Effect of docosahexaenoic acid-containing phospholipid on phase-separated lipid membrane analyzed by fluorescent derivatives

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Docosahexaenoic acid (DHA) is a polyunsaturated fatty acid abundant in the brain and retina, predominantly incorporated in phospholipids as acyl chains. DHA-containing phospholipids (DHA-PL) are typically composed of a saturated acyl chain and DHA, which are assumed to localize at the boundary between ordered (Lo) and disordered (Ld) membrane domains, thereby influencing the formation of lipid rafts.[1,2] In this study, to observe dynamics and behavior of DHA-PL in membranes, fluorescent derivatives of a representative DHA-PL, 1-palmitoyl-2-docosahexaenoyl-*sn*-glycero-3- phosphatidylcholine (PDPC), were synthesized by applying our established method for preparing fluorescent lipids.[3] For comparison, fluorescent derivatives of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC) were also synthesized. These fluorescent lipids were incorporated into phase-separated membranes composed of sphingomyelin (SM) / cholesterol (Chol) / 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC). Contrary to the above assumption, neither PDPC nor POPC accumulated at the boundary between the Lo and Ld domains.

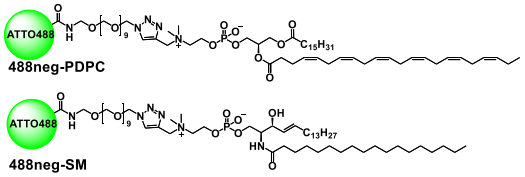

Fig. 1 Structure of 488neg-PDPC and -SM

Next, to further investigate the effect of PDPC on phase-separated membranes, we increased the PDPC ratios in SM / Chol / DOPC membranes and observed the lipid distributions between Lo and Ld phases using fluorescent PDPC and SM derivatives shown in Fig. 1. The results showed that, as the PDPC ratio increased, both PDPC (blue plots in Fig. 2) and DOPC distributions in the Lo domain increased, while the distribution of SM decreased (red plots in Fig. 2). Similar experiments were performed using POPC instead of PDPC, which showed no significant changes in the Lo/ Ld distributions of any of the lipids. This indicates that PDPC effectively fluidizes the Lo phase, changing the lipid distributions.

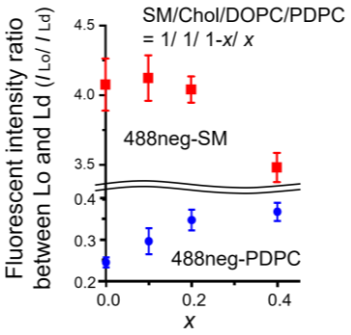

Fig. 2 The change in the Lo / Ld distribution of SM (red) and PDPC (blue) as the PDPC proportion increases.
Higher values on the vertical axis indicate a greater tendency to distribute into the Lo phase.

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[2] Pennington, E. R. *et al. J. Nutr.* **2024**
[3] Kinoshita, M. *et al. J. Cell Biol.* **2017**