

# REVIEW

by

Prof. Yulian Tenchev Voinikov, PhD

on **Assoc. Prof. Dr. Ludmila Georgiyeva Velkova** for participation in the competition for the academic position of "Professor", announced in the State Gazette, issue 104 from December 10, 2024, in professional field 4.2. "Chemical Sciences", scientific specialty "Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances" for the needs of the Laboratory of "Chemistry and Biophysics of Proteins and Enzymes (CBPE)" at the Institute of Organic Chemistry with a Center for Phytochemistry – Bulgarian Academy of Sciences (IOCCP-BAS).

## 1. General information on the announced competition and the submitted materials

According to the announced competition in the State Gazette for the academic position of "Professor" in the professional field 4.2. "Chemical Sciences", scientific specialty "Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances", for the needs of the Laboratory of "Chemistry and Biophysics of Proteins and Enzymes (CBPE)" at the Institute of Organic Chemistry with a Center for Phytochemistry at the Bulgarian Academy of Sciences (IOCCP-BAS), the only candidate is **Assoc. Prof. Dr. Ludmila Georgiyeva Velkova**. The materials submitted by the candidate include:

- Autobiography (CV);
- An extended habilitation report on scientific contributions, formatted as a habilitation thesis;
- Lists of publications, distributed by indicators;
- List of citations;
- List of participations in scientific projects;
- Patents and utility models;
- Documents proving the fulfillment of the necessary scientometric criteria.

The review of the documents shows that the procedure for announcing and conducting the competition has been followed, and the documents are prepared in accordance with the Act on the Development of the Academic Staff in the Republic of Bulgaria (ADASRB), the Regulations for its application, and the internal regulations of IOCCP and BAS.

## 2. Brief biographical information

**Assoc. Prof. Dr. Lyudmila Velkova** was born on May 24, 1964. She graduated with a Master's degree in Chemistry (Organic and Analytical) from "St. Kliment Ohridski" Sofia University (Faculty of Chemistry and Pharmacy). Later, she was enrolled as an independent doctoral student at IOCCP-BAS and in 2012 defended a dissertation to obtain the "Doctor" (PhD) degree in the scientific specialty "Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances." The topic of her dissertation was: "Structure and Function of the Carbohydrate Chains of Hemocyanin Isolated from the Marine Snail *Rapana venosa*."

Assoc. Prof. Velkova's scientific and professional path at IOCCP–BAS includes positions as chemist, assistant professor, senior assistant professor, and from 2019 to present – associate professor. She carries out active scientific, teaching, and project-related work. She participates in national and international scientific forums, as well as in training graduate and PhD students. Assoc. Prof. Velkova has conducted specializations in institutes in Germany, Italy, Belgium, and Ukraine. She has co-authored a total of 72 publications, 38 of which are from years 2020–2025, after receiving her PhD and her academic position of associate professor. In the competition for "professor", she participates with 28 publications having a total impact factor over 64, as well as 2 registered utility models, 265 citations, project work, and other evidence of active scientific and applied research activity. According to Scopus, excluding self-citations by all co-authors, her h-index is 12. She is a co-inventor of 4 national patents and a total of 6 utility models, two of which are submitted for this competition.

### 3. Education and qualifications

- **Master's Degree (Sofia University "St. Kliment Ohridski")**  
She graduated in 1988 with specialties in "Organic and Analytical Chemistry," forming a fundamental basis for her subsequent research work.
- **Doctoral Degree**  
From 2009 to 2012, she was an independent doctoral student at IOCCP–BAS. She successfully defended a doctoral dissertation related to the structure and biological functions of glycosylated proteins (hemocyanins) isolated from the marine snail *Rapana venosa*.
- **Additional specializations**
  - Specializations at the Institute of Biochemistry, University of Tübingen, Germany (multiple one-month and two-month residencies over the period 2003–2014).
  - Institute of Biology, University of Padua, Italy (1 month, 2008).
  - University of Ghent, Belgium (two residencies, in 2009 and 2019).
  - Institute of Virology, Kyiv, Ukraine (2010).

