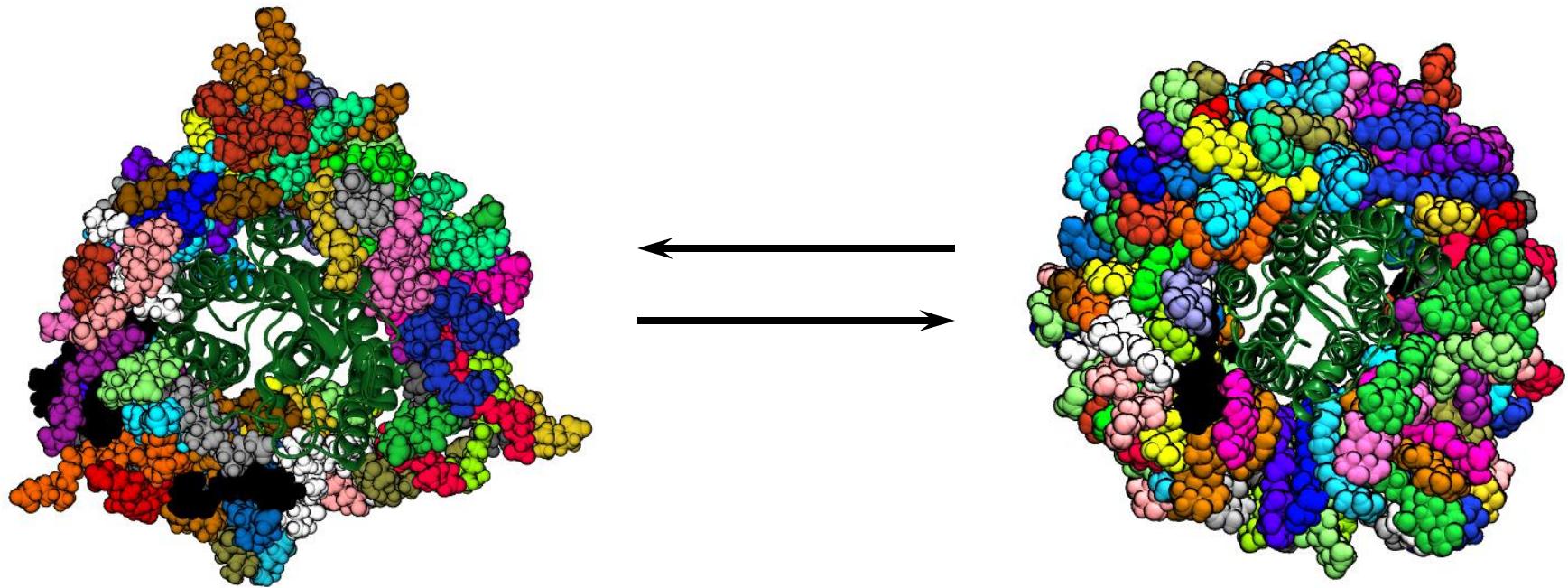


Using molecular dynamics to probe the activation of rhodopsin



Blake Mertz

June 16, 2014 WVU HPC Summer Institute

2012 Nobel prize in chemistry



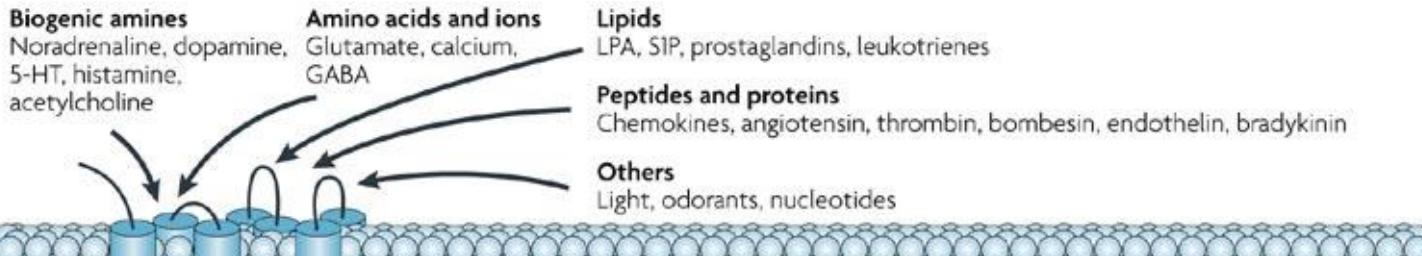
Robert J. Lefkowitz

Brian K. Kobilka

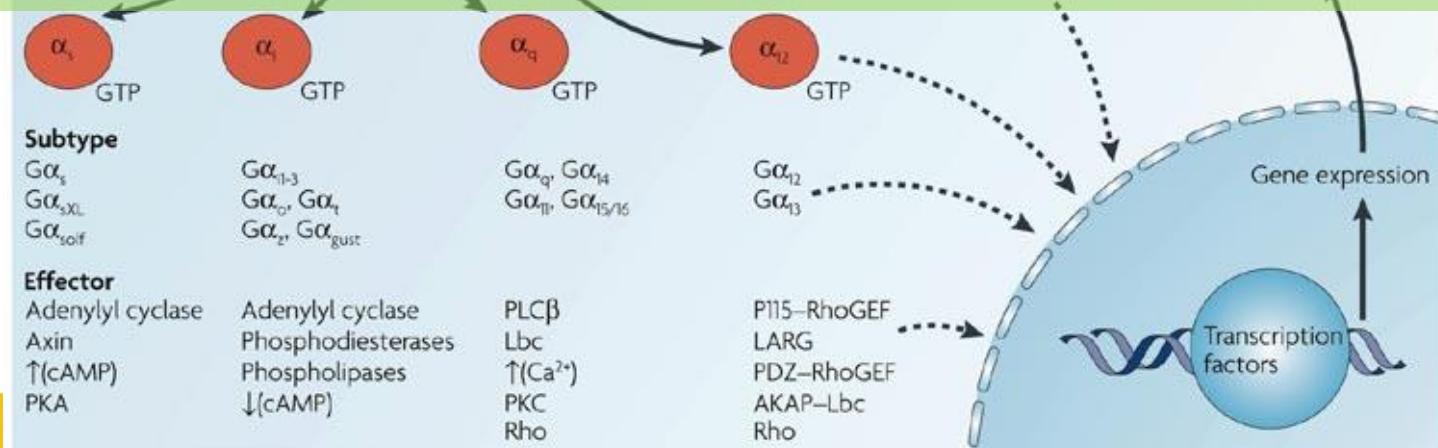
- Bob Lefkowitz (Duke) & Brian Kobilka (Stanford)
- Awarded for seminal work in establishing the role of **G protein-coupled receptors** (GPCRs) in cellular signaling



What is a GPCR?

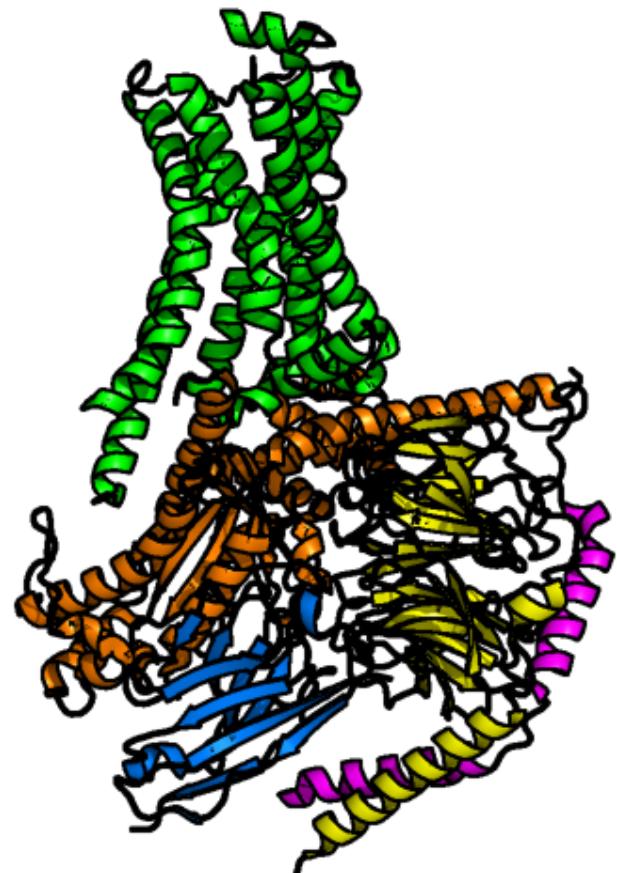


GPCRs represent almost 50% of all drug targets



And why are Lefkowitz & Kobilka so important?

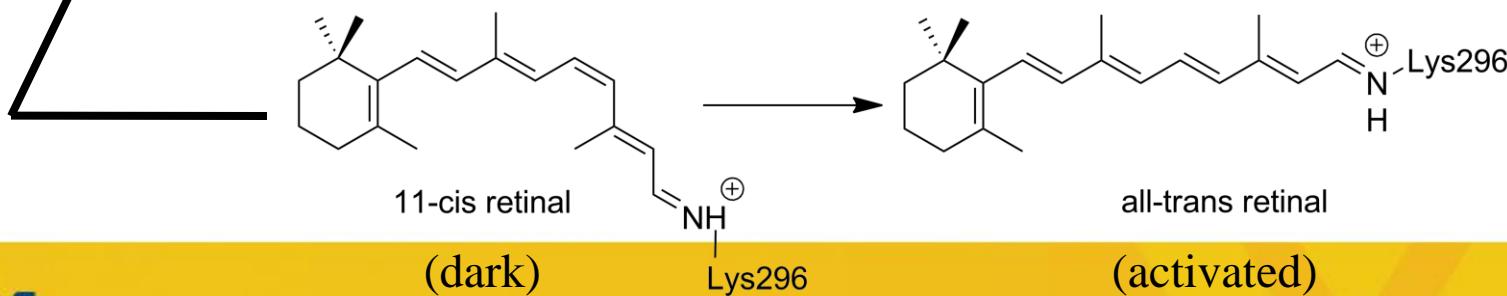
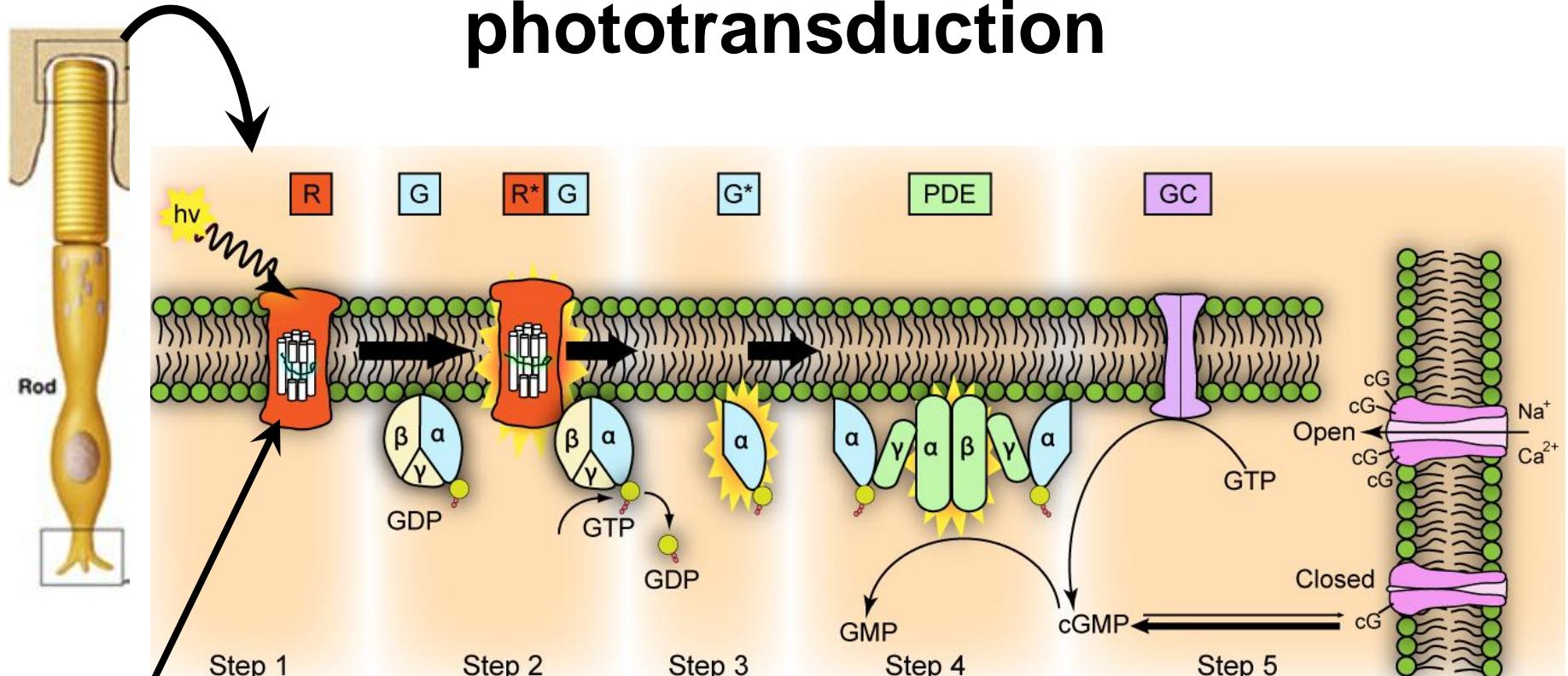
- Discovered GPCRs back in 1970s
- Kobilka isolated gene for β_2 -adrenergic receptor (activated by adrenaline) in 1980s
- Wasn't until 20+ years later (2011) that Kobilka published crystal structure of an active beta-adrenergic receptor with its cognate G protein, G_s



