ACADEMY OF SCIENCES OF THE CZECH REPUBLIC
J. HEYROVSKÝ INSTITUTE OF PHYSICAL CHEMISTRY
DOLEJŠKOVA 3, 182 23 PRAGUE 8, CZECH REPUBLIC, PHONE: (+420) 266 052 011, (+420) 286 583 014, FAX: (+420) 286 582 307

Advanced fluorescence techniques applied on biomolecules (lipid membranes and DNA)

Submitted by **Mgr. Lenka Beranova**

PhD advisor's opinion on the PhD work

Mgr. Lenka Beranová started her PhD program in my laboratory in October 2007. Her first project was to apply Fluorescence Lifetime Correlation Spectroscopy (FLCS) for unraveling the compaction mechanism of different sized DNA molecules. As FLCS is a rather demanding method, which was to a large extent developed in our laboratory, she was in this project closely collaborating with Dr. Jana Humpoličkova, who mainly advised her in the sophisticated data analysis. In that work Mgr. Lenka Beranová demonstrated that FLCS can distinguish between different compaction mechanisms of DNA molecules being even smaller than the resolution of a fluorescence microscope. The success of the unique technique is based on the fact that FLCS allows for characterizing the diffusion properties of the condensed forms exclusively in presence of uncondensed DNA molecules. She focused on the condensation mechanism of circular 10 and linear 49 kbp DNA induced by spermine and cetyltrimethylammonium bromide (CTAB) and showed that spermine induces an all-or-none transition, while the condensation with CTAB is gradual. All the FLCS experiments summarized in the J. Phys Chem 2008 paper were performed by Mgr. Lenka Beranová.

After finishing that project she got interested in the effect of lipid oxidation on the biophysics of lipid bilayers. Lipid peroxidation was observed already in 1970s, and profound effects on the biophysical properties, such as phase changes and phase separation in model membranes, were demonstrated. Most likely because of the complex and poorly controlled chemistry of the reaction of unsaturated lipids with oxygen free radicals, the area became

more or less forgotten for several decades. However, the interest in the effects of oxidized lipids on membrane biophysics has revived due to the commercial availability of well-defined oxidized phospholipids together with more recent advances in cell biology pointing to the involvement of oxidized lipids in processes such as inflammation and apoptosis and the molecular pathology of several neurodegenerative diseases. In this work Mgr. Lenka Beranová was able to define the impact of two truncated oxidized phospolipid species on the biophysics of supported lipid bilayers using z-scan FCS and the time-dependent fluorescence shifts methods. Here experimental findings were interpreted using Molecular Dynamic Simulations performed by Dr. habil. Lukasz Cwiklik. It might be for some members of the committee of interest that the resulting Langmuir paper published in 2010 received already 20 citations in Web of Sciences.

Her last main project is titled as "specific biophysical properties of a phospholipid bilayer influenced by heavy water". Heavy water is widely used as a replacement for normal water in certain experiments, and thus defined picture on a possible heavy water effect is desired. Her study provided direct experimental confirmation of the solvent isotope effect on phospholipid bilayers which was previously described in detail based on molecular dynamics simulations. Specifically, Mgr. Lenka Beranová used the time-dependent fluorescence shift method, fluorescence anisotropy and two-focus FCS and by that could draw a comprehensive picture on the heavy water effect on the structure and dynamics of lipid bilayers. Although the physical and chemical properties of both solvents are almost the same, when it comes to the phospholipid bilayer, the assembly is entirely dependent on the weak interactions between water and lipid molecules, and the slight alternation can cause measurable changes in the properties of the membrane. Especially when it comes to time-resolved physicochemical properties, the change can be significant.

It should be mentioned that beside of these three main papers, Mgr. Lenka Beranová co-authored further 3 papers. Remarkably, her contribution in *Chem listy* got awarded by the Karel Preis Prize 2009 for the best communication (*Chem listy, 103, 125-129, 2009)*

Just by summarizing her activities, it is evident that Mgr. Lenka Beranová has been a very good PhD student and I truly enjoyed working with her. Although she sometimes doubted whether science is the right place for her, I always found her attitude to scientific work professional and characterized by deep understanding of the experiments she is carrying

out. On top that, her very honest character is an ideal prerequisite for creating meaningful research. Thus I do a little bit regret that Mgr. Lenka Beranová left science. However, when recognizing her pedagogical ability, I believe that she decided correctly for her future.

There is absolutely no doubt that her PhD thesis should be accepted by the PhD examination board. For the case Mgr. Lenka Beranová will successfully defend her thesis, he should be awarded with the title PhD.

Prague, 27.9.2013

Prof. Dr. Martin Hof, DSc.