Dynamic Ligand-Protein Interactions Alter Rhodopsin's Conformational Ensemble: Simulations of Rhodopsin and Opsin

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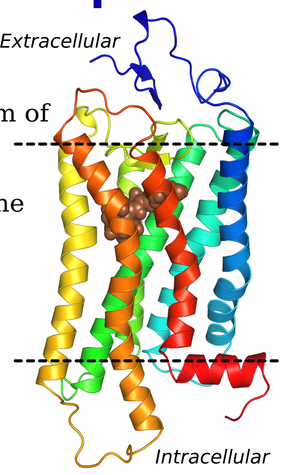
Poster PDF
http://tinyurl.com/rtn-rhod-dyna

Abstract

Given their function as tranducers of molecular signals across the cell membrane, G protein-coupled receptors (GPCRs) constitute a major target for drugs in a wide variety of physiological scenarios. Understanding the course of structural transitions that allosterically modulate their activation is therefore fundamental towards improving rational drug design. Here, we analyze unbiased microsecond-scale all-atom molecular dynamics simulations to characterize distinct ensembles of the class A GPCR rhodopsin that correspond to both active- and inactive-like conformations, in the presence and absence of the ligand. By monitoring the ligand's orientation and interactions within the binding pocket, we show that retinal adopts heterogeneous conformations that are consistent with ensemble-dependent dynamics.

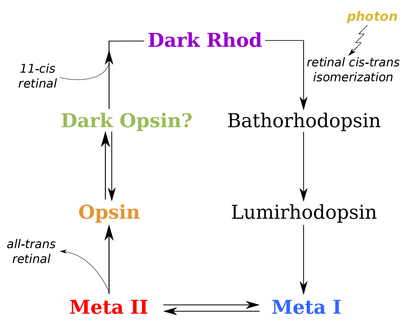
Class A GPCR Rhodopsin

- 7 transmembrane (7TM) α-helical proteins
- 800 GPCRs in the human genome
- Binding of G protein to the active form of the receptor induces:
  - Conformational change
  - Signal transduction across membrane
  - Signaling cascade
- Biomedically relevant
  - More than 30% drugs target GPCRs
- Rhodopsin is the mammalian dim-light receptor
  - Retinal: covalently bound
    - 11-cis: inverse agonist
    - All-trans: agonist



Rhodopsin Photocycle

- Retinal isomerizes from 11-cis to all-trans when a photon is captured by dark-state rhodopsin.



- Transient intermediates
- Meta I and Meta II are in equilibrium
  - Only Meta II activates G protein
- Opsin is apo form
  - Crystal looks active-like
  - Experimentally has minimal activity
- Dark Opsin is a proposed inactive-like, ligand-free state

Simulation Details

- To understand state-dependent dynamics and their correlation with retinal motions we analyzed the following all-atom MD simulations:

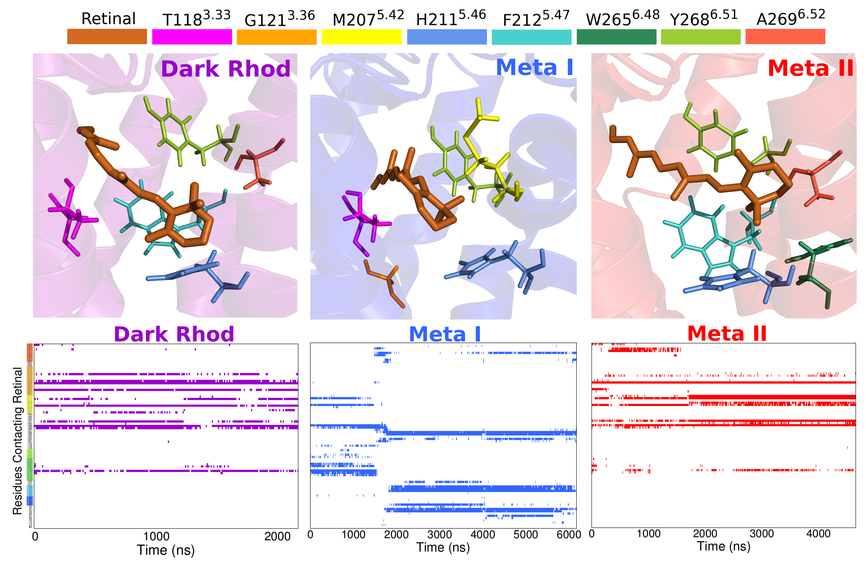
Ensemble	Structure	Simulation Time (μs)
Dark Rhod	1U19	6 runs x 1.8
Meta I	previous work[1]	6 runs x 6.0
Meta II	3PXO	6 runs x 4.5
Opsin	3CAP	6 runs x 4.5
Dark Opsin	1U19 (ligand removed)	6 runs x 4.5
	Total	≈ 130 μs

