A novel method for the stoichiometric analysis of molecular aggregates in living cells

Mario Brameshuber¹, Manuel Moertelmaier¹, Julian Weghuber¹, Verena Ruprecht¹, Hannes Stockinger², Gerhard J. Schütz²

¹ Biophysics Institute, University Linz, Altenbergerstr. 69, A-4040 Linz, Austria
² Department of Molecular Immunology, Center of Biomolecular, Medicine and Pharmacology, Medical University of Vienna, Lazarettgasse 19, A-1090 Vienna, Austria.
E-mail: mario.brameshuber@jku.at

The current picture of cellular plasma membrane is based on the existence of small stable structures which enable controlled aggregation and segregation of distinct sets of proteins. These structures, commonly termed lipid rafts, are too small and too close to be observed directly with fluorescence microscopy, too mobile for high resolution scanning techniques, and too fragile for reliable chemical purification.

We developed a novel method¹ (TOCCSL - Thinning Out Clusters while Conserving the Stoichiometry of Labeling) for the stoichiometric analysis of molecular aggregates in the cellular plasma membrane. We use selective photobleaching to erase all active fluorophores within a small region of the membrane, while conserving the stoichiometry of labeling in the remaining part of the membrane. At the onset of repopulation due to Brownian motion, single diffraction limited spots of individual aggregates can be resolved and quantified utilizing ultra sensitive fluorescence microscopy.

Figure 1. Principle of TOCCSL. Anti-DNP antibodies labeled with multiple FITC molecules were used to mimic stable clusters. A fluid supported lipid bilayer containing a fraction of DNP-labeled lipid provided the matrix for the experiment. On the left, the initial equilibrium situation is shown: a surface density of ~15 clusters per µm² makes direct observation of individual clusters impossible. Upon photobleaching for t_{bl}=200ms, clusters were allowed to diffuse into the bleached area. To the right, three images recorded after distinct recovery times are shown: after t_{rec}=0.5ms, no fluorescence signal can be observed; this image serves as control for complete photobleaching. After t_{rec}=500ms, individual clusters were clearly resolvable in the central part of the image, indicated by the dashed white circle; such single cluster signals are used for subsequent stoichiometric analysis. After t_{rec}=10s, the system has nearly reached equilibrium again¹.

To address the question of stable lipid rafts within the cellular plasma membrane, we applied TOCCSL to investigate the aggregation of a glycosyl-phosphatidyl-inositol (GPI) anchored monomeric green fluorescent protein stable expressed in living CHO cells. Besides monomers, we found a significant fraction of dimers diffusing freely in the plasma membranes. Those dimers were stable on a seconds time scale. With this study, one basis of the raft concept – the formation of stable platforms in the plasma membrane – has been confirmed.