Retinal Changes Conformation During the Early Stages of Rhodopsin Activation

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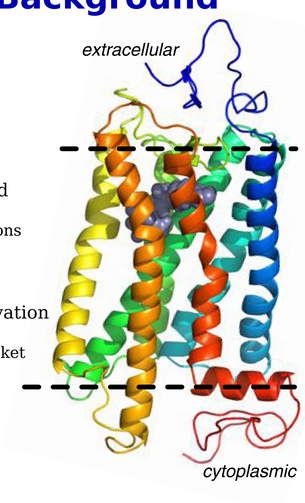
Abstract

Rhodopsin, the mammalian dim-light receptor, is one of the best-characterized G protein-coupled receptors—a pharmaceutically important class of membrane proteins that has garnered much attention due to the recent availability of structural information. Yet, the activation mechanism is not fully understood. Here, we combined solid-state NMR with three separate µs-scale all-atom molecular dynamics simulations to understand the transition between the dark and metarhodopsin I (Meta I) states. From the simulations, we directly computed NMR spectra for specifically deuterated methyl groups in retinal. The simulation-based results corroborated one of two competing hypotheses for Meta I formation, the complex-counterion mechanism. Further simulation analysis revealed striking differences in ligand flexibility between the two states; retinal was more dynamic in Meta I, adopting an elongated conformation. Surprisingly, this elongation also corresponded to a dramatic influx of bulk water into the hydrophobic core of the protein. Importantly, this enhanced retinal motion upon light activation may reconcile two recent crystal structures of active rhodopsin, which showed retinal in two distinct conformations.

Rhodopsin Background

- Integral membrane protein
  - 7 transmembrane (TM) α-helices
- Dim-light receptor
  - Covalently bound ligand (retinal)
  - Isomerized by photon
  - G protein binds cytoplasmic face
- Structurally well characterized
  - Recent active structures report retinal in two distinct conformations
    - · Elongated in both
    - · Differ by 180° rotation
- Two hypotheses for early activation
  - Complex-counterion hypothesis
    - · Negatively charged binding pocket
    - · Glu113 & Glu181 stabilize ligand in Meta I
  - Counterion-switch hypothesis
    - · Neutral binding pocket
    - · Ligand support switches from Glu113 to Glu181 in Meta I



Simulation Details

- BlueMatter - BlueGene/L
- Forcefield: CHARMM (c27)
- Timestep: 2 fs
  - RATTLE constrained bonds
- Ensemble: NVE
- Electrostatics: PME
  - Cutoff: 10 Å
- Membrane Composition
  - 49 SDPC lipids
  - 50 SDPE lipids
  - 24 cholesterols
- 7400 waters (TIP3P)
- 100 mM NaCl
- System Size: 43000 atoms

Simulation	Ligand State	Time
Dark State	11-*cis* retinal	1605 ns
Complex-Counterion	all-*trans* retinal	1470 ns
Counterion-Switch	all-*trans* retinal	2000 ns

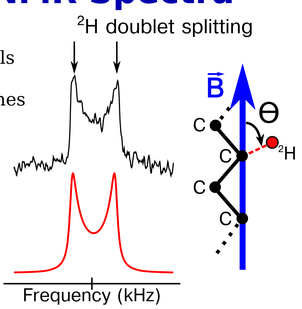
Deuterium NMR Spectra

Experiment

- Selectively deuterated methyls
  - C5, C9, and C13 in retinal
- Prepared in aligned membranes
- Spectra obtained at seven membrane tilt angles

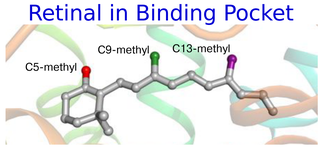
Simulation

- Spectra calculated from retinal orientation
  - Reconstruct spectra
  - Fit mosiac spread