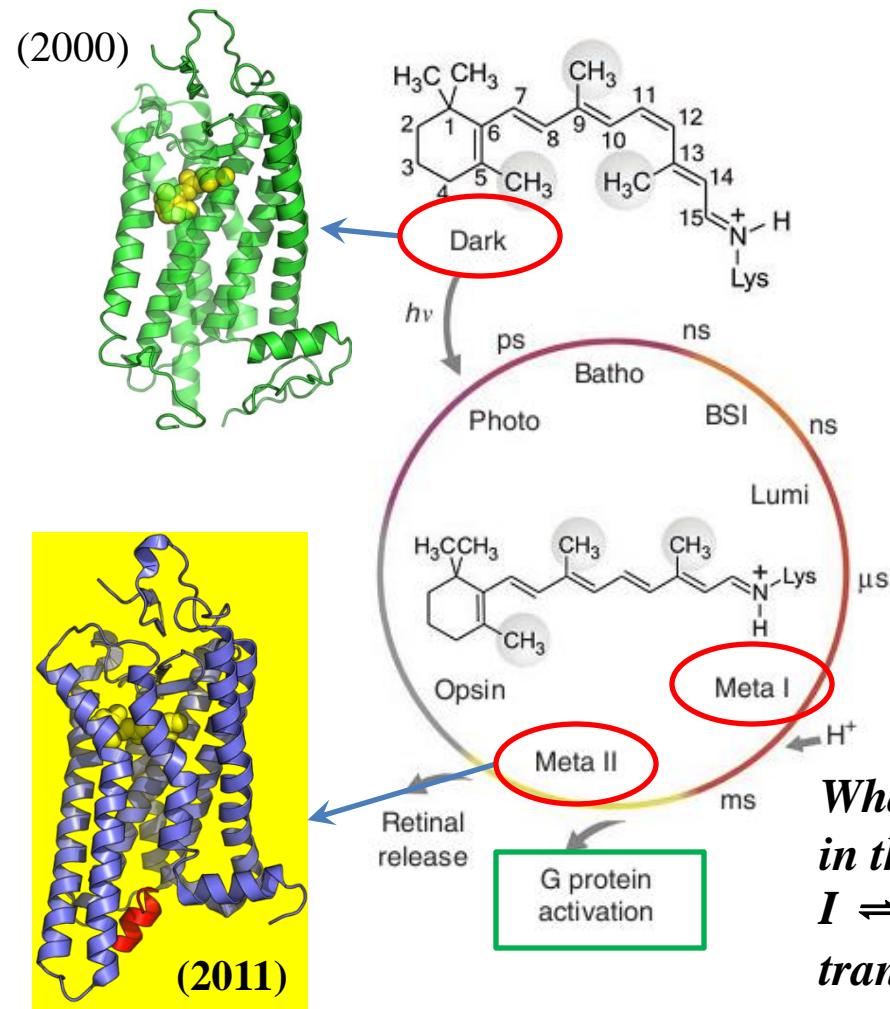
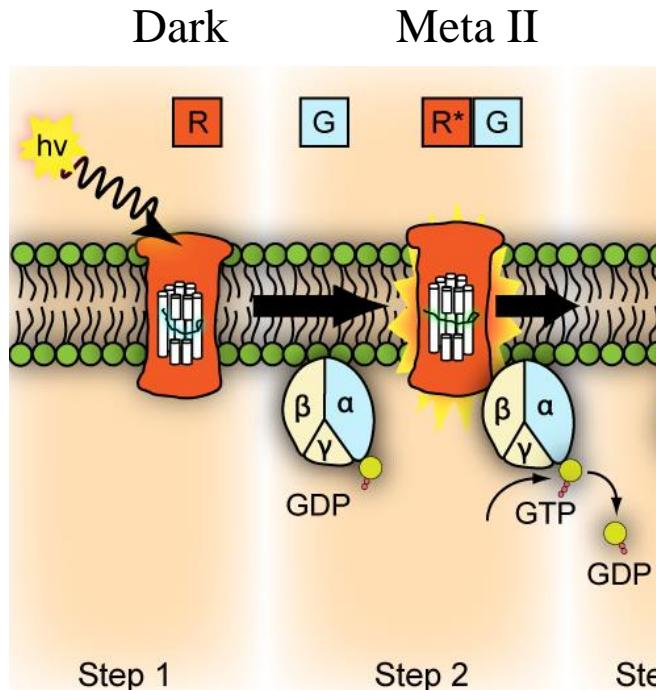
Rasmussen *et al.*, *Nature* (2011) 477: 549



Rhodopsin photocycle & vertebrate phototransduction



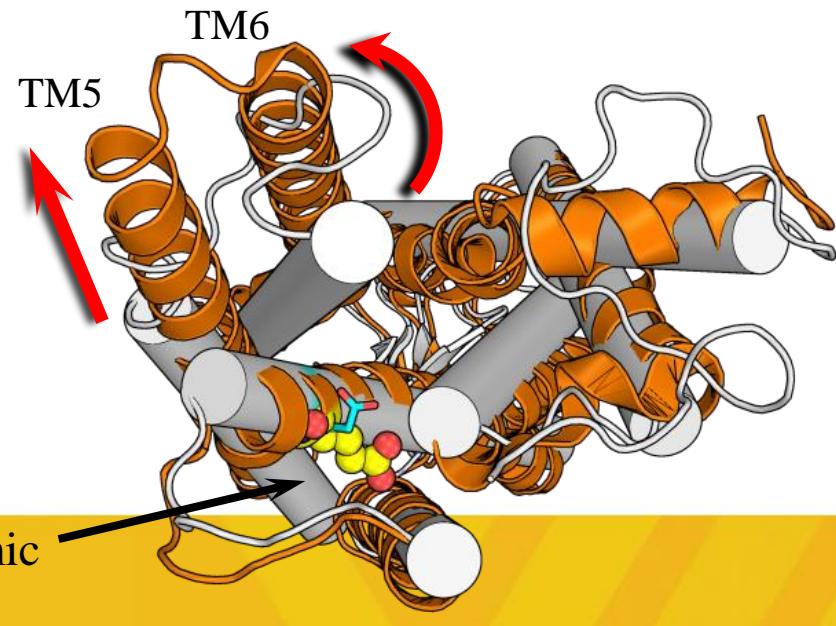
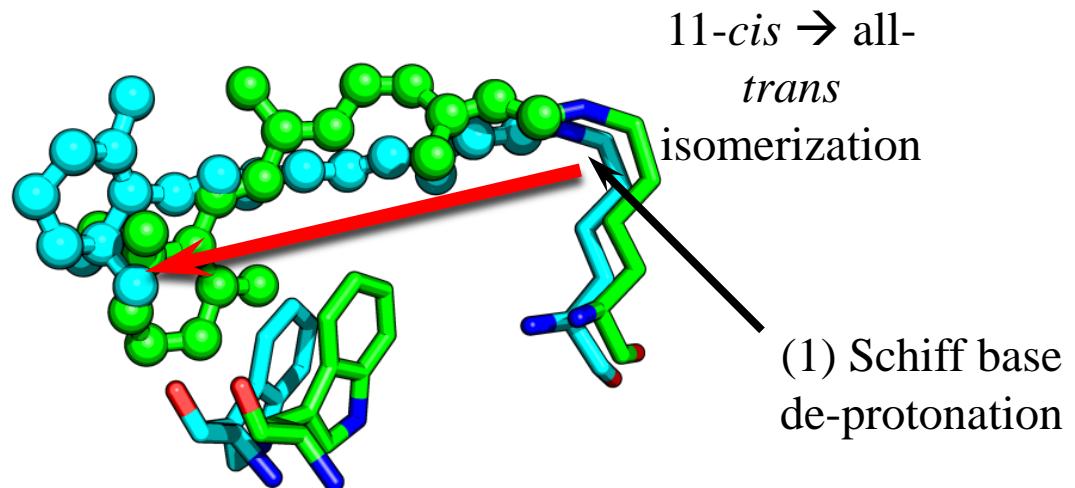
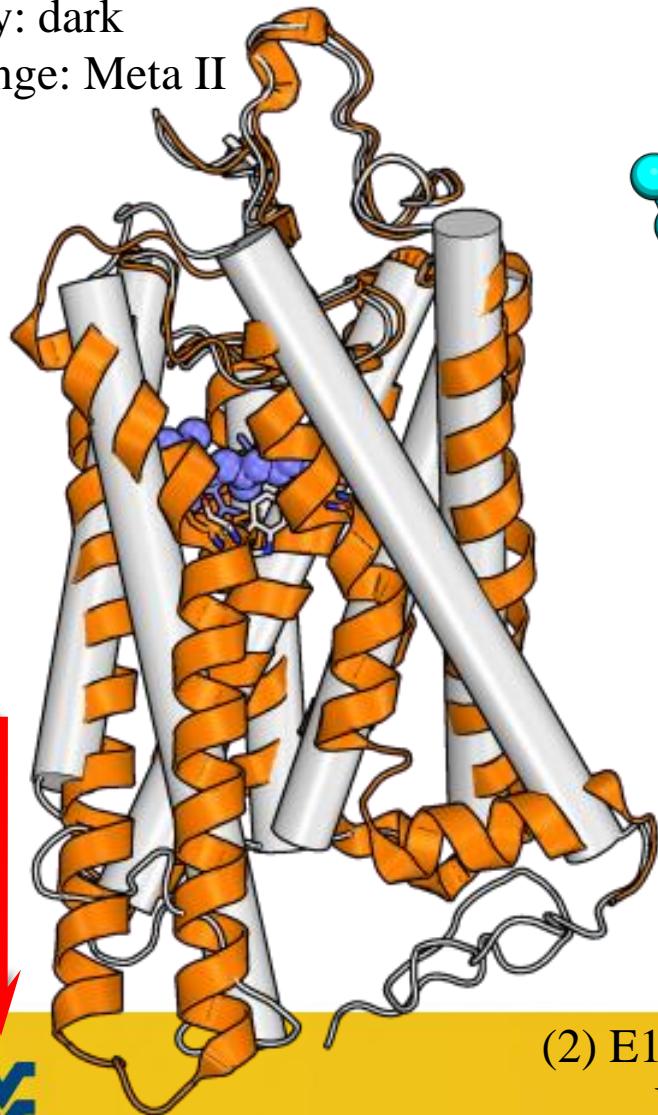
Structural features of rhodopsin activation



Structural features of rhodopsin activation

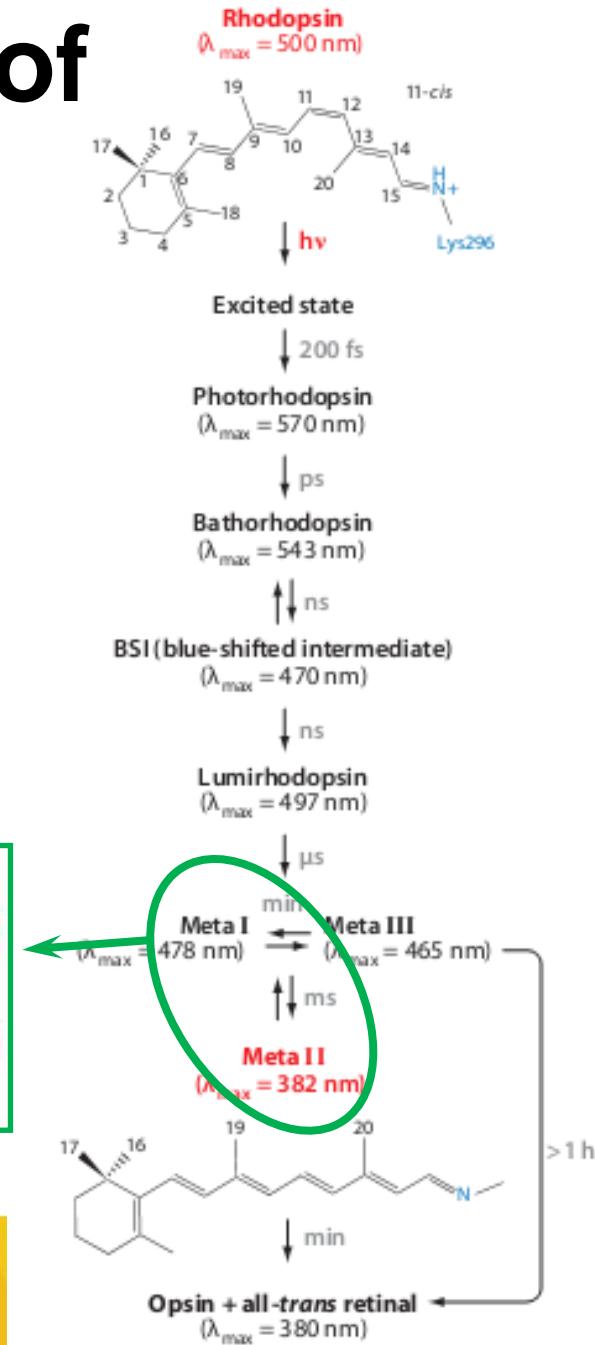
Grey: dark

Orange: Meta II



Spectroscopic features of rhodopsin activation

- Each photointermediate has specific absorption wavelength
- Markers for four distinct substates in $\text{Meta I} \rightleftharpoons \text{Meta II}$



