

# REVIEW

By Prof. Ivan Georgiev Ivanov, D.Sc., IMB-BAS

Regarding the competition for Full Professor position announced by the Institute of Organic Chemistry with Center for Phytochemistry at the Bulgarian Academy of Sciences

## 1. Common part

The competition for Full Professor in 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 is announced by the Institute of Organic Chemistry with Center for Phytochemistry at the Bulgarian Academy of Sciences (BAS) in State Gazette no. 104/10.12.2024. The only candidate is Dr. Lyudmila G. Velkova, Associate Professor at the same institute. Documents overview showed that the entire procedure for opening and announcing and execution of the competition has been conducted in accordance with the requirements of the Law on Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the regulations for its implementation and the internal rules of BAS.

## 2. Brief biographical information

Assoc. Prof. Lyudmila Velkova was born on 24.05.1964. She is a graduate of Sofia University "St. Kliment Ohridski", Faculty of Chemistry and Pharmacy, where she obtained a MS degree (1988) in Organic and Analytical Chemistry. During 2003-2009 she worked as a chemist at the Institute of Organic Chemistry, and from 2009 to 2012 she was a PhD student at the same institute. After defending her PhD thesis in Bioorganic Chemistry and Chemistry of Natural and Physiologically Active Substances she was appointed as an Assistant Professor (2013-2019). In 2019 she was promoter Associate Professor at the same laboratory.

During her career development, Dr. Velkova has specialized in a number of renowned research centers in Germany (Institutes of Biochemistry and Cell Biology, Tübingen), Italy (Institute of Biology, Padua), Ukraine (Institute of Virology, Kiev), Belgium (University of Ghent), etc.

She has been honored with 11 awards for scientific achievements. Dr. Velkova is a member of the Union of Bulgarian Scientists, the Bulgarian Peptide Society and the European Peptide Society.

## 3. Scientific publications

### 3.1. Overview of the publications

According to the presented list of papers, Assoc. Prof. L. Velkova is the author of 72 scientific papers with a total IF>100. Her papers have been cited more than 400 times and her h-index (Scopus) is 12. She is also a co-author of 4 national patents, 6 utility models, 2 monographs, 1 university textbook and 1 teaching aid.

In the present competition Dr. Velkova participates with 28 scientific papers of which 6 are under indicator B (Q1 - 5, Q2 - 1), 22 under D (Q1 - 5, Q2 - 6, Q3 - 8, Q4 - 3) and 2 utility models. In 3 of the publications she is the lead (first) author, and in 8 (B1-B6, D9 and D19) she is a corresponding author. All papers are in the field of Bioorganic Chemistry and therefore correspond to the scientific profile of the announced position. In my review, I will refer to the papers with their original numbering according to the original list of publications.

Dr. Velkova has reported her results at 31 national and international scientific forums. Their abstracts will be taken into consideration in my evaluation, however they will not be explicitly peer-reviewed.

### **3.2. Evaluation of the scientific papers**

The peer-reviewed papers of Assoc. Prof. L. Velkova are dedicated to the search for new natural medicinal substances with antimicrobial and antitumor activity from mucus and hemolymph of gastropods. According to their chemical nature, these substances could be peptides, proteins or glycoproteins. In addition to their chemical characterization, Velkova also studies their antimicrobial, antitumor, antioxidant and regenerative potential, as well as the mechanism of their action. Her papers can be classified into four thematic groups: a) *Isolation and characterization of biologically active substances from gastropods with antimicrobial activity*; b) *Antitumor potential of components isolated from the hemolymph and mucus of gastropods*; c) *Study of the mechanism of action of biologically active substances from mollusks through proteomic analysis*; d) *Characterization of biologically active substances from other natural sources*.

#### **a) Isolation and characterization of biologically active substances from gastropods with antimicrobial activity** (articles №: B1, B3, B4, B5, Г1, Г12, Г13, Г19 и Г22).

The studies of this category are inspired by the fact that gastropods are naturally resistant to bacterial infections and quickly regenerate after injury. This indicates for the presence of perfect defense mechanisms based (most likely) on highly effective antibacterial substances in their tissues and physiological fluids. One of her research objects is the garden snail *Cornu aspersum*, where Dr. Velkova isolated from its mucosa 5 peptide fractions with molecular masses ranging from 3 kDa to 20 kDa. They all demonstrated a well pronounced antibacterial activity against *Bacillus cereus*, *Propionibacterium acnes*, *Salmonella enterica*, *Enterococcus faecalis*, *Enterococcus faecium*, *Pseudomonas aureofaciens*, and also against some fungicide-resistant fungal strains. They are enriched in Gly, Leu, Val, Pro, Trp, Lys, Asp, Phe and Arg and show high homology (over 70%) with other natural antimicrobial peptides. Based on her studies, Dr. Velkova has invented an original medicinal composition with antibacterial activity against *P. aureofaciens*, *B. Laterosporus* and *E. coli* granted with a useful model.

