REVIEWER REPORT

From Dr. Petko Nedyalkov Denev, prof. at the Institute of Organic Chemistry with Centre of Phytochemistry (IOCCP), Bulgarian Academy of Sciences (BAS)

on the materials, presented in competition for acquisition of academic degree **"professor"** at the **IOCCP-BAS**

in area of higher education: 4. **"Natural sciences, mathematics and informatics"**
professional field: 4.2. **"Chemical sciences"**
scientific specialty: "**Bioorganic chemistry, chemistry of natural and physiologically active substances"**

1. General presentation of the received materials

By order No. РД-19-15 from 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 of "professor" at IOCCP-BAS in the field of higher education 4. "Natural Sciences, Mathematics and Informatics", professional direction 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" (CBPE) at IOCCP-BAS. Only assoc. prof. Dr. Lyudmila Georgieva Velkova from the CBPE lab at IOCCP-BAS submitted documents for participation in the competition, announced in the State Gazette, issue 104 from 10.12.2024. At its first meeting, the scientific jury unanimously decided that the candidacy of assoc. prof. Velkova meets the criteria of the IOCCP-BAS for holding the academic position of "professor", and I was selected to prepare a review report on the materials submitted for the competition.

The set of materials on paper and electronic media submitted by assoc. prof. Dr. Velkova is in accordance with the Regulations for the Development of the Academic Staff of the IOCCP-BAS for holding the academic position of "professor" and includes: Scientific CV; Reference (according to the model) for fulfilling the minimum requirements according to the Regulations for the conditions and procedure for acquiring scientific degrees and for holding academic positions at the IOCCP-BAS; Diplomas for acquiring the educational and scientific degree of "doctor" and for awarding the academic position of "associate professor"; Abstract of a dissertation for acquiring the educational and scientific degree of "doctor"; Extended habilitation reference for scientific contributions in Bulgarian and English; A complete list of scientific publications, patents, utility models and collective monographs; A list and copies of the publications participating in the competition as an equivalent number of articles for a habilitation thesis by group of indicators B; A list and copies of the publications participating in the competition by group of indicators Г; A list of utility models, accompanied by evidence; A list of participation in conferences, accompanied by evidence; A list of citations; A list of participation in research and applied projects; A list and evidence of diplomas and awards received.

Assoc. prof. Dr. Lyudmila Velkova has attached a list with a total of seventy-two scientific publications, ten patents and utility models, two collective monographs and two university textbooks, prepared in co-authorship throughout her professional career. She participates in this

competition with twenty-eight publications, of which six publications participating as equivalent to a habilitation thesis and twenty-two participating by group of indicators Г (according to Appendix 1 of the Regulations for the Development of the Academic Staff of the IOCCP). All twenty-eight scientific papers are on the topic of the competition, cover the period 2020 - 2025 and, accordingly, have not been presented in other competitions and procedures in which the candidate has participated. In addition, two utility models have been presented, which have also not been presented in other competitions and procedures. Therefore, all materials are accepted for review and are taken into account in the final assessment.

2. Short biography of the applicant

Lyudmila Velkova obtained a master's degree in "Organic and analytical chemistry" from the Faculty of Chemistry and Pharmacy of Sofia University "St. Kliment Ohridski" in 1988. In the period 2003 - 2009 she worked as a "chemist" at the IOCCP-BAS, and in 2012 she obtained the "doctor" degree after defending a dissertation in an independent form of study on the topic: "Structure and function of the carbohydrate chains of hemocyanin isolated from the sea snail *Rapana venosa*", prepared at the IOCCP-BAS. This doctoral dissertation largely shaped the future scientific interests of Dr. Velkova, mainly focused on characterizing the structure and biological activity of peptides, proteins and glycoproteins isolated from the mucus and hemolymph of gastropods, and investigating the possibilities for their application in practice. In 2013 briefly held the position of "assistant professor" at the IOCCP-BAS, after which she was elected as "chief assistant professor", and in 2019 as "associate professor" in the CBPE at the IOCCP-BAS, a position she holds to this day. In her professional career, assoc. prof. Velkova demonstrated very active participation in scientific and applied projects, including as a supervisor. Her scientific and innovative activity has been awarded a number of prestigious diplomas and awards, among which I would single out the "Pythagoras" award for a scientific team with successful exploitation and commercialization of scientific results, with the supervisor prof. P. Dolashka, and a number of awards from the National Exhibitions "Inventions, Technologies, Innovations". Assoc. prof. Velkova's educational activity includes supervision of five graduate students, four students on student internships, as well as supervision of postgraduates. She has led seminars on "Good laboratory and manufacturing practice in bioindustries", the master's program "Biobusiness and bioentrepreneurship" at the Faculty of Biology of Sofia University, and has led practical courses and lectures on the master's program "Food quality and safety" at the same faculty.

