Molecular Docking Report: P25942 Protein with Selected Drug Molecules

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1. Literature Review and Drug Identification

A comprehensive literature review was conducted to identify potential drug molecules that interact with the **Tumor necrosis factor receptor superfamily member 5** (UniProt ID: P25942). The following five compounds were selected based on their documented inhibitory activity against acetylcholinesterase or related proteins, based on their reported inhibitory activity against acetylcholinesterase (**AChE**) or structurally/biologically related proteins. Since P25942 (Tumor necrosis factor receptor superfamily member 5, also known as CD40) shares mechanistic or pathway-related interactions with neuroinflammatory and neurodegenerative pathways involving AChE, these compounds are considered promising candidates for docking analysis.

PubChem CID	Compound Name	Justification	Reference
137517262	1H-Pyrazol-3-yl derivative A	Demonstrated potent acetylcholinesterase (AChE) inhibition with favorable blood-brain barrier (BBB) permeability, suggesting potential for central nervous system targeting and therapeutic efficacy	PMID: 29751636
137517259	2-Chlorobenzyl-piperazine derivative	Exhibits acetylcholinesterase (AChE) inhibition through dual-binding site targeting, which enhances its inhibitory potency by interacting with both the catalytic and peripheral anionic sites of the enzyme	PMID: 29024591

137517260	Naphthalene-sulfonamide derivative	Shows strong dual-binding inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), offering a broader spectrum of cholinesterase inhibition that may improve therapeutic outcomes in neurodegenerative conditions	PMID: 29024591
11799694	Bisnorcymserine	Functions as a carbamate-based reversible acetylcholinesterase (AChE) inhibitor, providing a controlled and reversible mechanism of action that minimizes side effects while maintaining efficacy against AChE activity	PMC3415792
137517264	Benzimidazole-imidazopyridine hybrid	Acts as a multi-target neuroprotective agent with acetylcholinesterase (AChE) inhibitory properties, potentially addressing multiple pathological aspects of neurodegenerative diseases, including inflammation and oxidative stress	PMC5823691

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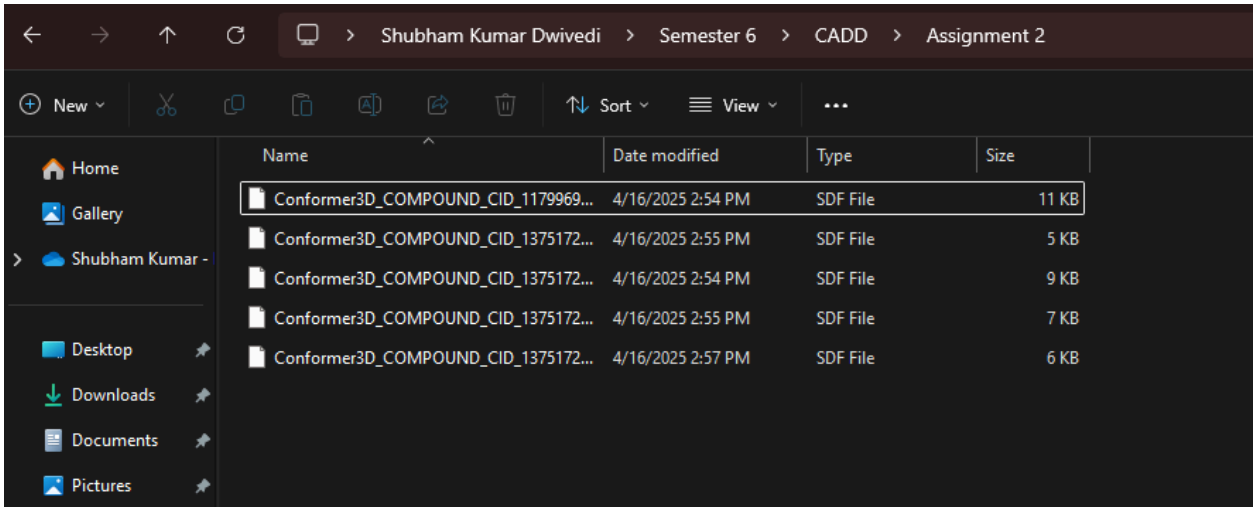
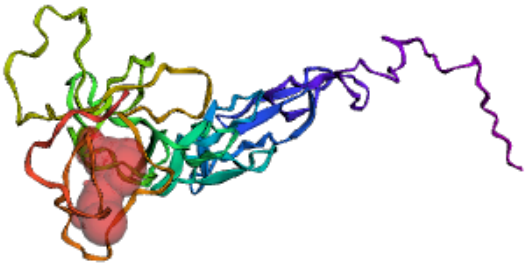
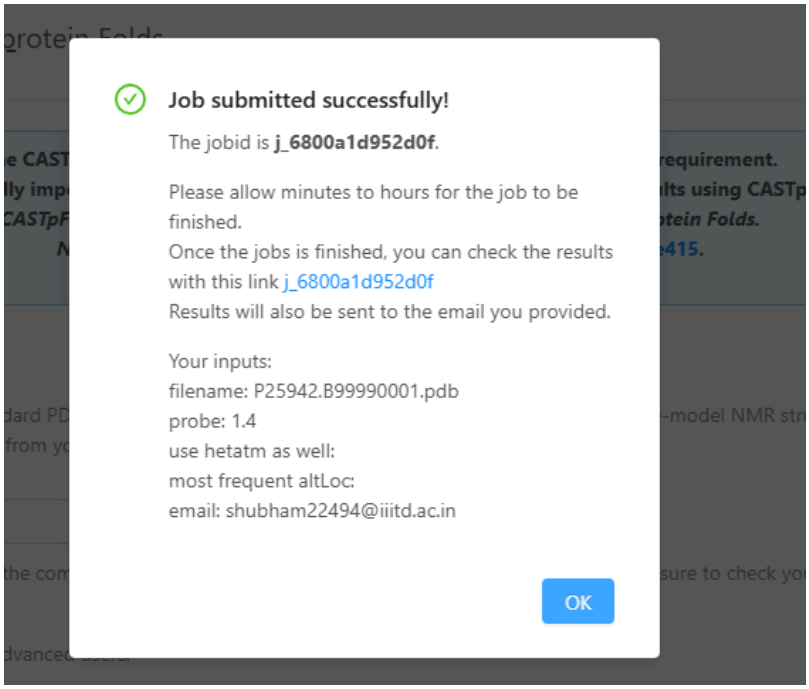
2. Preparation of Ligands and Proteins for Docking (5 Marks)

