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Fluorescence Lifetime of Certain Probes as an Effective Indicator of Cell Membrane Hydration

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Cellular membranes are dynamic entities whose characteristics depend on extrinsic and intrinsic factors such as hydration level and pH. Certain fluorescent probes are used to measure changes in membrane properties, as they may emit different light when excited, depending on the local environment. We used Atto 633 attached to DOPE lipid and TopFluor (TP) attached to either egg yolk sphingomyelin (SM) or cholesterol to study fluorescence lifetime changes in model cell membranes under dehydration. The membranes were composed of 14:1 PC, SM, and cholesterol (1:1:1 by weight) and analysed using Fluorescence Lifetime Imaging Microscopy (FLIM). The results indicate that TP's behaviour varies depending on the molecule to which it is attached: in fully hydrated membranes, its peak fluorescence lifetime of liquid phase was approximately 1.9 ns for SM-bound TP and 3.2 ns for cholesterol-bound TP. Dehydration increased these to 2.2 ns and 3.4 ns, respectively. FLIM also distinguished membrane phases: liquid phases showed shorter fluorescence lifetimes, while lipid rafts exhibited longer ones. In conclusion, fluorescence lifetime measurements of fluorescent probes play a crucial role in advancing cellular science, enabling the exploration of membrane properties, especially its hydration state, and phase separation architecture.

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