Molecular freeze dance - tracking the spatial orientation of molecules in biomimetic cell membranes

Popular science summary

There has been a significant progress in our understanding of the organisation and activity of lipid cell membranes, however many fundamental questions regarding how different membrane constituents are spatial arranged within the membrane and how specific orientation affects their activity remain elusive. There are convincing theoretical predictions that Laurdan, which is one of the most common fluorescent probes used to study the organization of model and native cell membranes, can take up very different orientations within lipid membrane. However, this hypothesis has not been addressed experimentally. In parallel, presence of orientational heterogeneity of transmembrane domains, which are part of many membrane associated proteins, in different lipid membrane systems is continuously debated. What these two molecular species, Laurdan and transmembrane domains, have in common is that no single, coherent picture about the relation between their spatial orientation and local properties of cell membrane is available.

The overall goal of this proposal is to investigate the spatial orientation of the selected guest molecules in model biological membranes and to provide molecular-level insights into the mechanisms that modulate the spatial orientation of these molecules.

Specifically, we wish to verify:

1. the existence of molecules/populations of molecules with different spatial orientations;

and determine:

2. the distribution of different orientations;
3. factors that induce the specific spatial organisation of the molecules of interest;
4. the consequences for molecules taking up different orientations;

We will map the spatial orientation of Laurdan and polypeptide transmembrane domains in biological model membranes both addressing the entire population of guest molecules as well as by detecting the molecules of interest one by one. The motivation here is not only to resolve spatial organisation of Laurdan and transmembrane domains in lipid membranes but also to understand the relation with the membrane properties (such as composition, lipid phase or hydration).

In the project we will deploy a palette of five sophisticated techniques, mostly based on fluorescence imaging. The experiments will be performed on two different membrane systems: supported lipid bilayers and giant unilamellar vesicles. Consequently, this project will expand the experimental protocol that might be used to detect and characterize spatial orientation of biologically relevant molecules in soft matter and as such will undoubtedly contribute to the development of the field of biophysical imaging.

This research project has a truly multidisciplinary character, bringing together state-of the-art microscopy for cutting-edge physical chemistry / biophysical research. Its novelty encompasses different aspects: for the first time we will map experimentally the spatial orientation of Laurdan in lipid membranes; we will investigate the spatial orientation of transmembrane domains; and we will systematically study the effect of environmental conditions and membrane structural properties on the orientation of membrane guest molecules.