3. General characteristics of the applicant's activities

Assessment of scientometric indicators

In **indicators group A** from of the Regulations for the Implementation of the Act for the Development of the Academic Staff in the Republic of Bulgaria (ADASRB), Assoc. prof. Velkova has presented an abstract of a dissertation for the acquisition of the educational and scientific degree "doctor", in the specialty "Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances" on the topic "Structure and Function of the Carbohydrate

Chains of Hemocyanin Isolated from the Sea Snail Rapana venosa". This brings her 50 points on this indicator.

Indicators group B: Six of the publications in the competition are presented by the candidate as equivalent to a habilitation thesis. They are of a high scientific level, with five of them in Q1 issues and one in Q2. Their topic is mainly on the characterization and biological activity of compounds and extracts from gastropods. As a remark on these publications, it should be noted the discrepancy between the impact factor (IF) indicated in the reference for publications B1, B3 and B4 with the real IFs of the publications from Journal Citation Reports™ (Web of Science). I attribute these errors to an oversight on the part of the candidate, since for one of the publications (B1) she indicated an impact factor of 4.717, which is significantly lower than the real one for this year – 6.081. The total IF of these publications is over 25, which is a very good certificate of their quality. These publications bring the candidate 145 points, which exceeds the required minimum of 100 points of the ADASRB and the regulations of the IOCCP. In all of them, assoc. prof. Velkova is listed as one of the corresponding authors, which also fulfills one of the specific requirements of the regulations of the IOCCP-BAS for holding the position of "professor".

Indicators group Г: Twenty-two publications are presented under this group of indicators, all of which have an impact factor and/or impact rank. Here, similarly to the publications equivalent to a habilitation thesis, there is a discrepancy between the impact factors of the publications (for publications Г1, Г2, Г8, Г13, Г16 and Г21) indicated in the reference and the actual ones. It is noteworthy that for a significant number of them, the IFs of the publications for the previous year are indicated. Publications Г4 and Г9 do not have an impact factor for the respective years of publication, but are in the same quartile by impact rank (Scimago Journal Rank) and carry the same number of points. The most significant error here is related to publication Г3, since for 2020 the journal *Lecture Notes in Computer Science* is in quartile Q3, and not in Q2 as indicated in the reference. Therefore, the points that this publication carries should be reduced from 20 points to 15 points. Thus, adding the points from the two registered utility models in which she is a co-inventor, assoc. prof. Velkova collects 446 points, which exceeds the required minimum of 250 points by over 78%. The high number of publications, for a relatively short period of 5 years after assuming the academic position of "associate professor", is indicative of the candidate's intensive publication activity and shows that she is an active researcher.

Indicators groups Д and Ж: A Scopus reference shows 345 citations with an H-index of 13 for the entire scientific career of assoc. prof. Velkova. Thus, this indicator exceeds the minimum requirements of the IOCCP-BAS for a minimum H-index of 10. In the competition, she applied with 265 citations beyond those submitted for the award of the academic position of "associate professor" and the "doctor" degree. These citations are mainly from foreign authors in foreign publications of large academic publishing houses and were received mainly in the last five years. The 530 points collected thus exceed the minimum legal requirements and those of the IOCCP-BAS by more than twice.

