Application of Molecular Dynamics for Development of Therapeutics Against Opioid Overdose

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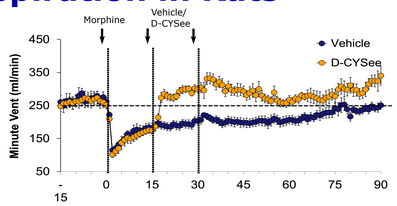
Abstract

Opioid overdose is a long-standing public health issue in the United States, which 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 such as naloxone are limited to emergency scenarios because they induce 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 has been identified that reverse OIRD without blocking analgesic effects or inducing withdrawal. The current hypothesis is that the cysteine derivatives function by binding β-arrestin, a downstream signaling partner of opioid receptors. The goal of my proposed work is to characterize this binding interaction to rationalize the trends observed in the preliminary data. Using molecular dynamics-based methods, I have identified unique potential binding sites for some members of this class of compounds to the inactive structures of β-arrestin 1 and β-arrestin 2. I have used 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. Additionally, experimental data shows super-additive physiological effects when these cysteine derivatives are combined, a phenomenon difficult to explain when the compounds are structurally very similar. We present a simple theory to explain these results.

Cysteine Derivatives Restore Respiration in Rats

- 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

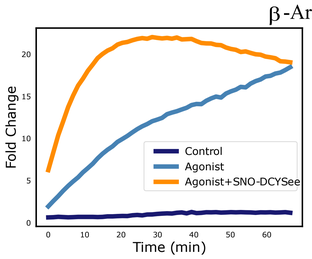
*Data collected by Drs. Getsy & Lewis

∗ = Statistically significant from baseline
† = Statistically significant from D-Cysteine and vehicle

M = minutes post morphine
D = minutes post experimental dose

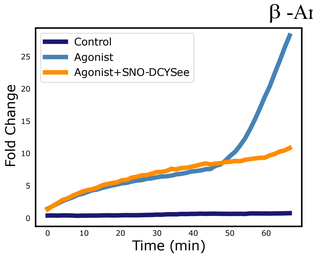
S-Nitrosylated compounds modulate arrestin recruitment

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

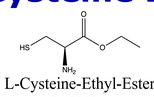
β -Arrestin 2



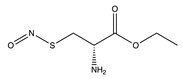
- Luminescence complementation between DOR and β-Arrestin 2
- DOR agonist results less association with β-Arrestin 2 than β-Arrestin 1 until 50 min post treatment
- Presence of SNO-DCYSee decreases association between DOR and β-Arrestin 2 at the 50 min time point compared to agonist alone

*Data collected by Dr. Seckler

Cysteine Derivative Structures



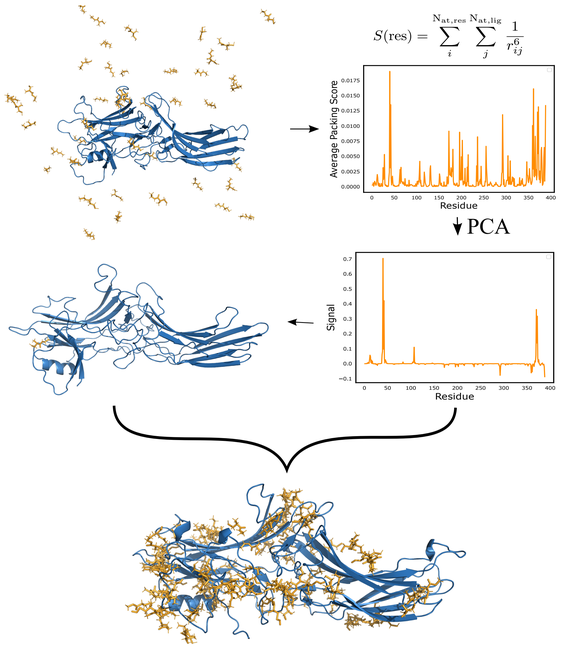
L-Cysteine-Ethyl-Ester (L-CYSee)



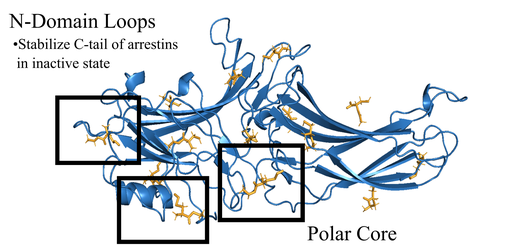
S-Nitrosyl-D-Cysteine-Ethyl-Ester (SNO-D-CYSee)

- One example each for L and D-Cysteine derivatives
- Testing other groups besides ethyl ester on same scaffold
  - Methyl Ester (CYSme)
  - Ethyl Amide (CYSea)
  - Methyl Amide (CYSma)
- Other cysteine derivatives with different structures
  - L-N-Acetyl-Cysteine
  - Glutathione-based esters
- Estimated pKa near 7, running charged and neutral forms

Candidate Binding Site Identification



Cluster to Identify Likely Binding Sites

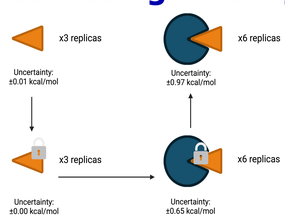


N-Domain Loops
- Stabilize C-tail of arrestins in inactive state

Three Element Interaction
- Holds arrestin in inactive state
- Disrupted by GPCR binding

