

REVIEW

competition for the academic position "associate professor", professional field 4.3. Biological Sciences - Biochemistry, announced for the needs of the Faculty of Biology at Sofia University "St. Kliment Ohridski" in State Gazette no. 30 from 15.04.2022

Reviewer: Academician Roumen Pankov, Faculty of Biology, University of Sofia St. Kliment Ohridski", appointed as a member of the scientific jury, according to order RD-38-279/07.06.2022. of the Rector of the SU.

The only candidate who submitted documents for participation in the announced competition is Ch. Assistant Professor Kirilka Stefanova Mladenova from the Department of Biochemistry at the Faculty of Biology of the SU. The submitted documentation fully meets the requirements set forth in the Regulations for the terms and conditions for acquiring scientific degrees and holding academic positions at SU "St. Kliment Ohridski".

Brief biographical details of the applicant

Ch. Assistant Professor Kirilka Mladenova was born in 1987 in Sofia. She completed her higher education at the Faculty of Biology at Sofia University "St. Kliment Ohridski", as a bachelor in Molecular Biology (2011) and a master in Cell Biology and Pathology in 2013 with excellent results. Her professional career began in 2015 when she joined the Department of Biochemistry at the Faculty of Biology of SU. In 2017, she successfully defended her dissertation on the topic "Investigation of the interaction of the bestrophin-1 protein with model membrane structures and its behavior in eukaryotic cells" and received the educational and scientific degree "Doctor". In the same year, after a competition, she was chosen as the Chief Assistant Professor in the Department of Biochemistry, where she works to this day. According to the submitted documents Ch. Assistant Professor Mladenova has over five years of work experience in the specialty, which fully meets the requirements of Art. 105 paragraph 1 of the Regulations on the terms and conditions for acquiring scientific degrees and holding academic positions at SU "St. Kliment Ohridski".

Dr. Mladenova is a member of the Union of Scientists in Bulgaria.

Scientific publications and analysis of scientometric data

Ch. Assistant Professor Mladenova has presented a complete list of scientific publications, including 23 articles in journals with an impact factor, 8 publications in journals without an impact factor and conference reports in full text, as well as 41 participations in scientific conferences.

In the current competition, Dr. Mladenova participates with 17 works, of which 16 articles and one chapter of a book, which were not reviewed in previous procedures. All 16 articles were published in international journals with IF and SJR, among which journals with a good impact factor such as *Colloids and Surfaces B: Biointerfaces* (IF 3.9), *Macromolecular bioscience* (IF 3,2), *European Polymer Journal* (IF 3,6), *Membranes* (3,1) and others make an impression.. Applying the adopted quartile

division, the distribution of these publications is as follows – 7 articles were published in journals with Q1, 4 – in journals with Q2, 3 – in Q3 and two - in journals with Q4. The total impact factor of the articles on the competition, according to the presented information, is 44.36, and the documented citations of the same publications are 31 in Web of Science and Scopus and 6 outside these databases. According to the information in Scopus, Dr. Mladenova's Hirsch index is 6. These scientometric data are an attestation of the good quality of the presented scientific production.

Tracking the publication activity over time, it is evident that all 17 scientific works with which Ch. Assistant Professor Mladenova participates in the current competition have been published in the last nine years, which is an average of 2 papers per year. Taking into account her workload, as a lecturer in the Faculty of Biology, I consider that not only the quality, but also the intensity of her research activity is very good.

The above-mentioned scientometric data fully meet, and in some indicators exceed, the criteria for awarding the academic position "Associate Professor", defined in the Regulations for the terms and conditions for acquiring scientific degrees and holding academic positions at SU "St. Kliment Ohridski". The required and achieved points from Ch. Assistant Professor Mladenova are summarized in the table below:

Indicator	Required minimum	Number of points achieved
A	50	50
B	100	104
Г	200	226
Д	50	62
Общо	400	442

Analysis of scientific contributions

The scientific interests of Ch. Assistant Professor Mladenova and her published scientific results fit entirely into the field of the announced competition, being concentrated in three main directions: 1) characterization of the transmembrane protein bestrophin-1 (hBest1); 2) description of the biological properties of nanoparticles; 3) study of the influence of biologically active substances on cell lines.

