Application of Molecular Dynamics for Development of Therapeutics Against Opioid Overdose

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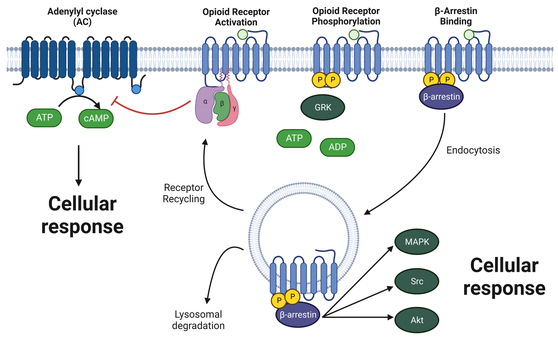
Poster available online

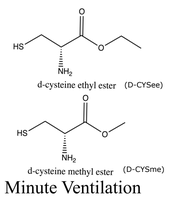
Abstract

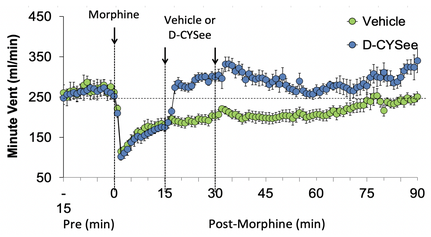
Opioid overdose kills 128 people every day in the United States. While there are treatments for opioid overdose, there are currently no therapeutic tools to prevent it. Moreover, these treatments are limited to emergency scenarios, most notably due to induction of withdrawal and loss of analgesia. Fatal opioid overdoses are primarily attributed to opioid-induced respiratory depression (OIRD). As part of an ongoing collaboration, a class of cysteine esters have been identified that reverse OIRD without blocking the analgesic effects of the opioid 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 primary goal of my proposed work is to characterize this binding interaction to (i) rationalize the trends currently observed in the preliminary data and (ii) suggest new compounds with superior performance. Specifically, I will apply molecular dynamics simulation techniques to elucidate the molecular interactions of these cysteine esters. The results of these simulations will aid in advancing the current understanding of their function in stopping the OIRD response. Using these techniques, I have identified preliminary binding sites of these cysteine esters to the inactive structures of β-arrestin 1 and β-arrestin 2. Going forward, I will use 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.

Opioid Signaling

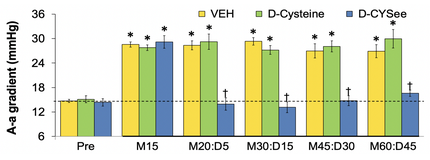


Cysteine Esters Restore Respiration in Rats





- Minute Ventilation
  - Volume of air moved per unit time
  - Same effect for:
    - Tidal Volume
    - Peak Inspiratory Flow
    - Inspiratory Drive
- A-a Gradient
  - Gas Exchange in the alveoli
  - Same effect for:
    - pCO_2
    - pO_2
    - Blood pH

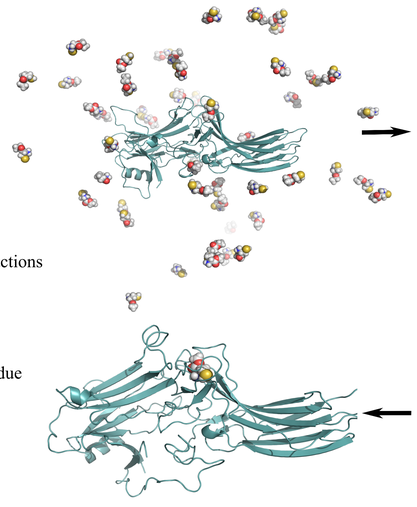


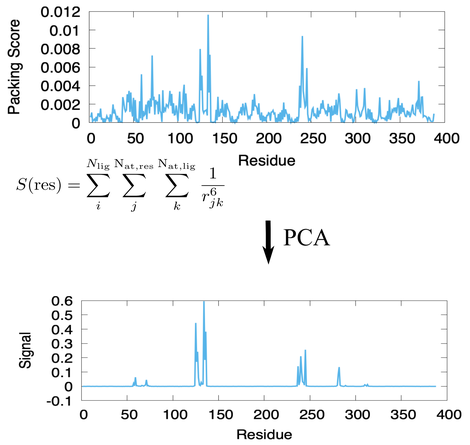
β-Arrestin as a Target

- Surface Plasmon Resonance
  - β-arrestin 2 bound to chip
  - D-CYSee flowed over chip
- Shows interaction but not where binding occurs

Flooding Molecular Dynamics

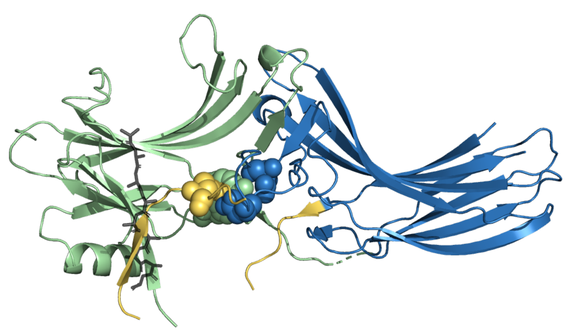
- All-atom simulation
  - OPC Water
  - Hydrogen Mass Repartitioning
  - 5 replicate runs 1.5μs per molecule
- Calculate packing score
  - Analogous to attractive van der Waals interactions
  - Generally finds surface of protein
  - Cannot identify binding mode alone
- PCA to Identify Binding Mode
  - Covariance matrix of packing score per residue

