Study of Ultra-Fast Rhodopsin Activation Dynamics with Molecular Dynamics Simulations

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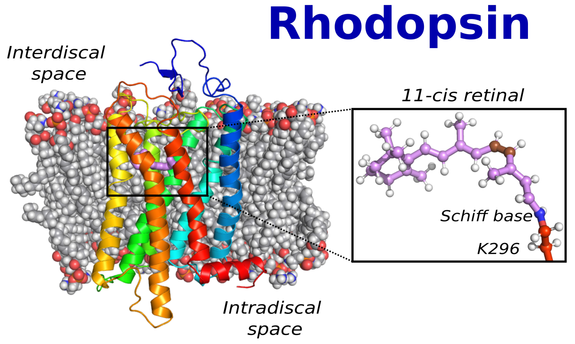
Poster PDF
http://tinyurl.com/ultra-fast-dyn

Abstract

Rhodopsin is a photo-sensitive membrane protein that, when excited by light, can activate the signaling pathway that leads to dim-light vision in mammals. Although this receptor is well-characterized, there is limited structural information about the events that occur immediately after light-excitation, as they take place in notably short time regimes (fs-ns) and are not easily captured experimentally. All-atom molecular dynamics (MD) simulations can be a powerful tool for the study of these ultra-fast dynamic events *in silico* and aid the interpretation of emerging experimental techniques, such as time-resolved small- and wide-angle X-ray scattering (TR-SWAXS) with free-electron lasers. Starting from well-equilibrated microsecond-scale dark-state bovine rhodopsin simulations, we run and analyze ~3,000 pairs of 100 ps trajectories in two conditions—dark and light-excited—to *1) model the process of energy dispersion across the protein after light-excitation* and *2) study the ultra-fast structural changes that occur upon activation*. We observe an increase in the radius of gyration of the receptor and a propagation of the light-induced perturbation that occurs roughly at the speed of sound.

Rhodopsin

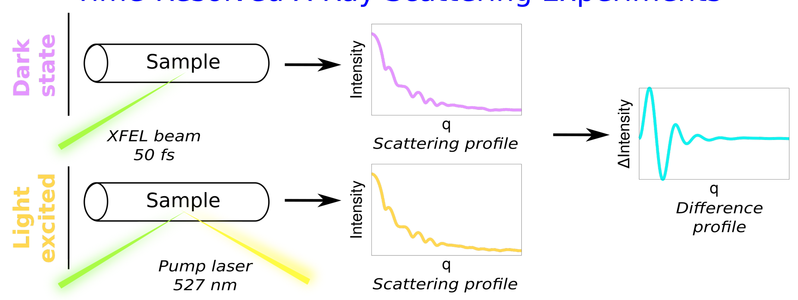


- Photoreceptor located in disk membranes of rod cells
- Mediates dim-light vision
- Light-excitation isomerizes ligand

Dark state + 1 hν
↓ 200 fs
Photorhodopsin
↓ ps
Bathorhodopsin
↓ ns
Blue-shifted intermediate
↓ ns
Lumirhodopsin
↓↓

- Early activation events occur at the sub-nanosecond timescale
- Limited information of ultra-fast dynamics

Time-Resolved X-Ray Scattering Experiments



- Samples are probed with 50-fs X-ray pulses
  - One sample is excited with a 527 nm laser
  - Scattering profiles are recorded at different time delays after light-excitation
- Experiment captures changes in size/shape at high time resolutions
  - Limited spatial/structural information
- Simulations give atomic level physical interpretation

Simulation Strategy

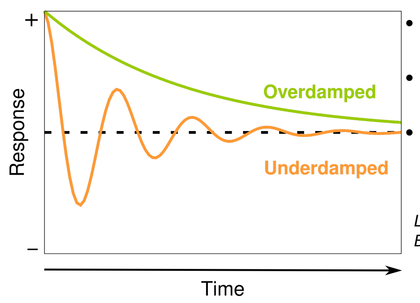
- System size: ~46,000 atoms
  - 123 SDPE; 8,000 waters; 0.1 M NaCl
  - Box size: 74 Å x 74 Å x 94 Å
- Force field: CHARMM27/36
- Barostat: 1 bar (Langevin)
- Electrostatics cutoff: 10 Å (PME)
- VDW cutoff: 10 Å
- Timestep: 2 fs (RATTLE)
- NAMD 2.8 on BlueGene/Q

