Scientific report for the visit to Hof Fluorescence Group (Sep 30 – Oct 25, 2012),

Biophysical Chemistry Department, Jaroslav Heyrovský Institute of Physical Chemistry,

Academy of Sciences of the Czech Republic (ASCR). Prague, Czech Republic

Visitor	Petteri Parkkila
Host	Prof. Martin Hof, PI, OXPL, EuroMEMBRANE
Supervisor	Prof. Paavo Kinnunen, PL, OXPL, EuroMEMBRANE

Aim of the visit

The visit to Hof Fluorescence Group lasted 18 working days. During my stay I worked in close collaboration with Ph.D. student Martin Stefl, Ph.D. Mariana Amaro and Professor Martin Hof. Our aim was to construct a setup for measuring lateral diffusion in the water-lipid monolayer interface using fluorescence correlation spectroscopy. After establishing a feasible system, we desired to measure diffusion in ternary lipid raft films with low concentrations of oxidized phospholipid. It is well known that oxidative stress alters several properties of lipid raft membranes. It was recently proposed by Volinsky et al. that oxidized phospholipids tend to stabilize ordered sphingomyelin-cholesterol domains in lipid monolayers [1]. We intended to quantify these results using diffusion measurements.

FCS-Monolayer Setup

The first task was to successfully combine Langmuir monolayer system (Kibron Inc.) with the confocal microscope required for measuring FCS. The details of FCS are further described in article by Machan et al. [2]. We soon found out that using long-distance objective (up to 2 mm), which is conventionally used with Langmuir monolayers, did not provide adequate efficiency of collection of photons. Thus, a reduction of subphase thickness was necessary and was achieved by elevating the cover glass window using a rubber spacer. Water-glass adhesion was further increased by saturating cover glass with bovine serum albumin. We were able to achieve subphase thickness which allowed us to use the objective with 0.28mm working distance. In addition, a pump constantly adding more water to the trough was required in order to counteract the effects of evaporation. Later the system was augmented by a plastic cover box for reducing surface flow.

Methods

We first tested the setup with pure POPC and PazePC (oxidized analog) lipid monolayers. Measuring FCS in constant surface pressure and surface area was found to be impossible because of the tendency of film relaxation after compression. Oxidation of POPC should not be the case

because isotherms were unaffected by several compressions and same effect (relaxation) was seen in PazePC monolayers. We found out that the most effective way to measure FCS was to perform multiple Z-scans (correlation spectra over the multiple points in z-axis) during the compression under non-equilibrium conditions. It was possible to perform approximately 2-3 Z-scans during one compression. We decided to calculate the average surface pressures corresponding to the points in Z-scan where lipid film was in focus. By doing this we avoided the need to take the slight changes in lipid concentrations into account (area per lipid molecule).

Results

The graphs presenting the main results are included as an appendix. The correspondence between diffusion time (average time which tracer molecule spends in focus) and surface pressure was linear in single lipid films. Results with POPC and POPC/PazePC 70:30 were similar, indicating that oxidation does not affect diffusion in these films (temperature 22 C). We then moved to the ternary mixtures comprising POPC/PazePC, sphingomyelin (porcine brain) and cholesterol in proportions of 1.5:1.5:1 (with labeled POPE-Atto488 1:300 000, temperature 25 C). Visual inspection of the FCS scans during compression revealed the sudden disappearance of the black (no signal) liquid ordered domains at certain surface pressures. According to these scans, labeled POPE was not able to partition into the Lo-phase. Therefore, diffusion data was obtained only from disordered phospholipid fraction. Nevertheless, substitution of POPC with PazePC (from 0 to 4.7 and 9.4 mol-%) seemed to shift 'miscibility transition' to the higher pressures. This further confirms the results of Volinsky et al. Surface pressure – Diffusion time curves show clear tendencies. At low pressures, average diffusion times of the dye molecules are much affected by neither surrounding Lo-domains nor level of oxidation. After the domains become undetectable, we can see very rapid increase in diffusion times. This could be attributed to the formation of one homogenous phase where dye molecules have increasingly difficult to diffuse laterally because of the intermolecular forces between dye molecules and other lipid components. As the monolayer becomes more tightly packed (> 25 mN/m), we cannot see much increase in diffusion times, which seems reasonable.