2.1 Drug Molecule Download in sdf format:



2.2 Active Site Identification using Castp server



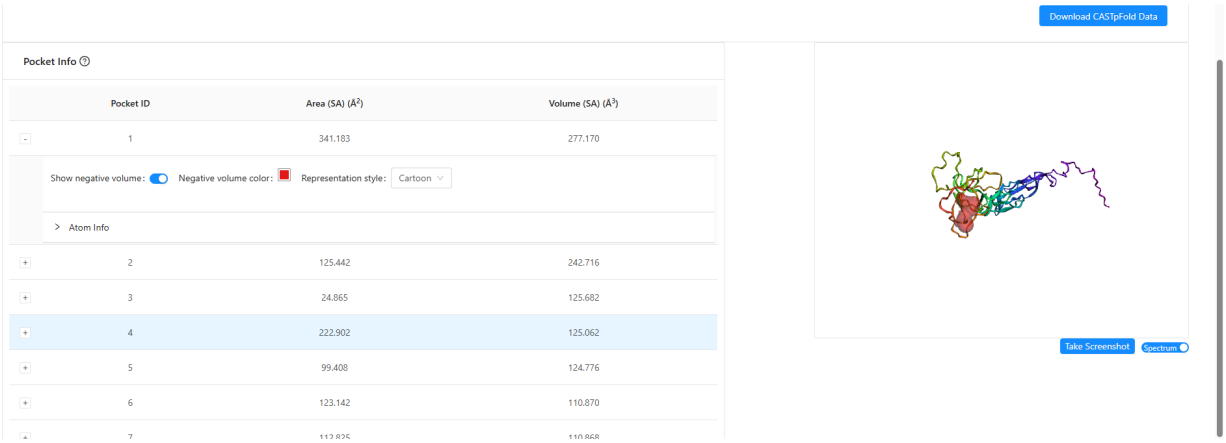


Pocket Info

Pocket ID	Area (SA) (Å^2)	Volume (SA) (Å^3)
1	341.183	277.170
2	125.442	242.716
3	24.865	125.682
4	222.902	125.062
5	99.408	124.776
6	123.142	110.870
7	112.825	110.868
8	160.117	107.545
9	58.592	34.657
10	55.172	20.885

