

REVIEW

from Cor. member Prof. Roumen Georgiev Pankov, DSc, Faculty of Biology, Sofia University "St. Kliment Ohridski"

of the materials submitted for participation in a competition for the academic position of **"Associate Professor" at Plovdiv University "Paisii Hilendarski"** in the field of higher education 4. Natural Sciences, Mathematics and Informatics; professional field 4.3. Biological sciences; scientific specialty **Cell Biology**, announced in the State Gazette, issue 57 of 26.06.2020. and on the website of Plovdiv University "Paisii Hilendarski" for the needs of the Department of Developmental Biology at the Faculty of Biology

1. General presentation of the received materials

By order № P33-4708 of 25.09.2020 of the Rector of University of Plovdiv "Paisii Hilendarski" (PU) I was appointed a member of the scientific jury of a competition for the academic position "Associate Professor" in PU in the field of higher education 4. Natural sciences, mathematics and informatics, professional field 4.3 Biological sciences (Cell biology), announced for the needs of the Department of Developmental Biology at the Faculty of Biology.

Only one candidate has submitted documents for participation in the announced competition - Ch. Assistant Professor Tsvetelina Georgieva Batsalova, PhD from the same department. The set of materials presented by the candidate on paper is in accordance with the Regulations for development of the academic staff of PU. The set includes documents described in the application of the candidate to the Rector of PU, dated 14.09.2020, in connection with admission to the competition. The candidate has submitted a list of 29 publications, 3 textbooks, 10 published abstracts and 12 participations in research projects covering the period 2009-2020. Twenty-one scientific papers have been submitted for participation in this competition, of which 16 articles in refereed scientific journals (14 with IF and 2 with SJR), 3 articles in journals without impact factor and 2 textbooks.

I accept for review all 21 submitted scientific papers, as they are not part of the dissertation and should be taken into account in the final evaluation for this competition.

2. Brief biographical data about the candidate

Ch. Assistant Tsvetelina Batsalova, PhD was born in 1983. She completed her higher education at the Faculty of Biology at Paisii Hilendarski University of Plovdiv, receiving a bachelor's degree in Molecular Biology in 2005 and a master's degree in Cell Biology in 2006. She started her professional career as a laboratory assistant at the Paisii Hilendarski University of Plovdiv (2005-2006), and in the next few years, she specialized abroad - in 2007-2008 at the Lund University, Sweden and at the Karolinska Institute, Sweden in 2008-2009. After returning to Bulgaria, she entered as a full-time doctoral student in Cell Biology at Paisii Hilendarski University of Plovdiv, where she successfully defended her dissertation entitled "The role of MHC class II and post-translational modifications of type II collagen for the cell communication in rheumatoid arthritis" in 2011. In the same year, she was elected an Assistant Professor at the Department of Developmental Biology, and since 2013 she is a chief assistant professor. Her total work experience now, which is also pedagogical, is already over 10 years, of which almost 8 years as a Ch. Assistant Professor. It should be noted that the entire scientific and teaching experience of Ch. Assistant Professor Batsalova is closely related to Cell Biology, which fully corresponds to the specialty of the announced competition.

Dr. Batsalova is a member of the Union of Scientists in Bulgaria, section Immunology and Biochemistry, Biophysics and Molecular Biology and the Bulgarian Cytometry Association (BAC).

3. General characteristics of the candidate's activity

Assessment of educational and pedagogical activity of the candidate

As a member of the academic staff of Plovdiv University "Paisii Hilendarski" Ch. Assistant Professor Batsalova has an intensive teaching activity. It includes practical courses in "Cell Biology" for Bachelor's degree students in "Ecology and Environmental Protection", "Biology", "Medical Biology", "Bioinformatics", "Molecular Biology". After taking the academic position of Chief Assistant, Dr. Batsalova was assigned to develop and conduct the lecture course and laboratory classes in "Animal in vitro cultures" of students majoring in "Molecular Biology" and the lecture course and exercises in "Cell and tissue cultures" (the part "Animal cell and tissue cultures") of the students from the specialty "Bioinformatics". In the education of part-time students, Ch. Assistant Professor Batsalova has participated in the development and implementation of the lecture course in "Cell Biology" for the specialties "Ecology and Environmental Protection" and "Biology". The elective course "Stem Cells and Regeneration" has been developed for the training of bachelor students in the specialty "Medical Biology".

