REVIEW

in a competition for the academic position of "Professor", Professional field 4.3. Biological sciences (Biochemistry), field of higher education 4. "Natural sciences, mathematics and informatics", announced in State Gazette no. 32/16.04.2021 for the needs of the Faculty of Biology at Sofia University "St. Kliment Ohridski".

Reviewer: Corr. Member of BAS Prof. Rumen Pankov DSc, Faculty of Biology, Sofia University "St. Kliment Ohridski", appointed a member of the Scientific Jury according to order № RD-38-246/19.05.2021 of the Rector of Sofia University "St. Kliment Ohridski".

The only candidate who submitted documents for the announced competition is Associate Professor Jordan Atanasov Doumanov PhD, from the Department of Biochemistry at the Faculty of Biology, Sofia University "St. Kliment Ohridski". According to the document submitted by the Human Resources Department, Associate Professor Doumanov has more than 14 years and 10 months of experience in the area of the competition, which fully satisfies the requirements set out in the Law and the Regulations for its application. The materials submitted by the applicant are accurately prepared and completed in accordance with the legal requirements.

I declare that I have no common publications with the candidate submitted for this competition.

Brief biographical data for the candidate

Associate Professor Jordan Doumanov was born in 1973 in the town of Bansko. He completed his higher education at Sofia University "St. Kl. Ohridski " in 1999, majoring in Biology and Master's degree in" Cell and Developmental Biology", and at the same time received a second specialization - " Biology Teacher". After graduating, he went to Germany, where he first worked as a researcher at the Institute of Human Genetics at the University of Greifswald and the Institute of Biochemistry, RWTH-Aachen, and since 2001 has become a doctoral student at the University of Hohenheim in Stuttgart. In 2006 he successfully defended his dissertation on "Identification of the basolateral sorting signal in the cytoplasmic domain of the interleukin-6 signal transporter gp130". In the same year, Assoc. Prof. Doumanov returned to Bulgaria and started working as an assistant professor in the Department of Biochemistry of Faculty of Biology at Sofia University. In this department he continues his professional development, where he successively passes through the positions of chief assistant (2011-2014) and associate professor (2015-present).

Assoc. Prof. Dumanov has specialized for two years at the Institute of Vision, Pierre and Marie Curie University, Paris, France and CABIMER, Seville, Spain for four months. He is a member of the Union of Scientists in Bulgaria, section Biochemistry, Biophysics and Molecular Biology.

Scientific production and analysis of scientometric data

The total scientific production presented by Assoc. Prof. Doumanov includes 47 publications, 2 textbooks, 1 dissertation abstract and 78 participations with reports and posters in national and international scientific forums. Of all the scientific articles, 34 have been published in international journals with IF and Q rank, and among them stand out prestigious journals such as *Human Mutation* (IF 6.9), *Cellular Signalling* (IF 4.9), *FEBS Journal* (IF 4.5), *Langmuir* (IF 4.4).) and others. Sixteen of these articles have been published in journals belonging to the highest

quartile (Q1) according to the Scimago Journal Rank (SJR). The total impact factor from the presented scientific production is over 80.4, and in the reference for the citations 124 titles without self-citations are indicated. According to the data from Scopus, the Hirsch index of Assoc. Prof. Doumanov is 7.

For his participation in the current competition, Assoc. Prof. Doumanov has presented 20 scientific publications, one textbook and 46 participations in scientific conferences and congresses, which have not been reviewed in previous competitions for awarding scientific degrees or academic positions. The scientific articles in this competition make up 42.5% of all his scientific output, and the analysis of the publishing activity over the years shows that after his habilitation in 2015 he has published an average of 4 articles per year. This definitely demonstrates a very intensive research activity, especially for a university lecturer, who is also engaged in intensive lecture work. Of the articles in this competition, 14 have been published in journals with impact factor (total IF 43.13), and 9 (64.3%) are in magazines with impact rank Q1, 4 (28.6%) in Q2, and one (7.1%) in - Q3. This distribution well illustrates not only the intensity but also the high quality of the research conducted by Assoc. Prof. Doumanov.

The presented scientific production and the achieved scientometric data fully correspond, and in many of the indicators exceed the minimum requirements for awarding the academic position "professor", defined in the Regulations on the terms and conditions for acquiring scientific degrees and holding academic positions at Sofia University. Data for meeting the requirements for indicators of group B (required 100 points), 235 points of group D (required 200 points) and 150 points of group E (required 100 points) are presented.

