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"Amyloid-like" fibers formed upon interaction of proteins and anionic lipids: Structure and dynamics from advanced FRET methodologies and microscopy.

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The phenomenon of protein/peptide self-assembly into highly ordered β-sheet-rich fibrils plays a key role in various diseases, e.g., Alzheimer, Parkinson and Type II diabetes mellitus. Specifically, it has been suggested that initial binding to anionic membrane lipids can promote the pathological conversion into amyloidogenic assemblies. Surprisingly, it has also been proposed that this type of membranes can trigger rapid "amyloid-like" fibril formation by several non-amyloidogenic proteins, such as lysozyme and cytochrome *c*. In order to elucidate the key factors that govern the formation of these lipid-protein supramolecular complexes, lysozyme was here used as a model protein.

The first step was the derivation of formalisms and data acquisition for the determination of the extent of protein-lipid interaction from FCS (Fluorescence Correlation Spectroscopy). Quantitative models taking explicitly into account in the correlation function for the Poissonian distribution of proteins over the lipid vesicle ensemble were derived. This was followed by the structural study of the fibers via advanced time-resolved FRET methodologies, and it was concluded that these supramolecular complexes are multi-stacked membranes with proteins in-between. FLIM (Fluorescence Lifetime Imaging Microscopy) was used to further characterize the fibers, and these revealed to be homogeneous at the meso scale. The protein is not monomeric within the multi-stacked membranes, and the formation of hexamers was detected both from the variation of fluorescence lifetime upon fiber formation of the derivatized protein lysozyme with the probe Alexa 488, and also from energy migration (homo-FRET); Quantitative models assuming a three-state species inter-conversion were derived. From FRAP (Fluorescence Recovery After Photobleaching) data, it was concluded that both the lipid and the protein diffusion in the fibers is very slow, as compared to the usual dynamics in GUV (Giant Unilamellar Vesicles), pointing out to the formation of very rigid structures. FLIM-FRET studies and 2PE generalized polarization measurements of Laurdan incorporated in the mixed lipid-protein fibers produced at a low L/P ratio will also be discussed.