Utilizing Molecular Docking and Mutagenesis of Lys-233 into Ala-233 to Analyze the Effect on the Binding of the Morphinian Antagonist to the μ-Opioid Receptor

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Abstract

Opium is one of the oldest known medicines. Its derivatives, morphine and codeine, are among the most utilized therapeutic treatments to relieve severe pain (Manglik et al., 2012). Opium was originally used to create the first crystal structure of the μ-opioid receptor: the primary receptor for opioids that regulate the body's response to pain (MedlinePlus, 2021). However, a method to increase the binding efficiency of its morphinian antagonist, β-funaltrexamine (β-FNA), is still unknown. In this study the residue, Lys-233, was mutated into Ala-233 to observe significant changes to binding. We used computational tools including AutoDock Vina (Eberhardt et al., 2021; Trott, et al., 2010) and PyMOL (The PyMOL Molecular Graphics System) to redock the ligand into the mutated protein. As a result, we found that the best RMSD value before mutation was 1.87 Å, but after mutation became 1.167 Å. On average, in the series of trials that occurred after the mutation, the nine poses produced better RMSD values. This led to the conclusion that mutating Lys-233 into Ala-233 enhances the binding of the morphinian antagonist to the μ-opioid receptor. Such research encourages the mutation of other opioid receptor residues in order to anticipate which variation provides the optimal result. This study suggests that many other combinations of mutations exist, through which the process of drug discovery can be improved.

Introduction

Being one of the earliest medicinal remedies to mitigate pain, opium has been used for several centuries (Manglik et al., 2012). Originating in the Middle East from opium poppy plants, it has been used exhaustively to alleviate the effects of a variety of illnesses. Through X-ray diffraction, opium was initially utilized to develop the first crystal structure of the μ-opioid

receptor (PDB ID: 4DKL) (Protein Data Bank Japan, 2021). The research group determined the structure of the μ-opioid receptor (μ-OR) from the crystals collected using meso crystallization (Manglik et al., 2012). The findings illustrated that μ-OR was bound to the morphinan antagonist β-funaltrexamine (β-FNA) (MedlinePlus, 2021). This morphinian antagonist is an amide of methyl fumarate with a naltrexone derivative (Fig. 3). It preserves the protein in its natural state, preventing it from inducing a signal continuously (UVA Collab: VirginiaTech, 2021). The μ-opioid receptor has been a good candidate to develop pharmaceuticals for opioid substance abuse disorder. Thus, understanding how this antagonist binds to and reacts to a mutation in its amino acid sequence might help researchers create more effective medicines.

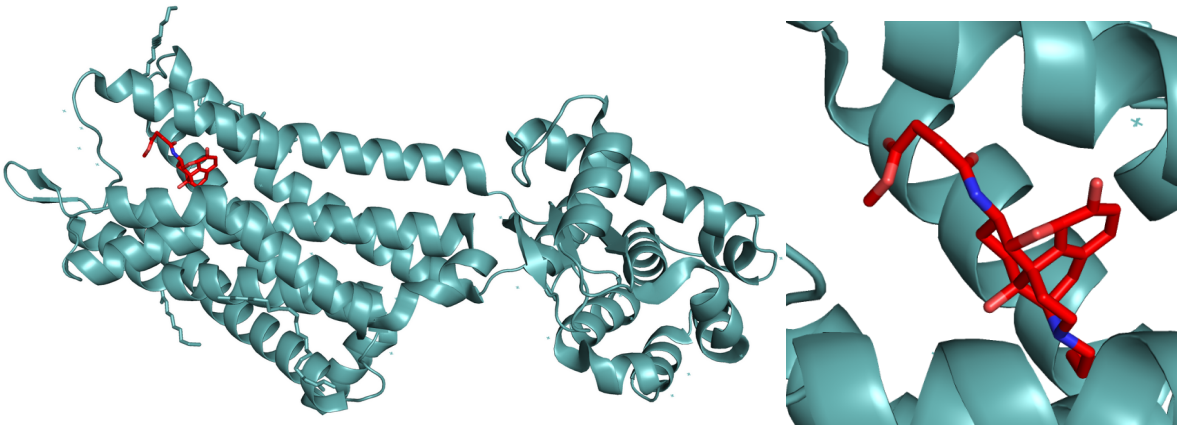


Figure 1 - *Structure of μ-opioid receptor and its ligand - BFO-601. Its PDB ID 4DKL was visualized using PyMOL. The μ-opioid receptor is shown in light teal, as a cartoon, and the BFO-601 ligand as red, colored by element, in the form of sticks.*

Lys-233, an amino acid part of the protein's original sequence, is positively charged. It is 1 angstrom away from the BFO-601 ligand (Fig 2). However, Alanine is traditionally a neutral, hydrophobic amino acid. The interactions between a protein and its ligand are often non-covalent, and these interactions stabilize the ligand when it binds to a protein. Therefore, it was

predicted that hydrophobic interactions between the ligand and amino acids are crucial, because it allows for more favorable interactions between the ligand and the residues. Thus, mutating the hydrophilic Lysine into a more neutral hydrophobic amino acid would improve the stability of the protein-ligand complex, when redocking the ligand.

