Drug-receptor interactions in inflamed tissue

Structural determinants of opioid signaling

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In Short

- Protonation-coupled dynamics of μ-opioid receptors and their ligands in the low pH environment of inflamed tissues
- Investigating of amino acid residue single-point mutations on μ-opioid binding and receptor signaling
- Binding of *morphine-like* molecules to the μ-opioid receptor
- Receptor dynamics in realistic lipid membrane models.

In this research we propose to understand how opioid drugs used in the management of severe pain bind to the membrane-embedded μ-opioid G-protein coupled receptor (GPCR) and how the receptor propagates the signal inside the cell (Figure 1). The μ-opioid receptor is part of the opioid receptor group - also consisting of the δ- and κ-opioid receptors - that is most important in conferring analgesia as well as most of the unwanted side effects [1].

Opioid drugs are the most potent known painkillers used in medicine, however they can be highly additive, while exhibiting dangerous side-effects. It is therefore of great interest to understand how opioid compounds bind and activate opioid receptors and how this knowledge can be used in the design of novel and less addictive analgesic drugs.

Due to the recent development of selective opioid drugs that are active only in acidic inflamed tissue [1], we will study the protonation-coupled dynamics of opioid receptors in low pH environments, as this is a topic that is poorly understood. Moreover, current experimental studies on cell cultures shows that specific mutations of conserved amino acid residues in the μ-opioid receptor binding site lead to changes in receptor signaling [2]. By performing numerical simulations on systems containing such mutations, we wish to understand this on a molecular level. Besides the specifics in the receptor structure itself, the composition of lipid membranes in which the receptors are embedded can have a significant influence on receptor dynamics [3]. To extend the knowledge on this topic we will perform simulations in realistic lipid environments.

Recently, we published the force-field parameters for the strong opioid agonist fentanyl, which enables reliable atomistic simulations of systems containing this drug [4]. To date, no such parameters exist for morphine and morphine-like drugs. Therefore, we wish to develop a fast, but reliable automated procedure for obtaining parameters for such opioids, and validate these parameters by performing extensive molecular dynamics simulations.

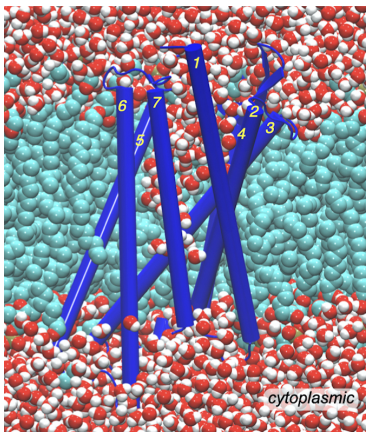

***Figure 1:** The μ-opioid receptor embedded in a solvated lipid membrane. The blue pipes depict the seven trans-membrane helices of the receptor. Cyan balls show the phospholipids of the lipid bilayer. Red and white balls represent the oxygen and hydrogen atoms, respectively, which form the water molecules. We can observe a continuous water channel which transverses the receptor.*

As a first step, we plan to study how protonation states characteristic for low pH change the hydrogen-bond (H-bond) networks of the receptor. Specifically, we will study how the protonation of Asp147, crucial for opioid binding, impacts the overall shape of the H-bond network spanning the receptor. Moreover, we will study the protonation of the Asp164, which is part of the conserved DRY motif (Figure 2), which acts as a ionic lock, that constrains the receptor in an inactive conformation until ligand activation.

Next, we will be interested in the impact of the His297Ala mutation. We will try to explain if this mutation leads to changes in downstream receptor signaling due to changes in ligand binding, or due to specific changes in H-bond networks. In the previous year we focused only on fentanyl as a ligand. Now we wish to expand out studies to morphinne and moprhine-like ligands heroin and oliceridine (Figure 3), as they likely exhibit a common binding pattern; which is distinct when compared to fentanyl.

To date, the membranes used in our simulations were composed from a single lipid type - POPC.

