Computational Analysis of a Mutated μ-opioid Receptor Bound to a Morphinan Antagonist

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Abstract

Opium is a depressant drug, derived from the opium poppy (*Papaver somniferum L.*), and holds one of the earliest records of medicinal plant use. Derivatives of opium, codeine and morphine, are extensively used in therapeutic pain relief treatments[1],[2].The mu (μ) opioid receptor is produced from the *OPRM1* gene and acts as the primary receptor for most opioid drugs, which are highly addictive. Narcan is used to counter the effects of an opium overdose; if Narcan binds to the receptor more effectively, its inhibitory effect will increase and it can bind more favorably than opium or opium derivatives in the event of an overdose. Researchers know that differences in the receptors' structure and function influence how the body responds to opioids.[3] In this study, we mutated the Tryptophan-318 residue into an Arginine residue in order to observe whether it would result in a more ideal binding arrangement of narcan. We performed molecular docking in order to obtain the RMSD and affinity values for each predicted position of narcan with and without the investigated mutation. On average, the Y318R μ-opioid receptor produced poses with higher binding affinity than the original protein. The optimum affinity value for the Y318R protein was also lower than that of the original protein. This led to the conclusion that mutating Tryptophan-318 into Arginine enhances the binding of the morphinian antagonist to the μ-opioid receptor, making it more effective at countering the effects of an opium overdose. This research encourages further experimentation regarding the mutation of additional residues and in turn the process of drug discovery can be improved.

Keywords: μ-opioid receptor, morphinan antagonist, molecular docking

1. Introduction

Opioids are drugs often used as pain relievers for those who have chronic pain or recently had surgery. However, opioids are one of the most abused drugs due to their easily addictive nature. In recent years the number of opioid overdoses have greatly increased.

The human body produces natural opioids called β-Endorphins, which are a type of neurotransmitter that send signals throughout the body. β-Endorphins can bind to the μ-opioid receptor and send a reward signal to our brains for doing activities like eating or exercising. However, when opioids such as fentanyl and morphine are taken, they bind tighter to the μ-opioid receptor. This elicits a larger increase in positive emotions, which can lead to opioid dependency [4]. This occurs because for affected individuals it can be difficult to find something else that produces the same feeling.

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Opioid dependency has led to an epidemic that continues to be a prevalent issue in society. In addition, the opioid epidemic has only been exacerbated by the lack of universal mental health resources. There have been 115,000 opioid induced deaths worldwide in 2017. Specifically in the United States, opioid deaths have increased by 120% between 2010 and 2018 according to the World Health Organization [5].

Narcan is an effective drug that binds to the μ-opioid receptor and acts as an antagonist by blocking opioids from binding to the μ-opioid receptor. Narcan, or Naloxone, has been crucial to combating the opioid epidemic as it has proven effective at stopping opioid overdoses in 75-100% of cases [6]. Seeing how Narcan binds to the μ-opioid receptor is essential to decreasing the amount of overdose deaths because a stronger binding arrangement would increase the effectiveness of Narcan.

Mutagenesis of the μ-opioid receptor, the initial protein before ligand is bound, can enhance or reduce the agonists' effect. The agonist can bind to the μ-opioid receptor to inhibit the function of addictive opioids such as fentanyl, oxycodone, and morphine. Certain mutations in the μ-opioid receptor are found to contribute to an increased chance of addiction to alcohol, nicotine, and exogenous opioids. Even though most of these substances don't directly bind to the receptor, they affect the pathways that the opioid receptor is involved in [7].

The μ-opioid receptor is a G-protein coupled reactor. Arginine has been found to have antiinflammatory properties that create a more conducive binding response [8]. This should increase affinity and make narcan more effective. Changing the amino acid, Tryptophan 318, to Arginine should create a shorter distance between the Narcan and μ-opioid receptor. Narcan is one of many potential ligands that could bind to the protein. Some of these ligands include β-Endorphins, fentanyl, morphine, etc.

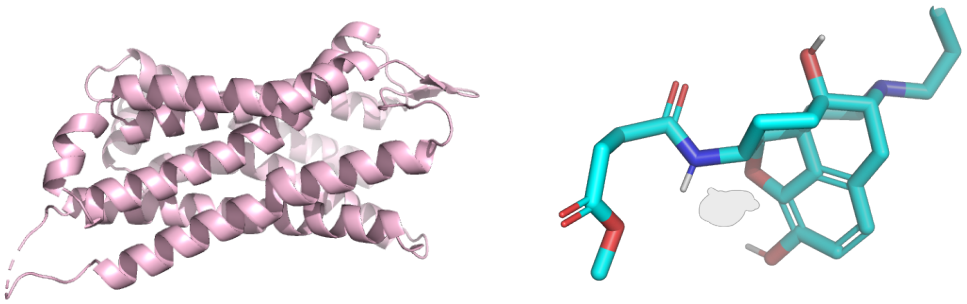


Figure 1- Shown above is the μ-opioid receptor (pink). It is made up of loops on each end and alpha helices spanning the middle. Narcan (blue) before it is bound to the μ-opioid receptor to block the binding of opioids.

