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

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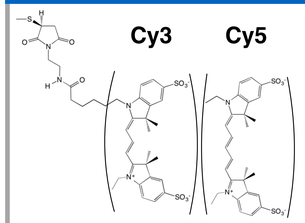
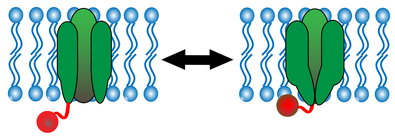
Background



Common Fluorescent Probe

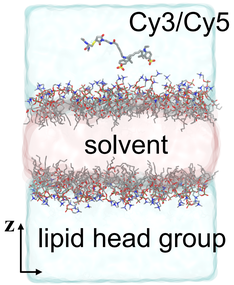
- (Sulfo-)Cyanine (Cy3) and Cyanine 5 (Cy5) are widely used in fluorescent microscopy for studying protein functions.
- Negatively charged (-1)
- Could interact with lipids **[1,2]**.



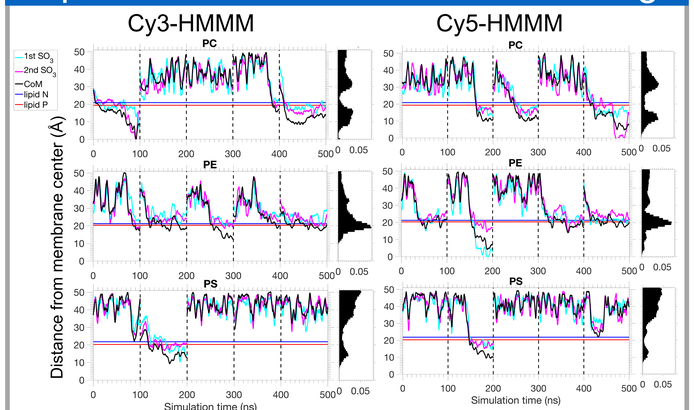
How do fluorophores affect the natural dynamics of membrane proteins?

Molecular Dynamics to Study Cy3/5-Lipid Interactions

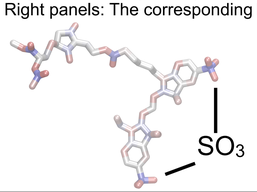
- The highly mobile membrane mimetic (HMMM) model **[3]** allows fast sampling of interactions with lipid.
- Hydrophobic core of lipid bilayer is replaced by organic solvent (DCLE).
- Bias-exchange umbrella sampling (BEUS) method was used to characterize binding energy



Spontaneous Membrane Partitioning

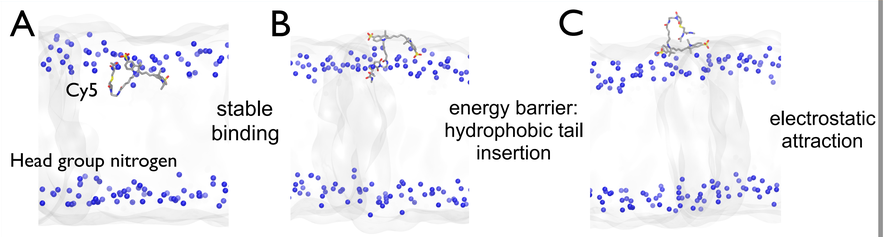


Trajectories of Cy3/5 z-position for PC, PE and PS:PC 1:1 mixture bilayer. Right panels: The corresponding histograms for center of mass

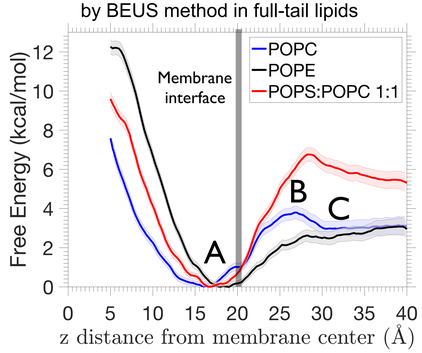


Molecular structure of a Cy3 molecule: positive partial charges shown in **blue** and negative charges shown in **red**

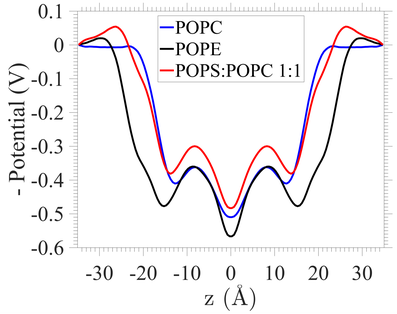
Electrostatics-Stabilized Membrane Binding



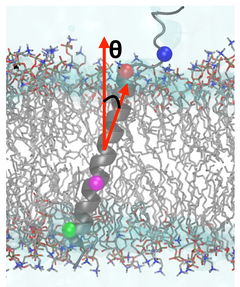
Membrane binding free energy of Cy5 calculated by BEUS method in full-tail lipids



The (negative) electrostatic potential of lipids, water molecules and ions in membrane normal direction

