Unraveling Allostery with Simulations of Rhodopsin and Opsin

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Poster PDF
tinyurl.com/rhod-sim

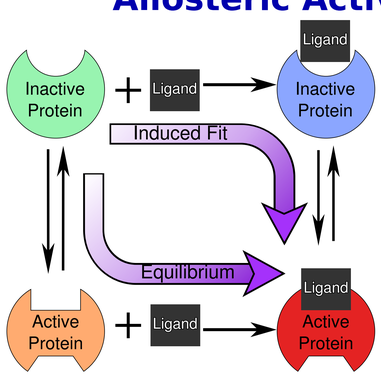
Abstract

G protein-coupled receptors (GPCRs) are biomedically important integral membrane proteins that allosterically transduce signal across the lipid bilayer; structural changes cascade through the protein to modulate activity in a mechanism that is not fully understood. Rhodopsin, the mammalian dim-light receptor, is a model GPCR that provides a unique test case for understanding allostery. The ligand-bound protein acts as a two-state switch with minimal basal activity. However, its apo-form (opsin) is outside the activation cycle and may behave differently. Structural data reveal an active-like opsin, but physiologically it has only minimal activity. We explore opsin's ability to fluctuate between states and test the ligand's role in activation. We performed an ensemble of microsecond-scale all-atom simulations (~100 μs in all) using four systems: two with ligand present and two without. Opsin's fluctuations suggest that both active-like and inactive-like structures may be part of its conformational ensemble. Opsin trajectories appear better able to sample both conformations, although all four ensembles are still statistically converging.

GPCR Background

- Integral membrane proteins
  - 7 transmembrane (TM) α-helices
- Molecular transducer
  - Ligand enters extracellular side
  - Binds in hydrophobic core (class A GPCRs)
  - G protein binds cytoplasmic face
- Ligand does not enter cell
  - Allosteric activation process
- Most GPCRs: basal activity
  - Three classes of ligand:
    - Agonists: increase signaling
    - Inverse agonists: lower signal
    - Antagonists: do not alter signal
- Rhodopsin: photoreceptor
  - Ligand: retinal
    - Agonist and inverse agonist
- Opsin: apo-rhodopsin
  - Outside photocycle
  - Low activity

Allosteric Activation



- Ligand binding site & active site are distant
- Induced fit
  - Ligand drives conformational change
  - Active participation
- Conformational equilibrium
  - Protein fluctuates
  - Ligand binds single conformation
  - Passive participation
- Simulate states
  - Understand dynamics
  - Effect of ligand
  - Identify transitions

