Abstract

The interplay and communication between cells build the foundation of life. Many signaling processes at the cell surface and inside the cell, as well as the cellular function itself depend on protein-protein interactions and the oligomerization of proteins. In the past, we presented an approach to single out interactions of fluorescently labeled membrane proteins by combining photobleaching and single-molecule microscopy. With this approach, termed "Thinning Out Clusters while Conserving Stoichiometry of Labeling" (TOCCSL, Moertelmaier et al. *Appl Phys Lett* 2005), oligomerization can be detected even at high surface densities of fluorescently labeled proteins. In TOCCSL, an aperture-restricted region of the plasma membrane is irreversibly photobleached by applying a high-intensity laser pulse. During a recovery time, in which illumination is turned off, non-photobleached molecules from the non-illuminated area of the plasma membrane re-populate the aperture-restricted region. At the onset of this recovery process, these molecules can be detected as well-separated, diffraction-limited signals and their oligomerization state can be quantified.

Here, I used extensive Monte Carlo simulations to provide a theoretical framework for quantitative interpretation of TOCCSL measurements. I determined the influence of experimental parameters and intrinsic characteristics of the investigated system on the outcome of a TOCCSL experiment. I further identified the diffraction-affected laser intensity profile and the diffusion of molecules at the aperture edges during photobleaching as major sources of generating partially photobleached oligomers. They are falsely detected as lower order oligomers and, hence, higher order oligomers might be prevented from detection. The amount of such partially photobleached oligomers depends on the photobleaching and the recovery time, on the mobility of the molecules and – for mixed populations of oligomers – on mobility differences between different kinds of oligomers. Moreover, I quantified random colocalizations of molecules after recovery, which are falsely detected as higher-order oligomers.

In a set of *in vitro* experiments I applied the TOCCSL method to a model dimer system to observe the discrepancies between ground-truth oligomeric state and the results obtained from TOCCSL experiments that I had previously seen in my simulations. The model dimer system was realized by incorporating fluorescently labeled divalent streptavidin in a mobile supported lipid bilayer (SLB). Different models were applied for the brightness analysis of recovered molecules to determine the fraction of detected dimers. Additionally, intrinsic characteristics of the model system (diffusion coefficient, molecules density, photobleaching probability, ground truth fluorescent state) were determined experimentally. Eventually, I performed a TOCCSL simulation based on the intrinsic characteristics of the model system and the experimental parameters (photobleaching time, recovery time, aperture size) used in the *in vitro* TOCCSL assay, to quantitatively compare the fraction of detected dimers in *in vitro* and *in silico* TOCCSL experiments. I could show how partial photobleaching influences the outcome of both *in vitro* and *in silico* oligomerization studies based on TOCCSL. I could further show how TOCCSL simulations based on experimentally determined parameters/characteristics can be used to get a good quantitative estimate of the outcome of an *in vitro* TOCCSL experiment.