

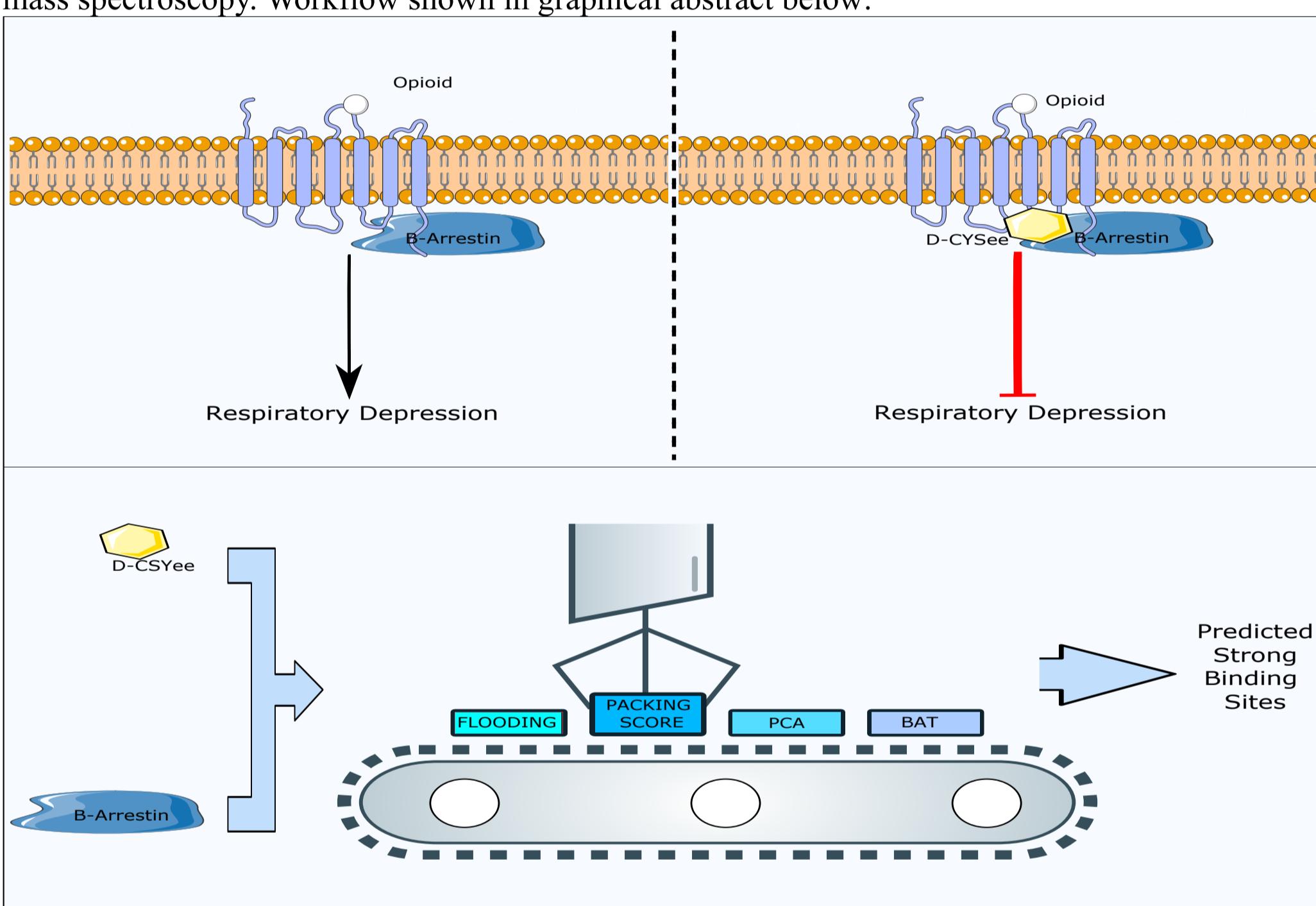
# Application of Molecular Dynamics to Elucidate Key Binding Interactions for the Development of Therapeutics Against Opioid Overdose

