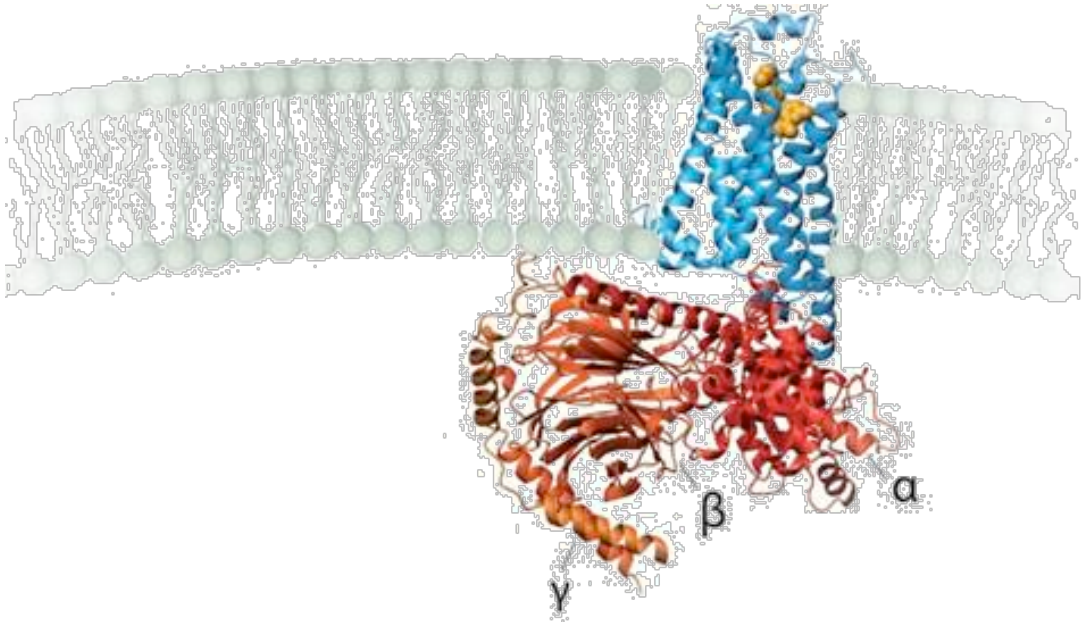
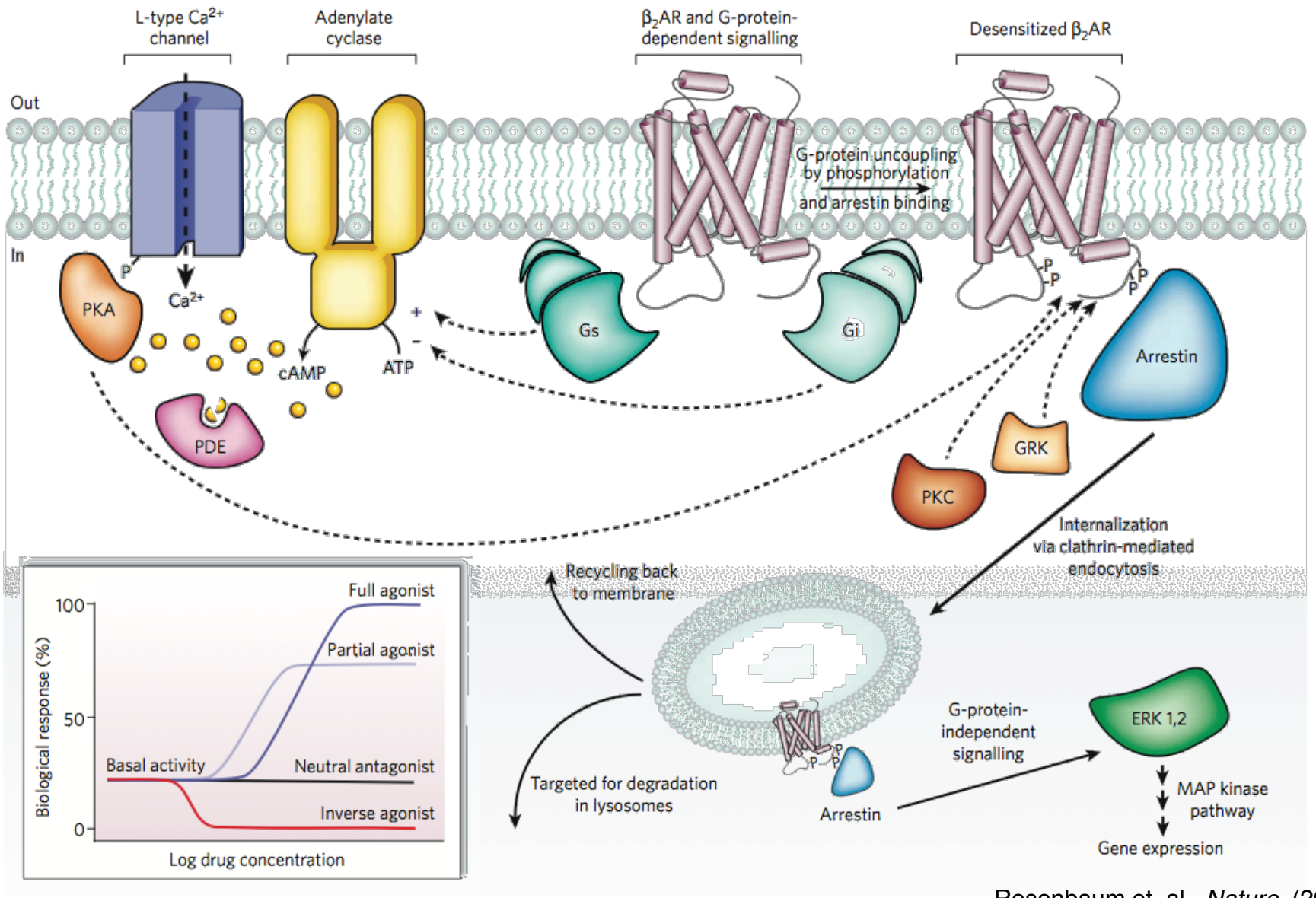
Investigating Ligand Modulation of GPCR Conformational Dynamics in the Membrane

Morgan Lawrenz, Kai Kohloff,
Diwakar Shukla, Greg Bowman,
Russ Altman, and Vijay Pande

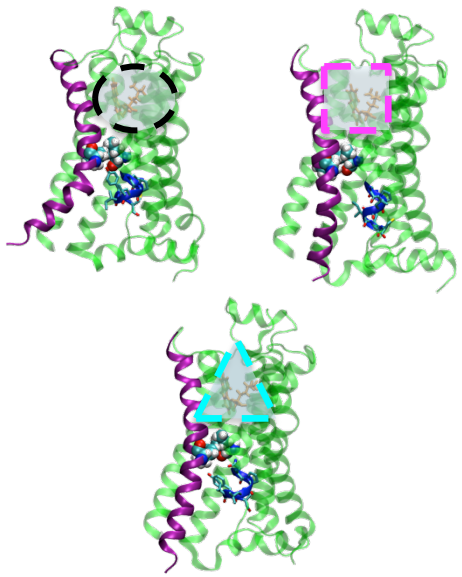


G-protein coupled receptors (GPCRs) are key regulators of signal transduction

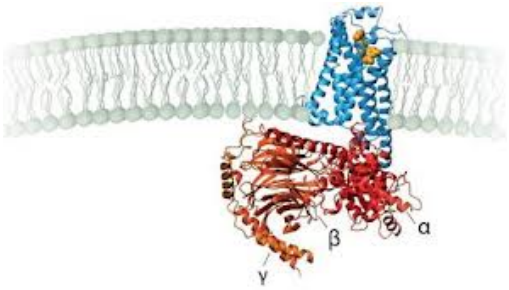


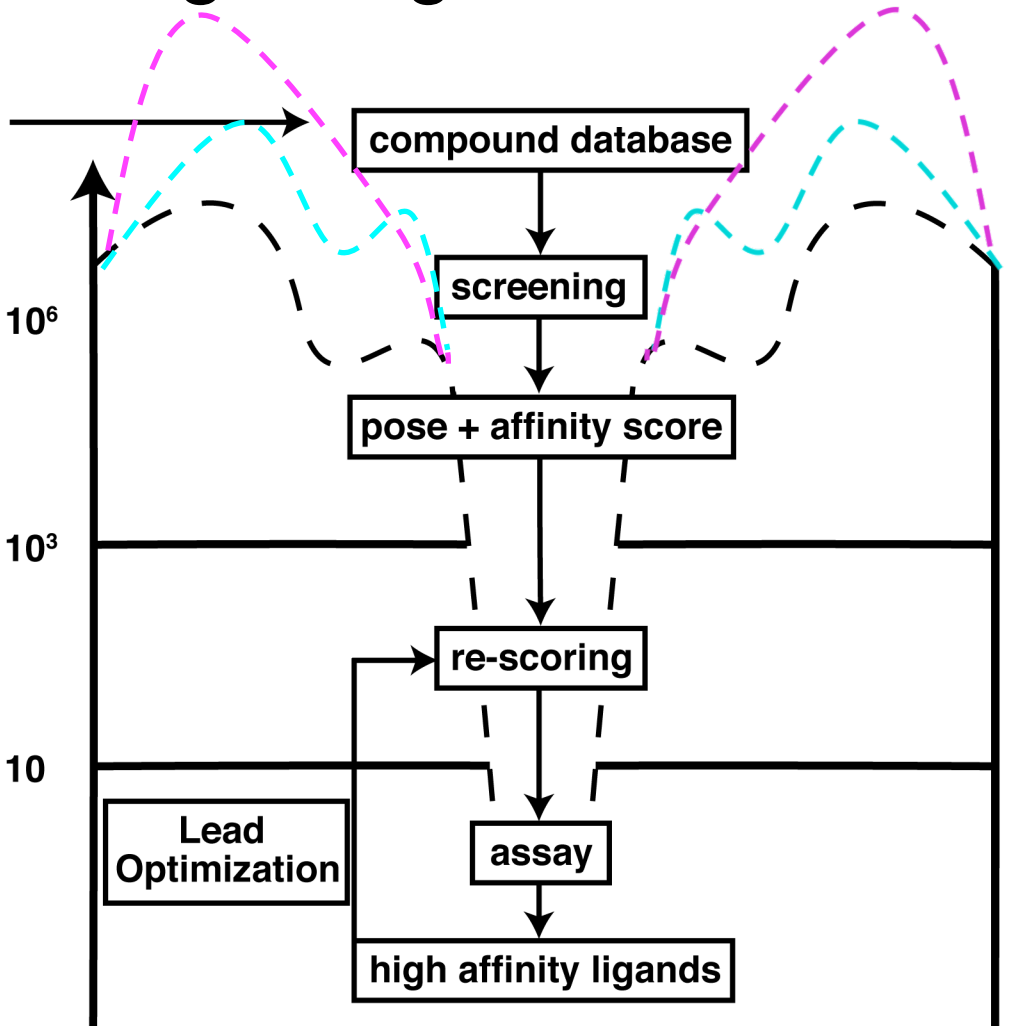
Rosenbaum et. al., *Nature*, (2009)

Incorporate GPCR conformational ensemble into structure-based drug design workflows