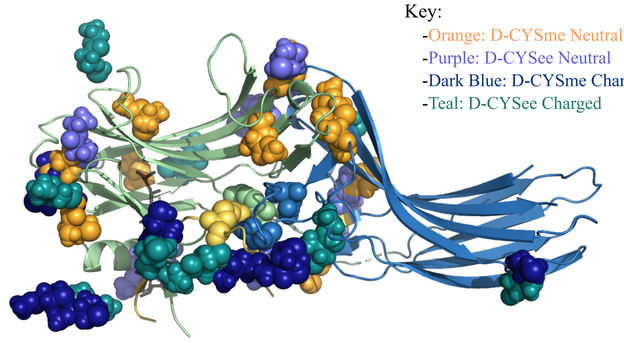




$$S(\mathrm{res}) = \sum_{i}^{N_{lig}} \sum_{j}^{N_{at,res}} \sum_{k}^{N_{at,lig}} \frac{1}{r_{jk}^{6}}$$

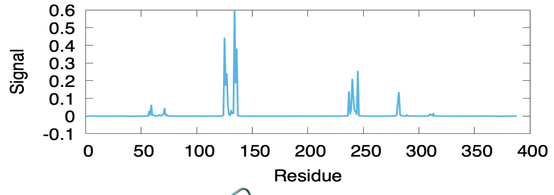
Candidate Sites in Functionally Important Regions

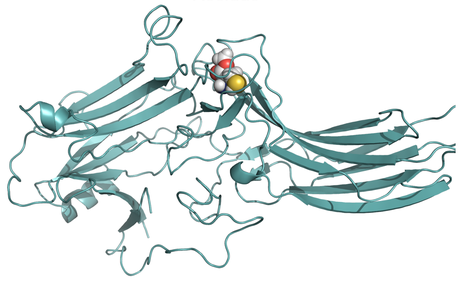




Key:
- Orange: D-CYSme Neutral
- Purple: D-CYSee Neutral
- Dark Blue: D-CYSme Charged
- Teal: D-CYSee Charged

Preliminary Binding Free Energy Calculation

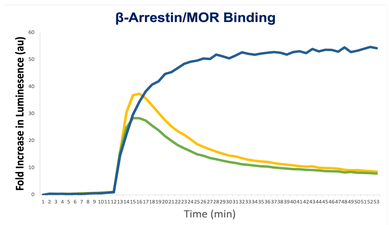




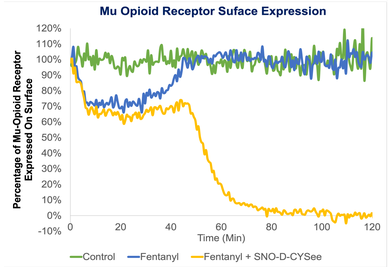
- Site near important cysteine residue
  - D-CYSme binding β-arrestin 2
  - Nitrosylated CYS253 inactivates arrestin
  - Site involves flexible C-terminal loop
    - Loop was modeled into structure
    - Would have been missed by docking
- Simultaneous decoupling recoupling
- MBAR for extracting free energies
- 3 Replicas of Free Energy Calculation
  - -1.15 kcal/mol
  - -1.12 kcal/mol
  - -3.75 kcal/mol

S-Nitrosyl-D-Cysteine Ethyl Ester May be Active Form

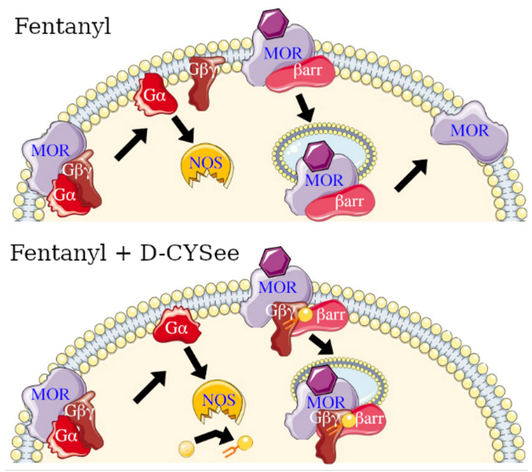
- Luminesence assay of β-arrestin MOR Complex
- Fentanyl leads to complex formation
- Over time complex separates for downsteam siganling
- D-CYSee reduced β-arrestin binding to MOR
- SNO-D-CYSee extends lifetime of MOR/β-arrestin complex



- Fentanyl caused MOR to be trafficked to the endosome and recycled to the cell surface
- SNO-D-CYSee caused enhanced endosomal trafficking followed by degradation rather than recycling



Hypothesized Mechanism



Future Directions

- Flooding of SNO-D-CYSee and SNO-D-CYSme
- Free energy calculations on candidate sites
- Repeat protocol on active β-arrestin
- Experimental validation with SPR and HDX-MS

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