[1] Grossfield et al., *J. Mol. Biol.* 2008, 381: 478-486

- System size: ~46,000 atoms
  - 123 SDPE lipids
  - ~ 8,000 waters
  - 100 mM NaCl
- Box size: 74 Å x 74 Å x 90Å
- Forcefield: CHARMM27/36
  - Retinal parameters obtained from the Feller lab
- Ensemble: NPγT
  - γ = 30 dyn/cm
- Thermostat: Langevin
  - 310 K
  - 1 bar
- Electrostatics: PME
- VDW cutoff: 10 Å
- Timestep: 2 fs
  - RATTLE
- Software:
  - NAMD 2.8
  - BlueGene/Q

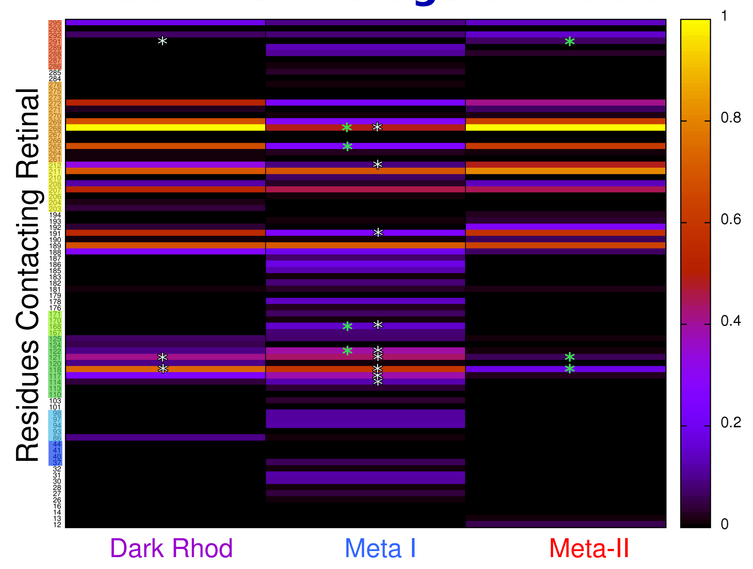
Retinal Dynamics Distinguish Protein State

Retinal-Protein Contacts



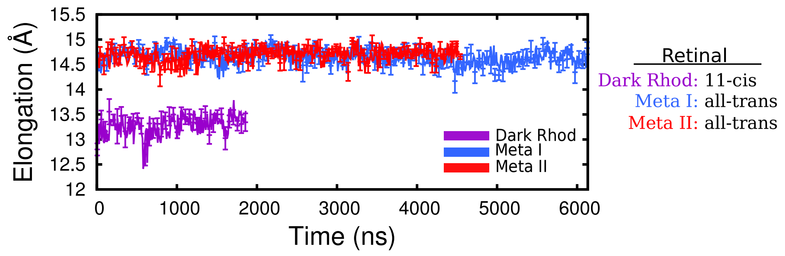
- Retinal-protein interactions
  - *Contact:* Residue-retinal centroid-to-centroid distance < 8 Å
  - Retinal taken as a single residue
- Illustration: Five highest occupancy contacts
  - Ballesteros-Weinstein numbering in superscript
- One representative time series per ensemble
  - Residue number colored by helix
  - Ensemble-dependent interactions
  - Contacts vary on the timescale of hundreds of ns
- Average occupancy
  - Residue number colored by helix
  - Meta I is the most heterogeneous