Associate Professor Doumanov actively participates in supervising doctoral students and in the development of research projects. His participation in 8 projects (one international and 7 national), funded by the Bulgarian Research Fund, is noted in the presented materials. The report states that he was the leader of 2 of these projects. The total amount of the attracted project funds from the applicant is BGN 250,000. As a result of this active work, Assoc. Prof. Doumanov collects over 190 points from group E (required 150 points).

Analysis of scientific contributions

The scientific interests of Assoc. Prof. Doumanov and the published scientific results are entirely in the field of the announced competition and are focused on the characterization of the protein bestrofin and studies of newly synthesized nanoparticles and natural biologically active substances. The results presented in the non-reviewed publications so far are summarized in groups B and D, according to Appendix 1 of PPZRASRB. I will consider the presented articles as they are structured by the candidate, and in a summarized form I will present the most important scientific achievements, from which the scientific contributions follow.

In group "B" are presented 4 publications (all belonging to Q1) summarizing the results, which are a continuation of the main topic established by Assoc. Prof. Dumanov before his habilitation, namely - the study of the membrane protein bestrofin 1. In this sense they are quite correctly assigned to group "B". The published new studies exploit the advantage that his group achieved back in 2013, when the MDCK-hBest1 cell line was created and the methodology for isolating its expressed recombinant human bestrophin 1 (hBest1). Having the purified and functionally active protein, Assoc. Prof. Doumanov successfully uses the opportunity to expand the knowledge about the molecular structure of bestrophin, as well as its interaction with membrane lipids and its association with lipid rafts. These studies have been conducted in both

cellular and non-cellular model systems - a unique opportunity that has not yet been achieved by other research groups in the world.

The conducted researches enable Assoc. Prof. Doumanov to establish the structure and surface characteristics of bestrofin 1 in Langmuir monolayers, as well as the role of Ca^{2+} in the formation of the elements of its secondary structure. In addition, atomic force microscopy of Langmuir-Blodgett films has produced images of "pure" hBest1 for the first time in the world. Expanding the in vitro model systems by using combinations of the studied protein and different lipids in the formation of Langmuir monolayers, Assoc. Prof. Doumanov shows that phospholipids (POPC) can significantly change the organization and activity of hBest1 in cell membranes. The results demonstrate that the incorporation of hBest1 into the subphase of the monolayer does not lead to deep penetration of the protein, but rather the interaction takes place in the area of the heads of the lipid monolayer. Using sphingomyelin (SM) and mixed hBest1/SM Langmuir monolayers and agents involved in the normal functioning of hBest1 - calcium, glutamate (Glu) and gamma-aminobutyrate (GABA), it has been demonstrated that the addition of protein improves the order of the mixed Langmuir monolayers and the miscibility between hBest1 and sphingomyelin is a thermodynamically advantageous process, which is a prerequisite for strong protein-lipid interactions in biological membranes as well. Applying Brewster angle microscopy (BAM), the lateral phase separation and domain formation in binary hBest1/SM Langmuir monolayers were investigated in real time, and it was demonstrated that mixing and intermolecular interactions increase with increasing surface pressure. In the same article, the association of hBest1 with membrane domains in living cells has been investigated. By using stably transfected MDCKII-hBest1 cells and vital Laurdan staining, the presence of hBest1 was shown for the first time to cause an increase in the liquid disordered domains in the cell membrane. The protein has been shown to preferentially associate with liquid-disordered domains (65%) and is present in significantly smaller amounts in the liquid-ordered (35%) membrane domains. These results have been confirmed using another approach - treatment of membranes with detergents to isolate detergent insoluble and detergent soluble membrane fractions.

The papers from the second group - group G, with which Assoc. Prof. Doumanov participates in this competition include 10 scientific articles and one chapter of a book. Of all the articles, 5 (50%) were published in magazines belonging to Q1, 4 (40%) were from Q2 and one (10%) - from Q3. The articles from this group summarize the results of research, which can be attributed to three main thematic areas.

The first direction is expanding the studies on bestrophin, which are presented in a recently published article and a chapter of a collective monograph published in 2019. To bring the above-described model systems for in vitro study of hBest1 closer to biological membranes, pure protein (hBest1) or protein/lipid (hBest1/POPC, hBest1/SM) Langmuir monolayers in combination with one of the main membrane components - cholesterol are studied. Through these experiments, Assoc. Prof. Doumanov demonstrated that cholesterol has a condensing effect on the monolayers of "pure" hBest1, mainly in the presence of Ca^{2+} ions and induces a condensing effect in binary hBest1/POPC and hBest1/SM films.

