Unraveling Allostery with Simulations of Rhodopsin and Opsin

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Poster PDF
tinyurl.com/gpcr_allostery

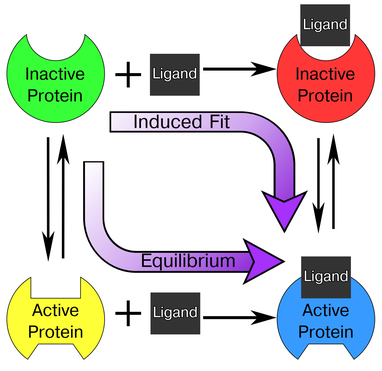
Abstract

G protein-coupled receptors (GPCRs) are a biomedically important class of membrane proteins, accounting for roughly one third of FDA approved drugs. They act as molecular transducers, allosterically passing signals across the cell membrane. This modulation of GPCR signal is vital to their pertinence as drug targets, but the details of this mechanism are not fully understood. Two prominent hypotheses exist to describe how ligands affect changes in signaling. In the current work we are using unbiased, all-atom molecular dynamics simulations of the GPCR rhodopsin to test the relevancy of these hypotheses. Rhodopsin, the visual photoreceptor, is a unique test case; both the active and inactive protein bind the same ligand, retinal. In addition, opsin, the apo-form of rhodopsin, is outside the normal functional cycle. Using simulations of four systems (apo- and holo-protein in the active and inactive states) we will comment on the applicability of these allosteric models and the steps involved in the activation of this model GPCR.

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
- GPCRs act as guanine exchange factors
  - GDP exchanged for GTP
  - G protein dissociates
- GPCRs basally active
  - 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 photo cycle
  - Low activity

Allosteric Activation



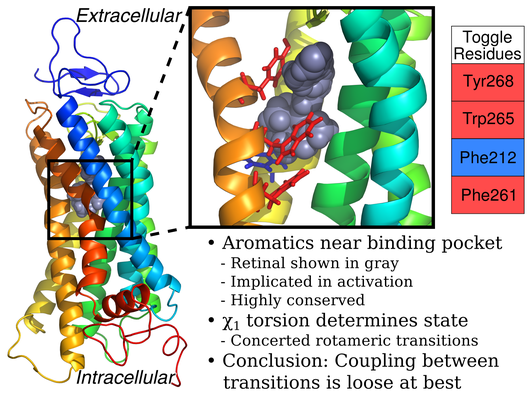
- Ligand binding site & active site are distant
- Induced fit
  - Ligand drives conformational change
  - Active participation
- Conformational equilibrium
  - Protein fluctuates
  - Ligand stabilizes conformation
  - Passive participation
- Simulate states
  - Understand dynamics
  - Identify transitions between states

Bahar et al., Annu. Rev. Biop. (2010), 39:23-42

Simulation Details

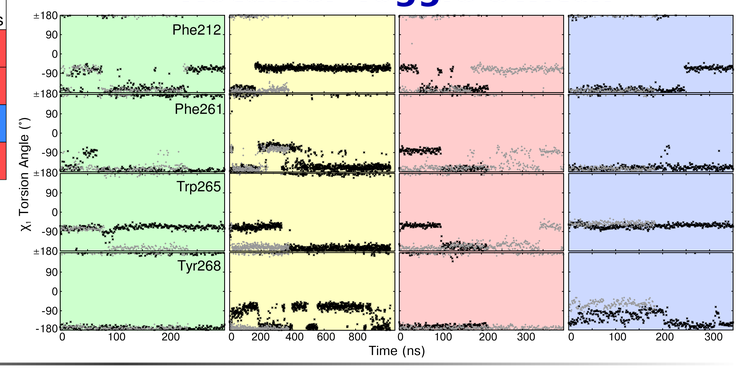
- Forcefield: CHARMM36
  - Retinal parameters provided by S. Feller
- Timestep: 2 fs
- Ensemble: NPγT
  - γ = 30 dyn/cm
- Thermostat: Langevin
- Electrostatics: PME
  - Cutoff: 10 Å
- NAMD 2.8 - BlueGene/P
- Size 74x74x90 Å
- 123 SDPE lipids
- ~7000 waters
- Neutralizing ions
  - Additional 100 mM NaCl
- System Size: ~46000 atoms
- Glu113 & Glu134 protonated
- Conditions favor Meta-II

