Probing local viscosity and protein mobility through enhanced oxidative green-to-red photoconversion of EGFP

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Protein dynamics plays a key role in live cell functioning, stimulating the development of new techniques for studying protein transport phenomena. Here, we introduce a relaxation method, which is based on the rapid formation of a non-equilibrium concentration profile of the enhanced green fluorescent protein (EGFP) across a sample by its oxidative green-to-red photoconversion. Following the irradiation of a part of a sample containing EGFP and an oxidant, the diffusion-controlled response of a system is monitored. Changes in the concentration of the initial green-emitting and oxidized red-emitting forms are simultaneously tracked by fluorescence lifetime measurements using the time-correlated single photon counting. A theoretical model, describing the diffusion-induced concentration changes in the center of the irradiated spot, is introduced to fit the experimental data using the protein diffusion coefficient as a parameter. To verify the method, we show that the obtained diffusion coefficient of EGFP in water is in good agreement with the previously published data. Moreover, we show that the photoconversion efficiency can be enhanced by mode-specific excitation of the chromophore using the 465 nm light irradiation, thus revealing a nuclear-driven light-induced electron transfer mechanism of the oxidative green-to-red photoconversion of EGFP. Our approach opens a way for the studies of intracellular viscosity changes combined with sensing of elevated levels of reactive oxygen species. This work is supported by the RSF (project no. 22-13-00126).