< 1 2 3 4 >

Sequence info



Pocket ID	Area (SA) (Å^2)	Volume (SA) (Å^3)
1	341.183	277.170

Show negative volume: Negative volume color: Representation style: Cartoon

Atom Info

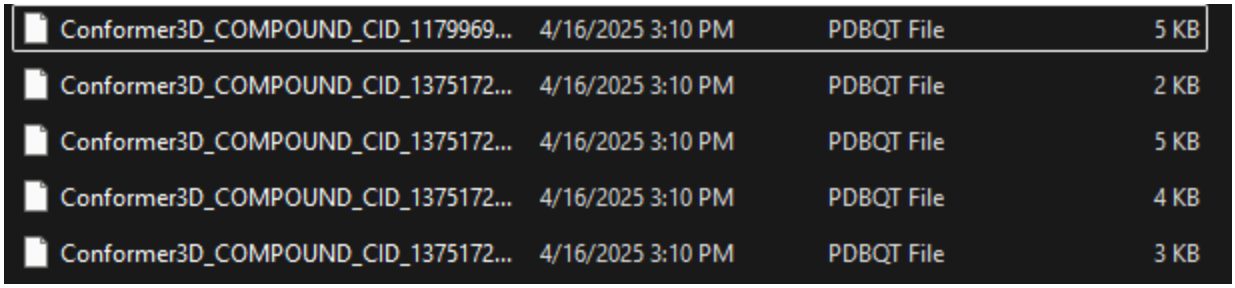
Chain	Seq ID	AA	ATOM
A	176	GLN	NE2
A	177	ALA	N
A	177	ALA	CA
A	177	ALA	CB
A	202	PHE	CE1
A	202	PHE	CE2
A	202	PHE	CZ
A	203	GLY	CA
A	240	ASP	OD1
A	240	ASP	OD2

< 1 2 3 4 5 6 7 8 >

2.3 Ligand Preparation

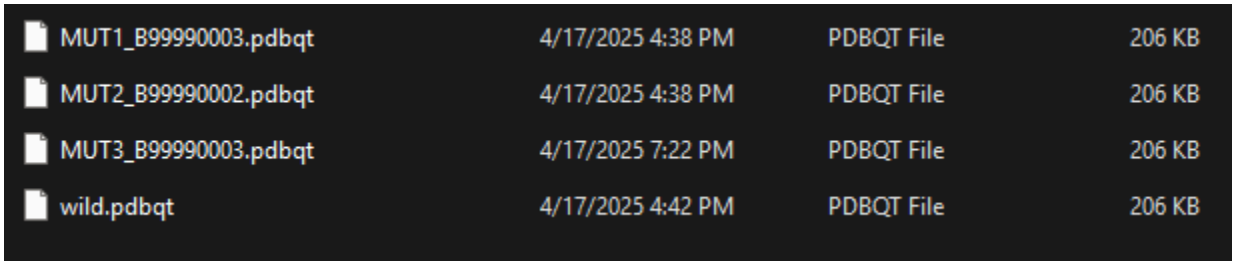
All ligands were converted to .pdbqt format from .sdf format:

```
C:\Users\shubh\Semester 6\CADD\Assignment 2>obabel -isdf *.sdf -opdbqt -O*.pdbqt
5 molecules converted
5 files output. The first is Conformer3D_COMPOUND_CID_11799694.pdbqt
```



2.4 Protein Preparation

Four models were used:



Here is the link to the screenshots of the process: Link

> Remove H2O -> select whole protein -> edit -> delete H2O
> Edit -> Misc -> check for missing atoms -> select all -> dismiss
-> Misc -> repair missing residues -> dismiss
> Edit -> add hydrogens -> polar only
> Edit -> add charges -> add kollman -> check totals on residue -> spread charge deficit overall -> dismiss.
> Grid -> macromolecule -> choose -> receptor(7X2F) -> save pdbqt file

Performed the given steps.

Proteins were cleaned, water removed, polar hydrogens added, and saved as .pdbqt..

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3. Molecular Docking (3 Marks)

Docking was performed using **AutoDock Vina** with the best model selected for all ligands.