Conformational Ensemble

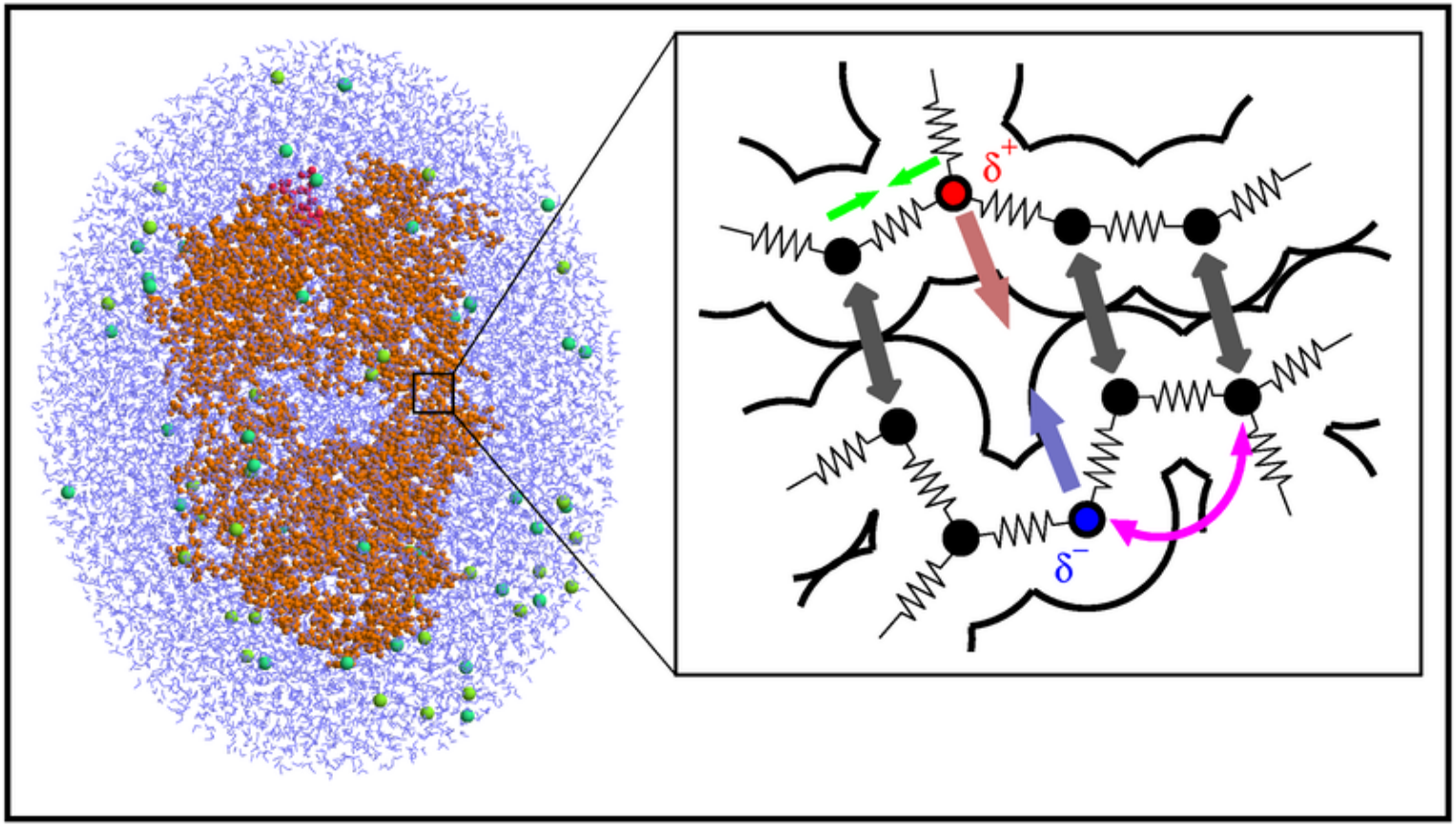




Rasmussen, et al. *Nature*. (2011)

Molecular dynamics simulations

To study GPCR dynamics in the membrane

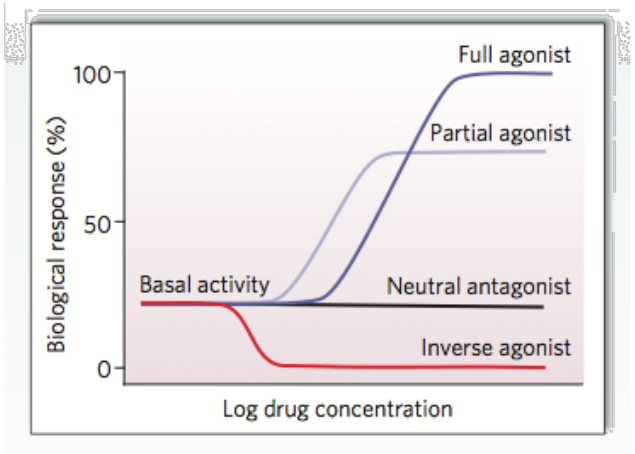


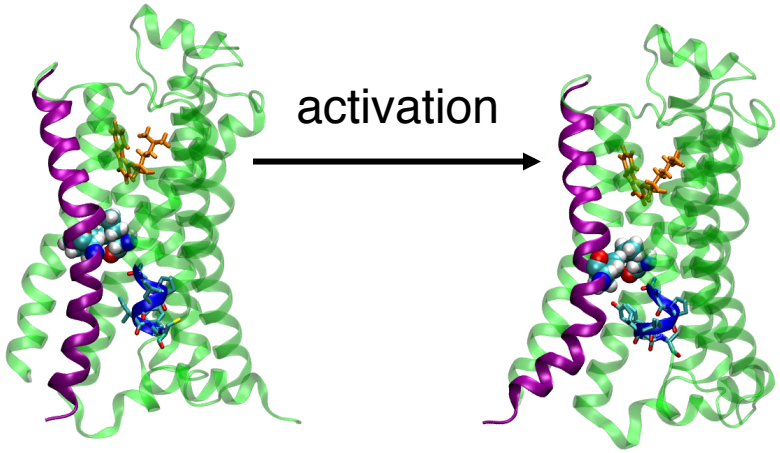
$$m_i \frac{d^2}{dt^2} \vec{x}_i = \vec{F}_i(\vec{x}_1, \ldots, \vec{x}_N) \qquad i = 1 \ldots N$$

Bert de Groot, http://www.gromacs.org

Use MD simulations to understand:

- What are the structural changes that connect active and inactive GPCR states?
- How do different ligands affect GPCR conformational dynamics?
- What are determinants of ligand selectivity?

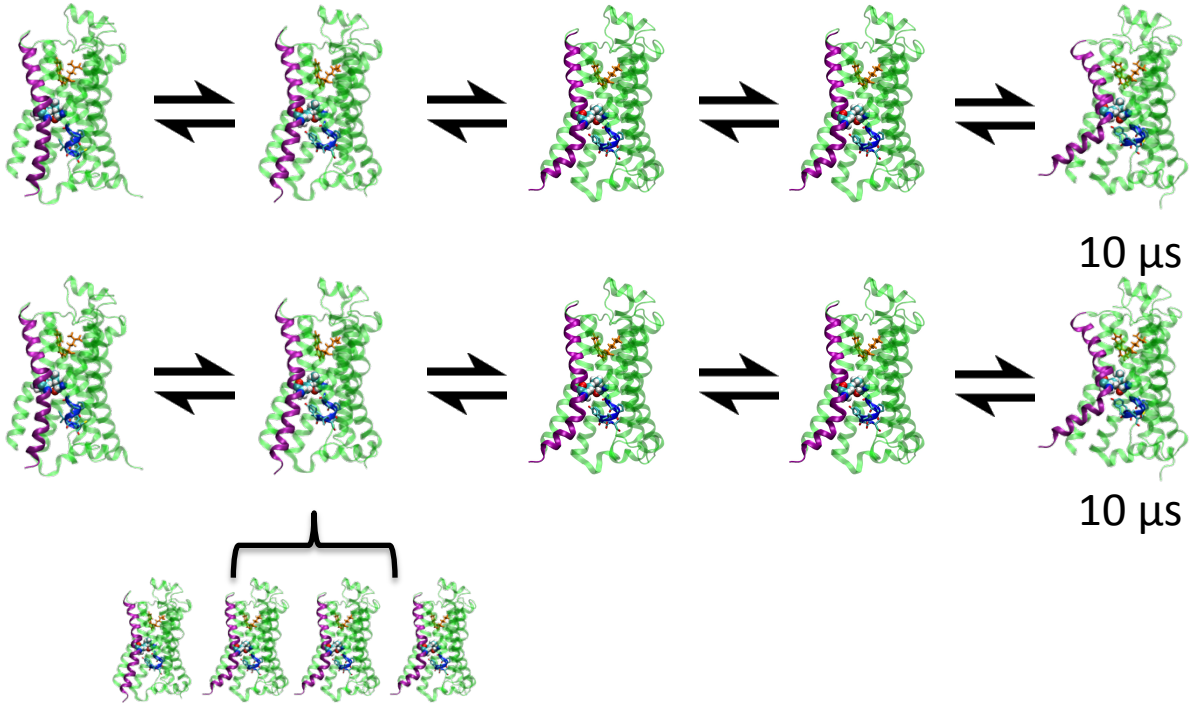




Dror, et al PNAS (2011).
Vanni, et al PLoS Comput. Biol (2011)

Synergistic Sampling Strategies

Long simulations from supercomputing can seed massively parallel simulations



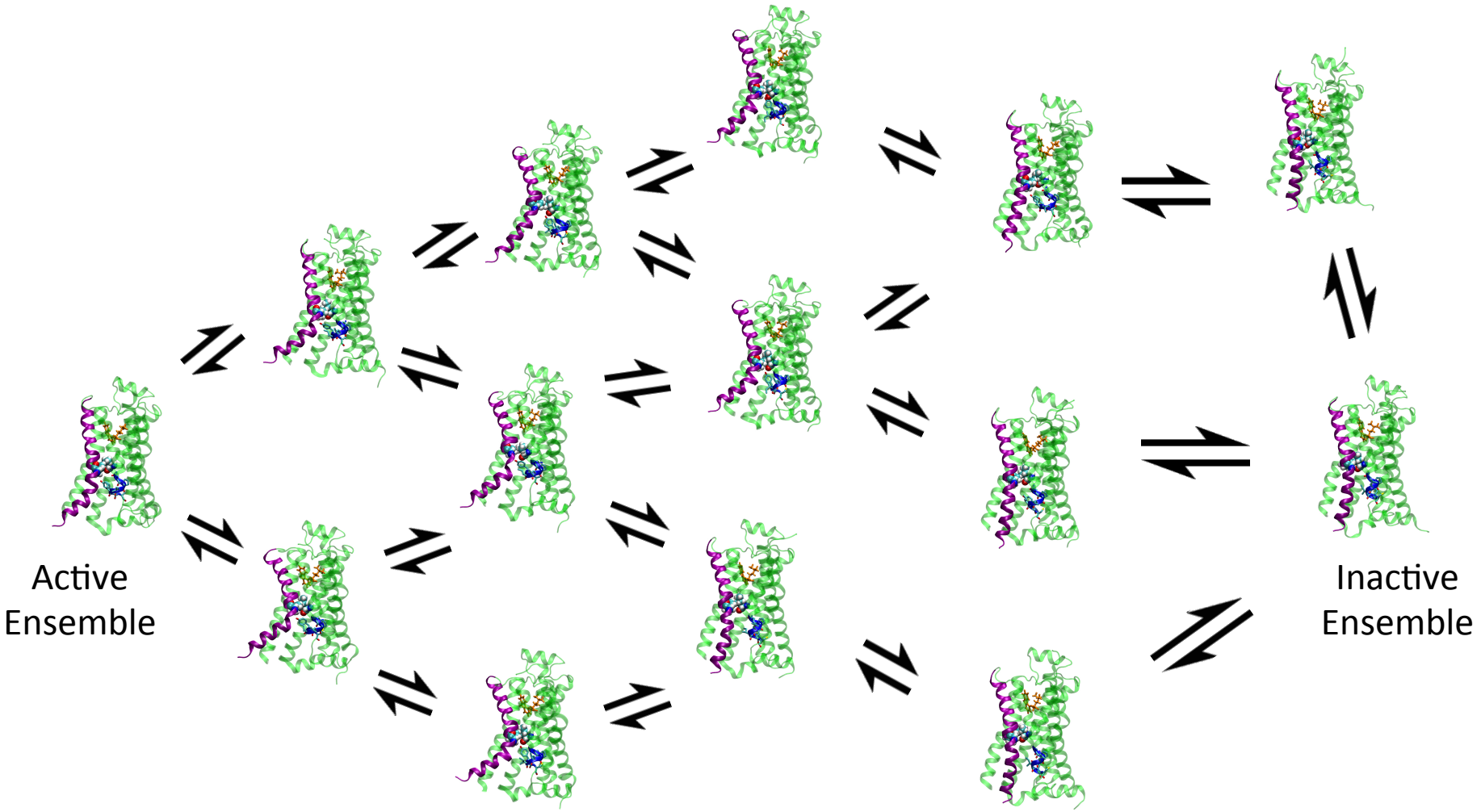
BLUE WATERS
SUSTAINED PETASCALE COMPUTING