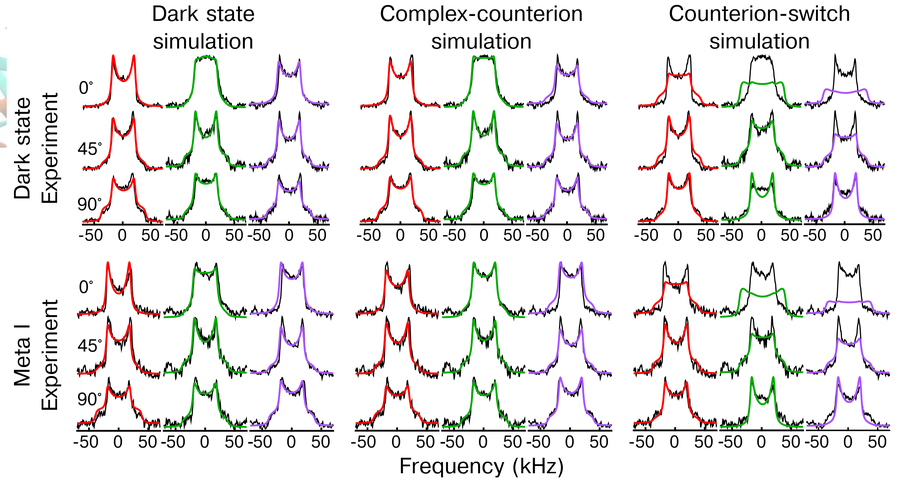


Simulation and NMR Corroborate Complex-Counterion

Retinal in Binding Pocket

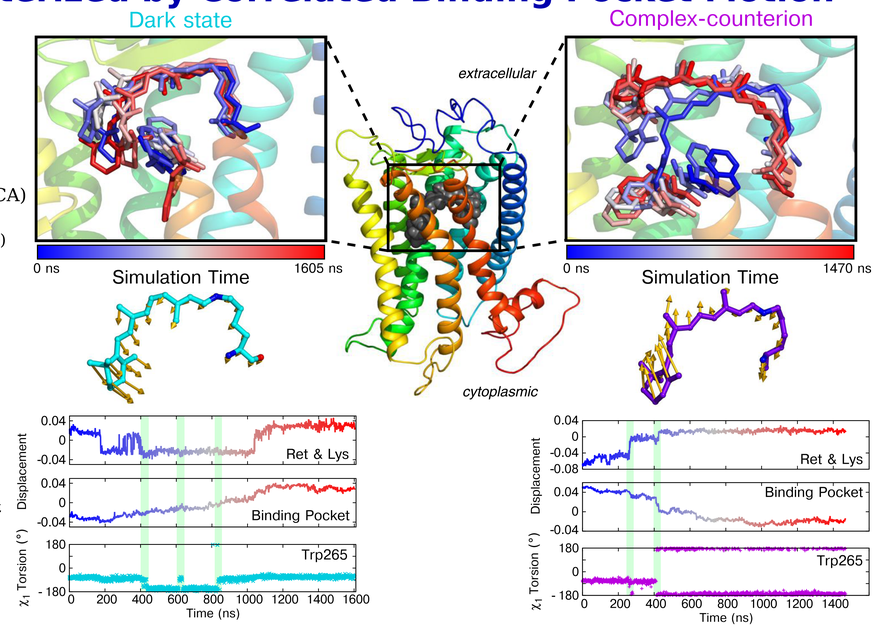


- MD compared to ssNMR
  - Simulation spectra in color
    - · Three simulation conditions
    - · Last 500 ns used to calculate spectra
  - Experimental spectra in black
    - · Two NMR conditions
    - · Both NMR spectra are similar
- Dark state simulation matches experiment
- Complex-counterion simulation matches experiment
- Counterion-switch simulation DOES NOT match experiment