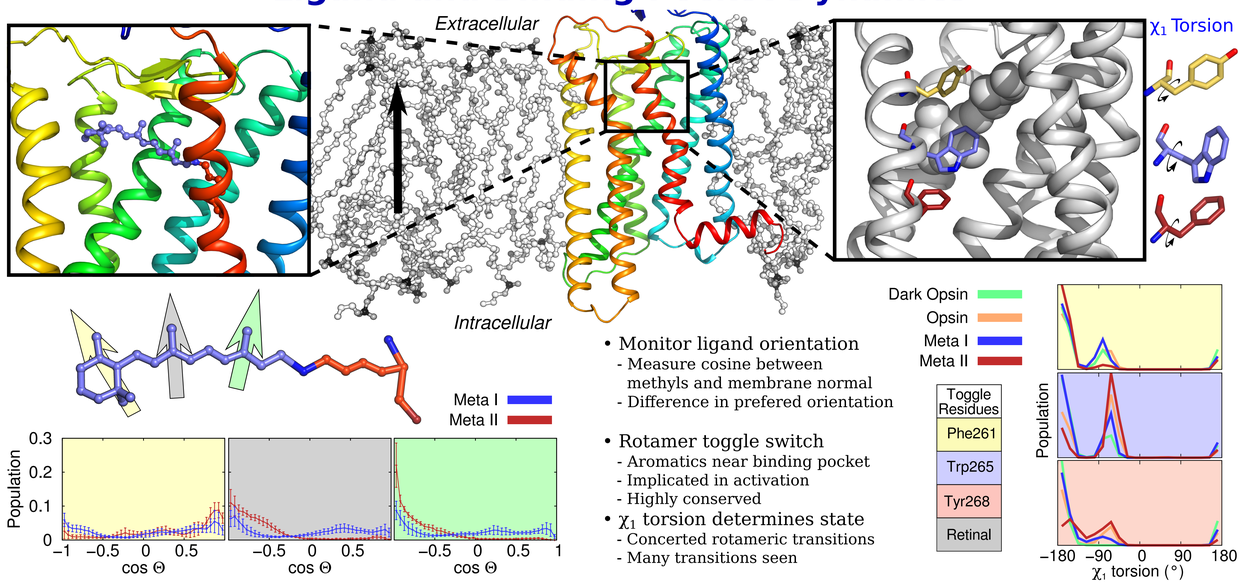
Simulation Details

- Forcefield: CHARMM27/36
  - Retinal parameters provided by S. Feller
- Timestep: 2 fs
- Ensemble: NPγT
  - γ = 30 dyn/cm
- Thermostat: Langevin
- Electrostatics: PME
  - Cutoff: 10 Å
- NAMD 2.8 - BlueGene/Q
- Size 74x74x90 Å
- 123 SDPE lipids
- ~7000 waters
- Neutralizing ions
  - Additional 100 mM NaCl
- System size: ~46000 atoms
- Low pH conditions
  - Glu113 & Glu134 protonated
  - Favors Meta II

System	Structure	Notes	Simulation Time (μs)
Dark-opsin	1U19	retinal removed	6× 4.0
Opsin	3CAP		6× 4.0
Meta I	"Meta I"	from previous simulation	6× 4.7
Meta II	3PXO		6× 4.0
		Total	≈100 μs

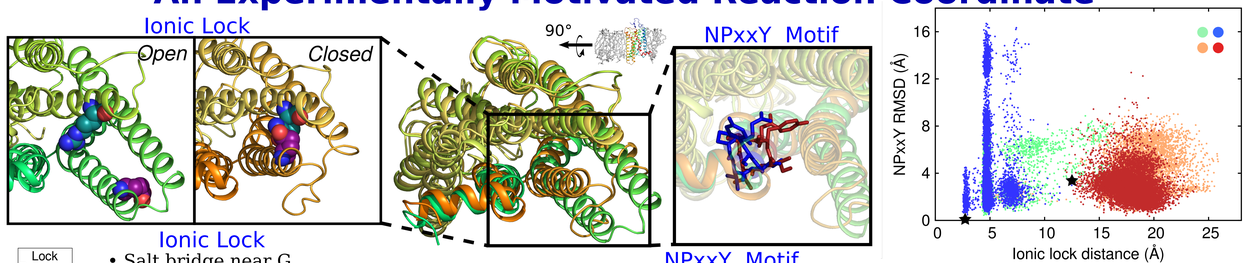
Work done in LOOS (Lightweight Object Oriented Structure analysis library), an open source C++ library designed and maintained by the Grossfield lab. LOOS provides a concise, adaptable framework for designing analysis tools that interfaces with native formats of most simulation packages.
http://loos.sourceforge.net

Ligand and Binding Pocket Dynamics



- Monitor ligand orientation
  - Measure cosine between methyls and membrane normal
  - Difference in prefered orientation
- Rotamer toggle switch
  - Aromatics near binding pocket
  - Implicated in activation
  - Highly conserved
- χ_1 torsion determines state
  - Concerted rotameric transitions
  - Many transitions seen

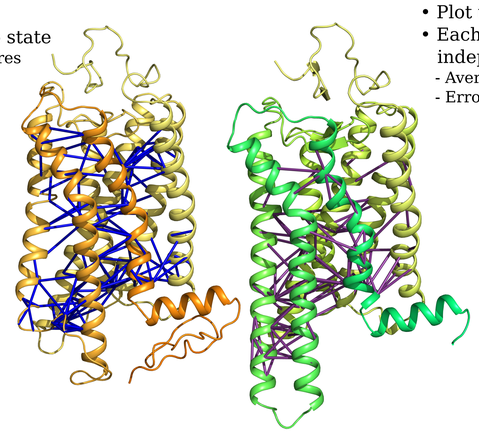
An Experimentally Motivated Reaction Coordinate