Indicators group E: In this group of indicators, assoc. prof. Velkova presents data on leadership and very active participation in international and national scientific projects. She is the

head of three projects at the National Science Foundation (two of which are ongoing), for the implementation of which significant financial resources have been attracted to the IOCCP. In addition, she has participated in seventeen national and fifteen international projects financed by various funding organizations. The 602 points obtained under this group of indicators exceed four times the minimum of 150 points from the IOCCP-BAS regulations and are further evidence of the intensity of her scientific research activity.

Thus, the candidate's summarized points for the individual groups of indicators are as follows:

Indicator	Minimal requirements of IOCCP-BAS	Points of the candidate
А	50	50
Б	-	-
В	100	145
Г	250	446
Д	200	530
Е	150	602
Ж (H-index)	≥ 10	13

As is evident from this summarized information, the scientific output of assoc. prof. Velkova not only covers, but also significantly exceeds the minimum scientometric requirements set out in the ADASRB and the Regulations of the IOCCP-BAS and the Bulgarian Academy of Sciences for the academic position of "professor", and the points collected for indicators in groups Д and Е are several times higher.

Evaluation of contributions

All publications presented by assoc. prof. Velkova correspond to the topic of the competition. The main scientific contributions of these scientific papers are related to the development of new approaches for isolating and characterizing active components from the mucus and hemolymph of gastropods, clarifying the relationship between structure, function and biological activity, as well as establishing the mechanism of action of the active components. I find the research of assoc. prof. Velkova, aimed at discovering new antimicrobial peptides, proteins and other biocomponents from gastropods with antimicrobial activity in order to reduce the spread of antimicrobial resistance, to be extremely interesting and relevant. These studies are largely pioneering, and the group in which she works is recognizable in our country and abroad with this topic. As a result of the research in this direction, various fractions of the mucus of the garden snail *C. Aspersum* have been isolated, which have been structurally characterized by means of mass spectrometric analyses. The potential of peptide fractions from the mucus of this snail as a source of new antibacterial agents has been revealed and hypotheses have been proposed for the relationship between their structure and antimicrobial activity. For the first time, the antibacterial potential of 5 peptide fractions obtained by ultrafiltration of a purified extract from the mucus of the garden snail *C. aspersum* has been demonstrated against a number of Gram+ and Gram- bacteria [В1; В5; Г12]. It has been established that a fraction with a molecular mass (MM)

< 10 kDa exhibits high activity against *Escherichia coli* NBIMCC 8785 [B1; Г12], and the fraction with a molecular mass below 20 kDa demonstrates a wide spectrum of antibacterial properties [B5, B1]. The primary structures of over 30 new peptides with a MM below 3 kDa with a rich variety of amphipathic structures have been identified, and it has been shown that this is essential for their selectivity and antibacterial activity [B1; B5; Г13]. Based on the results of these studies, a utility model for a composition including a peptide fraction with a molecular weight below 10 kDa and carbon nanoparticles with antibacterial activity against *Pseudomonas aureofaciens* AP9, *Brevibacillus laterosporus* BT271 and *Escherichia coli* NBIMCC 878 has been registered, bringing these studies closer to implementation in practice. In another study, it was found that the rich diversity of cationic, anionic and neutral peptides with a molecular weight below 3 kDa in the mucus of *C. aspersum* is a prerequisite for spontaneous interaction between them and the formation of oligomeric structures before the targeted interaction with the bacterial membrane [B4]. A hypothesis has been proposed for the spontaneous formation of peptide nanostructures (clusters) in the mucus of *C. Aspersum*, which has been experimentally proven by UV-Vis spectroscopic and fluorescence studies and in vitro tests of the antibacterial activity of two peptides: p1 (KVKDNQWRP) and p3 (LFGGHQGGGLVGGLWRK) and their binary mixture.