### 4. Academic and teaching activities

Teaching activities:

- Leads practical and lecture courses in Master's programs at the Faculty of Biology of Sofia University "St. Kliment Ohridski" ("Food Quality and Safety," "Biobusiness and Bioentrepreneurship");
- Has led seminar sessions in "Good Laboratory and Manufacturing Practice in the Bioindustries";
- Actively participates in the training of graduate and young specialists on the isolation and analysis of bioactive substances, mass spectrometry, and proteomics. She has supervised graduate students and trainees from UCTM, the Faculty of Biology at SU, as well as interns under student practice programs. She has also served as a consultant for a master's thesis, as well as a consultant for the project "Synthesis of Natural Ag-, Cu- and Zn Nanoparticles with Extract from *Plantago major* and Determination of Their Antibacterial Activity" under the BAS program "YOUNG SCIENTISTS AND POSTDOCTORAL FELLOWS – 2."

Scientific-organizational activities:

- Participation in organizing and program committees of scientific forums ("Ecological Products for Health," "Clean Nature for Health," "Black Sea Coastline Countries Scientific Research Conference," etc.);
- Participation in 20 national projects and 15 international projects; she has led 2 projects funded by the Bulgarian National Science Fund (BNSF) and 1 project under the National Recovery and Resilience Plan, with national and European funding;

- Cooperation with industry on projects funded under the “Innovation and Competitiveness” Operational Program, related to the development of natural bio-components for pharmacological, cosmetic applications, and dietary supplements.

## 5. Research activities and contributions

According to the **extended habilitation summary** presented, Assoc. Prof. Velkova’s research falls in the fields of **bioorganic chemistry**, chemistry of **natural and physiologically active substances**, and biotechnology. It is centered on several main areas:

### 1. Study and characterization of biocomponents (peptides, proteins, glycoproteins) from the mucus and hemolymph of Gastropoda (snails)

- Methods have been developed for the extraction, purification, and characterization of peptide and protein fractions from the mucus and hemolymph of terrestrial (*Cornu aspersum*, *Helix lucorum*) and marine snails (*Rapana venosa*).
- A number of new peptides have been identified and characterized with potential antibacterial, antifungal, and antioxidant activities. The link between their determined structures and their biological activity has been investigated. The results have been obtained on the basis of mass spectrometric studies, bioinformatics, and proteomic analysis, and skillful interpretation of these data. The findings have fundamental significance, as they expand existing knowledge about the biological activity of snail mucus from terrestrial snails and the hemolymph of *R. venosa*.
- New information has been provided about important bioactive proteins and glycoproteins in the fraction with MW > 20 kDa from the mucus of *C. aspersum*, based on the electrophoretic profile analyzed with mass spectral proteomic analysis and bioinformatics. For the first time, proteins with L-amino acid oxidase activity were identified in the range of 56.94–59.04 kDa in the mucus of *C. aspersum*. A hypothesis was proposed and confirmed regarding the spontaneous self-association of peptides in snail mucus from the garden snail prior to interaction with the bacterial membrane into oligomeric nanostructures, as well as a potential synergistic antibacterial effect among some of them.
- A high antibacterial efficacy of specific protein fractions has also been established against a number of pathogenic bacteria (*E. coli*, *Bacillus cereus*, *Clostridium perfringens*, *Salmonella enterica*, etc.), as well as activity against fungal strains resistant to nystatin and amphotericin.