Ensemble-Average Contacts



*: p-value vs. Dark Rhod < 0.05
*: p-value vs. Meta II < 0.05

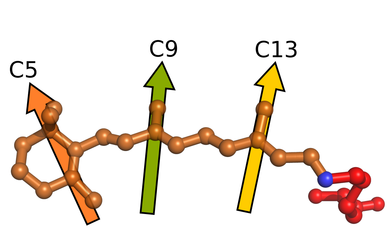
Retinal Elongation

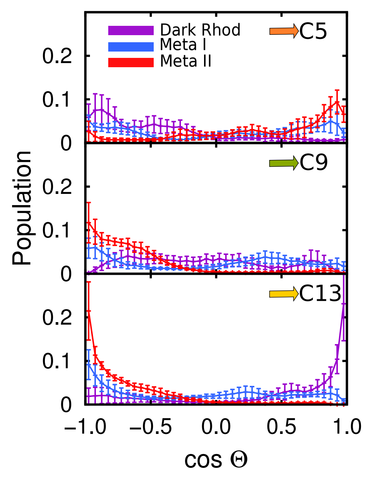


Retinal
Dark Rhod: 11-cis
Meta I: all-trans
Meta II: all-trans

- C3-NZ distance vs. time
  - Ensemble average over 6 trajectories
- Retinal rapidly elongates after isomerization

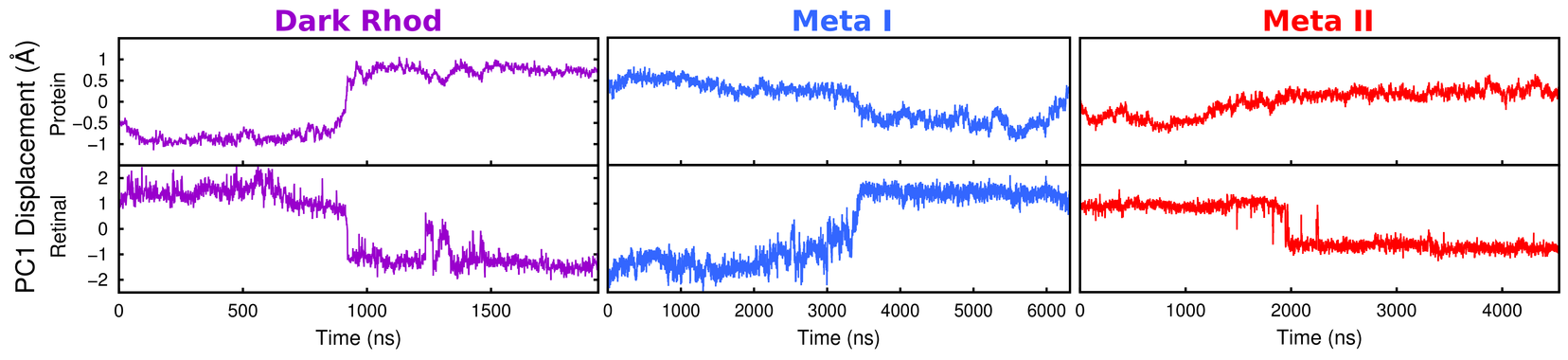
Retinal Orientation Varies with Ensemble



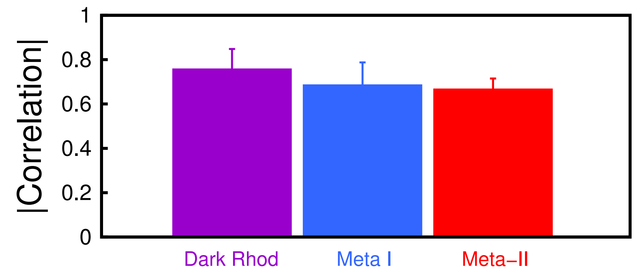


- Cosine of methyl orientation with membrane normal
  - Meta II and Dark Rhod: retinal orientations distinctly polarized near C5 and C13
  - Meta I: more heterogeneous conformations