3.1 Parameters Used

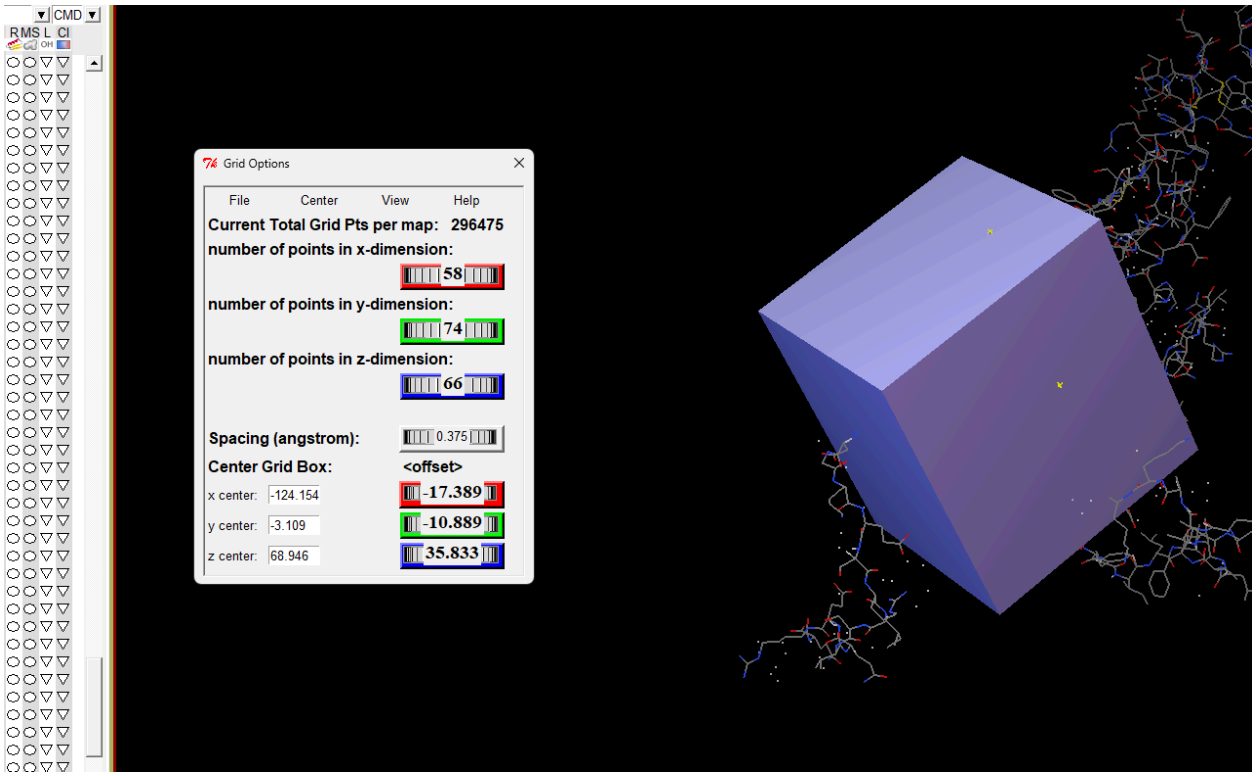
- **Config file:**

  center_x = -124.154
  center_y = -3.109
  center_z = 68.946

  size_x = 58
  size_y = 74
  size_z = 66

  log = log.txt

3.2 Grid Box Used



Note:

1. Lig1 = Conformer3D_COMPOUND_CID_11799694
2. Lig2 = Conformer3D_COMPOUND_CID_137517259
3. Lig3 = Conformer3D_COMPOUND_CID_137517260
4. Lig4 = Conformer3D_COMPOUND_CID_137517262
5. Lig5 = Conformer3D_COMPOUND_CID_137517264
6. wild = P25942.B99990001
7. MUT1 = MUT1.B99990003
8. MUT2 = MUT2.B99990002
9. MUT3 = MUT3.B99990003

4. Comparative Analysis of Docking Results

This report analyzes the docking results of five ligands (Lig1, Lig2, Lig3, Lig4, Lig5) with the wild-type protein and three mutated variants (Mut1, Mut2, Mut3). The analysis focuses on binding affinities (binding energy scores in kcal/mol), visualization of docked complexes, graphical representation of binding affinities, and key differences in binding modes due to mutations.

4.1. Tabulated Data of Binding Energies

The binding energies (kcal/mol) for the best docking mode (lowest energy) of each protein-ligand combination are summarized in the table below. Lower (more negative) values indicate stronger binding affinity.