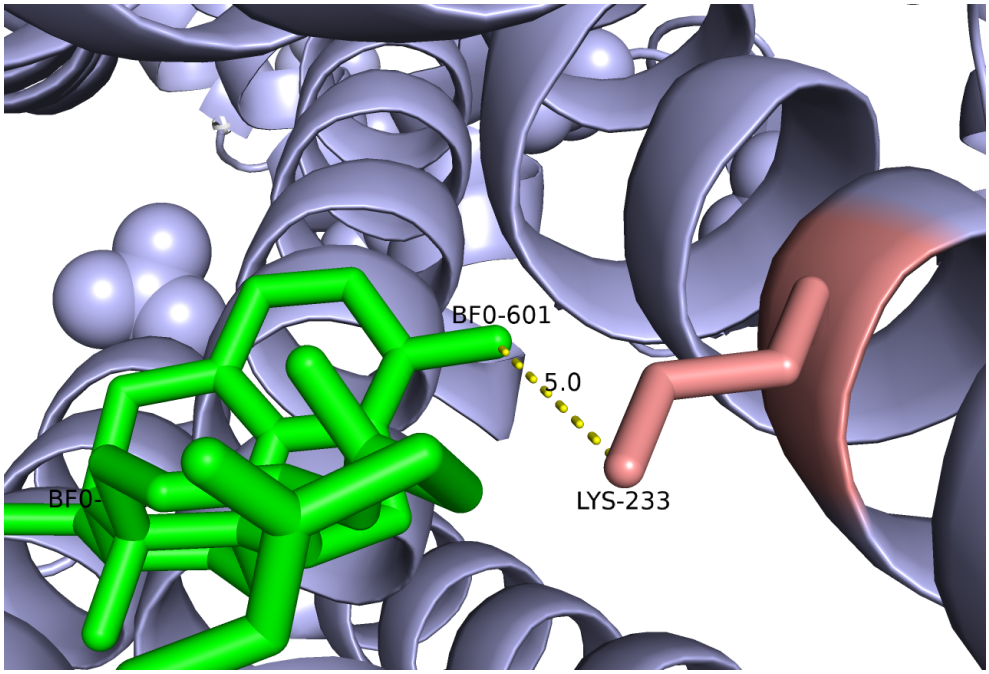


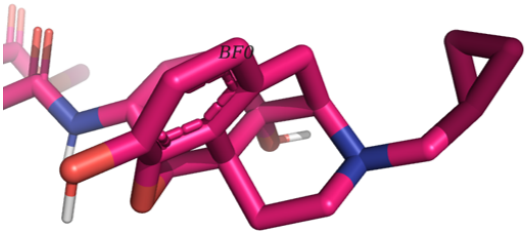
Figure 2 - *Visualization representing distance (5.0Å) from Lys-233 residue to BFO-601 ligand.*

Therefore, it was hypothesized that if Lys-233 is mutated to Ala-233, the binding of the morphinan antagonist β-funaltrexamine (β-FNA) to the μ-opioid receptor will be enhanced.

Methods

First, the opioid receptor protein (4DKL) was loaded from the Protein Data Bank into PyMOL (Manglik et al., 2012). Its ligand, BFO-601, was extracted and saved as a separated PDB file. It was cleaned by removing all water molecules and ions. Then, polar hydrogens were added to the molecules. Some hydrogens on compounds are polar, some are nonpolar. Only polar hydrogens participate in hydrogen bonding, therefore only the polar hydrogens were

added to the μ-opioid receptor and the ligand.



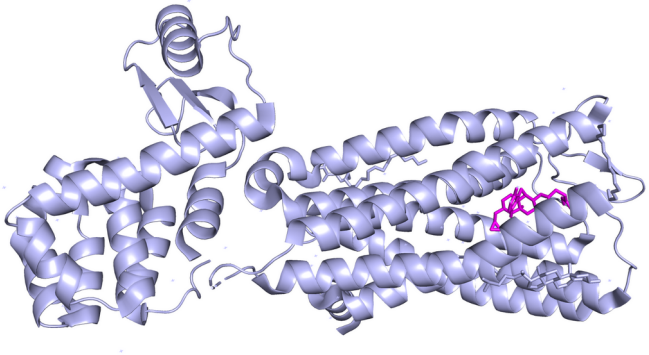
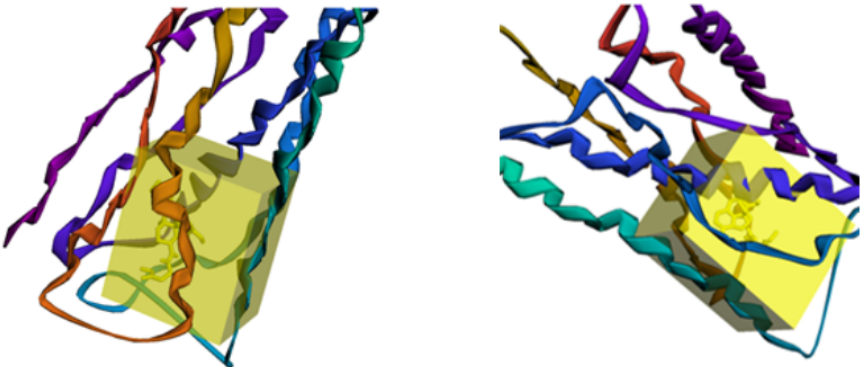


