

Time-Resolved Microscopic and Spectroscopic Measurements of Membrane Dynamics in Living Cells

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Membranes of living cells were characterized by laser-assisted fluorescence microscopy, in particular a combination of microspectrofluorometry, total internal reflection fluorescence microscopy (TIRFM) and fluorescence decay kinetics in the sub-nanosecond range. The generalized polarization (GP), characterizing a spectral shift which depends on the phase of membrane lipids, as well as the effective fluorescence lifetime τ_{eff} and fluorescence anisotropy $r(t)$ of the membrane marker laurdan revealed to be appropriate parameters for membrane stiffness and fluidity. For a deeper understanding of the cellular processes involved, we used U373-MG human glioblastoma cells as a model system for studies of membrane dynamics. GP generally decreased with temperature between 16 °C and 41 °C and was always higher for the plasma membrane than for the intracellular membranes. An increase of GP occurred after enrichment a decrease of GP was observed after depletion of cholesterol in cell membranes. The measurement of fluorescence lifetime and the fluorescence anisotropy $r(t)$ give us numerous informations about the microenvironment of specific molecules. Fluorescence decay kinetics and fluorescence lifetime images were recorded using an image intensifying camera system with time gates of 200–1000 ps duration and adjustable delay times. Two fluorescence images were recorded within two time gates, the first one immediately after the laser pulse and the second one $\Delta t = 3$ ns later. Out of these two images the effective fluorescence lifetime τ_{eff} could be calculated. At $T = 24$ °C all effective fluorescence lifetimes τ_{eff} were uniformly spread over all cells, whereas at temperatures above 30 °C domains with shorter and longer lifetimes could be distinguished. Measurements of fluorescence polarization as a function of temperature are subject of present investigations. Rotational diffusion depends on the time interval between excitation and fluorescence detection, since during the lifetime of their excited states, many molecules change their orientation by rotation. In a more viscous medium this rotation is expected to be slower. A decrease of the time constant τ_r of fluorescence anisotropy with temperature indicates an increasing fluidity of cell membranes.