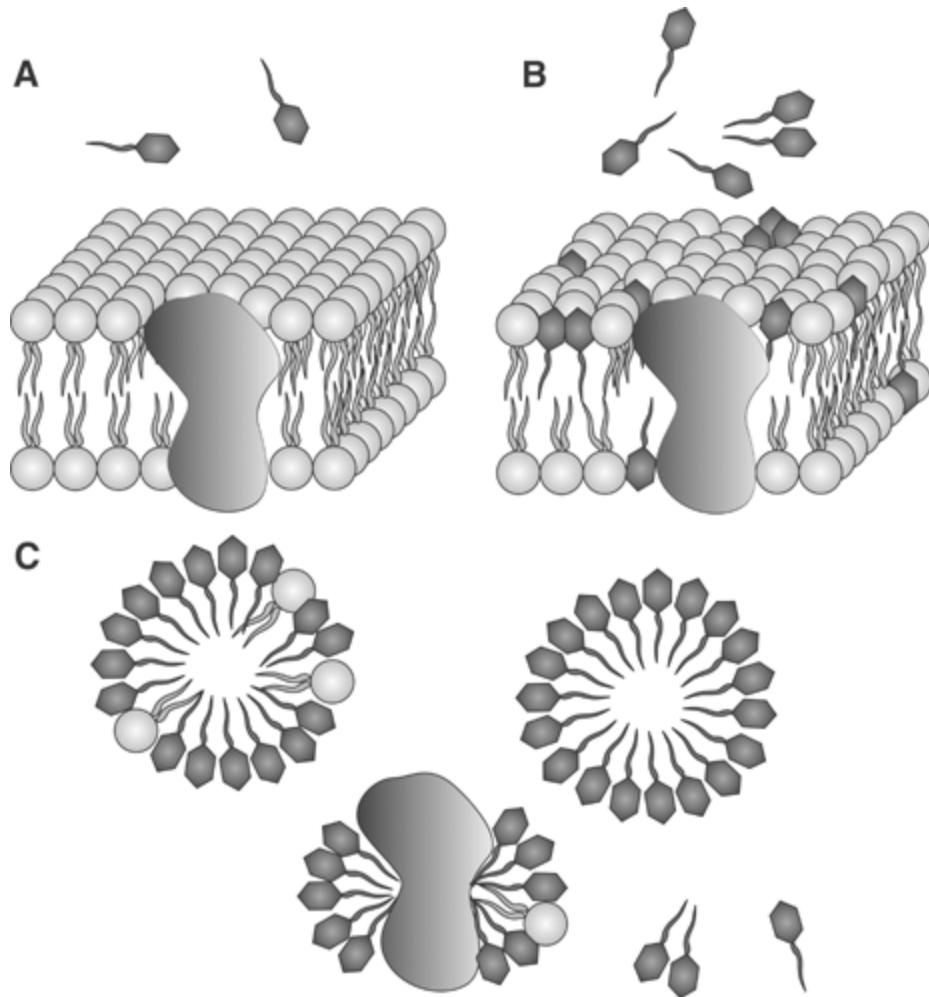
Issues to address

1. How can we get past timescale limitations from the Meta I \rightleftharpoons Meta II transition (ms)?
2. What are the dynamical processes that occur during rhodopsin activation? (Meta I \rightleftharpoons Meta II transition)
3. Why is the retinal polyene chain flipped in the Meta II structure?



Soap & GPCRs

- Integral membrane proteins such as GPCRs need a membrane-like environment to stay properly folded and functional
- Lipid bilayer makes it impossible to diffract high-resolution crystals of GPCRs
- Amphiphilic (both hydrophobic & hydrophilic) detergents mimic this environment and make it possible to diffract crystals

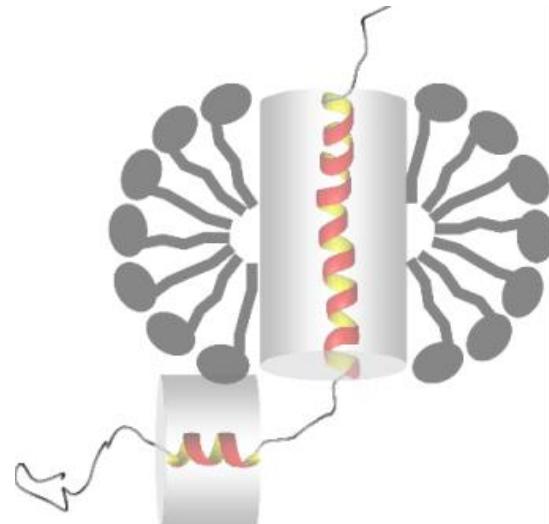


Arnold & Linke, *Curr. Prot. Prot. Sci.* (2008)

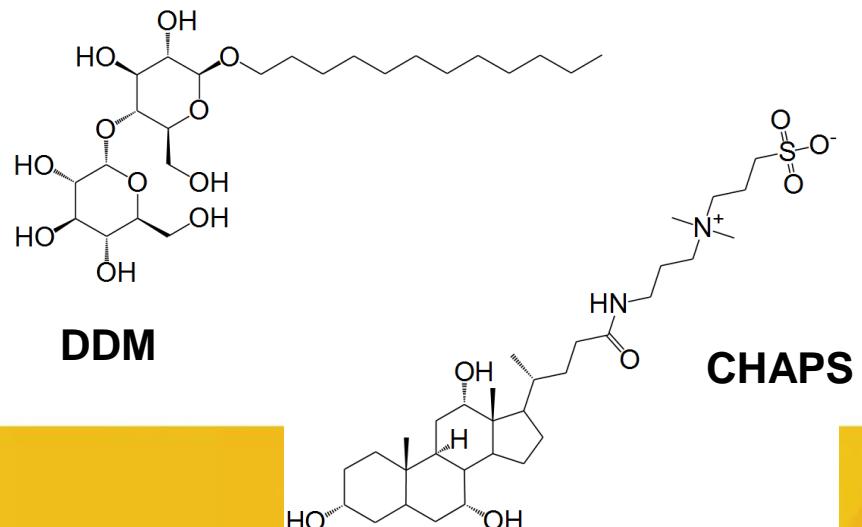


Our idea....

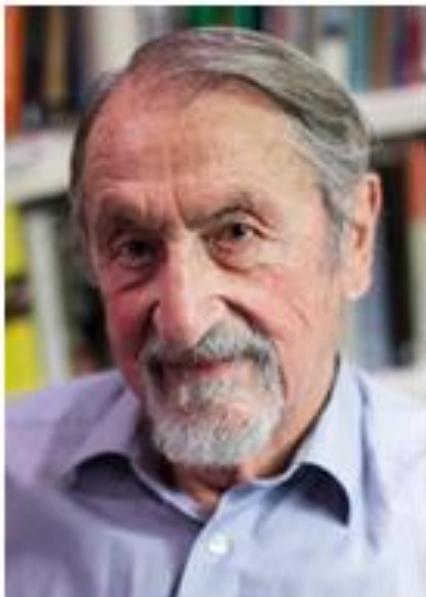
- Use rhodopsin in detergent micelle to duplicate experimental conditions
- Choose specific detergents to manipulate function of rhodopsin
 - 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate (**CHAPS**): backshifts towards Meta I
 - Dodecyl β-maltoside (**DDM**): forward shifts towards Meta II



http://www2.fz-juelich.de/isb/isb-3/topics/virus_proteins/cd4



2013 Nobel Prize in Chemistry



© Nobel Media AB

Martin Karplus



Photo: Keilana via
Wikimedia Commons

Michael Levitt

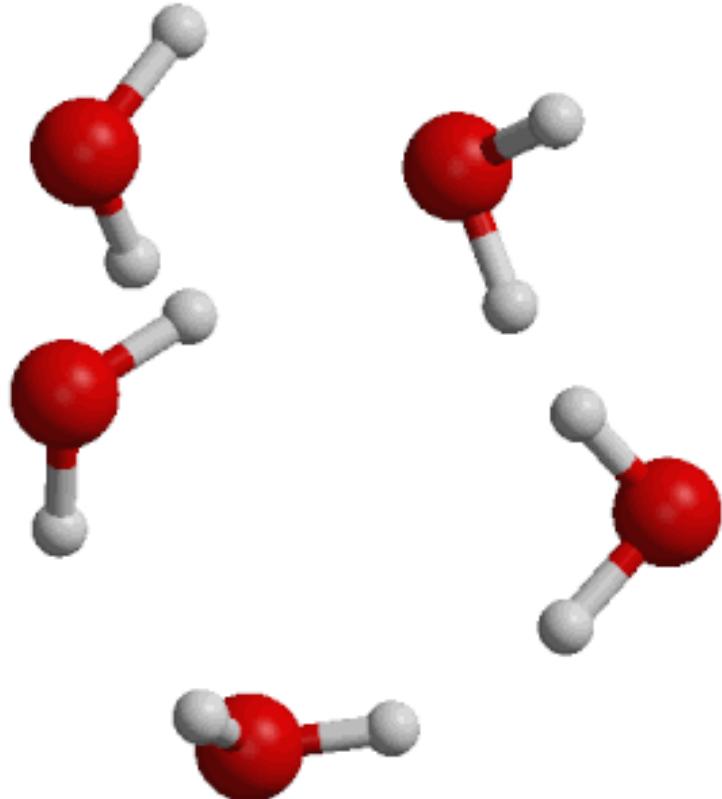


Photo: Wikimedia
Commons

Arieh Warshel

The Nobel Prize in Chemistry 2013 was awarded jointly to **Martin Karplus**, **Michael Levitt** and **Arieh Warshel** *"for the development of multiscale models for complex chemical systems"*.

Molecular dynamics



$$\mathbf{F} = m\vec{a}$$

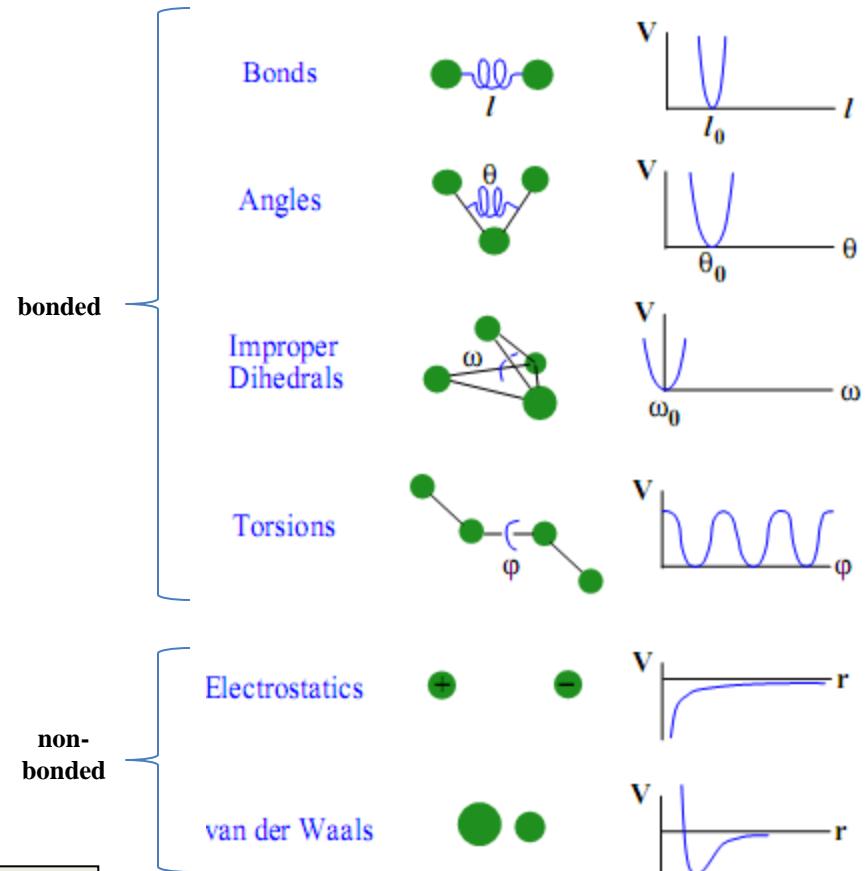
Molecular dynamics primer

$$F_i = m_i \cdot a_i = m_i \cdot \frac{dv_i}{dt} = m \cdot \frac{d^2r_i}{dt^2}$$

$$F_i = -\nabla_i V$$

- Solving Newton's 2nd equation of motion to examine molecular motions and thermodynamics
- Force related to acceleration, which can be related to potential energy
- Model potential energy with a molecular mechanics force field that includes bonded and non-bonded interactions
- Dihedral parameter models 1-4 interactions

$$\boxed{E = E_{bond} + E_{angle} + \\ E_{dihedral} + E_{vdW} + E_{electrostatic}}$$



Steinbach, P.J. http://cmm.cit.nih.gov/intro_simulation



Simulation details

- Rhodopsin structure: Meta II (PDB 3PXO)
- Detergent parameters: Abel *et al.* (2011) *J. Phys. Chem. B* (DDM) and paramchem.org (CHAPS)
- Packmol for micelle-rhodopsin system construction
- NAMD 2.9
- Each simulation is in *NPT* ensemble (constant number of atoms (*N*), constant pressure (*P*), constant temperature (*T*))
- CHAPS: 80 detergent molecules, 142 ns
- DDM: 153 detergent molecules, 276 ns