The first and main direction in Dr. Mladenova's research is related to the study of the human protein bestrophin-1, which is a calcium-dependent chloride channel expressed on the basolateral surface of the retinal pigment epithelium. The interest in this protein is driven not only by the lack of sufficient knowledge about it, but also by the accumulated data showing that mutations in the gene develop specific and incurable pathological conditions - bestrophinopathies, which lead to progressive vision loss. The presented research is a continuation and upgrade of the long-standing interest of Ch. Assistant Professor Mladenova to this protein, dating back to the elaboration of her diploma work and dissertation thesis.

Through studies on the stably transfected cell line MDCK II expressing human Best1, Dr. Mladenova showed that exogenous bestrophin-1 has a correct basolateral localization and does not interfere with cell growth and mitotic index of transfected cells. Its forced expression leads to an increase in fluid-disordered membrane domains (in which it is preferentially located) compared to control cells and leads to differences in

cell polarization compared to control cells not expressing the protein. Using the same experimental approach, Dr. Mladenova demonstrates that mutation of the arginine at position 25 (R25W), which is highly conserved and lies between two potential sorting motifs, causes an increase in the apical localization of the protein, but is unable to completely reversed its polarity.

The availability of a cell line stably expressing human bestrophin-1 makes it possible to isolate and purify it. Thus, having a purified and functionally active protein, the team of Prof. Dumanov from the Department of Biochemistry and with the active participation of Ch. Assistant Professor Mladenova can focus on its detailed characterization. In a series of studies, Dr. Mladenova exploited this possibility, and by modeling Langmuir monolayers, Brewster angle microscopy, atomic force microscopy and infrared spectroscopy, obtained and presented new data on the morphology and some physicochemical characteristics of hBest1, which can be summarized as follows:

- The secondary structure of hBest1 includes 51.1% helical regions, α -helices (16.3%), short helices (7.6%) and β -turns and loops (32.2%), and the presence of Ca^{2+} leads to an increase in helical structures (59.2%);

- Addition of Ca^{2+} and the neurotransmitters glutamate (Glu) and γ -aminobutyric acid (GABA) to the substrate in monolayers of pure hBest1 leads to a change in the areas of the isotherms at the same surface pressure and a change in the area per molecule in the order $A_{\text{Ca}^{2+}} < A_{\text{NaCl}} < A_{\text{Glu}} < A_{\text{GABA}}$;

- Phospholipids affect the oligomerization and activity of hBest1 at the plasma membrane. The result is based on the established ability of PORS (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) to “mask” the effect of Ca^{2+} , Glu and GABA on surface dynamics in hBest1/POPC films and surface conformational changes, structure, self-organization and surface dynamics of hBest1 upon addition of the mentioned agents to mixed hBest1/SM monolayers;

- Addition of cholesterol at different concentrations to hBest1/SM and hBest1/POPC monolayers induces a condensing effect;

- The morphology of hBest1 molecules is reported for the first time - the pure protein has lateral dimensions of about $100 \times 160 \text{ \AA}$ and a height of about 75 \AA . In the presence of Ca^{2+} , hBest1 molecules form dimeric structures $\sim 200 \times 670 \text{ \AA}$ in size and 220 \AA in height and trimeric structures in size $200 \times 990 \text{ \AA}$ in 220 \AA in height.

