

## STATEMENT

By: Assoc. Prof. Dr. Ivanka Georgieva Tsacheva,

Department of Biochemistry, Sofia University "St. Kliment Ohridski",  
member of the scientific jury appointed by order No RD 38-595/31.10.2023  
of the Rector of Sofia University "St. Kliment Ohridski",

of the habilitation thesis of Prof. Dr. JORDAN ATANASSOV DOUMANOV, entitled "**Organization and surface features of hBest1 protein in models of biological membranes**" for the scientific degree "Doctor habilitatus" in professional area 4.3. Biological Sciences (Molecular Biology)

### **Characteristics of the habilitation thesis**

The subject of the research work is human bestrophin-1 (hBest1), a protein that is expressed by the retinal pigment epithelium of the eye. Mutations in the BEST1 gene are known to cause diseases called "bestrophinopathies", which are associated with retinal degenerations. The scientifically defined problem at the basis of the dissertation work is establishing the relationship between the structure and function of the hBest1 channel protein and its role in the occurrence and development of eye pathophysiology. The research is extremely timely, in the context of a lack of molecular biological characterization of hBest1 to guide the creation of approaches to treat or counteract clinical symptoms. The scientifically defined problem at the basis of the research work is establishing the structure - function relationship of the hBest1 channel protein and its role in the occurrence and development of eye pathophysiology. The research is extremely timely, in the context of a lack of molecular biological characterization of hBest1 to guide the creation of approaches to treat or counteract clinical symptoms.

The specific aim of this dissertation is to investigate the physiological role of hBest1 in the cell - identifying elements of its structure and organization, its functions, and the molecular mechanisms leading to bestrophinopathies.

The research spans a significant period of time - the first article cited in relation to the thesis was published in 2013. The research work includes collaborations with national and European research institutions. The author has an in-depth knowledge of the methodological approaches of the research carried out, and his two-year experience at the "Institut de la Vision", Paris, played no small role in shaping his research strategy.

The dissertation is written in compliance with the requirements of the Act for the Development of the Academic Staff in Republic of Bulgaria (ADASRB), the Regulations for its implementation, and the Regulations for the conditions and the order for acquiring scientific degrees at Sofia University, and has a relatively standard structure, including an Introduction, Literature Review, Aim and Objectives, Methodological Section, Results and Discussion, in which the results are critically and analytically discussed, and Conclusions. The work covers 183 pages, with over 44% devoted to the results of the experimental work. The research strategy is based on a multidisciplinary approach and includes biochemical, immunological, molecular biological, physicochemical and cell biological methods. A large part of the research has been carried out in cell cultures as a model system and another part in model biological membranes. As a result of this work, two hBest1 expressing cell lines, RPE-1 and MDCK II, were established, with stable hBest1 expression achieved in the latter. In the same cell line, hBest1 sorting, transepithelial resistance, lipid composition in membranes and association of hBest1 with lipid rafts were investigated. The successful eukaryotic expression and purification of hBest1 are the basis of complex experiments performed to identify elements of the protein's secondary structure and to investigate its surface properties in Langmuir monolayers and Langmuir-Blodgett films, in which a number of properties of hBest1 were identified, such as topology, molecular area, "fluidizing" effect and increased elasticity of hBest1/POPC films, decreased elastic modulus of SM monolayers, spontaneous miscibility with lipids in hBest1/POPC/Chol and hBest1/SM/Chol monolayers, and increased resistance to the enzymatic action of PLA2. The influence of some of the mutant forms of the protein has also been identified, which is reflected in disturbances in their sorting and localization, and hence disturbances in hBest1 function.

This research work has fundamental, scientific and methodological contributions to the world and national literature.

### **Scientific metrics related to the habilitation thesis**

The results of the experimental work related to the dissertation are included in 18 publications of which 16 with a total impact factor of 62.303 and 22 communications at scientific forums in Bulgaria and abroad. The research articles date from 2013 to 2022, with 9 of them published in prestigious Q1 journals, three in Q2, and two each in Q3 and Q4. These publications have been cited 44 times. The publications related to this dissertation are part of a sound academic

achievement of 44 articles with a total impact factor of 156.07 (including 36 articles in Scopus and 183 citations in Scopus excluding half-citations and self-citations). For the time period of the research work prof. Doumanov was a scientific co-supervisor of 3 successfully defended PhD theses and two master theses, as well as a scientific supervisor of 7 successfully defended master theses. For the same period prof. Doumanov was the head of two projects at the National Research Fund and was the team leader of the Faculty of Biology's team in two others. In addition, he has led 4 projects at Research Fund of Sofia University, co-led 1 and participated in 3 others. He is also a participant in 1 Scientific Infrastructure project "Cellular Technologies in Biomedicine". The number of all publications, the referenced ones, as well as their specific citations significantly exceed the requirements of the ADASRB and the specific additional criteria for obtaining the degree of "Doctor habilitatus" at Sofia University "St. Kl. Ohridski" in the professional area "Biological Sciences" (Molecular Biology).

## **CONCLUSION**

Prof. Dr. Jordan Doumanov presents an original, comprehensive and in-depth scientific and applied work with an outstanding fundamental and methodological contribution, which also has clinical relevance. Dr. Doumanov has demonstrated the qualities of a well-established scientist and scientific leader of research projects, with a serious publication activity that has found wide echo in the citations of the international scientific community. Because of the above, I confidently give my positive assessment of the research, presented by the above-reviewed dissertation and author summary, results and contributions, and I propose to the esteemed scientific jury to award the degree of "Doctor habilitatus" to Prof. Dr. Jordan Atanassov Doumanov.

05.12.2023

Assoc. Prof. Dr. I. Tsacheva