- Retinal isomerization induced by temporally modified potential
  - 60 kcal/mol barrier to cis conformation
  - Isomerization occurs in less than 0.5 ps
- Energy of 527 nm photon added by rescaling ligand velocities
  - Thermostat turned off to preserve correct short time dynamics

[1]*Salas-Estrada et al., Biophys. J. 2018, 114: 355-367*

Rhodopsin's Radius of Gyration Increases After Light-Excitation

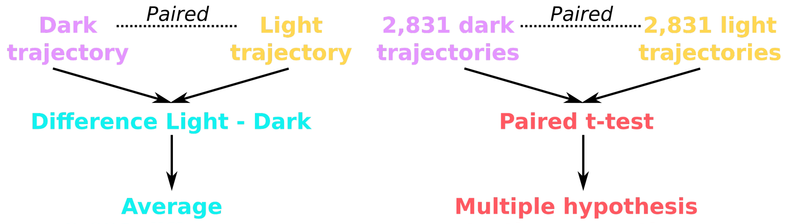
What Happens to Proteins After Being Struck by a Force?



- Generally accepted notion of overdamped oscillations/decay
- Ultra-fast conformational changes → *protein quake*
- Myoglobin:
  - TR-SWAXS → underdamped
  - MD → overdamped

Levantino et al., Nat. Comm. 2015, 6: 6672-8
Brinkmann et al., PNAS 2016, 113: 10565-70

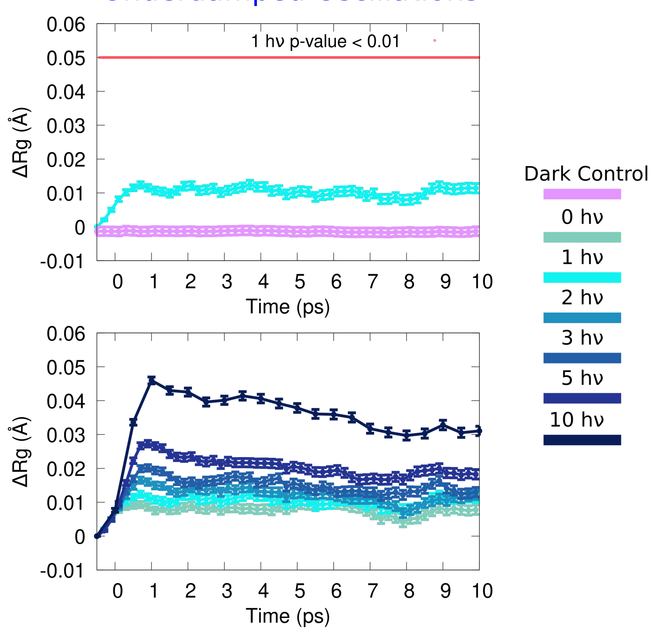
Calculating Light-Induced Changes from Simulations



- Error bars are the standard error of the mean
- Dark controls calculated by randomly pairing dark trajectories with replacement

- Multiple hypothesis testing with Holm-Šídák algorithm
  - Implementation from `StatsModel`
  Seabold et al., Proceeding of the 9th Python in Science Conference, 2010

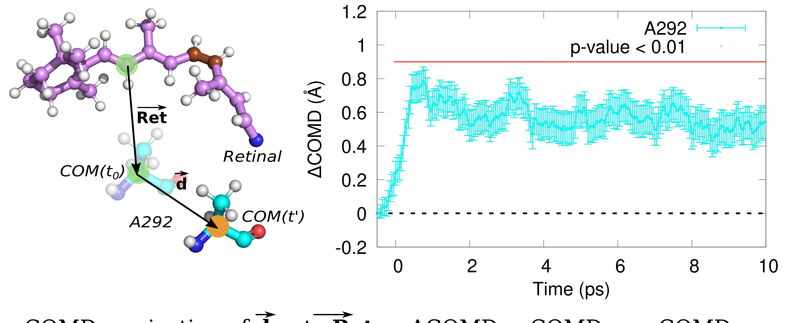
Rhodopsin Does Not Seem to Undergo Underdamped Oscillations



- Light-excitation produces an increase in rhodopsin's R_g
  - ΔRg statistically significant for t > 0.1 ps (p-value < 0.01)
  - Agreement with experiments
  - No evidence of underdamped oscillations or decay
- Multiple photons produce larger change that decays monotonically
- ΔR_g is overdamped when adding extra energy

