Cy3/Cy5 Fluorophore-Lipid Interactions and Their Effects on Membrane Protein Dynamics

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How do fluorophores affect the natural dynamics of membrane proteins?

- Hydrophobic core of lipid bilayer is replaced by organic solvent (OCCE).
- Bias-exchange umbrella sampling (BEUS) method was used to characterize binding energy.

Spontaneous Membrane Partitioning

- Cy3 and Cy5 fluorophore structures and parameters were taken from the CHARMM-GUI membrane builder. 5 independent copies of angle fluorophore were placed 10 Å above pre-equilibrated HMMM bilayers (PC/PE/PS:PC). Each copy was simulated for 100 ns with 150 mM of NaCl and all simulations were performed using full-tailed lipids with 3 copies.

Electrostatics-Stabilized Membrane Binding

- Membrane binding free energy of Cy5 calculated by BEUS method in full-tail lipids.
- The (negative) electrostatic potential of lipid, water molecules and ions in membrane normal direction.

Effects on a Single-Pass Transmembrane Helix

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Summary

- Cy3/Cy5 show fast binding to lipids, stabilized by electrostatics and hydrophobic interactions. Negatively charged lipids favor more stable binding.
- Diffusion and conformation of small membrane proteins could be largely affected by Cy5-lipid interactions.
- Pore radius and hence the conductance of channels could be affected by Cy5, but effect may depends on the Cy5-tagging position.

Simulation Methods

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References