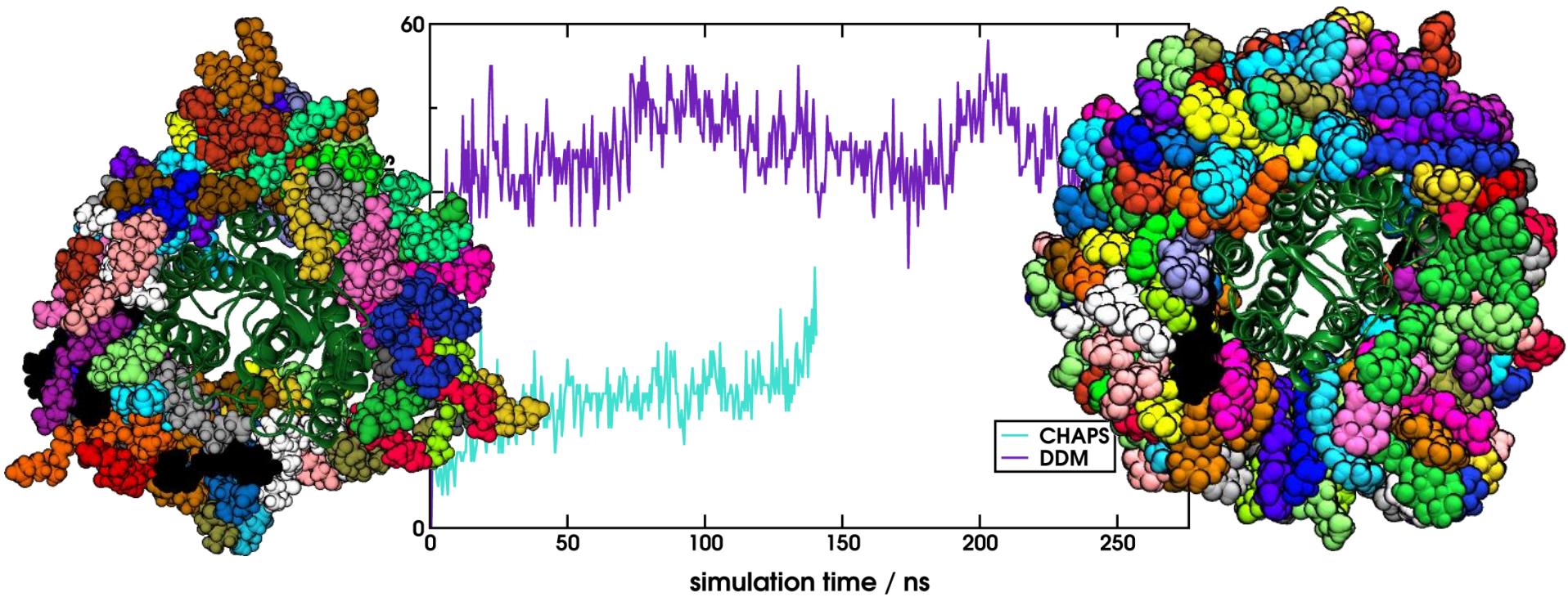
Key protonations in rhodopsin Meta states				
		Schiff base	Glu113	Glu134
CHAPS	Meta I	+	-	-
DDM	Meta II	-	+	+



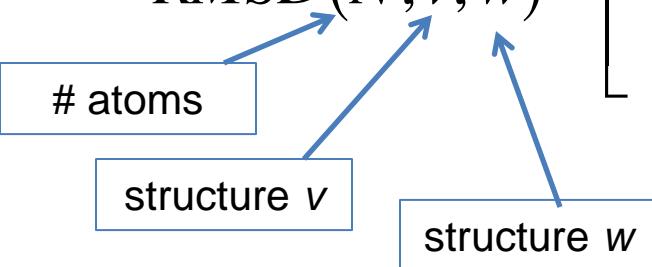
Analyzing MD results



Micelle formation

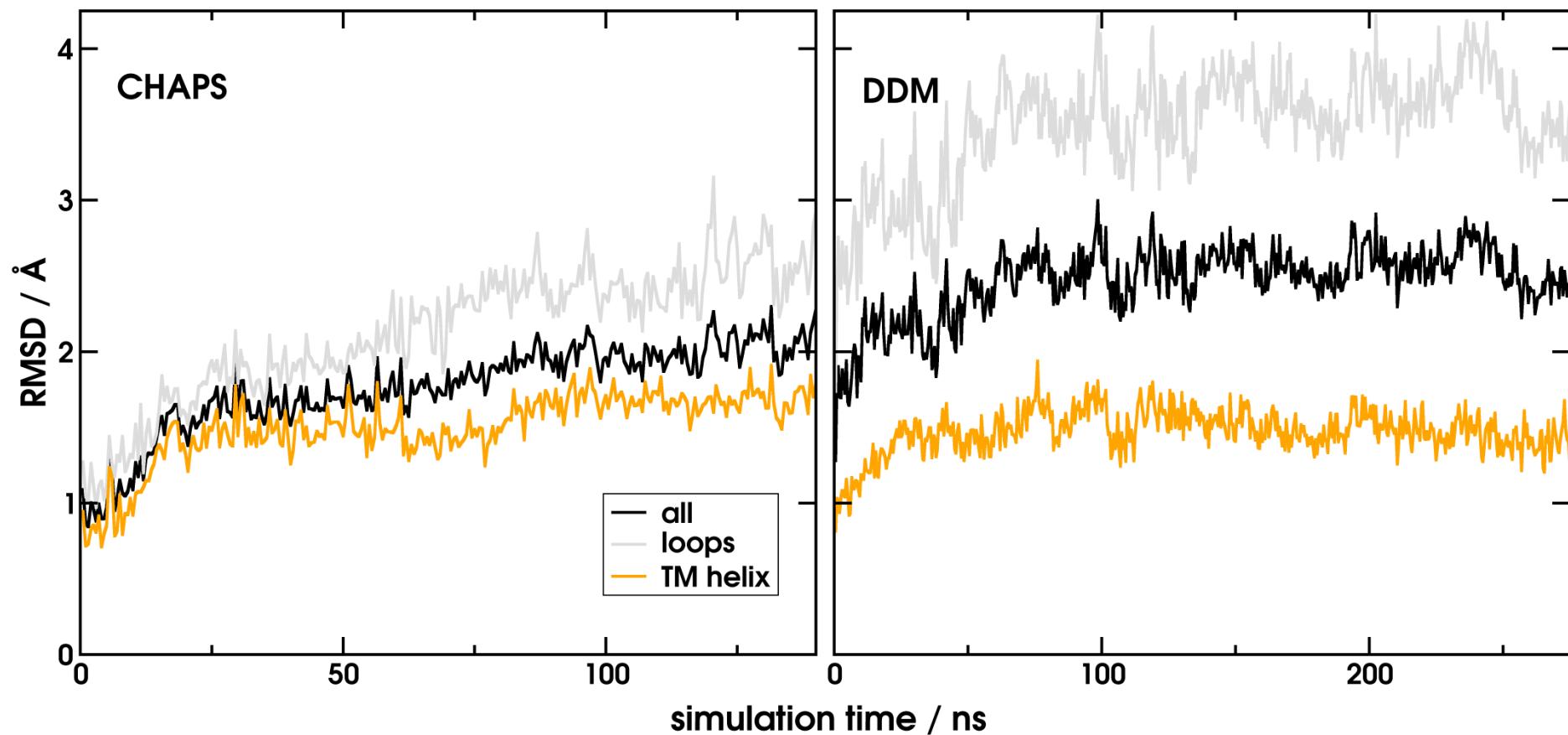


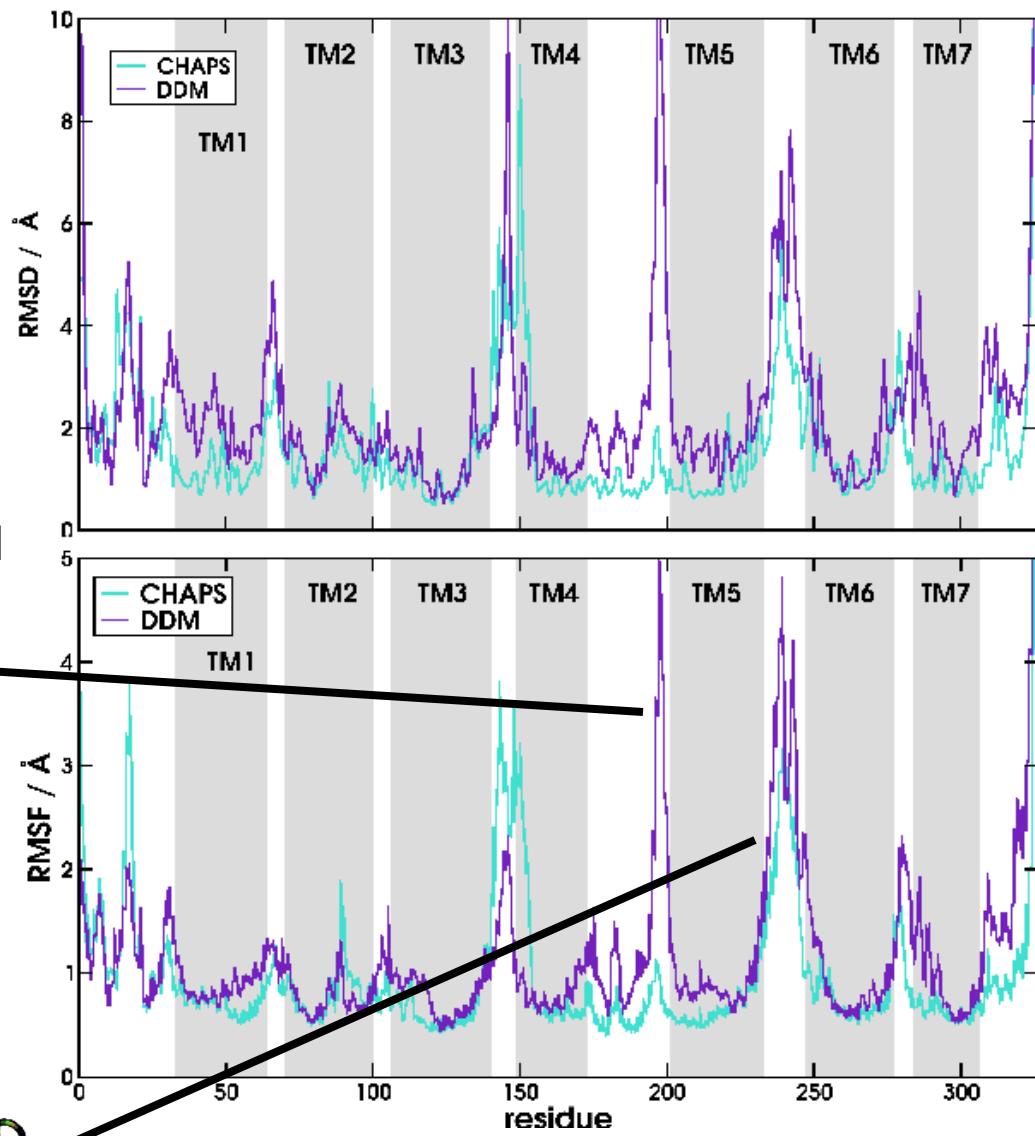
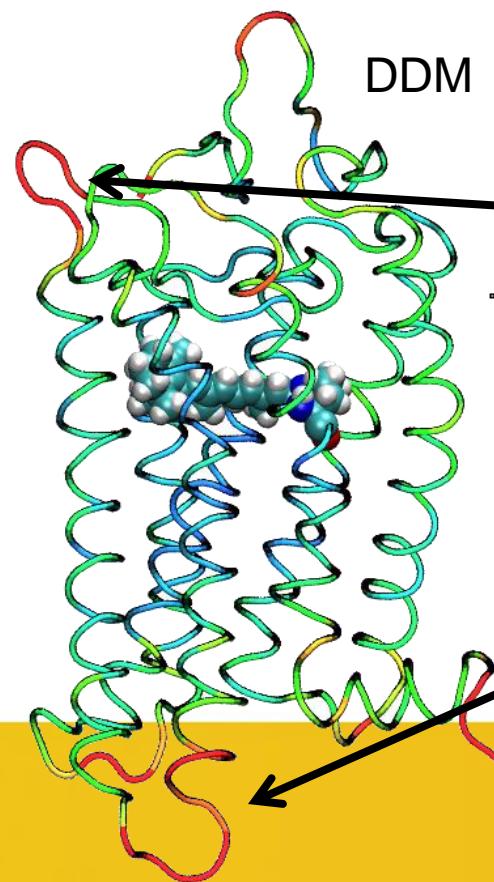
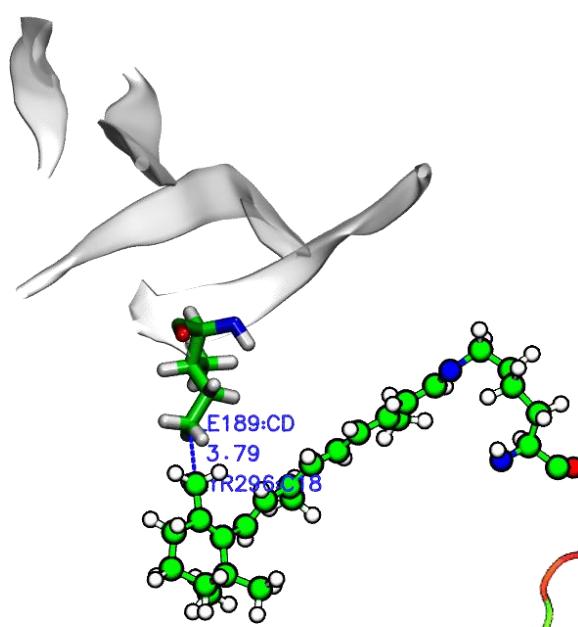
RMSD

