Imaging plasma membrane domains in signal transduction pathways

Pezzarossa, A.

Summary

The cell membrane separates the inside of the cell from the outside world. It acts as a barrier that controls the passage of substances (ions, organic molecules) from the outside to the inside. The membrane of a cell, also referred as plasma membrane, is composed of various lipids organized in a bilayer with different kinds of proteins embedded. The first model describing the plasma membrane was proposed in the '70s by Singer and Nicolson. According to this model, named the mosaic-fluid model, the membrane is a uniform bilayer, where proteins are homogeneously distributed and free to move. Subsequently, this model was challenged by experimental data that suggested a more complex picture of the membrane. In the new model the various kinds of lipids are organized in nanometer-sized structures called membrane domains. These domains differ in size (from few tens to few hundreds of nanometer) and lipid composition.

The cell membrane is involved in many processes: cell-cell adhesion, ion conductivity and cell signaling. It is widely accepted that membrane domains play a functional role in regulating these processes, in particular signal transduction. Signal transduction is the process by which a cell translates a signal from the outside world into a response from the cell interior. During signal transduction the signaling molecule (sometimes referred as ligand) binds to a specific receptor on the cell outer surface. Binding triggers a set of responses, which lead to a physiological response. These processes are highly regulated in the cells, and the organization of membrane receptors in domains could increase the efficiency of the signaling process.

In this thesis, I study the existence and the role of membrane domains in living cells through single-molecule fluorescence microscopy. This technique uses a combination of laser excitation, and specific fluorescent labeling of the proteins of interest to pinpoint the position of each molecule in the membrane with extremely high accuracy.

In chapter 2 I apply this technique to study the distribution of a membrane-anchored protein, HRas, in the inner leaflet of the plasma membrane. This protein is involved in many different cellular processes, particularly cell growth, proliferation and differentiation. From the positions of all single-molecules I reconstruct a "map" of the protein distribution along the membrane. Statistical analysis of the observed distribution reveals that HRas is dynamically localized in membrane domains, varying in size from 80 to 250 nm. These domains are localized in the inner leaflet of the membrane, but their shape and size is related also to the lipid organization on the outer leaflet.

A different approach to study domain localization relies on tracking single proteins as they diffuse in the membrane. We apply this approach to study the influence of membrane structuring in the assembly of a two-component receptor, type I interferon receptor. This receptor is of fundamental importance in the response of the cell to infection and uncontrolled growth, like in cancer invasion. This receptor is formed by two separate subunits, ifnar1 and ifnar2, which upon binding of the ligand, interferon, form the signaling complex. However, there are only few copies of the subunits spread overall the cell membrane, and ifnar1 and ifnar2 need to find each other before signaling is initiated. If the subunits have to find each other along the whole membrane, it would take considerable time before signaling. Restriction to nanometer sized domains makes this process faster and more effective. By looking at the diffusive behavior of the components, I observed that they are confined in domains. Moreover, the relative concentration and the interplay btween the two components is fundamental to maintain the lateral organization of ifnar subunits in the plane of the membrane.

Single-molecule tracking is an extremely powerful technique to study protein motility, however it is limited in its temporal resolution to a few milliseconds. In chapter 4 of this thesis I present an advanced technique which allows following single-molecules down to a few microseconds. After validating the technique on DNA molecules, I applied it to protein diffusion, and the results are reposted in Chapter 5. Studying the diffusion of a small protein, a GPI-anchor protein, at microsecond time resolution I was able to confirm the existence of small domains, which influence the diffusion at very short times, which have not been observed with regular tracking techniques.