OPINION

by Prof. Dr. Genoveva Nacheva
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Regarding: Competition for the academic position "Professor" at the Institute of Organic Chemistry with a Centre for Phytochemistry at BAS (IOCCP-BAS), announced in the State Gazette No. 104/10.12.2024

By order # RD-09-15/30.01.2025 of the Director of IOCCP-BAS, I am appointed as a member of the scientific jury in a competition for the academic position "Professor" at the same institute in Professional field: 4.2 Chemical Sciences, Scientific specialty: "Bioorganic chemistry, chemistry of natural and physiologically active substances", for the needs of the Laboratory "Chemistry and biophysics of proteins and enzymes". One candidate has submitted documents for participation in the announced competition – Dr. Lyudmila Georgieva Velkov, Associate Professor at the same institute. I was provided with all the documents required by the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for its implementation (in force from 05/09/2023) and the relevant regulations of the Bulgarian Academy of Sciences and the of Organic Chemistry with a Centre for Phytochemistry at BAS in electronic form.

Brief biographical data

Assoc. Prof. Velkova obtained her PhD in "Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances" in 2013 at the Organic Chemistry with a Centre for Phytochemistry of the Bulgarian Academy of Sciences with the thesis topic: "Structure and Function of Carbohydrate Chains of Hemocyanin Isolated from the Sea Snail *Rapana venosa*" with scientific supervisor Assoc. Prof. (now Prof.) Dr. Pavlina Dolashka. At IOCCP-BAS Assoc. Prof. Velkova has successively held the academic positions of "Assistant" from 2003 to 2009, "Assistant Professor" from 2013 to 2019 and "Associate Professor" from 2019 to the present. During her scientific career at IOCCP-BAS, the candidate has enriched her competences in the field of bioorganic chemistry through 10 short-term (up to 2 months) specializations in prestigious institutes and universities in Germany, Italy, Belgium and Ukraine. For her scientific and applied scientific activities, Dr. Velkova has been awarded 10 awards, including the prestigious Pythagoras (2018), "Inventor of the Year 2012", a gold and silver plaque/medal from the National Exhibition "Inventions, Technologies, Innovations - ITI" (2011, 2014 and 2016) and others. The candidate is a member of the Bulgarian Society of Chemical Engineers, the Bulgarian and European Peptide Societies. *These brief biographical data show that in the course of her career development, Dr. Velkova has acquired extensive theoretical and practical experience in the specialty of the announced competition "Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances" and has received high praise for her scientific and scientifically applied achievements.*

Scientometric indicators, research directions and contributions

Dr. Velkova participates in the current competition with 28 journal articles and 2 recognized and operating useful models registered in the Bulgarian. The total JCR-IF of all articles is high - 72.047, and the articles are distributed by quartiles as follows: Q1 – 10 pcs., Q2 – 7 pcs., Q3

– 8 pcs. and Q4 – 3 pcs., from which it can be seen that among the articles those with the highest quartiles Q1 and Q2 dominate (61%).

The scientific articles with the candidate's participation have been cited a total of 265 times, but 212 of them are in publications indexed in WoS and Scopus. In accordance with the requirement of the LDASRB, I recognize these 212 citations as a number.

Assoc. Prof. Velkova has participated in more than 100 scientific forums, 31 of which have not been presented in previous procedures. Of these, 21 are national and 10 international, in which the candidate has participated mainly as a lecturer (21 conferences or 68%).

In terms of the subject, Assoc. Prof. Velkova's publications fully correspond to the specialty "Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances" of the current competition. The entire scientific career of the candidate has been spent at the IOCCP-BAS, which allows her to work and develop in a clearly defined scientific direction, namely the biochemistry of natural and physiologically active substances and biotechnology. The candidate focuses her research on the isolation and characterization of components from hemolymph and mucus of the class *Gastropoda* with potential antimicrobial, antitumor and antioxidant properties.

The contributions in the publications of Assoc. Prof. Velkova can be attributed to the following **main** research areas:

1. **Isolation and characterization of biocomponents with antimicrobial properties.** The main object of these studies is the mucus of the garden snail *Helix aspersa* (also called *Cornu aspersum*). The candidate has developed a wide range of methods for the isolation and identification of peptide fractions and proteins including chromatographic and mass spectrometric analyses, ^{1}H-NMR spectroscopy, *de novo* sequencing, two-dimensional electrophoresis and bioinformatics analyses. As a contribution to these studies, the identification and characterization of 5 low-molecular peptide fractions with pronounced antimicrobial activity against *E. coli* NBIMCC 8785, *Bacillus cereus* 1085, *Propionibacterium acnes* 1897, *Salmonella enterica* 8691, *Enterococcus faecalis* 3915, *Enterococcus faecium* 8754, *Pseudomonas aureofaciens*, as well as against nystatin- and amphotericin-resistant fungal strains can be noted. The proteins included in the composition of the higher molecular weight fraction (> 20kDa) were analysed and homology with other known proteins that exhibit antimicrobial properties was shown. Some of them, such as proteins with L-amino acid oxidase activity, were determined for the first time in the mucus of *C. aspersum*, which is a contribution to the research of Assoc. Prof. Velkova. In addition to the mucus of the garden snail, 3 main types of proteins with high antimicrobial properties were identified in the hemolymph of the sea snail *Rapana venosa*. Assoc. Prof. Velkova's contributions of an exclusively applied nature include the protected utility model for a composition with potential antibacterial activity against *Pseudomonas aureofaciens* AP9, *Brevibacillus laterosporus* BT271 and *Escherichia coli* MCC 878, including a peptide fraction of garden snail mucus with a molecular weight below 10 kDa and carbon nanoparticles.

