Seeing the invisible – elucidating the nature of sterol aggregates in biomimetic cell membranes using modulation transfer microscopy

Popular science summary

Sterols belong to the group of lipids (fats), and are the key components of the eukaryotic cell membranes. The main membrane sterol present in animal cells and necessary for the proper course of life processes is cholesterol. By interacting with the adjacent phospholipids, cholesterol regulates both fluidity and the structure of the cell membrane. Its level is tightly controlled by the organism, and any imbalance has serious health consequences.

The studies performed on both native and model membranes have proven that under conditions of increased cholesterol content, two-dimensional domains composed exclusively of cholesterol are formed within the membrane. They are hypothesized to serve as the nucleation centers for larger, three-dimensional crystals, which are eventually responsible for various pathological conditions. For example, cholesterol crystals are present in advanced atherosclerotic plaques and it is recognized that their further growth may be the cause of a heart attack. Moreover, recent findings suggest that cholesterol crystallites inhibit the nerve repair in multiple sclerosis disease. Consequently, the possibility to visualize the cholesterol crystals and better understanding of their formation mechanisms are of the utmost importance. Current research on the biomembranes oversaturated with cholesterol has undoubtedly made a significant progress in recent years. However, many of the **fundamental questions** regarding the sterol crystals morphology, mechanism of aggregation and location of their growth as well as possible factors that trigger their formation, still **remain unanswered**. This is mainly due to the **lack of universal experimental technique**, which, in addition to the possibility of detecting aggregate forms of sterols, would allow for their systematic imaging and studying their basic physicochemical properties. This project faces the above challenges.

The main goal of the project is to understand, at the molecular level, the mechanisms of formation and structure of cholesterol crystallites in model cell membranes. To achieve this goal we will use the novel microscopy technique, which makes it possible "to see the invisible", that is, to visualize molecules exhibiting very weak, undetectable fluorescence.

The proposed research will allow us to determine the relationship between the intrinsic membrane properties, external environmental conditions and the structure of sterol crystallites with unprecedented sensitivity and chemical specificity. To achieve the goal formulated above, we will use both the naturally occurring fluorescent cholesterol analogue and very difficult to visualize, non-fluorescent but native to our cells cholesterol. The use of the novel technique - modulation transfer microscopy, combined with conventional fluorescence microscopy, will give us a unique possibility to answer the following questions without the need of using external fluorescent labels:

1. **What is the structure** of cholesterol crystallites in the biomimetic cell membranes? Do different structural forms coexist?
2. **Where are** the sterol crystals located? Are they embedded within the membrane or adsorbed onto the membrane surface?
3. **What is the mechanism** of cholesterol crystals formation? How does it depend on the composition, structure of the biomimetic membrane and environmental factors?

The proposed research aims to answer these questions and to fill in the gaps in our knowledge on the nature and formation mechanisms of the various types of sterol aggregates in the cell membrane. Providing this information is intended to help the scientific community, firstly, to understand how, where and what structural forms of crystals are formed in cell membranes, and secondly, in the longer perspective, to help develop new strategies to prevent the formation of pathological conditions in which the growth of cholesterol crystals plays a significant role.