The second direction summarizes research carried out in the implementation of two national projects (DFNI-T02/7, 2014 and ДН19/8 from 10.12.2017,) and dedicated to researching the influence of newly synthesized nanoparticles, created to deliver nucleic acids in target cells. The results of these studies can be summarized as follows:

- The internalization and transfection efficiency in target cells of polyplex nanoparticles synthesized on the basis of polyethyleneimine (IPEI20-comb20-IPEI96; IPEI66-comb7-IPEI66; and IPEI96-comb5-IPEI48) 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 are more promising as transfection systems;

- 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, entering the

cell particles can release the transferred plasmid, which remains intact and functionally active;

- When nanoparticles, built on the basis of the 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;

- Nanoparticles based on conjugates between DNA and a synthetic polymer (Nucleic acid-polymer conjugates, NAPCs) are biocompatible, non-toxic, have increased uptake by cells and are stable in the presence of nuclease enzymes.

The third direction in research of Ch. Assistant Professor Mladenova is related to the study of the biological activity of substances isolated from snake venom or present in plant extracts from some medicinal plants.

Studies on vipoxin, the main toxic component in the venom of the Bulgarian viper *Vipera ammodytes meridionalis*, elucidate the different cellular effects that its individual subunits have. The PLA2 subunit was found to induce cytotoxicity, affect cytoskeletal structure, and induce early apoptosis in a concentration-dependent manner and this was related to its enzymatic activity, while vipoxin and vipoxin acid component (VAC) did not affect cell viability, but exhibit a high degree of genotoxicity.

Studies on extracts of the medicinal plant *Haberlea rhodopensis* (Rhodope silyvriak) demonstrate that at concentrations up to 2 mg/ml the extracts affect the cell periphery, penetrate the membrane and disrupt the mechanical contacts of HaCat keratinocytes, the effect being more pronounced in actively dividing cells. The results suggest that these extracts may be a good candidate for use in the complex treatment of pathological dermatological conditions.

Studies on extracts from two other types of medicinal plants - *Inula* (White oman) and in vitro cultured *Lamium album* L. (white dead nettle) have also shown promising results in different cytotoxicity against non-cancerous and cancerous cells, making them a suitable target for further searches for potential anti-tumor effects.

Teaching activity

As a member of the teaching staff of the Faculty of Biology Ch. Assistant Professor Kirilka Mladenova is also engaged in active teaching. Her auditory activity, according to the presented report, is 654.5 hours in 2020 and 868.5 hours in 2021, and the total teaching activity is 828.2 and 1013.20 hours, respectively. She conducts practical classes on Biocatalysis and Basics of Biochemistry for undergraduate students from the Faculty of Biology and Physics. Participates in the preparation of master's students from three master's programs in the Faculty of Chemistry and Physics by conducting practical classes in Biochemistry. She also conducts the Cell Polarization exercises for Masters from the Cell Biology and Pathology and Biochemistry master's programs at the Faculty of Biology. On Ch. Assistant Professor Mladenova, the lectures on Biochemistry for bachelors from the specializations Agrobiotechnologies and Biology (correspondence study) were also assigned. She also supervised the development of two diploma theses, which have already been successfully defended. The presented

data undoubtedly characterize Ch. Assistant Professor Mladenova as a teacher with very intensive teaching work.

Conclusion:

As a long-time member of the team of the Faculty of Biology and a reviewer of her dissertation work, I know the scientific and teaching work of Ch. Assistant Professor Mladenova. She is a teacher and scientist with high professional training with prominent scientific interests and an active attitude towards the learning process. The scientific production with which she is presented in the current competition legitimizes her as an established and productive researcher in the field of modern biochemistry. The presented results are original, have been published in prestigious scientific journals and have been well received by the international and our scientific communities. The achieved scientometric indicators fully cover and in some cases exceed the requirements specified in the Regulations for the conditions and procedures for acquiring scientific degrees and occupying academic positions at SU "St. Clement. All this gives me reason to express my positive assessment and to recommend to the Scientific Jury to support the election of Chief Assistant Professor Dr. Kirilka Stefanova Mladenova for the academic position "Associate Professor" in professional field 4.3. Biological Sciences, specialty "Biochemistry".

Sofia, 4/8/2022.

Reviewer:
Acad. Roumen Pankov