Conclusions

Despite the challenges, we succeeded in constructing a functional system for investigating lateral diffusion in lipid monolayers by FCS. The data we obtained shows that FCS-monolayer system provides with an effective way to compare how the changes in molecular properties influence model membranes. We have also considered continuing the experiments with labeled oxidized phospholipids. However, the current results should be carefully comprehended in order to gain more understanding of the biophysical problem at hand.

Appendix I: Main results

[1] Volinsky, Paananen, Kinnunen: *Oxidized Phosphatidylcholines Promote Phase Separation of Cholesterol-Sphingomyelin Domains*. **Biophysical Journal (2012)** Vol 103:1-8

[2] Machan, Hof: *Lipid diffusion in planar membranes investigated by fluorescence correlation spectroscopy*. **Biochimica et Biophysica Acta (2010)** Vol 1798:1377-1391

[3] Gudmand, Fidorra, Bjornholm, Heimburg: *Diffusion and Partitioning of Fluorescent Lipid Probes in Phospholipid Monolayers*. **Biophysical Journal (2009)** Vol 96:4598-4609

Appendix I: Main results

Figure 1: Diffusion time as a function of Surface pressure in

POPC and POPC/PazePC 70:30 monolayers

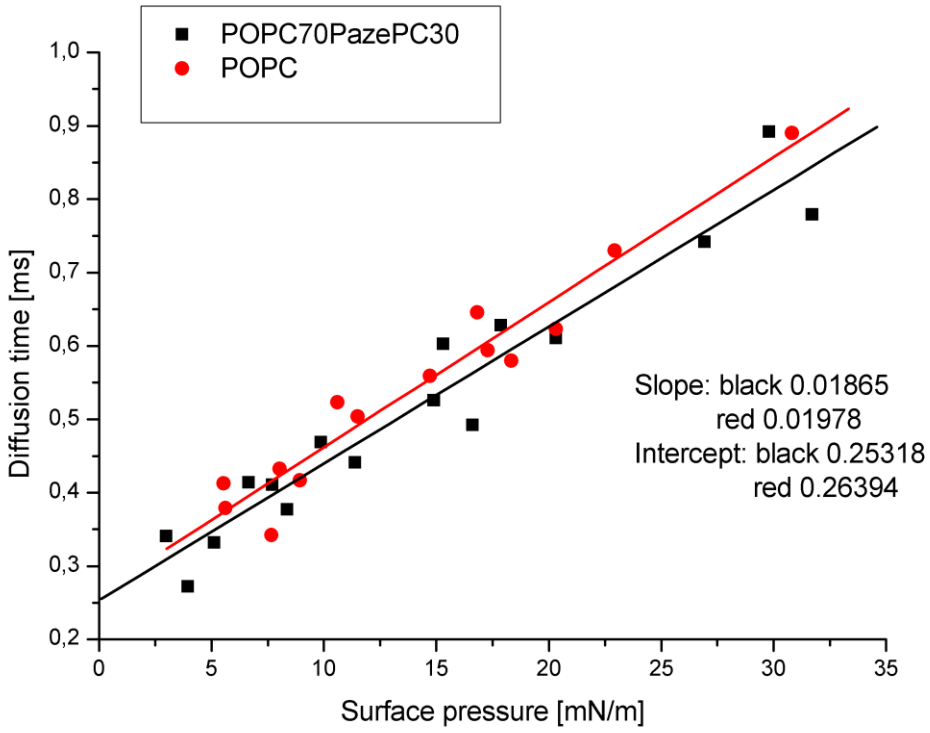

Figure 2: Diffusion time as a function of Surface pressure in POPC/pSM/Chol 1.5:1.5:1 ternary monolayers and mixtures with POPC partially replaced by PazePC (mol-% as indicated)