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# Long-term quantification of membrane biophysical properties using an exchangeable polarity-sensitive sensor

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Polarity-sensitive fluorescent sensors constitute useful tools to quantify key biophysical properties of biomembranes, such as lipid order. However, prolonged acquisitions using these dyes are universally limited by dye photobleaching, i.e. the irreversible destruction of the sensor by the excitation light. This problem is aggravated in the case of super-resolution microscopy, due to the high laser powers used. Exchangeable membrane dyes have been recently proposed as a remedy to photobleaching, avoiding it by only temporarily binding to their target. Here, we show NR4A, a new exchangeable membrane dye, reports on the molecular order and dynamics of lipid membranes with no photobleaching-induced signal loss. We use super-resolution fluorescence correlation spectroscopy (STED-FCS) to simultaneously quantify membrane dynamics and lipid packing, which correlate in model and live cell membranes. Last, to showcase potential applications of polarity-sensitive exchangeable dyes, we use live 3D-STED imaging to monitor lipid exchange during membrane fusion (Figure 1).

**Primary author:** CARRAVILLA, Pablo (Leibniz Institute of Photonic Technology)

**Co-authors:** DASGUPTA, Anindita (Leibniz Institute of Photonic Technology); ZHURGENBAYEVA, Gaukhar; DANYLCHUK, Dmytro I.; KLYMCHENKO, Andrey S.; Dr SEZGIN, Erdinc (Assistant Professor in Biophysyics at Karolinska Institutet, Sweden); EGGELING, Christian (Leibniz Institute of Photonic Technology)

**Presenter:** CARRAVILLA, Pablo (Leibniz Institute of Photonic Technology)

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