Tryptophan is a neutral aromatic residue, meaning the protein-ligand interaction is hydrophobic. Tryptophan-318 is close to the binding pocket, but mutating it to arginine will place it in a closer, possibly more secure place for binding. If the change from a hydrophobic interaction to an electrostatic interaction results in a strong binding arrangement, then the inhibitory effects of the antagonist should increase. If the amino acid, Tryptophan 318 is mutated to Arginine, a positively charged amino acid, an electrostatic interaction between oxygen and nitrogen will occur.

Since the morphinan antagonist of interest is relatively rare in terms of bioavailability to perform *in vivo* and *in vitro* experiments, the methods chosen to perform the first stage of the tests were a

series of computational experiments. By exploring the ligand and the receptor structure virtually, it's possible to get a clearer idea of the behavior of the interactions between them to check whether further experiments are worthy to be performed. For short, the digital experiments are going to be used as a low-cost set of techniques before getting to an actual wet lab in order to fully confirmate this hypothesis.

2. Methods

The experiments mainly consisted in computational simulations of the most important changes within the mu-opioid receptor throughout the mutation process and its interactions with the ligand. The testing routine consisted in 4 main steps: redocking, mutating the residue, docking and viewing.

2.1. The Control

Protein-Ligand Docking is a virtual process applied to get information about how two compounds (ligand and receptor) bind together in nature. The protein (receptor) is kept still and the ligand is the one molecule changing positions according to its energy levels. The docking performed is responsible for energy ranking all the possible places the ligand might be able to occupy when normally interacting with the protein (pose). This method is used to determine if a compound would naturally bind to a protein and also analyze its interaction with the protein amino acids.

Before performing the actual docking to analyze the influence of the edited residue in the narcan interactions, it is necessary to first be sure of the precision of our experiment by first applying a technique called redocking. The redocking is placed in order to confirm the accuracy of our models as compared to the base PDB file (4DKL) disposed in RCSB Protein Data Bank. The referred file contains the ligand and the receptor of interest in this research.

To perform the redocking, the first step is setting a box size that will narrow the binding site to a specific place in the protein receptor we want the program to focus on. In this experiment, a biased box would be more adequate to show details about the bond between the specific residue (Tryptophan-318) and the ligand. A box size was created to encompass the binding pocket (fig. 2).

a)

	Center	**Size**
x	**-29.775**	**16**
y	**-12.009**	**16**
z	**-10.623**	**16**

b)

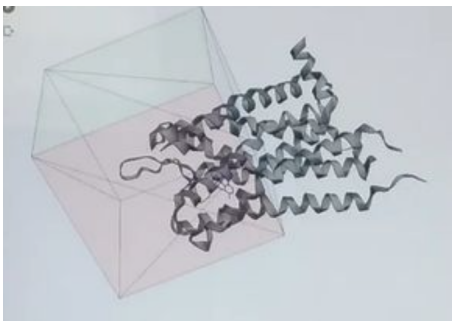


Figure 2 - (a) The coordinates of the docking box size for the Mu-opioid receptor and antagonist ligand biased redocking box. (b) A visual of the Mu-opioid receptor (PDB 4DKL) is provided using GINNA.

This step consists in separating the Narcan molecule from the original PDB file and docking it again to its original place using our own measurements for box sizing. The main goal is to check the Root Mean Square Deviation (RMSD) values that compare the new and the original file superposed to see if the chosen sizes are showing results closer enough to reality. It is expected that the RMSD values are as close to 0 as possible, so results below 2A are considered a success.

2.2. Mutating the Residue

Another step is actually making the necessary edits on the mu-opioid receptor to make it look like the mutations proposed in the hypothesis. There is supporting evidence that Trp318 might be an important residue in the receptor that facilitates the selectivity of morphinan antagonists such as narcan[7]. In other words, this specific amino acid may have a significant part in making their reaction channel overwhelmingly energetically favorable. With that in mind, by mutating this Tryptophan into an arginine it is expected that a stronger bond is created by electromagnetic interactions.

