

REVIEW

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About the competition for the scientific position “Professor” in professional field 4.3
“ Biological Sciences (Biochemistry) for the needs of the Department of Biochemistry,
Faculty of Biology, Sofia University “ St. Kliment Ochridski” announced in the State
Gazette issue . 32/16.04.2021

The only candidate in the competition is Assoc. Prof. Yordan Atanasov Doumanov, PhD. The candidate was born in 1973 in Bansko. In 1999 he graduated from Sofia University "St. Kliment Ohridski "master degree in Biology with specializations - Cell Biology and Biology of Reproduction as well as a Teacher of Biology. Since 2001 he is a PhD student at the University of Hohenheim, Stuttgart, Germany, where he successfully defended his PhD thesis on "Identification of the basolateral sorting signal in the cytoplasmic domain of the interleukin-6 signal transporter gp130" in 2006. After defending his PhD thesis he started his professional carrier in the Department of Biochemistry, Sofia University "St. Kliment Ohridski". Since 2015 he has been an associate professor in the same department. Assoc. Prof. Doumanov has been abroad several times on specializations in leading laboratories in the field of biochemistry and molecular biology in Germany, France and Spain, which has had a positive impact on his scientific qualification.

The scientific papers with which Assoc. Prof. Y. Doumanov applied for the competition are:14 scientific articles in journals with IF, with a total IF 43. 131, one chapter of a book, 5 articles in refereed journals without IF and one textbook with a team - Biochemistry Protocol Notebook. A number of participations in scientific events at Bulgaria and abroad have been applied also. He has been the leader and participant in a number of contracts funded by Bulgarian sources. Under his supervision, 4 PhD thesis were defended. He was also the supervisor of 8 diploma theses. More than 124 citations of his scientific papers have been noted. 75 of them are on the scientific papers with which he applied in the competition.

According to the attached materials, the candidate exceeds the minimum requirements of the Law for the Development of the Academic Staff of the Republic of Bulgaria and the relevant regulations for its implementation of Sofia University.

The main scientific fields in which Assoc. Prof. Yordan Doumanov works are : biochemistry, molecular and cell biology. Most of his scientific contributions are related to the elucidation of the molecular basis of some socially significant pathologies such as hereditary degenerative diseases of the retina; nanoparticles loaded with specific RNA, as well as the interrelationships between the structure, function, and cellular localization of certain proteins. Part of the research is related to the mechanism of interactions of biologically active substances with different cell types in order to elucidate their pharmacological application.

On my opinion, the main scientific contributions of the candidate can be divided into three groups:

1. Investigations related to the structure, lipid interactions and localization of hBest1 in the cell membrane.

- The molecular basis of Best's disease, which is an autosomal dominant inherited disease affecting central vision, has been studied. It should be noted that in recent years these are the only studies in Bulgaria dedicated to the protein bestrofin-1 mutations which are one of the main causes of this pathology. These studies have been made possible by the success of the team of Assoc. Prof. Doumanov to develop and introduce an original methodology for obtaining in sufficient quantities of purified, functionally active, recombinant protein bestrofin-1 from a cell line stably expressing this protein..

- The structure and surface characteristics of bestrofin-1 in Langmuir monolayers were established. The elements of the secondary structure of hBest1 were determined by Fourier transform infrared spectroscopy (FTIR) as well as the role of Ca^{2+} for their formation. The study of the surface properties of the protein by Langmuir monolayers, showed that Ca^{2+} , Glu and GABA strongly change the morphology and area of the monolayers. The Langmuir-Blodgett films made possible to visualize the protein by atomic force microscopy, and for the first time in the world, images of "pure" hBest1 protein were obtained. The size of the protein molecule and the changes that occur when interacting with Ca^{2+} , Glu and GABA have been proven.

- Evidence of the interactions of the Best-1 protein with phospholipid monolayers has been presented for the first time, in order to elucidate the mechanism of protein interaction with membrane lipids, since the correct orientation, conformation and reorganization of the protein for optimal activity depends on it. POPC has been shown to eliminate the influence of Ca^{2+} , Glu and GABA on the surface dynamics of the films, which is an evidence that phospholipids can significantly alter the behavior of the protein in cell membranes. The interactions of the protein with sphingomyelin and cholesterol, the main lipids of *raft*-domains in membrane bilayer, were also studied. Sphingomyelin and protein have been shown to mix spontaneously. The condensing effect of cholesterol has been proven in pure and mixed monolayers of protein and phospholipids,

- The investigation on the association of protein with cell membrane domains, show that 30% of hBest1 is localized in *raft*-domains. In addition, protein expression has been shown to increase fluid-disordered regions in protein-expressing cells compared to those non-protein-expressing. Therefore, the association of hBest1 with the Lo and / or Ld domains is fundamental to its structure, conformational dynamics, oligomerization and is related to its biological role as a transmembrane channel.