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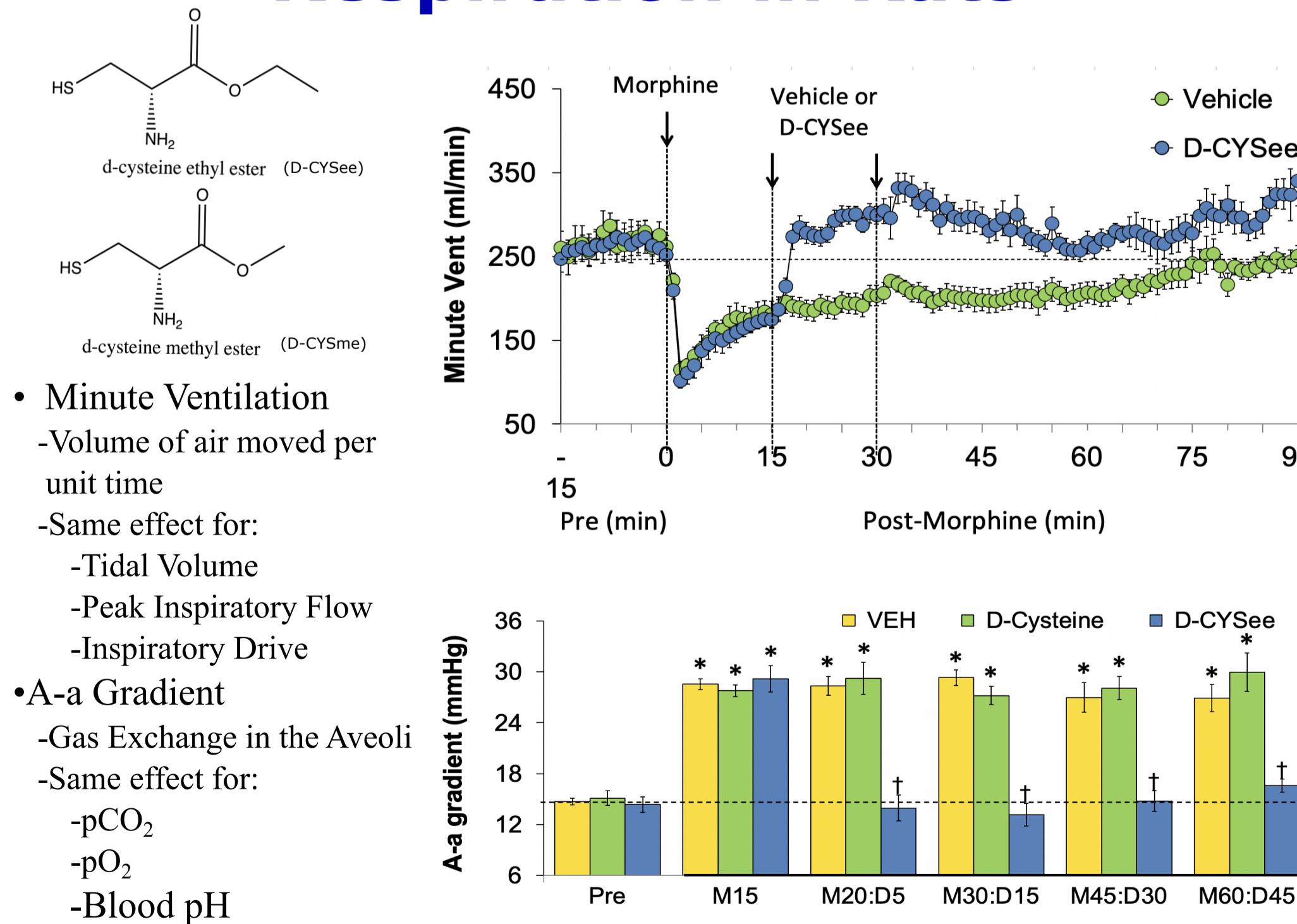