In addition to mucus polypeptides, the protein fractions from the hemolymph of *C. aspersum* also express high antibacterial potential comparable to that of vancomycin. Using a new approach, representing a combination between PAG-electrophoresis and the ImageQuant™ TL v8.2.0 software, Dr. Velkova found that many hemolymph components of molecular mass higher than 20 kDa share high homology with certain proteins and glycoproteins from the mucus and hemolymph of other gastropods, thus implying a common evolutionary origin. The chemical analyses of the snail's physiological fluids also led to the identification of proteins with L-amino acid oxidase activity. Homology was found also with other proteins and glycoproteins such as glutathione S-transferase, H-type lectins, including agglutinin, different forms of hemocyanin, several types of collagen and mucins. The new approach allowed the author to isolate and characterize 3 antibacterial proteins (93.088 kDa, 62.100 kDa and 50.230 kDa) from the hemolymph of the sea snail *Rapana venosa*. In addition to their antibacterial activity, they also demonstrated antifungal effects against *Aspergillus niger* and *Penicillium griseofulvum*.

#### **b) Antitumor potential of components isolated from the hemolymph and mucus of gastropods** (B2, B3, Г2, Г14, Г18, Г19 и Г21).

This group of papers is devoted to the antitumor activity of different isoforms and functional units of hemocyanins from *H. lucorum*, *H. aspersa* and *R. venosa*, as well as fractions of hemolymph of *R. venosa* (with molecular masses of 10 - 50 kDa and 50 - 100 kDa), and fractions of mucus of *C. aspersum* against various cancer cell lines. Promising results have been obtained with polypeptide fractions of 50-100 kDa and also with both RvH1 and RvH2 isoforms of the hemocyanin of *R. venosa* against 6 breast cancer cell lines. It is shown that the fraction HRv (50-100 kDa) demonstrates the highest antitumor potential, which is due to apoptosis, and in some cases also to autophagy. Dr. Velkova proves for the first time that the combination of HRv (50-100 kDa) with cisplatin and/or tamoxifen has a synergistic effect, thus opening up new perspectives for the application of the molluscs proteins in oncotherapy. The observation that the N-glycosylated functional unit  $\beta$ c-HIH-h from *H. lucorum* hemocyanin exhibits antiproliferative effect comparable to that of doxorubicin opens new avenues for treatment of human bladder carcinoma.

**c) Study of the mechanism of action of biologically active substances from mollusks through proteomic analysis (B2, B6 и Г16).**

The articles belonging to this section are dedicated to the mechanism of action of some biologically active components of the mucus and hemolymph of mollusks through modern proteomic analysis. Dr. Velkova observed a significant change in the proteome of human bladder carcinoma cells treated with the  $\beta$ c-HIH-h subunit of *H. lucorum* hemocyanin. It affected the expression of proteins from the glycolytic pathway, lysosomes, proteasomes as well as some components of the cytoskeleton and extracellular matrix.

Similar approach has been applied also to study the mechanism of action of extracts from the garden snail mucus on Alzheimer's disease animal models. Dr. Velkova found that the 20 kDa fraction affected expression of memory related cortex proteins some of which are potential targets for Alzheimer's-type dementia treatment.

**d) Characterization of biologically active substances from other natural sources (Г1, Г4, Г7, Г9, Г15 и Г21).**

These group of papers aim to identify and study biological activities of short peptides (with a molecular mass lower than 1 kDa) and secondary metabolites from *H. lucorum* hemolymph as well as of cyclolipopeptides from *Bacillus velezensis*. The main contribution of these studies is related to the development of an original methodology for their isolation, purification and molecular characterization.

#### **4. Scientific projects**

Assoc. Prof. L. Velkova has participated in 20 scientific projects with national and foreign funding in 3 of which she was the principle investigator. She was also a scientific consultant for 1 project under the young scientists supporting programme of BAS. She works in close cooperation with researchers from various Bulgarian institutions, as well as with scientists from the universities of Tübingen, Germany; Mainz, Germany; Padova, Italy; Ghent, Belgium; Kiev, Ukraine, etc.

#### **5. Pedagogical activity**

Assoc. Prof. L. Velkova has supervised 5 graduates and 8 postgraduates from the Faculty of Chemistry and Faculty of Biology of Sofia University. She has also led seminars and lab work for MS students in the Faculty of Biology under the disciplines "Good Laboratory and Production Practice in Bioindustries" and "Food Quality and Safety".

## 5. Summaries assessment

The summarized assessment according to the LDASRB in the field of Natural Sciences for the academic position of Full Professor is presented in the table below.

- Indicators A: 50 points
- Indicators B: 0 points
- Indicators C: 145 points
- Indicators D: 401 points
- Indicators E: 530 points
- Indicators F: 602 points

**Total: 1 728 т.** (640 points required)

Presented data show that Assoc. Prof. Dr. Lyudmila Velkova exceeds more than two times times the formal criteria required by the law for the academic position "Professor" in the Field of Natural Sciences.

**Conclusion:** Assoc. Prof. Dr. Lyudmila Velkova is a prominent researcher in the field of Bioorganic Chemistry and Chemistry of Natural Products. She has published a total of 72 scientific articles in peer reviewed scientific journals with a total IF 112.629 cited so far more than 400 times. She has also 4 patents and 6 utility models and is co-author of two university textbooks. The summary assessment of her scientific and teaching activity shows that they are more than two times higher than the formally required by the LDASRB for the academic position "Full Professor" in the field of Natural Sciences. All this gives me a reason to recommend to the distinguished Scientific Jury as well as to the Scientific Council of the Institute of Organic Chemistry with Center of Phytochemistry to award to Dr. L. Velkova the Academic Position "Full Professor" in Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances.

Sofia, 17.03.2025

Reviewer:

/ Acad. Ivan Ivanov/