Computational prediction of the kinetics and pathways of opioid dissociation from μ-opioid receptor

Paween Mahinthichaichan[1,2], Quynh Vo[1,2], Lidiya Stavitskaya[1], Christopher R. Ellis[3], Jana Shen[2]

[1]Division of Applied Regulatory Science, Office of Clinical Pharmacology, FDA Center for Drug Evaluation and Research, Silver Spring, MD

[2]Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, MD

[3]United States Army, DEVCOM Chemical Biological Center, Aberdeen Proving Ground, MD

Abstract

Synthetic opioids are powerful painkillers but are also among the most abused drugs. Computational predictions and detailed molecular understanding of opioid receptor binding kinetics and mechanisms may help determine the appropriate naloxone dose needed to adequately compete at the receptor and reverse opioid overdose. Towards this goal, an advanced molecular simulation called metadynamics was applied to study the dissociation pathways and kinetics of fentanyl and its derivatives from the μ-opioid receptor (mOR), the primary target of clinically relevant opioids. Large-scale simulations were performed to retrospectively predict the dissociation times of fentanyl, fentanyl's derivatives, and buprenorphine. Remarkably, the simulations uncovered two types of unbinding mechanism, one of which involves a new binding pocket, located deep in the receptor and below the well-known "orthosteric site". The results of the study, together with the available X-ray crystal structures of mOR, suggest that deep pocket binding is a major contributor to the long residence time and high binding affinity of those opioid ligands. The developed protocol will be used to prospectively predict relative dissociation times of newly identified opioids that continue to emerge from illicit manufacturing and determine an appropriate naloxone dose to reduce opioid overdose mortality.

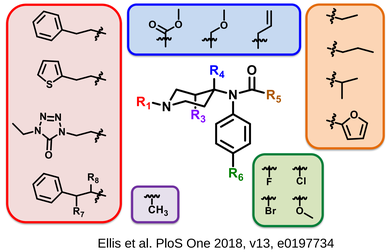
Background and Problems

Opioids, especially fentanyl and its derivatives, are major contributors to recent increase in drug overdose related deaths in the United States.

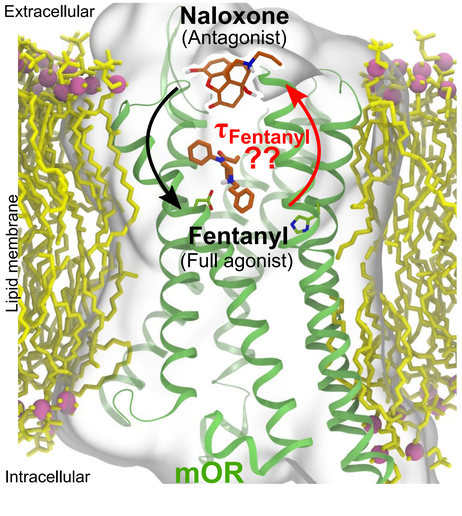
Fentanyl is 100 times more potent than morphine.

Fentanyl derivatives may have different pharmacological profiles despite their high structural similarity.

Fentanyl's derivatives



Ellis et al. PloS One 2018, v13, e0197734



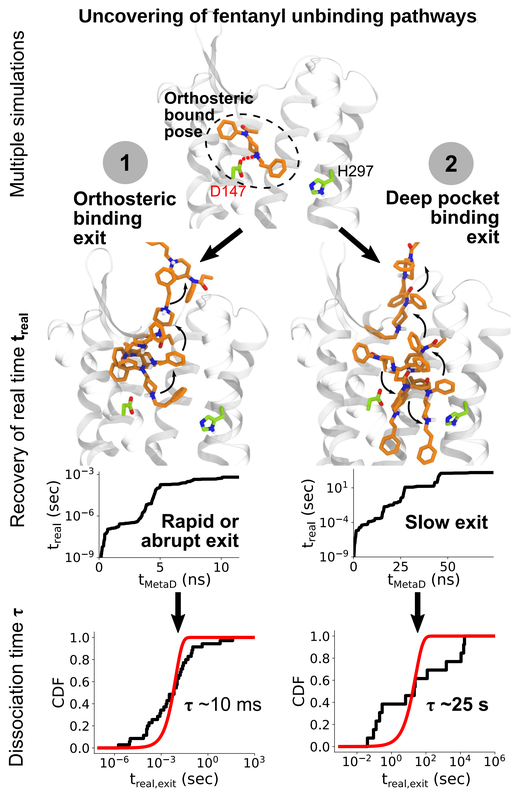
The μ-opioid receptor (mOR), a G-protein coupled receptor, is the primary target of clinically used opioids, such as morphine and fentanyl.

The duration of opioid binding in mOR can affect the ability of naloxone (Narcan®) to reverse an overdose.

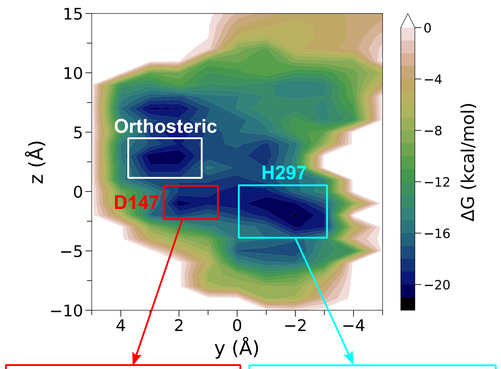
The objective is to develop a computational protocol that fully elucidates structural dynamics associated with the binding of opioids to the mOR and effectively predicts their unbinding kinetics.

