

OPINION

by Prof. Albena Vladimirova Alexandrova, Ph.D., National Sports Academy "Vasil Levski", Department of "Physiology and Biochemistry"

Regarding: the candidacy of Assistant Professor Kirilka Stefanova Mladenova, Ph.D., for participation in a competition for filling the academic position of "Associate Professor" in professional field 4.3 Biological Sciences, Scientific specialty Biochemistry, for the needs of the department of "Biochemistry" at Faculty of Biology at Sofia University "St. Kliment Ohridski", announced in the Official Gazette no. 30/15.04.22

1. General overview of the submitted documentation

Assistant Professor Kirilka Mladenova, Ph.D. is the only candidate, who submitted documents in the competition for the academic position "Associate Professor", announced for the needs of the Department of Biochemistry at the Faculty of Biology of the Sofia University "St. Kliment Ohridski" within the statutory deadline. The submitted documents and a set of materials for the competition have been prepared according to the requirements of the Law on the Development of the Academic Staff of the Republic of Bulgaria, the Regulations for its application, and the Regulations for the development of the academic staff of the Sofia University "St. Kliment Ohridski" and meet the criteria of the Faculty of Biology for holding the academic position of "associate professor".

2. Career development of the candidate

The candidate, Kirilka Mladenova, graduated in 2011 with a bachelor's degree in "Molecular Biology", and in 2013 graduated with a master's degree in the master's program "Cell Biology and Pathology" at the Faculty of Biology of Sofia University "St. Kl. Ohridski". In 2017, Mladenova defended her Ph.D. thesis on "Investigation of the interaction of the bestrophin-1 protein with model membrane structures and its behavior in eukaryotic cells" and held the educational and scientific degree "Philosophy Doctor" in 4.3. Biological Sciences (Molecular Biology – Cell Polarization and Membranes). From 2015 until now, she has been working in the "Biochemistry" department of the Faculty of Biology of the Sofia University "St. Kl. Ohridski" initially as an Assistant (2015-2017), and from 2017 as Assistant Profesor.

3. Evaluation of the candidate's educational and teaching activities

Kirilka Mladenova has a teaching experience since 2015 at the Department of Biochemistry, where she leads practical classes on Biocatalysis for students from the Faculty of Biology (bachelor's study programs Molecular Biology and Biotechnologies) and practical classes on Biochemistry for students from the Faculty of Physics (bachelor's study program Optometry and Pharmacy), as well as a summer school in Biochemistry for students from the Faculty of Biology (master's study program Molecular Biology). She also leads practical classes in Biochemistry for students from the Faculty of Chemistry and Pharmacy (master's study program Pharmacy) and from the Faculty of Physics (master's study programs Optometry and Pharmacy and Medical Physics), as well as practical classes in "Cell Polarization and Membranes" for master's degree students from the Faculty of Biology of the Sofia University.

Dr. Mladenova gives lectures on Biochemistry to the students of the Agrobiotechnologies and the Biology bachelor's study programs (part-time study) at the Faculty of Biology.

She was the supervisor of 2 successfully defended diploma theses: 1) "Effects of polyplex nanoparticles containing gold on epithelial cells" by Svetoslava Genadieva Stoycheva, graduate of the Master's program "Cell Biology and Pathology" and 2) "Effects of cholesterol

on Langmuir cells monolayers containing hBest-1” by Jung Hoon Park, a graduate of the Molecular Biology Bachelor Program.

4. Characteristics of the candidate's scientific activity

Kirilka Mladenova has a scientific output including a total of 31 scientific publications, of which 22 are published in scientific refereed and indexed journals, and 9 publications are in non-refereed journals. She participated in a total of 41 scientific conferences, 23 of which were international or national with international participation.

In the current competition, the candidate participated with 17 publications published in refereed and indexed journals, of which 7 are in journals with Q1 and 4 are in journals with Q2 and respectively with a high impact factor, including FEBS journal (Q1, IF 4.53); Colloids and Surfaces B: Biointerfaces (Q1, 4.295); European Polymer Journal, (Q1, IF 3.621); Membranes (Q2, IF 3.094). Dr. Mladenova's scientific articles have been cited 37 times, with 31 of the spotted citations being in publications in journals referenced in Scopus and Web of Science, and 6 in other publications. The total impact factor of the candidate is 44.356 and the h-index (by Scopus) is 5.

The presented scientometric indicators of Dr. Mladenova fully cover and even exceed the minimum national requirements for the academic position "Associate Profesor".

