Antibody development against μ-opioid receptor

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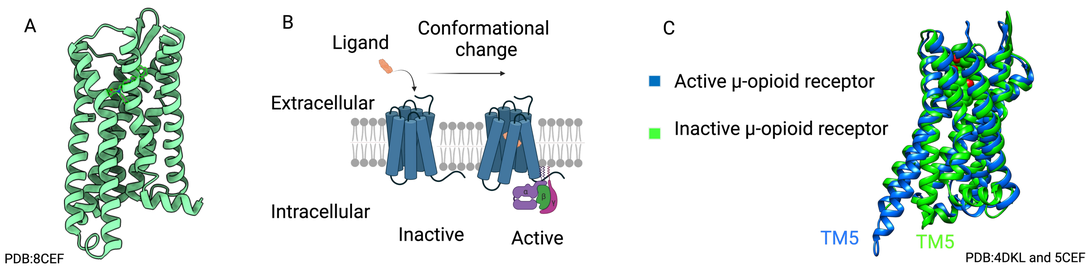
Introduction

Opioids are analgesic agents commonly used for pain management. They can interact with the opioid receptor as agonists, antagonists or partial agonists. The opioid receptor family consists of four main receptors: delta (DOP), kappa (KOP), mu (MOP) and nociception receptors (NOP). All are class A, G-protein-coupled receptors (GPCRs), comprised of seven transmembrane helices linked by three intracellular and extracellular loops.

Opioid receptors are activated by native ligands such as opiates, which bind directly to the transmembrane domain and initiate signaling pathways. While μ-opioid receptor agonists are the most effective pain relievers, they also carry a high risk of addiction. In addition, these medications do not discriminate among all opioid receptors throughout the body, resulting in adverse effects such as constipation, nausea, apnea and sedation. The most painful conditions generally arise from inflammation, causing tissue acidosis and an increase in reactive oxygen species, which may alter GPCR function and interaction with ligands. Thus, targeting opioid receptors at the site of inflammation could lead to drugs that alleviate pain without unwanted side effects.

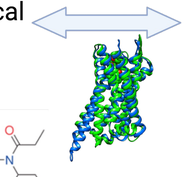


Conformational change in GPCR upon ligand binding. A) Structure of human μ-opioid receptor. B) Schematics of the μ-opioid receptor activation on ligand binding. C) Overlay of active ligand-bound μ-opioid receptor and inactive μ-opioid receptor.

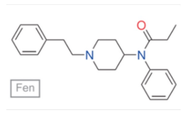
Objective

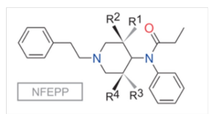
Goal: To combine *in vitro* biochemical analysis and computational modeling to understand the dynamics of human μ-opioid receptors in both physiological and pathological conditions.

Healthy
Physiological
pH ~7.4



Pathological
Inflamed
Low pH (acidosis)
ROS/RNS(Free radicals)





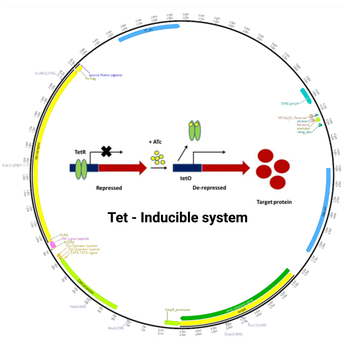
- **Purify the μ-opioid receptors from mammalian cells.**
- **Develop confirmational-specific antibodies against the μ-opioid receptors.**
- **Establish the membrane protein Antibody Discovery Platform.**

The fentanyl derivative NFEPP only binds to μ-opioid receptors under acidic conditions at inflamed regions, which may open new avenues for opioid drug development.

