Application of Molecular Dynamics for Development of Therapeutics Against Opioid Overdose

Emily Weed-Nichols[1], James M. Seckler[2], Alexander J. Crook[1], Stephen J. Lewis[2], Alan Grossfield[1]

[1]University of Rochester Medical School, Rochester, NY, USA

[2]Case Western Reserve University, Cleveland, OH, USA

Poster available online

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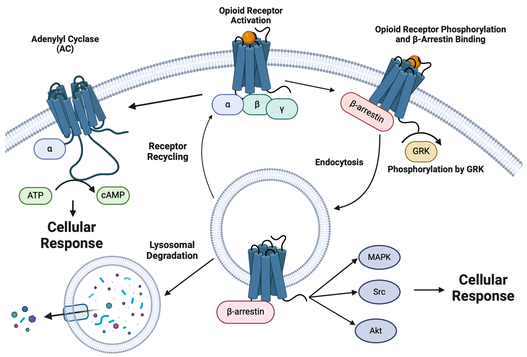
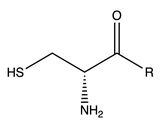
Abstract

Opioid overdose is a long-standing public health issue in the United States. This crisis has only been exacerbated by the Covid-19 pandemic, with overdose deaths involving opioids increasing from an estimated 70,630 in 2019 to 107,941 in 2022. While there are treatments for opioid overdose, currently no therapeutic tools exist to prevent it. Moreover, treatments are limited to emergency scenarios due to induction of withdrawal and loss of analgesia. Fatal opioid overdoses are primarily attributed to opioid-induced respiratory depression (OIRD). As part of an ongoing collaboration, a class of cysteine derivatives have been identified that reverse OIRD without blocking analgesic effects or inducing withdrawal. The current hypothesis is that these esters function by binding β-arrestin, a protein that signals downstream of the opioid receptors. The goal of my proposed work is to characterize this binding interaction to rationalize the trends observed in the preliminary data. I use molecular dynamics simulation techniques to elucidate the molecular interactions of these cysteine esters. Using these techniques, I have identified potential binding sites for some members of this class of compounds to the inactive structures of β-arrestin 1 and β-arrestin 2. I am using alchemical free energy calculations to determine the affinity of the candidate binding sites; the results will be tested experimentally by collaborators using surface plasmon resonance and hydrogen-deuterium mass spectroscopy. These techniques will be repeated looking at the active structures of these proteins to help further characterize these binding interactions and form hypotheses toward mechanism of action.

Opioid Signaling

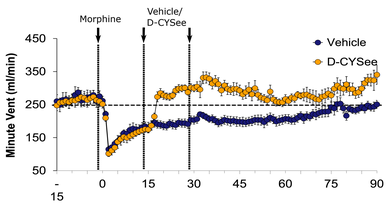


Cysteine Derivatives Restore Respiration in Rats

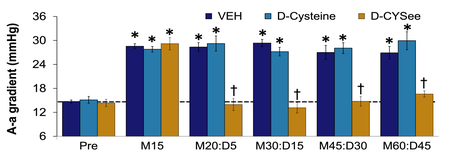


- Looking specifically at the D-form cysteine structure
- R groups currently being tested:
  - Ethyl Ester (D-CYSee)
  - Methyl Ester (D-CYSme)
  - Ethyl Amide (D-CYSea)
  - Methyl Amide (D-CYSma)
- Estimated pKa near 7, running charged and neutral forms

•Minute ventilation
- •Volume of air moved per unit time
- •Same effect for:
  - •Tidal volume
  - •Peak inspiratory flow
  - •Inspiratory drive

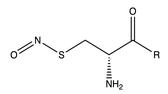


•A-a gradient
- •Gas exchange alveoli
- •Same effect for:
  - •pCO2
  - •pO2
  - •Blood pH