Ch. Assistant Professor Batsalova also participates in the second level of student education. She has prepared lecture courses for students from various master's programs including "Molecular-biological methods in clinical diagnostics" (for the specialty Biodiagnostics); "Cytological methods in clinical diagnostics" (for the specialty Biodiagnostics); "Application of cell and tissue cultures in reproductive biology" (for the specialty Reproductive Biology); "In vitro cell cultures and their application in genetics" (for the specialty Genetics); "Flow cytometry" (for the specialty Biodiagnostics).

Dr. Batsalova was the supervisor of 7 successfully defended graduates and co-supervisor of one doctoral student. Her participation as an academic mentor of 16 students, implemented under the project "Student Internships", funded by OP "Human Resources Management" is also noted.

It is evident from the submitted documentation that currently Ch. Assistant Professor Batsalova gives 160 hours of lectures in Bachelor's and Master's degrees. The fact that she has been assigned such a significant number of lecture courses is a very good testament to her qualities as a teacher. Ch. Assistant Professor Batsalova is a co-author of three textbooks – "Practical classes in cell biology", published in 2010 and as a supplemented and revised edition in 2014 and "Manual for laboratory classes in animal cell cultures".

Evaluation of the scientific activity of the candidate

As noted, in the current competition, Ch. Assistant Professor Batsalova participates with 21 scientific papers, of which 16 articles in peer-reviewed scientific journals, 3 articles in journals without impact factor and 2 textbooks. Of all the articles, 14 have been published in international journals with an impact factor and some of them include such prestigious journals as *J. Am. Chem. Soc.* (IF 9.9), *J. Immunol.* (IF 5.7), *Carbohydr Polym.* (IF 5.2), *Arthritis Res. Ther.* (IF 4.3) and others. According to the accepted division into quartiles, half of the articles with impact factor (7) fall in the highest Q1, 4 - in Q2, 1 - in Q3 and 2 - in Q4. The total impact factor, calculated from the presented scientific production is over 45, and the reference for the citations of the articles in the competition includes 76 titles. According to Scopus, Dr. Batsalova's total citations are 143 and

her Hirsch index is eight. These high scientometric indicators undoubtedly outline the high quality of her scientific output.

The chronological analysis of Dr. Batsalova's publishing activity shows that out of all 21 scientific papers, 18 (or 86% of all) were published after her election as Chief Assistant Professor, which reflects the intensification of her research activities. This activity is combined with intensive project work. The presented materials document her participation as a leader of 2 research projects (one funded by the NSF and 1, supported by the University of Plovdiv) and as a participant in 10 projects.

It should be noted that the above scientometric data significantly exceed the criteria of the Faculty of Biology of the University of Plovdiv for awarding the academic position of "Associate Professor". Dr. Batsalova has presented 19 publications (15 required), of which 16 in referenced journals (10 required), and the publications with IF are 14 (7 required). There is a significant excess in the other criteria as well. Instead of the required 20 citations (10 of which in international peer-reviewed journals), 76 were achieved, all in peer-reviewed publications. With a requirement for 5 defending graduates, Dr. Batsalova has led 7, and her participation in research projects is 12, with a required 2. There is also exceeding the minimum national criteria set out in the Regulations for the implementation of the Academic Staff Development Act in the Republic of Bulgaria. The presented data show completion of the required 100 points from section B, achieved 214 points from section D (200 points required) and 152 points from section E (50 points required).

Scientific contributions

The scientific research presented in the hitherto unreviewed publications, with which Chief Assistant Professor Batsalova participates in this competition are summarized in groups B and D, according to the Regulations for implementation of Academic Staff Development Act in the Republic of Bulgaria. I will consider the presented articles as they are structured by the candidate, and in a summarized form will present the most important scientific achievements.