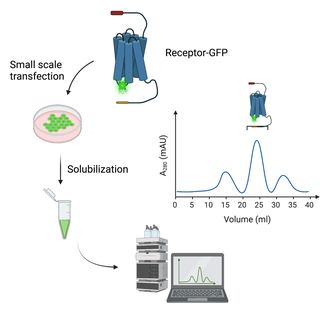
Spahn, V et al. "A nontoxic pain killer designed by modeling of pathological receptor conformations." Science.vol. 355, (2017): 966-969. doi:10.1126

Screening

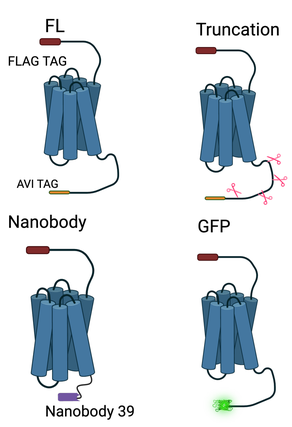
A) Expression vector



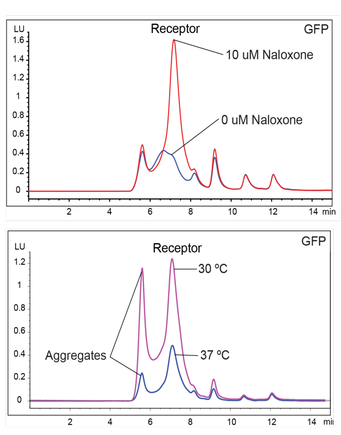
B) FSEC



C) μ-opioid receptor constructs



D) Optimization of expression



A) Plasmid design for GPCRs expression.

B) Fluorescent size exclusion chromatography (FSEC). By monitoring the GFP signal in HPLC, we can assess the quality and the biochemical properties of the protein.

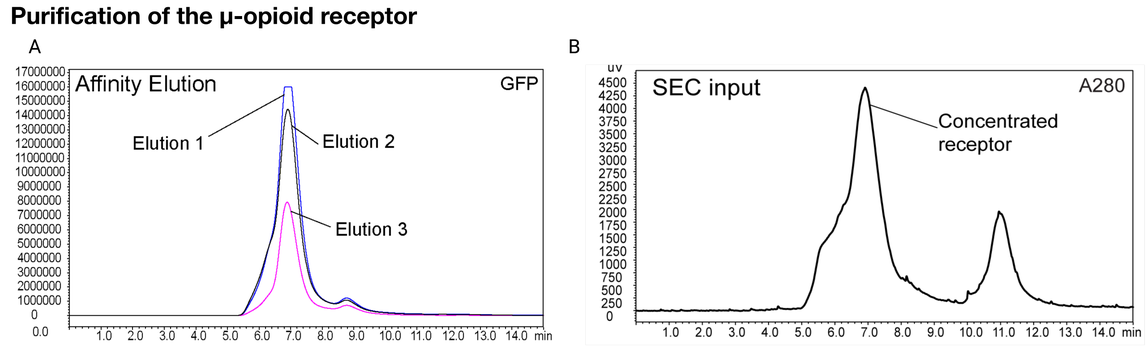
C) Design of the μ-opioid receptor constructs. The construct features an N-terminal FLAG tag and C-terminal Avi tag. GFP fusion constructs do not contain an Avi tag. The nanobody construct has NB 39 fused to its C-terminus.

D) The GFP fusion constructs were expressed with and without naloxone. The expression of the receptor increased at lower temperatures.

Das, Atze T et al. "Tet-On Systems For Doxycycline-inducible Gene Expression." Current gene therapy vol. 16,3 (2016): 156-67.

