Naloxone Binding Sites on the μ-Opioid Receptor

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Abstract

Naloxone is a high-affinity competitive antagonist for the μ-opioid receptor (μOR) used to reverse opioid overdose. The molecular conformation of the two transmembrane binding sites for Naloxone on the μOR has been identified through a cell-based photocrosslinking technique. Both hydrophobic and hydrophilic amino acid residues comprising the binding sites interact with Naloxone to stabilize the receptor in a deactivated conformational state. To visualize the binding sites and conformational changes induced by Naloxone in the μOR, we designed a preliminary 3D-printed model. Photocrosslinking was to be demonstrated within the transmembrane domains by using two colors of UV paint to represent the hydrophilicity/hydrophobicity of the interacting residues within the two Naloxone binding sites; due to limitations caused by the COVID-19 pandemic, the 3D model was redesigned into a digital model. In conclusion, our model successfully demonstrates how photocrosslinking can be used to identify specific Naloxone binding site residues within the μOR.

Model Refinement

The receptor and naloxone molecule models were scaled to properly reflect the size, in Angstrom, of the cellular representations. Issues that arose throughout the process revolved mainly around the assembly of the individually printed subunits along with the ability to accurately display the photoreactivity observed within the experiment. Two thin sheets of 3D printing polymer around the model shown above act as layers of the phospholipid bilayer while demonstration of photoreactivity was solved via integration of UV paint cells that when contacted with the Naloxone molecules would leave artificial residues that could be observed with a black light. Due to restricted access of the UNC MakerSpace 3D printers, which were utilized for model creation, and other complications caused by COVID-19 a final model was unable to be created. The digital model above was constructed as a solution to these complications. Both the visualization and photocrosslinking models were created to improve understanding of naloxone to μ-Opioid interactions.
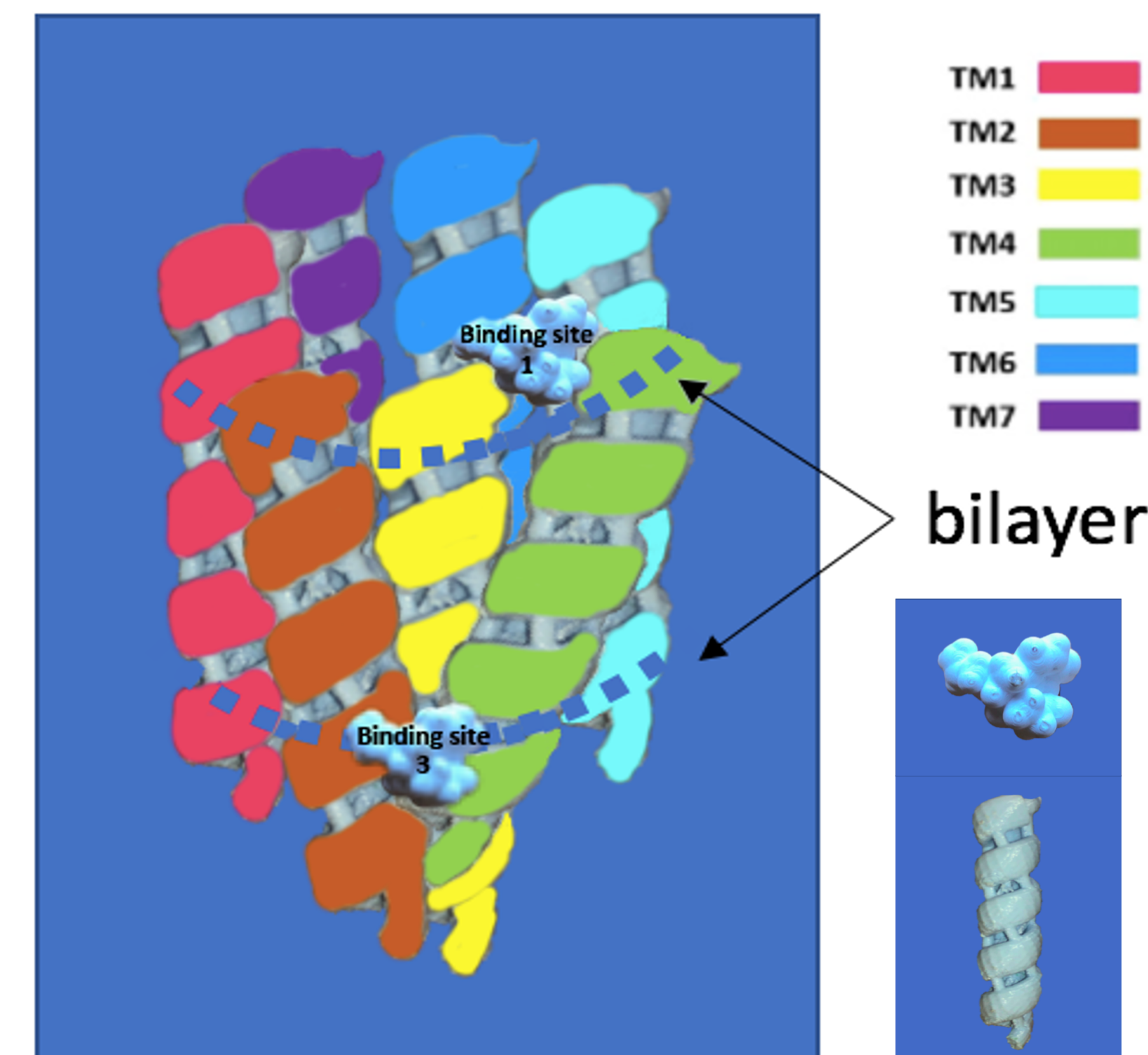
Visualization

