ÚFE Institute of Photonics and Electronics
The Czech Academy of Sciences

Doctoral thesis review from the reviewer Michal Cifra

Title of the doctoral thesis:
Membrane organization and dynamics of glycosphingolipid nanodomains

Author of the doctoral thesis:
Barbora Svobodová

This thesis offers a comprehensive and insightful exploration of ganglioside nanodomains, shedding new light on their structure, organization, and dynamics. By focusing on the unique role of bulky sugar headgroups in ganglioside clustering, the research provides strong evidence on how the number of sugar units and sialic acid residues significantly influence mutual interactions among gangliosides. The discovery of GM2's distinctive behavior in disrupting hydrogen bonding networks and the critical role of sphingomyelin in stabilizing nanodomains are particularly noteworthy. The combination of experimental data with molecular dynamics and Monte Carlo simulations adds depth and robustness to the findings, enhancing our understanding of membrane organization at the nanoscale.
Moreover, the development and application of innovative fluorescence techniques such as MC-FRET and STED-FCS demonstrate the candidate's ability to push methodological boundaries. These techniques allowed for nanometer-scale resolution in analyzing interleaflet nanodomain coupling and provided new insights into the dynamic behavior of these domains. The thorough analysis of STED-FCS diffusion law plots not only validated the experimental approach but also enriched the interpretative framework for studying mobile membrane nanodomains. Overall, this work represents a significant contribution to the field of membrane biophysics, offering valuable methodologies and insights that will undoubtedly inspire future research on ganglioside-containing membranes.
The thesis is well-written, with the results clearly described with high level of graphics quality. The typographical errors and description omissions are rare (e.g. missing description of “a” in eq. 1.2)

Questions:

1. In page 24, you write “Under the dynamic limit conditions assuming the dipole reorientation is much faster than energy transfer”. Could you clarify what dipole is considered here and the assumed range of time scales for the dipole reorientation and energy transfer ?
2. “Possible scenarios of probe localization in a nanoscopically heterogeneous membrane according to partition coefficient” e.g. in Fig. 2.3: What would be an effect of swapping positions of FRET acceptors or donors ? Would that be useful for any analysis ?
3. Conclusion, p. 56 “An interesting observation from this work is that the size of ganglioside nanodomains generally scales with the size of the glycan headgroup, suggesting a direct correlation between ganglioside structure and domain size.” In the biological tissue scenario, where the cells are tightly packed, could the steric effects limit the formation of nanodomains with very large glycan headgroup ?

Overall, the thesis is of a very high quality, delivers proof of candidate's capability to carry out independent creative work. I recommend defense of this thesis.

Prague, 21th November, 2024
Ing. Michal Cifra, PhD.

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Signature