- Root-mean-square deviation:
$$RMSD(N; v, w) = \left[\frac{\sum_{i=1}^N \|v_i - w_i\|^2}{N} \right]^{\frac{1}{2}}$$

- Used to understand how much your structure changed during the simulation
- Typically, the RMSD on heavy-atoms should not change more than 3 Å during an MD simulation
- Important for this particular system because we don't know how unstable protein will be in detergent micelle

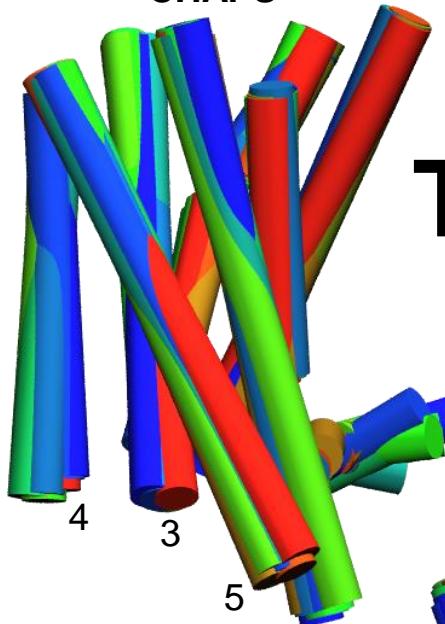


RMSD



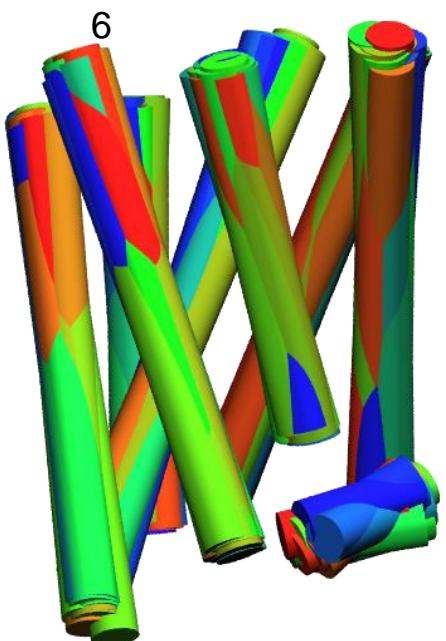


CHAPS

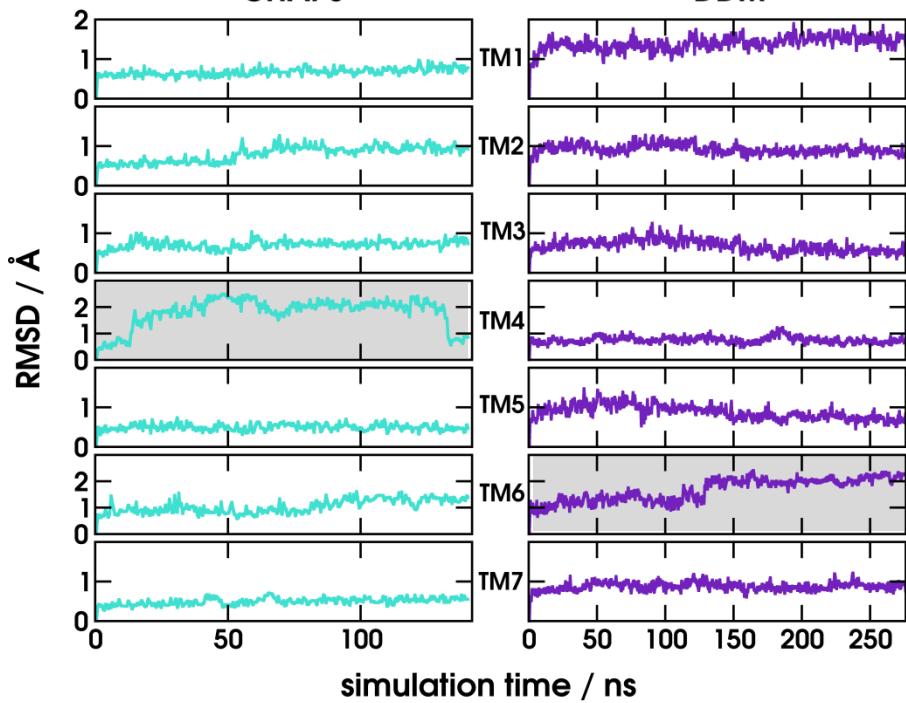


TM RMSD

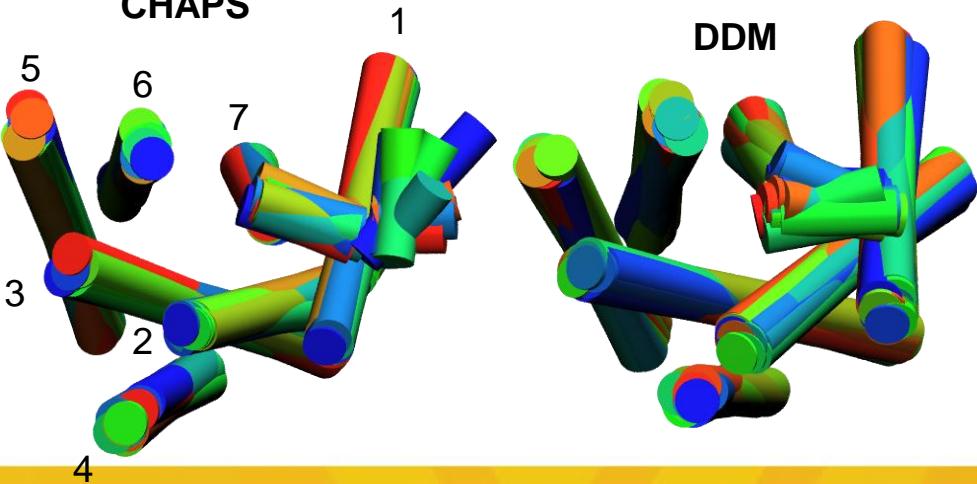
DDM



CHAPS



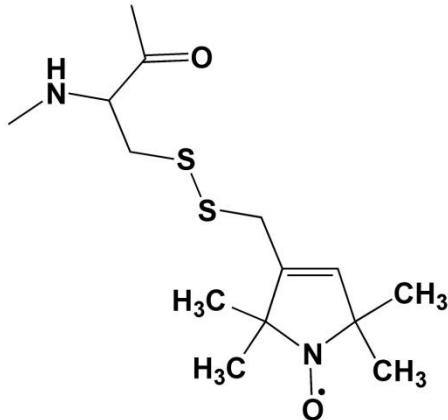
CHAPS



DDM



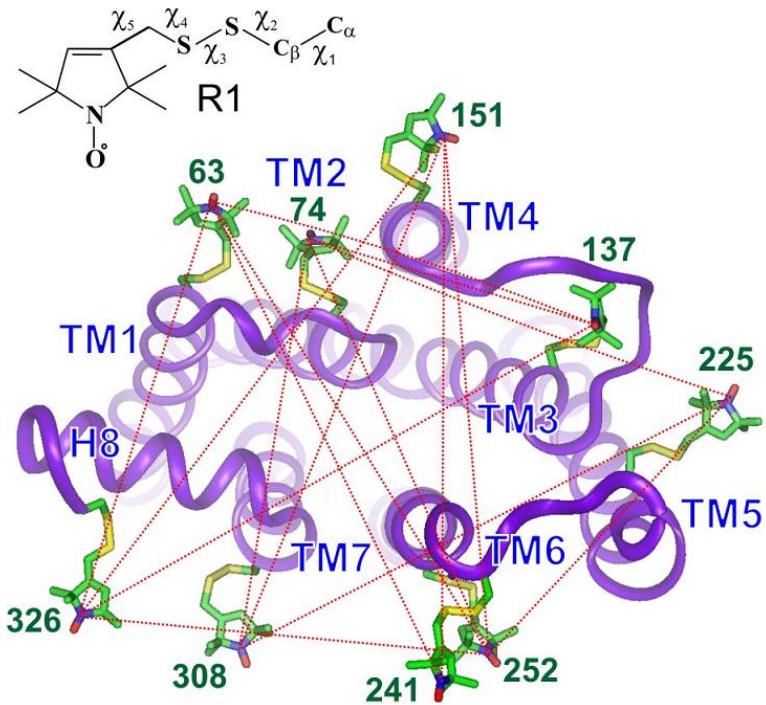
Double electron-electron resonance (DEER) spectroscopy



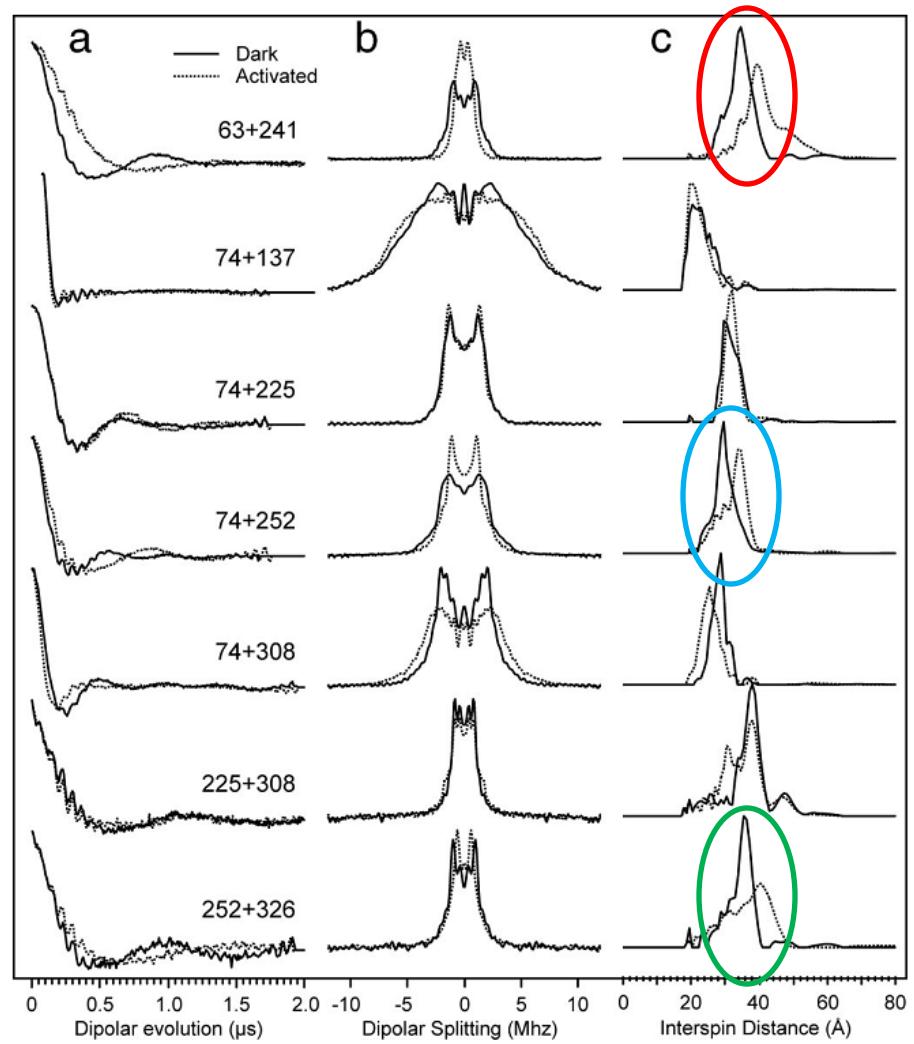
- Two separate microwave frequencies to examine coupling between two electron spins to make a distance measurement, typically between two nitroxide spin labels
- Interaction distances can be detected in 18-60 Å range
- Useful for membrane proteins