Fig. 1
3D Receptor Model

TM1
TM2
TM3
TM4
TM5
TM6
TM7

An external 3D model of the μOR was developed in Adobe Photoshop by combining images of a 3D printed alpha helix and naloxone molecule to visualize the receptor and binding sites of naloxone. We color-coded the helices according to the original study and illustrated the phospholipid bilayer of the receptor.

Future Directions

Future efforts should focus on 3D-printing a moveable physical model of the μOR, before and after Naloxone binding, featuring visual markers for interacting amino acid residues. Such a model could aid further elucidation of the structure-activity relationships (SAR) between the μOR and its ligands to support the design of more potent antagonists, or less potent agonists, for specific signaling pathways such as β-arrestin-2 whose activation induces fatal opioid overdose symptomatology, such as respiratory depression[2].

Photocrosslinking

μORs were genetically engineered with p-benzoyl-L-phenylalanine (BzF) to produce a photoreactive model that revealed Naloxone specific binding sites. To visualize the photocrosslinking method, a digital model was constructed to identify the crosslinks between the μOR and Naloxone after 365 nm UV irradiation.

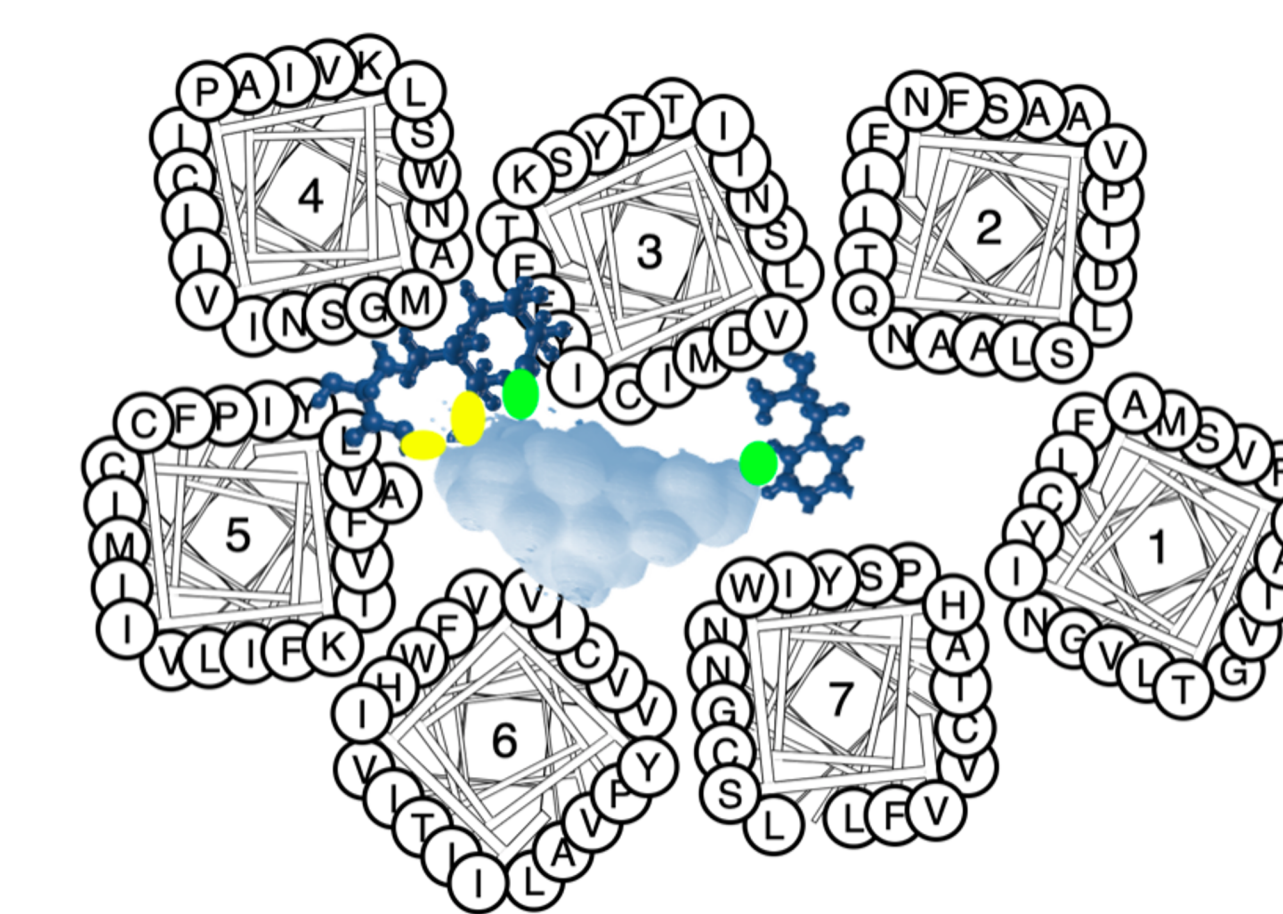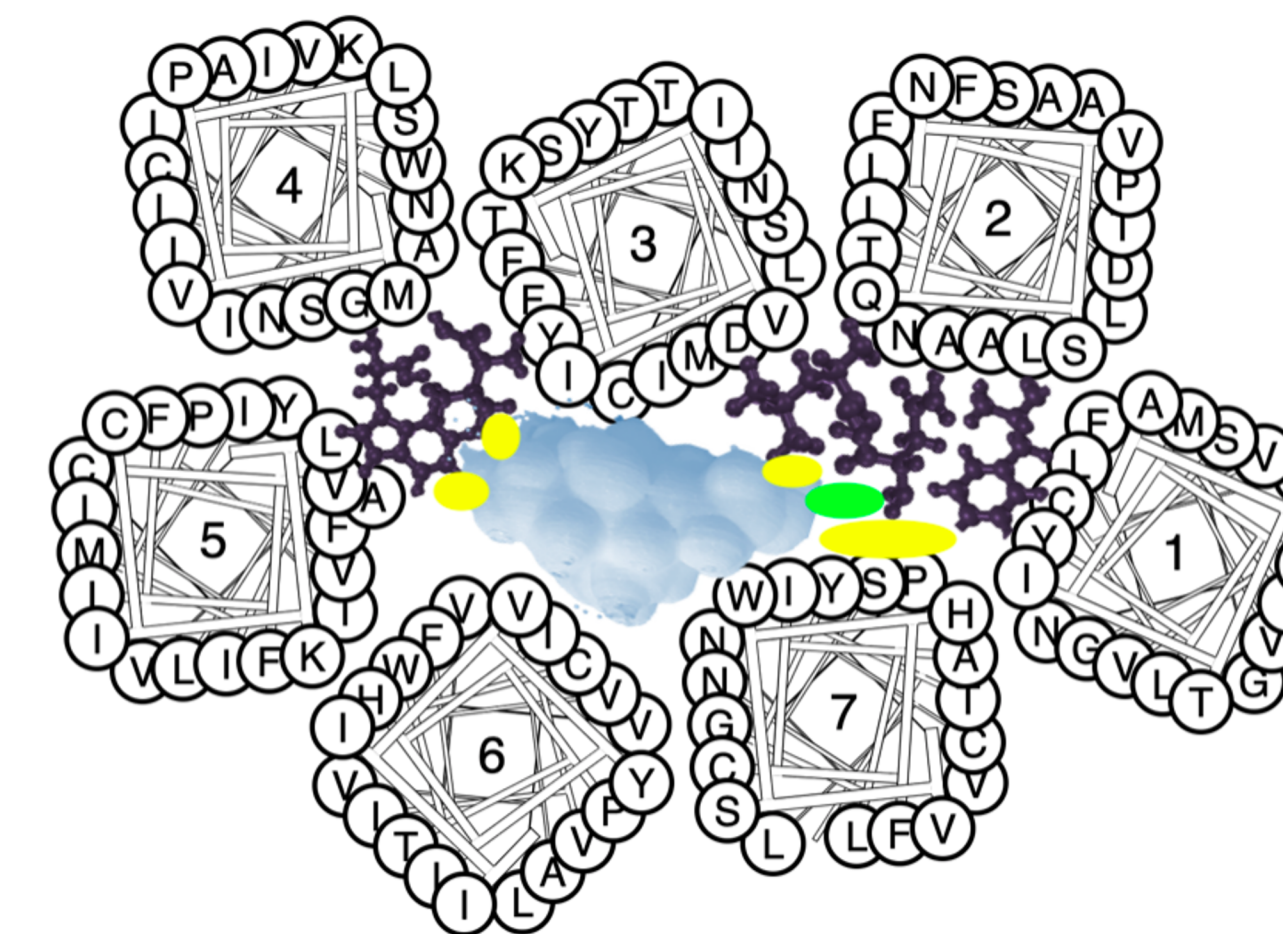*Fig. 2* Binding Site 1

Fig. 3 Binding Site 3

Identification of residue specific binding sites within the μOR was accomplished through photo- crosslinking. The digital models (Fig. 2 & 3) represent how the technique forms covalent bonds between BzF and Naloxone. To improve the understanding of the binding sites, two colors of UV markers were used to reveal the hydrophilic and hydrophobic residues within the binding sites.

References

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2. Groer, C. E., Tidgewell, K., Moyer, R. A., Harding, W. W., Rothman, R. B., Prisinzano, T. E., & Bohn, L. M. (2007). An Opioid Agonist that Does Not Induce μ-Opioid Receptor—Arrestin Interactions or Receptor Internalization. *Molecular Pharmacology*, *71*(2), 549–557. https://doi.org/10.1124/mol.106.028258