Exploring Rhodopsin-Bilayer Interactions Via Coarse-Grained Molecular Dynamics Simulation

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Abstract

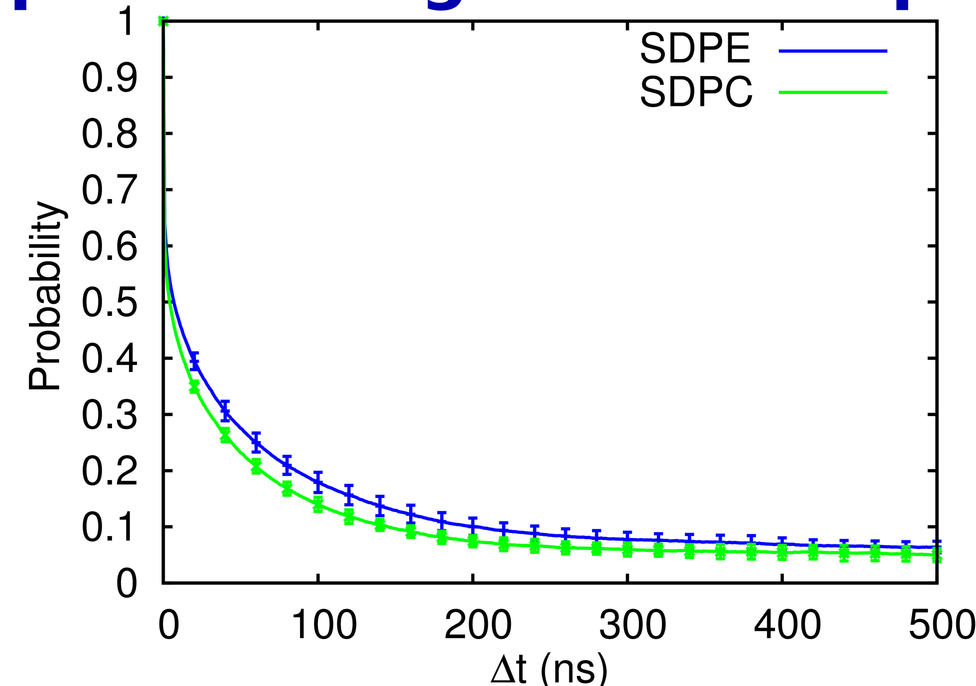
Proteins are dynamic in structure, with molecular motions dictated primarily by local physical forces. Integral membrane proteins differ in the sense that the heterogeneous environment plays a major role in protein flexibility and, in turn, function. Rhodopsin, a G protein-coupled receptor, is a membrane protein whose function is dependent on major environmental factors, including lipid composition, cholesterol concentration, and the ionic strength of the surrounding solvent. In this work, we further explored these effects by utilizing coarse-grained molecular dynamics to simulate large, native-like membranes for long timescales. We discovered clear preferences at the surface of the protein for polyunsaturated lipid tails, an effect that has been explored before with all-atom simulation, though not at the timescales present in this work. We also noted preferential binding regions for cholesterol and SDPE lipids.

Rhodopsin Models

Rhodopsin (1U19) **Opsin (3CAP)**

- MARTINI forcefield
- Palmitoyls on C322/C323
- GROMACS 4.5.4
  - 10 fs timestep
  - 300 K with Nosé-Hoover
  - 1 bar Parrinello-Rahman
  - LJ shifted 9 Å to 12 Å
  - Electrostatics cutoff 12 Å
- Restrain native structure
  - Between C_α 2 Å to 10 Å apart
  - 800 kJ/mol·nm^2
  - Match fluctuations to all-atom
- 32 independent simulations
  - 16 each for rhodopsin and opsin
  - 1.6 μs each
  - 51 μs total
- 180 SDPE lipids
- 180 SDPC lipids
- Each lipid has two tails
  - Polyunsaturated docosahexanoyl (DHA)
  - Saturated stearoyl (STEA)
- 90 cholesterols (CHOL)
- 19,000 water beads (4 waters per bead)

