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Review of the habilitation thesis
„Membrane at the nanoscale – molecular insights by fluorescence microscopy"
submitted by Dr. Radek Šachl

Lipid membranes are one of the most important structural constituents of living cells. They serve as boundaries between a cell's exterior and interior, but also between different cellular organelles and compartments, but contain also innumerable embedded proteins that regular matter exchange or play important roles in cellular sensing. Thus, the study of the physical and chemical properties of lipid membranes (lipid bilayers) and their interaction with diverse proteins play an important role in modern biophysics. This exciting field of research is at the center of Dr. Šachl's habilitation thesis, where he uses advanced fluorescence spectroscopic methods and modelling for gaining deeper inside into membrane organization and protein-membrane interaction.

In particular, the thesis presents two new powerful fluorescence spectroscopic techniques, Förster resonance energy transfer with Monte-Carlo simulations (MC-FRET), and dual(+1) fluorescence correlation spectroscopy (2+1-FCS).

One of the most studied questions in membrane biophysics is the existence and role of so-called nano-domains in such membranes (also called sometime "lipid rafts"). The hypothesis about the existence of lipid rafts was first formulated by Singer & Nicolson [1] who considered the possibility of small membrane domains (~100 nm at most) in the fluid cell membrane bilayer. Ever since, researchers around the world have desperately tried to confirm (or reject) the existence of lipid rafts. Due to their postulated smallness and associated high dynamics, conventional microscopic techniques are fundamentally unable to resolve such small structures, but even modern super-resolution microscopy techniques will fail due to the expected high dynamics and possibly transient nature of the supposed rafts. As a result, innumerable strongly contradicting papers have been published that either supported or rejected the raft hypothesis. Here, the method of MC-FRET developed and applied by Dr. Šachl proved to be extremely powerful to yield quantitative results about lipid nanodomains. The core idea is to perform FRET measurements on membranes densely labeled with donor and acceptor dyes, and to accompany these measurements with clever Monte-Carlo simulations that allow for a

quantitative interpretation of the data. This research resulted in several excellent publications [2–7] demonstrating the ability to resolve and quantify nanometric highly dynamics nanodomains. To my knowledge, this is the most precise and convincing study about lipid nanodomains in biological membranes so far, and I consider this work as a landmark study for the field of membrane biophysics. Moreover, the method MC-FRET allowed Dr. Šachl and his colleagues to not only resolve and size nanodomains, but also to quantify surface coverage of labeled proteins and lipids, and to study leaflet-leaflet interaction. This last topic is another highly important question in membrane biophysics: How large is the coupling between the leaflets of a lipid bilayer, and how is this coupling modulated by lipid composition and embedded proteins? Here again, Dr. Šachl made significant contributions in detecting and quantifying interleaflet coupling [4,8,9].

The second important method that was developed by Dr. Šachl is dual(+1)-FCS. It uses a three-wavelength excitation for enabling cross-correlation measurements between two colors and using the third color for checking the intactness of vesicles [10]. This measurement technique is optimized for studying protein-membrane interactions, and was used by Dr. Šachl to correlate the oligomeric state of the fibroblast growth factor 2 protein with membrane pore formation, both statically and in a time-resolved manner. In another study, he employed the method for looking at peptide dimerization during membrane fusion [11].

In the above exposition of Dr. Šachl's work, I have only cited the most prominent papers. Dr. Šachl is an outstandingly prolific scientist who has made important contributions to the biophysics of biological membranes, both by the development of powerful new techniques as by their application to important questions. In our University, the work presented by Dr. Šachl in his habilitation thesis would fully meet all requirements for a successful habilitation defense. Thus, I highly recommend acceptance of his thesis by your faculty, and I congratulate you that you enlist such an excellent and internationally visible scientists among your members.

Sincerely yours,

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