## Abstract

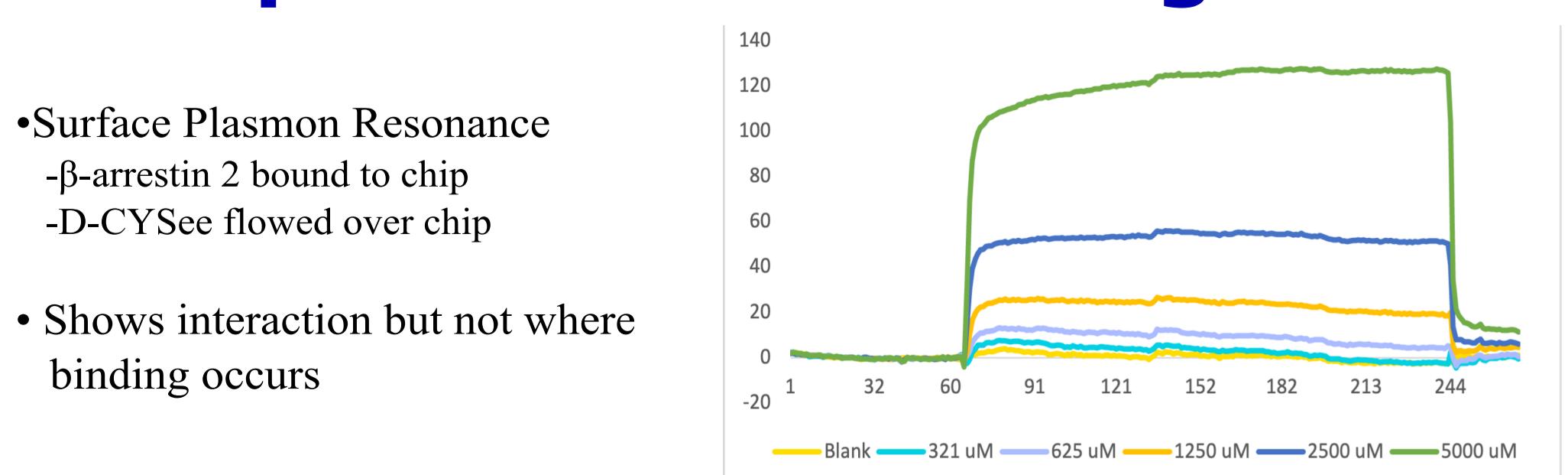
Opioid overdose kills 128 people every day in the United States. While there are treatments for opioid overdose, there are currently no therapeutic tools to prevent it. Moreover, these treatments are limited to emergency scenarios, most notably due to induction of withdrawal and loss of analgesia. Fatal 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  $\beta$ -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  $\beta$ -arrestin 1 and  $\beta$ -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. Workflow shown in graphical abstract below:



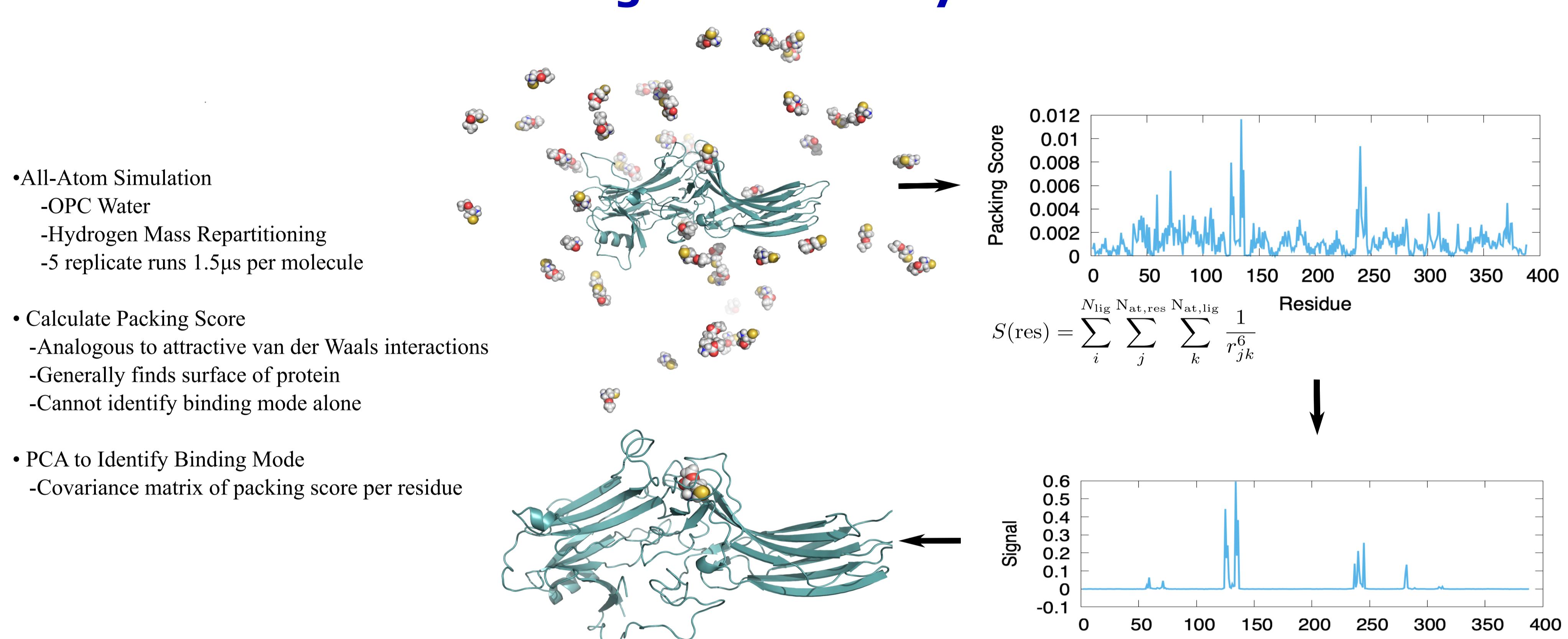
## Cysteine Esters Restore Respiration in Rats