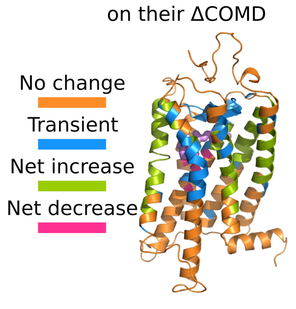
Light-Induced Perturbation Propagates as a Pressure Wave

Signal Propagation in the Protein After Light-Excitation

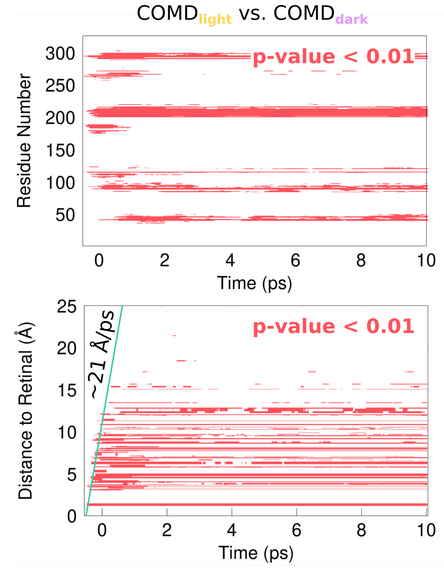


- COMD: projection of $\vec{\mathbf{d}}$ onto $\overrightarrow{\mathbf{Ret}}$
  - COMD < 0: residue gets closer
  - COMD > 0: residue gets farther way
- $\Delta COMD = COMD_{light} - COMD_{dark}$
  - A292 gets farther away from retinal COM
  - Change significant after isomerization

Residues classified based on their ΔCOMD

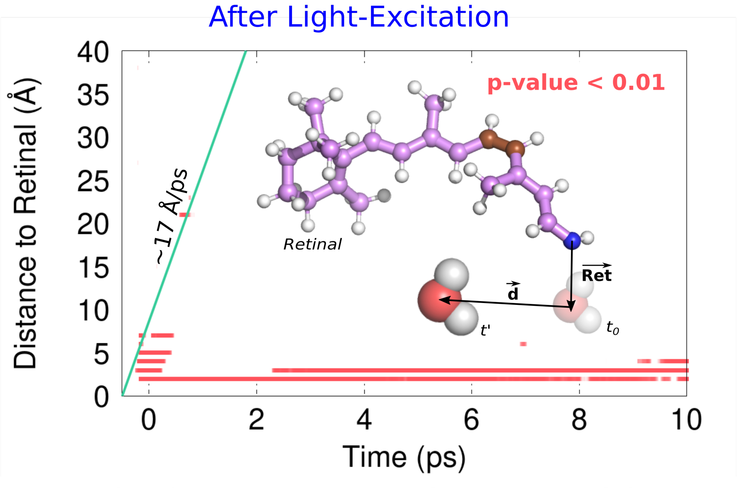


Residues with significantly different $COMD_{light}$ vs. $COMD_{dark}$

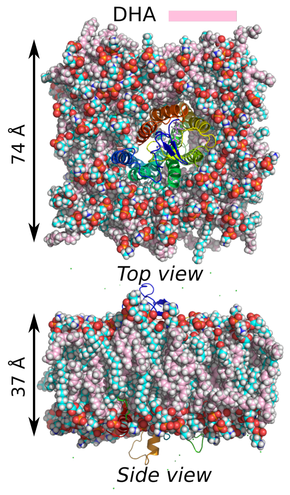


- Residues reordered by their distance to retinal
- Light-induced perturbation propagates at ~21 Å/ps, roughly the speed of sound in proteins
- Signal propagates through protein as a pressure wave

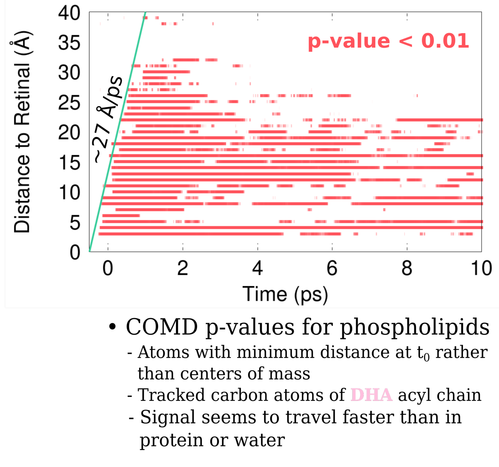
Signal Propagation in the Rest of the System After Light-Excitation



