Rhodopsin's Ultra-Fast Activation Dynamics in Micelle and Bilayer Environments

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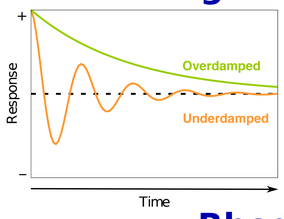
Poster PDF
http://tinyurl.com/rhod-act-dyn

Abstract

Dark-adapted vision in mammals starts with the absorption of a photon and the activation of rhodopsin, a G protein-coupled receptor. The early stages of rhodopsin activation involve the cis-to-trans isomerization of the receptor's ligand (retinal) and a relaxation process that drives the receptor through several non-equilibrium intermediates. The structural information available that describes the femtosecond-to-picosecond scale changes involved is limited. Time-resolved small- and wide-angle X-ray scattering with free electron lasers can provide insights into the functional protein dynamics that take place at these timescales. However, extracting structural information from scattering data is challenging. Here, we use all-atom dynamics simulations to aid the interpretation of this type of experiment. Starting from well-equilibrated dark-state simulations of bovine rhodopsin, we run and analyze thousands of 10 ps trajectories in two environments—micelles and bilayers—and two conditions—dark and light-excited—to model the process of energy dispersion across the receptor after light-excitation.

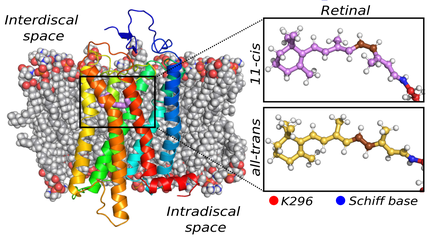
What Happens to Proteins After Being Struck by a Force?



- Proteins dissipate energy in the form of waves → proteinquake
- Relaxation thought to result in overdamping of global motions
- Myoglobin:
  - TR-SWAXS → underdamped
  - MD → overdamped

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

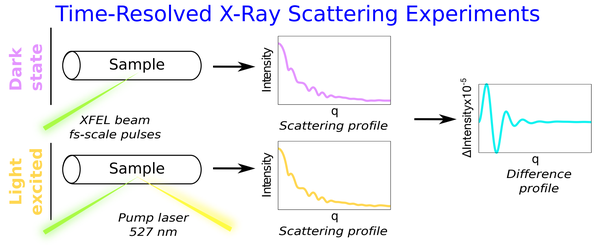
Rhodopsin



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

- Photoreceptor in stacked membranes of rod cells in the retina
- Mediates dark-adapted vision
- Light-excitation isomerizes ligand
- Early activation events occur at the sub-nanosecond timescale
- Limited information on ultra-fast dynamics

Time-Resolved X-Ray Scattering Experiments

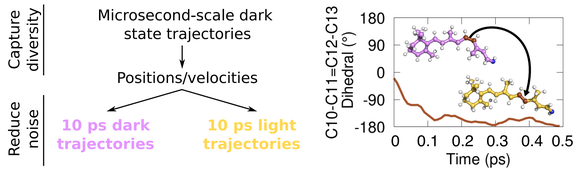


- Samples probed with fs-scale X-ray pulses
  - One sample excited with a 527 nm laser
  - Scattering profiles recorded at different time delays after light-excitation
- Experiment captures changes in size/shape at high time resolutions
  - Limited spatial/structural information
- Simulations give atomic-level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

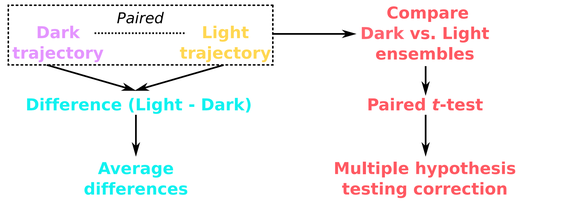
Simulation Details

- System composition:
  - Micelles: dark rhodopsin; 40 CHAPS; 39,378 waters; 0.15 M NaCl
  - Bilayers: dark rhodopsin; 123 SDPE; 8,000 waters; 0.1 M NaCl
- Box size:
  - Micelles: 109 Å x 109 Å x 109 Å
  - Bilayers: 74 Å x 74 Å x 94 Å
- Force field: CHARMM27/36
- Barostat: 1.01 bar (Langevin)
- Electrostatics cutoff: 10 Å (PME)
- VDW cutoff: 10 Å
- Timestep: 2 fs (RATTLE)
- Simulation package:
  - Micelles: NAMD 2.13 on Summit
  - Bilayers: NAMD 2.8 on BlueGene/Q