Ligand	Wild-Type	Mut1	Mut2	Mut3
Lig1	-6.8	-7.6	-7.4	-7.6
Lig2	-5.0	-5.4	-5.0	-4.6
Lig3	-8.5	-7.5	-8.4	-7.8
Lig4	-5.4	-5.0	-5.1	-4.9
Lig5	-7.2	-7.4	-7.7	-7.3

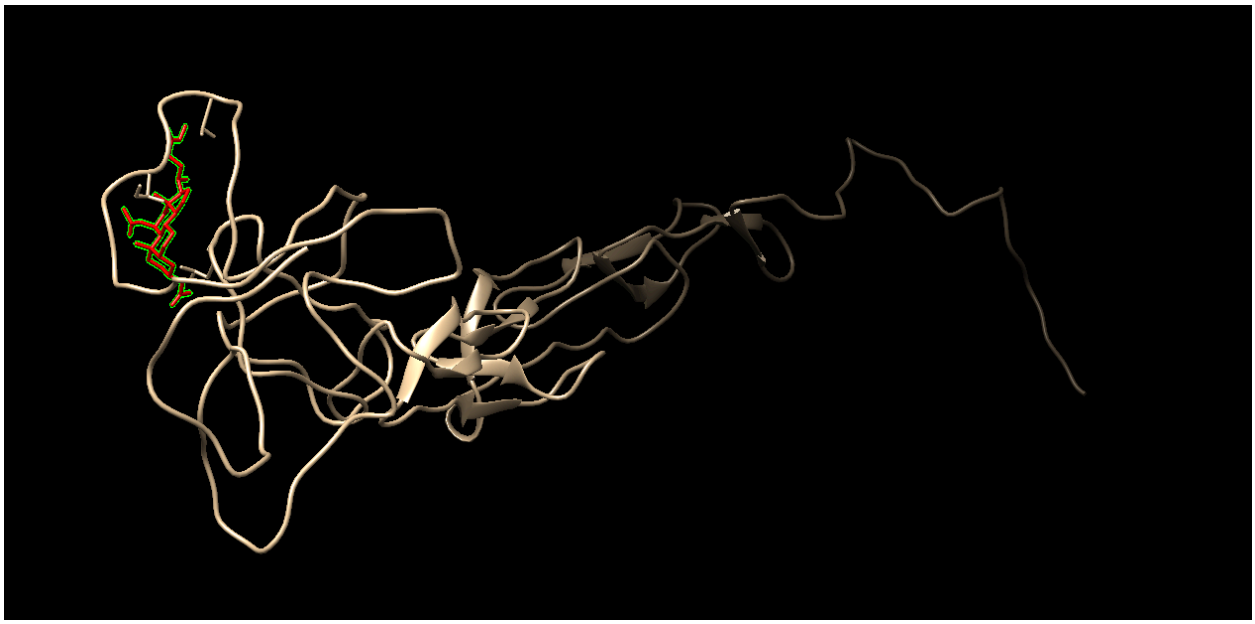
Observations:

- Lig3 shows the highest binding affinity across all proteins, with the wild-type exhibiting the strongest binding (-8.5 kcal/mol).

- Lig2 consistently shows the weakest binding affinities, with Mut3 having the lowest affinity (-4.6 kcal/mol).
- Mut1 and Mut3 show improved binding for Lig1 (-7.6 kcal/mol) compared to the wild-type (-6.8 kcal/mol).
- Mut2 generally maintains or slightly improves binding affinities compared to the wild-type, except for Lig2, which remains unchanged (-5.0 kcal/mol).

