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Review
of a doctoral thesis
prepared by **Sakshi Sareen**
titled **"Biophysical Symptoms of Cellular Stress"**
under the supervision of **Prof. Robert Holyst**
and **Dr. Karina Kwapiszewska** (auxiliary supervisor)
at the Institute of Physical Chemistry, Department of Condensed Soft Matter
Polish Academy of Sciences

1. Formal Assessment of the Dissertation

The dissertation comprises a 106-page document. It includes a 20-page introduction outlining the research topic and current scientific challenges related to tracking cellular changes under induced stress. The main body of the work presents the research findings, a comprehensive discussion, and concluding remarks. The research methodology and a list of abbreviations were provided in the form of appendices.

The dissertation is organised into three chapters, contains an abstract in two language versions, and has a bibliography listing 158 references. The results are illustrated with 37 figures and 8 tables.

The dissertation has been well-organised and prepared. Particular attention should be given to high-quality microscopy images, the coherent and consistent formatting of the graphs, and the inclusion of active hyperlinks to the list of abbreviations. Only a single formatting error was identified throughout the document: reference number 130 was not formatted as a superscript (page 54, line 2).

Based on the Scopus database, Sakshi Sareen has authored one scientific article published in Nanoscale (RSC, IF 5.8, 140 MEiN score), one review article published in Chemical Communications (RSC, IF 4.3, 200 MEiN score) and one conference paper published in

Progress in Biomedical Optics and Imaging - Proceedings of SPIE. Sakshi Sareen is the first author of all of the aforementioned publications.

2. Assessment of the Dissertation's Objective and Relevance of the Topic

The research conducted by the PhD student investigates the stress-induced changes in the biophysical properties of cancer cells during long-term starvation and mitochondrial stress. The studies were supported by the National Science Centre, Poland, within the grant OPUS UMO-2019/33/B/ST4/00557 and by the Polish National Agency for Academic Exchange and Genetics division, Department of Medicine at Brigham and Women's Hospital and Harvard Medical School.

Understanding how cells respond to stress at the microscopic scale is fundamental in advancing the knowledge of cellular dynamics under pathological conditions. Analysis of biophysical changes, i.e. intracellular viscosity, diffusion coefficients and fluorescence lifetime, provides crucial information on how cells adapt, survive and die. This insight is critical to the context of cancer, where stress-induced changes often disrupt normal cellular function, may promote malignant transformation, and contribute to the progression of disease.

These findings can potentially develop novel strategies for preventing and diagnosing diseases such as cancer, thereby significantly contributing to advancing chemistry, materials engineering and medicine.

3. Substantive Assessment of the Dissertation

The introduction is well-written and presents the main findings connected to biophysical changes in selected cells induced by two types of stress: cellular starvation and staurosporine-induced apoptosis (mitochondrial stress). The PhD student selected biophysical properties that, in her opinion, can be utilised as label-free markers to investigate and understand physiological changes occurring within the cell, such as macromolecular crowding, autofluorescence activity, as well as nanoviscosity and diffusion alterations. The introduction also describes the methods applied to study the physiological changes induced by the stress within the cells.

To sum up, the introduction is well-written and shows that the author understands the current scientific challenges. **It is logically structured and coherent, effectively guiding the reader through the study's background, objectives, and rationale.** Each subsection ends with a summary or interesting comment highlighting the importance.

The scientific hypothesis assumes that starvation causes biophysical changes in the cells and finally leads to their death. Prolonged starvation result in a decrease in ATP level inside cells and increased nanoviscosity (or decreased diffusion of molecules).

The thesis applied validated mathematical models to analyse intracellular diffusion and knockout HeLa cells during induced starvation. The selection of models and their detailed description can be considered both accurate and methodologically sound.
The manner in which the results were presented, in addition to the thoroughness and depth of their analysis and subsequent discussion, merits particular commendation.

The key findings from the presented results indicate that cell starvation reduces GFP molecular diffusion in the cytoplasm and affects transport and molecular interactions. Moreover, starvation changes the cytoplasmic mesh structure, causing cell shrinkage and is linked to water efflux. The findings further demonstrate how cells modulate stress across distinct cellular compartments, ensuring the protection of the nucleus, which plays a vital role in preserving cellular integrity and function. It also indicates that the knockout of a transmembrane protein involved in cell volume homeostasis leads to alterations in the cytoplasmic nanostructure, thereby affecting the diffusion patterns of GFP within the cell.
In case of the techniques applied, the results indicate that drug-induced mitochondrial stress increases autofluorescence in cancer cells. Fluorescence lifetime analysis of FAD, an intrinsic autofluorescent molecule, enables the differentiation of viable cells from those experiencing stress or undergoing cell death. This approach offers a valuable tool for monitoring mitochondrial dysfunction at the single-cell level.

Does the author intend to continue the research by searching for other proteins that could serve as markers for studying/monitoring cell functions?

Is there a plan to transfer the experiments and findings to the *ex vivo* or *in vivo* level?

4. Final remarks

The doctoral dissertation by Sakshi Sareen, entitled *Biophysical Symptoms of Cellular Stress*, contributes to science in terms of cognitive value and practical relevance. **Through the research presented in the dissertation, the doctoral candidate has demonstrated her mastery of fundamental scientific knowledge and expertise in chemistry, materials engineering and medical sciences.** She has shown considerable scientific and technical creativity in planning and executing the research, at a level that confirms her ability to conduct independent scientific investigations. **Particular attention should be given to the exemplary use of biophysical, chemical, and advanced molecular biology methods.**

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In conclusion, I confirm that the work meets the requirements for a doctoral dissertation, particularly those specified in Article 13 of the Act of 14 March 2003 on Scientific Degrees and Academic Titles and Degrees and Title in Art (Journal of Laws No. 65, item 595). I therefore request that Sakshi Sareen be admitted to the public defence. Due to the very high scientific quality, I recommend the doctoral dissertation for **distinction**.

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