Reviewer's Report on Doctoral Thesis

"Development of dual-(+1)-Fluorescence Correlation Spectroscopy for Monitoring Protein Oligomerization Leading to Membrane Pore Formation"

Author: Vandana Singh, J. Heyrovský Institute of Physical Chemistry of the Czech Academy of Sciences, Dolejškova 3, 182 23 Prague, Czech Republic

This PhD thesis presents an interesting and innovative contribution to the field of membrane biology, specifically focusing on the mechanisms of protein oligomerization and membrane pore formation. The author introduces a novel experimental approach, demonstrated through the case study of Fibroblast Growth Factor 2 (FGF2), which holds potential for future research. The dual-(+1)-FCS method developed in this dissertation is an advanced and sophisticated technique for correlating protein oligomerization with membrane permeability in free-standing membranes. The method's ability to analyze brightness and diffusion properties of fluorescently labeled proteins in giant unilamellar vesicles (GUVs) offers insights into protein behavior on the nanoscale.

The thesis is well-structured, with a clear division between the methodological development and its application. The first part demonstrates the power of the newly developed fluorescence assay in distinguishing functional oligomers from non-functional aggregates, addressing a critical challenge in the study of membrane proteins. This assay's ability to provide time-resolved analysis makes it especially valuable for tracking dynamic processes like pore formation. In the second part, the author applies this methodology to the specific case of FGF2, providing key insights into the oligomerization states responsible for membrane permeabilization. The clear distinction between functional and non-functional protein aggregates is a significant achievement and opens up new avenues for studying other membrane-associated proteins.

Overall, this PhD thesis is a robust piece of research that advances both the methodological and theoretical aspects of membrane protein studies. The clarity of the writing, the rigor of the experiments, and the novelty of the approach all contribute to making this dissertation a significant contribution to the field. The

author's work has the potential to influence future research in membrane biology and protein oligomerization for years to come.

In summary, the PhD candidate, Vandana Singh, has conducted a substantial and insightful body of research, producing original findings that significantly enhance our understanding in membrane biology and protein oligomerization. The doctoral work demonstrates a high level of scientific rigor, and all key results have been published in reputable peer-reviewed journals. Based on the quality and depth of the research presented in the dissertation, Vandana Singh is fully deserving of the PhD degree.

For the discussion, I propose the following questions:

1) Could you briefly summarize the key discrepancies you've identified in the existing literature that have shaped the direction, objectives, and focus of your research?
2) Can author provide a critical insight into the limitations of the study in the context of using artificial in vitro systems mimicking biological cells?
3) Is there any competing hypothesis contradicting unconventional protein secretion (UPS) pathways in context of FGF2 secretion?

Sincerely,

Oleg Lunov, PhD
Head of the Laboratory of Biophysics,
Department of Optic and Biophysical Systems,
Institute of Physics of the Academy of Sciences of the Czech Republic
Na Slovance 2, Prague, Czech Republic
Telephone: +420-266-05-2875
Email: lunov@fzu.cz
Web: https://www.fzu.cz/en/research/divisions-and-departments/division-4/department-21/laboratory-2102

Institute of Physics
of The Czech Academy
of Sciences
Na Slovance 1999/2
182 21 Prague 8
Czech Republic
Tel.: +420 266 052 110
Email: info@fzu.cz
Web: www.fzu.cz