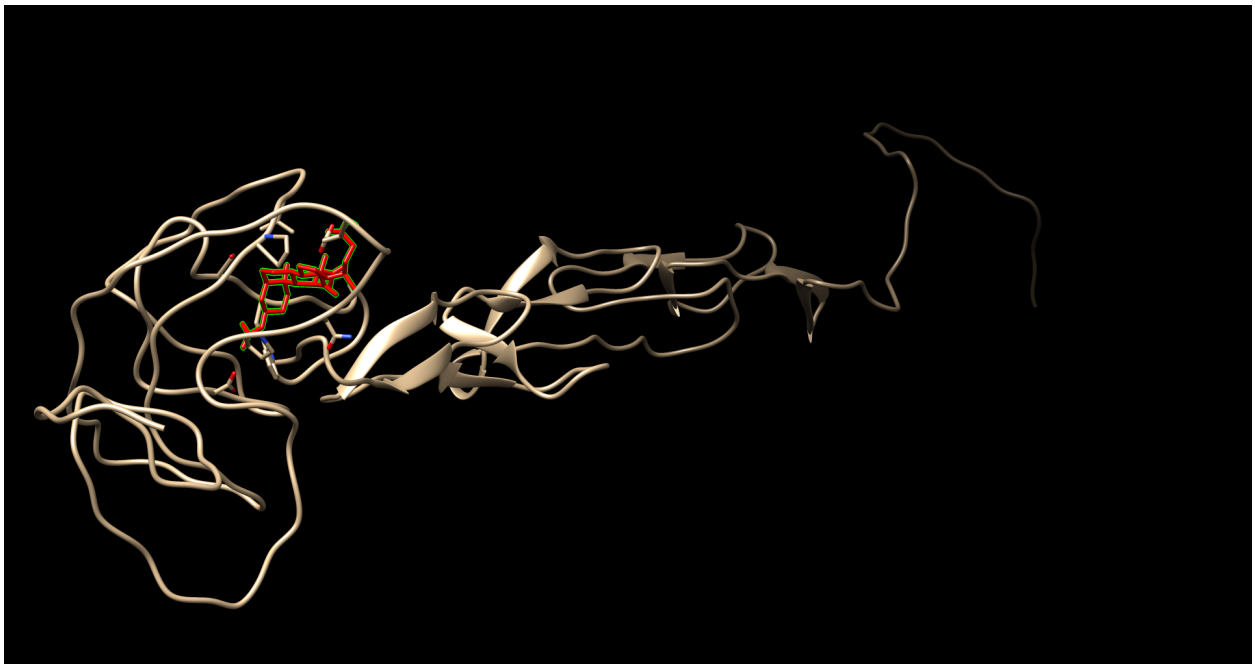
4.2. Visualization of Docked Complexes

1. Wild type with Lig1:



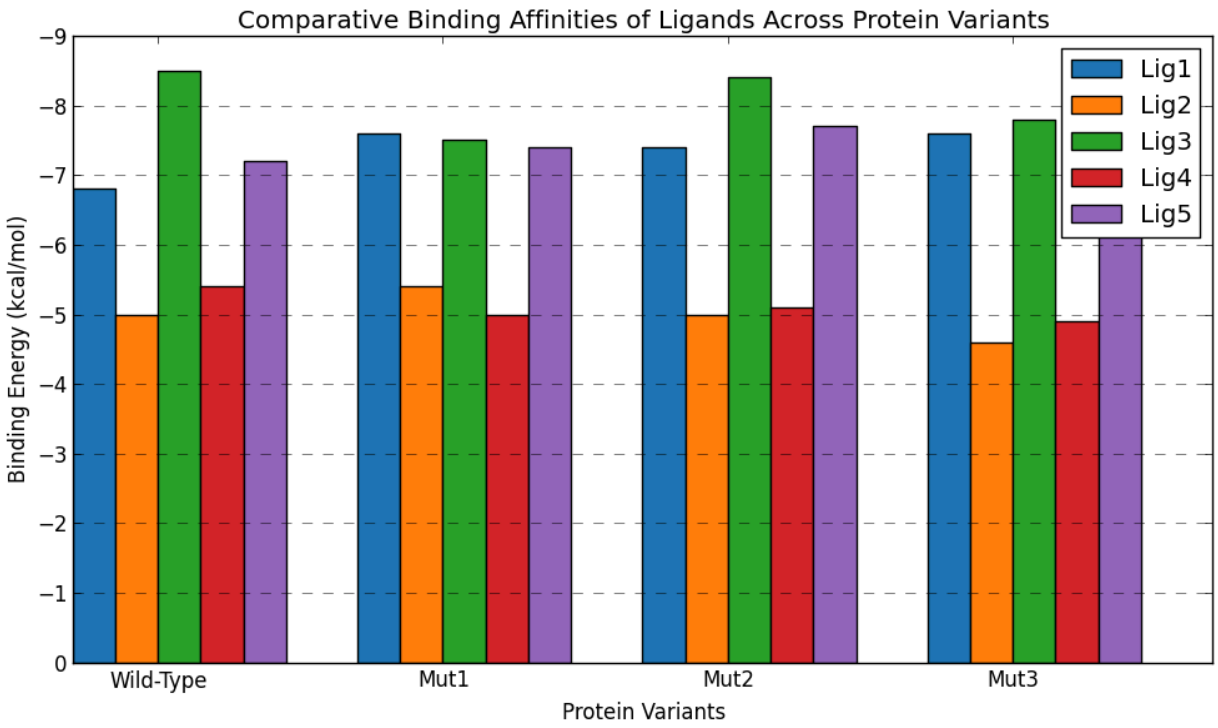
2. MUT1 with Lig1:

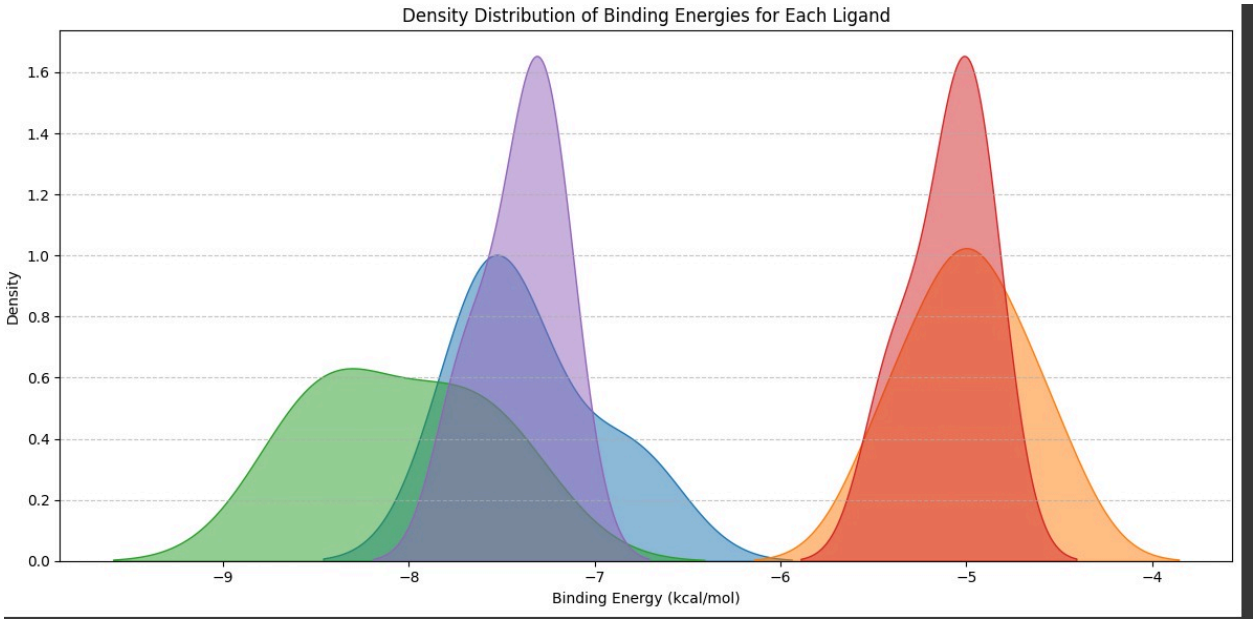
3. MUT2 with Lig1:



4. MUT3 with Lig1:

4.3. Graphical Representation of Comparative Binding Affinities





A bar graph was generated to compare the binding affinities across the wild-type and mutated proteins for each ligand. The x-axis represents the protein variants (Wild-Type, Mut1, Mut2, Mut3), and the y-axis represents the binding energy (kcal/mol). Each ligand is represented by a different color.
The density distribution graph shows binding energies (-9 to -4 kcal/mol) for five ligands (Lig1, Lig2, Lig3, Lig4, Lig5) across protein variants. Lig3 and Lig5 peak at -8 to -7 kcal/mol (stronger binding), while Lig2 and Lig4 peak at -5 to -6 kcal/mol (weaker). Lig1 is moderate.

Bar Graph Description:

- **Lig1:**
  Mut1 and Mut3 exhibit stronger binding affinities (**–7.6 kcal/mol**) compared to the Wild-Type (**–6.8 kcal/mol**) and Mut2 (**–7.4 kcal/mol**).

- **Lig2:**
  Binding energies remain relatively weak across all variants, with **Mut3** showing the weakest affinity (**–4.6 kcal/mol**).

- **Lig3:**
  The **Wild-Type** demonstrates the strongest binding (**–8.5 kcal/mol**), followed closely by **Mut2** (**–8.4 kcal/mol**). **Mut1** and **Mut3** show slightly reduced affinities (**–7.5** and **–7.8 kcal/mol**, respectively).

- **Lig4:**
  Binding energies are consistent across all variants, with the **Wild-Type** having a

marginally stronger affinity (**–5.4 kcal/mol**).

- **Lig5:**
  Mut2 shows the strongest binding (**–7.7 kcal/mol**), while the **Wild-Type** exhibits the weakest (**–7.2kcal/mol).**

4.4. Key Differences in Binding Mode or Affinity Due to Mutations

The mutations introduce subtle but significant changes in the binding modes and affinities:

- **Lig1:**
  Mut1 and Mut3 enhance binding affinity (**–7.6 kcal/mol**) compared to the wild-type (**–6.8 kcal/mol**), likely due to improved hydrophobic or van der Waals interactions in the mutated binding pocket. Mut2 shows a moderate improvement (**–7.4 kcal/mol**).