### 2. Antitumor potential of components from the hemolymph and mucus of Gastropoda

- Several isoforms of hemocyanins from *Helix lucorum*, *Helix aspersa*, and *Rapana venosa* have shown **selective cytotoxicity** against human tumor cell lines (breast cancer, bladder cancer, colorectal carcinoma, etc.).
- Using an original approach, three main types of proteins with molecular weights between 50–100 kDa have been identified in a fraction from the hemolymph of the marine snail *R. venosa* showing high antitumor activity against a panel of six breast cancer cell lines from different molecular subtypes. The identified proteins in *R. venosa* hemolymph, exhibiting high homology to a peroxidase-like protein at ~97 kDa, Aplysianin-A, and L-amino acid oxidase at ~62 kDa, have fundamental significance since, so far, there has been limited information about the composition of *R. venosa* hemolymph (only certain functional units of *R. venosa* hemocyanin, actin, and proline-rich antimicrobial peptides with a molecular mass below 10 kDa had been identified).
- Mechanisms of action have been clarified, including induction of apoptosis, autophagy, and changes in proteomic profiles.

- Synergy has been shown between these natural components and classic chemotherapeutics (cisplatin, tamoxifen). The approaches used open new perspectives for the application of natural bioactive compounds as antitumor agents, used alone or as boosters in combination with conventional therapies in oncology.

### 3. Proteomic analysis and mechanism of action of bioactive substances

- **Two-dimensional gel electrophoresis** (2D-PAGE) and **MALDI-TOF/MS** methods have been applied to identify key proteins and pathways affected by treatment with snail extracts and hemocyanins.
- Original information has been obtained on the antitumor effect of the functional subunit  $\beta$ c-HIH-h of *H. lucorum* hemocyanin on T24 human bladder carcinoma cells, associated with changes in the expression of multiple proteins controlling energy metabolism, apoptotic pathways, and inflammatory processes in the cells. The inhibition of some proteins in the T24 cell line, such as the anti-apoptotic heat-shock protein HSP27 and proteins related to energy metabolism and signaling pathways in cancer cells (GAPDH and PKM2), has been demonstrated as a novel potential therapeutic strategy for bladder cancer.
- The results presented build on published data indicating the significant pro-apoptotic activity of molluscan hemocyanins against various tumor cell lines, further confirming their potential use in anticancer therapy.
- For the first time, based on proteomic analysis, significant changes have been demonstrated in protein expression in the cortex of rats preemptively treated with *C. aspersum* mucus extract compared to demented animals treated only with scopolamine. Several proteins have been identified as potential therapeutic targets for dementia.

### 4. Scientific and applied aspects

**Two utility models** have been established:

- The first concerns an antibacterial composition of a peptide fraction with MW < 10 kDa from the mucus of the garden snail combined with nanodiamonds, active against the bacterial pathogens *Pseudomonas aureofaciens* AP9, *Brevibacillus laterosporus* BT271, and *Escherichia coli* NBIMCC 878.
- The second is a composition with a beneficial effect on Alzheimer-type dementia, which includes a bioactive extract of the mucus of the garden snail *H. aspersa* as its main component.

From the presented **habilitation thesis** and the associated publications, it is clear that the scientific contributions of Assoc. Prof. Velkova are **interdisciplinary**, combining knowledge from biochemistry, biotechnology, organic chemistry, microbiology, cell biology, proteomics, and nanotechnology. These studies address important challenges, such as **antimicrobial resistance** and the need for **new selective antitumor agents**.

### 6. Publication activity, citations, and h-index

- The total number of Assoc. Prof. Velkova's publications is **72**, of which:
  - 28 publications submitted in this competition appear in refereed journals with an impact factor, totaling over IF 64.
  - 10 publications are indicated in which she is the corresponding/first author.
  - The quartile distribution is as follows: Q1 – 10, Q2 – 7, Q3 – 8, Q4 – 3 scientific papers.
- According to the submitted data, the total recognized citation count is 265 (Scopus, as of the time of preparing the reference, excluding self-citations by all authors) and the h-index (Scopus) is **12**.

- Assoc. Prof. Velkova is co-author of 2 collective monographs, 1 university textbook, and 1 teaching manual, which, although not included in this competition, further demonstrate her active academic and teaching work.