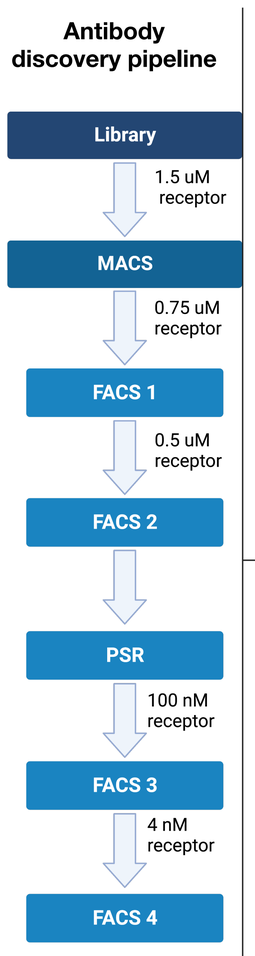
Results

Purification of the μ-opioid receptor

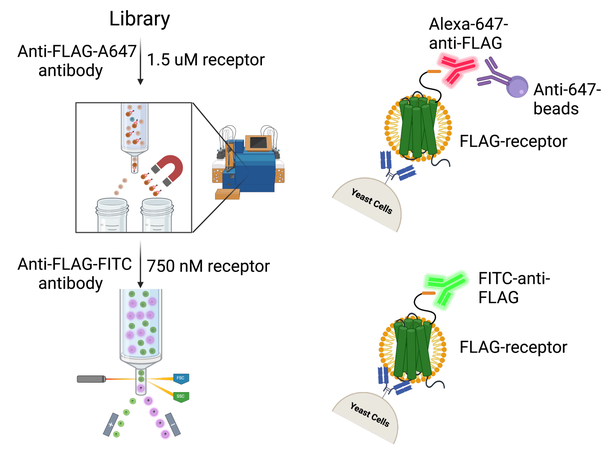


A) Affinity elution from anti-FLAG M1 antibody resin. B) The concentrated receptor from affinity elution. C) The SEC profile. D) HPLC and SDS-PAGE showing the purified receptor. PNGase F treatment removes the N-glycans on the receptors.

Antibody discovery pipeline

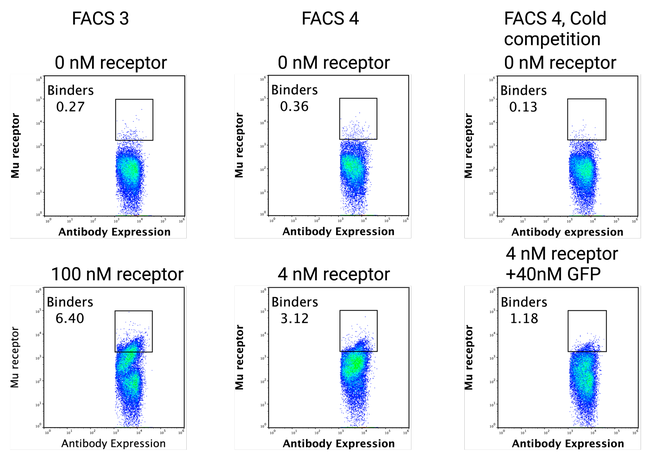


General schematic of the antibody discovery process



In our workflow, we employed a FLAG tag-based selection method using an Alexa-647/FITC-labeled anti-FLAG antibody. In the MACS round, positive binders targeting the μ-opioid receptor were selected using anti-A647/FITC magnetic beads, followed by successive rounds of FACS selection with decreasing concentration.

Figure depicting the FACS selection rounds 3 and 4



In FACS rounds 3 and 4, we reduced the protein concentration to the nanomolar range. Significant binders were observed in both FACS 3 and FACS 4 when the μ-opioid receptor was present. Additionally, a cold competition assay using non-labeled GFP at a 10-fold concentration effectively removed any GFP-positive binders.

Future Plans

- Continue our antibody discovery effort targeting the human μ-opioid receptor.
- Collaborate with our partners to validate our antibodies and gain insight into the receptor's conformational mechanisms.

Acknowledgment

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Dr. Marcus Weber, Zuse Institute Berlin, Germany.

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Victor Miranda, Kruse Lab, Harvard Medical School, Boston.

Want To Collaborate With Us?

We have generated antibodies to more than 200 cell surface receptor targets.

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