Unraveling Allostery with Simulations of Rhodopsin and Opsin

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Poster PDF
tinyurl.com/rhod-allostery

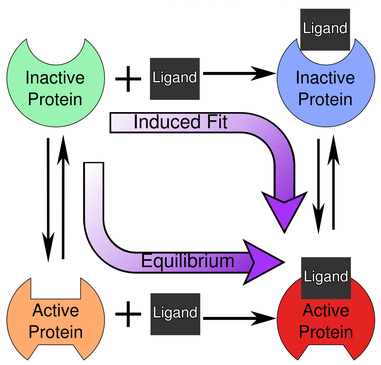
Abstract

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 (~90 microseconds 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. The underlying allosteric process is clearly not a simple lock and key or conformational equilibrium mechanism, but some combination of both.

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



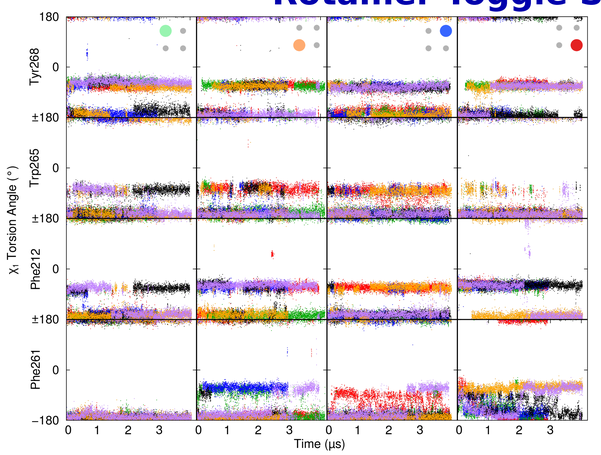
- Ligand binding site & active site are distant
- Induced fit
  - Ligand drives conformational change
  - Active participation
- Conformational equilibrium
  - Protein fluctuates
  - Ligand binds single conformation
  - Passive participation
- Simulate states
  - Understand dynamics
  - Effect of ligand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

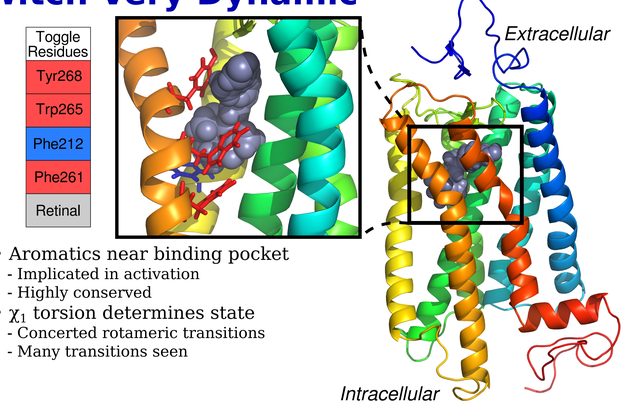
Simulation Details

- 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 (ns)
Dark-opsin	1U19	retinal removed	3x3000 3x4000
Opsin	3CAP		3x3000 3x4000
MI-MII	"Meta-I"	from previous simulation	6x4000
Meta-II	3PXO		3x3000 3x4000
		Total	≈87,000ns

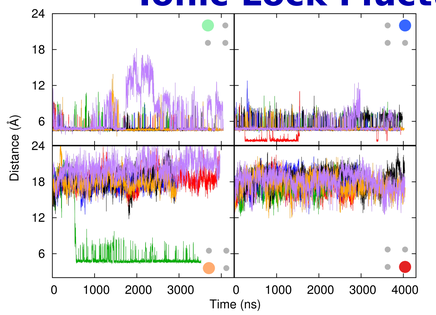
Rotamer Toggle Switch Very Dynamic



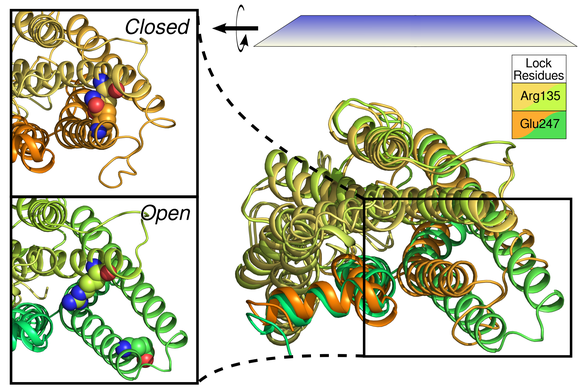


- Aromatics near binding pocket
  - Implicated in activation
  - Highly conserved
- χ_1 torsion determines state
  - Concerted rotameric transitions
  - Many transitions seen