Lipid Exchange Well-Sampled

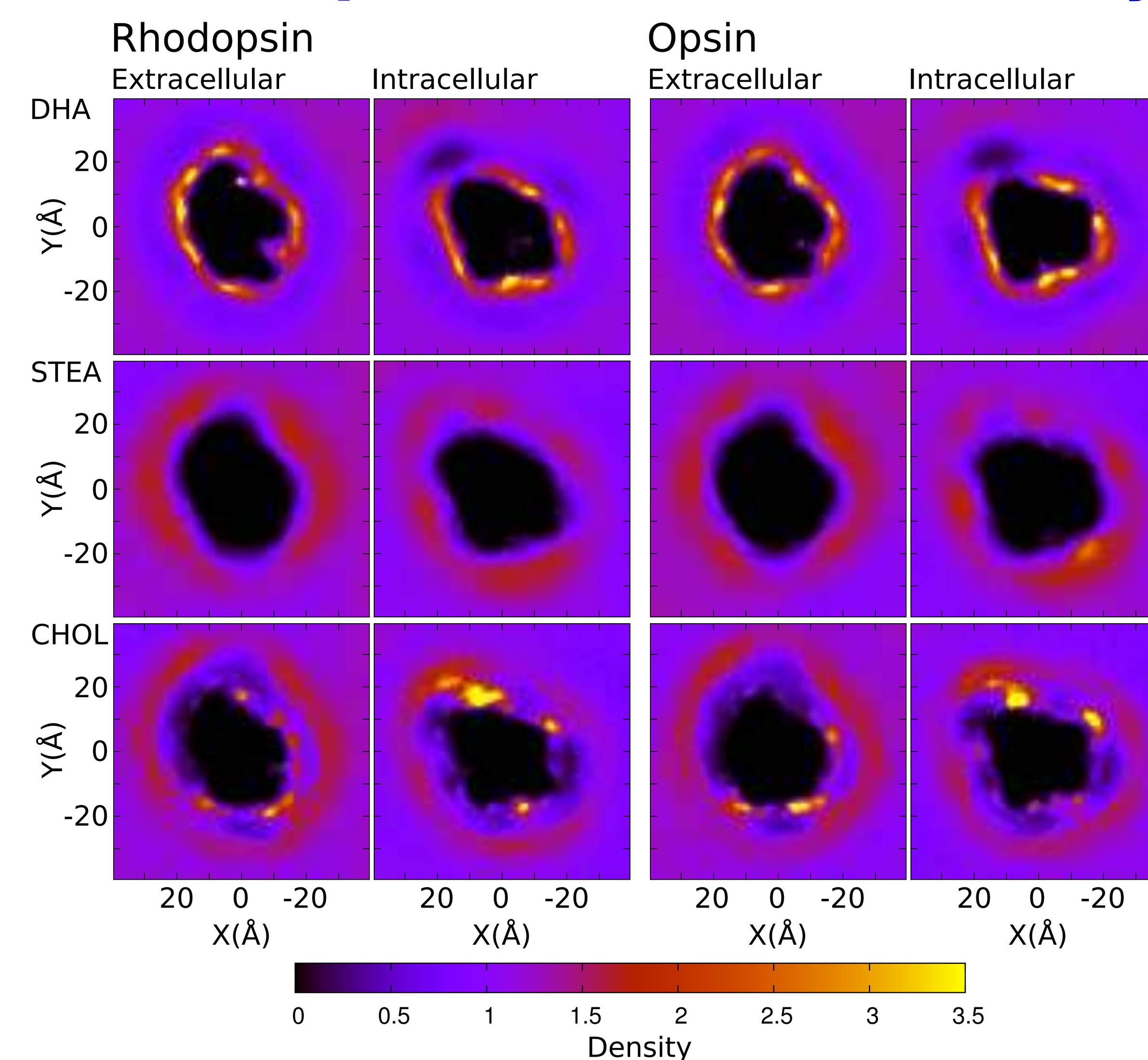


- Probability a lipid will remain at protein surface
  - $P_{bound}(\Delta t)$ given bound to protein at t=0
  - Lipid bound if it is within 6 Å of protein
- Can be fit to a double exponential
  - Fast decay time of <1 ns, slow decay time of 60-80 ns
  - Fast decay the result of on/off "flicker"
  - Simulations are much longer than slow decay time, implies good sampling

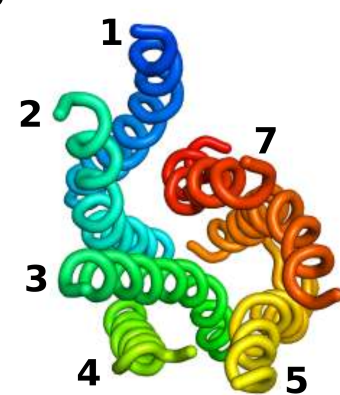
For reference, use the code to the left to access a digital copy of this poster. To learn more about this work or to contact the authors, visit us at:

http://membrane.urmc.rochester.edu

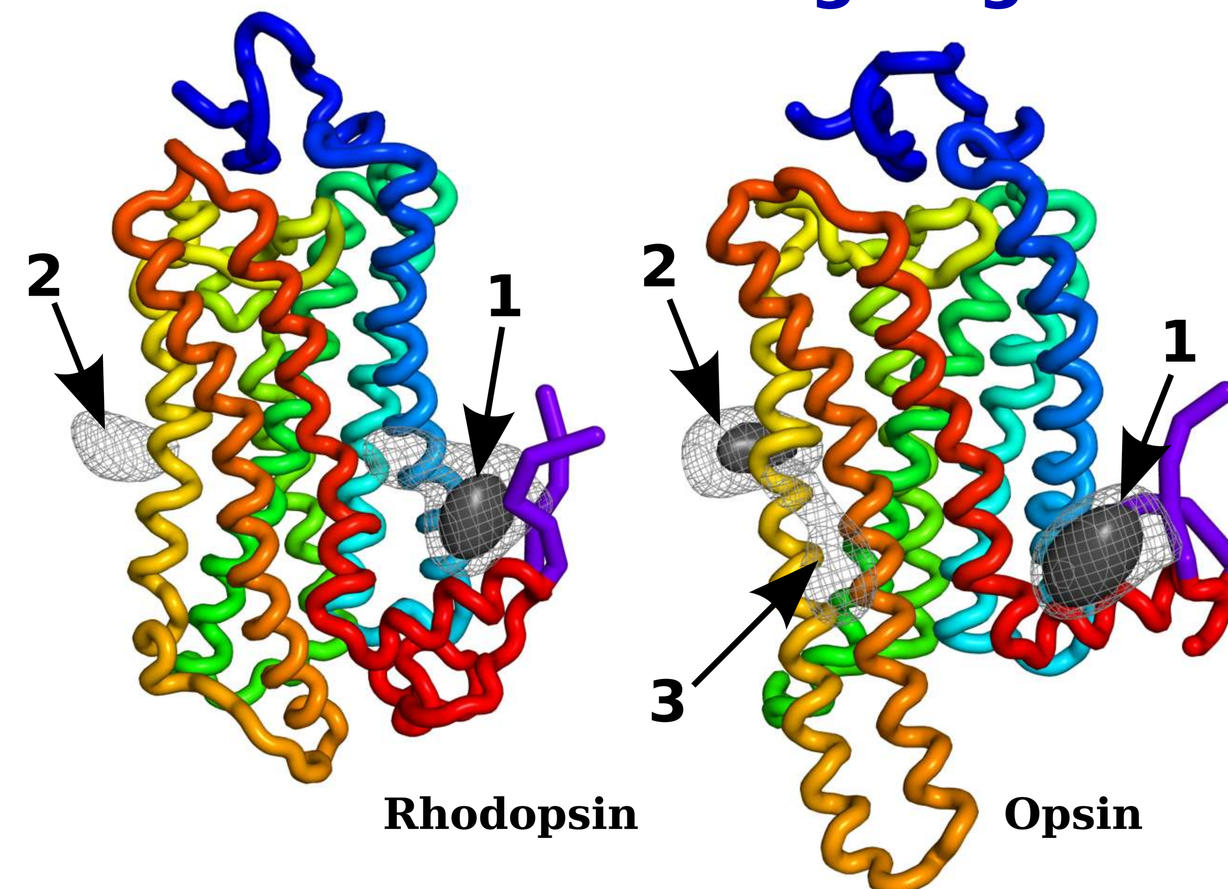
Heatmap Shows Chain Density



- Measures density of lipid components in plane of the leaflet
- Orientation of protein shown at right (from extracellular side)
- Large dark region in middle is protein occlusion
- DHA has a bright ring against protein surface
- Stearoyl has a diffuse outer ring around DHA
- Cholesterol is generally excluded from the protein surface
  - There are cholesterol hotspots on the protein