Folding@home distributed computing

Google
Exacycle for Visiting Faculty

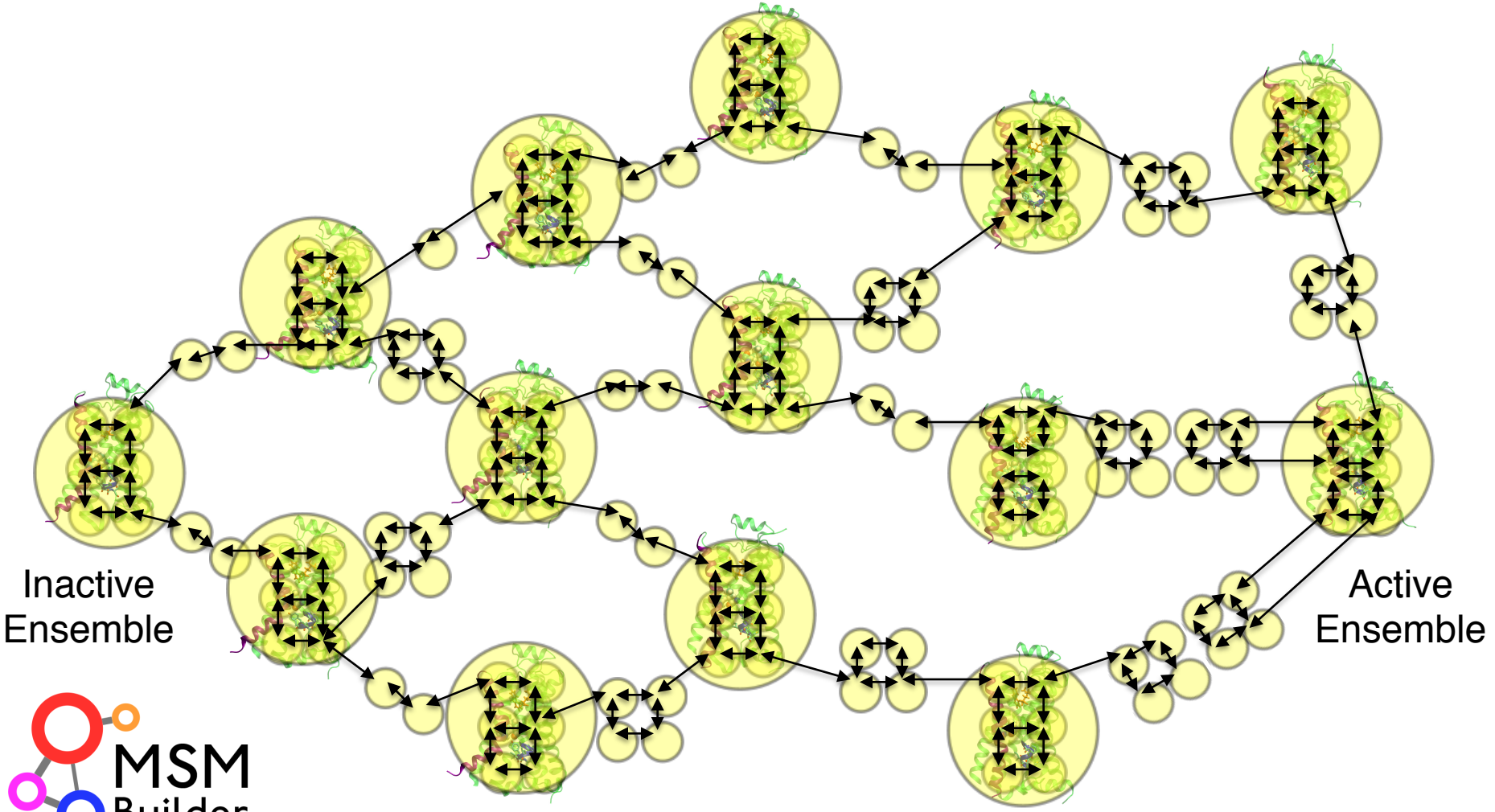
Stitching together simulations

~2 milliseconds of aggregate simulation time for the β_2AR bound to agonist, inverse agonist, and apo



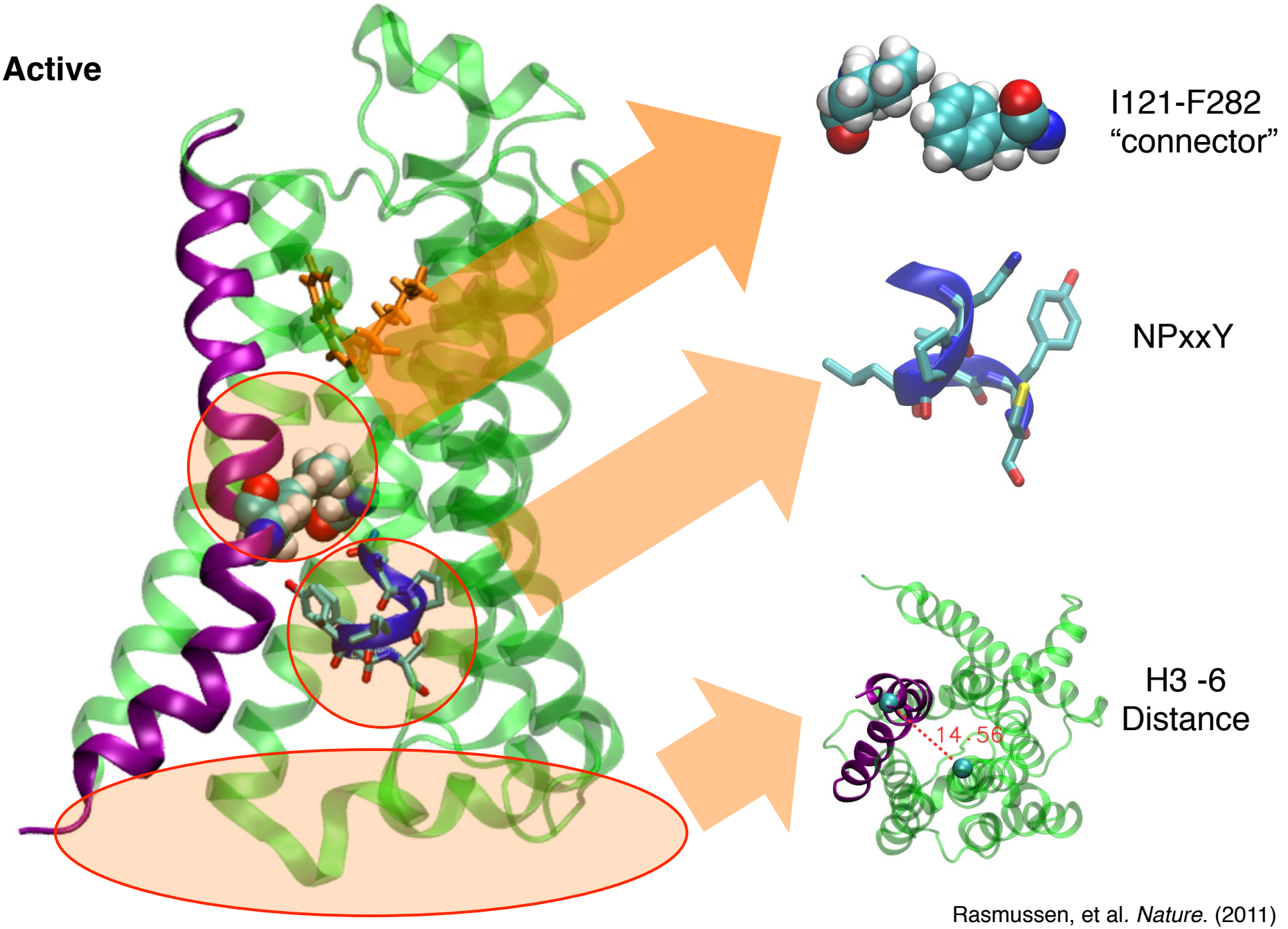
Stitching together simulations

Transitions counted between geometric clusters to determine Markov states with a transition matrix Tij that maps out state connectivity, and gives kinetic information and equilibrium state probabilities.



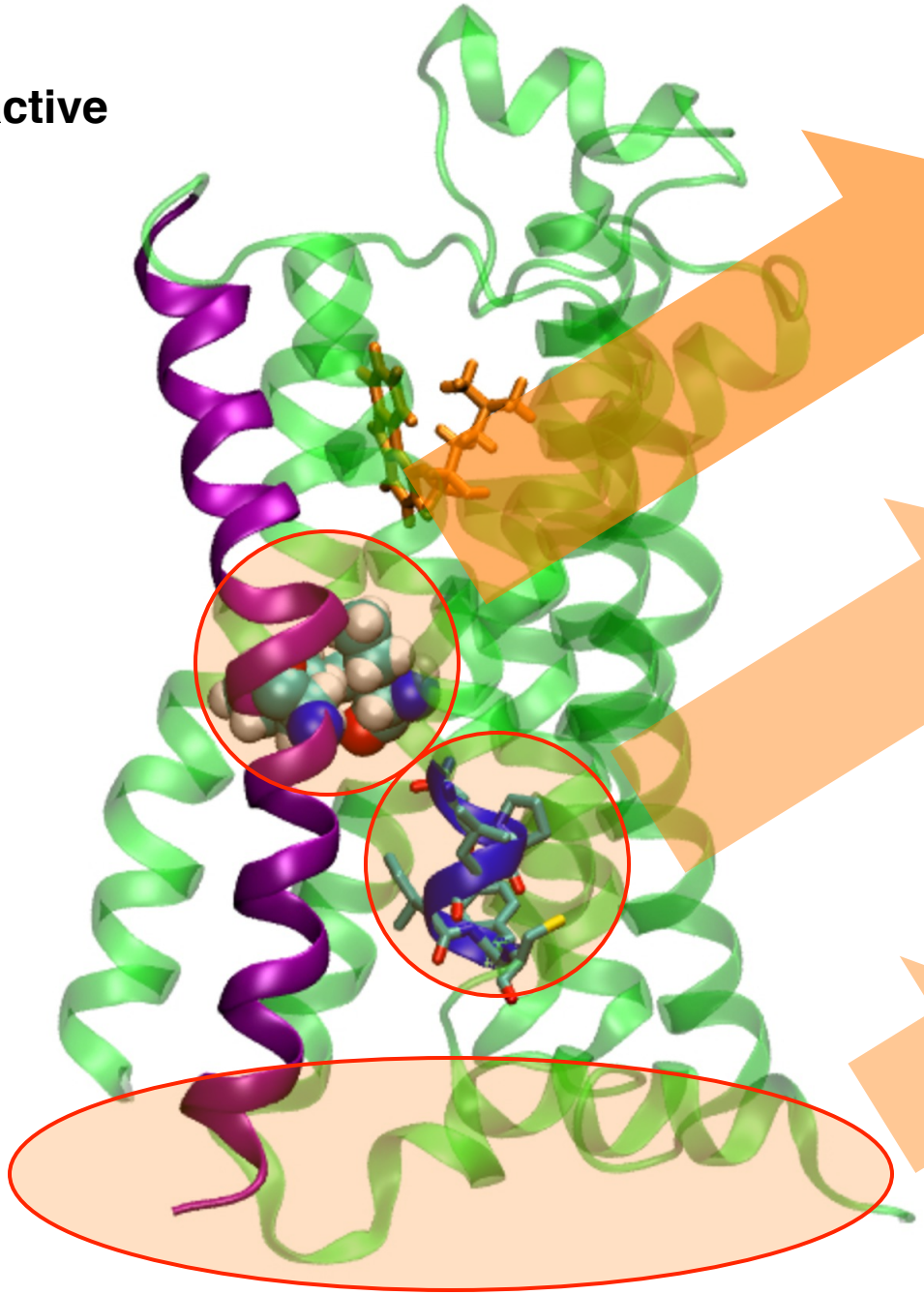
MSM Builder

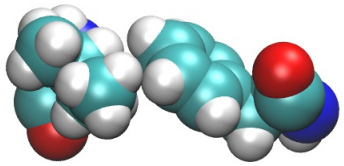
Beauchamp, et al. *JCTC* (2011)



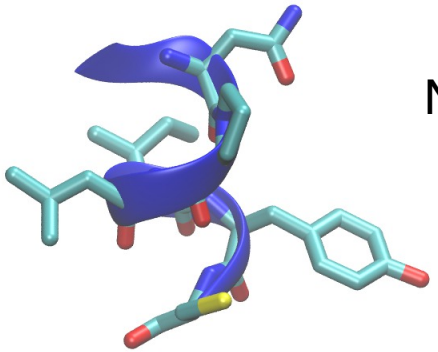
Rasmussen, et al. *Nature*. (2011)
Dror, et al *PNAS* (2011).