For the first time, information has been obtained on the antibacterial activity of a protein fraction with a molecular weight > 20 kDa from the mucus of *C. aspersum* against a number of aerobic bacterial pathogens such as *Bacillus cereus* 1085, *Propionibacterium acnes* 1897, *Salmonella enterica* 8691, *Enterococcus faecalis* 3915, *Enterococcus faecium* 8754 [B5], *Pseudomonas aureofaciens* and the anaerobic bacterium *Clostridium perfringens* [B1], and new fundamental information has been provided on the composition of the proteins and glycoproteins in this fraction. The antibacterial activity of the protein fraction (at concentrations between 32 and 128 μg/mL) was shown to be comparable to the antibiotic activity of vancomycin, but without cytotoxicity. The antibacterial activity of a protein fraction isolated from the hemolymph of the sea snail *Rapana venosa* was also investigated and it was found that the fraction with a MW of 50-100 kDa exhibited high antibacterial activity against *E. coli* NBIMCC 8785 [Г19]. It was hypothesized that the high antibacterial activity of this fraction is due to the interaction of three main types of proteins, homologous to a peroxidase-like protein (detected at 93.088 kDa), aplycyanin A and L-amino acid oxidase (at 62.100 kDa), and functional units with a MW of ~50 kDa of *R. venosa* hemocyanin, which demonstrate different mechanisms of bactericidal and/or bacteriostatic action [Г19]. For the first time, the antifungal activity of two fractions from gastropods against fungal strains resistant to nystatin and amphotericin has been evaluated. The results show a significant fungicidal effect of the two studied fractions against *Aspergillus niger* and *Penicillium griseofulvum*, which is a very interesting result, given the fact that a limited number of natural molecules exhibit effective antifungal properties, compared to those with antibacterial activity [Г22].

In another series of studies, the antitumor activity of different isoforms and some functional units of hemocyanins from *H. lucorum* (HlH), *H. aspersa* (HaH) and *R. venosa* (RvH), as well as fractions of the hemolymph of *R. venosa* with MM 10-50 kDa and 50-100 kDa and fractions of the mucus of *C. aspersum* with MM over 20 kDa and with MM over 50 kDa, against cancer cell lines of diverse origin and different genetic profiles has been demonstrated [Г2; B2; Г14; B3; Г18]. It has been established that the antiproliferative activity of the tested hemocyanins and fractions is mainly associated with the induction of apoptotic and to a lesser extent late apoptotic or necrotic changes in tumor cells, and in some cases with the induction of autophagy

[Г2; В2; Г14; В3; Г18], which emphasizes their advantages for influencing resistant neoplastic processes. It has been hypothesized that the established antiproliferative changes in tumor cells after treatment with different forms of hemocyanins are probably related to the specific oligosaccharide structures located on the surface of the functional and structural subunits of hemocyanins [Г2; Г14], and also that the antitumor activity of the fractions of the mucus of *C. aspersum* and the hemolymph of *R. venosa* is probably due to the interaction between proteins with different functions that induce cell death through different mechanisms of action [В3; Г18]. For the first time, the mechanism of antiproliferative action of the functional unit βc-HlH-h of H. lucorum hemocyanin on the T-24 cell line of human bladder carcinoma has been studied by proteomic analysis [В2]. Forty proteins were identified in the studied tumor cell line, which changed their expression after treatment with βc-HlH-h [В2]. For the first time, information was provided on the ability of a standardized extract of the snail mucus *C. aspersum* to improve memory and cognitive abilities in scopolamine-induced dementia of the Alzheimer type in rats [Г16]. It was found that the extract exhibits moderate antioxidant properties and modulates the content of monoamines in brain structures related to memory [Г16]. It was shown that some secondary metabolites and some peptides and proteins related to the antioxidant and antimicrobial properties of the mucus are responsible for the established effect. Based on these results, a utility model was protected [Г(2)] for a composition including the extract of the garden snail *H. aspersa* mucus as the main component with a beneficial effect in Alzheimer type dementia. The primary structures of seventeen new peptides in the fraction with a molecular weight below 1 kDa from the hemolymph of the garden snail *H. Lucorum*, which exhibits antioxidant activity, have been determined [Г4; Г9]. It has been established that this activity is mainly due to peptides with low molecular weight and specific amino acid sequences, mainly including Leu, Val, Phe, His, Pro, Lys and Tyr residues. The obtained results confirm that the higher proportion of hydrophobic amino acid residues compared to hydrophilic ones is a key factor for the increased ability to capture hydroxyl radicals [Г4; Г9]. A methodology has been developed for the isolation, purification and characterization of cyclolipopeptides from cell-free supernatants of *B. velezensis* R22 [Г21].