2. **Isolation and characterization of biocomponents with antitumor potential from mucus and hemolymph of *Gastropoda*.** Fractions with different molecular mass, isolated from hemocyanins of *H. lucorum*, *H. aspersa* and *R. venosa*, as well as fractions from the hemolymph of *R. venosa* and fractions from the mucus of *C. aspersum* with antitumor activity against cancer cell lines of diverse origin and genetic profiles, have been identified. It has been shown that

their action is mainly associated with induction of apoptosis and in some cases with autophagy. Here, the established synergism between the action of fractions from the hemolymph of *R. venosa* with cisplatin and/or tamoxifen has a contributing character, as this combination is three times more effective compared to treatment with the classical chemotherapeutic agent alone. Using proteomic analysis, the regulation of key proteins for tumour cell proliferation was studied and inhibition of a number of them was shown upon treatment of cells with the functional unit βc of hemocyanin from *H. lucorum*. It was found that glycosylation plays a key role in the antiproliferative effect of the functional unit. These results open up prospects for future research on the application of the identified biocomponents as antitumor agents.

3. **Isolation and characterization of biocomponents from garden snail mucus with potential for the treatment of neurodegenerative diseases.** It was found that garden snail mucus extract, enriched with a fraction above 20 kDa, has a beneficial effect on memory and cognitive processes in a rat model of Alzheimer's disease induced with scopolamine. Through proteomic analysis of two-dimensional electrophoresis of rat cortex extracts, the expressions of various proteins related to memory and cognitive functions were identified. As a scientific contribution, the established difference in the expression of the genes of ubiquitin carboxyl-terminal hydrolase isozyme L1, calbindin, vacuolar ATP synthase catalytic subunit A, tropomyosin beta chain, 14-3-3 zeta/delta, kinesin-1 heavy chain and Stathmin-4 in rats treated with mucus extract compared to demented animals can be considered. It has been hypothesized that these brain proteins may be potential therapeutic targets for the treatment of Alzheimer's-type dementia. In connection with this topic, a utility model has been protected for a composition with a beneficial effect on Alzheimer's-type dementia, including a bioactive extract from the mucus of the garden snail *H. aspersa* as the main component, which can be considered as a contribution of Assoc. Prof. Velkova of an exceptionally applied nature.

4. **Isolation and characterization of peptides from the hemolymph of garden snail and rapana with antioxidant properties.** The characterized peptides are distinguished by a higher proportion of hydrophobic amino acids, which determines their antioxidant properties. It has been suggested that the antioxidant properties of the hemolymph fraction of rapana are due to the high content of proline.

Assoc. Prof. Velkova has also participated in *in silico* studies on the self-formation of peptide clusters in garden snail mucus, which may explain the mechanism of their antimicrobial action. The candidate also has a study dedicated to the characterization of secondary metabolites from *Bacillus velezensis* R22 as inhibitors of fungal pathogens, which in the future can be applied as plant protection products.

Compliance with the Law on the Development of the Academic Staff in the Republic of Bulgaria

In the table below, I have presented the compliance of the group of indicators from A to E of Assoc. Prof. Velkova with the national minimum requirements, adjusted for BAS and IOCCP by indicators C and D. As can be seen from the table, the total number of points of the candidate exceeds the required minimum by more than twice. This is due to the overall scientific, scientific-applied and project activities of the candidate.

Indicator group	Minimum number of points	Candidate points
A	50	50
B	100	145

C	200 (220 for BAS, 250 for IOCCP)	451
D	100 (120 for BAS, 200 for IOCCP)	424
E	150	602
Sum	**750**	**1672**

Project activity

Assoc. Prof. Velkova has presented an extensive list of participations in scientific research and applied scientific projects. She was the head of 2 projects at the National Science Foundation and one project at the Recovery and Sustainability Plan in the field of green technologies with attracted funds of over 410,000 leva. She was a participant in another 17 scientific and infrastructure projects with national funding and another 15 with international funding. She also participates in contracts with companies for conducting analyses. *Assoc. Prof. Velkova's rich experience in the implementation and management of scientific research projects is extremely valuable and represents a solid foundation for her successful implementation of the new academic position.*

Organizational and pedagogical activities

Assoc. Prof. Velkova demonstrates excellent organizational and pedagogical skills. She has participated in the organization of 5 national and international scientific events. She has led seminars and exercises in master's programs at the Faculty of Philosophy of Sofia University. She has been the supervisor of five master's theses at the University of Sofia and the Faculty of Philosophy of Sofia University, of 9 postgraduate students and a consultant to 1 graduate student.

CONCLUSION

The candidacy of Assoc. Prof. Dr. Lyudmila Velkova fully meets and significantly exceeds the requirements of the LDASRB, BAS and IOCCP-BAS for occupying the academic position of "Professor". The candidate has significant scientific and applied scientific contributions, which is evidenced by the publication of her research in prestigious scientific journals and patents and by the repeated citation of her works. She has solid methodological training and a clearly outlined scientific research profile in the scientific specialty "Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances" of the current competition. All this gives me reason to confidently recommend to the esteemed members of the Scientific Council of IOCCP-BAS to vote positively for the election of Assoc. Prof. Dr. Lyudmila Georgieva Velkova to the academic position of "Professor".

Date: April, 2, 2025 Signature:

/Prof. G. Nacheva/