System	Structure	Notes	Simulation Time
Dark-opsin	1U19	dark-state, retinal removed	234 ns 302 ns
Opsin	3CAP		377 ns 1041 ns
Meta-I	"Meta-I"	from previous simulation	185 ns 359 ns
Meta-II	3PXO		408 ns 217 ns
Total Simulation Time:			3123 ns

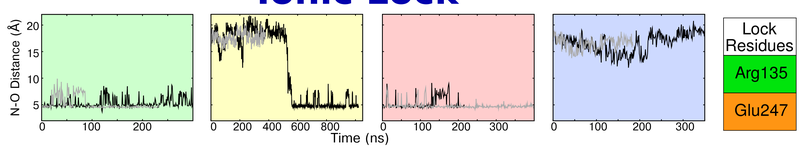


- Aromatics near binding pocket
  - Retinal shown in gray
  - Implicated in activation
  - Highly conserved
- χ_1 torsion determines state
  - Concerted rotameric transitions
- Conclusion: Coupling between transitions is loose at best

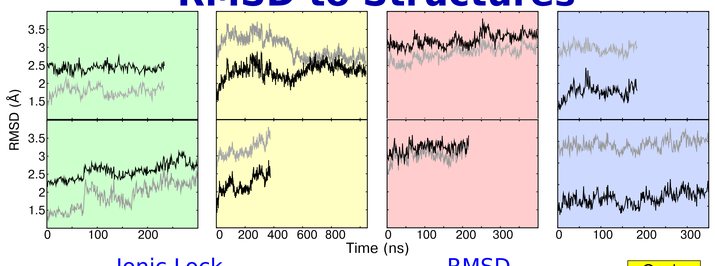
Rotamer Toggle Switch



Ionic Lock



RMSD to Structures



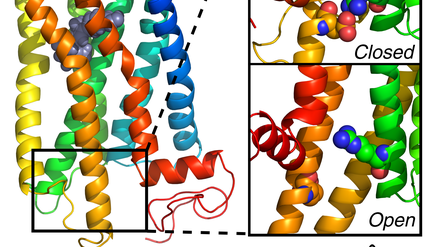
Ionic Lock

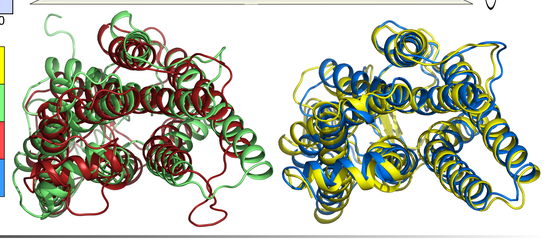
- Salt bridge between TM3 & TM6
  - Implicated in activation
  - Highly conserved Arg135 & Glu247
- Broken in active structures
  - Active-like simulations: flexible
  - Inactive-like simulations: stable
- Only forms in opsin simulation
- Not broken in dark-like simulations

RMSD

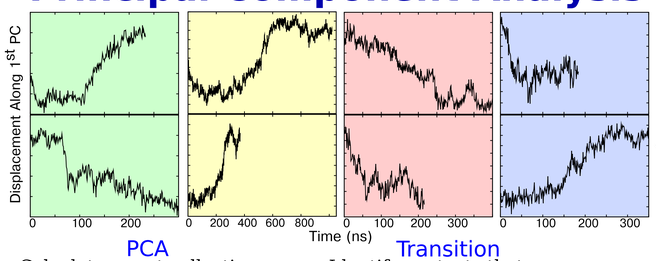
- Reports on overall structure
  - RMSD to inactive (gray)
  - RMSD to active (black)
- Opsin: RMSD to inactive decreases after lock forms
  - Lower than value at t=0
- Coarse measurement
  - Transition not clear