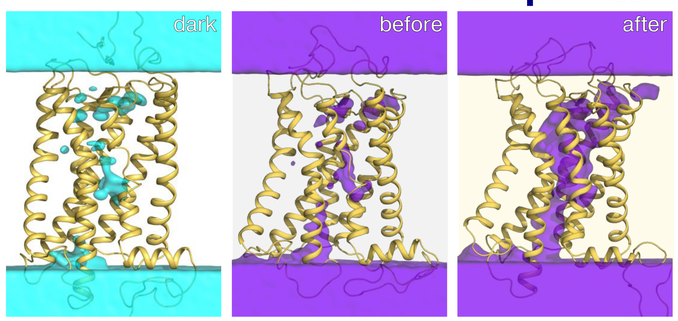


Activation Characterized by Correlated Binding Pocket Motion

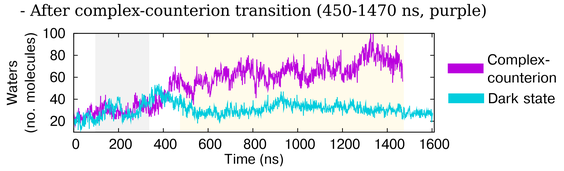
- Time course of retinal dynamics
  - Dark state (left):
    - · Subtle motion
    - · Similar starting/ending conformations
  - Complex-counterion (right):
    - · β-ionone moves upward
- Principal Component (PC) Analysis (PCA)
  - Extracts concerted motions
  - Direction of most concerted motion (yellow)
    - · Dark state (left, cyan)
    - · Complex-counterion (right, purple)
- Displacement along PC
  - Shows when transitions occur
    - · PCA of retinal and Lys296 (top)
    - · PCA of binding pocket (middle)
- Trp265 also implicated in activation
  - χ_1 torsion dynamic in both simulations
    - · Dark state: reorients sporadically
    - · Complex-counterion: reorients concurrent with concerted transitions
  - Adopts distinct preferred orientation between simulations



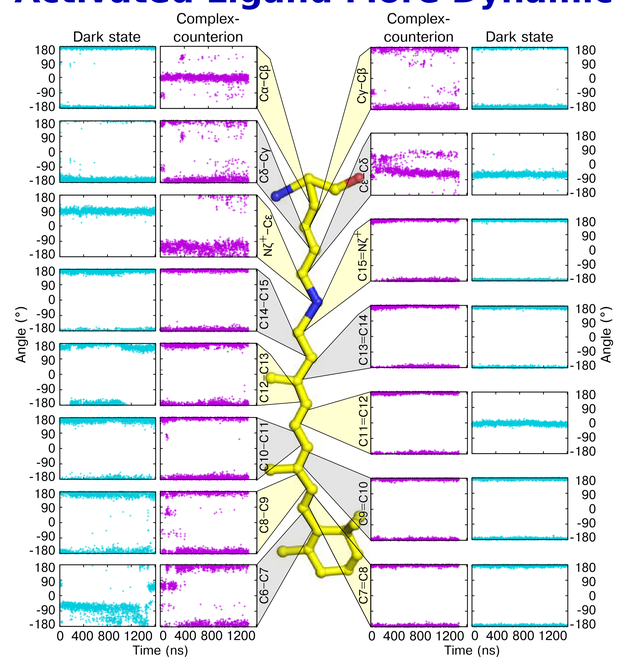
Water Influx Accompanies Binding Pocket Motion



- Internal hydration increases on activation
  - Hydration level constant in dark state simulation
- Water density shown contoured at 25% bulk
  - Averaged over whole dark state simulation (cyan)
  - Before complex-counterion transition (100-250 ns, purple)
  - After complex-counterion transition (450-1470 ns, purple)



Activated Ligand More Dynamic

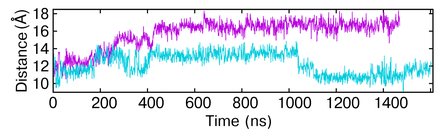


Torsional Dynamics

- Lys296 torsions more flexible during activation
  - C11=C12 isomerization
  - Many more transitions
  - Changes in dominant orientation for:
    - Cα–Cβ
    - Nζ–Cε
    - C6–C7

Ligand Elongation

- Retinal elongates only in complex-counterion simulation
  - Monitor Cα to C3 distance
  - Similar time as concerted transition
  - No transition in dark state



Conclusions

- MD and NMR identified complex-counterion mechanism
  - Simulation matched experimental spectra
  - Counterion-switch spectra did not match
- Retinal makes a concerted transition after isomerization
  - β-ionone moves toward extracellular face of rhodopsin
  - Largest collective motion during trajectory
  - Trp265 χ_1 torsion reorients during collective motions
- Internal hydration increases in complex-counterion simulation
  - Increased after retinal's concerted transitions
- Retinal becomes more dynamic after isomerization
  - Increased torsional flexibility (especially in Lys296)
  - Ligand elongates as seen in active crystal structures

Future Work

- Track specifics of water interactions
  - Involved in H-bonding network?
  - Separate effects of bulk water
  - Active vs. inactive features

Related Work:
GPCR Activation - poster B37 (today)
AA Rhodopsin - poster B292 (Mon)
Analysis Tools - poster B812 (Wed)

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