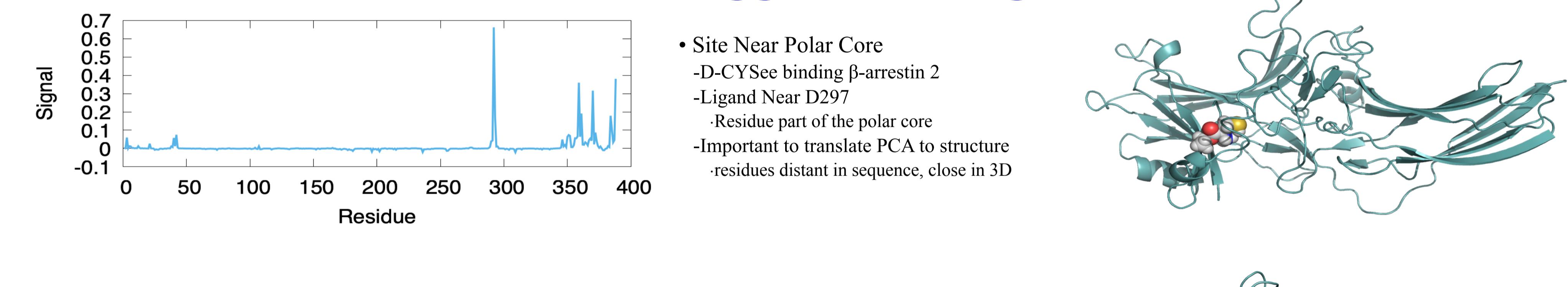
## $\beta$ -Arrestin as a Target



## Flooding Molecular Dynamics

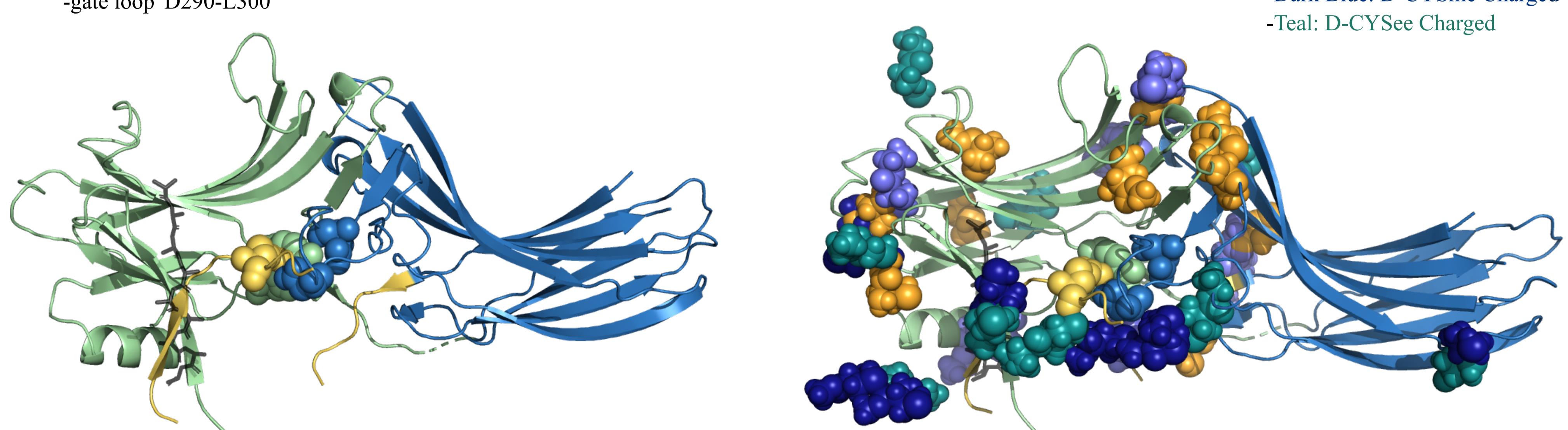


## PCA Modes Suggest Binding Sites



- Site near important Cysteine Residue  
- D-CYSme binding  $\beta$ -arrestin 2  
- nitrosylated CYS253 inactivates arrestin  
- Site involves flexible C-terminal loop  
- Loop was modeled into structure  
- Would have been missed by docking