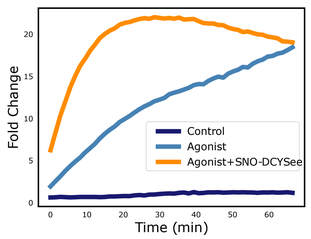
∗ = Statistically significant from baseline
† = Statistically significant from D-Cysteine and vehicle
M = minutes post morphine
D = minutes post experimental dose

S-Nitrosylated Cysteine Derivatives May Be Active Forms



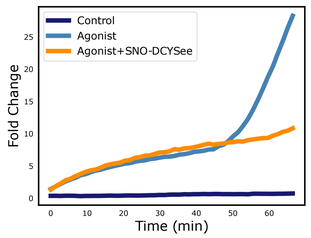
- •Neuronal nitric oxide synthase (NOS) activated by opioid receptor
- •NO → s-nitrosylation
- •Explains why serine derivatives are inactive

β-Arrestin 1

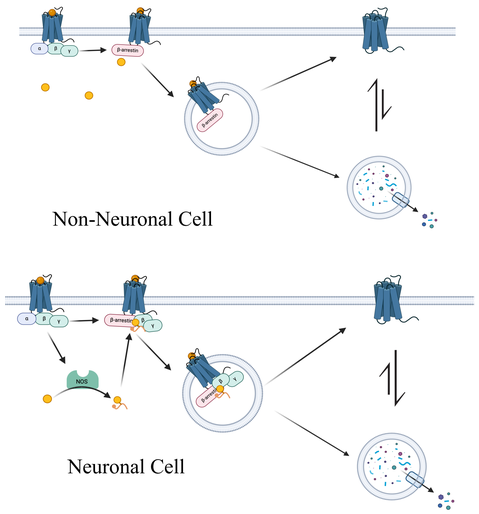


- •Luminescence complementation between delta opioid receptor (DOR) and β-arrestin 1
- •DOR agonist results in association with β-Arrestin 1
- •Presence of SNO-DCYSee increases association between DOR and β-Arrestin 1