The discovery of new, effective and selective drug molecules of natural origin is an important and interesting scientific task, which determines the relevance of assoc. prof. Velkova's scientific research. The main objects of her research work (mucus and hemolymph of various gastropod organisms) are complex, multicomponent mixtures, including substances with different biochemical characteristics and properties, and their detailed characterization is a difficult and challenging task. Even more valuable is that these studies not only demonstrate for the first time new activities of the studied biocomponents, but also shed light on their potential mechanism of action, which is a prerequisite for their potential application in anti-cancer therapy and the reduction of antimicrobial resistance. The significance of her scientific output is also enhanced by the fact that a number of studies with her participation are protected by utility models and patents, and some of them have already been implemented in practice and have reached consumers in the form of cosmetic products and food supplements.

4. Evaluation of the candidate's personal contribution

The scientific contributions presented in the competition are the result of interdisciplinary research at the border between bioorganic chemistry, molecular biology, biotechnology and microbiology, and all scientific publications are co-authored. The fact that assoc. prof. Velkova is the first and/or corresponding author in ten of them leaves no doubt about her essential role and personal contribution to their development and publication. The presented habilitation reference very well outlines the personal contribution of assoc. prof. Velkova in these multidisciplinary studies.

5. Critical comments and recommendations

The documents for the presented competition are well-structured and arranged, which facilitates their analysis and evaluation. The habilitation reference is meaningful and presents well the scientific and applied contributions of assoc. prof. Velkova. My only significant remark on the presented materials is related to the errors in the references for the scientometric indicators of the scientific publications of assoc. prof. Velkova, but this remark is rather technical and in no way affects the significance of the results and contributions in these publications.

6. Personal impressions

I know assoc. prof. Velkova as a colleague from the IOCCP-BAS, as well as from some presentations of her scientific and applied research within the framework of various initiatives at the Institute, but until now I did not have a comprehensive view of her scientific work. My impression is that she is a friendly and responsive colleague, and after a detailed acquaintance with her scientific production, I can say that she is a serious and active researcher with an affinity for applied research and development.

CONCLUSION

The documents and materials submitted by the sole candidate in the competition, assoc. prof. Dr. Lyudmila Velkova, meet all the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its implementation, the Regulations for the Implementation of the ADSRB at the Bulgarian Academy of Sciences and the Regulations of the IOCCP-BAS. She has submitted a sufficient number of scientific publications beyond those used in the procedures for the defense of the doctoral dissertation and the acquisition of the academic position "associate professor". All scientific papers are of a high scientific level and contain original fundamental scientific contributions to the elucidation of the structure, properties, biological activity and mechanism of action of peptides, proteins and glycoproteins isolated from the mucus and hemolymph of various species of gastropods. In addition, these studies have practical applicability, and their significance is enhanced by the fact that a number of them are protected by utility models and patents, and have already been implemented in practice. It is clear from the documents submitted that assoc. prof. Velkova is an active researcher who has

achieved significant and interesting results that significantly exceed the specific requirements of the Regulations of the IOCCP-BAS for holding the academic position of "professor".

Because of all this, after a thorough acquaintance with the materials and scientific works presented in the competition, an analysis of their significance and the scientific and applied contributions contained in them, **with full conviction I give my positive assessment** and recommend to the Scientific Jury to prepare a report-proposal to the Scientific Council of the IOCCP-BAS for the election of assoc. prof. Dr. Lyudmila Georgieva Velkova to the academic position of "professor" at the IOCCP-BAS in professional field 4.2. "Chemical Sciences", scientific specialty "Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances" for the needs of the CBPE laboratory at the IOCCP-BAS.

22.04.2025 Reviewer: .....

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