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Evaluation report of the doctoral thesis

Author of the doctoral thesis: Barbora Svobodová
Title of the thesis: Membrane organization and dynamics of glycosphingolipid nanodomains
Reviewer: Radek Šachl

Gangliosides, specific lipids composed of a hydrophobic ceramide backbone and a bulky hydrophilic sugar headgroup, which are naturally found in neuronal plasma membranes, have diverse biological functions based on their ability to interact with biologically active molecules. A key feature of gangliosides is their tendency to cluster and form nanoscopic structures called ganglioside nanodomains, which serve as platforms for extracellular ligands to interact with the membrane. Disruption of these interactions has been linked to various human pathologies, including epilepsy, Alzheimer's disease, Parkinson's disease, and multiple sclerosis.

Our laboratory has a long-standing focus on ganglioside research. In 2012, we developed a novel fluorescence microscopy technique called MC-FRET (Förster Resonance Energy Transfer analyzed by Monte-Carlo simulations), which allows for the characterization of membrane nanostructures with nanometer precision. Early in her PhD, Barbora became familiar with this technique. She collaborated with colleagues to investigate the role of ganglioside headgroups in membrane clustering (Sarmento et al., Biophys. J., 2021) and identified the key components responsible for organizing gangliosides into nanodomains (Davidović et al., J. Phys. Chem. Lett., 2023).

In 2019, our team demonstrated that the MC-FRET could achieve single-leaflet nanometer resolution in characterizing lipid nanodomains (Vinklárek et al., J. Phys. Chem. Lett., 2019), enabling the study of interleaflet nanodomain coupling. However, it became clear that achieving such high resolution requires careful optimization of experimental conditions. Building on this work, Barbora focused on finding the optimal experimental conditions for studying interleaflet nanodomain coupling using MC-FRET and Monte-Carlo simulations. She successfully identified several critical parameters that when properly set enable the detailed characterization of inter-leaflet nanodomain coupling (Chmelová et al., Biophys. J., 2023). Related to this, she also co-authored a book chapter summarizing the principles and applications of the MC-FRET method (Chmelová et al., The Analysis of In-Membrane Nanoscopic Aggregation of Lipids and Proteins by MC-FRET, Springer Series on Fluorescence, 2023).

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In my view, Barbora's most significant contribution lies in her recent work, where she introduced a new quantitative approach to analyzing STED-FCS diffusion law plot dependencies for nanoscopically heterogeneous membranes (Svobodová et al, manuscript). This method has shown promise as a tool for studying lipid dynamics within nanoscopically heterogeneous membranes, but has so far provided only qualitative insights into membrane lipid dynamics (Eggeling et al., Nature, 2009). By applying this new quantitative methodology to ganglioside nanodomains, Barbora successfully determined lipid diffusion coefficients both within and outside of the nanodomains, as well as the diffusion coefficient of the nanodomains themselves. Furthermore, she used this approach to accurately measure nanodomain size and the surface concentration of gangliosides in giant plasma membrane vesicles and the plasma membranes of living cells, providing new fundamental insights into the dynamics and nanoscale organization of gangliosides in cellular membranes.

In conclusion, while Barbora's PhD thesis makes a solid contribution to the study of ganglioside nanodomains, her work also opens potential avenues for future research in membrane organization and dynamics. I believe her thesis meets the requirements for a doctoral dissertation, and I recommend it for defense. I am confident that her defense will further illuminate the significance of her findings and her ability to contribute to scientific research.

Radek Šachl

In Prague, 18.11. 2024