Section "B" presents 4 publications (all belonging to Q1), summarizing the results of research, which can be considered as a continuation of the main topic developed by Chief Assistant Professor Batsalova since the preparation of her dissertation, namely - research on autoimmune diseases and establishing the cell-molecular mechanisms of autoimmune diseases. Published new results deepens research on the mechanisms of rheumatoid arthritis and the main candidate autoantigen - type II collagen. The main contributions can be summarized as follows:

Experiments with the Ncf1 mutant mouse gene line have shown that the presence of normal levels of reactive oxygen species (ROS) is important for establishing immunological tolerance and protection against the development of arthritis induced after immunization with glycosylated collagen II 259-273 peptide in complex with MHC II. Due to the similarities between the model system used and some human immunological characteristics, the results obtained could be used in the treatment of patients with rheumatoid arthritis.

Following the creation of a new "humanized" mouse model (B10.DR4.Ncf.1*/*) by introducing a mutant Ncf1 gene, that enhances susceptibility to autoimmune diseases, in transgenic mice co-expressing HLA-DR4 and the human CD4 receptor, the collagen-specific II T-cell and antibody reactivity is clarified during different periods of development of collagen-induced arthritis. The results convincingly demonstrate that: collagen II-specific and T cell and antibody reactivity increases significantly after the onset of clinical symptoms of collagen-induced arthritis and persists during the disease; and the different T-cell specificity in transgenic DR4 mice is not due to the level of glycosylation of collagen II.

A methodology has been developed that combines, for the first time, structure-based virtual screening, ligand-based statistical molecular design, and biological studies to investigate interactions between glycosylated peptides and receptors. Using this methodology a library of collagen II 259-273 glycopeptides with variations in positions, relevant to binding to Aq and DR4 molecules are created. Following synthesis and biological evaluation, glycopeptides are grouped according to the immunological response. Some of the generated groups are of particular interest because of the possibility of their use in the development of specific vaccines for collagen-induced arthritis. The developed strategy is suitable for the design of new ligands of any type of protein-ligand system, for which there is an established structural model.

The effect of disrupting the hydrogen bonds between the collagen II 259-270 glycopeptide and the murine A^q MHC class II molecules has been investigated using (E)-alken- and ethylene-amide-linked isosters. Changes in these interactions have been shown to be critical for the MHC class II/peptide/T-cell receptor signaling system.

In half of the presented articles (2 out of 4) in this section, Ch. Assistant Professor Batsalova is the first author, and in one - the second. These leading positions show the significant personal contribution of the candidate in the conducted research. The assessment of the professional community for these articles is very good and can be illustrated by the achieved citation - 31 citations are documented.

The articles from the second section - G, with which Chief Assistant Professor Batsalova participates in this competition include 15 articles, of which 3 are from Q1, 4 - from Q2, one is from Q3, two - from Q4, two articles in journals with SJR and three articles without IF or SJR. The candidate has appropriately grouped his contributions from these articles into five groups:

The first group summarizes studies published in 6 articles aimed at elucidating the biological activity of extracts, essential oils and new molecules from various plant species and microalgae. The studies demonstrate the antitumor activity of *Vitex agnus-castus* essential oils, *Clinopodium vulgare* extracts and newly isolated furostanol saponins from *Smilax aspera*. Analyzes on polysaccharides - pectins from *Tilia tomentosa* and polysaccharide samples from *Portulaca oleracea*, *Lavandula angustifolia* and *Tilia tomentosa* for the first time proved the immunomodulatory properties of pectin and confirmed the possibility of using polysaccharides in therapies aimed at restoring compromised immune system.

Although at an early stage, studies on microalgae from the *Cyanoprokaryota* are also of some interest as sources of biologically active substances. The cytotoxic and antioxidant activity of *Fischerella major* extracts has been reported for the first time, and studies on the action of microcystin-LR have shown for the first time the protective effect of captopril against the toxic potential of this cyanobacterial toxin.

The second group includes studies published in two articles aimed at elucidating the role of various chemokines in pollen allergies. Decreased serum levels of four CC chemokines (CCL2/MCP-1, CCL3/MIP-1 α , CCL4/MIP-1 β and CCL5 RANTES) and elevated levels of IL-8/CXCL8 compared to healthy controls were reported in individuals with an allergy to ragweed. The study was conducted outside the pollen season. This study, as well as the presented similar study of individuals, allergic to birch pollen, provide an opportunity to develop appropriate biomarkers for more accurate assessment of the status of patients with allergy to pollen, applicable out of season, as well as a basis for the development of new therapies.