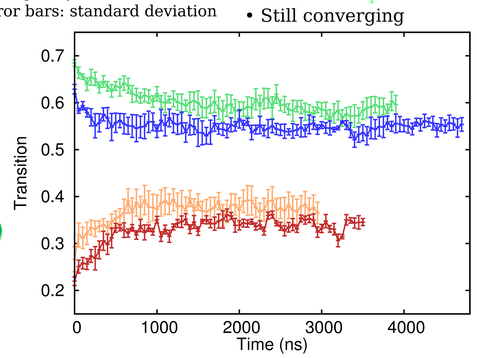
- Ionic Lock
  - Salt bridge near G protein binding site
    - Conserved residues
    - Implicated in activation
    - Two-state switch
      - Closed: inactive
      - Open: active
- Monitor distance through simulations
  - Use N-O distance
  - Only 1-2 transitions seen
- NPxxY Motif
  - Highly conserved residues
  - Rotates into protein core
  - May stabilize active conformation
    - Use RMSD from inactive structure
    - Many transitions seen
- Combine measures
  - Used by Dror et al. (*PNAS*, 2011)
  - Track by simulation
  - NPxxY transitions not coupled to Ionic Lock motion
- Crystal structures indicated by black stars

Contact-Based Coordinate

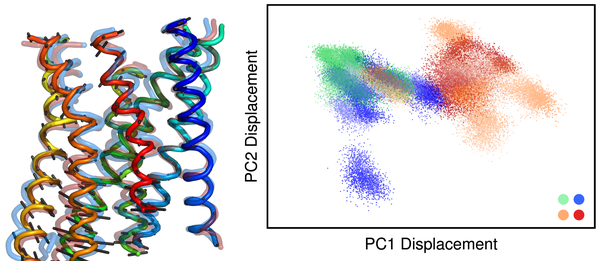
- Identify contacts unique to state
  - Contacts from crystal structures
    - Active (1U19)
    - Inactive (3PXO)
  - Normalized number of:
    - Active contacts broken
    - Inactive contacts formed
  - Side-chain centroids within 8Å
- Contacts form network across extracellular and intracellular regions
  - Illustration shows activation
  - Left: broken contacts on inactive structure
  - Right: formed contacts on active structure



- Plot transition in simulations
- Each trajectory calculated independently
  - Averaged by initial state
  - Error bars: standard deviation
- Opsin more inactive than Meta-II
- MI-MII more active than Dark-opsin
- Still converging



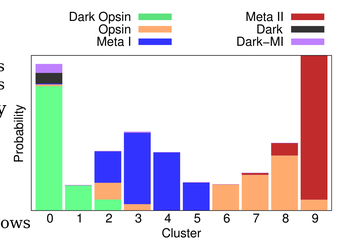
Principal Component Analysis

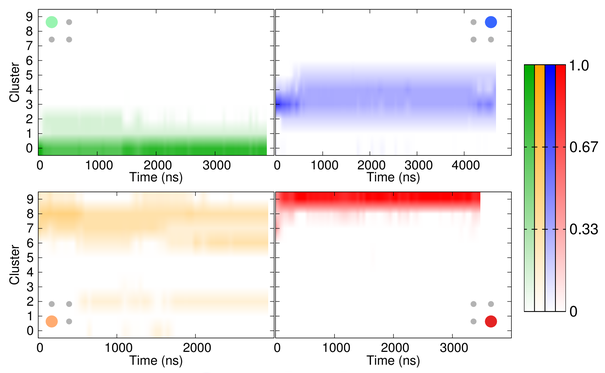


- Calculate collective motions
  - Use all trajectories in aggregate
    - PC-space defines all simulations
  - Transmembrane Cα's only
- Plot PC1 vs. PC2 displacement
  - Individual trajectories plotted
    - Colored by ensemble
  - PC1 captures activation
- PC1 illustration
  - Average structure: rainbow
  - Black vectors: PC1
  - Active structure in red
  - Inactive structure in blue

Clustering Reveals Structural Overlap

- Cluster on Cα position
  - K-means algorithm
  - First pass: K = 10 clusters
  - Use all 24 systems
    - Add 2 published simulations
    - Dark & Dark-MI simulations
- Plot normalized probability
  - Bars grouped by cluster
  - Color indicates initial state
- Time course of cluster population
  - Points averaged in 50ns windows
    - All trajectories in ensemble considered





Conclusions

- Only a handful of transitions seen
  - Confirmed by multiple metrics
  - Structural overlap observed
  - Best analyses let data speak for itself
  - Need enhanced sampling/bias
- To what extent does the ligand impact activation?
  - Changes whole protein dynamics
    - More data needed to quantify changes satisfactorily