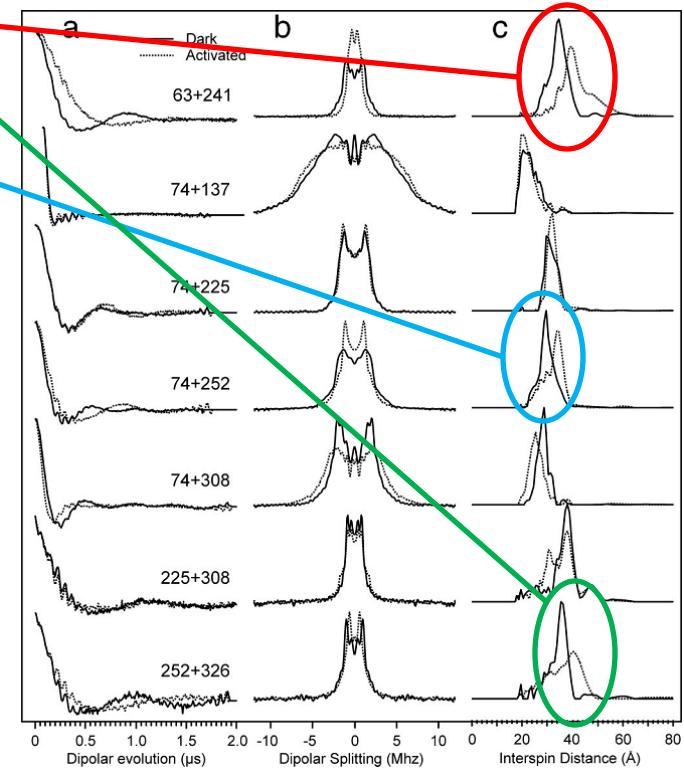
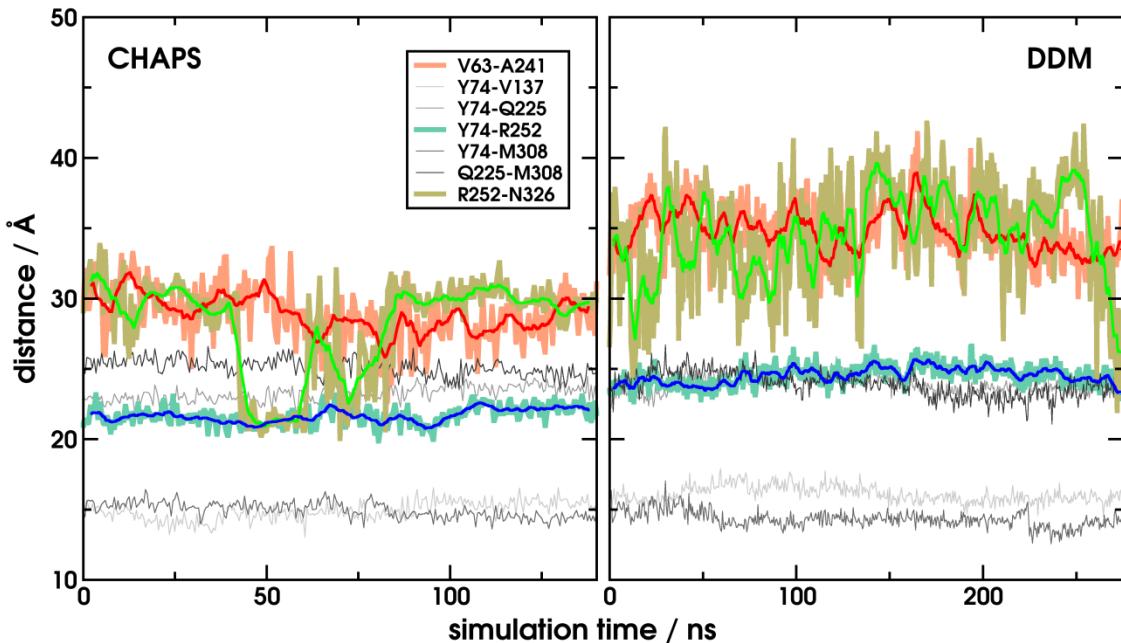
Rhodopsin studies using DEER



- Altenbach *et al.* (2008) PNAS 105: 7439. (Wayne Hubbell group, UCLA)
- DEER study on rhodopsin in dark and Meta II states
- TM5 & TM6 specific area of focus



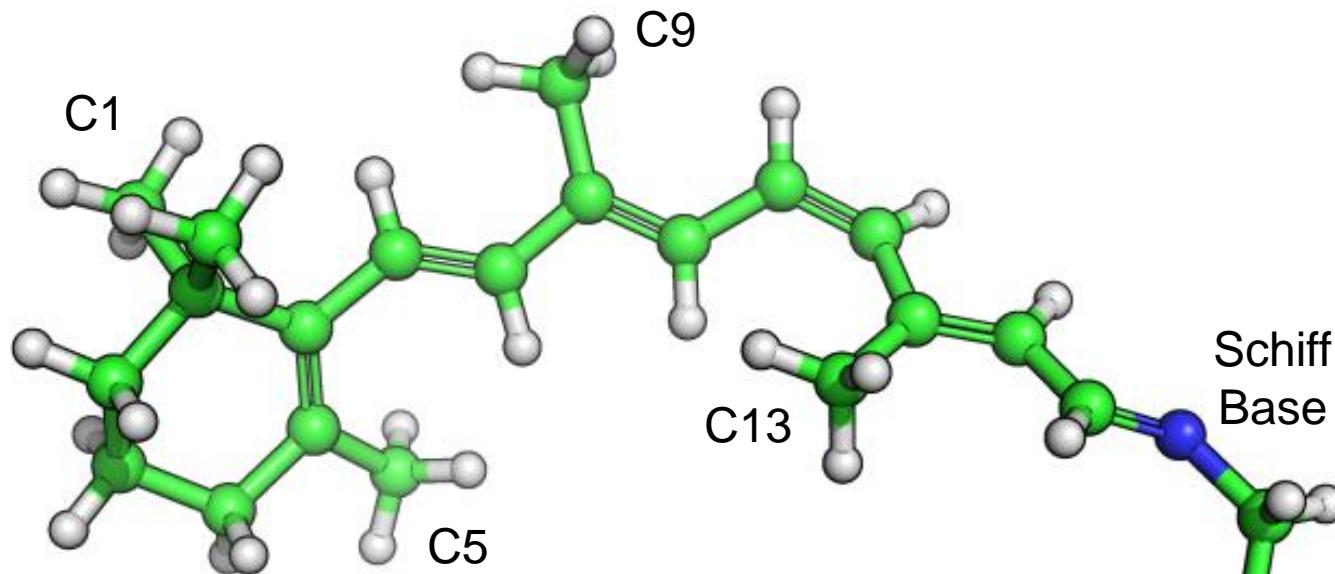
Rhodopsin studies using DEER



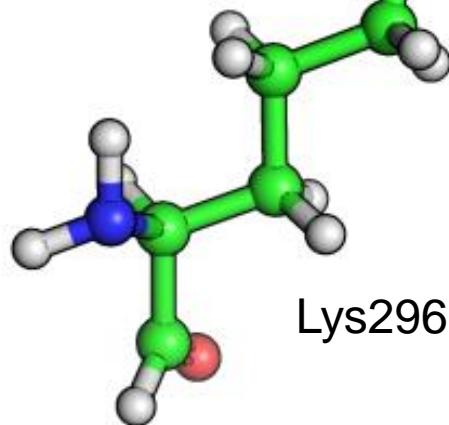
- Outward rotation of TM6 and helical tilt of TM5 do NOT occur in Meta I
- Should expect decrease in distances towards more dark-like state



Retinal as a rhodopsin marker

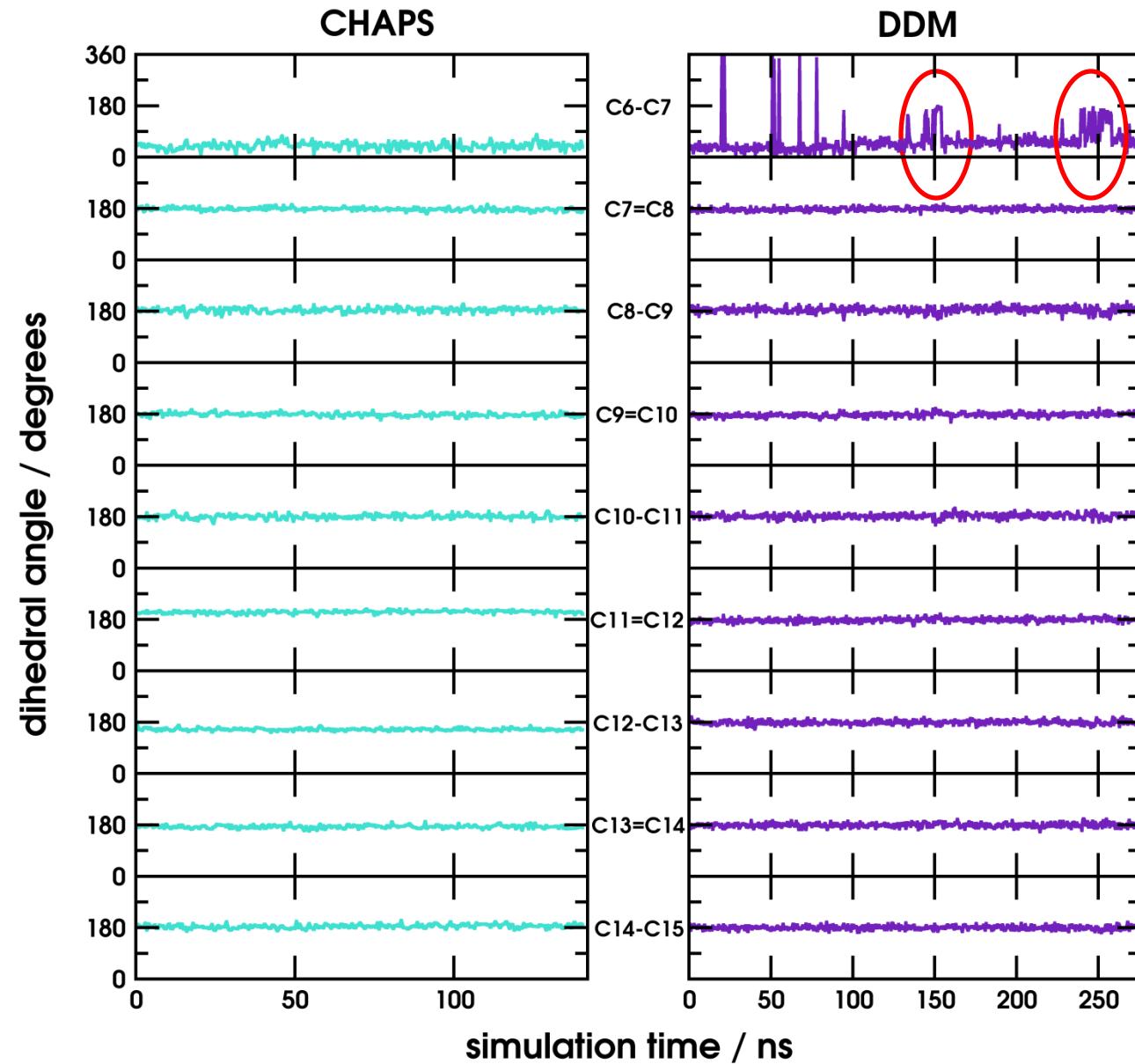


- Polyene dihedrals
- Methyl bond orientation

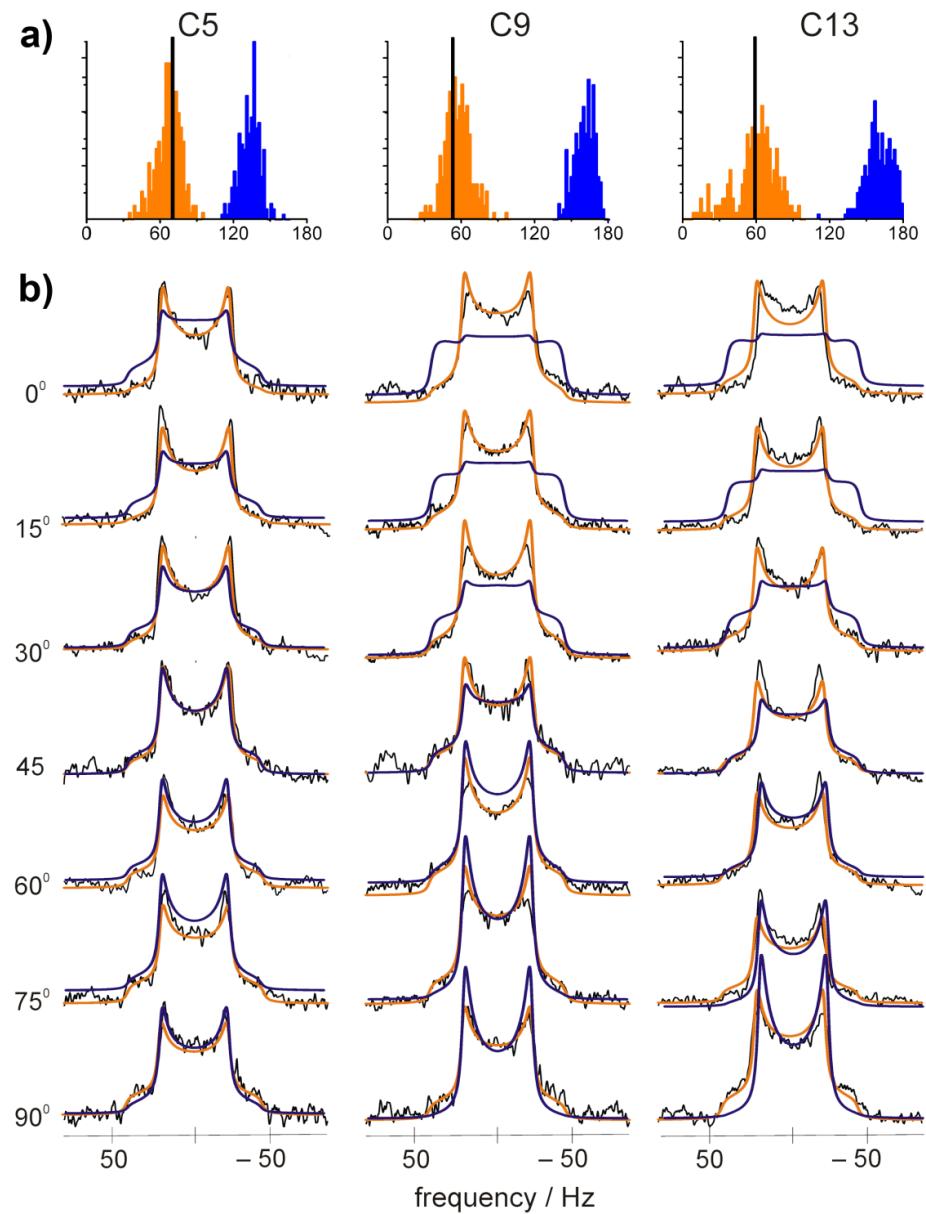
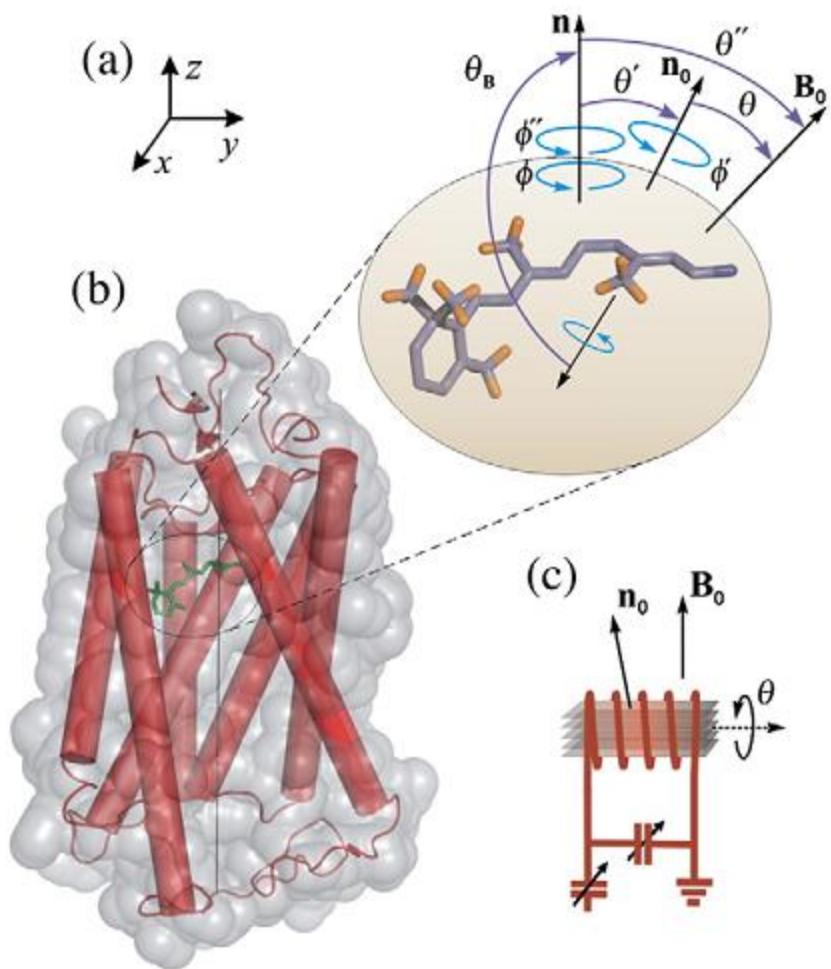