The second direction summarizes research conducted in collaboration with researchers from the Institute of Polymers at BAS and dedicated to the characterization of newly synthesized nanoparticles designed to efficiently deliver nucleic acids to target cells. The contributions of these studies can be summarized as follows:

- It has been shown that the pathways of internalization and transfection efficiency in eukaryotic cells of polyplex nanoparticles synthesized on the basis of polyethyleneimine (lPEI20-

comb20-lPEI96; lPEI66-comb7-lPEI66; and lPEI96-comb5-lPEI48) and those containing poly (2-ethyl-2-oxazoline) (LPEI-comb-PEtOx), depend on the topology and shape of the polymer chain. Polyplexes with a denser structure have been shown to be more promising as transfection systems.

- It was found that nanoparticles containing POEGMA-b-PLL (poly (ethylene glycol) methacrylate (POEGMA) and poly (L-lysine) (PLL) blocks) diblock copolymer and DNA can penetrate directly through the plasma membrane, avoiding the endosomal pathway. The particles trapped in the cell can release the transferred plasmid, which remains intact and functionally active.

- It has been shown that when nanoparticles based on amphiphilic poly (2- (dimethylamino) ethyl methacrylate) - block - poly (ε - caprolactone) - block - poly (2- (dimethylamino) ethyl methacrylate) (PDMAEMA20-b- PCL70-b-PDMAEMA20) triblock copolymer, are in the form of cationic micelles and capsules they are not toxic to eukaryotic cells.

- Spherical nucleic acid nanoparticles (SNAs) containing poly (ethoxyethyl glycidyl ether) -block-poly (propyleneoxide) -block-oligonucleotide conjugate and oligonucleotide-block-poly (ε-caprolactone) -block oligonucleotide despite differences in size, morphology and structure have the typical SNAs toxicity, biocompatibility, increased cell uptake without the need for transfection agents and improved nuclease stability

The third direction in the research of Assoc. Prof. Doumanov is related to the study of the biological activity of substances isolated from snake venom or present in various plant extracts.

- Studies on vipoxin - the main toxic component in the venom of the *Vipera ammodytes meridionalis* clarify the various cellular effects that have its individual subunits. The PLA2 subunit has been shown to induce cytotoxicity, cytoskeletal rearrangement and early apoptosis in a concentration-dependent manner and to be related to its enzymatic activity, while vipoxin and the vipoxinic acid component (VAC) do not affect cell viability but show a high degree of genotoxicity.

- Extracts of *Haberlea rhodopensis* have been shown to affect the cell periphery and to disrupt tight contacts in keratinocytes.

- It has been found that methanol extracts of 6 species of *Inula* containing chlorogenic acids, as well as extracts containing flavonoid glycosides, flavonoid aglycones, phenolic acids and sesquiterpene lactones have different cytotoxicity to non-cancerous and cancer cells, which makes them suitable subject to further studies of potential anti-tumor effects.

- Extracts of in vitro cultured white dead nettle (*Lamium album L*) have been shown to have a well-defined antitumor effect.

Teaching activity

As a habilitated lecturer, Assoc. Prof. Doumanov is also engaged in active teaching. He conducts lectures from 11 lecture courses for full-time and part-time students at the Faculty of Biology and Physics at Sofia University, of which 7 in bachelor's and 4 in master's degree programs. A contribution to his educational activity is also the co-authored textbook Protocol Notebook in Biochemistry of the University Publishing House “St. Kliment Ohridski”. Assoc. Prof. Doumanov has been the supervisor of eight graduates and participated in the supervision of four doctoral students, three of whom have already successfully defended their dissertations.

The presented data characterize Assoc. Prof. Doumanov as a lecturer with intensive teaching work in the field of biochemistry, covering all aspects of this activity - from the development and implementation of new courses, through writing textbooks needed to prepare students to individual work with them. Assoc. Prof. Doumanov is respected by his students and colleagues and enjoys the name of a highly qualified and erudite lecturer.

Conclusion

I have the pleasure to personally know Assoc. Prof. Jordan Doumanov and for years to witness his success as a researcher and lecturer in the Department of Biochemistry. He is an established and sought-after specialist with high professional qualification, who maintains active research in the field of biochemistry. His scientific output is significant in volume and quality and exceeds the requirements for awarding the academic position of "professor", referred to in the regulations. He has extensive experience in leadership and teamwork, preparation of PhD students and graduates, competencies and skills for conceptualization and implementation of scientific publications and projects. This gives me reason to give confidently my positive assessment and to recommend to the Scientific Jury to choose Assoc. Prof. Jordan Atanasov Doumanov as a "professor" in the Professional field 4.3. Biological sciences, with a scientific specialty "Biochemistry".

Sofia, 28/07/2021

Reviewer:
Corr. Member Prof. Rumen Pankov DSc