STATEMENT

by Prof. Dr. Ivaylo Vladimirov Dimitrov,
Institute of Polymers - Bulgarian Academy of Sciences

regarding the dissertation of Prof. Dr. Jordan Atanasov Doumanov entitled: *"Self-organization and surface properties of hBest1 in models of biological membranes"* for awarding the scientific degree "Doctor of Sciences" in the area of higher education 4. "Natural Sciences, Mathematics and Informatics", professional field 4.3. "Biological Sciences (Molecular Biology)"

This statement was prepared on the basis of the Order of the Rector of Sofia University (SU) "St. Kliment Ohridski" No. RD 38-595 from 31.10.2023 and a decision from the meeting of the scientific jury from 06.11.2023. It complies with the requirements of the Development of the Academic Staff in the Republic of Bulgaria Act (DASRBA), the Regulations for its Implementation (RIDASRBA) and the Rules on the Conditions and Procedure for Acquiring Science Degrees and Holding Academic Positions in SU “St. Kliment Ohridski”.

Prof. Doumanov has submitted all the necessary documents regarding the procedure in accordance with the requirements of the DASRBA, RIDASRBA and the University Rules.

Brief professional biography

Prof. Jordan Doumanov graduated from the SU "St. Kliment Ohridski" with a master's degree in "Cell Biology and Developmental Biology". In the period 1999-2001, he worked in Germany as a researcher successively at the Institute of Human Genetics at the University of Greifswald and at the Institute of Biochemistry at the RWTH-Aachen University. In 2006, he defended his doctoral thesis at the University of Hohenheim, Stuttgart, Germany on the topic "Identification of the basolateral sorting signal in the cytoplasmic domain of the interleukin-6 signal transmitter gp130". The scientific career of Prof. Jordan Doumanov in the Department of Biochemistry at the Faculty of Biology of the SU St. Kliment Ohridski” began later that year, holding the academic positions from assistant professor to a full professor for the period 2006-2021. He was on a two-year post-doctoral specialization at the Vision Institute, University of Pierre and Marie Curie, Paris, France (2008-2010) and on a 4-month specialization at the Andalusian Molecular Biology and Regenerative Medicine Centre (CABIMER), Seville, Spain (2009).

Scientific indicators

Prof. Jordan Doumanov is a co-author of 64 scientific publications with a total impact factor of 157.06, which have been cited more than 180 times. He was coordinator or team-member of 20 research projects. Under his (co)supervision, 3 doctoral students and 9 graduate

students successfully defended their theses. Prof. Doumanov has presented a list of 18 papers related to the topic of his dissertation, published in specialized scientific journals. Sixteen of them were published in journals with an impact factor, and the remaining 2 – in journals without an impact factor. The total impact factor of these publications is 62.303. In most of the presented publications, Prof. Doumanov is the leading author, which is a proof of his main role in the research carried out. Results related to the dissertation have been presented at 22 scientific forums. Four of the diploma theses defended under the supervision of Prof. Doumanov and the three PhD theses for the acquisition of the educational and scientific degree "doctor" concern separate issues related to the subject of the present work.
From the presented materials, it can be seen that Prof. Jordan Doumanov meets the minimum national requirements for all the indicators specified in the DASRBA and in the Regulations for its application, even exceeding them for one of the indicators.

Dissertation and scientific contributions

Prof. Doumanov's dissertation is devoted to studies on the transmembrane protein bestrophin-1 (hBest1) in cells and in models of biological membranes. The research is aimed at determining the main characteristics of the protein, including its structure, its interaction with the essential membrane lipids, and its association with the lipid rafts in cell membranes. The interdisciplinary research approach applied by the author is aimed at establishing the relationship between the structure and functions of hBest1, which is of fundamental importance for elucidating the molecular mechanisms underlying the severe retinal degenerative diseases (bestrophinopathies).

The dissertation is written on 196 pages, containing 69 figures, 3 tables and appendices (presented on 30 pages). A total of 294 literature references are cited.

The aim of the work is formulated clearly and precisely, namely to follow and study the role of hBest1 in the cell, its structure, organization and functions, the relationship between structure and functions, as well as the molecular mechanisms leading to bestrophinopathies. In order to achieve the aim specific tasks divided into two directions have been formulated.