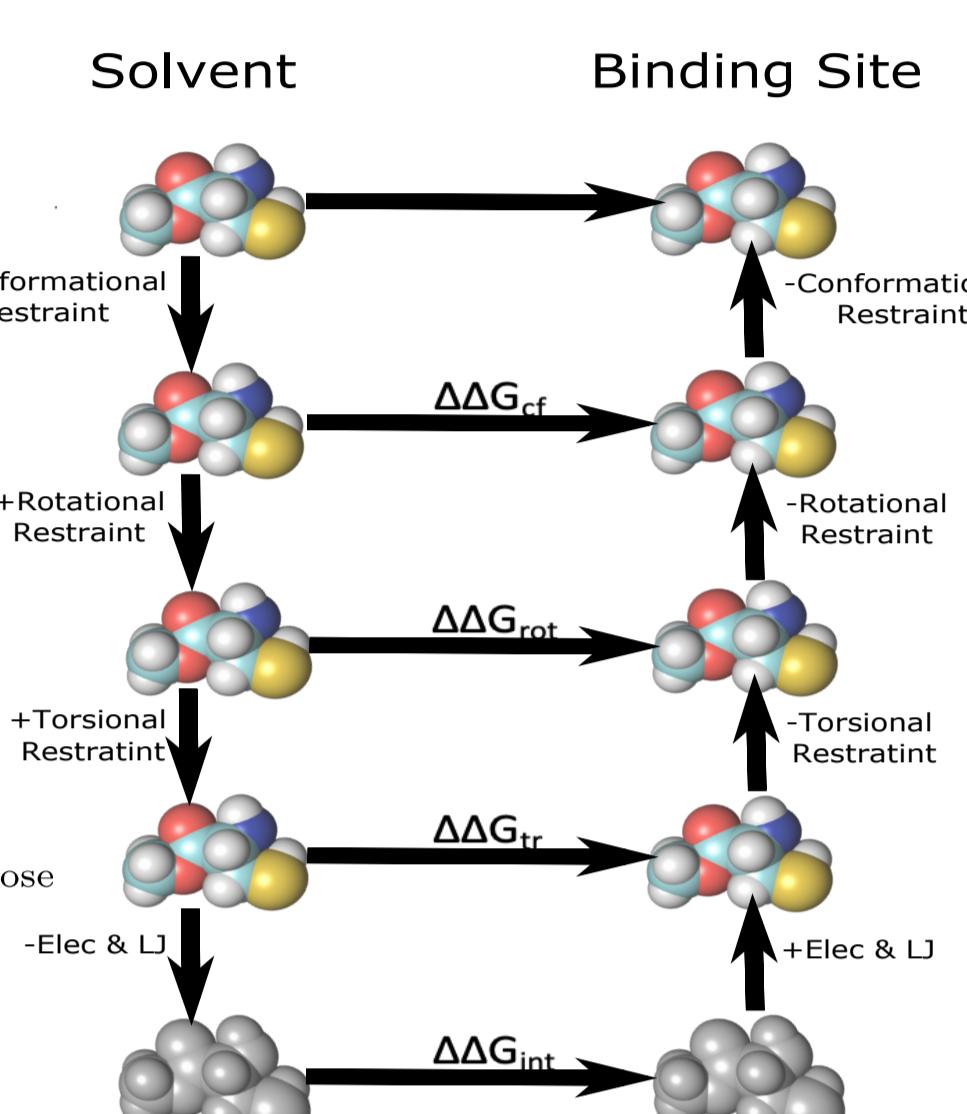
## Binding Sites in Functionally Important Regions



## Alchemical Binding Free Energy

- Cannot calculate affinities from standard MD  
- run times too short for on/off rates
- Alchemical simulations overcome this issue  
- Free energy as a state function  
- all equilibrium paths give the same answer  
- can take non-physical path in computer

