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Phasor-FLIM for a direct investigation of Transportan 10 interactions with model membranes

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Transportan 10 (TP10), a short and positive charged peptide, belonging to the family of the cell penetrating peptides has gained increasing attention for its antimicrobial and anticancer activity but also for its applications in drug delivery as it is able to translocate therapeutic molecules in cellular environment. Due to the complexity of the phenomena involved in cellular uptake and following processes, which strongly depend on the membrane lipid composition, structural details of the peptide (e.g., charge, hydrophobicity, steric hindrance) and environmental conditions, it is not easy to understand the general rules governing them. Here, we combine spectroscopic techniques and fluorescence lifetime imaging microscopy (FLIM) to investigate (i) the fate of the TP10 in the presence of model membranes, analyzing its conformational changes occurring at membrane interface and distinguishing peptide adsorption from insertion into the lipid bilayer (ii) the changes of the fluidity of the membrane and the formation of pores into the latter induced by TP10 interaction. In addition, thanks to the use of the environment sensitive fluorescence dyes, Laurdan and di-4-ANEPPDHQ, and of the phasor approach to analyze FLIM data, we were able to monitor in real time fine events at different depths of phospholipid bilayers.