

## Fluorescent Lipids with Selective Partitioning to Liquid Ordered Membrane Domains

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Microscopic tracking of cellular processes is vital to understanding pathogen invasion, disease progression, and delivery of therapeutic material. Fluorescent labeling of phase separated membrane domains, also known as lipid rafts, are an important aid in this area but phase specific labels are few and their performance can be inconsistent. We have developed series of lipids that provide insights into fluorescent lipid probe design for selective partitioning to liquid ordered (Lo) membrane phases. Here, we investigated the role of the lipid's spacer region and the fluorophore situated at the head group position. With a lipid structure consisting of a glycerol backbone and two palmityl tails for favorable packing into ordered regions of the membrane, we found that hydrophobicity of the fluorophore plays a determining role in the lipid's partitioning behavior. Hydrophilic fluorophores, which display little membrane association (e.g., Atto488, OG488), enable the lipid tails to direct the lipid's partitioning towards the Lo phase. Hydrophobic fluorophore (e.g., rhodamine B), on the other hand, steers the lipid to the disordered phase (Ld). Using a polyethylene glycol (PEG) spacer can buffer some of the partitioning effect induced by the hydrophobic fluorophores, but only to an extent. For example, we find that fluorescein-labeled lipids partition to the Ld phase when the PEG spacer is short but partition well to the Lo phase at a molecular weight of 2000, however, rhodamine B partitions strongly to the Ld phase regardless of PEG length. Both red and green fluorescent labeled lipids that partition to the Lo phase will be described.

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