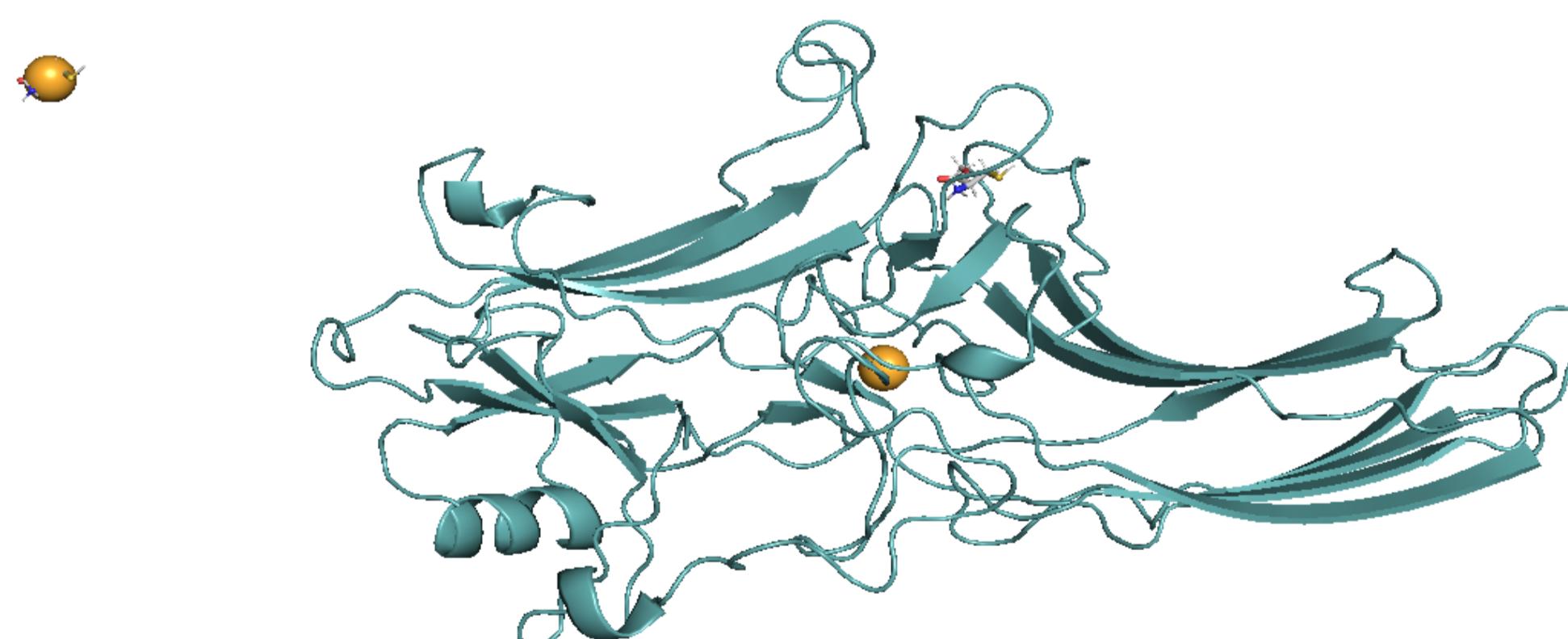
$$\Delta G_b^0 = \Delta\Delta G_{cf} + \Delta\Delta G_{rot} + \Delta\Delta G_{int} + \Delta\Delta G_{close}$$
$$\Delta\Delta G_{int} = \Delta\Delta G_{elec} + \Delta\Delta G_{LJ}$$



## Protocol

Simulation Step	Windows	Equip Sim Time	Prod Sim Time	Free Energy Term
Attachment of Receptor Conformational Restraints	16	1ns	2ns	$\Delta G_{p,cf, att}$
Attachment of Ligand Conformational Restraints	16	1ns	2ns	$\Delta G_{l,cf, att}$
Attachment of Ligand Torsional Restraints	16	1ns	2ns	$\Delta G_{l,tr, att}$
Simultaneous Decoupling/ Recoupling	23	1ns	2ns	$\Delta G_{elec}$
Simultaneous Decoupling/ Recoupling of Ligand Charge Interactions	23	1ns	2ns	$\Delta G_{LJ}$
Release of Ligand Conformational Restraints	16	1ns	2ns	$\Delta G_{l,cf, rel}$
Release of Receptor Conformational Restraints	16	1ns	2ns	$\Delta G_{p,cf, rel}$

## SDR Box Setup



## Future Directions

- Free energy simulations currently running
- Repeat protocol on active  $\beta$ -arrestin
- Experimental validation with SPR and HDX-MS



U01DA051373

BAT.py

Poster available online:

