Direct imaging of nanoplatforms in the live cell plasma membrane

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Abstract

Based on single molecule fluorescence microscopy we developed techniques, which enable the detection of mobile nanometer sized platforms or molecular aggregates diffusing in the plasma membrane of living cells. By utilizing a single molecule FRAP-approach termed TOCCSL ('Thinning Out Clusters while Conserving the Stoichiometry of Labeling')[1,2] combined with quantitative brightness analysis, nanoplatforms are detected by their property to confine fluorescent labels on a timescale of seconds. A special photobleaching protocol is used to reduce the surface density of labeled mobile platforms down to the level of well-isolated diffraction-limited spots, without altering the single spot brightness. The statistical distribution of probe molecules per platform is determined by single molecule brightness analysis. As platform marker we chose monomeric GFP linked via a GPI (glycosyl-phosphatidylinositol) anchor to the cell membrane. We found cholesterol-dependent homo-association of mGFP-GPI in the plasma membrane of living CHO cells, thereby demonstrating the existence of small, mobile, stable platforms hosting these probes[3]. Raising the temperature to 39°C resulted in a clear disintegration of nanoplatforms. A further temperature increase completely inhibited nanoplatform assembly.
The strong homo-association could also be released by addition of 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine (POVPC) or 1-palmitoyl-2-glutaroyl-sn-glycero-3-phosphocholine (PGPC), two oxidized lipids typically present in oxidatively modified low density lipoprotein. We found a clear dose-response relationship of mGPF-GPI nanodomain disintegration upon addition of POVP, correlating with the signal of an apoptosis marker. Applying similar concentrations of lyso-lipid did not dissolve nanodomains. To understand the mechanism behind nanodomain disintegration we inhibited the activation of acid sphingomyelinase (aSMase), which activates several apoptotic signaling pathways via ceramide generation. Inhibition of aSMase by NB-19 before addition of POVPC completely abolished the nanodomain-disintegration effect of oxidized phospholipids, thereby proofing a rather indirect effect of oxidized phospholipids on lipid nanodomains.
TOCCSL was further enhanced by implementing a dual-color based co-localization approach[4]. This allows for detection of very rare events and better estimations of nanoplatforms size and stability by utilizing Förster resonance energy transfer (FRET) as readout.

[1] Moertelmaier, Brameshuber et al., APL 2005, 87(26): 263905
[2] Brameshuber and Schütz, Methods Enzymol. 2012, 505: 159-86
[3] Brameshuber et al., JBC 2010, 285(53): 411765-71
[4] Ruprecht, Brameshuber et al., Soft Matter 2010, 6(3): 568-81