The amino acid was edited directly in PyMOL, a molecular modeling software used for making renders of biochemical structures. We used its tools to edit the selected amino acid, the TRP 318, into ARG. By doing so, we could generate a PDB file containing only the mutated protein, as in image 03 and 04. This will be further used to integrate the final file in the docking series.

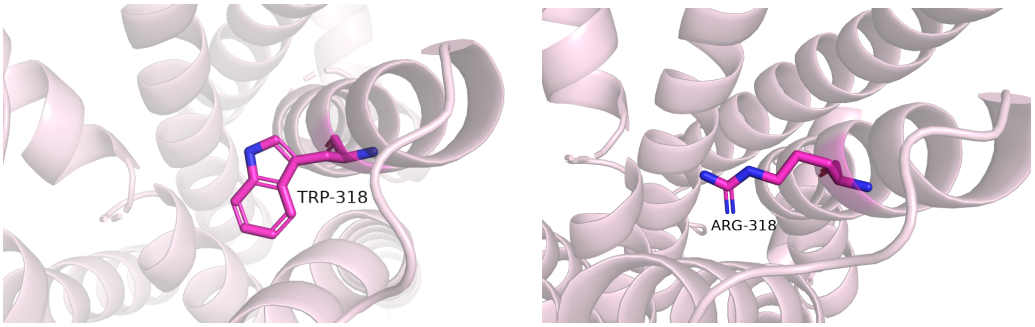


Figure 3 - Structure of μ-opioid receptor with original (TRP-318) and mutated residue (ARG-318)respectively. Its PDB ID 4DKL was visualized and edited using PyMOL. The μ-opioid receptor is shown in light pink, as a cartoon, and both amino acids are shown in magenta, colored by element, in the form of sticks.

2.3. Docking

After mutating the residue, we performed the docking procedure using the same box-sizing as in topic 2.1 in order to measure an expected distance between the ligand and the new amino acid. By doing that, it's expected that the distance would shorten from the original pdb file (4.4A to 5.6A), or at least be less than 5A to possibly indicate successful results.
To proceed, we separated the mutated mu-opioid file from the ligand and used GNINA, a docking software from Dr. Koes' lab at the University of Pittsburgh, to predict how the narcan binds to the protein receptor with a known 3D structure. Following the same process applied to the redocking. If the hypothesis is to be confirmed, the expected result is that the lowest energy poses will be relatively closer to the changed residue than compared to the original amino acid, meaning a stronger and more stable binding. After the docking sequence is finished, the measurements obtained are going to be analyzed in PyMOL.

2.4. Calculations

To complete the set of experiments, PyMOL was used again to calculate the measurements resulting from both dockings. This technique was preferred in order to make clear 3D visualization of the entire receptor as well as the binding cavity. The program contains a function capable of obtaining simulations of real-life distance measurements, which is a very important feature to determine the nature of the binding.
During this step, we measured the distance between possible binding atoms in the top ranked poses to compare it with the measures in the control protein-ligand complex expecting to see the most probable binding sites.

3. Results

The results obtained consist mainly in tables provided by GNINA, the docking software used in this study. The tables compare different characteristics of the poses the ligand may occupy. The most important ones to define are energy affinity (kcal/mol) in docking and redocking tables and also the RMSD values at the redocking table.

The energy affinity values, in both docking and redocking, measure the internal energy the ligand will need to dispose of in order to occupy a certain pose within the receptor. It analyzes the most likely poses to happen according to GNINA`s algorithm considering that the most stable ones to be the ones that require the least amount of energy to be achieved. While looking at those values, the lowest energy affinity defines the pose closer to the real site the ligand might occupy.

The RMSD values stand for Root Mean Square Deviation. This particular aspect compares the position of the crystal structure ligand model to the redocked one. It measures the distance between the real life experiments and the computational simulations in order to check if the current parameters can be used as means to reproduce experimental results with precision. The lower the RMSD value is, the closer it is to reality, showing whether the program is more or less accurate. Usually, the best results stay below the line of 2A for RMSD.

3.1. The Control

Table 1 listed below is the one to analyze the RMSD values received from the redocking. Out of the 9 poses obtained using the biased box, 2 of them reveal promising results for the accuracy of the project as highlighted in the table. Even though not all of the poses stay below 2A, this box sizing was the most accurate one in four tries with different box sizing. These numbers could be improved by rotating the box coordinates and narrowing it, but since the results obtained in this procedure were still not abnormal, the parameters were also used in docking.