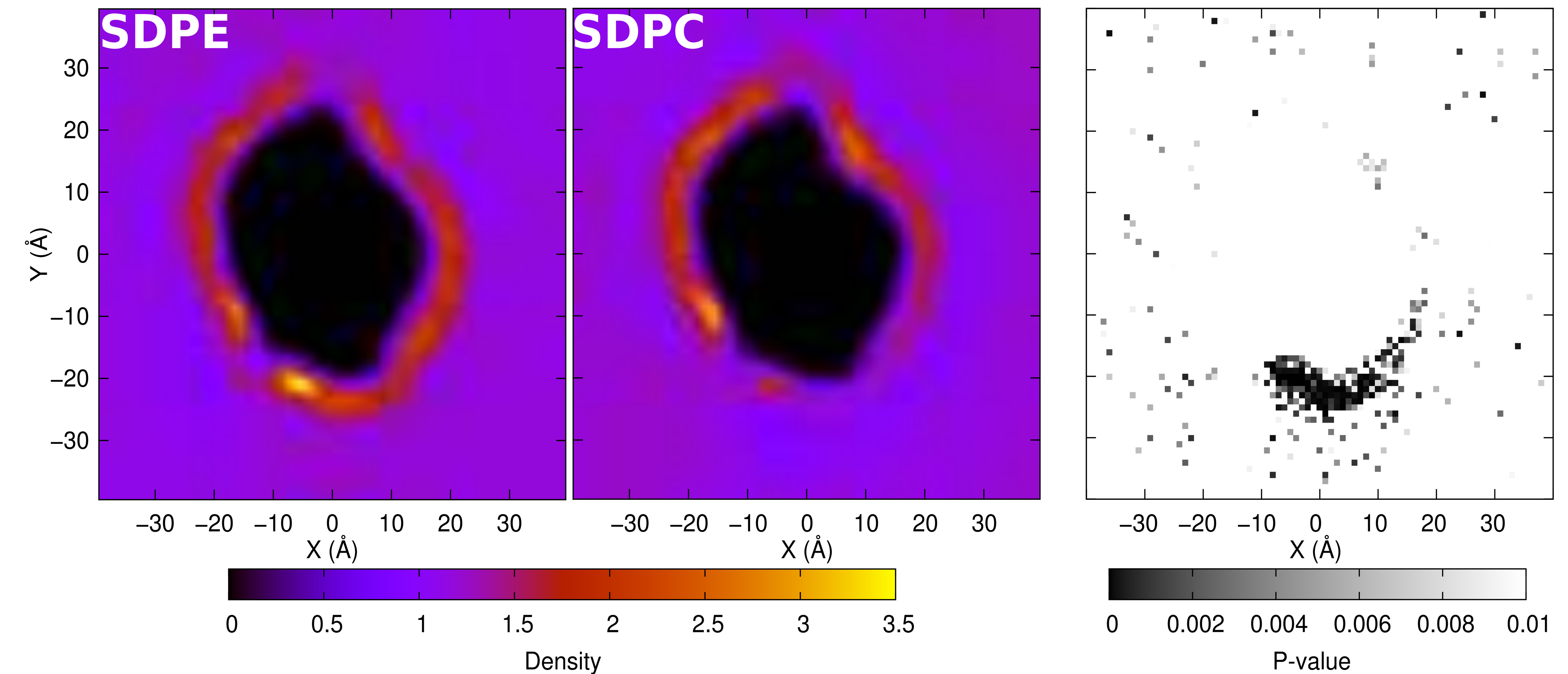


Cholesterol Binding Regions



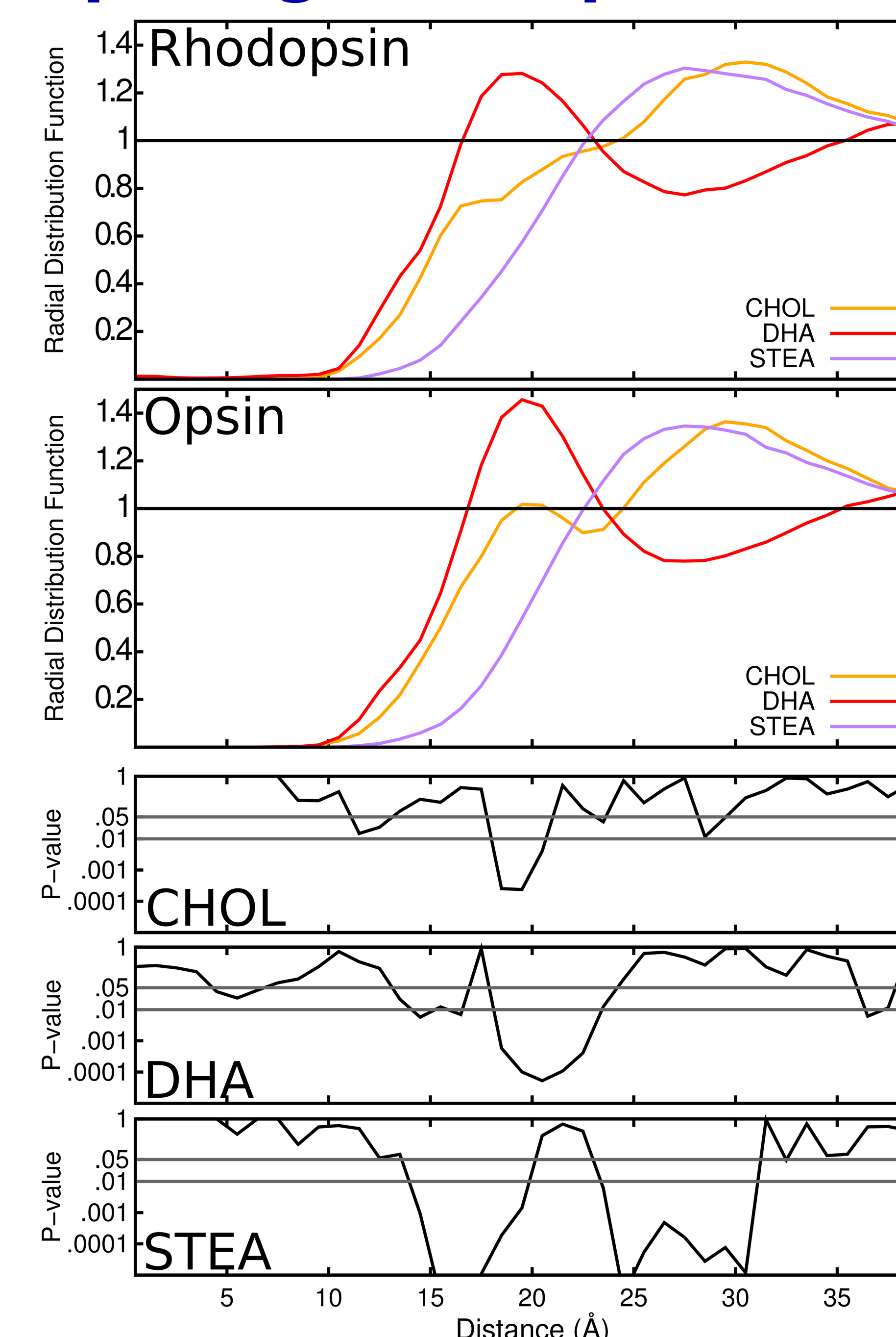
- Regions of cholesterol high density contoured at two levels
  - High density in black, low density in gray mesh
- Highest (#1) behind the palmitoyls on helix 8 (purple)
  - Packed against protein surface of helices 1 and 7 (red and blue helices)
- Second region (#2) packs on the opposite side of the protein
  - Interface of helices 3, 4, and 5 (yellow to green helices)
- Opsin has a third region (#3)
  - Packed against helices 5 and 6 (yellow to orange)