Calculating Light-Induced Changes from Simulations



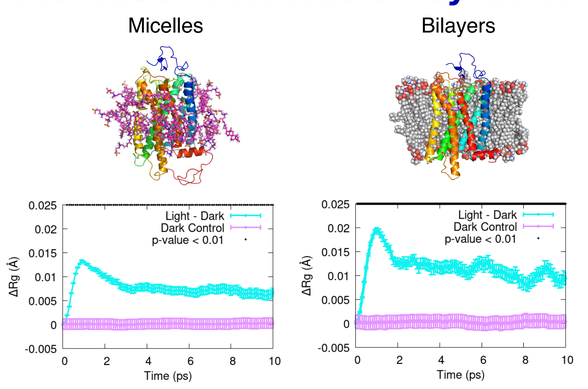
- Photon absorption modeled classically
- Quantum excitation only lasts ~10-100 fs
- Retinal isomerization induced by barrier to cis conformation<0.5 ps
- Energy of 527 nm photon added by rescaling ligand velocities



- 8,016 micelle pairs and 2,954 bilayer pairs
  - Error bars: standard error of the mean
  - Dark controls: randomly pairing dark trajectories with replacement
- Multiple hypothesis testing with Holm-Šídák algorithm
  - Implementation from `StatsModel`

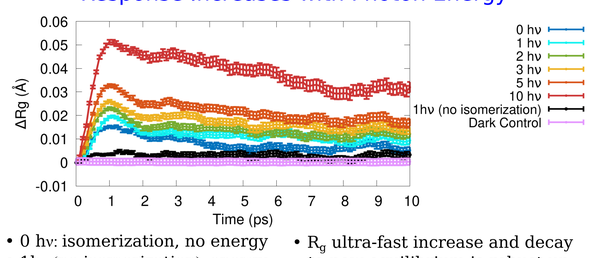
Seabold et al., Proceeding of the 9th Python in Science Conference, 2010

Light-Excitation Induces Increase in Radius of Gyration



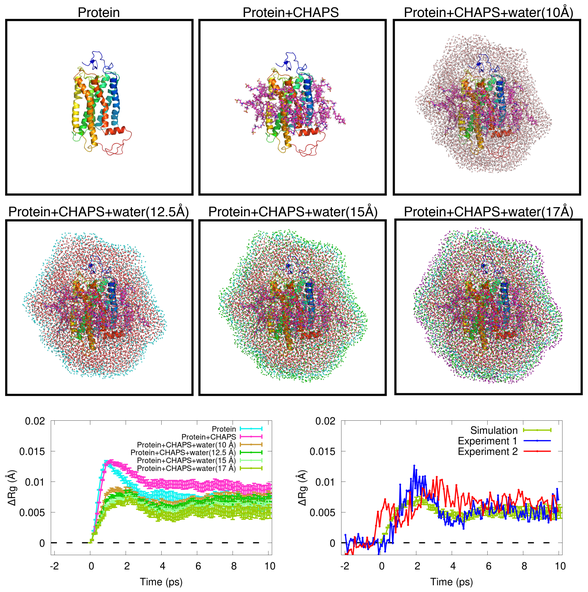
- Light-excitation produces an ultra-fast increase in protein's R_g
  - ΔR_g statistically significant after 100 fs (p-value < 0.01)
- Protein ΔR_g peaks at 900 fs, then decays monotonically to new mean
  - No evidence of underdamped oscillations or decay

Response Increases with Photon Energy



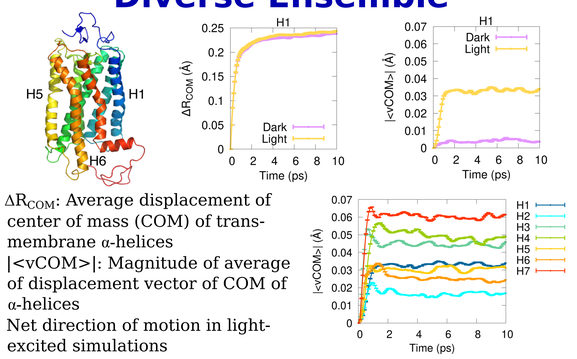
- 0 hν: isomerization, no energy
- 1hν (no isomerization): energy added without isomerization
- R_g ultra-fast increase and decay to new equilibrium is robust up to several hν's worth of energy