Retinal polyene dihedrals

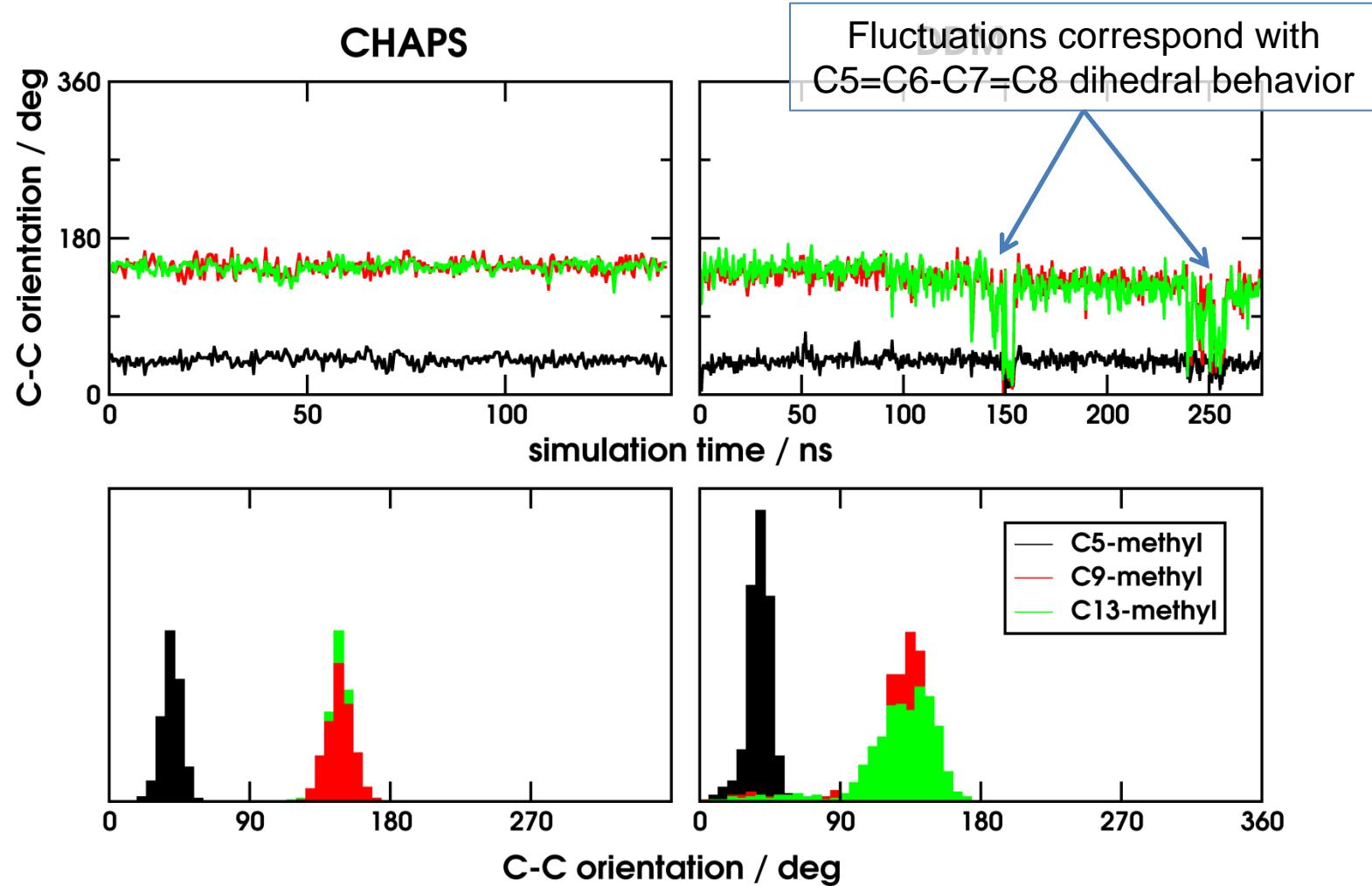
Orientation of β -ionone ring has effect on protonation state of PSB (Feller *et al.* *J. Amer. Chem. Soc.* 2013 135:9391)



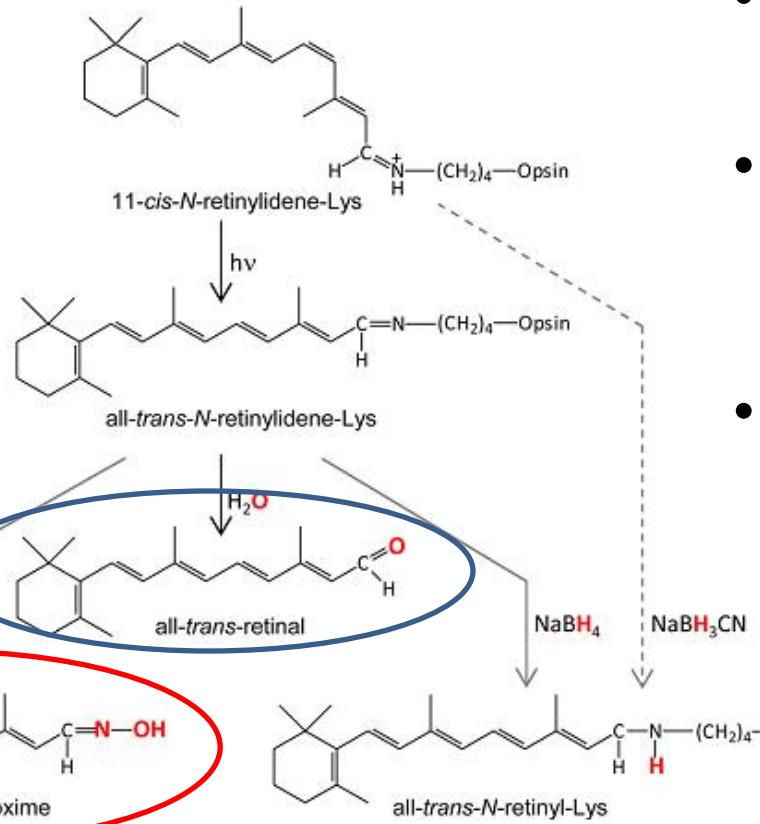
Comparison to ^2H NMR



Retinal methyl bond orientation

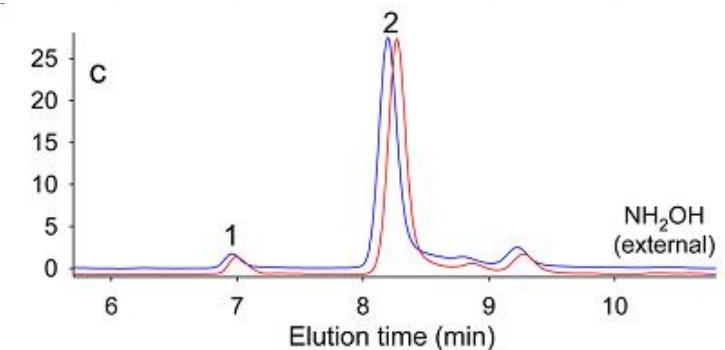


Water influx in rhodopsin activation



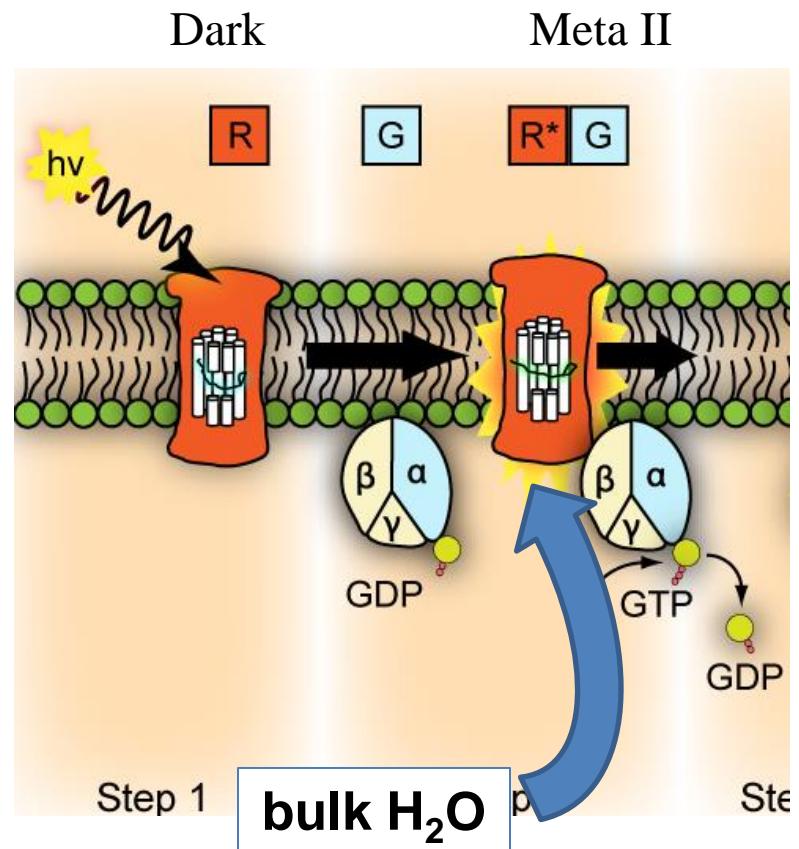
Jastrzebska (2011) *J. Biol. Chem.* 286:18930.

- Rhodopsin activation: dark → Meta II (fully active)
- Hydroxylamine (NH_2OH) cannot hydrolyze retinal until Meta II
- *Key question:* how does this happen?

