Application of Molecular Dynamics for Development of Therapeutics Against Opioid Overdose

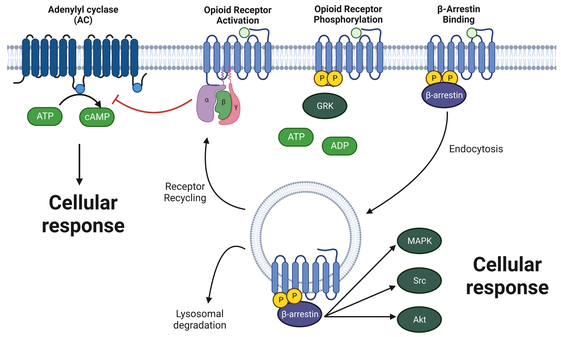
Emily N. Robinson, James M. Seckler, Alexander J. Crook, Stephen J. Lewis, Alan Grossfield
University of Rochester Medical School, Rochester, NY, USA
Case Western Reserve University, Cleveland, OH, USA

Poster available online

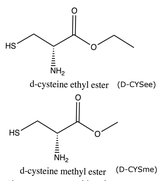
Abstract

Opioid overdose has been a long-standing public health issue in the United States, which has only been exacerbated by the Covid-19 pandemic. 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 derivatives have been identified that reverse OIRD without blocking the analgesic effects of the opioid or inducing withdrawal. The current hypothesis is that these compounds function by binding β-arrestin, a protein that signals downstream of the opioid receptors. Here, we present molecular dynamics simulations intended to characterize this binding interaction and rationalize the trends currently observed in the preliminary data. Specifically, we combine conventional simulations of the protein in the presence of high ligand concentrations – "flooding" simulations – with alchemical free energy calculations to estimate the binding affinities. We perform these calculations for both inactive apo β-arrestin and a model for the active form bound to a peptide mimic for the opioid receptor's C-terminal tail. The results will be used to suggest new experiments for our collaborators, including mutagenesis to β-arrestin and new compounds to test.

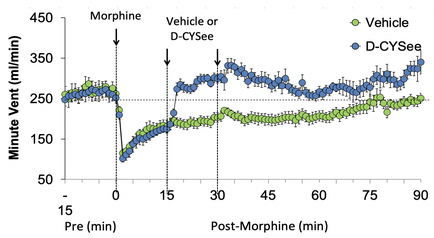
Opioid Signaling

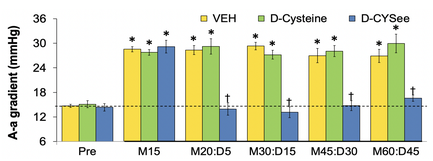


Cysteine Esters Restore Respiration in Rats



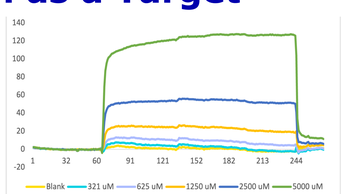
- Minute ventilation
  - Volume of air moved per unit time
  - Same effect for:
    - Tidal volume
    - Peak inspiratory flow
    - Inspiratory drive
- A-a gradient
  - Gas exchange in the alveoli
  - Same effect for:
    - pCO_2
    - pO_2
    - Blood pH





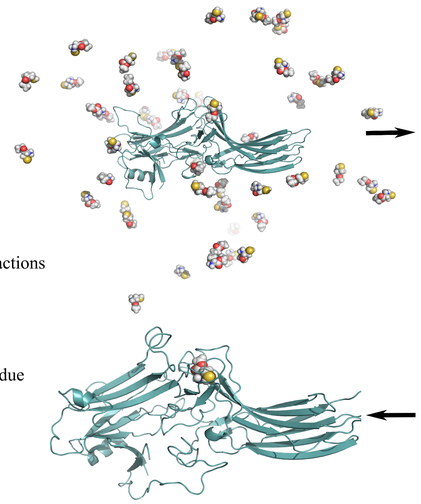
β-Arrestin as a Target

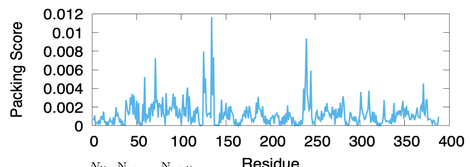
- Surface plasmon resonance
  - β-arrestin 2 bound to chip
  - D-CYSee flowed over chip
- Shows interaction but not where binding occurs



Flooding Molecular Dynamics

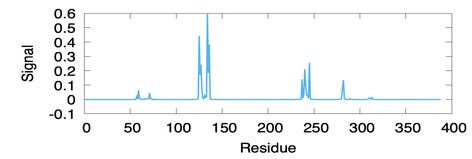
- All-atom simulation
  - OPC water
  - Hydrogen mass repartitioning
  - 5 replicate runs 1.5μs per molecule
- Calculate packing score
  - Analogous to attractive van der Waals interactions
  - Generally finds surface of protein
  - Cannot identify binding mode alone
- PCA to identify binding mode
 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



