Lipid Rafts Mechanical Properties Revealed by AFM

Lipid Rafts Mechanical Properties Revealed by AFM: We present a method based on atomic force microscopy which permits to detect and measure the mechanical properties of lipid rafts in living neurons. This technique, based on the interpretation of force distance curves, confirmed that rafts have a diameter lower than 70 nm and that they are stiffer than their surrounding membrane.

Lipid Rafts Play Primordial Role

Lipid rafts model postulates that cholesterol and sphingolipids of the outer leaflet of the cellular membrane are not distributed homogeneously but cluster in an ordered phase called lipid raft [1;2]. Depending on their physico-chemical properties some proteins locate in, while other are excluded from the rafts. For example glycosylphosphatidylinositol-anchored proteins (GPI-AP) are known to reside preferentially into, whereas the transferring receptor is known to be excluded from the rafts [3]. The biological relevance of these structures is suggested by the finding that rafts are associated to signal transduction pathways present in the inner leaflet and play a primordial role in several physiological and pathological mechanisms such as the setup of the cellular polarity, cell adhesion or infection by viruses and prions [4-6]. In the recent years, it has been demonstrated that the Brownian motion of proteins present into the cholesterol enriched domains is significantly reduced when compared with those which are excluded from the rafts [3]. This finding suggests that lipid rafts posses a peculiar mechanical property and its study was the main topic of the present research.

Atomic Force Microscopy

The strategy we developed to measure living cells membrane properties was based on the use of an atomic force microscope [7] operating in the so called force volume mode. In this mode the AFM tip is periodically approached to and retracted from the sample while scanning. During the process, the microscope records the deformation of the cantilever as a function of its position. These curves are referred to as force distance curves. This operating mode is widely used in the AFM
community to detect specific protein-protein interactions [8;9] or to measure the mechanical properties of the sample [10].

In this project, the localization of lipid rafts was achieved by covalently attaching aerolysine onto the AFM tip and recording force volume files all over the sample. The spots where aerolysine molecules of the tip interacted with GPI-AP, which are, as mentioned above, preferentially located into lipid rafts, were characterized by a rupture event on the retraction segment of the force distance curve. This signal, which is depicted onto figure 1, is induced by the downwards movement of the AFM cantilever during the retraction part of the force distance curve. It occurs when the AFM cantilever stretches the bond which has been formed between the proteins on the tip and those in the membrane. When the retraction force of the cantilever overcomes the adhesion between the two proteins, the bond breaks and the cantilever returns to its rest position, giving rise to the signal on the force distance curve. The detection of this characteristic signature has been done by a dedicated software based on fuzzy logic recognition [11].

**Mechanical Properties of Rafts**

Similarly, the analysis of the extension segment of the force distance was used to determine the mechanical properties of the rafts. It was done by fitting the indentation curve with the Hertz model [12]. The indentation curve, which is calculated from the force distance curve, basically indicates the force that one requires to push (i.e. indent) the AFM tip to a certain distance into the sample. However, the whole indentation curve integrates the mechanical contribution of all the components of the sample which are located along the indentation path of the AFM tip and therefore "blurs" the subtle contribution of the cell membrane. In order to get rid of this effect, only the very first nanometers of the force distance curves were considered. This technique has already been tested and successfully applied onto living cells to distinguish the mechanical contributions of the different
Relative Stiffness of Spots

An additional innovation of the project consisted in measuring the relative stiffness of spots where aerolysine-GPI-AP interaction occurred. This was achieved by comparing the stiffness of spots where rafts were detected with those located in its immediate vicinity as depicted on figure 2. This methodology has several advantages: it is insensitive to large scale stiffness variations all over the surface of the sample and permits to compare data recorded on different cells at different time and different experimental conditions. The "absolute" stiffness of the membrane was determined by processing the extension segment of the force distance curve according to the Hertz model as depicted on figure 3.

Merging the different data recorded during force volume scans permits to display the topography, the stiffness as well the spots were rafts have been detected (see fig. 4).

This methodology permitted for the first time to demonstrate that rafts are 20-30% stiffer than their surrounding membrane and that their size is smaller than 70 nm [14].

Figure 5 shows the result of five independent experiments recorded on rats hypocampal neurons. This time lapse experiment shows the effect of cholesterol extraction on the lipid rafts. Before the injection of the cholesterol extracting chemical (cylodextrin) the relative stiffness of the rafts is about 30 % stiffer than the surrounding membrane. After cyclodextrin injection, the relative stiffness of the lipid rafts dropped to 100% which corresponds to the average stiffness of the membrane outside the rafts.

The measurements were confirmed by numerous controls designed to verify the aerolysine-GPI-AP binding specificity, the importance of tip contamination during several hours long scanning on living cells or the effect of cholesterol extraction on the stiffness of the rafts. Furthermore the experimental procedure was repeated on different cell types and by targeting the transferrine receptor, which is known to localize outside the lipid raft domains. All these controls confirmed that rafts are stiffer than the membrane located in their vicinity and definitely validated the experimental setup.
This new methodology offers the possibility not only to detect specific domains in the cellular membrane of living cells but also to follow their mechanical properties as a function of time and/or chemical that are added to the imaging medium.

References:

Please ask the author for further references.

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