Figure 3 - *BFO-601 ligand*

Figure 4 - *μ-opioid receptor protein*

The PDB files were then uploaded onto Webina and converted into PDBQT files. The coordinates of the box center and size are shown in the table below.



Box Center	-25	-13	-8
Box Size	14	21	17.45

X, Y, and Z coordinates of the docking-box center

Figure 5 - *Visualization of Webina box and dimensions.*

We obtained an output file consisting of nine poses of the protein-ligand complex.

Mode	Affinity (Kcal/mol)	Dist from rmsd l.b.	Best mode rmsd u.b.
1	-9.1	0.000	0.000

2	-8.3	1.993	2.455
3	-8.2	2.091	4.041
4	-7.8	1.810	3.993
5	-7.4	3.804	8.093
6	-7.0	1.902	3.051
7	-6.9	2.931	8.041
8	-6.8	3.329	8.494
9	-6.7	2.705	7.813

Table 1 - *Command Line/Terminal Vina*

Through a text editor, a configuration file for the opioid receptor protein (4DKL) was created. The file was used in conjunction with AutoDock Vina (1.1.2).

The Root Mean Square Deviation (RMSD) value of each of the nine poses was calculated to assess how far away the docked ligand was from the native ligand. The higher the RMSD, the farther the docked ligand was. For example, a redock with an RMSD of 0Å would mean that the docked ligand bound to the protein 100% accurately, however such an idealistic scenario is impossible. Therefore, an RMSD of less than 2.0Å was considered successful.

Results

Model 1	2.32
Model 2	2.88
Model 3	1.873
Model 4	6.38
Model 5	5.79

Model 6	6.37
Model 7	7.83
Model 8	5.16
Model 9	7.95

Table 2 - *RMSD values of the nine redocked ligand poses before mutating Lys-233.*

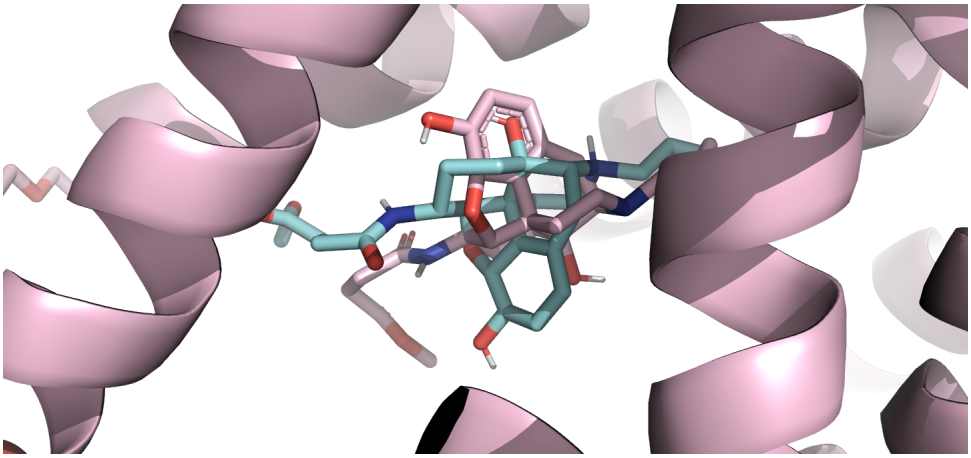


Figure 6 - *Redocked ligand before mutated Lys-233 residue of model 3 shown in light blue, encompassed in light pink protein structure.*

As illustrated in the table above, Model 3 had the best RMSD value of 1.87 Å. This meant that the redocked BFO-601 ligand was able to redock, but its RMSD value could be improved upon. One of the other trials resulted in the best RMSD value being at a similar value: 1.796 Å. However, the mutation of Lys-233 into Ala-233, resulted in improved docking.

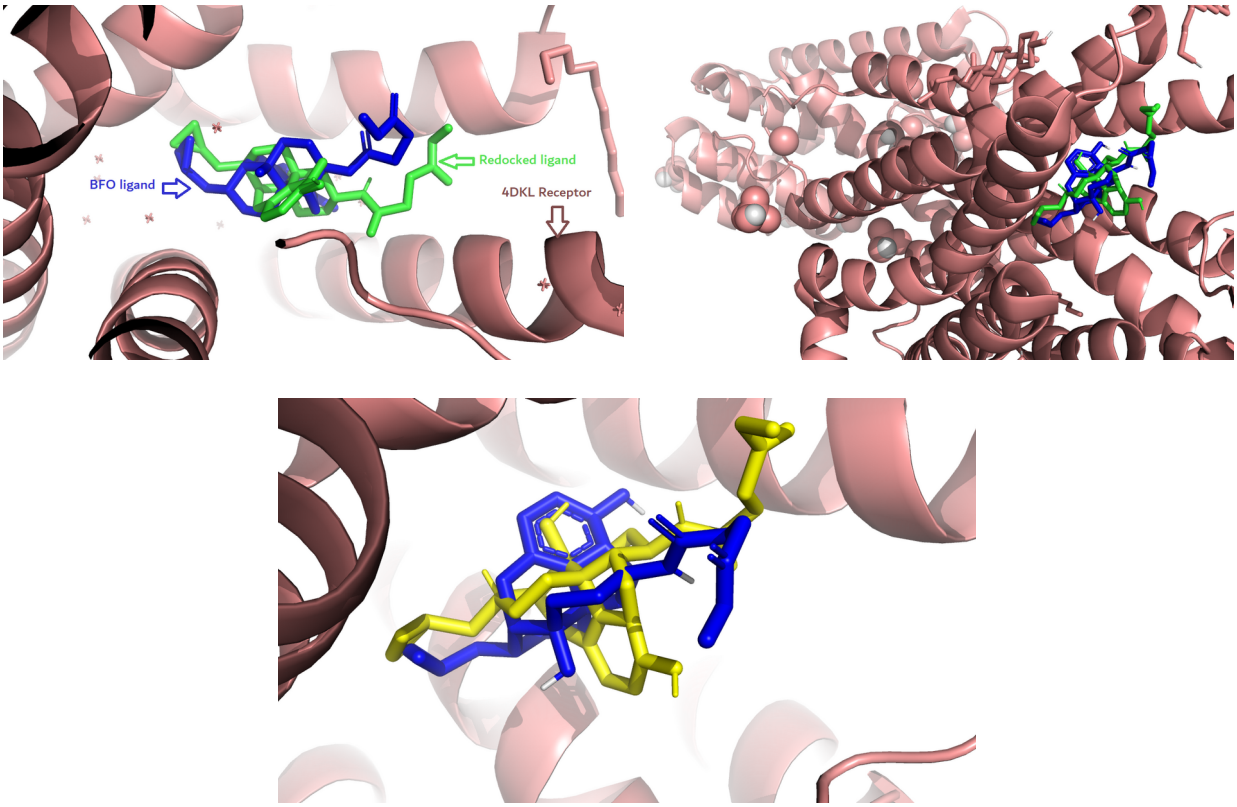


Figure 7 - *Redocked ligand AFTER mutating Lys-233. Ligands (native BFO ligand and newly docked green ligand) overlap very closely.*

The table below illustrated an *improved* best RMSD value from 1.87 Å to 1.167 Å (angstroms) in Model 3.

Model Number	**RMSD**
Model 1	2.60
Model 2	2.11
Model 3	1.16
Model 4	2.60
Model 5	4.26
Model 6	6.33
Model 7	7.85

Model 8	7.79

Table 3 - *Redocked ligand & lower RMSD values after mutation. The pose with the lowest RMSD value was Model 3.*

Discussion/Conclusions

Analyzing the RMSD values and illustrated models in PyMOL for the redocked ligand before and after the mutation, allows one to conclude that mutating Lys-233 to Ala-233 produces better RMSD values. As a hydrophobic, neutral amino acid, alanine provided a stronger binding affinity and improved hydrophobic interactions with the ligand, than the positively charged, hydrophilic Lysine. This mutation provides insight into how such modification would allow for enhanced binding of the morphinian antagonist. It validates the previously stated hypothesis and encourages simulating the mutation of other residues to predict which alteration produces the best result. This could imply that the opioid receptor could potentially have an enhanced ability to respond to antagonists. Such objectives could be applied to patients struggling with withdrawal symptoms, as enhanced binding of the antagonist could mean increased absorption of medication. Such research on the opioid receptor's binding capabilities, after mutating an amino acid, hints at the wide range of possibilities to be explored in using mutagenesis to improve docking. Understanding how this antagonist binds to and reacts to a mutation in its amino acid sequence might help researchers create more effective medicines.

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