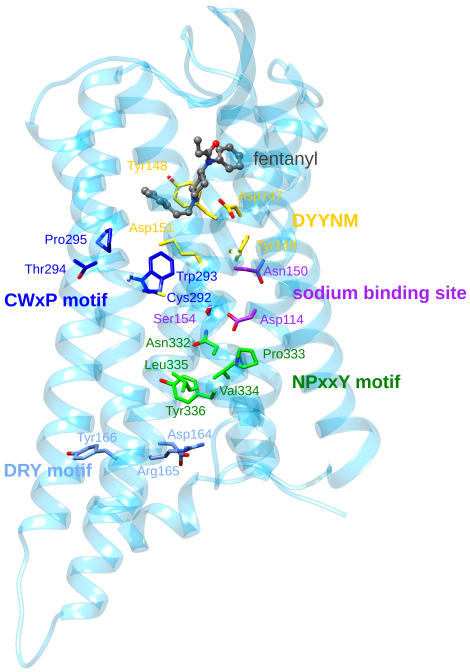

Figure 2: *Conserved motifs that are crucial in signal transduction between the the outside and the inside of the cell. The μ-opioid receptor is depicted with cyan cartoons. The binding site motif is presented with yellow, CWxP motif with blue, sodium binding side purple, NPxxY motif green, and the DRY motif with light-blue sticks. Fentanyl bound to the receptor is shown in balls-and-sticks depiction with grey carbon atoms.*

We want to study the impact of using more realistic, multi-component membrane compositions, containing other lipid molecules, *e.g.* cholesterol and sphingolipids which are able to stabilize conformations corresponding to active receptors.

To study the H-bond networks of our simulation systems we will use the software Bridge, which was developed in our laboratory [5]. Bridge uses the concept of graph theory to enable visualization and study of large H-bond graphs obtained after prolonged simulations (Figure 4). Applying measurements of graph theory (e.g. measurements such as betweenness centrality) enables us to identify important protein groups and long-distance signal transduction pathways.

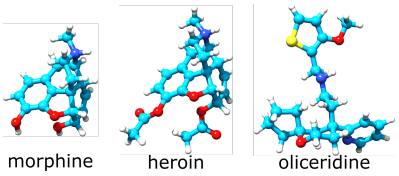

Figure 3: *3D structures of strong opioid agonists morphine, heroin and oliceridine*

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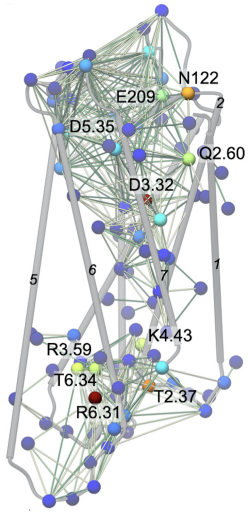

Figure 4: *H-bond network computed for an opioid receptor in MD simulations. Protein groups are indicated as spheres and colored by their number of connections (blue to red, with red representing the highest number of connections). The figure is taken from the Master Thesis of Éva Bertalan: Atomistic insight into dynamic water mediated networks and sodium binding in opioid receptors.*

More Information

[1] V. Spahn , G. Del Vecchio, D. Labuz, A. Rodriguez-Gaztelumendi, N. Massaly, J. Temp, V. Durmaz, P. Sabri, M. Reidelbach, H. Machelska, M. Weber, C. Stein *Science* **355**, 966-9698 (2017). doi:10.1007/s00018-005-4527-3

[2] J. Meyer, G. Del Vecchio, V. Seitz, N. Massaly C. Stein *British Journal of Pharmacology* **176.23**, 4510-4520 (2019). doi: https://doi.org/10.1111/bph.14810

[3] A. N. Bondar, J. C. Smith, *Photochemistry and photobiology* **93.6**, 1336â1344(2017). doi: 10.1111/php.12790

[4] S. Lešnik, M. Hodošček, U. Bren, C. Stein, A. N. Bondar, *Journal of Chemical Information and Modeling* **60.7**, 3566â3576(2020). doi: https://doi.org/10.1021/acs.jcim.0c00185

[5] M. Siemers, M. Lazaratos, K. Karathanou, F. Guerra, L. S. Brown, A. N. Bondar, *Journal of chemical theory and computation* **15.12**, 6781â6798(2019). doi: https://doi.org/10.1021/acs.jctc.9b00697

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