The interest of Ch. Assistant Professor Batsalova to cyanobacteria is not limited to the establishment of new biologically active substances. As a separate contributing study, She has also presented studies on 86 cyanobacterial species that demonstrated that outer membrane efflux

protein (OMER) could be used as a molecular marker to elucidate the phylogenetic and taxonomic status of closely related cyanobacteria, with OMER being more the appropriate marker than the 16S rRNA gene.

The fourth group summarizes studies that contribute to a better understanding of the effect of prebiotics on probiotic bacterial strains. By culturing different strains of *Lactobacillus brevis* in the presence of different prebiotics (xylooligosaccharides, inulin, pectin and chitosan), the immunomodulatory properties of the strains have been shown to be species-specific and prebiotic-dependent. Continuing the research in this direction, Chief Assistant Professor Batsalova showed that the adhesion of three bacterial species (*Lactobacillus rhamnosus* 1010, *Lactobacillus acidophilus*11 and *Lactobacillus paracasei* 8458) after treatment with six different prebiotics (inulin, pectin, chitosan, galactooligosaccharides, xylooligothacaribanic saccharides) undergo prebiotic-dependent changes. Beta-glucan and chitosan treatments have a negative effect, while xylooligosaccharides and galactooligosaccharides enhance the adhesion of lactobacilli to enterocyte-like cell lines.

The last group combines research in the field of bionanotechnology and, in particular, studies on cytotoxicity and the possibility of use in the diagnosis of autoimmune diseases of iron oxide nanoparticles. These studies demonstrate that iron oxide (II, III) nanoparticles (ION) inhibit the cell growth of both the studied algae and human cells. Decreased ATP production and cell motility were also reported. The obtained results prove the need for a more detailed study of the biological activity of ION in view of their widespread biomedical and industrial application. A further study showed that the same nanoparticles could be used to develop a rapid and sensitive diagnostic test for rheumatoid arthritis after biofunctionalization with molecules used to detect biomarkers for this disease. The effectiveness of the developed methodology is similar to the conventional enzyme-linked immunosorbent assay.

This section, combining 15 publications Ch. Assistant Professor Batsalova also demonstrates a significant personal contribution. In seven of the articles she is the lead author (in six articles - the first author and in one article - the last), and in four she is the second author. The articles in this section have already been cited 45 times and it should be noted that one third of them have been published in the last three years, a very short period to expect their active citation.

4. Critical remarks and recommendations

I have no critical remarks. The submitted documentation is complete, well organized and meets all legal requirements.

5. Personal impressions

I have no personal impressions of Ch. Assistant Professor Batsalova, except for her dissertation, for which I had the pleasure of being a reviewer. Now, I can confidently say that my positive assessment was correct and Chief Assistant. Batsalova has developed as a skilled scientist with active research and teaching.

CONCLUSION

Chief Assistant Dr. Batsalova is an established specialist with a clearly defined research profile in the field of Cell Biology. She has extensive experience in teamwork, competencies and skills for conceptualization and implementation of scientific publications. Her scientific output is significant in volume and quality and exceeds the requirements referred to in the regulations. The results obtained are original, have been published in a series of prestigious scientific journals and

have been well received by the international and our scientific communities. At the same time, Ch. Assistant Professor Batsalova is a respected lecturer who maintains intensive teaching activities and actively participates in the organization and improvement of the learning process. This gives me reason to confidently give my positive assessment and to recommend to the Scientific Jury to prepare a report-proposal to the Faculty Council of the Faculty of Biology for election of Ch. Assistant Professor Tsvetelina Georgieva Batsalovaq PhD to the academic position of ‘Associate Professor’ at the University of Plovdiv “P. Hilendarski” by professional field 4.3. Biological sciences, scientific specialty Cell biology.

18.11.2020

Reviewer:
/Cor. member Prof. Roumen Pankov, DSc/