Polar Core
- Important in conformational change apon activation

Estimating Binding Affinities

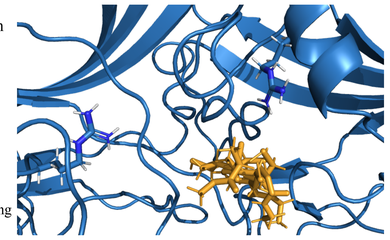


- Free energy calculations done with BFEE2 (Fu et. al, JCIM, 2021)
  - Systems run using NAMD3
  - OPC3 water
  - Colvars for restraints

Clustering All Sites Reveals Specificity Between β-arrestins

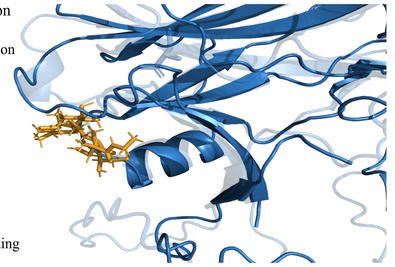
β-Arrestin 1-Specific Cluster

- 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
  - **-4.04 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
  - **-5.63 kcal/mol**

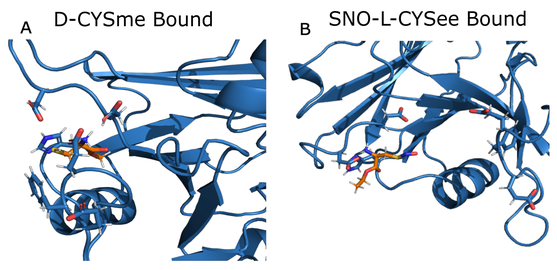


Ligand Affinities

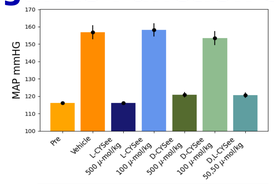
ΔG values in kcal/mol

Target Protein	D-CYSee	L-CYSee	D-CYSme	D-CYSea	SNO-D-CYSee	SNO-L-CYSee
β-arrestin 1	-5.76 (±0.13)	-5.02 (±0.15)	-2.77 (±0.70)	-5.47 (±1.08)	-0.36 (±1.19)	-4.84 (±0.66)
β-arrestin 2	-1.07 (±0.36)	-13.13 (±0.38)	-5.96 (±0.56)	-9.59 (±2.63)	-0.87 (±2.51)	-3.18 (±1.12)

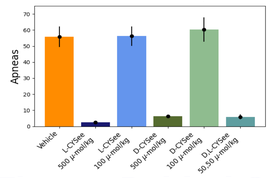
SNO Ligand Activates Arrestin



Ligands Function Super-Additively



- D-CYSee and L-CYSee mitigate naloxone induced withdrawl at high doses only
  - 9 rats per study group
  - 36 hr IV Morphine
  - Naloxone HCl (1.5 mg/kg, IV)
  - Mean arterial blood pressure (MAP) and apnea events reduced
  - Similar trend observed in other withdrawl metrics

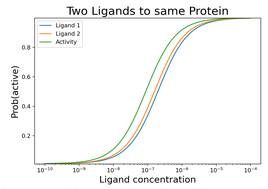


- Low dose mixture performs similarly to high dose single enantiomer
  - Similar trend observed in other withdrawl metrics

*Data collected by Drs. Getsy & Lewis

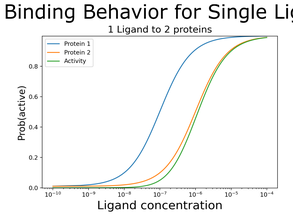
Theory Behind Super-Additivity

Binding Behavior for Two Ligands to One Binding Site



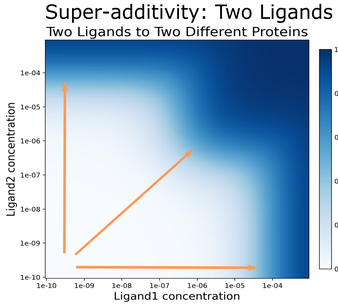
- D, L-CYSee are very similar structures
- Assumption: same binding site, possibly with different affinity
- Outcome: additive results

Binding Behavior for Single Ligand with Two Binding Sites



- Assume ligand is high affinity to one protein and lower for the other
  - ex: Kd_1 = 1nM, Kd_2 = 10nM
- To see activity, both pathways must be active
- Total observed activity requites saturating low affinity site
- Explains why D-CYSee and L-CYSee only work at high concentrations

Super-additivity: Two Ligands and Two Distinct Sites



- Assume Lig_1 is high affinity to site 1 and low affinity for site 2
  - ex: Kd_1 = 1nM, Kd_2 = 100nM
- Assume Lig_2 is high affinity to site 2 and low affinity to site 1
  - ex: Kd_1 = 100nM, Kd_2 = 1nM
- Assume total activity reqires both pathways activated
- Activity only observed at high concentration with one ligand present
- Activity observed at lower concentrations with both ligands

Google Colab where you can see derivation and test with your own Kd values
Scan QR code or available at: https://shorturl.at/N8HJz

Future Directions

- Complete relative free energy calculations for all ligands tested to 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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