

STATEMENT

on dissertation thesis for the acquisition of the scientific degree "Doctor of Sciences" in Higher Education District 4. "Natural Sciences, Mathematics, and Informatics", Professional Direction 4.3. Biological Sciences (Molecular Biology)

Dissertation topic: "Self-organization and surface properties of hBest1 in models of biological membranes"

Author: Prof. Jordan Atanasov Doumanov

The statement was prepared by: Prof. Albena Vladimirova Alexandrova, Ph.D., Department of Physiology and Biochemistry, Vasil Levski National Academy of Sciences, member of the scientific jury, appointed by order of the Rector of the University of St. Kliment Ohridski" No. RD 38-595/31.10.2023

1. PRESENTATION OF THE PROCEDURE AND THE CANDIDATE

The documents presented by Prof. Jordan Doumanov for the defense procedure: dissertation thesis and accompanying documentation, fully meet the requirements of Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for the terms and conditions for acquiring scientific degrees and occupying academic positions at Sofia University "St. Kliment Ohridski".

Prof. Yordan Doumanov completed his higher education at the Faculty of Biology of Sofia University "St. Kliment Ohridski" with a master's degree in Cell Biology and Developmental Biology and a second specialization Teacher in Biology (1999), as well as Information and communication technologies in Education (2011). In 2006, he successfully defended his PhD at the University of Hohenheim in Stuttgart, Germany on "Identification of the basolateral sorting signal in the cytoplasmic domain of the interleukin-6 signal transmitter gp130".

His professional career began in 1999 as a researcher at the Institute of Human Genetics at the University of Greifswald, Germany (1999-2000) and the Institute of Biochemistry, RWTH - Aachen, Germany (2001). In 2001-2006, he was a PhD student at the University of Hohenheim in Stuttgart, Germany. In 2006, he was appointed as an assistant in the Department of Biochemistry of the Faculty of Biology at Sofia University. There, he successively held the positions of senior assistant (2007-2011), chief assistant (211-2014), associate professor (2015-2021), and professor (2021- until now, reg. no. 43841).

He has specialized in leading scientific institutes in the field of ophthalmology in France (Institute of Vision, Pierre and Marie Curie University, Paris) and Spain (CABIMER, Seville). These specializations are related to in-depth research on the bestrophin-1 protein and bestrophinopathies. Prof. Doumanov's interests in bestrophinopathies are developing consistently and successfully, which is reflected in his works and the introduction of this direction in the Faculty of Biology at Sofia Univerisity "St. Kliment Ohridski". The scientific group of the "Biochemistry" department is the only one in Bulgaria that researches hBest1.

As a professor in the Faculty of Biology, Doumanov is the lead lecturer of the mandatory courses for the Bachelor's degree in Biochemistry in full-time study form of the specialties "Biology", "Ecology and Environmental Protection", "Optometry", as well as of the "Biological Membranes" course for the "Molecular Biology" in distance learning form. He is a leading lecturer in the mandatory courses for the Master's degree in "Biochemistry", "Cell Biology and Pathology", "Fundamentals of Biochemistry", "Protein Sorting and Cell Polarization", as well as the elective course in "Model Membranes".

Prof. Doumanov participated in the supervision of 9 successfully defended diploma theses, 7 of which he was supervisor and 2 co-supervisor. He was also the co-supervisor of 3 successfully defended PhD theses at the Department of Biochemistry.

Prof. Doumanov was a participant in a total of 11 successfully completed scientific research projects, of which he was the head of 2 and a participant in 9 projects, financially supported by the National Scientific Research Fund. He was also a participant in 8 research projects (he was the head of 4 projects, the co-head of 1 project, and a participant in 3 of them), financially supported by SU "St. Kliment Ohridski", as well as a participant in 1 project for Scientific Infrastructure "Cell Technologies in Biomedicine".

2. STRUCTURE OF THE DISSERTATION

The dissertation thesis presented to me for opinion is well structured in a volume of 196 pages and contains the following sections: Introduction (2 pages), Literature review (23 pages), Aim and tasks (1 page), Materials and methods (22 pages), Results and Discussion (81 pages), Conclusions (3 pages), Contributions (1 page), Future Research (1 page), List of publications related to the dissertation topic (3 pages, 16 impact articles factor and 2 without an impact factor), List of participations in scientific forums, where results related to the topic of the dissertation were presented (4 pages, 21 forums in the country and 1 abroad), Appendices (30 items), references (294 titles in Latin). The dissertation follows the accepted requirements for this type of scientific work.

3. RELEVANCE OF THE DISSERTATION, EVALUATION OF THE RESULTS AND SCIENTIFIC CONTRIBUTIONS

The relevance of the dissertation topic "Self-organization and surface properties of hBest1 in models of biological membranes" is determined by the widespread of bestrophinopathies, which lead to progressive vision loss and are currently incurable. The bestrophinopathies are associated with more than 200 mutations in the bestrophin-1 (BEST1) gene and affect approximately 1 in 16,500 to 1 in 21,000 people. Human bestrophin-1 is a calcium-dependent chloride channel that is expressed on the basolateral surface of the retinal pigment epithelium. Other functions such as a γ -aminobutyric acid and glutamate transporter in the nervous system, as well as a connection with neurodegenerative diseases such as Alzheimer's, Parkinson's, epilepsy, etc., have been suggested for hBest1. The obtained results of the research and the acquired data of the thesis provide new knowledge and understanding of the surface organization of hBest1, its interactions with the main membrane lipids, and its relationship with the different microdomains in the cell membranes. They are fundamental to understanding the modulation and regulation of the activity of hBest1 channel molecules in cells.

