“Protein-protein interactions at the plasma membrane: new techniques & new views”

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Abstract:

The organization and dynamics of proteins and lipids in the plasma membrane, and their role in membrane functionality, have been subject of a long-lasting debate. Specifically, it is unclear to what extent membrane proteins are affected by their immediate lipid and protein environment and vice versa. To study protein interactions directly in the live cell plasma membrane we developed a method for in vivo micropatterning, and used it in combination with single molecule tracking to quantify the influence of a glycosylphosphatidylinositol- (GPI-) anchored protein – a typical marker of liquid ordered phases – on its molecular environment directly in the live cell plasma membrane (*1*). We found that the captured proteins merely acted as bulky obstacles to the diffusion of other membrane constituents, but did not influence their membrane environment over distances past their actual physical size. Our results imply that the outer leaflet of the plasma membrane is in a homogenous single phase regime under physiological conditions, ruling out the presence of “raft” phases associated with lipid-anchored proteins.

Next, we applied single molecule tracking combined with brightness analysis to study the oligomerization of membrane proteins. For a GPI-anchored GFP we observed cholesterol-dependent homodimerization (*2*). Similar lipid-dependent oligomerization was observed for the Serotonin transporter (SERT) (*3*). Surprisingly, oligomerization kinetics were completely different at the plasma membrane versus the ER membrane, indicating that oligomerization is equilibrated at subcellular membranes, but kinetically trapped at the plasma membrane.

Finally, we aimed at the direct imaging of protein nanoclusters at the plasma membrane using superresolution techniques. Previous results indicated the presence of a variety of protein nanoclusters was revealed, which lead to speculations whether nanoclustering was a general feature of plasma membrane proteins. Recently, however, doubts were raised whether imaging artifacts inherent to PALM/STORM might have influenced or even caused the observation of some of those protein clusters. To approach these concerns, we developed a method to robustly discriminate clustered from random distributions of molecules detected with single molecule localization microscopy-based techniques like PALM and STORM (*4*). I will present the application of superresolution techniques to different proteins expressed at the T cell plasma membrane.

1. E. Sevcsik *et al.*, GPI-anchored proteins do not reside in ordered domains in the live cell plasma membrane. *Nat Commun* **6**, 6969 (2015).
2. M. Brameshuber *et al.*, Imaging of Mobile Long-lived Nanoplatforms in the Live Cell Plasma Membrane. *J Biol Chem* **285**, 41765-41771 (2010).
3. A. Anderluh *et al.*, Direct PIP_2 binding mediates stable oligomer formation of the serotonin transporter. *Nature Communications* **8**, 14089 (2017).
4. F. Baumgart *et al.*, Varying label density allows artifact-free analysis of membrane-protein nanoclusters. *Nat Meth* **13**, 661-664 (2016).