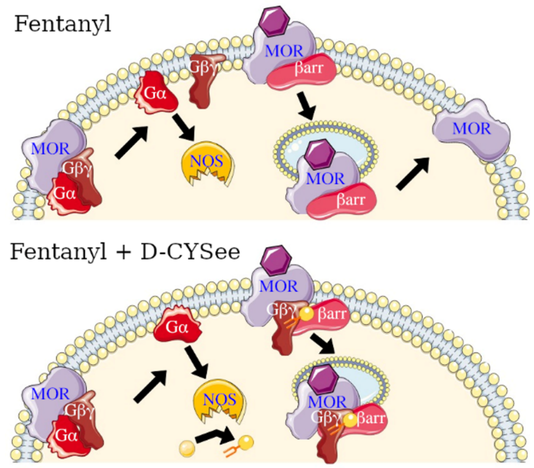


$$S(\mathrm{res}) = \sum_{i}^{N_{\mathrm{lig}}} \sum_{j}^{N_{\mathrm{at,res}}} \sum_{k}^{N_{\mathrm{at,lig}}} \frac{1}{r_{jk}^{6}}$$

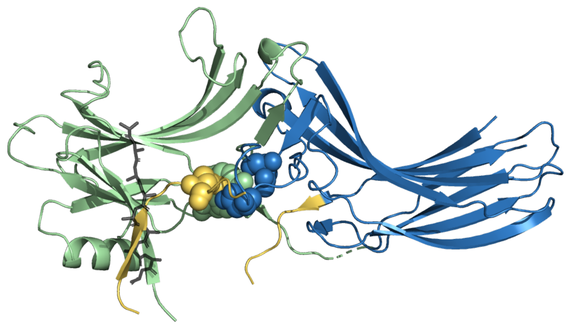
PCA

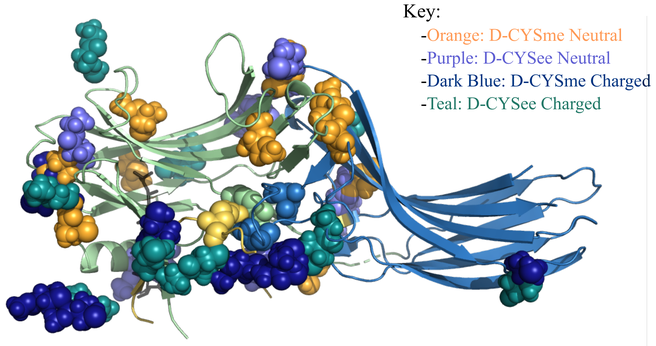


Hypothesized Mechanism



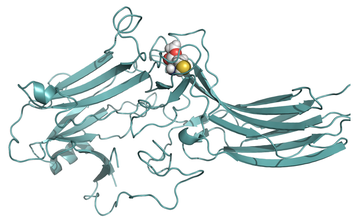
Candidate Sites in Functionally Important Regions





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

Preliminary Binding Free Energy Calculation

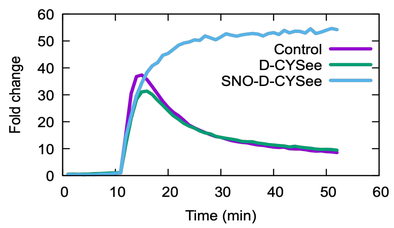


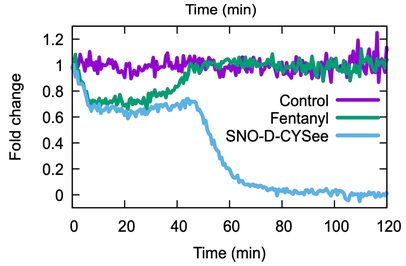
- Site near important cysteine residue
  - D-CYSme binding β-arrestin 2
  - Nitrosylated CYS253 inactivates arrestin
- 3 replicas of free energy calculation
  - -1.15 kcal/mol
  - -1.12 kcal/mol
  - -3.75 kcal/mol

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

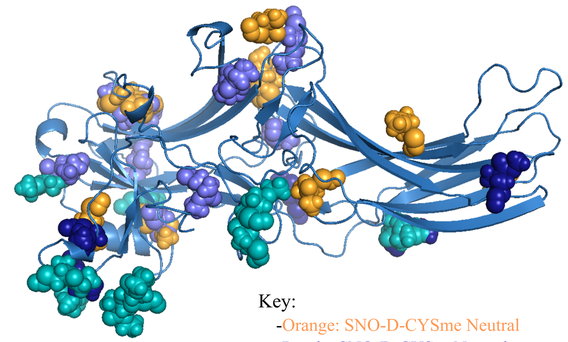
- Test sytem using beta adrenergic receptor (β2AR) and isopretenrol
- Isopretenerol causes (β2AR - β-arrestin complex to form
- D-CYSee reduced β-arrestin binding to β2AR
- SNO-D-CYSee extends life of β2AR β-arrestin complex

- 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





S-Nitrosyl-D-Cysteine Ethyl Ester Candidate Binding Sites



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

Future Directions

- Free energy calculations on all candidate sites
- Repeat protocol on modeled active β-arrestin structures
- Experimental validation with SPR and luminesence complementation assays

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