Prof. Doumanov has more than 10 years of experience in research to elucidate the structure and functional activity of hBest1, and this logically determines the purpose of the research presented in the dissertation, namely, based on the structure-function relationship, to reveal the

molecular mechanisms in detail, leading to bestrophinopathies. The approach to achieving this goal requires the preservation of hBest1 translation by retinal epithelial cells in vitro. To achieve the goal, Prof. Doumanov has set himself the following tasks: 1) to create cell lines from transfected with normal and mutant forms of hBest1 eukaryotic cells with proven expression of hBest1 proteins in them and determining the influence of hBest1 on cell development and their metabolism and 2) in biological membrane models that are directly dependent on the isolation and purification of hBest1 from stably transfected MDCK II - hBest1 cells, to determine the surface physicochemical characteristics of Langmuir monolayers and Langmuir-Blodgett films of purified hBest1, as well as of two- and three-component Langmuir monolayers and Langmuir-Blodgett films containing hBest1 and combinations of the major membrane lipids phosphatidylcholine, sphingomyelin, and cholesterol. To realize the tasks set out in the dissertation, Prof. Doumanov applies an extremely wide range of modern and classic research methods using cell cultures and models of biological membranes (including Real-Time PCR, cytometry, immunofluorescence, molecular sieve chromatography, affinity chromatography, Fourier-transform infrared spectroscopy, Brewster-angle microscopy, atomic force microscopy, etc.).

Prof. Doumanov 's dissertation in-depth studies with cell cultures on the sorting of hBest1 and its mutant forms show that at least three sorting signals and phosphorylation are decisive for the correct targeting of hBest1 to the basolateral membrane; the expression of hBest1 in cells leads to a change in lipid metabolism, and the localization of the protein in the plasma membrane causes its "fluidization" and a decrease in the size of the ordered areas in it; the detected partial association (~ 30 %) of hBest1 with lipid rafts defines the main role of the different microdomains for the surface (self) organization and activity of the protein.

A significant number of new data was obtained in the development through the application of various methodological approaches and visually presented through tables, graphs, and photos (including a large part of them were exported as appendices - 30 pieces), enable a reliable and objective assessment of the functional activity of hBest1 depending on structural features. The interpretation of the obtained results convincingly shows the wide knowledge of the problem and the high professionalism of Prof. Doumanov. As a result of the conducted research, 18 conclusions and 7 contributions were formulated (3 of a fundamental nature, 2 of a scientifically applied nature, and 2 of a methodological nature), with which I fully agree. Of these, I would like to highlight the creation of two new, stably transfected with hBest1, cell lines and the original method of purification and isolation of hBest1 from the cells of the newly created MDCK II - hBest1 cell line, which gives the possibility to conduct studies with model membranes to understand the molecular mechanisms of interaction of hBest1 with basic lipids determining the surface properties, localization, (self)organization and functions of hBest1 in cell membranes. A very good impression is made by the planned future research of the author, which confirms his stable scientific interest in the problem and aspiration to obtain practically applicable strategies (the creation of nanoparticles with the participation of hBest1) for the transport and integration of the protein in cell membranes and the restoration of membrane functions.

4. EVALUATION OF THE SCIENTIFIC INDICATORS

Prof. Doumanov has presented a list of 18 publications on the topic of the dissertation work. Of these, 16 are in journals with an impact factor, and 2 are in journals without impact factor. The total impact factor of these publications is 62,303, and their observed citations are 44. Taking into account the distribution of publications by quartiles, it should be noted that out of 16 publications,

more than half - 9 publications are in journals with Q1. In journals with Q2 there are 3 publications, in journals with Q3 there are 2 publications, and in journals with Q4 there are 2 publications. Prof. Doumanov 's scientometric indicators are in full compliance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria - 390 points, which exceeds the minimum required number of points for this degree.

5. ABSTRACT

The abstract includes a significant part of the obtained results and their discussion. Essentially, the abstract reflects all the main conclusions and contributions of the dissertation work.

CONCLUSION

In the dissertation work of Prof. Jordan Doumanov, the original results of extensive innovative research, obtained by applying appropriately combined state-of-the-art methods, are presented. The paper is written precisely, illustrated with appropriate evidentiary material, and the data is interpreted expertly. The set specific tasks have been completed and the set goal has been achieved. During the development of the dissertation, significant results were achieved, which have a significant contribution, both in theoretical and practical aspects. The work of Prof. Doumanov finds a wide response among specialists in the field and characterizes the author as a highly qualified specialist in the field of molecular biology. The dissertation fully satisfies the criteria of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its implementation, and the Regulations of the SU on the conditions and procedure for acquiring the scientific degrees and academic positions of Sofia University for obtaining the scientific degree "Doctor of Sciences". The above gives me the reason to give a positive assessment of the work and to recommend to the respected members of the Scientific Jury to award Prof. Jordan Atanassov Doumanov the scientific degree "Doctor of Sciences" in Professional field 4.3 Biological Sciences (Molecular Biology).

12/12/2023

Prepared the statement:

/Prof. Albena Alexandrova/