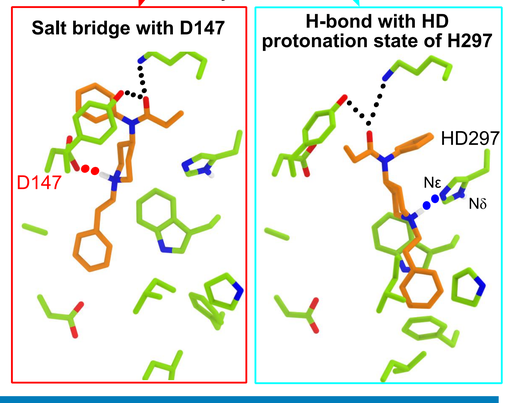
Results and Discussion

Uncovering of fentanyl unbinding pathways

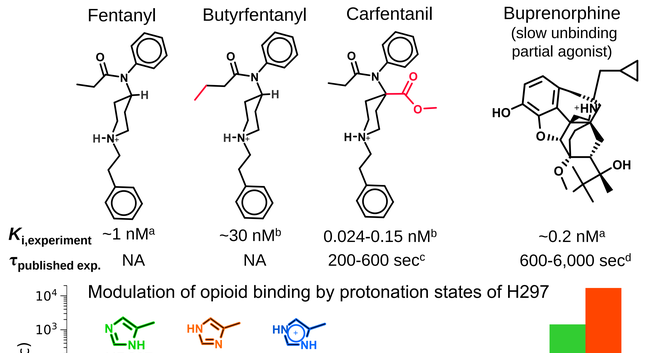


Newly identified deep pocket binding modes of fentanyl



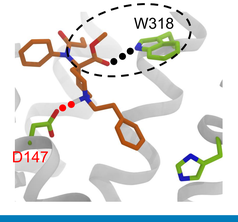


Predicted opioid—mOR dissociation times (τ)

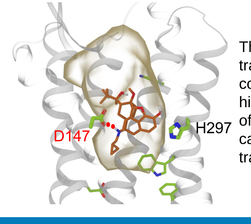


References: a) Volpe et al. Reg. Toxicol. Pharm. 2011; b) Wilde et al. Front. Pharmacol. 2019; c) Titeler et al. Eur. J. Pharmacol. 1989; d) Pederson et al. Neuropharmacolgy 2020 & Yassen et al. Clin. Pharmcol. Therapeau. 2007

Additional H-bond strengthens carfentanil binding



Buprenorphine also penetrates deep in mOR

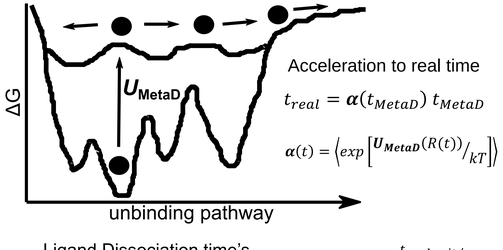


The potato-like transparent surface corresponds to a highly dense region of buprenorphine calculated over 20 trajectories.

Methods and Materials

Metadynamics molecular simulation

Laio & Parrinello. PNAS 2002; Barducci et al. Phys. Rev. Lett. 2008; Tiwary & Parrinello. Phys. Rev, Lett. 2013



Acceleration to real time

$$t_{real} = \alpha(t_{MetaD})\, t_{MetaD}$$

$$\alpha(t) = \left\langle exp\left[{U_{MetaD}(R(t))}/{kT} \right] \right\rangle$$

Ligand Dissociation time's approximation

$$CDF = 1 - e^{-{t_{real,exit}}/{\tau}}$$

System setup and protocols

Each simulation system was made of a ligand-bound mOR protein embedded in a solvated POPC lipid bilayer and was empirically represented by the CHARMM36 force field. Simulations were carried out with 2-fs integration time, and at 1 atm and 310 K.

The agonist BU72-bound X-ray structure (PDB:5C1M) was used as the protein model. The top docked pose of a ligand (from molecular docking) was used as its initial bound configuration.

Metadynamics simulations were performed using ligand's z-position and ligand-protein's number of contacts to make up the reaction coordinate for ligand unbinding with U_{MetaD} deposited in every 10 ps.

Software

- NAMD2 –simulations
- CHARMM –preparation
- VMD –preparation, analysis and visualization
- PLUMED –analysis and reweighting

Summary and Perspectives

Metadynamics effectively predicted opioid-mOR dissociation times.

The present study :

1) Identified a new binding region potentially relevant for slow agonist release;
2) Showed how residue protonation state (i.e., H297) modulates ligand binding;
3) Illustrated a correlation between ligand structure and dissociation time.

Are ligand's dissociation time, stability of activated conformational states of mOR, and opioid tolerance connected?
Can an advanced molecular dynamics or biophysical technique structurally and quantitatively distinguish between a full agonist and a partial agonist?

Acknowledgements

OAK RIDGE INSTITUTE FOR SCIENCE AND EDUCATION To P. M. & Q. V.

NIH To J. S.

We appreciate Pratyush Tiwary (U. Maryland, College Park) for helpful discussions regarding metadynamics.