SDPE Preferred vs SDPC at Protein Surface



- Density maps of SDPE and SDPC on rhodopsin extracellular side
- Difference quantified at the right with plot of p-values
  - Each point in maps is average of 16 trajectories
  - Welch's t-test performed to compare means
- One region of statistically significant difference
  - Corresponds to helices 3, 4, and 5
  - Likely a binding region that prefers SDPE

Comparing Rhodopsin to Opsin



- Lateral radial distribution function (first two panes)
  - In plane of membrane as function of distance from protein
  - Clear enrichment of DHA at short range, exclusion of stearoyl
  - DHA peak is more dramatic in opsin systems
  - Cholesterol penetrates more deeply in opsin systems
- P-values comparing means of different components (panes 3-5)
  - Peak differences are statistically significant between opsin and rhodopsin
  - More "open" opsin structure has more surface area for lipid packing

Conclusions

- Clear preference for polyunsaturated chains (DHA) at surface
  - Entropically driven
  - *Grossfield et al., J Phys Chem B, 2006; 110(18):8907-8909.*
  - Each bound DHA has accompanying stearoyl, diffuse second ring
- Cholesterol binding sites exist, the greatest beside helix 8
- SDPE density is enhanced in specific regions
- More open opsin structure allows for greater surface area
  - Cholesterol penetrates more deeply between helices
  - More DHA packed against the protein
- Coarse-graining allows good sampling and statistics
  - P-values can be calculated to assess differences

Future Work

- Explore GPCR dimerization
  - *Periole et al., JACS, 2007; 129(33):10,126-132.*
  - *Johnston et al., PLOS Comp Bio, 2012; 8(8):e1002649.*
  - *Knepp et al., Biochemistry, 2012; 51(9):1819-1821.*
- Vary cholesterol concentrations
  - Explore effects of cholesterol on bilayer and rhodopsin activation
- Quantify the role of lipid interactions in rhodopsin function
  - Need a forcefield that does not require restraints
  - Longer timescales required to assess protein motions
- Couple these simulations to detail-rich all-atom simulations
  - MARTINI limits our ability to capture specific interactions

Simulation details and results are in press: Horn et al., in G Protein-Coupled Receptor Modeling and Simulation, ed. Marta Filizola, Springer, 2013.

Analysis done using LOOS (Lightweight Object Oriented Structure analysis library), an open source C++ library designed and maintained by the Grossfield lab. LOOS provides a framework for designing analysis tools that interface with file formats of most simulation packages.

http://loos.sourceforge.net