6. Summary

First of all, the issue whether and to what extent lipid mobility in the bilayer is altered when the bilayer is being deposited on the solid support was addressed. We designed a method that allows a direct comparison of the diffusion coefficients of labelled-lipids in the free-standing lipid bilayer of GUVs and that of the bilayer interacting with the mica surface (SPBs) in the glucose solution. The lateral diffusion analysis strongly indicate that the observed phenomenon is an unrestricted 2D diffusion. The results clearly show that the diffusion is slowed by more than 2 times for the interaction with the support. Moreover, we believe that the quantitative comparison of lipid diffusion in two frequently used model membranes can be helpful when comparing data reported in the literature.

Additionally, the obtained data clearly indicate the existence of an inter-leaflet coupling. The lateral diffusion of investigated membrane probes and phospholipid analogues depend on the character and structure of the used fluorescent probe. The obtained lateral diffusion of the flavone probe, F2N12S is significantly faster than that of the fluorescent lipid analogues under study. Moreover, flavone probe exhibits approximately an order of magnitude faster flip-flop in comparison to the Atto633-DOPE lipid analogue in SPBs. We may speculate that interactions of the F2N12S probe with surrounding lipids are weaker and hence the flip-flop motion energy barrier is comparatively lower than in the case of the fluorescent phospholipid analogues. Weaker dye-lipid interactions are likely to be responsible for the faster lateral diffusion of the F2N12S dye within the SPBs. The weaker interactions and faster diffusion of F2N12S are probably because it bears only one hydrophobic chain (dodecyl) in contrast to the lipid derivative Atto633-DOPE that bears two longer chains (dioleoyl).

The results presented herein are the first trial to indirectly determine the chromophore location within the SPBs by use of the lifetime tunning approach, exploiting the optically active support containing a thin layer of ITO.

Moreover, a novel and straightforward fluorescent assay has been devised and introduced to investigate the trans-bilayer movement of head-labelled fluorescent lipid analogues across confluent supported phospholipid bilayers. Up to our knowledge, it is the first suitable method for flip-flop studies of SPBs prepared by SUVs fusion.

We found that the lateral diffusion of alexa633-BP is coupled with the diffusion of atto488- DOPE. However, BP is diffusing considerably slower than the lipids. No differences in the lateral diffusion within both layers of the used SLBs were observed. Thus, there is no evidence for significantly faster diffusion within the layer facing the support compared to diffusion within the lipid layer binding BP, suggesting that the reason for the difference in D values between BP and lipids might be connected with the mechanism of BP binding.