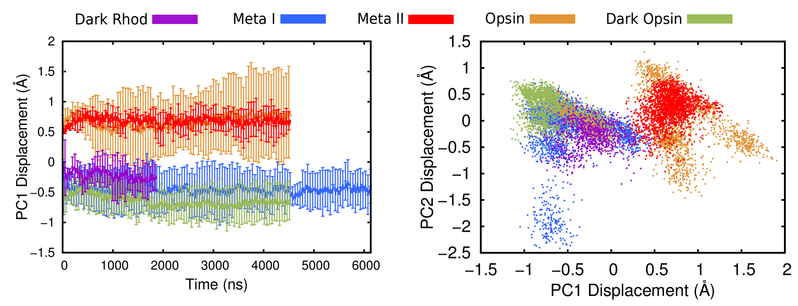
Retinal Motions Correlate with Overall Protein Dynamics



- Correlation between rhodopsin and retinal's most collective motions
  - Principal component analysis (PCA)
  - Each trajectory of every ensemble for ligand and receptor
  - Alignment on transmembrane Cα's
- Representative time series of PC1 for protein and retinal
- Protein-retinal correlation per ensemble
  - Compute PCA for protein and retinal for each trajectory
  - Correlation between PC1 for each
  - Average over 6 trajectories per ensemble
  - Differences are not statistically significant (p-value > 0.05)
  - Retinal remains strongly coupled

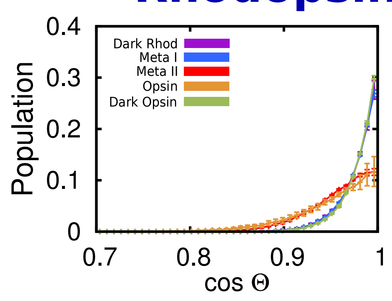


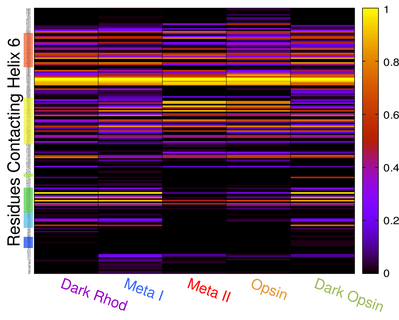
Large-Scale Protein Motions Vary Between Ensembles



- PCA calculated on aggregate basis set
  - All 5 ensembles
  - Transmembrane Cα's only
  - Trajectories downsampled to 5 ns resolution
- Average displacement by ensemble
  - PC1 distinguishes active-like from inactive
  - Amplitude of fluctuations: diversity
- Ensemble projected onto PC1 and PC2
  - Every dot represents a single structure in the corresponding ensemble
- Opsin overlaps with all four ensembles
- Dark Rhod and Meta II are compact
- Meta I explores some unique states

Helix 6 Dynamics Characterize Rhodopsin Ensembles





- Helix 6 principal axis cosine with membrane normal
  - Cytoplasmic half of the helix
  - Opsin and Meta II more tilted, broader range
- Average helix 6 interactions
  - *Contact:* Centroid-to-centroid distance < 10 Å
  - Helix 6 taken residue by residue

Conclusions

- Retinal changes during activation
  - More transient contacts in Meta I
  - Prefered orientation switches upon isomerization
  - Retinal elongates in Meta I and Meta II
  - Retinal dynamics are strongly coupled to protein motions
- Transitions involve protein's most collective motions
- H6 orientation and contacts are ensemble-dependent
- Overall slow relaxation times
  - Events require hundreds of ns
  - Multiple simulations essential

Future Directions

- Internal solvation
  - H-bonding patterns by ensemble
    - Water and salt
- Lipid-protein interactions
- Predict ^{2}H NMR spectra for retinal
- Enhanced sampling using Markov State Models
- Other ligands
  - Better agonists
  - Weaker inverse agonists
- Extend accessible timescales with simple models
  - Structure-based potentials
  - Transitions in equilibrium

Data analysis was performed using LOOS (Lightweight Object-Oriented Structure library), an open source C++ library designed by the Grossfield lab. LOOS is adaptable and compatible with all major simulation packages, providing a leveled and friendly platform for developing analysis applications. The source code is available at:
http://loos.sourceforge.net