Fluorescence Correlation Spectroscopy (FCS) in photosynthetic research - *how to untangle proteins diffusion, oligomerization and photochemistry on microscopic level*

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Photosynthesis is light driven process localized in thylakoid membrane (TM). Thylakoids contain a heterogeneous mosaic of proteins that facilitates various functions from light absorption to electron/proton transport. To fulfill their functions, proteins are precisely co-localized, in a form of nanoscale super-complexes (<0.25 μm in size) or larger microdomains areas (0.25 μm - 1.5 μm) represented by granal/stromal thylakoids or microdomains in cyanobacteria [1, 2]. The membrane heterogeneity and its dynamics can be studied either by standard confocal microscopy or newly by semi-single-molecule confocal method called **F**luorescence **C**orrelation **S**pectroscopy (FCS). We applied the method to study proteins mobility of a free TM protein native cyanobacterial thylakoids (PetC1-GFP [3]) and to resolve *in vitro* oligomerization process of light-harvesting antenna (LHCII) isolated from plant thylakoids *[4]*. The *in vitro* FCS data resolved a pH and low detergent induced process of LHCII oligomerization; it depicted plausible sizes of LHCII oligomers and their possible importance for light-harvesting *in vivo.* Further, we were able to proof a fast diffusion of a free thylakoids membrane protein – PetC1. It shed light on importance of protein-protein in restriction of proteins mobility in thylakoids. In conclusion, the FCS methods, that is suitable to characterize time-distribution of "events" (e.g. proteins diffusion, blinking or oligomerization) inside nano-scale volume; it is a very useful tool to study sub-cellular photosynthesis in the heterogeneous mosaic of thylakoids.

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