- COMD p-values for water
  - Atoms with minimum distance at t_0 rather than centers of mass

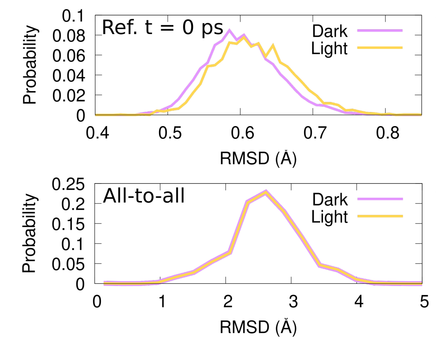


- Signal seems to travel slower than in protein
  - Speed of sound in water is ~15 Å/ps



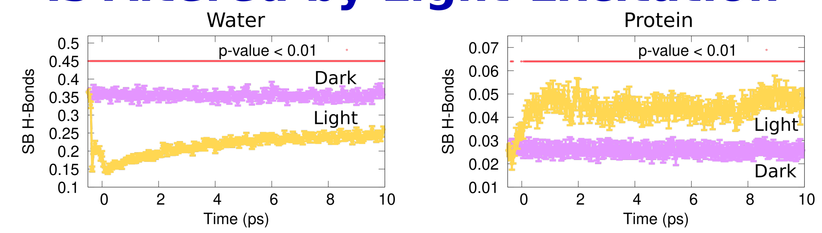
- COMD p-values for phospholipids
  - Atoms with minimum distance at t_0 rather than centers of mass
  - Tracked carbon atoms of DHA acyl chain
  - Signal seems to travel faster than in protein or water

Light-Excitation Shifts Diverse Ensemble

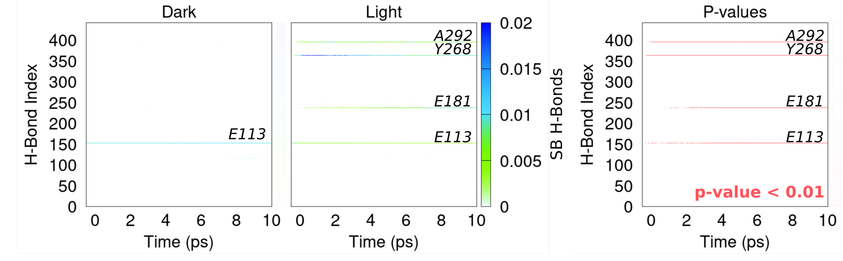


- RMSD of α-helices at 10 ps vs. 0ps
  - Dark = 0.603 Å ± 9E-4
  - Light = 0.619 Å ± 1E-3 (p-value 1E-30)
  - RMSD significantly different
  - Light-excited distribution shifts to the right
- All-to-all RMSD of α-helices at 10 ps
  - Dark = 2.608 Å ± 1E-7 (Std. Dev. 0.555)
  - Light = 2.615 Å ± 1E-7 (Std. Dev. 0.557)
  - Underlying protein ensemble is diverse
  - Well-equilibrated trajectories of dark state required to capture diversity

Binding Pocket H-Bond Network Is Altered by Light-Excitation



- H-bonds between retinal's Schiff base and water molecules
  - H-bonds decrease after light-excitation
- H-bond between retinal's Schiff base and protein oxygen atoms
  - H-bonds increase after light-excitation



- Schiff base H-bonds with protein change after light-excitation
- H-bonds with A292, Y268, E181 only observed after light-excitation

Conclusions

- TR-SWAXS experiments can capture ultra-fast events
  - Extracting structural information is hard
- MD simulations aid experiment interpretation
  - High temporal and spatial resolution
  - Short timescales → many trajectories
- Light-excitation causes an increase in rhodopsin's radius of gyration
  - No underdamped oscillations
- Light-induced perturbation is propagated at the speed of sound
- Light-excitation alters the binding pocket's H-bond network

Future Directions

- Simulate rhodopsin in micelles
  - Experiment → detergent micelles
  - Simulation → lipid bilayer
- Compute scattering profiles from simulations
  - Compare to experimental profiles
  - Calculate solvent contributions
- Compare bilayer vs. micelles simulations
  - Analyze dark state conformational ensemble in micelles
  - Seed thousands of new pairs of dark/light simulations
  - Do we observe the same effects?

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Data analysis was performed using the Lightweight Object-Oriented Structure Library (LOOS), an open source C++ and Python library for MD analysis developed by the Grossfield lab:
https://github.com/GrossfieldLab/loos