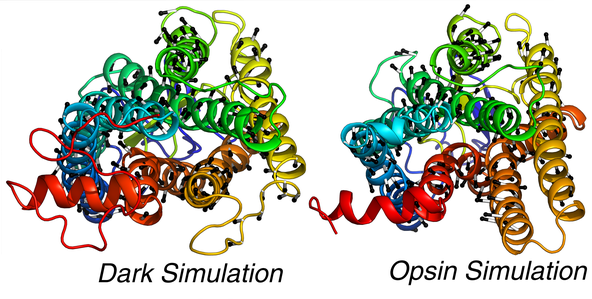
Opsin Start
Opsin End
1U19
3PXO





Principal Component Analysis





PCA

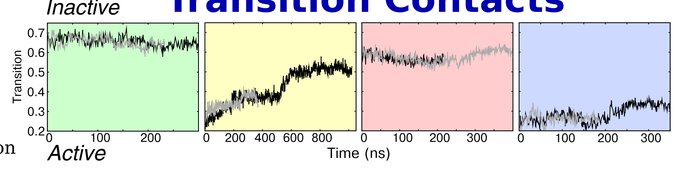
- Calculates most collective motions in the trajectory
  - Figure: vectors for dark-state* & long opsin simulations
- Plot normalized displacement along first PC
- Opsin first PC captures bulk of transition to inactive-like state

*Grossfield et al., JMB (2008), 38(2):478–486

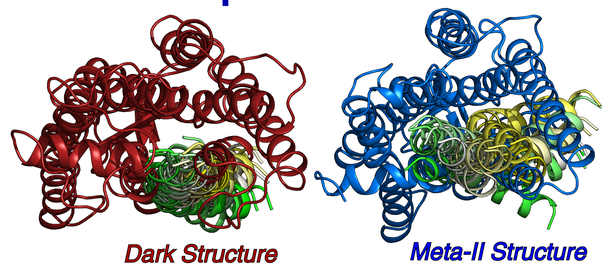
Transition

- Identify contacts that differ between states
  - Contacts from 1U19 & 3PXO
  - Normalized number of:
    Active contacts broken
    Inactive contacts formed
  - Side-chain CoM calculated
  - 8Å cutoff
- Only opsin makes transition

Transition Contacts

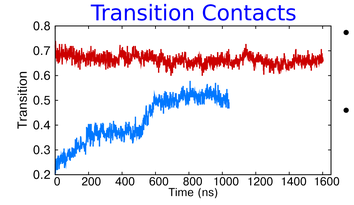


Helix 6: Opsin vs. Dark-state



- Dark-state simulation using inactive protein
- TM6 motion shown vs inactive structure (red)
  - Simulation time lapse shown in yellow to green
  - TM6 stays closed
- Long opsin simulation
- TM6 motion shown vs active structure (blue)
  - Simulation time lapse shown in yellow to green
  - TM6 transitions open to closed

Transition Contacts



- Dark-state simulation significantly different from inactive structure
- Opsin simulation
  - Drift at start: contacts broken
  - New contacts when ionic lock forms

Conclusions

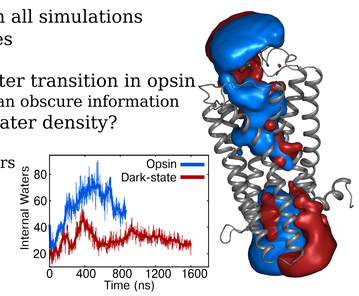
- Opsin capable of transition to dark-like structure
  - Only happened once
  - Took significant simulation time (~600 ns)
  - Reverse transition not seen
- No other transitions
- Rotamer toggle switch coupling loose at best
  - Trp265 not flipped in opsin or Meta-II structures
- Ionic lock may be an indicator
  - Need more transitions
- Coarser measurements corroborate transition

Future

- Need to run longer
  - More trajectories
  - BlueGene/Q resources
- Retinal/counterion involvement
  - Other structural motifs
- Changes in hydrogen bonding

Internal Hydration

- Many internal waters in all simulations
- Number of waters varies
  - Density pattern varies
- Hydration decreases after transition in opsin
  - Simply counting waters can obscure information
- Are there patterns in water density?
  - Long lived waters
  - Hydrogen bonding partners
- Use pattern matching algorithms to identify similarities



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