

## Opponent's review of the dissertation thesis

**Title of the Thesis:** Membrane organization and dynamics of glycosphingolipid nanodomains

**Author:** Ing. Barbora Svobodová

**Supervisor:** doc. RNDr. Radek Šachl, Ph.D.

**Institution:** Institute of Physics of Charles University, J. Heyrovsky Institute of Physical Chemistry  
CAS

**Study branch:** Biophysics, Chemical and Macromolecular Physics

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### Introduction and Significance of the Thesis

The dissertation by Ing. Barbora Svobodová addresses a critical area of biophysical research, specifically the organization and dynamics of glycosphingolipid nanodomains in cellular membranes. The topic is highly relevant, as nanodomains play a fundamental role in many cellular processes including intercellular communication, adhesion, and differentiation. The work is scientifically significant due to its introduction of novel analytical methods and their application to important biological systems.

### Main Objectives and Methods

The objectives of the thesis include characterizing nanodomains formed by gangliosides, identifying key molecular structures responsible for their formation, and quantifying the dynamics of these structures. The use of advanced fluorescence techniques such as MC-FRET and STED-FCS marks an important step forward. Additionally, the author has contributed new quantitative tools for data interpretation.

### Evaluation of Content and Scientific Quality

The thesis is logically structured and well-written. The chapters cover the theoretical background, methodology, results, and discussion comprehensively. The findings offer new insights into the formation of nanodomains and their physical properties, with potential implications not only for biophysics but also for fields like therapeutic development.

A key strength of the thesis is the extensive use of experimental data combined with computational simulations. The author demonstrated the ability to apply complex methods to biological systems and interpret results within the context of current scientific knowledge.

### Formal and Linguistic Quality

The thesis is professionally prepared, with clear and high-quality graphical representations of results. A few typos or formal shortcomings, including inconsistent placement of references, are noted but do not significantly impact the work.

### Comments and Questions for the Defence

1. Could the author elaborate further on the limitations of the methods used? How could the identified methodological limitations be mitigated in future studies? Are there complementary techniques that could enhance the robustness of the findings?
2. What are the potential applications of the proposed quantitative tools in other areas of biophysics or pharmacology?
3. Can the results of this work be extended to other types of lipids or biological membranes?

### Conclusion

The dissertation by Ing. Barbora Svobodová meets all the requirements for a scientific work of this kind. The author has demonstrated the ability to conduct independent research, advance scientific knowledge, and present findings at a high level. I recommend the thesis for defence and propose awarding the Ph.D. degree.

Barbora Špačková  
Institute of Physics of the Czech Academy of Sciences