Inactive

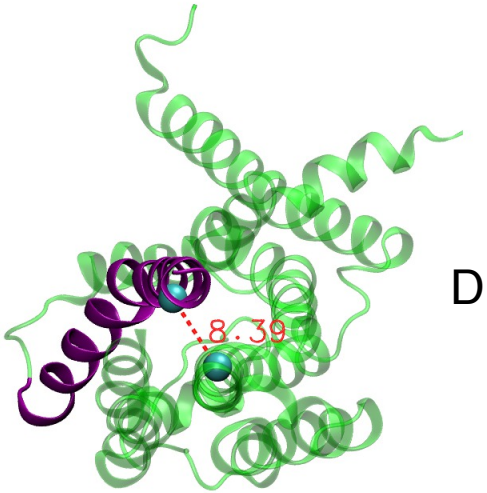




I121-F282
"connector"



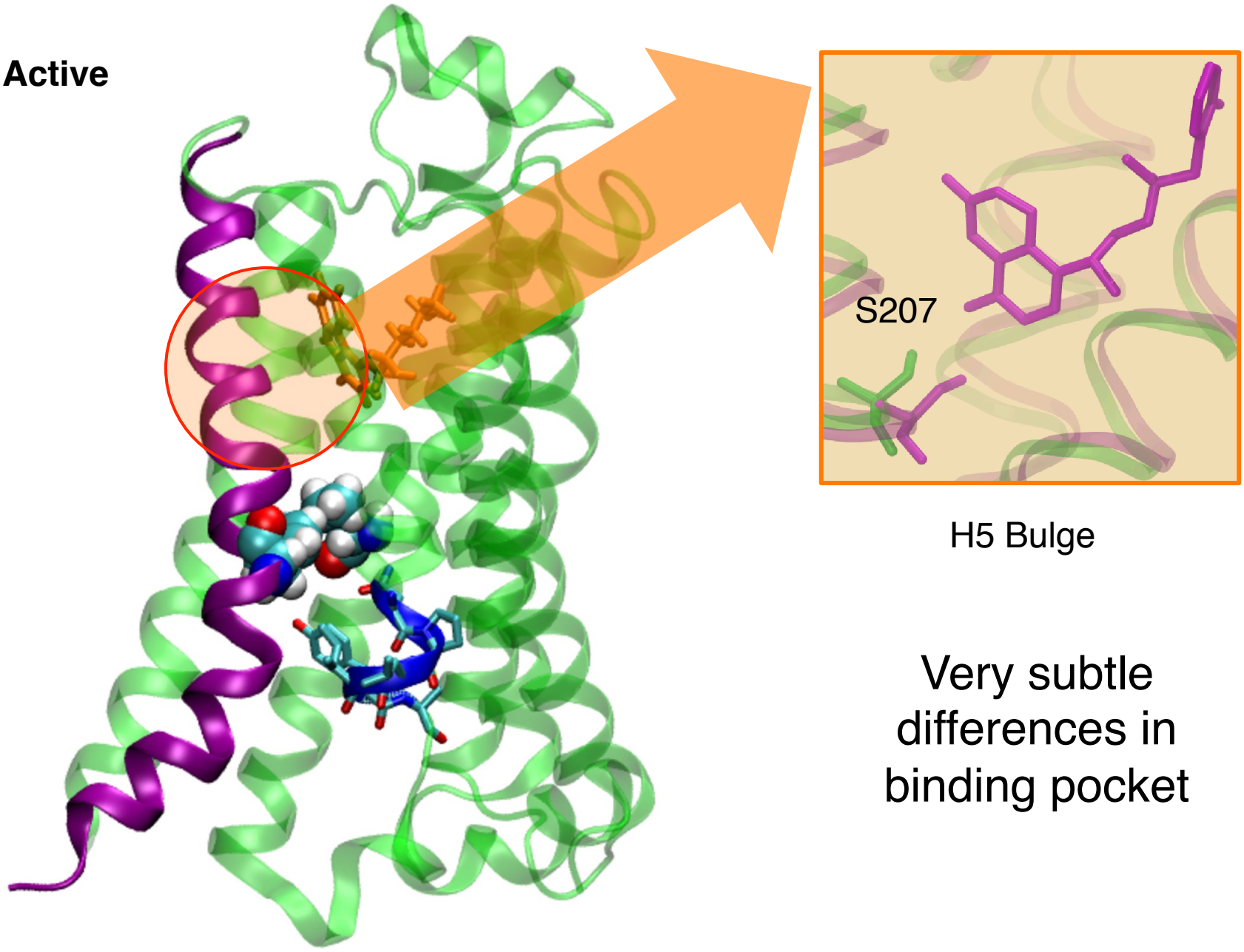
NPxxY



H3 -6
Distance

Rasmussen, et al. *Nature*. (2011)
Dror, et al *PNAS* (2011).

Active



H5 Bulge

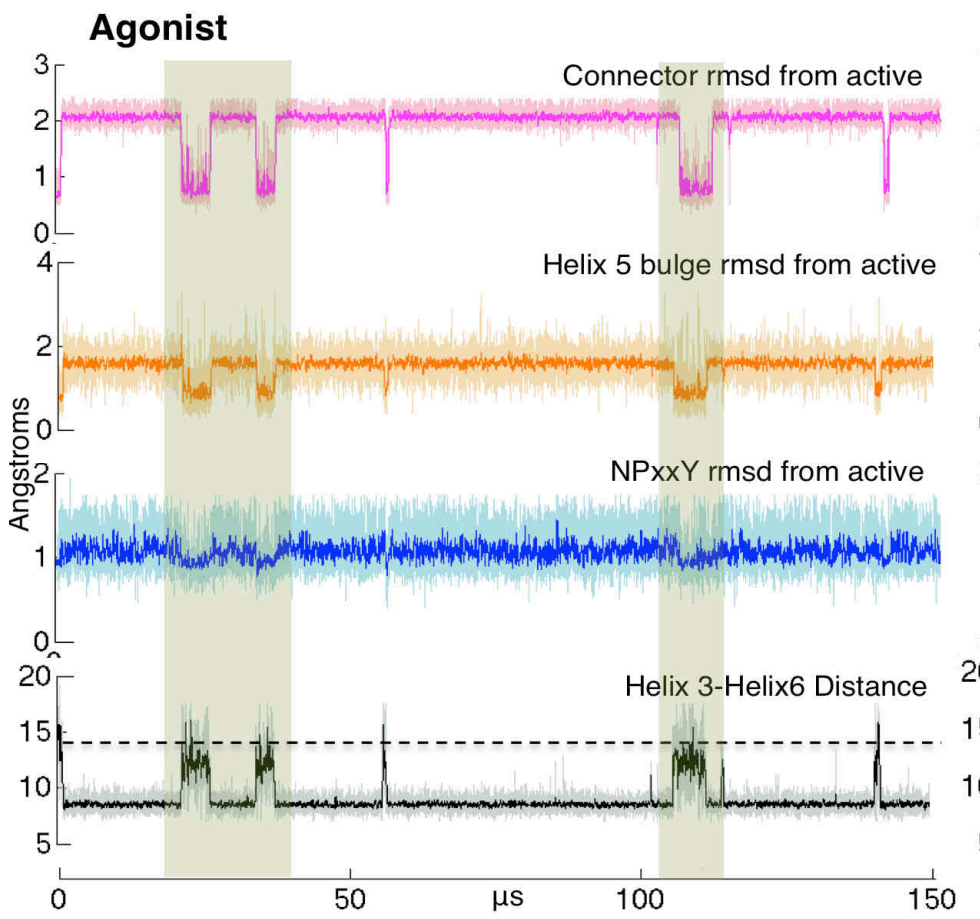
Very subtle differences in binding pocket

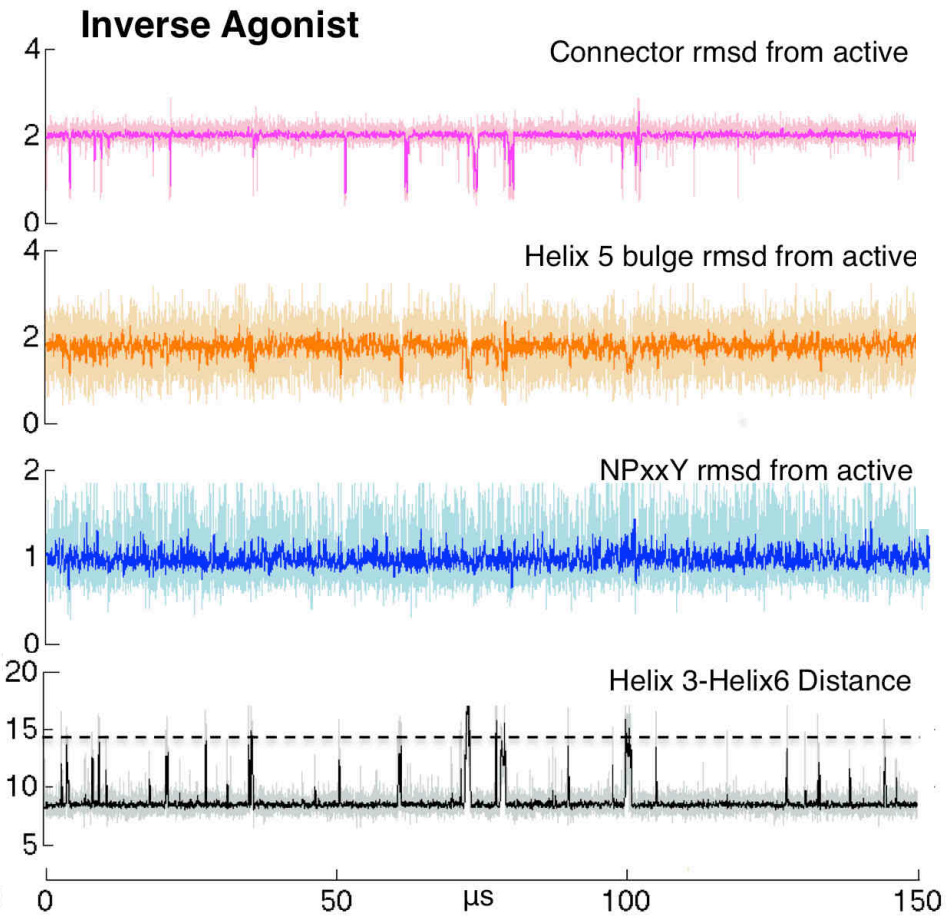
Rasmussen, et al. *Nature*. (2011)
Dror, et al *PNAS* (2011).

MSM Activation Trajectories

Monte Carlo sampling of Tij creates 150 μs activation trajectories

	Active State Duration (μs)
Agonist	5.25
Inverse agonist	n/a



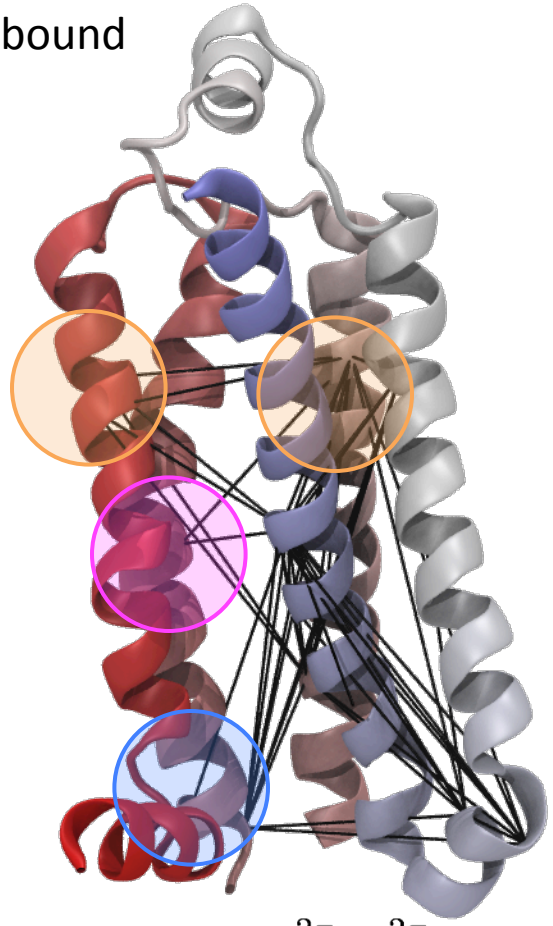


K. Kohloff,* D. Shukla,* M. Lawrenz,* et al. *Nature Chem.* (2013)

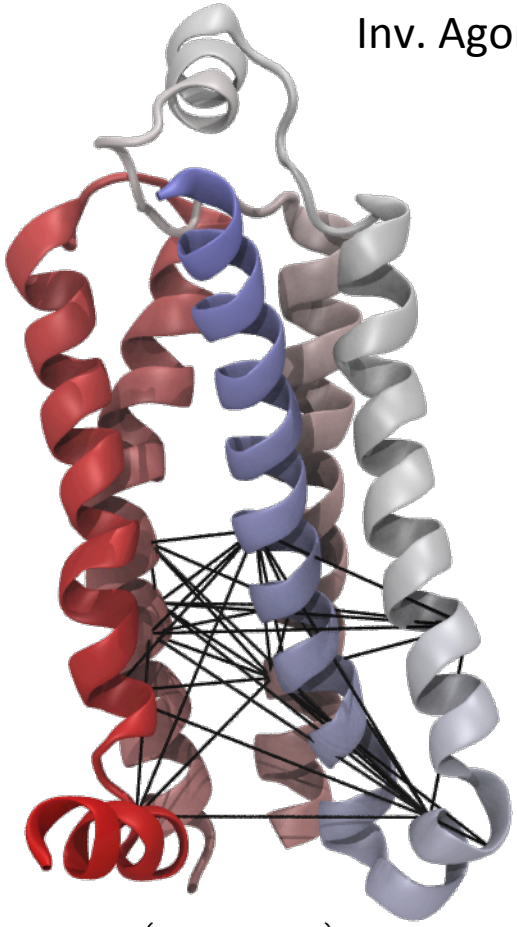
Networks of Dynamically Correlated Residues