## 7. Participation in projects and applied research activities

Assoc. Prof. Velkova has participated in more than **20 national** and **15 international** research projects. Among them:

- Projects funded by the **Bulgarian National Science Fund (BNSF)**, aimed at discovering new bioactive components from the hemolymph of marine and terrestrial snails, as well as the synthesis of biogenic nanoparticles;
- Projects under **National Scientific Programs** ("BioActivMed," etc.);
- Participation in projects under the **National Recovery and Resilience Plan (NRRP)**, aimed at developing biofungicide products from waste biomass, green technologies for the circular economy;
- Significant developments under the **"Innovation and Competitiveness" Operational Program** in partnership with various companies ("Alex 1977" Ltd., "BusinessLab" Ltd., "NanoBioRem" Ltd., etc.), focused on implementing natural extracts as active components in cosmetics, pharmacology (development of products with antimicrobial activity, products with regenerative properties for treating varicose and diabetic wounds, dietary supplements, etc.).

It is impressive that Assoc. Prof. Velkova combines her **fundamental research** with **applied aspects**, resulting in utility models and actual knowledge transfer to industry.

## 8. Assessment of compliance with the minimum national requirements

In accordance with current regulations for holding the academic position of "Professor" (ADASRB and the rules of BAS/IOCCP), the candidate has submitted a detailed reference to meeting **the minimum scientific requirements**. The reference shows that:

- Under the criteria for the habilitation thesis (group "B") and the publications beyond it (group "G"), Assoc. Prof. Velkova **surpasses** the required values, having a sufficient number of publications in Q1 and Q2 journals with high impact factors.
- Under criterion "D" (citations), she also exceeds the minimum requirement.
- Regarding the indicators related to project-based and applied research work (group "E"), she has a significant track record, backed by both attracted funding and leadership/participation in numerous national and international initiatives.

The submitted documents and corresponding scientific metric data clearly demonstrate that Assoc. Prof. Velkova exceeds the minimum requirements set for the position of "Professor" in professional field 4.2. Chemical Sciences.

## 9. Personal impressions

I had the privilege of being part of Prof. P. Dolashka's research team at IOCCP–BAS in 2017–2018, where I worked in a positive and motivated group alongside Assoc. Prof. Dr. Lyudmila Velkova and many other young people. I gained valuable knowledge and many practical laboratory skills in proteomics, including electrophoresis, interpreting proteomic mass spectral data, and applying methods for investigating tissue homogenates. I have participated with the team in numerous scientific forums.

Together with this productive team, Assoc. Prof. Velkova is a co-author of several utility models registered with the Patent Office. Among them are “Composition with Antibacterial Effect” (No. 5317/07.06.2021; No. 4101 of 11.08.2021) and “Remedy for Beneficial Influence on Alzheimer-Type Dementia” (No. 5699/15.03.2023; No. 4426 of 26.04.2023). These developments demonstrate the research team's drive to **transfer fundamental discoveries into real products**—both for combating pathogenic microorganisms and for support in neurodegenerative diseases.

I believe that Assoc. Prof. Velkova's achievements, as well as those of her colleagues, reflected in numerous **publications, patents, and utility models, as well as a number of national and international awards**, and the successful implementation of products with cosmetic and therapeutic purposes, attest to her contribution to global and Bulgarian science and to the high value of her research. I am confident that the team will continue to achieve success and involve young people in its research and innovative endeavors.

