Understanding the Effect of Polyunsaturated Fatty Acids on Rhodopsin Using All-Atom Molecular Dynamics Simulations

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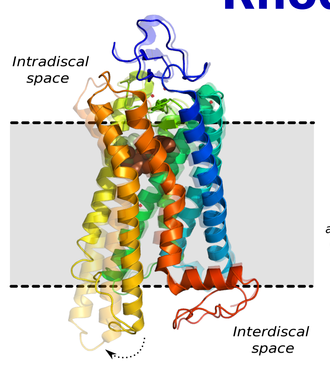
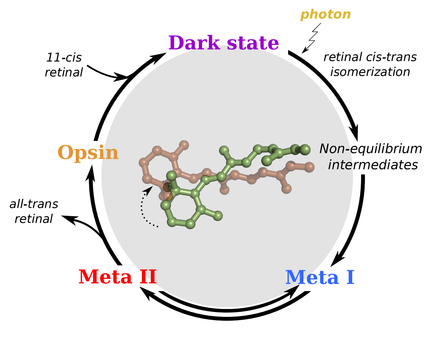
Poster PDF
http://tinyurl.com/rhod-in-SDPE

Abstract

G protein-coupled receptors (GPCRs) are membrane proteins that can transduce external stimuli across lipid bilayers via conformational changes, which modulate their interactions with different binding partners. This mechanism and their involvement in the mediation of many signaling pathways make them important drug targets. The functional versatility of these receptors is thought to be governed by their dynamics and structural plasticity, which can be influenced by several factors including the membrane surrounding them. Here, we analyze microsecond-scale all-atom molecular dynamics simulations to investigate how the conformational dynamics of this prototypical GPCR can be modulated by its lipid environment. We observe that PE headgroups, DHA and STEA fatty acid chains interact differently with active and inactive ensembles of the receptor and thus may play a role in stabilizing different protein states.

Rhodopsin

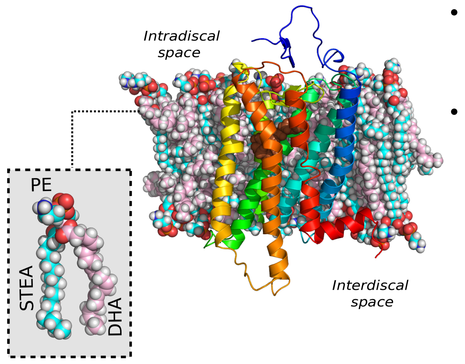




- 7 transmembrane (7TM) α-helical protein
- Located in disk membranes of rod cells in the retina
- Highly efficient photoreceptor
- Involvement in retinal diseases and model GPCR

- Mediates the visual signaling cascade
  - Ligand: covalently bound chromophore
    - *11-cis* retinal: inverse agonist
    - *All-trans* retinal: agonist
  - Light signal transduced across lipid bilayer via conformational changes
  - Binds G protein transducin

Bilayer Composition Modulates Rhodopsin's Dynamics



- Proposed mechanisms of action:
  (1) Bulk membrane properties
  (2) Direct protein-lipid interactions
- ROS disk membranes are enriched in:
  - DHA acyl chains
    - From 35 to 60%
    - Favor Meta II formation
    - Annular lipids
  - PE headgroups
    - Around 41%
    - Favor Meta II formation