Provide mechanistic insights into functional differences between ligands

Agonist-bound



Inv. Agonist-bound

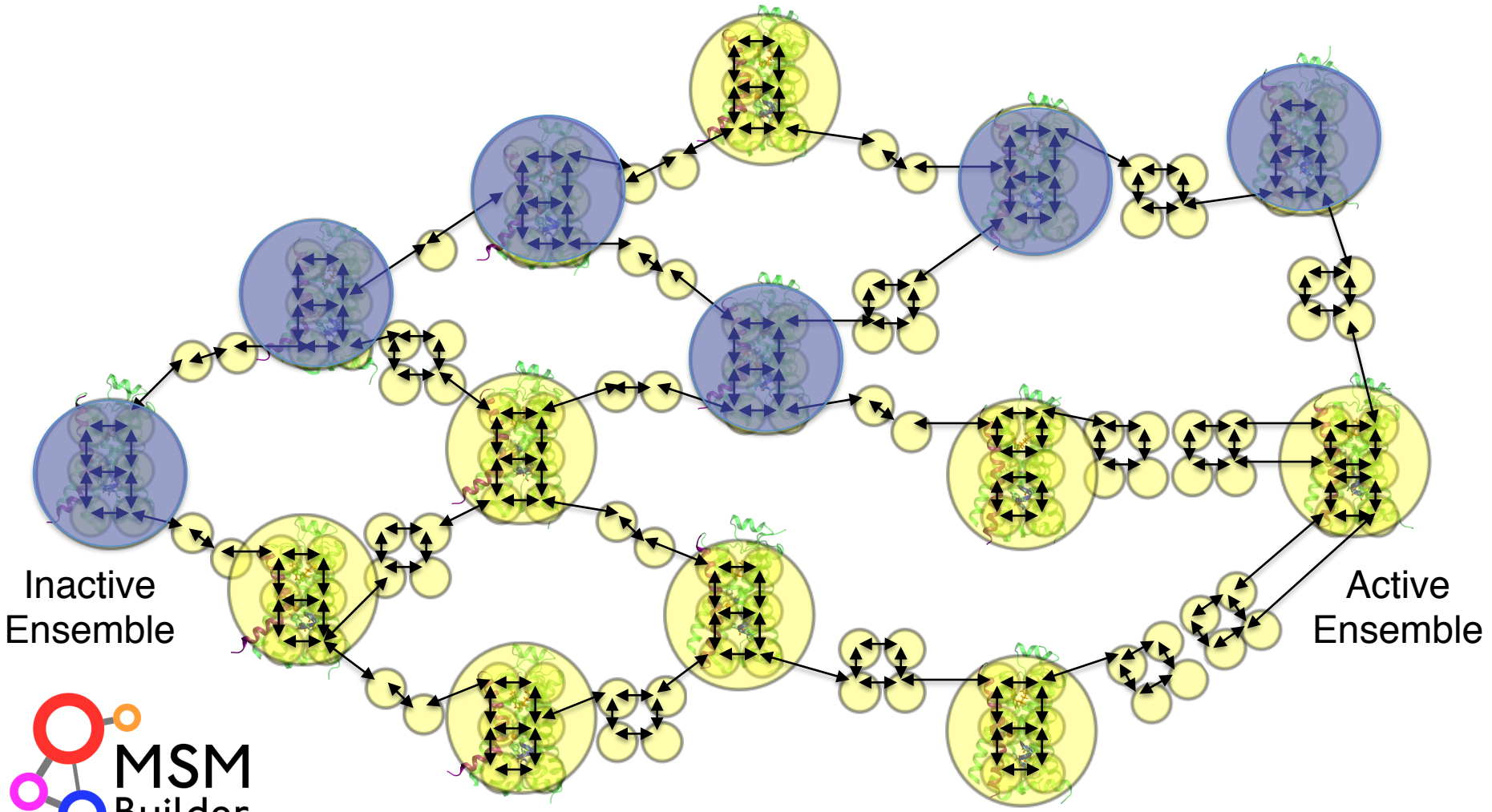


$$MutInf_{R1,R2} = \int_0^{2\pi} \int_0^{2\pi} p(\chi_{R1}, \chi_{R2}) \ln \frac{(\chi_{R1}, \chi_{R2})}{(\chi_{R1})(\chi_{R2})} d\chi_{R1} d\chi_{R2}$$

K. Kohloff,* D. Shukla,* M. Lawrenz,* et al. *Nature Chem.* (2013)

Transition Path Theory

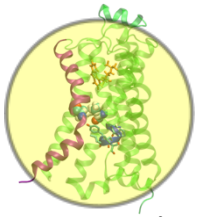
Determine highest probability pathways between active and inactive states



MSM Builder

Noe, et al. *PNAS.* (2009)

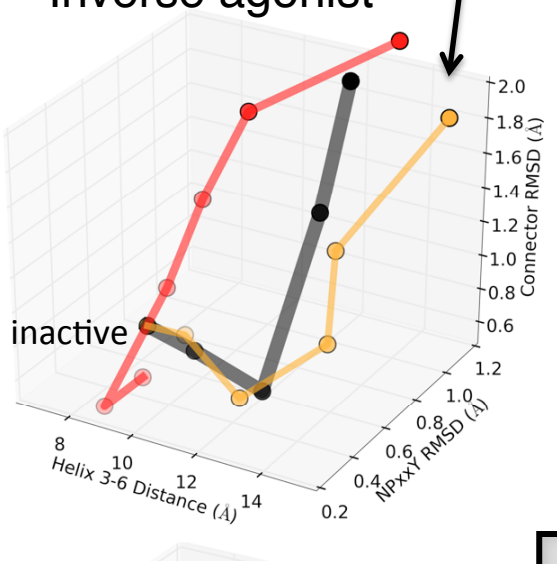
Ligands modulate receptor dynamics to prefer different pathways



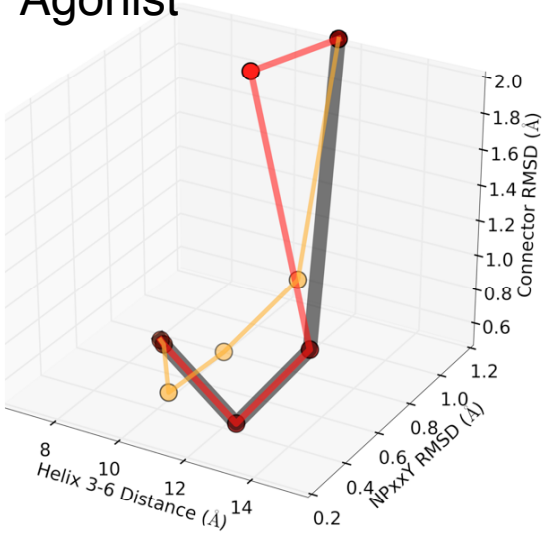
active

Connector (I121-F282) RMSD

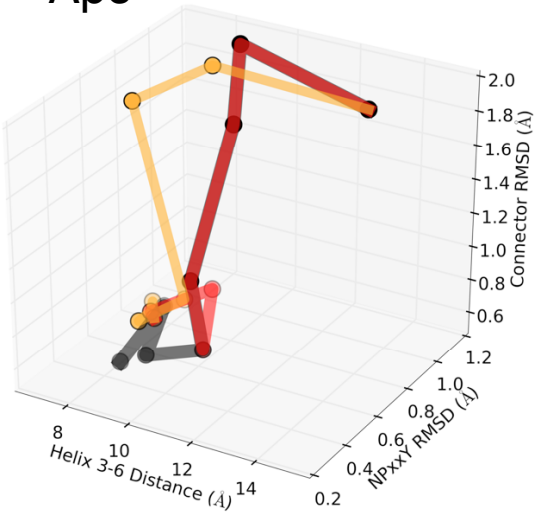
Inverse agonist



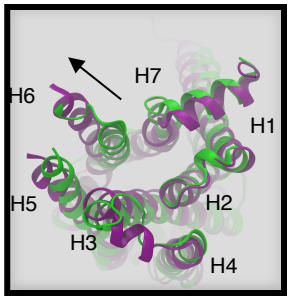
Agonist

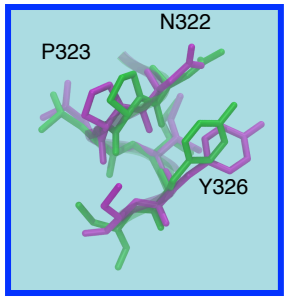


Apo



H3-H6 Distance (R131-L273 C_α distance)





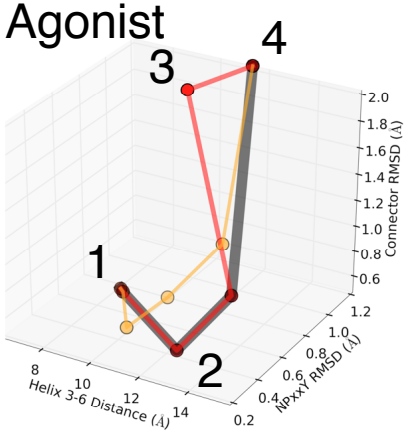
NPxxY (N322-C327 in H7) RMSD

Noe, et al. *PNAS.* (2009)

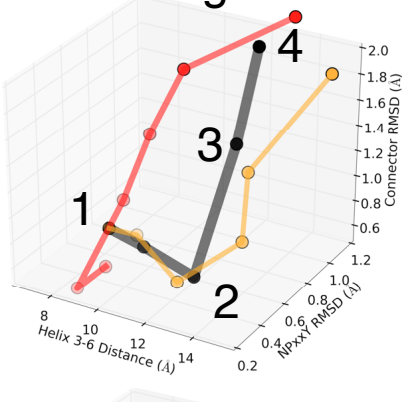
Activation structural dynamics along pathways

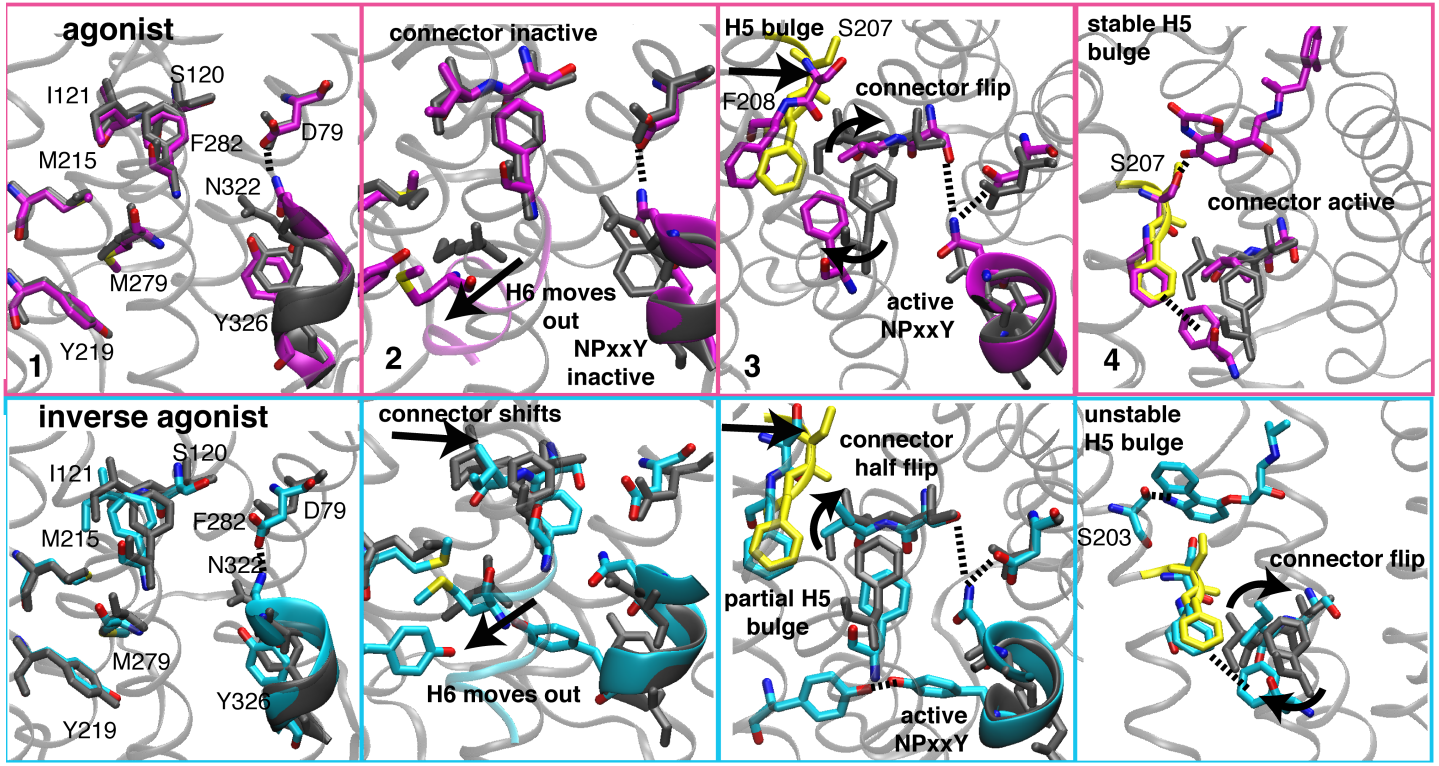
MSM trajectory reproduces a variety of previous experimental and computational results and give new insight into ligand modulation of the GPCR conformational landscape

