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Interplay between lipid lateral diffusion, dye concentration and membrane permeability in a model lipid bilayer

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Lipid lateral diffusion in membrane bilayers is a fundamental process exploited by cells to enable complex protein structural and dynamic reorganizations. For its importance, lipid mobility in both cellular and model bilayers has been extensively investigated in recent years, especially through
the application of time-resolved, fluorescence-based, optical microscopy techniques. However, one caveat of fluorescence techniques is the need to use dye-labeled variants of the lipid of interest, thus potentially perturbing the structural and dynamic properties of the native species. Generally, the effect of the dye/tracer molecule is implicitly assumed to be negligible. Nevertheless, in view of the widespread use of optically modified lipids for studying lipid bilayer dynamics, it is highly desirable to well assess this point. Here, fluorescence correlation spectroscopy (FCS) and molecular dynamics (MD) simulations have been combined together to uncover subtle structural and dynamic effects in DOPC planar membranes enriched with a standard Rhodamine-labeled lipid. Our findings support a non- neutral role of the dye-labeled lipids in diffusion experiments, quantitatively estimating a decrease
in lipid mobility of up to 20% with respect to the unlabeled species. Moreover, results highlight the existing interplay between dye concentration, lipid lateral diffusion and membrane permeability, thus suggesting possible implications for future optical microscopy studies of biophysical processes occurring at the membrane level.

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