REPORT

Considering the competition for the academic position "**Assoc. Professor**" in the area of higher education 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological Sciences, scientific specialty "Biophysics" announced in the State gazette issue 69 dated August 16, 2024, for the needs of the Institute of biophysics and biomedical engineering, Bulgarian Academy of Sciences, department of Lipid protein interacvtions".

with candidate in the competition **Assistant Professor Dr. Rusinova Lachezarova Hazarosova-Dimitrova**

by Prof. DSc Stefka Germanova Taneva, Institute of biophysics and biomedical engineering, Bulgarian Academy of Sciences

General presentation of the materials received for review

Assistant Professor Rusina Lachezarova Hazarosova-Dimitrova, Ph.D., is the only candidate in the competition. The materials submitted by the candidate have been prepared in accordance with the Regulations for the Scientific Development of the Academic Staff of the IBFBMI-BAS and the criteria for occupying the academic position "Associate Professor".
The total number of points on the scientometric indicators is 494 (indicator A - 50, indicator B - 117, indicator D - 231, indicator D - 96 and indicator E - 120), with minimum national requirements and the regulations of ZRAS - IBFBMI-BAN for taking the academic position "Associate Professor" - 430.

Education and career development

Dr. Rusina Lachezarova Hazarosova-Dimitrova holds a bachelor's degree in biology from the New Bulgarian University, Sofia, and a master's degree in "Cell Biology and Pathology", Faculty of Biology, Sofia University "St. Kliment Ohridski". She defended a dissertation on the topic: "Influence of biologically active molecules on membrane organization" in 2016.
During the period 2004-2007 she worked as a biologist, from 2007 to 2011 as an assistant, and from 2017 until now as an assistant professor in the "Lipid-protein interactions" department, IPhFBMI-BAS. From 2012 to 2014, she worked as a medical representative at Fabric Bank Sadasa, Sofia.

Scientific indicators

Dr. Hazarosova has a total of **23** publications. She participates in the competition with **19** publications of which **6** were included in the habilitation work (2 with Q1, 2 with Q2, 1 with Q3 and 1 with Q4) and **13** were outside the habilitation work (3 with Q1, 3 with Q2, 4 with Q3, and 3 with Q4). 14 of the publications have IF (total IF 41.02) and 5 publications SJR. The publications were cited 48 times, h-index 5 (Scopus). Dr. Hazarosova is first author of 1 publication, second of 6 and last author of 1 publication. She participated in 44 scientific forums (national and

international). She was coordinator of 1 project and participated in 12 national and 2 international scientific projects.

Scientific Contributions

The research activity of Dr. Hazarosova is focused on studying the interaction of biologically active substances with membrane lipids and lipid domains, their influence on the structural organization and biophysical properties of cell membranes, the role of lipid rafts in pathological conditions.

The habilitation work (indicator B) includes 6 publications focused on the mechanism of action of natural antioxidant agents on biomimetic model membranes and cell lines.
Studies with the secondary metabolite phenyl glycoside myconoside (isolated from the plant Haberlea rhodopensis) on normal canine kidney epithelial (MDCKII) cells, as a model for polarized epithelial monolayers, showed interaction of myconoside with the cell plasma membrane and apical adhesive complexes of MDCKII cells. A change in membrane organization and adhesion complexes and cell viability was demonstrated depending on the concentration of myconoside, which was explained by a myconoside-induced hormetic effect. On the other hand, myconoside was found to interact with plasma membranes and concentration-dependently reorganize the lipid bilayer and change the fraction of lipid rafts in biomimetic membranes, and exhibit a cytotoxic effect on the viability of human lung adenocarcinoma A549 cells.

The ability of the polyphenolic compound resveratrol to modulate membrane organization has been demonstrated. The analysis of the action of resveratrol on the metabolism of sphingolipids in A549 cells and the level of raft-forming sphingomyelin, as well as on the enzymes responsible for maintaining the levels of sphingolipids with high physiological activity which determines the balance between apoptosis and survival of A549 cells, revealed possibilities for the combined use of the polyphenol with specific antiproliferative agents in complex antitumor therapies. Resveratrol was shown to differentially affect lipid ordering depending on the degree of fatty acid unsaturation at the sn-2 position of phosphatidylcholine (PC)-containing model lipid membranes and the bending elasticity in mono- and polyunsaturated PC-containing matrices. The results have implications for understanding the mechanism of action and pharmacological action of this antioxidant.

Non-habilitation publications (indicator G)

The publications summarize results of research on membrane organization, physicochemical properties of biomimetic membranes and changes in these characteristics induced by various factors.