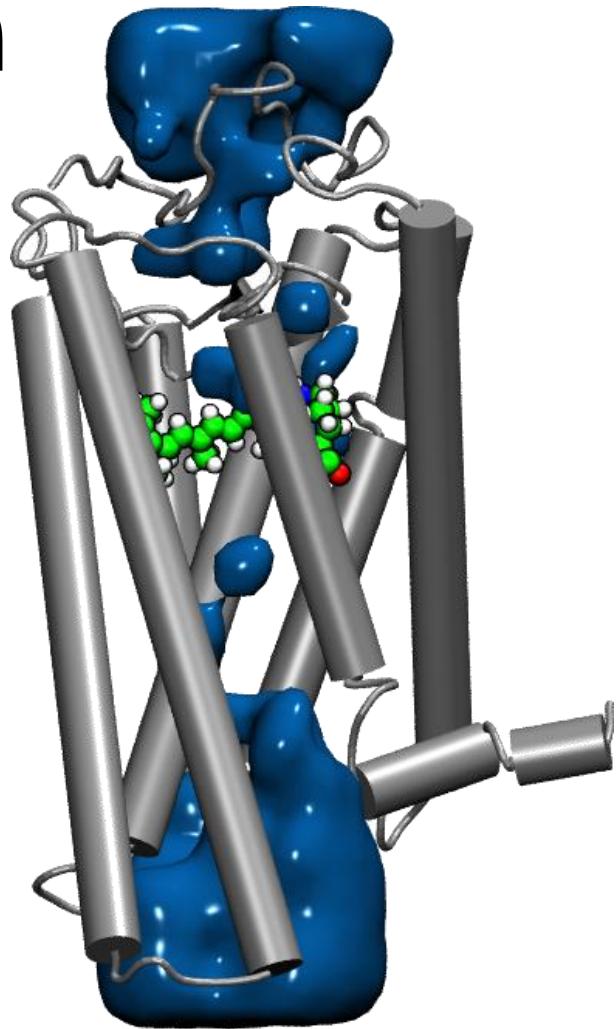
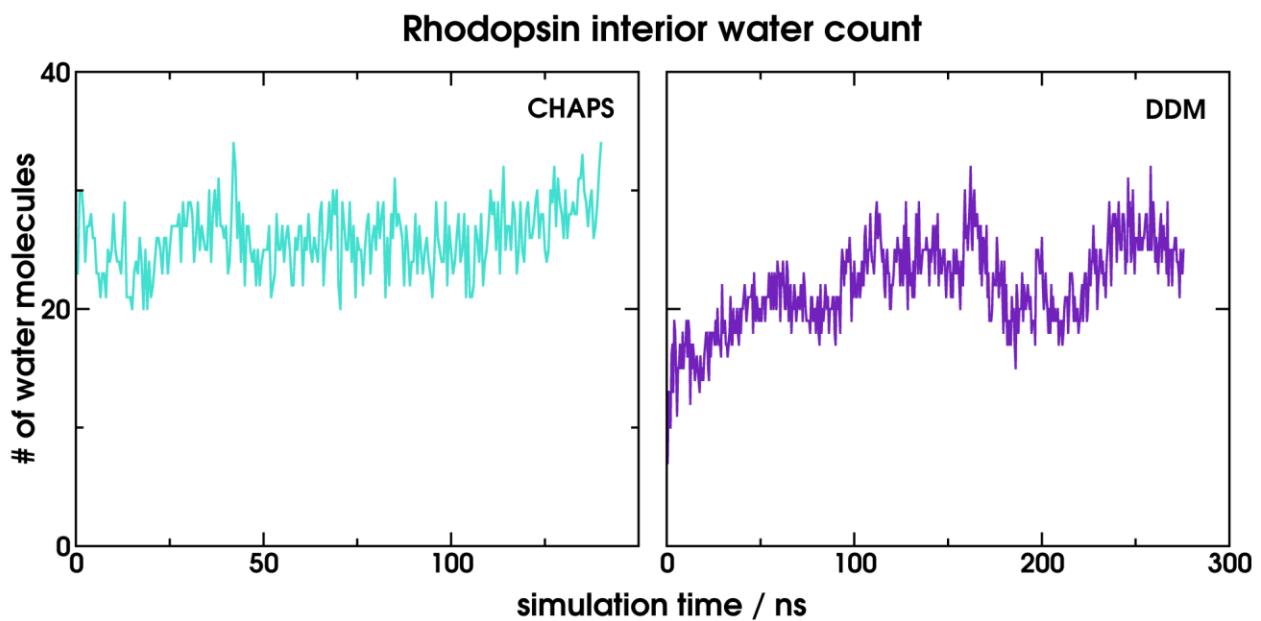


Role of H₂O in rhodopsin activation

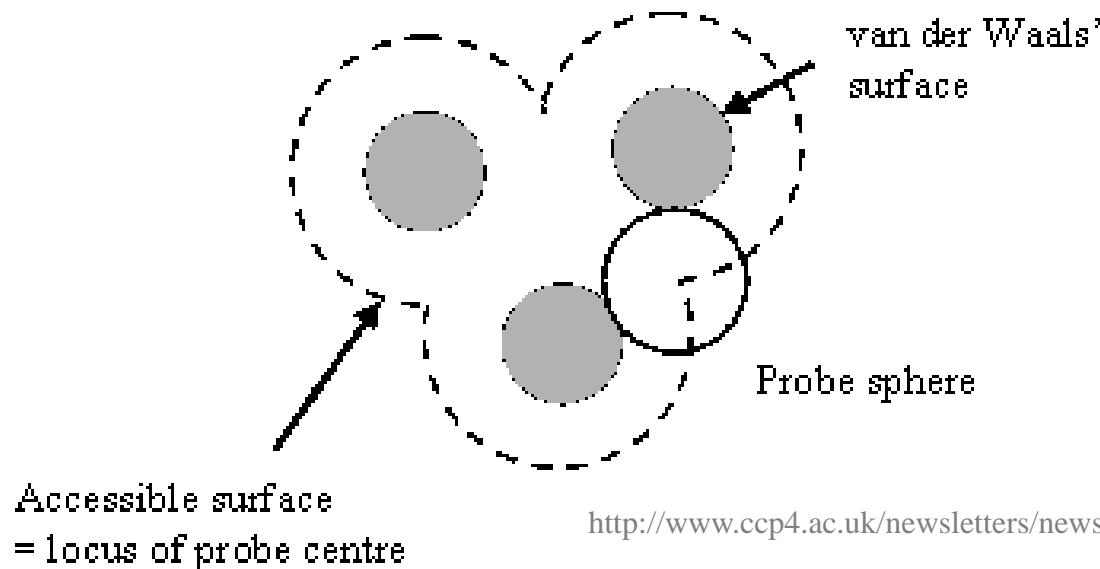
- Jastrzebska *et al.*, established that H₂O influx must come from cytoplasm during Meta II formation
- Experiments couldn't provide atomic details of this process, but MD simulations have this capability...



Internal hydration



SASA data for retinal

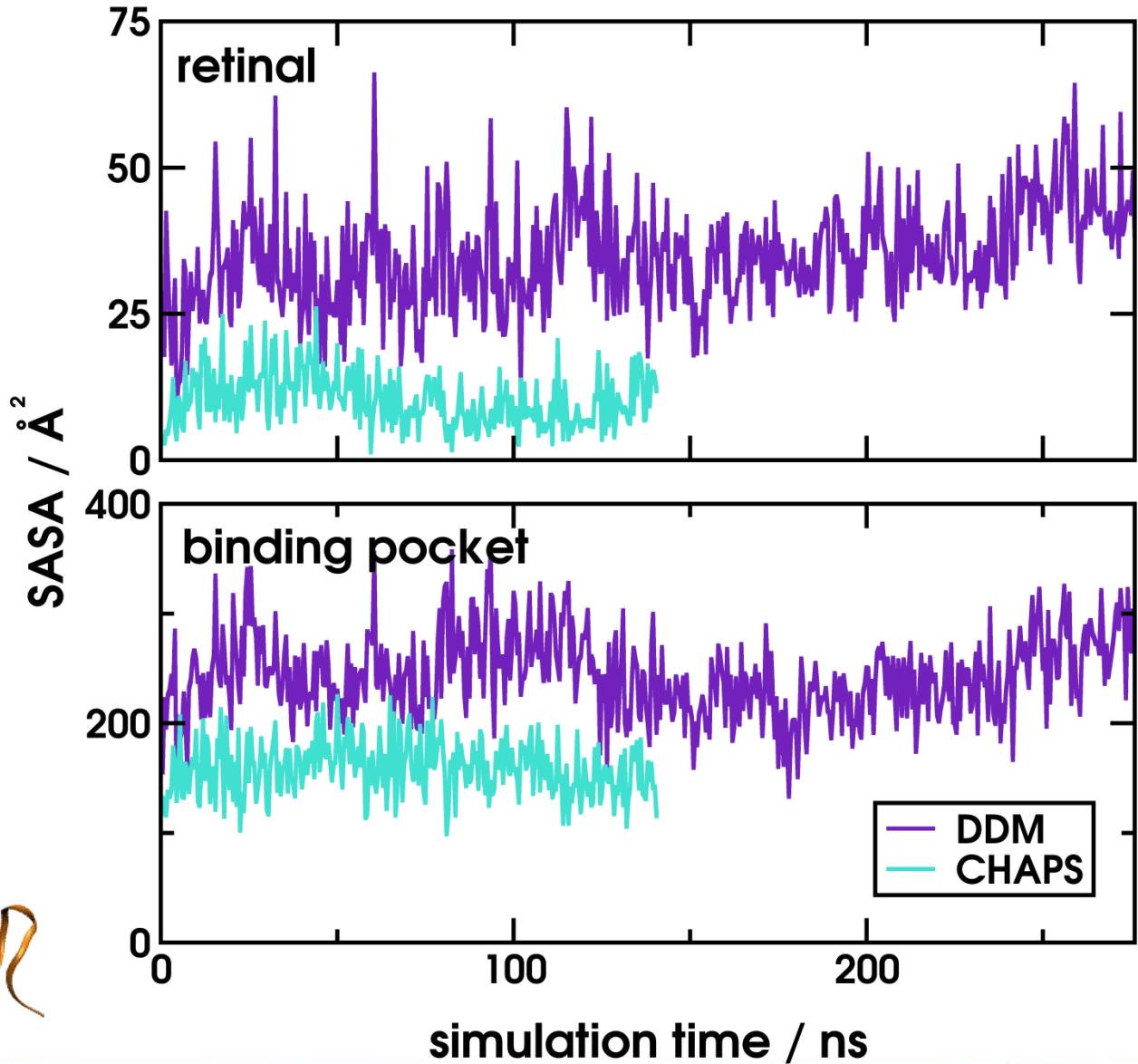
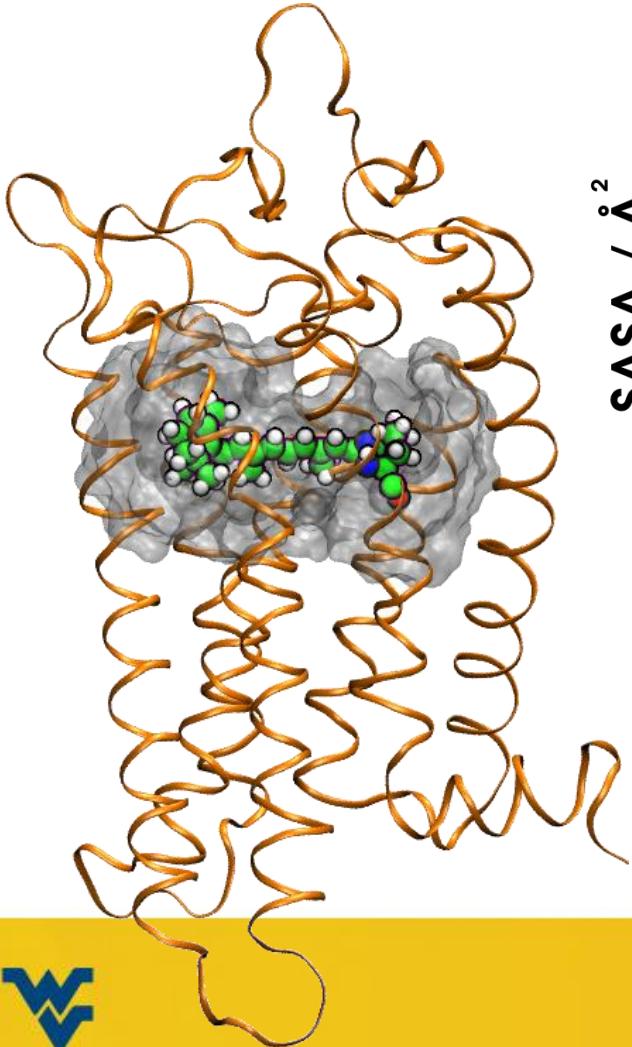


http://www ccp4.ac.uk/newsletters/newsletter38/03_surfarea.html

Solvent-accessible surface area: measure of surface over which molecule of interest and solvent contact can occur



Solvent-accessible surface area



Conclusions

1. How can we get past timescale limitations from the Meta I \rightleftharpoons Meta II transition (ms)? *With detergents?....perhaps*
2. What are the dynamical processes that occur during rhodopsin activation? (Meta I \rightleftharpoons Meta II transition) *Potential for decrease in hydration.*
3. Why is the retinal polyene chain flipped in the Meta II structure? *Experimental artifact or the real thing?* Need more time....



So where are we? Future directions...

- Need to simulate longer – systems haven't converged (typical membrane proteins are on μ s-ms timescale)
- Consider other detergents (MNG-3 is one used in β_2 -adrenergic crystal structure)
- Look for other ways to compare results with experimental data (EPR, solid-state NMR, QM calculations of IR & UV-vis spectra)



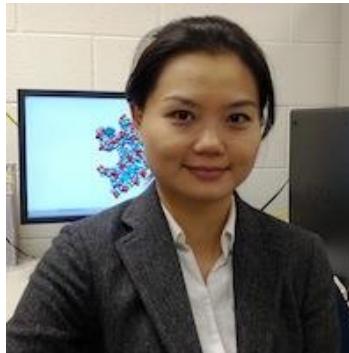
Acknowledgments



D E Shaw Research



Extreme Science and Engineering
Discovery Environment



Dr. Jun Feng



Michael Brown



Ed Lyman



Mertz Group @ WVU



Chitrak Gupta



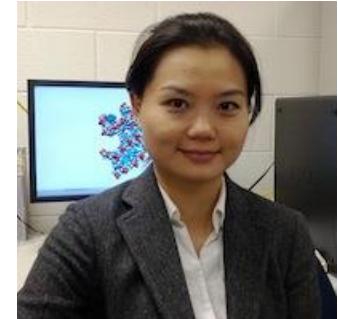
Yue Ren



Sadegh
Faramarzi



Dr. Choongkeun Lee



Dr. Jun Feng



Brandon
Neeley



Afsheen
Misaghi

Questions?

CRL Rm 463

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