Agonist



Inverse agonist

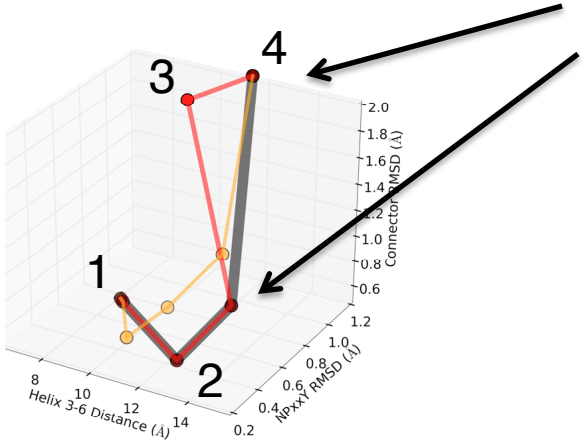


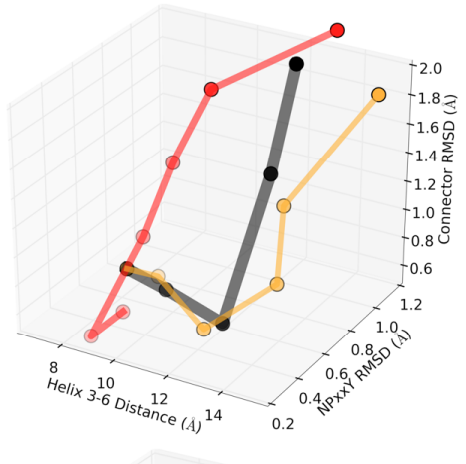


K. Kohloff,* D. Shukla,* M. Lawrenz,* et al. *Nature Chem.* (2013)

Ligand binding site dynamics

Mine rich structural dataset for interesting drug leads

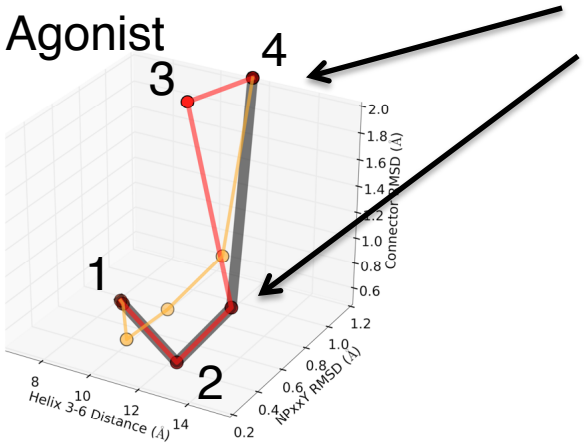


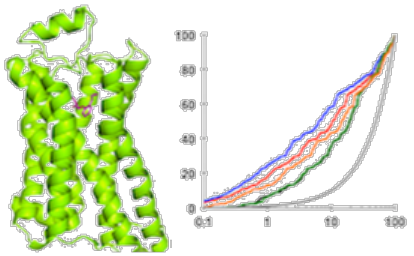


- Target states from pathways with small molecule docking
- Characterize ligands with molecular 3-D similarity calculations
- Millions of parallelizable calculations: would take ~2 months to run final protocol with single user allocation on local Stanford cluster (10 nodes, 12 CPUs)

BLUE WATERS
SUSTAINED PETASCALE COMPUTING

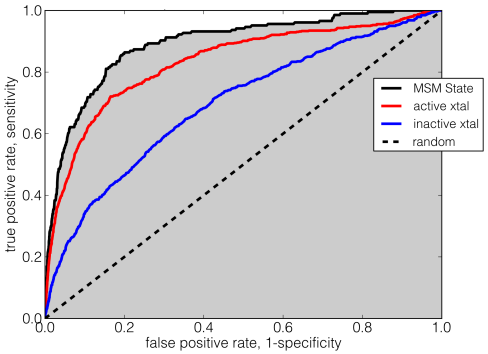
Ligand binding site dynamics
Virtual screen of GPCR ligands and decoys





GDD
GPCR Decoy Database

- Evaluate performance of docking to MSM states
- Characterize ligand types selected by intermediate states
- Obtain biophysical insight into binding site based on predicted ligand poses

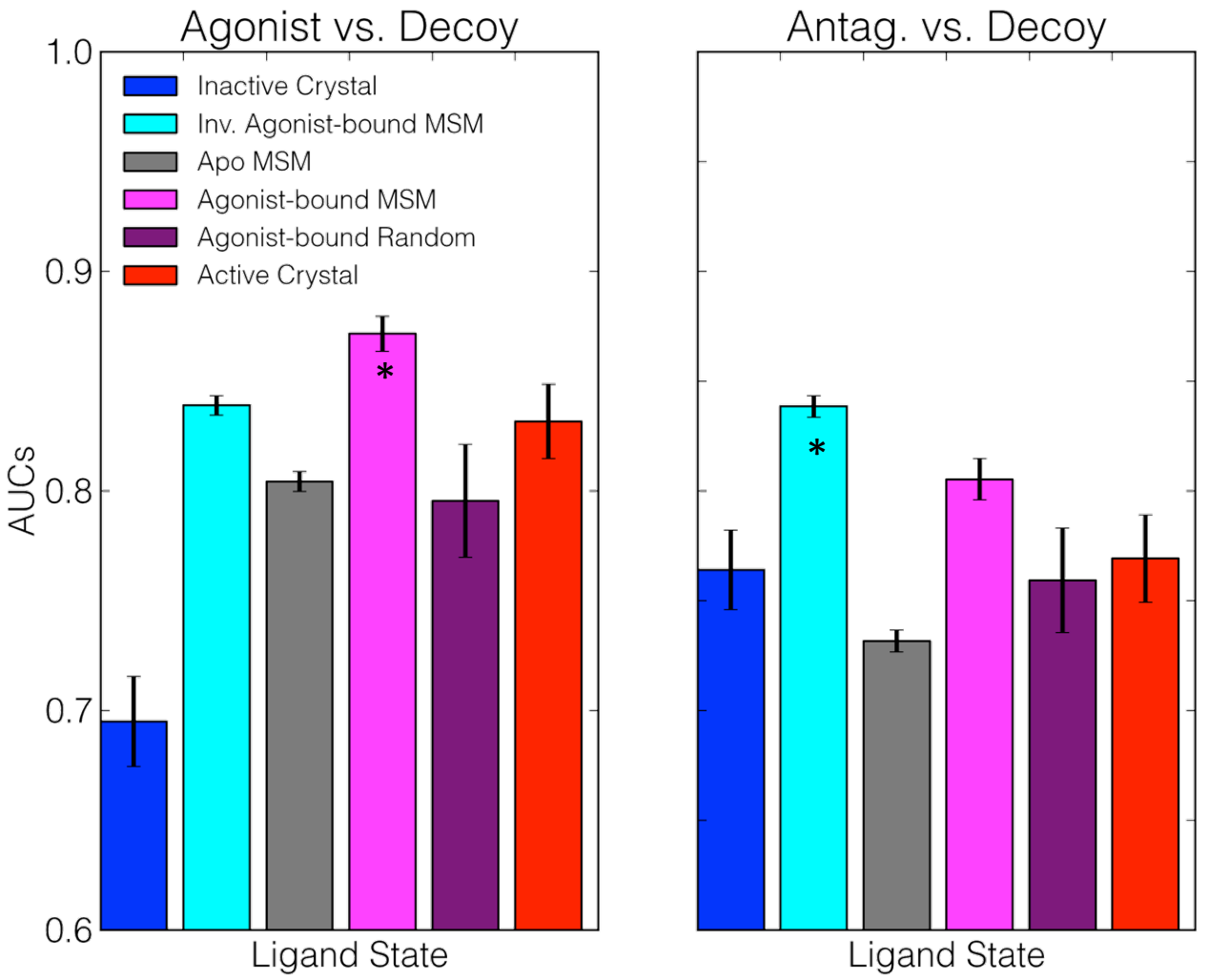


AUC = Area under curve of ROC plot

Ajay Jain. *JCAMD* (2007)
Gatica and Cavasotto *J. Chem. Inf.* (2012)

MSM state docking is effective

Significantly improved performance compared to crystal structure and random MD docking

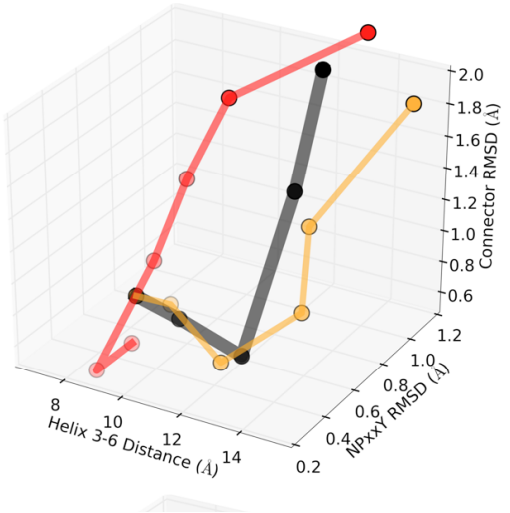


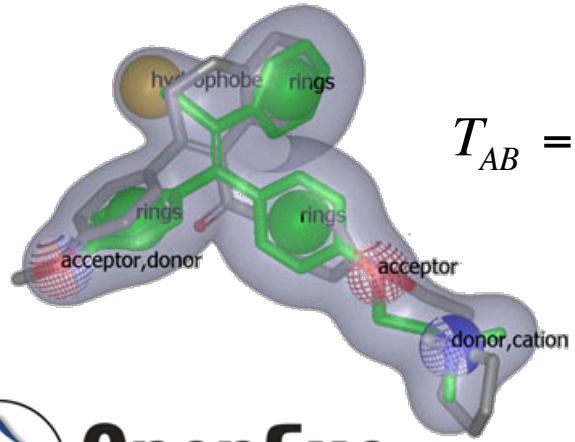
K. Kohloff,* D. Shukla,* M. Lawrenz,* et al. *Nature Chem.* (2013)

Evaluate diversity of ligands enriched by MSM states

Cluster ligand chemotypes that are highly ranked by inactive, intermediate, and active MSM states

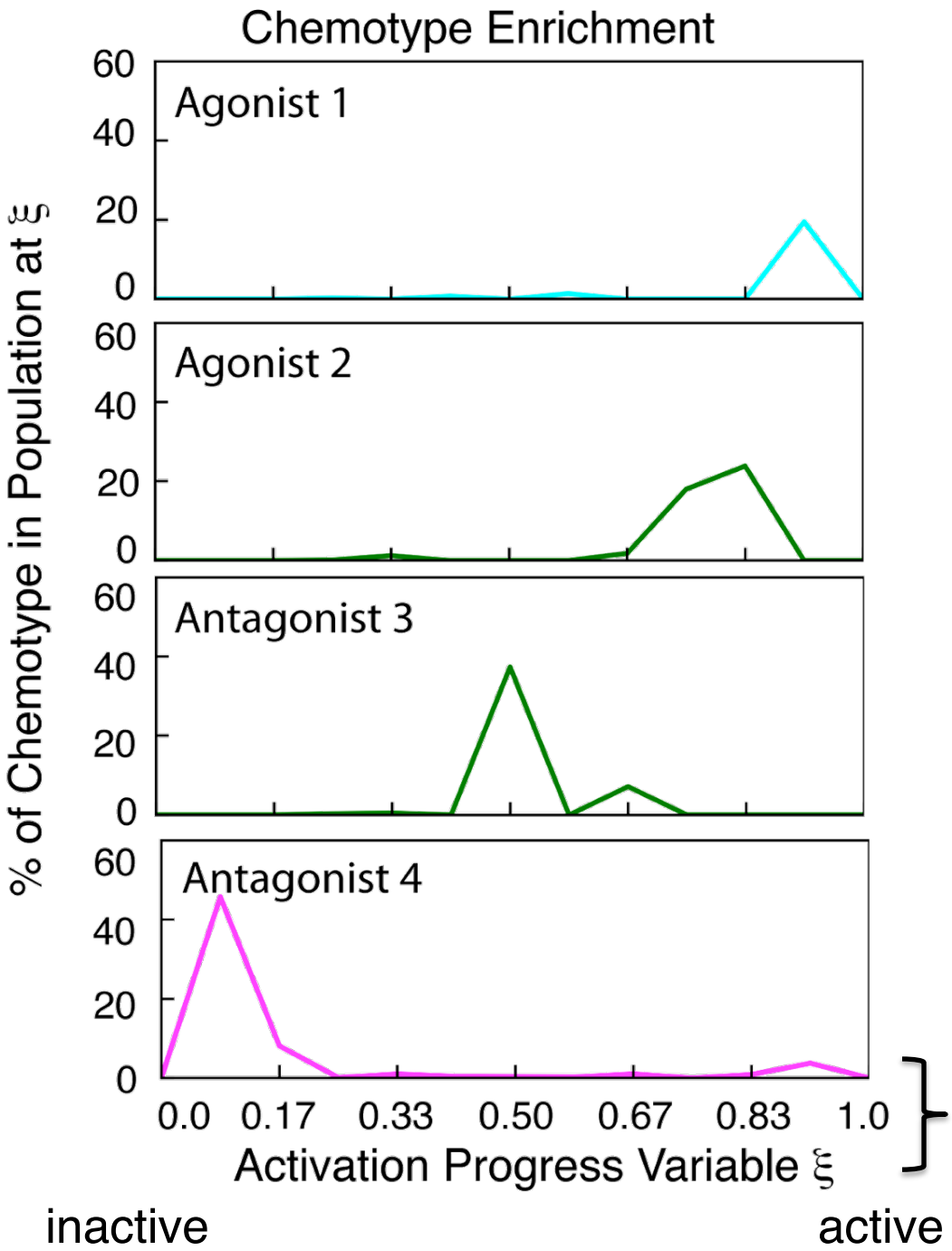
Linear combination score of MSM metrics → Progress Variable ξ → Cluster top 10% scoring true ligands from MSM states along ξ