Table 01 - Control protein-ligand complex redocking accuracy. Root Mean Square Deviation in all poses in comparison to the PDB 4DKL. Calculations were made using GNINA. The best results are highlighted in yellow.

POSE	RMSD
1	**2.67059**
2	**1.62769**
3	7.50734

4	5.41178
5	7.00374
6	5.80491
7	6.74309
8	3.92087
9	8.80626

The second table describes the energy affinity values in the interactions between the original crystal structure and the ligand before the mutation was performed. This table was used as a comparative item to discover whether the mutated protein carried less energy requiring poses in it, meaning that it could possibly establish more stable positions. As highlighted in the table, the lesser value of the affinity encountered was -8,96 kcal/mol in the top ranked pose in GNINAs algorithm, it indicates that this one is the most stable the redocking could find and by comparing it to the RMSD values of 2,67A its possible to be a pose we could get from *in vivo* and *in vitro* experiments furthermore.

Table 02 - Control protein-ligand complex redocking poses and respective energy affinities. Calculations were made using GNINA. The best result is highlighted in yellow.

POSE	AFFINITY (kcal/mol)
1	**-8,96**
2	-8,41
3	-8.60
4	-6.97
5	-7.36
6	-7.30
7	-7.35
8	-6.63
9	-5.18

3.2. Docking

The docking results consisted in the energy affinity table since it's not possible to obtain RMSD values without a comparative archive. Even so, it is possible to compare the stability of the poses based in GNINA's ranking algorithm, by doing that, it is possible to compare the average of stabilities of poses with in both procedures to determine which residue provided the best interactions.

The average affinity in the redocking was -7.42 kcal/mol while in the redocking it was -7.67 kcal/mol, so the measures resulted in a slight change from each other and the docking exhibited some degree of variability with a standard deviation of 1.0689. Also, in the docking set of poses, the lowest affinity values were obtained with the mark of -9.11 kcal/mol.

Table 03 - Mutated mu-opioid receptor and ligand docking poses energy affinity. The table was obtained in GNINA. The best result is highlighted in yellow

Pose	AFFINITY (kcal/mol)
1	**-8.72**
2	**-8.48**
3	**-8.98**
4	**-9.11**
5	**-7.47**
6	**-6.87**
7	**-6.85**
8	**-6.72**
9	**-5.79**

3.3. Calculations

Using pymol, we measured some of the possible electrostatic interactions (represented by blue-red bonds). In pose 4, the most stable position, the distance obtained was 4.2A, while for pose 2, the distance was 5.6A . This number indicates that the two atoms are possibly sharing electrons, because they are differently charged and close enough to do so. Therefore, it's possible that the two of them are in an electrostatic bond.

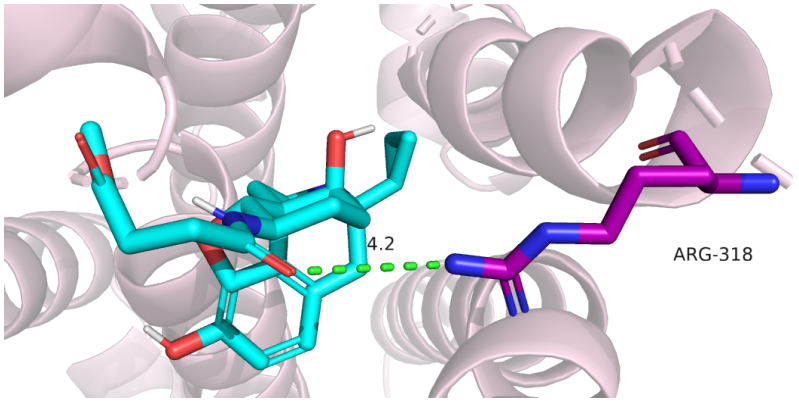


Figure 4: Electrostatic bond between mutated residue and ligand (pose 4). The PDB 4DKL was visualized and edited using PyMOL. The μ-opioid receptor is shown in light pink, as a cartoon, and the binding amino acid ARG-318 is shown in magenta, colored by element, in the form of sticks.