The results obtained in the first direction are from studies of hBest1 in cell cultures as model systems. Conducting these studies becomes possible thanks to the development by a team led by Prof. Doumanov of a new, stably transfected with hBest1 cell line MDCK II - hBest1 (originating from kidney epithelium). In this cell line, growth characteristics, metabolic activity, morphology and polarization were found to be unaffected by the stable protein expression. hBest1 was shown to localize to the basolateral membrane in 6-day-old polarized MDCKII - hBest1 cells. The protein interaction with membrane lipids was also investigated and it was found that the expression of hBest1 in cells is associated with the increased accumulation and/or biosynthesis of non-lamellar at the expense of lamellar lipids. The association of the protein with the membrane domains of stably transfected MDCKII - hBest1 cells was further investigated and it was established that the presence of the protein caused an increase in liquid-disordered phase in the cell membrane. Applying two different approaches, it was shown that hBest1 preferentially associates with liquid-disordered (65%)

compared to liquid-ordered (35%) membrane regions. This distribution has a direct impact on the structure, oligomerization and function of hBest1.

The second direction of hBest1 evaluations, presented in Prof. Doumanov's thesis, is related to conducting of extracellular experiments in models of biological membranes. The key step for their successful implementation is the developed by Prof. Doumanov's group original methodology for isolation and purification of functionally active protein expressed by the MDCK II - hBest1 cell line. This enables the study and establishing the structure and surface characteristics of hBest1. Applying Fourier-transform infrared spectroscopy, the secondary structure of the protein was analyzed and refined. For the first time, hBest1 was visualized by atomic force microscopy (AFM). The same technique was used to demonstrate the protein aggregation in the presence of Ca^{2+} as well as its conformational changes in the presence of γ-aminobutyric acid (GABA) or glutamate (Glu). The studies related to the determination of the surface physico-chemical characteristics of two- and three-component Langmuir monolayers and Langmuir-Blodgett films containing hBest1 and the major membrane lipid components phosphatidylcholine (POPC), sphingomyelin (SM) and cholesterol (Chol) are of particular interest. Chol was found to enhance and stabilize the mixing of the components in hBest1/POPC/Chol and hBest1/SM/Chol films, respectively. The interactions of the protein with lipids, as well as the demonstrated condensing effect of cholesterol, have a direct impact on the association of hBest1 with the cell membrane domains, its conformation, surface organization and functions.

In the final part of his dissertation, Prof. Doumanov presents results from the biological characterization of spherical nucleic acids (SNA), based on a non-phospholipid nucleolipid, which is an original hybrid biomacromolecule composed of a lipid-like hydrophobic residue, chemically linked via two alternative methods to an oligonucleotide chain. The self-associated in aqueous media vesicular structures characterized with a dense oligonucleotide shell, were investigated for resistance to enzymatic degradation and cell internalization, as the stability and rapid internalization are hallmark properties of the SNAs. The obtained results can be considered as a basis for future research related to the application of various nanostructures as hBest1 carriers for its intercalation in the cell membrane and restoration of the membrane's transport functions.

The 18 conclusions and 7 contributions (3 of a fundamental nature, 2 of a scientific-applied nature and 2 of a methodological nature) formulated by the author correctly reflect the results of the research conducted and presented in the thesis.

The presented dissertation undoubtedly represents an original scientific study that fully achieves the set goals and objectives. Moreover, the obtained results provide an excellent opportunity for Prof. Doumanov to continue his innovative research related to hBest1.

Conclusion

The dissertation of Prof. Jordan Doumanov entitled "Self-organization and surface properties of hBest1 in models of biological membranes" fully meets the requirements for the acquisition of the scientific degree "Doctor of Sciences" according to the Development of the

Academic Staff in the Republic of Bulgaria Act, the Regulations for its Implementation and the Rules on the Conditions and Procedure for Acquiring Science Degrees and Holding Academic Positions in SU “St. Kliment Ohridski”. Based on this and the above-described analysis, ***I confidently give my positive assessment and recommend to the respected members of the Scientific Jury to award Prof. Dr. Jordan Atanasov Doumanov the scientific degree "Doctor of Sciences" in the area of higher education 4. Natural Sciences, Mathematics and Informatics", professional field 4.3. "Biological Sciences (Molecular Biology)".***

Author of the statement:

Sofia,
14.12.2023 /Prof. Ivaylo Dimitrov, PhD/