Elucidation of the role of membrane-bound receptors involved in signaling events to maintain the transmembrane distribution of cholesterol and the formation and stabilization of lipid rafts. The level and transmembrane localization of cholesterol in the plasma membranes of β1 integrin-expressing (β1) and β1 integrin-deficient (β1 null) cells were found to be influenced by membrane-bound integrin receptors. A higher content of cholesterol as a component of lipid rafts and of sterol in the outer monolayer was determined in β1 cells. In both cell lines, the level of cholesterol in the outer monolayer of the membrane decreases when the level of sphingomyelin is reduced, but only in β1 cells the asymmetric distribution of

cholesterol is preserved. Depletion of sphingomyelin by sphingomyelinase was shown to reduce cholesterol levels in the outer membrane monolayer in both cell lines, but asymmetric cholesterol distribution was maintained only in β1 cells, suggesting a role for integrin receptors. These data indicate a role for certain proteins in maintaining the uneven transmembrane distribution of cholesterol in the formation and stabilization of lipid rafts.

Effect of oxidized lipids on membrane organization in mono- and polyunsaturated lipid matrices. The oxidized lipid palmitoyl-oxovaleroyl-phosphatidylcholine (POVPC) was found to affect the physicochemical properties of model membranes mimicking the lipid composition of rafts and the lipid ordering, size and dynamics of the raft domains depending on the nature of the lipid matrix (poly- or monounsaturated). The effect of POVPC was more pronounced in mixtures containing monounsaturated POPC compared to those containing polyunsaturated PDPC. The results suggest a protective role of polyunsaturated docosahexaenoic acid, from the family of beneficial Ω-3 fatty acids, against the generation of oxidized lipids during membrane-bound oxidative processes in cells.

Effect of oxidized lipids on lipid ordering and activity of secretory phospholipase A2. Oxidized lipids POVPC and PGPC were found to affect the composition, structure, and organization of the membrane bilayer in mono- and polyunsaturated PC vesicles, PGPC to a greater extent than POVPC in both types of vesicles. It has been shown that the activity of sPLA2 depends on the degree of unsaturation of the fatty acid at the sn-2 position in the glycerophospholipid molecule and on the type of oxidized lipid. POVPC inhibited sPLA2 activity in mono- and polyunsaturated PC matrices, whereas PGPC had a bimodal effect on sPLA2 activity depending on the type of PC matrix and lipid mixing protocol. For the first time, the formation of model systems (lipid hydration and mixing protocol) composed of polyunsaturated and oxidized glycerophospholipids was found to be essential for establishing the membrane lateral organization and activity of sPLA2.

Effect of the biologically active opioid peptide VV-hemorphin-5 (valorphine) and its analogues on the structural organization, mechanical and electrical properties of model lipid membranes. Unlike endogenous unmodified valorphine, its analogues increase the bending modulus and electrical capacity of the lipid membrane. The highest dipole potential of lipid bilayers was induced by the V-Dap peptide, and the Ile-V peptide induced lipid dehydration at the level of the glycerol backbone in membranes. These data have applications in the development of liposome-based strategies involving hemorphins as therapeutic agents and biomarkers in neuropharmacology, oncology, or inflammation.

Molecular mechanism of interaction of chitosan nanoparticles with biological membranes. Evidence is presented for changes in the lipid ordering and organization of zwitterionic biomimetic membranes (giant lipid vesicles mimicking the lipid architecture of plasma membranes) as a result of their interaction with chitosan nanoparticles regardless of the state of the lipid phase. The most significant increase in lipid order was observed for the Ld phases, while the lowest for the Lo phase. Chitosan has insignificant effect on the raft domains due to the high order due to cholesterol.

Ultrastructural changes, proliferation and ability of alveolar A549 cells to recover after treatment with the anesthetic halothane. A study of the effect of the inhalation anesthetic halothane on the ultrastructural organization and physiology of human lung adenocarcinoma A549 cells showed a decrease in the viability and suppression of mitotic activity, accompanied by DNA damage, disturbances in the

structure of nuclei and nucleoli, and a most pronounced negative effect on lamellar bodies. The results demonstrate a lack of direct interaction of halothane with DNA and irreversible damage to the cellular genome even at low anesthetic concentrations. These results indicate that halothane has genotoxic and cytotoxic effects on alveolar cells in vitro, which may modulate lung functions.

Critical notes and suggestions

I have no critical comments on the scientific works and the submitted documents.

CONCLUSION

The candidacy of Assistant Professor Rusina Lachezarova Hazarosova-Dimitrova meets the requirements for the academic position "Assoc. Professor". The relevance of the scientific topic and the quality of the scientific works give me reason to recommend to the members of the Scientific Jury and the Scientific Council of IBPhBMI-BAS to award Dr. Rusina Lachezarova Hazarosova-Dimitrova the academic position "Assoc. Professor" in the field of higher education 4. "Natural sciences, mathematics and informatics", professional direction 4.3. Biological Sciences, specialty "Biophysics".

Sofia
20.11.2024 г.

/Prof. DSc Stefka Germanova Taneva/