$$T_{AB} = \frac{O_{AB}}{O_{AA} + O_{BB} - O_{AB}}$$

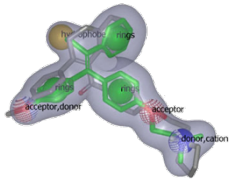
OpenEye Scientific Software



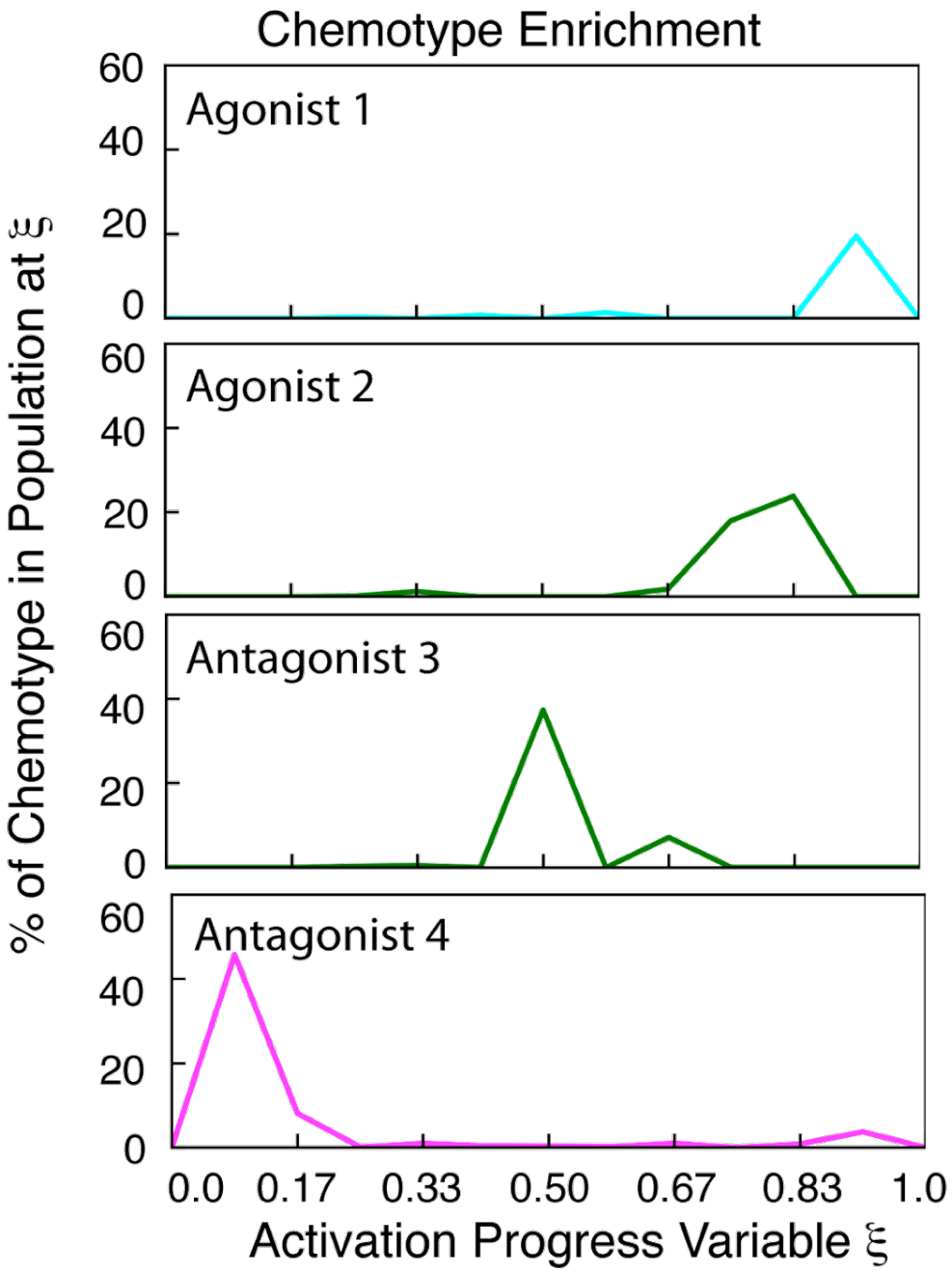
MSM states along activation pathway enrich diverse chemotypes

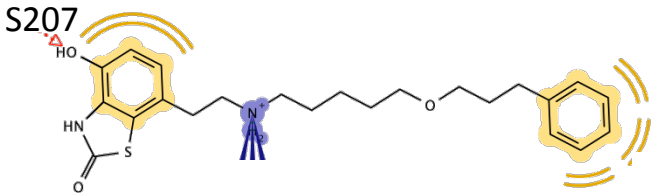
Intermediates select chemotypes undiscovered by active or inactive structures

(all types are known in this retrospective case)

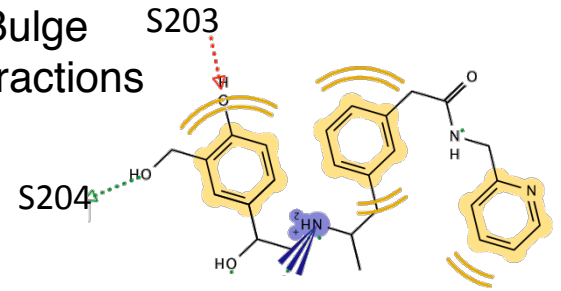


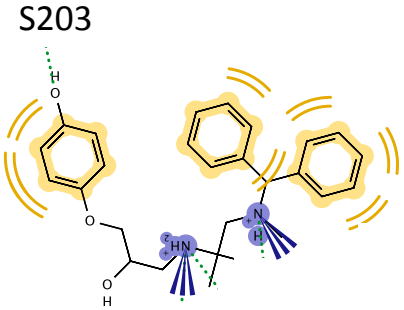
Linear combination score of structural metrics

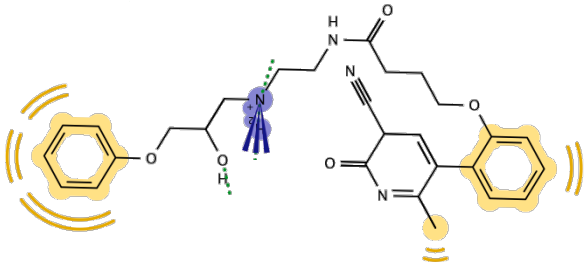




Polar H5 Bulge Interactions

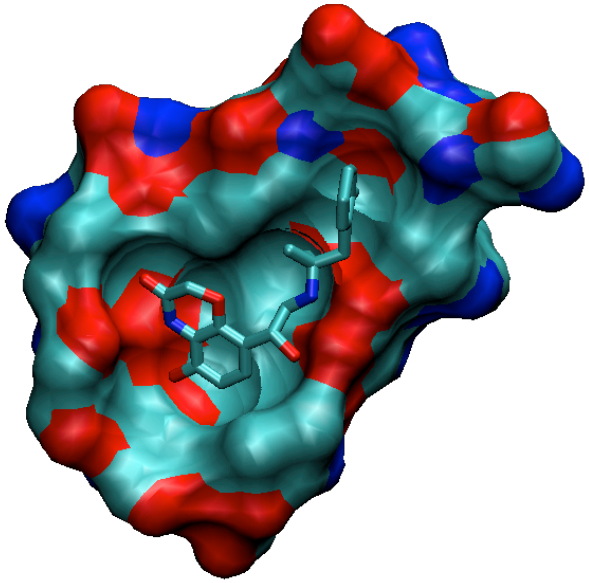




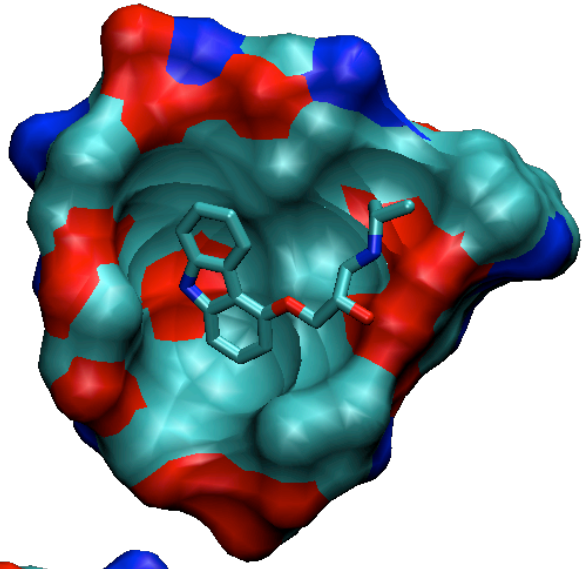


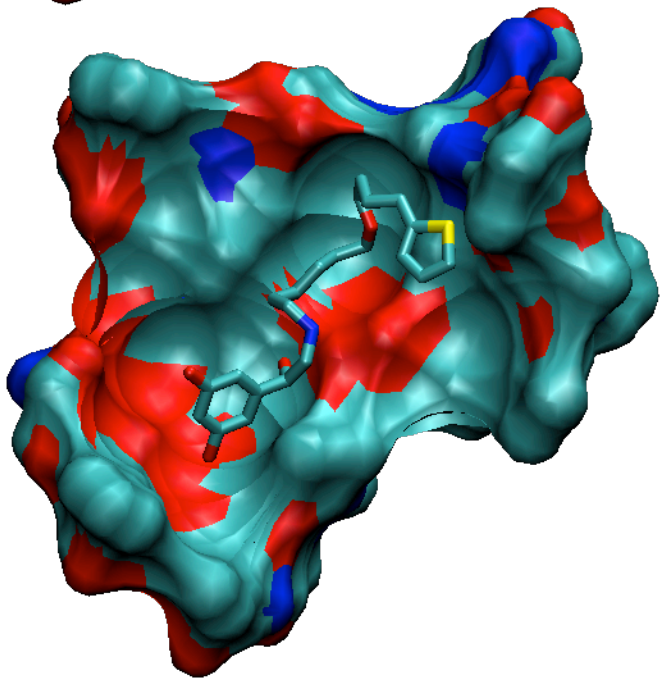
Active crystal structure

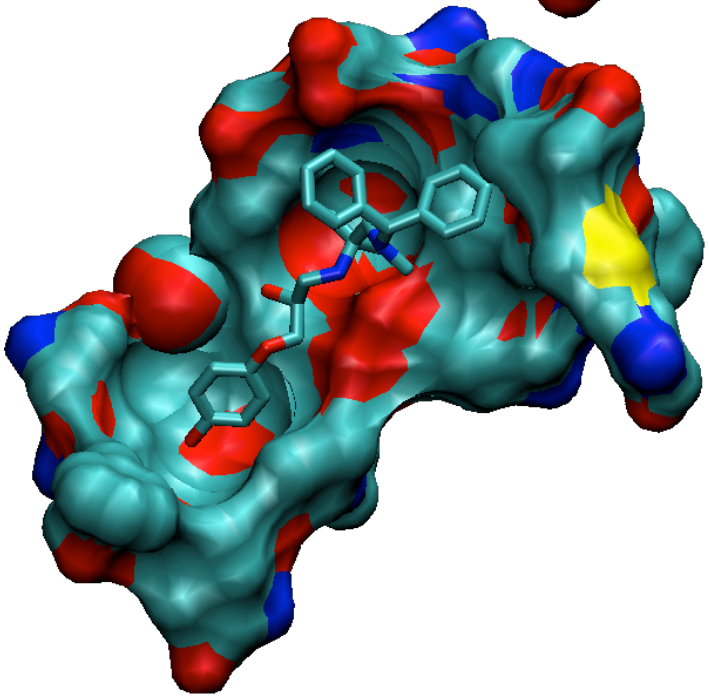
Inactive crystal structure



Kinetically stable MSM states capture binding pocket conformations that accommodate diverse chemotypes