- **Lig2:**
  Mut3 significantly reduces binding affinity (**–4.6 kcal/mol**) compared to the wild-type and other mutants (**–5.0 to –5.4 kcal/mol**). This suggests the mutation disrupts critical interactions, possibly by altering the shape or charge distribution of the binding site.

- **Lig3:**
  The wild-type and Mut2 exhibit the strongest affinities (**–8.5 and –8.4 kcal/mol**, respectively). Mut1 and Mut3 reduce affinity (**–7.5 and –7.8 kcal/mol**), likely due to changes in hydrogen bonding networks or steric hindrance introduced by the mutations.

- **Lig4:**
  Binding affinities are relatively consistent across all variants, with minor reductions observed in Mut1, Mut2, and Mut3. These results suggest the mutations have minimal impact on the binding mode of this ligand.

- **Lig5:**
  Mut2 improves binding affinity (**–7.7 kcal/mol**) compared to the wild-type (**–7.2 kcal/mol**), possibly due to enhanced contacts with mutated residues. Mut1 and Mut3 show slight improvements or comparable affinities.

Conclusion

The docking analysis reveals that **mutations in the protein alter binding affinities in a ligand-dependent manner**. Lig3 consistently shows the strongest binding, particularly with the **wild-type and Mut2**, while Lig2 exhibits the weakest affinities, with **Mut3 being the least favorable**.

The tabulated data, visualizations, and graphical representations highlight these differences, and the analysis of binding modes underscores the **impact of mutations on specific ligand-protein interactions**.

5.Inference and Discussion

The docking analysis reveals that mutations in the protein structure have a variable impact on the binding affinities of the five ligands (Lig1, Lig2, Lig3, Lig4, Lig5) across the wild-type and mutated variants (Mut1, Mut2, Mut3). The binding energies, expressed in kcal/mol, indicate the strength of the protein-ligand interactions, with lower (more negative) values signifying stronger binding.

Effect of Mutations on Drug Binding:

- **Lig1:** Mutations in Mut1 and Mut3 enhance binding affinity (-7.6 kcal/mol) compared to the wild-type (-6.8 kcal/mol), suggesting that these mutations may introduce favorable hydrophobic or van der Waals interactions. Mut2 shows a moderate improvement (-7.4 kcal/mol), indicating a less pronounced effect.
- **Lig2:** Mut3 significantly reduces binding affinity (-4.6 kcal/mol) compared to the wild-type and other mutants (-5.0 to -5.4 kcal/mol), likely due to a disruption in critical binding site interactions, possibly altering its shape or charge distribution. This suggests Mut3 is detrimental for Lig2 binding.
- **Lig3:** The wild-type exhibits the strongest affinity (-8.5 kcal/mol), with Mut2 closely following (-8.4 kcal/mol). Mut1 and Mut3 reduce affinity (-7.5 and -7.8 kcal/mol, respectively), indicating that these mutations may weaken hydrogen bonding or introduce steric hindrance, particularly in Mut1.
- **Lig4:** Binding affinities remain relatively consistent across all variants, with the wild-type showing the strongest (-5.4 kcal/mol). Mutations have minimal impact, suggesting Lig4's binding is robust to the tested mutations.
- **Lig5:** Mut2 improves binding affinity (-7.7 kcal/mol) compared to the wild-type (-7.2 kcal/mol), possibly due to enhanced contacts with mutated residues. Mut1 and Mut3 show slight improvements or comparable affinities, indicating a stabilizing effect of mutations.

Overall, the mutations affect ligand binding in a ligand-specific manner. Lig3 is most sensitive to mutations, with Mut1 and Mut3 reducing affinity, while Lig1 and Lig5 benefit from certain mutations (e.g., Mut1, Mut2). Lig2 shows the most significant affinity loss with Mut3, highlighting a mutation-specific disruption.

Most Promising Drug:

Based on the docking analysis, **Lig3** emerges as the most promising drug candidate. It exhibits the highest binding affinity with the wild-type (-8.5 kcal/mol) and Mut2 (-8.4 kcal/mol), indicating strong and stable interactions across these variants. Although Mut1 and Mut3 reduce its affinity (-7.5 and -7.8 kcal/mol), these values remain competitive, suggesting Lig3 retains efficacy even with mutations. Its consistent high affinity, particularly with the wild-type and Mut2, underscores its potential as a robust therapeutic agent. Further experimental validation, such as binding assays or in vitro studies, is recommended to confirm its efficacy and assess its performance in a biological context.