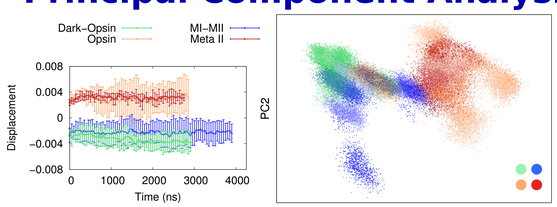
Ionic Lock Fluctuations



- 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
  - May be transient



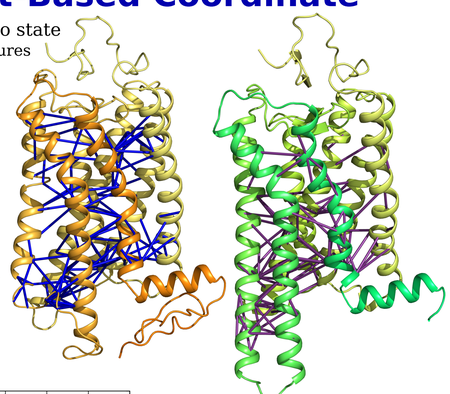
Principal Component Analysis

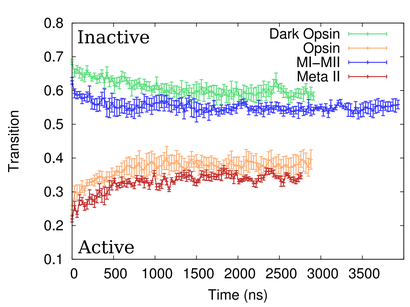


- Calculate most collective motions in the trajectory
  - Use all trajectories in aggregate
    - PC-space defines all simulations
  - Transmembrane Cα's only
- Displacement along PC1 vs. simulation time
  - Average +/- std. dev.
  - Group by starting strucutre
- PC1 vs. PC2
  - PC-space spans all simulations
  - Individual trajectories plotted
    - Colored by ensemle
  - Saturation increases with simulation time
  - Overlap between ensembles
- PC1 illustration
  - Average structure: rainbow
  - Black vectors indicate direction and relative magnitude of PC1
  - Active crystal in red
  - Inactive crystal in blue

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





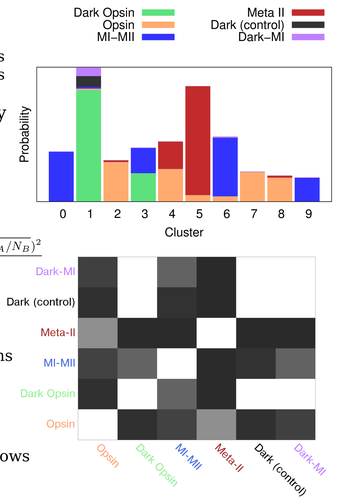
- Plot transition in simulations
- Each trajectory calculated independently
  - Averaged by initial state
  - Error bars: standard deviation
- Still converging
- Opsin more inactive than Meta-II
- MI-MII more active than Dark-opsin
- Normalized

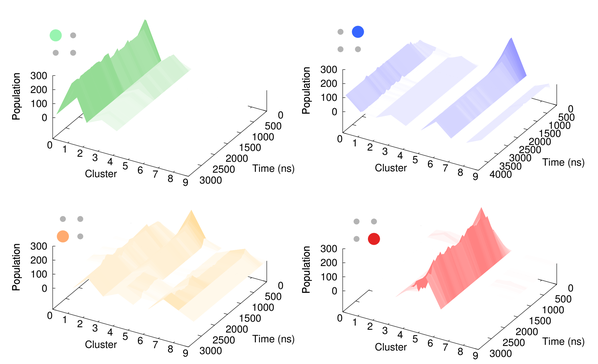
Clustering Reveals Structural Overlap

- 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
- Measure similarity of cluster populations

$$\chi^2 = \sum_{i}^{N_{clusters}} \frac{\left(n_A(i)\sqrt{N_B/N_A} - n_B(i)\sqrt{N_A/N_B}\right)^2}{n_A(i)+n_B(i)}$$

  - Lighter → more similar
- Endpoints: Meta II and Dark
- Opsin and Dark-opsin: high overlap with other simulations
- MI-MII also similar to others
- 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
- Whole protein involved in transitions
- Allosteric hypotheses not mutually exclusive
  - Two extremes on continuum
  - *To what extent does the ligand impact activation?*
- Ligand changes protein dynamics
  - More data needed to quantify changes satisfactorily

Future

- Need more data
  - More trajectories?
  - "Control" simulations
  - Enhanced sampling
  - Simple models
- Define allosteric network
  - Data-derived metrics
  - Quantify concertedness
- Retinal/counterion involvement
  - Other structural motifs
- Changes in hydrogen bonding
  - Ion-mediated interactions
  - Water-mediated interactions
- Contribution by lipid environment
- Bulk effects of water

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