

Fluorescence energy transfer between DPH and the chromophore of bacteriorhodopsin: Determination of the distance of closest approach and effect on fluorescence depolarization

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Evidence is presented for fluorescence energy transfer from diphenylhexatriene (DPH) donors embedded in the lipid phase of bacteriorhodopsin-dimyristoylphosphatidylcholine vesicles to the acceptor retinal of bacteriorhodopsin. When the surface concentration of acceptors is increased by raising the protein to lipid ratio, the mean fluorescence lifetime of the donors decreases by up to a factor of four. When the acceptor concentration is reduced in a controlled way at fixed protein to lipid ratio by bleaching of the chromophores, the lifetime increases and reaches approximately the value observed in protein-free vesicles when the bleaching is complete. Above the phase transition temperature of the lipids, the shape of the decay curve and the dependency of the mean lifetime on the surface concentration of acceptors are in agreement with theoretical predictions for a two-dimensional random distribution of donors and acceptors. From this analysis a distance of closest approach between donors and acceptors of about 23 Å is obtained, suggesting that retinal is deeply buried in the interior of bacteriorhodopsin. Energy transfer has a pronounced effect on the fluorescence anisotropy. At every temperature the steady-state fluorescence anisotropy in vesicles with bacteriorhodopsin is higher than in vesicles with bleached bacteriorhodopsin in which no energy transfer occurs. From a comparison of the steady-state fluorescence anisotropy in these two vesicle systems at various lipid to protein ratios we conclude that this intrinsic membrane protein increases the lipid order. Energy transfer experiments between fluorescent lipids and suitable acceptors on membrane proteins, in combination with fluorescence depolarization experiments, provide a promising way to investigate lateral inhomogeneities in the structural and dynamical properties of membrane lipids.