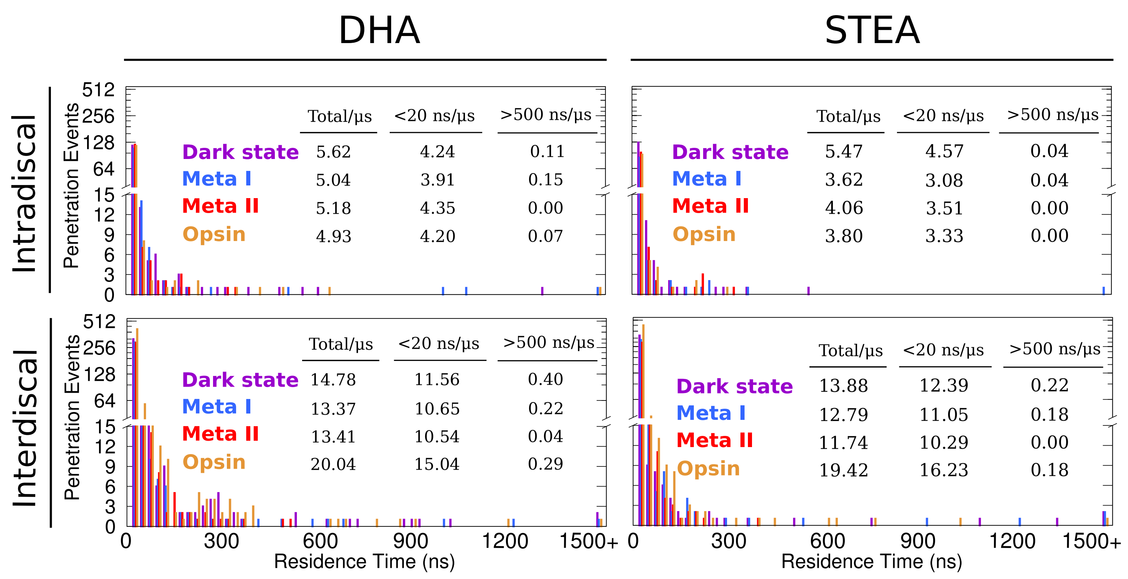
Simulation Details

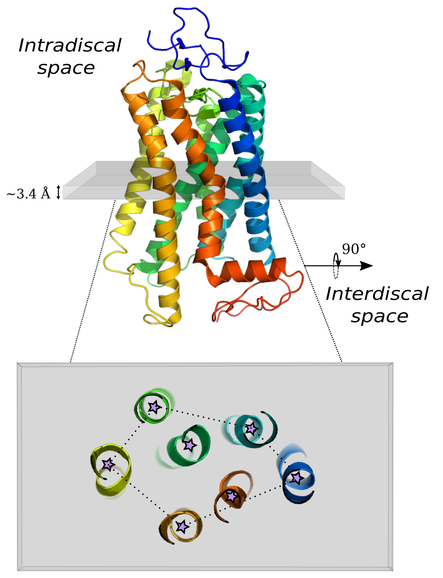
Protein State	Structure	Simulation Time (μs)
Dark state	1U19	6 runs x 4.8
Meta I[(1)]	previous work[(2)]	6 runs x 8.5
Meta II[(1)]	3PXO	6 runs x 4.6
Opsin[(1)]	3CAP	6 runs x 4.6
	Total	≈ 135 μs

[(1)] Leioatts et al., *Biophys. J.* 2015, 109: 608-617
[(2)] 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
- Force field: CHARMM27/36
  - Retinal parameters obtained from the Feller lab
- Ensemble: NPγT
  - γ = 30 dyn/cm
- Electrostatics: PME
- VDW cutoff: 10 Å
- Thermostat: Langevin
  - 310 K
- Barostat: Langevin Piston
  - 1 bar
- Timestep: 2 fs
  - RATTLE
- Software:
  - NAMD 2.8
  - BlueGene/Q

Lipid Penetration into Protein Core is State-Dependent

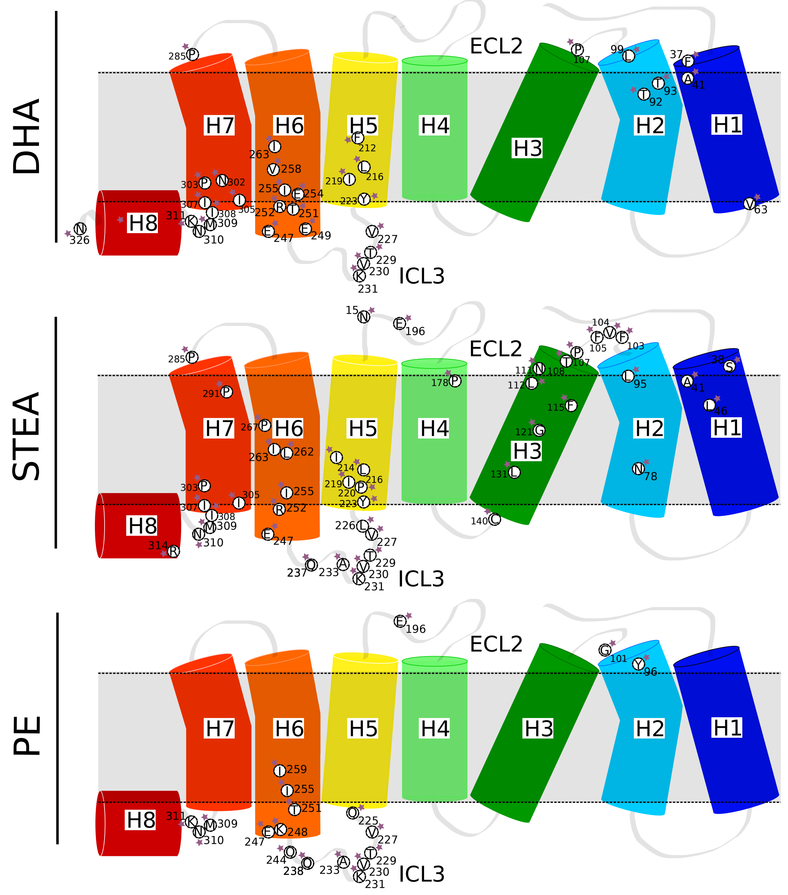




- Quantify fatty acid penetration events into protein core
  - Protein sliced along membrane normal (~3.4 Å)
    - Convex hull computed every frame using helix centroids in slice
  - Counted instances of acyl tails inside hull (≥ 1 heavy atom)
  - Independently calculated and histogrammed by initial state
- Residence times and number of events are state-dependent
  - Long-lived penetration events are more likely in inactive-like states
    - 37 events in **Dark state** and **Meta I** vs. 16 in **Meta II** and **Opsin** overall
  - Most events occur in the interdiscal side of the protein
    - **Opsin** has more events, but they tend to be short-lived
- More DHAs penetrate the core and for longer times

