UNIVERSITY OF CHEMISTRY AND TECHNOLOGY, PRAGUE
Faculty of Chemical Engineering
Department of Physical Chemistry

Oponentský posudek disertační práce

Adéla MELCROVÁ: Model membranes studied by advanced fluorescence techniques and molecular dynamics simulations.

In her PhD thesis, by means of experiment and simulations, Adéla Melcrová, investigated the structural changes and underlying kinetics in (and near) model membranes, when exposed to changes in the environmental conditions (mono- and divalent ions), or when bio-relevant molecules (cholesterol, trans-membrane domains of membrane proteins) are introduced in the membrane. In order to tackle the problem systematically, neutral (POPC) and mixed (POPC+POPS) membranes are investigated. This is a core of her work on signaling events. In order to achieve a solid and consistent micro- and macroscopic pictures in these systems, the research was performed in collaboration with foreign laboratories (prof. Cremer, Pennsylvania State University, US).
The final chapter of this thesis presents the theoretical investigation of the structural changes in the realistic, yet microscopic model of tear film lipid layer in case of dry eye disease. Moreover, the mechanism of potential positive effect of certain 'drug molecules' (e.g., CKC) on tear film lipid layer stability was modelled and analysed. This work may have in the near future even practical consequences for every day life.

Overall, the thesis is well build, chapters are systematically ordered, and the text clearly written with only small number of typos (page 35). Though, I would appreciate a bit more detailed explanation of the advanced florescence techniques, ideally with an illustrative interpretation of an established (well behaving) system. Similarly, as the charge-scaling methodology is a central approach in the atomistic membrane simulations, I would appreciate to see the comparison of the failure of the past-attempts in the field vs. the successful application of the current simulation approach.

The candidate and her work benefited from the expertise of the two excellent laboratories. Being part of the Prof. Hof group is visible in high quality experimental data, which allows to probe the kinetics and structural properties of the lipid bilayer. The solid computational approach, which conveniently switches between all-atom and coarse-grained description, builds on the experience of Dr. Cwiklik in the field, and supplement the microscopic insight.

This PhD thesis is backed by 5 directly related publications in peer-reviewed journals (*Scientific Reports, Chemical Communications, or Journal for Modeling in Opthalmology*). I must appreciate that the scientific scope is rather broad and covers three related, but different topics, which must have required significant time to adopt. Clearly the candidate can only profit from this fact in her future scientific career.

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To summarize, I must clearly state that Adéla Melcrová conducted independently very good joined experimental and theoretical research. This thesis and her scientific work fulfills all criteria for obtaining PhD degree. Thus, I am happy to recommend Adéla Melcrová being awarded the PhD degree.

Prague, June 12, 2019

RNDr. Mgr. Jan Heyda, Ph.D.

Questions for the defence that should be addressed by the candidate:

1. When dealing with divalent Ca^{2+} cation, scaled (ECC) charges were employed (also on Cl^-). On the other hand the other constituents of the system, such as POPC, POPS lipids were used with their original charges (full Berger). I see a certain inconsistency in such description. Does the literature, or the candidate, report some systematic comparison for combination of full and ECC charges? Were the Lennard-Jones parameters readjusted? Finally, which physico-chemical properties of membrane-systems are usually used to benchmark the force-field parametrization?

2. MARTINI force-field is significantly coarse-grained, thought in the TFLL project, the findings are very robust and convincing. In order to stabilize TF, it was found that the length of the introduced molecule (CKC vs. BAK) must be compatible with deficient polar lipids. This appears to be an excluded volume effect. How much of chemical detail, such as polar and charged moieties, can be described/introduced by MARTINI force-field? How qualitative, or quantitative are the predictions from MARTINI simulations?

3. The experimental techniques (zeta-potential measurement, total spectral shift, $\Delta\nu$, relaxation time, and general polarization, etc.) are well complemented with computer modelling (density distributions of individual species, their diffusion, etc.) and analysis. Could the candidate summarize, which experimental and simulated properties qualitatively match together? Which one may be good candidate for even a quantitative match?

4. From a physical-chemistry point of view, the stabilization of a biomolecule, can be caused by osmotic pressure (i.e., salt depletion from the vicinity of the protein native state), or by bridging interactions between the additive and the surface of the biomolecule (Parsegian et al., PNAS, 2000, 97, 3987-3992). In the former case, this is due to excess hydration, while in the latter case by surface excess of an additive (of the electrostatic origin for ions, or due to hydrophobic forces for surfactants). Would this view be applicable also for the observed action of Na^+ (screening + depletion) vs. Ca^{2+} (tight binding on the (sub)surface) on membranes? Is it supported by some of the obtained experimental, or simulation data?

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