The research activities of Assistant Professor Mladenova are interdisciplinary and cover the scientific fields of biochemistry, molecular biology, biocatalysis, biophysical chemistry, and cell biology. Dr. Mladenova's main research and publications are related to the physicochemical characterization of the transmembrane protein bestrophin-1 (hBest1) (publications 1, 2, 6, 7, 8, 15, and 16). Another narrower scientific topic, in which the candidate is actively working, is the biological properties of nanoparticles (publications 5, 12, 13, 14, and 17). She has studied the cytotoxicity, cellular entry routes, and stability in the presence of biologically active molecules of polyethyleneimine-based nanoparticles; of nanoparticles based on a diblock copolymer of poly(ethylene glycol) methacrylate (POEGMA) and poly(L-lysine) and plasmid DNA; of nanoparticles based on a triblock copolymer of poly(2-(dimethylamino) ethyl methacrylate) – block-poly (ϵ -caprolactone) – block – poly(2-(dimethylamino) ethyl methacrylate) containing DNA and of nanoparticles based on conjugates between DNA and a synthetic polymer. Mladenova's other research is related to establishing the influence of biologically active substances from animals (secretory enzymes isolated and purified from viper snake venom - *Vipera ammodytes meridionalis*) (publications 3, 9, and 10) and plants (extracts from *Haberlea rhodopensis* and from white dead nettle - *Lamium album* L.) origin (publications 4 and 11) on cell lines.

5. Evaluation of research contributions

The review of the candidate's scientific output shows that the results of the conducted research represent certain contributions in the scientific areas in which she works. The main contributions that I rate as significant and original are the result of research on the following scientific topics:

1. Study of the human transmembrane protein bestrophin-1 (hBest1), mutations in the gene of which lead to the development of currently incurable pathological conditions (bestrophinopathies) with progressive vision loss.

- Of methodological contribution is the preparation and characterization of a line of MDCK II cells that stably expresses the gene for hBest1. Obtaining this line enables its further isolation, purification, and detailed study.

- Substantial contribution is the obtaining of completely new data on the elements of the secondary structure of hBest1 and the influence of Ca^{2+} on their formation.

- The obtained new data on the interactions of hBest1 with lipids through model membrane systems (mixed hBest1/lipid monolayers with/without the participation of Ca^{2+} ,

Glu, and GABA) and determining its influence on cells are an important contribution in the field. It was found that hBest1 did not alter cell morphology, growth rates, and mitotic index, but altered the physicochemical characteristics of the plasma membrane and cell polarization.

- For the first time in the world, the morphology of hBest1 molecules was determined by atomic force microscopy applied to Langmuir-Blodgett films.

2. Research related to determining the biological activity of newly synthesized polymer nanoparticles, developed as drug delivery systems (including genes/DNA molecules).

- The evaluation of the ways of entering the cells of nanoparticles based on polyethyleneimine and the efficiency of the delivery of intact and functionally active DNA is of a contributing character.

- New data were obtained on the passage into the cell and release of an intact and functionally active plasmid from nanoparticles based on a diblock copolymer of poly(ethylene glycol) methacrylate (POEGMA) and poly(L-lysine) and plasmid DNA.

- New data and evidence were obtained for the lack of cytotoxicity of nanoparticles based on a triblock copolymer of poly(2-(dimethylamino) ethyl methacrylate) – block-poly (ϵ -caprolactone) – block – poly(2-(dimethylamino) ethyl methacrylate)), containing DNA when applied to eukaryotic cells in the form of cationic micelles and capsules.

- A significant scientific contribution is the assessment of toxicity, uptake by cells, and stability in the presence of biologically active molecules (nuclease enzymes) of nanoparticles based on conjugates between DNA and a synthetic polymer.

3. Study of the biological activity of substances of animal (vipoxin, isolated and purified from snake venom of *Vipera ammodytes meridionalis* Boulenger, 1903) and plant origin (extracts of *Rhodopis silivriak* (*Haberlea rhodopensis* Friv) and white dead nettle (*Lamium album* L.)).

- The assessment of the pharmacological activity of the toxic component vipoxin, composed of secretory phospholipase A2 (GIIA sPLA2) and non-enzymatic subunit VAC (Vipoxin Acidic component), is of a contributing character. Original data were obtained on the cytotoxicity of PLA2 and the genotoxicity of vipoxin, PLA2, and VAC.

- New data on the biological activity of *H. rhodopensis* extracts were obtained and the potential for application in the complex treatment of pathological dermatological conditions was demonstrated.

- New and original data were obtained on the effect of chloroform and methanol extracts of white dead nettle on cell membrane permeability, cell adhesion properties, and cancer cell morphology, and a potential anticancer effect was established.

6. Project activity

According to the provided documentation, Dr. Mladenova participated in 16 research projects, and she was the coordinator of 2 of them (thematic projects financed by Sofia University "St. Kliment Ohridski"). The participation and management of scientific projects is not a requirement for acquiring the academic position of "Associate Professor".

7. Opinions, recommendations, and remarks

I have no critical remarks about the materials presented to me for evaluation and the candidate's achievements.

8. Conclusion

Based on the overview, the presented scientific production and teaching activity of Assistant Professor Kirilka Mladenova fully meets the requirements for holding the academic position of "Associate Professor". I evaluate Dr. Mladenova's overall activity positively and, based on the characteristics made above, I am convinced to vote "FOR" holding the academic position of "Associate Professor" in Biochemistry in the professional field 4.3. Biological

Sciences and I propose to the respected members of the esteemed Scientific Jury to vote positively and unanimously to award the academic position of "Associate Professor" to Assistant Profesor Kirilka Stefanova Mladenova, Ph.D.

27/07/2022

Signature:
(Prof. Dr. Albena Alexandrova)