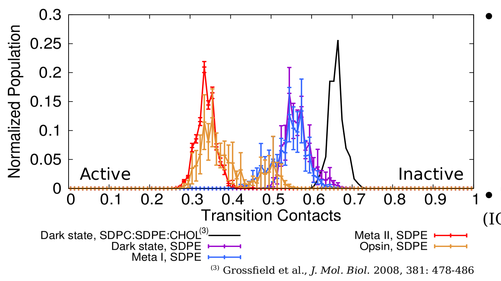
Protein-Lipid Interactions Vary Among States

p-values (<0.05): ★ **Dark state** vs. **Meta II**

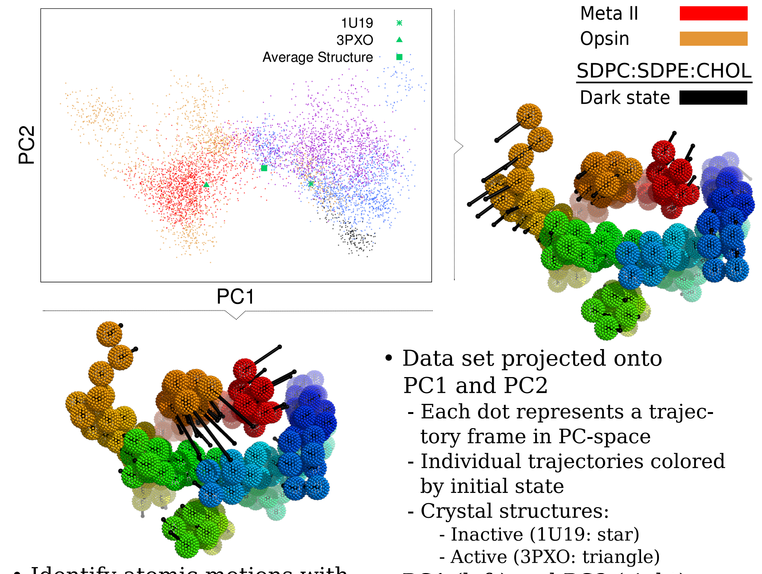


- Identify protein residues that interact differently with lipids
  - Occupancy: ≥ 1 lipid atom within 6 Å of side-chain centroids
  - t-test using 6 independent trajectories per initial state
- DHA: Most significant differences at interdiscal end of H5-H7
- STEA: Varies the most with protein state
  - Differences also span H1-H3
- PE: Most significantly different residues between active and inactive states at ICL3 and interdiscal end of H5-H6