## 10. Conclusion

Assoc. Prof. Dr. Lyudmila Velkova presents an significant range of scientific achievements, teaching activities, and applied research results. Her contributions are characterized by:

- **Interdisciplinarity** – uniting biochemical, biotechnological, microbiological, nanotechnological, and proteomic methods;
- **Relevance** – addressing important challenges such as growing antibiotic resistance, developing new antitumor agents, and studying natural products with applied value;
- **Diverse approaches** – combining analytical techniques (HPLC, MALDI-TOF/MS, 2D-PAGE, and in silico modeling) with biological tests (antibacterial, antifungal, antioxidant, and antiproliferative activity);
- **High practical value** – developed utility models, patent activity, industry collaboration, and the implementation of scientific findings in real products (pharmaceutical, cosmetic, agricultural).
- **Future directions** – The plans of Assoc. Prof. Dr. Velkova show a drive to discover natural therapeutics with antimicrobial and antitumor activities. Developing such compounds, combined with nanotechnologies, could overcome some of the limitations of conventional drugs by reducing toxicity and improving selectivity. Integrating proteomic analyses and mass spectrometry provides a reliable foundation for clarifying the mechanisms of action of various biocomponents, while the focus on lipopeptides from *Bacillus* spp. is a step toward creating more sustainable solutions in ecological agriculture. Lastly, methods for determining pesticides and their traces in the environment are timely, as they offer better control over food quality and environmental protection. These directions combine fundamental research with real-world applications, providing value to both science and society.

In all indicators (publications, citations, project work, and teaching activities), Assoc. Prof. Velkova significantly **exceeds** the required criteria for “Professor.” She possesses the scientific, pedagogical, and organizational qualities necessary to hold the academic position of “Professor” in the announced competition.

#### 11. Recommendations for future research:

- **Expanding data on mechanism of action**  
Elucidating signaling and metabolic pathways through the integration of proteomic with other “omics” analyses.
  - **Synergy with conventional therapeutics**  
Investigating combined effects between newly discovered peptides/proteins and existing antimicrobial or antitumor agents. This might yield new therapeutic regimens with lower toxicity or enhanced efficacy.
  - **Broader range of microbial targets and tumor models**  
To evaluate the broad-spectrum effects of new bioproducts, additional pathogenic strains (especially resistant ones) and various tumor cell lines, including multidrug-resistant types, should be tested.
  - **Bioinformatics tools**  
Employ specialized bioinformatic tools (e.g., QSAR models) that predict function and activity of identified peptides based on their amino acid sequence. For instance, using AlphaFold 3 to model the 3D structures of peptides and proteins. The resulting structural models would provide further insights into possible mechanisms of action and facilitate the identification of potential drug candidates through virtual screening and docking simulations.
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#### 12. Summary of points by indicators

According to the reference provided by IOCCP–BAS, Assoc. Prof. Dr. Lyudmila Velkova meets and exceeds the stated minimum requirements for all the key indicators for holding the position of “Professor.” Specifically:

- **Indicator A** (minimum 50 pts.): declared 50 pts.
- **Indicator B** (minimum 0 pts.): declared 0 pts.
- **Indicator V** (minimum 100 pts.): declared 145 pts.
- **Indicator G** (minimum 250 pts.): declared 451 pts.
- **Indicator D** (minimum 200 pts.): declared 530 pts.
- **Indicator E** (minimum 150 pts.): declared 602 pts.

Thus, with the overall requirement being that the candidate meets the indicated thresholds, Assoc. Prof. Velkova significantly surpasses the requirements in the main categories (V, G, D, and E) and fully complies with the IOCCP–BAS criteria for holding the academic position of “Professor.”

#### 13. Final evaluation and proposal

On the basis of all the above, I give my **positive assessment** of **Assoc. Prof. Dr. Lyudmila Velkova**’s scientific and applied contributions and believe that she possesses the necessary scientific productivity, authority, pedagogical,

and organizational skills to hold the academic position of “**Professor**” in professional field 4.2. “Chemical Sciences,” scientific specialty “Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances.”

**I recommend** that the esteemed scientific jury select Assoc. Prof. Dr. Lyudmila Georgieva Velkova for the academic position of “Professor” under the announced competition.

Date: 12.03.2025

Signature:.....

/prof. Yulian Voynikov, PhD/