
4. Drug5 shows less consistent results. In M2, its affinity drops to -6.787, which is worse than WT -6.902.

## **5. Inference and Discussion: Discuss the effect of mutations on drug binding and propose the most promising drug based on your docking analysis.**

### **Effect of Mutations on Drug Binding:**

1. Mutations enhanced ligand binding in most cases, likely due to better residue interactions or more favorable conformations in the binding pocket.
2. Drug3 and Drug4 were the most stable binders across all protein forms, suggesting broad-spectrum compatibility with mutant forms.

### **Most Promising Drug:**

Drug3 emerges as the most promising candidate:

1. Highest binding energy in M1 (-9.044).
2. Consistently strong across WT and all mutants.

Drug4 is a close second with very stable affinity across all forms.

### **NOTES:**

Uniprot ID	Protein Name	Mutation 1	Mutation 2	Mutation 3
P25942	Tumor necrosis factor receptor superfamily member 5	P227A	I208V	T112M