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Review of the doctoral dissertation by Sakshi Sareen entitled „Biophysical symptoms of cellular stress"

Contemporary cell biology increasingly uses tools and concepts derived from physics to understand and quantitatively describe the complex processes occurring in living organisms at the molecular level. A cell is not merely a collection of biochemical reactions, but a dynamic system in which the movement of molecules, changes in volume, interactions between macromolecules, and the properties of the intracellular environment—such as viscosity, density, and degree of order—play a key role in regulating life processes. The developing field of cellular biophysics not only enriches our understanding of the fundamentals of life, but also offers potential diagnostic and therapeutic tools, particularly in the context of diseases associated with cellular homeostasis disorders, such as cancer, neurodegenerative diseases, and viral infections. The doctoral thesis submitted to me for review by Sakshi Sareen, completed at the Department of Condensed Soft Matter Institute of Physical Chemistry PAS, under the supervision of Prof. Robert Hołyst and Dr. Karina Kwapiszewska, fits perfectly into this research trend, and its aim was to understand the response of cells to cellular stress. This may be crucial for discovering how cellular processes change in disease states. The thesis focuses on the analysis of biophysical factors such as intracellular viscosity, diffusion coefficients, and fluorescence lifetime in order to draw conclusions about the mechanisms of cell adaptation, survival, and death. The doctoral thesis has a classic structure, typical of experimental works at

the intersection of biology and physics. The dissertation is written at a high level of expertise, but in a very concise manner, which will be appreciated by busy reviewers. The 83 pages of this dissertation describe the theoretical introduction, followed by the obtained results. In addition, two appendixes are included, containing the abbreviations used and descriptions of the experimental procedures.

In terms of content, the doctoral dissertation on the biophysical consequences of cellular stress is an ambitious and timely attempt to understand the adaptive mechanisms that occur within cells in response to external factors that disrupt homeostasis. The author focuses on two fundamental forms of stress—cellular starvation and mitochondrial dysfunction—which affect all living organisms regardless of their level of organization. The research described is part of one of the key trends in contemporary cell biology and biophysics, which is the quantitative capture of dynamic changes occurring in the dense, heterogeneous, and highly organized intracellular space. The use of advanced fluorescence methods, such as fluorescence correlation spectroscopy (FCS) and fluorescence lifetime imaging (FLIM), allowed the author not only to assess changes in molecule diffusion (using GFP as an example), but also to draw conclusions about the structural and functional reorganization of cells under stress conditions. It is particularly important to show how stress affects phenomena such as reaction compartmentalization, molecular transport control, and cell volume maintenance—parameters that have a direct impact on cell survival. It is also worth noting that the author supplements the biophysical analysis with a functional approach, using genetic engineering (CRISPR-Cas9) to study the role of anion channels in maintaining diffusion and volume homeostasis. This combination of modern measurement methods and genome editing is a strong asset of the dissertation and demonstrates the doctoral student's high level of research maturity.

The doctoral candidate's scientific achievements related to the dissertation include two papers published in Nanoscale and Chemical Communications, in both of which she is the first co-author. Two further papers are in preparation. The paper published in Nanoscale shows how prolonged starvation leads to acute stress, inducing a state of cellular rest and reducing energy consumption. The doctoral student demonstrated that the lack of nutrients stops the movement of large ribosome subunits, and attributes this effect to water loss by cells, which causes a significant reduction in their volume. The second paper, published in Chemical Comm. is essentially a critical commentary (with experimental eviences) on another paper published in the same journal in 2019 describing the fluorimetric detection of ATP in water using an

imidazole hydrazine-based sensor. Based on their own research, the doctoral student and her colleagues question the use of bisanthrone as an ATP sensor in aqueous solutions with a pH close to physiological pH.

The doctoral candidate's most important achievements include the following:

1. Contribution to the understanding of cellular biophysical parameters under stress conditions.

2. Innovative approach: The work shifts the focus from traditional molecular research to the biomechanical and biophysical aspects of cellular response to stress, which is a unique and less explored approach.

3. Accurate characterization of changes in viscosity and diffusion at the subcellular level allows for the quantitative capture of adaptive responses that are not visible at the level of classical biomarkers.

4. Discovery of a differentiated spatial response of cells to stress. It has been shown that the cytoplasm and nucleus respond differently to starvation stress — the nucleus remains relatively

5. Observations leading to the conclusion that cellular stress leads to the remodeling of the cell nanostructure, as documented by the transition of the cytoplasm structure from liquid to gel and a reduction in pore size, which affects the mechanical properties of the cell (rigidity, contractility).

6. Introduction of autofluorescence and fluorescence lifetime as markers of mitochondrial stress. The PhD student demonstrated that FAD fluorescence lifetime can serve as a sensitive, non-indicator marker of mitochondrial dysfunction, which has potential diagnostic and prognostic significance. The consistency of the results obtained in different cell lines confirms the universality and reproducibility of the method.

7. Interdisciplinarity - The work combines methods of cell biology, biophysics, and advanced microscopy, demonstrating the doctoral student's high level of methodological competence.

The biggest doubt that arose while reading the thesis is to what extent the research presented in the dissertation, conducted on cell lines, will be reflected in whole tissues. For example, it is difficult for me to imagine that in a living organism, cells under starvation would get rid of such significant amounts of water to reduce their volume by half (assuming that weight loss would be straightforward). I would ask the doctoral student to present several ideas for verifying the most important theses of her work during the public defense, but not on isolated cells, but on more complex biological systems.

I have no doubt that the experimental material obtained during the work is valuable for cognitive reasons and, in the future, perhaps also for diagnostic purposes. The doctoral student has made a significant scientific contribution to the field of cell stress research by presenting an original, quantitative approach to describing the biophysical changes that occur in response to stress conditions. Her work is characterized by conceptual maturity, a high methodological level, and application potential, which fully justifies the positive assessment of her achievements and the high rating of her dissertation. The dissertation makes a significant contribution to the methodology of research on the influence of cellular stress on the biophysical parameters of cells. I believe that the research techniques developed during the work will allow for the improvement of research on cellular processes and the development of diagnostic tests. In conclusion, I am fully convinced that the doctoral dissertation submitted for my assessment meets all the conditions specified in the Act of July 20, 2018, on higher education and science (Journal of Laws of 2018, item 1668, as amended), the Act of July 3, 2018, Introductory Provisions to the Act on Higher Education and Science (Journal of Laws of 2018, item 1669, as amended), and I request the Scientific Council of the Institute of Physical Chemistry of the Polish Academy of Sciences to admit Sakshi Sareen, M.Sc., to the next stages of the doctoral procedure.

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Elektronicznie podpisany przez Jacek Jemielity; Uniwersytet Warszawski
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