The obtained data on the interactions of hBest1 with POPC, SM, Chol, show that the structure and biological functions of the protein depend on changes in the physicochemical characteristics of the "lipid" environment, and not only on the direct interaction with certain "types" of molecules.

2. Contributions to research on newly synthesized nanoparticles

The new methods of treatment of genetic diseases by delivering nucleic acids to target cells with specific carriers, different types of polymer nanoparticles have been studied to establish their cytotoxicity and internalization pathways in eukaryotic cells. ---

- The internalization of polyethyleneimine-based comb-like polyplexes has been studied and has been shown that the pathways of this process depend on the topology and shape of the polymer chain and more solid polyplexes being considered "promising" gene transfection systems in eukaryotic cells. The study of the internalization and transfection efficiency in eukaryotic cells of polyplexes containing POEGMA-b-PLL diblock copolymer demonstrated that these particles can penetrate directly across the plasma membrane thus avoid the endosomal pathway, releasing an intact and functionally active plasmid capable of expressing EGFP. These results are evidence that the newly synthesized POEGMA-b-PLL diblock copolymer can be used as a "good" candidate for DNA delivery.

3. Contributions to the research of natural biologically active substances

- Studies have been conducted on vipoxin, which is the main toxic component in the venom of the Bulgarian *Vipera ammodytes meridionalis*. Vipoxin is a heterodimeric postsynaptic ionic complex consisting of a basic and toxic PLA₂ enzyme subunit (GIIA secretory PLA₂) and an acidic, enzymatically inactive subunit - vipoxin acidic component (VAC). Vipoxin and its individual subunits affect the integrity and viability of eukaryotic cells in different ways due to differences in their pharmacological activity. The PLA₂ subunit induced cytotoxicity, cytoskeletal rearrangement, and early apoptosis in a concentration-dependent manner that was related to its enzyme activity. Vipoxin and VAC did not affect cell viability, but showed a high degree of genotoxicity, while DNA damage caused by the PLA₂ subunit was moderate and might not be related to its catalytic activity.

- Extracts from the endemic plant *Haberlea rhodopensis* have been found to affect cell peripheral properties, membrane permeabilization and disrupted mechanical junctions in keratinocytes. This has led to the suggestion that the extracts might be a good candidate in the complex treatment of pathological dermatological conditions.

- The study of the effect of methanolic extracts of 6 types of *Inula* containing chlorogenic acids, as well as extracts containing flavonoid glycosides, flavonoid aglycones, phenolic acids and sesquiterpene lactones on normal and cancer cells has shown that they show different degree of cytotoxicity against non-cancerous and cancerous cells, which may be the basis for further research on their potential anti-tumor properties.

-The study of the biological activity of extracts of *Lamium album L* has proved their well-defined antitumor action.

The main characteristic of the scientific contributions of Associate Professor J. Doumanov is that they are systematically aimed for clarifying the structure and function of some proteins and of the molecular mechanisms of socially significant pathologies, hereditary degenerative diseases of the retina. Very significant results have been obtained about the causative agent hBest1 protein, which have been published for the first time in the scientific literature. The results on polymer nanoparticles for their use as carriers of NA in the treatment of a number of hereditary pathologies should also be noted.

Assoc. Prof. Doumanov has a teaching activity of about 400 hours, which include basic lecture courses at the Department of Biochemistry, Faculty of Biology and Faculty of Physics at Sofia University. The candidate also has expert activities. He participated in the State Examination Commissions for the defense of diploma theses, at the Department

of Biochemistry and was a member of the jury for the election of Senior assistants at Sofia University, Medical University -Varna, Institute of Molecular Biology, Bulgarian Academy of Sciences, as well as a reviewer of an international journal. He is a member of the Council of specialties at Faculty of Biology and Optometry (Faculty of Physics), as well as a member of the Management Body of the Scientific research sector, Sofia University.

On my opinion all the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria have been met in the announced competition.

Conclusion: The publications of Associate Professor Yordan Doumanov PhD presented for the competition are on the topic of the competition and represent original scientific contribution in the field of biochemistry and molecular biology. The number of publications and citations exceed the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria. He has the necessary teaching activity for academic position of PROFESSOR and have proven his ability to lead and train young people. All this gives me grounds strongly recommend to the honorable Scientific jury Assoc. Prof. Yordan Doumanov PhD to be elected for the administrative and academic position of PROFESSOR in professional field 4.3 Biological Sciences (Biochemistry) for the needs of the Faculty of Biology, Sofia University "Kliment Ohridski".

22.07.2021

Reviewer:

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