SDPE Lipids Shift Rhodopsin's Conformational Distribution

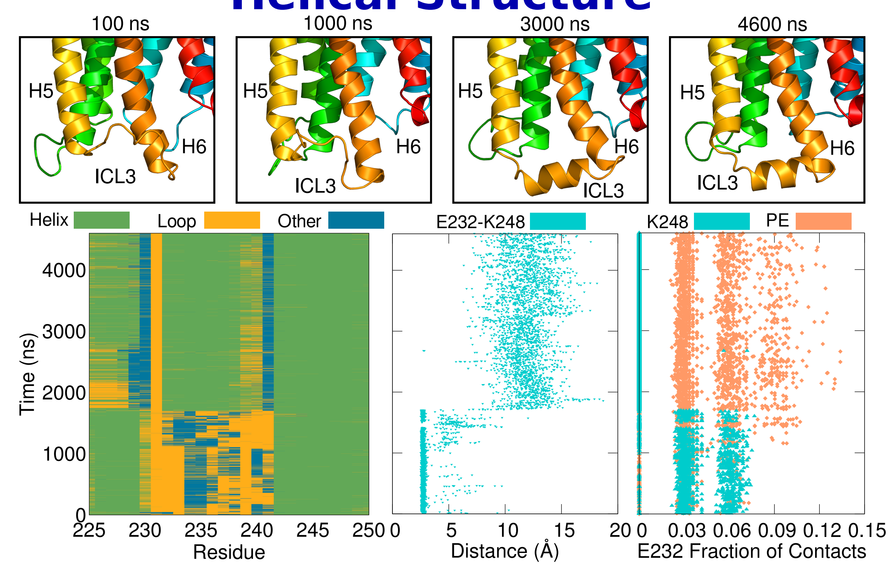


- Quantify state-specific contacts
  - Unique contacts from crystal structures (3PXO: AC; 1U19: IC)
  - Side-chain centroids within 8 Å
- Transition contacts:
  (IC formed + AC broken)/(IC + AC)
  - *1:* all IC formed & all AC broken;
  - *0:* none IC formed & none AC broken
  - Histogrammed by trajectory and averaged by initial state
  - Error bars: SEM of 6 trajectories
- 2-sample Kolmogorov-Smirnov test *(p-value < 0.05)*:
  - **Dark state** significantly different from all
- **Dark state** is left-shifted with respect to **Dark state**
  - SDPE membranes: No CHOL and larger PE content

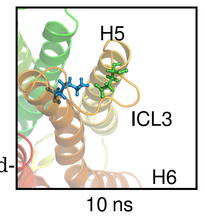


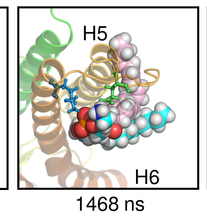
- Identify atomic motions with largest covariance
  - Principal component analysis on TMCAs of aggregate data set
- Data set projected onto PC1 and PC2
  - Each dot represents a trajectory frame in PC-space
  - Individual trajectories colored by initial state
  - Crystal structures:
    - Inactive (1U19: star)
    - Active (3PXO: triangle)
- PC1 (left) and PC2 (right) projected onto average structure
  - PC1: Interdiscal ends of H6-H7
  - PC2: Interdiscal end of H5 and ICL3

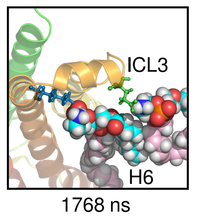
Intracellular Loop 3 Gains Helical Structure



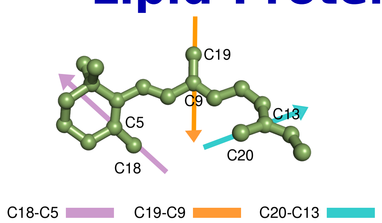
- Secondary structure of ICL3 in **Dark state**
- E232-K248 salt bridge is disrupted concomitantly with ICL3 structuring
- PE headgroups favor the open state of the salt bridge, stabilizing the helix

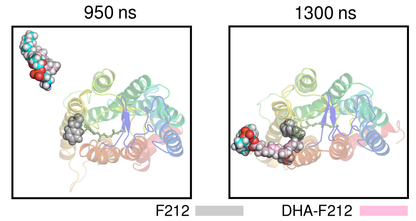


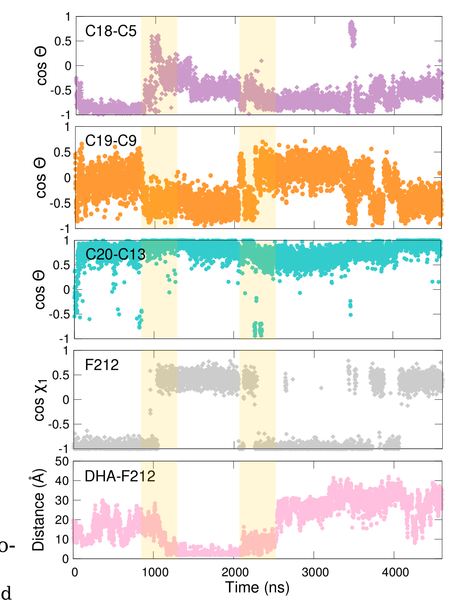




Retinal Orientation Is Altered by Lipid-Protein Interactions







- Retinal methyl orientations in **Dark state** vs. membrane normal
  - *cos θ* = 1: inter-; *cos θ* = -1: intradiscal
- Side-chain conformations of binding pocket residues can be affected by lipids
  - Retinal orientation is concurrently altered

Conclusions

- Direct protein-lipid interactions can alter protein structure and ligand behavior
  - Specific residues differentially interact with lipids among protein states
- PE headgroups can stabilize ICL3 helical structure
- Retinal undergoes lipid-mediated transitions
  - Binding pocket residues involved

Future Directions

- Characterize effect of retinal analogs
  - Predict ^{2}H NMR spectra
- Study other bilayer compositions
- Extend accessible timescales with simple models
  - Structure-based potentials
  - Use data set to seed trajectories

Data analysis was performed using LOOS (Lightweight Object-Oriented Structure library), an open source C++ library designed by the Grossfield lab for developing analysis tools. Available at:
http://loos.sourceforge.net
https://github.com/GrossfieldLab/loos