Abstract

Title: Development of dual-(+1)-Fluorescence Correlation Spectroscopy for Monitoring Protein Oligomerization Leading to Membrane Pore Formation.

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This dissertation introduces on the example of fibroblast growth factor 2 (FGF2) protein, a new statistical approach that can differentiate 'functional' membrane-inserted oligomers from 'non-functional' protein aggregates associated with membranes. Its application extends not only to FGF2 but also to many other membranes associated proteins that induce the formation of membrane pores. The principle of this approach is based on dual-color fluorescence correlation spectroscopy (FCS) applied to single giant unilamellar vesicles (GUVs). By analyzing the brightness and diffusion properties of fluorescently labeled proteins, it provides crucial insights into the protein oligomeric size, diffusion coefficients, surface concentrations, and membrane permeability on free-standing membrane parts of GUVs. It operates at a broad range of protein surface concentrations, allowing for a deeper exploration of protein oligomerization. Specifically tailored for studying membrane proteins, the dual-(+1)-FCS method stands out for its ability to comprehensively analyze multiple parameters in a single experiment. Overall, our methodology provides a robust tool for correlating membrane protein oligomerization with membrane pore formation and opens new avenues for understanding multimodal distributions of oligomeric states commonly obtained by single-molecule microscopic methods.

Keywords: dual-(+1)-FCS, FGF2, Oligomerization, membrane, pore formation.