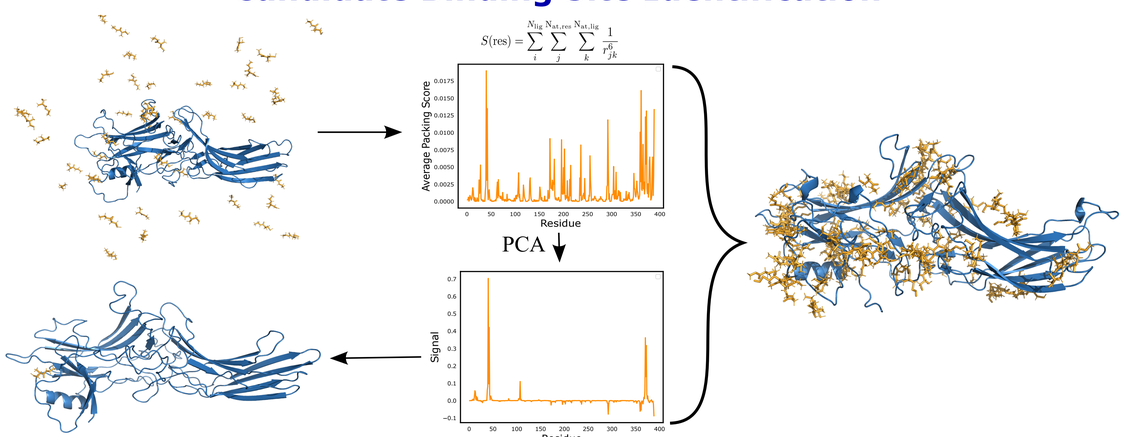
β-Arrestin 2



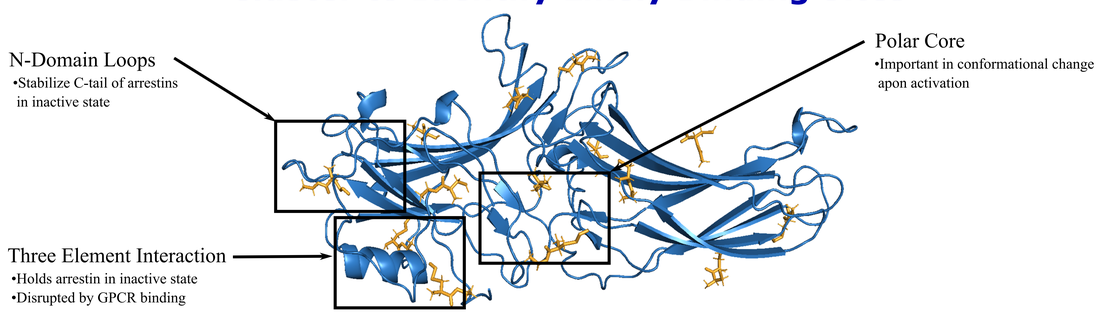
- •Luminescence complementation between DOR and β-Arrestin 2
- •DOR agonist results less association with β-Arrestin 2 than β-Arrestin 1 until 50 min post treatment
- •Presence of SNO-DCYSee decreases association between DOR and β-Arrestin 2 at the 50 min time point compared to agonist alone



Candidate Binding Site Identification

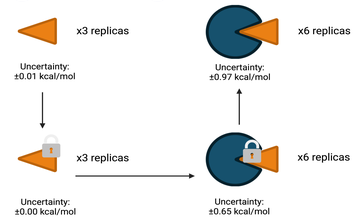


Cluster to Identify Likely Binding Sites



Estimating Binding Affinities

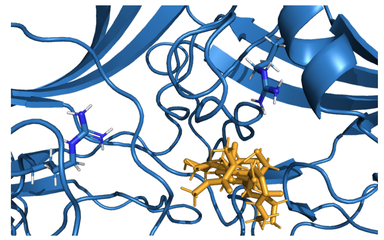
- •Free energy calculations done with BFEE2 (Fu et. al, JCIM, 2021)
  - •Systems run using NAMD3
  - •OPC3 water
  - •Colvars for restraints



Clustering All Sites Reveals Specificity Between β-arrestins

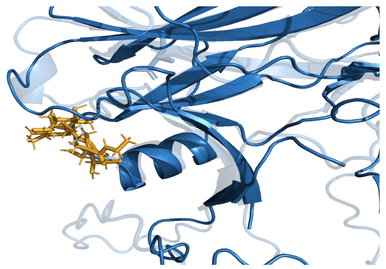
β-Arrestin 1-Specific Cluster

- •Cluster in Polar Core region
  - •Displacement of polar core required for activation
  - •Near two proposed arginine switches
- •Ligands present in cluster:
  - • D-CYSme charged
  - • D-CYSma charged
  - • D-CYSea charged
  - • SNO-L-CYSee charged
- •Average Free Energy of Binding
  - • **-5.64 kcal/mol**



β-Arrestin 2-Specific Cluster

- •Cluster in N-Domain region
  - •Alpha-helix important in GPCR binding and activation
  - •Near CYS409
    - •Only in β-arrestin 2
    - •Nitrosylation here inactivates β-arrestin 2
- •Ligands present in cluster:
  - • D-CYSea charged
  - • SNO-L-CYSee charged
  - • D-CYSma charged
  - • D-CYSme neutral
  - • D-CYSme charged
- •Average Free Energy of Binding
  - • **-6.25 kcal/mol**



Future Directions

- •Free energy calculations on all candidate sites in promising clusters
- •Complete relative free energy calculations for all ligands tested for sites of interest
- •Repeat protocol on modeled active β-arrestin structures
- •Experimental validation with SPR and luminescence complementation assays
- •Use structural information to design new ligands to improve affinity

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Grossfield Laboratory

LOOS Lightweight Object Oriented Structure Analysis

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