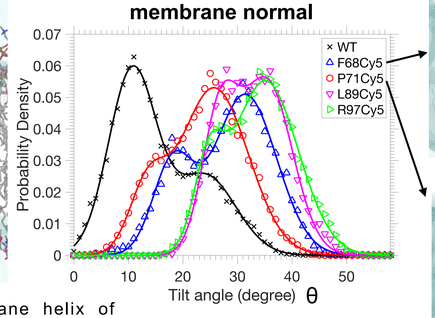


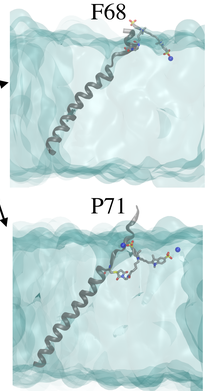
Effects on a Single-Pass Transmembrane Helix



Monomeric transmembrane helix of Glycophorin A (GpA) in POPC bilayer (Cy5 positions labeled by colored spheres)

Helix tilt angle with respect to membrane normal

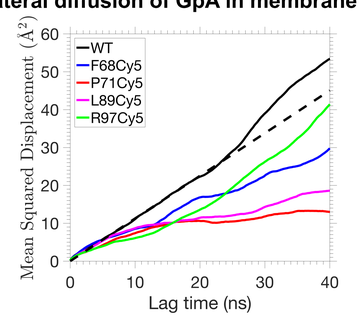




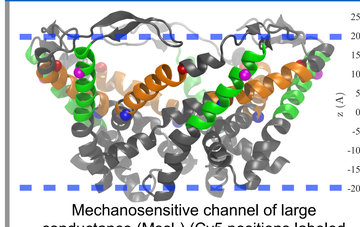
- Tilt angle was right-shifted by at least 15°, favoring a deep-sitting position in membrane.
- Lateral diffusion of transmembrane helix was hindered by Cy5-lipid interactions.

	WT	F68	P71	L89	R97
Diffusion Constant ($\mu m^2 s^{-1}$)	3.35	1.82	0.92	1.21	2.36

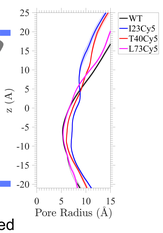
Lateral diffusion of GpA in membrane

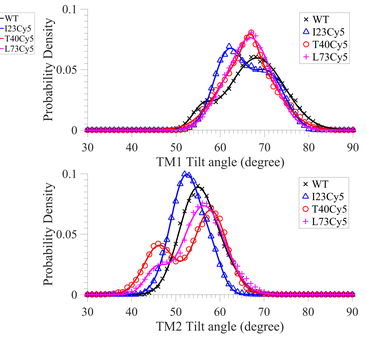


Effects on a Multi-Passes Channel

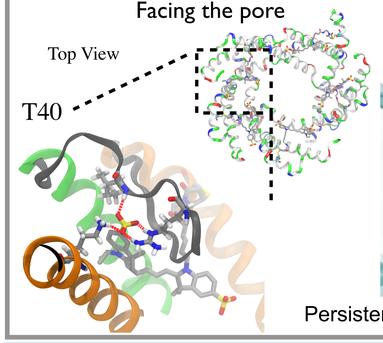


Mechanosensitive channel of large conductance (MscL) (Cy5 positions labeled by colored spheres)
Orange: TM1 helix, **green**: TM2 helix

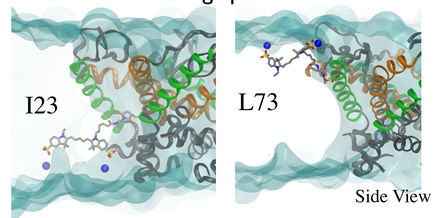




Facing the pore



Facing lipids



Persistent contacts with protein residues/lipids

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 affect by Cy5, but effect may depends on the Cy5-tagging position.

Simulation Methods

- Cy3 and Cy5 fluorophore structures and parameters were taken from the CHARMM-GUI membrane builder. 5 independent copies of single fluorophore were placed 10 Å above pre-equilibrated HMMM bilayers (PC/PE/PS:PC). Each copy was simulated for 150 ns. Biase-exchange umbrella sampling simulations were performed using full-tailed lipids with 40 windows.
- The 5 independent copies of monomeric GpA transmembrane helix (pdb: 1AFO) were embedded in full-tailed POPC bilayer using CHARMM-GUI. Each copy was equilibrated for 20 ns and simulated for 100 ns in NPT ensemble.
- The 4 independent copies of open state MscL **[4]** were embedded in full-tailed POPC bilayer using CHARMM-GUI. Each copy was equilibrated for 20 ns and simulated for 150 ns in NPT ensemble, with surface tension of 35 dyn/cm applied to keep the channel open.
- Common to all simulations: Solvated with TIP3P water and ionized with 150 mM of NaCl and all simulations performed using NAMD2.12 **[5]**.

References

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Acknowledgment

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