Solvent Contributions Dominate Ultra-Fast Response



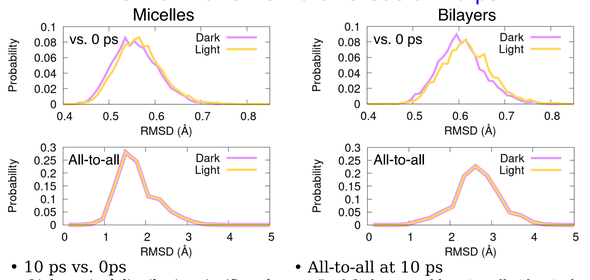
- Solvent slows down response
  - Blast wave behavior after light-excitation
  - Protein dynamics AND environment contribute to signal
- Experimental analysis is complex
  - Experiment 1 and Experiment 2: two different protocols to analyze the same experimental data
  - Analysis still under way

Light-Excitation Shifts Diverse Ensemble



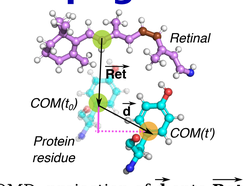
- ΔR_{COM}: Average displacement of center of mass (COM) of trans-membrane α-helices
- |<vCOM>|: Magnitude of average of displacement vector of COM of α-helices
- Net direction of motion in light-excited simulations

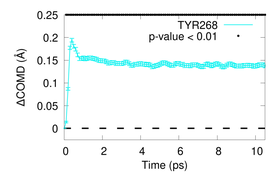
RMSD of Transmembrane Cαs at 10 ps



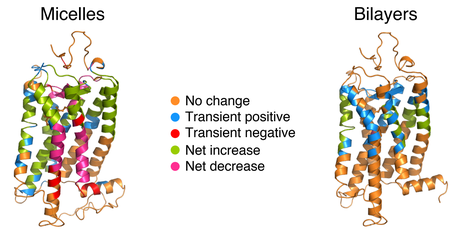
- 10 ps vs. 0ps
  - Light-excited distribution significantly shifted to the right (p-value < 1E-30)
  - Pairs needed to capture small change
- All-to-all at 10 ps
  - Dark/light ensembles virtually identical
  - Dark state diversity is larger than perturbation

Light-Induced Perturbation Propagates as Pressure Wave

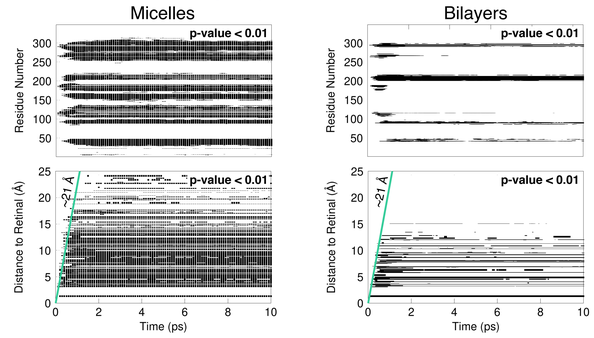




- COMD: projection of $\vec{\mathbf{d}}$ onto $\overrightarrow{\mathbf{Ret}}$
  - COMD < 0: residue gets closer
  - COMD > 0: residue gets farther way
- $\Delta COMD = COMD_{light} - COMD_{dark}$
  - Residues classified based on their ΔCOMD and mapped onto structure of dark state



Statistically Significant Perturbation



- Residues with significantly different $COMD_{light}$ vs. $COMD_{dark}$
  - By number or reordered by distance to retinal
- Light-induced perturbation propagates at ~21 Å/ps
- Speed of sound in proteins

Conclusions

- Rhodopsin relaxation occurs in an overdamped fashion
- Photoactivation is characterized by an ultra-fast increase in R_g that decays to a new mean in a few ps
- Solvent modulates and slows down response
- Activation induces propagation of pressure wave
  - Excess energy is dissipated at speed of sound in the protein
- Dissipative process also occurs under more physiologically relevant conditions

Future Directions

- Compute scattering profiles from simulations
  - Compare to experimental profiles
- Does retinal isomerization alter correlation of residue motions?
  - Ligand-binding pocket? Rest of protein?
- Use higher levels of detail and QM calculations to describe excited state of receptor
  - Light-excitation of chromophore is intrinsically a QM process
  - Do we observe the same effects?

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