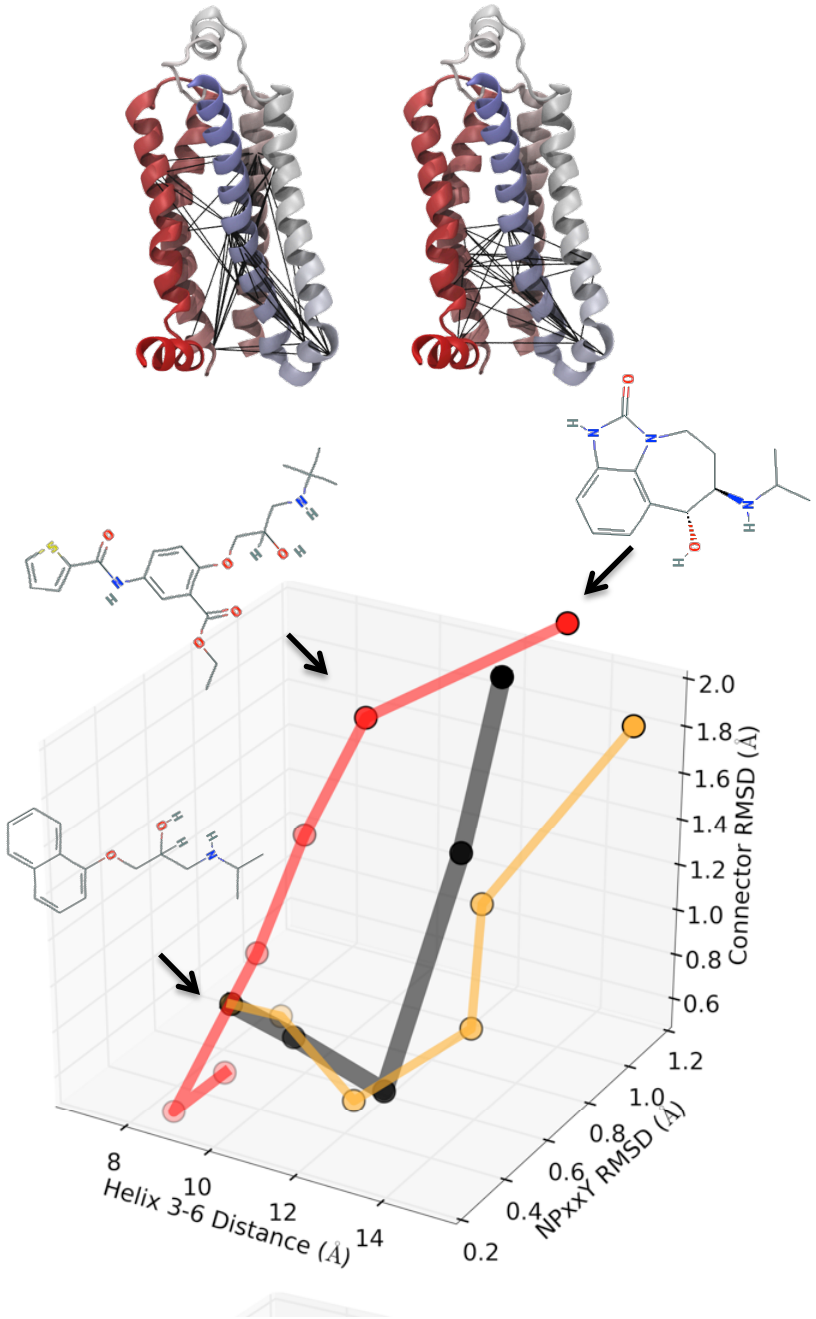


Active-like MSM State with agonist 1

Intermediate MSM State with antagonist 3

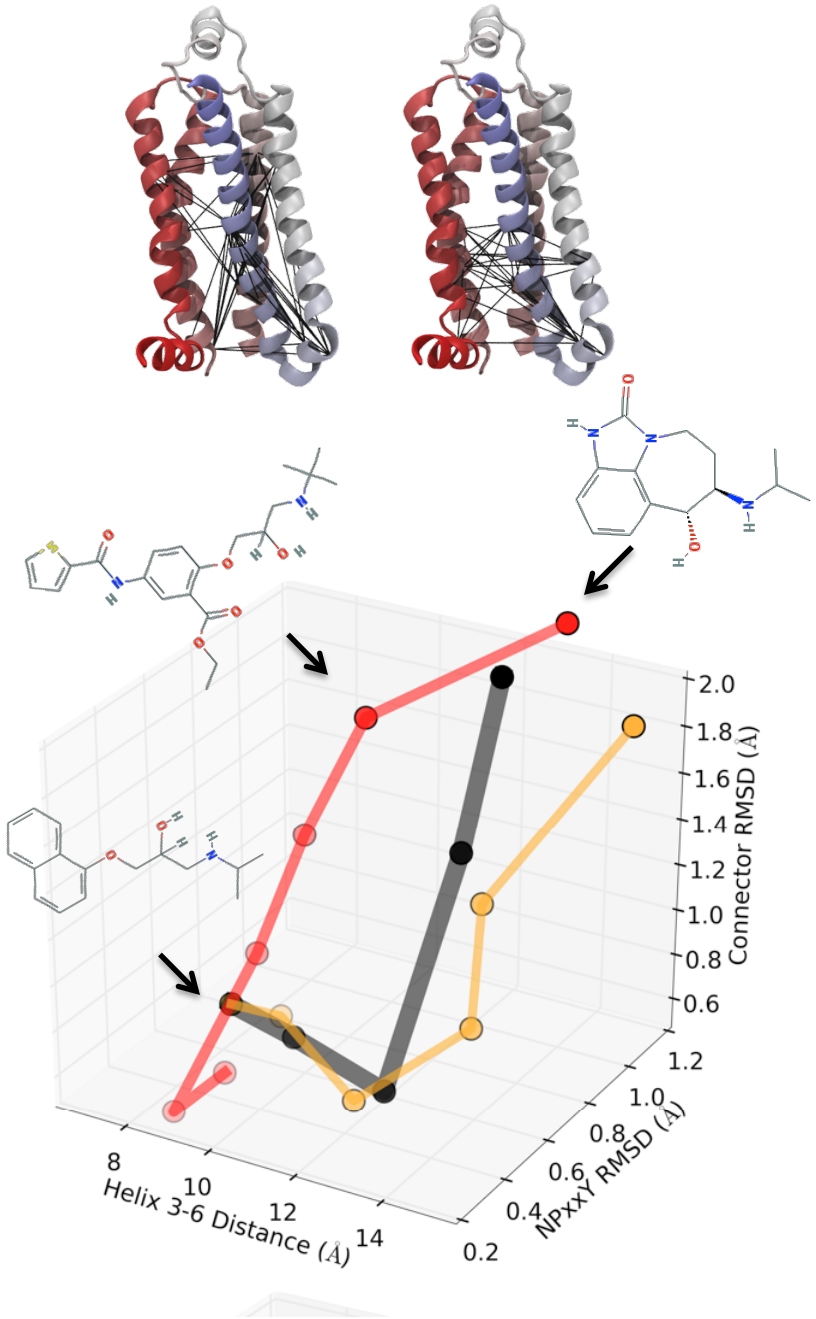
Accomplishments

- Parallel MD simulations can be stitched together with MSMs to reproduce long timescale protein dynamics
- Different correlated residue networks in the ß$_2$AR transduce ligand interactions into intracellular structural changes
- Ligands modulate structural dynamics to prefer different deactivation pathways
- Docking to MSM states indicate a correspondence between ligand types and kinetically stable intermediate receptor conformations

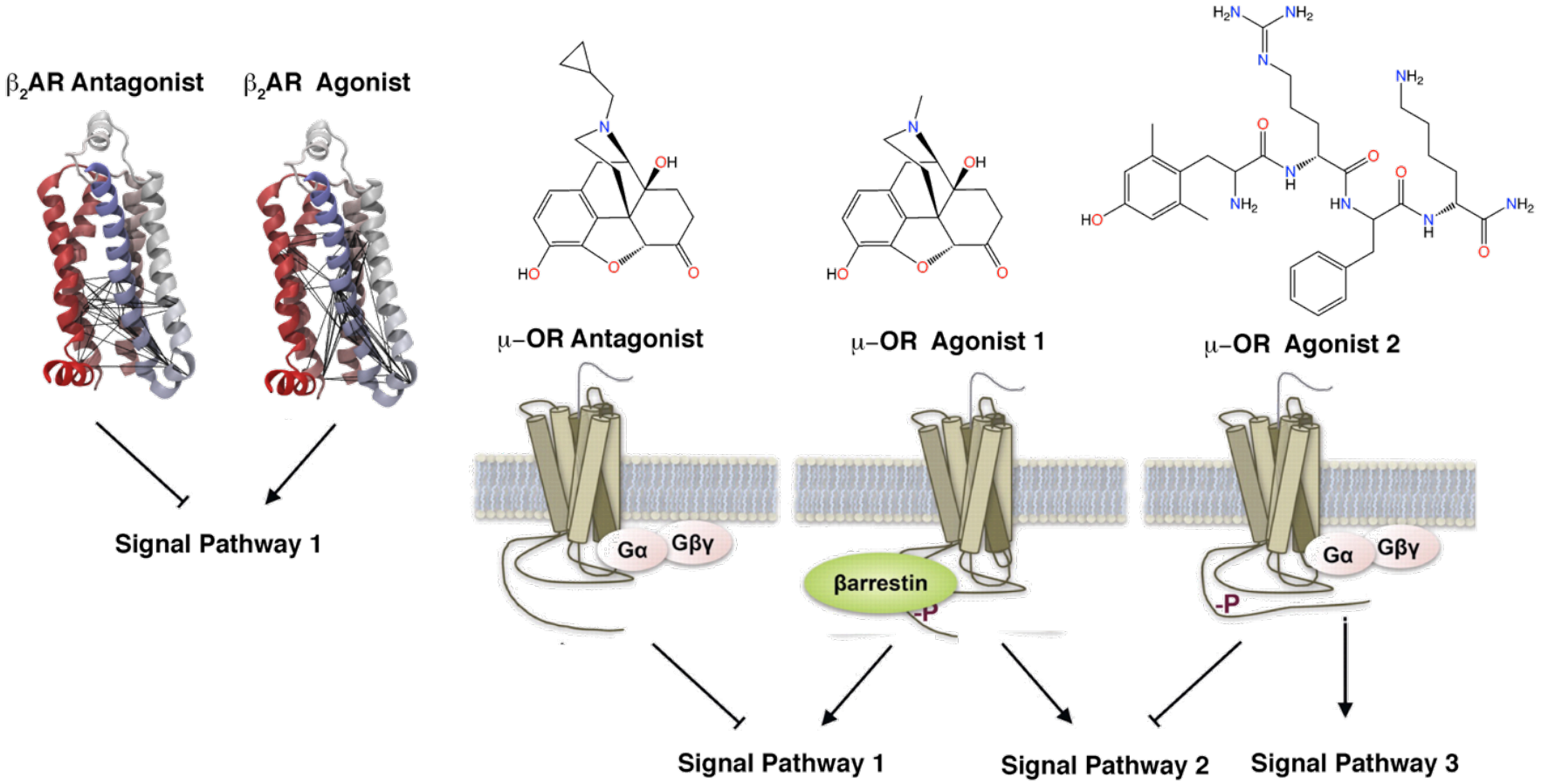


Why it matters

- Large datasets created on diverse architectures need analysis tools to extract pertinent information for researchers
- Knowledge of residues involved in functional dynamics can give testable predictions for the mechanism
- We hope to increase effectiveness of virtual screening for these receptors, which encompass 40% of all drug targets, and give predictions for ligands that may isolate rare intermediate conformations



Work in Progress: μ-opioid receptor is a central regulator of pain signaling



Opiates bind and modulate *μ*-OR dynamics to elicit different downstream signals which can lead to analgesic tolerance and addiction cycles

Acknowledgements

- Vijay Pande (PI), Diwakar Shukla, Greg Bowman, Russ Altman, Kai Kohloff, David Konerding, and Dan Belov
- MSMBuilder software team

https://github.com/SimTk/msmbuilder

- Blue Waters support

NCSA

NATIONAL INSTITUTES OF HEALTH

simbios

Google
Exacycle for Visiting Faculty