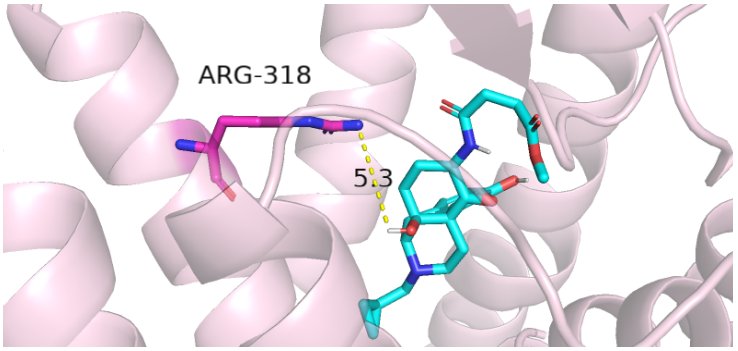
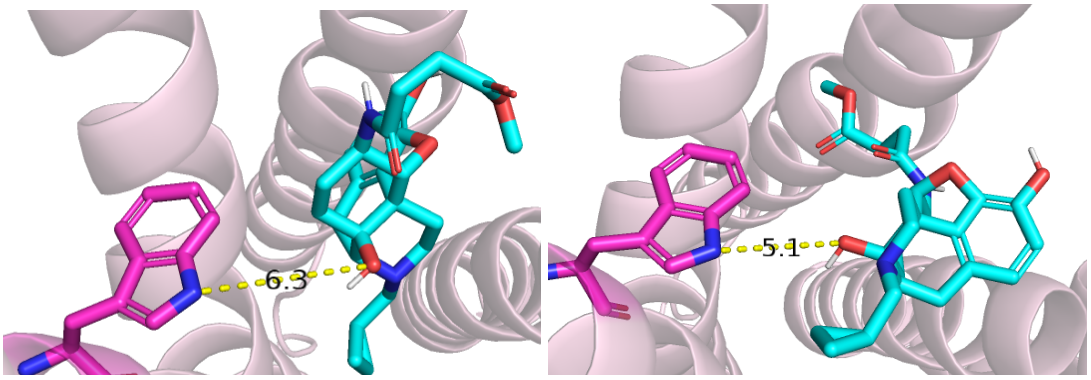


Figure 5 - Electrostatic bond between mutated residue and ligand (pose 2). The PDB 4DKL was visualized and edited using PyMOL. The μ-opioid receptor is shown in light pink, as a cartoon, and the binding amino acid ARG-318 is shown in magenta, colored by element, in the form of sticks.

Compared to the original crystal structure, the distance doesn't seem to differ much, but, by analyzing the energy affinity and the nature of the bond, there is still some evidence that the bond can be stabler and harder to undo.



Pose 1 **Pose 7**

Figure 6 - Electrostatic bond between control protein-ligand complex. The PDB 4DKL was visualized using PyMOL. The μ-opioid receptor is shown in light pink, as a cartoon, and the binding amino acid ARG-318 is shown in magenta, colored by element, in the form of sticks. The measurements are highlighted in yellow.

4. Discussion

In the present study we investigated mutating the Tryptophan-318 residue into an Arginine residue and its effect on the binding affinity to the μ-Opioid Receptor with naloxone. Tryptophan-318 is a neutral aromatic residue, and thus increases the number of hydrophobic interactions with the ligand. Arginine is a positively charged amino acid, and therefore can participate in electrostatic interactions with the ligand. The μ-Opioid Receptor (PDB: 4DKL) is a G-coupled protein receptor which can be found embedded in the cellular membrane of neurons.While it is known that different opioids bind to different residues of the receptor, the exact mechanism of ligand binding for many opioids is unknown.[9] However, after analyzing the nine poses and associated energy values before and after the mutation, we can conclude that mutating Tryptophan-318 residue into an Arginine residue makes for a more ideal binding arrangement. This mutation therefore can possibly experimentally increase the inhibitory effect of narcan and make it more effective in preventing an overdose.

5. Conclusion

This study focused on the mutagenesis of the μ-opioid receptor, specifically mutating Tryptophan-318 into Arginine, with the aim of improving the binding arrangement with a morphinan antagonist, Narcan. Through a series of computational tasks, including docking and redocking, the research provided valuable insights into the interaction between the receptor and ligand.

The results indicated that mutating Tryptophan-318 into Arginine led to more favorable binding arrangements, as evidenced by lower energy requirements for ligand binding. The transition from a hydrophobic interaction to an electrostatic one between the ligand and the mutated residue appeared to enhance the stability of the binding. This finding suggests that such mutations could potentially increase the inhibitory effect of Narcan, a crucial opioid antagonist used to counteract opioid overdoses.

This study represents a significant step in understanding the molecular interactions between opioid receptors and antagonists. Further research involving mutations of additional residues may provide a more comprehensive understanding of the receptor's behavior and could contribute to the development of more effective opioid antagonist drugs. Ultimately, these findings hold promise for improving the process of drug discovery in the context of opioid abuse and addiction, which continues to be a critical public health concern.

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