ASSESSMENT REPORT

by Dr. Valya Nikolova Vassileva, Professor at the Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences
regarding the competition for the academic position of Associate Professor at the Institute of Molecular Biology "Acad. Roumen Tsanev", Bulgarian Academy of Sciences
Field of higher education: 4. Natural Sciences, Mathematics, and Informatics
Professional field: 4.3 Biological Sciences
Scientific specialty: Molecular Biology

1. General information on the competition and the submitted documents

The competition for the academic position of Associate Professor was announced in the State Gazette (*Issue 21/10.12.2024*) to meet the needs of the Laboratory "Genome Stability" at the Institute of Molecular Biology (IMB) "Acad. Roumen Tsanev" of the Bulgarian Academy of Sciences. The sole candidate for this competition is Assistant Professor Dr. Radoslav Alexandrov Alexandrov, currently a researcher at the same laboratory. The documentation of the Candidate has been prepared in full compliance with the requirements of the Act for the Development of the Academic Staff in the Republic of Bulgaria (ADASRB), its Implementing Regulations, and the Rules for the Conditions and Procedures for Acquiring Academic Degrees and Occupying Academic Positions at IMB. A complete set of required documents has been submitted. The thematic focus of the scientific publications, which include original research achievements, is fully aligned with the scope of the competition and the research profile of the laboratory. The submitted materials include a curriculum vitae, a copy of the PhD diploma, an abstract of the dissertation, a certificate of professional experience, a list of scientific publications, brief and detailed reports on the fulfillment of the requirements for the academic position of Associate Professor, concise and extended habilitation references outlining the scientific contributions, abstracts of the publications in Bulgarian and English, the publications submitted for the competition and a copy of the competition announcement from the State Gazette.

2. Information on the career development and research interests of the Candidate

Dr. Radoslav Alexandrov is a graduate of the Faculty of Biology at Sofia University (SU) "St. Kliment Ohridski", where he consecutively earned a Bachelor degree in Molecular Biology (2012) and a Master degree in Biochemistry (2014). During his Master studies, he participated in an Erasmus exchange program at the University of Poitiers, France, where he developed a thesis focused on the mechanisms of sugar signaling in plant cells. Scientific career of Dr. Alexandrov is closely associated with IMB. In October 2014, he began working as a biologist at the Laboratory of "Genome Stability". In August 2015, he was enrolled as a full-time PhD student under the supervision of Associate Professor Dr. Stoyno Stoynov, and in December 2018, he successfully defended his doctoral dissertation on the topic "*Dynamics and sequence of protein binding involved in DNA repair*". The main results of his dissertation have been published in leading journals, including *Molecular Cell* and *Nature Communications*. Following the completion of his PhD, Dr. Alexandrov continued his research at the same laboratory, initially as a postdoctoral researcher under the National Program "Young Scientists and Postdoctoral Researchers" (2019–2022), and since February 2022, as an Assistant Professor. His research interests are focused on the molecular mechanisms that maintain genome stability in eukaryotic cells, with particular emphasis on the action of PARP inhibitors and other molecules influencing DNA repair systems.

3. Overview of the research activity of the candidate

3.1. Publication activity

Dr. Radoslav Alexandrov is a young researcher with an impressive scientific output, having authored 16 scientific publications in prestigious international journals with a total impact factor of 140.065. His

scientific achievements have been highly recognized in the academic community: in 2018, 2021 and 2024, publications where he is first or corresponding author were awarded as the best publications of IMB. The candidate has received several prestigious awards, including the "Marin Drinov" Award for Young Scientists from BAS (2019), the award for best publication by a young scientist in the competition dedicated to the 150th anniversary of the Academy, and the Award "Roumen Tsanev" for young scientist from the IMB (2019). These honors show his established academic reputation and active contribution to the research activities of IMB and BAS. For the current competition, the Candidate has submitted 14 publications with a total impact factor of 113.227, grouped into two categories. The first group includes four publications presented as part of his habilitation work, all published in Q1 (first quartile) journals – *Cell Reports*, *Cell*, *Biomedicines* and *International Journal of Molecular Sciences*. This reflects the significance of the research results of the Candidate and his ability to publish in leading international journals. The second group consists of ten publications, indexed in internationally recognized scientific databases Web of Science and Scopus (WoS/Scopus), separate from the habilitation work. These articles explore diverse aspects of cellular and molecular biology, including DNA replication dynamics, chromatin regulation, specific biochemical interactions and biomedical applications. Notably, they include papers published in prestigious journals such as *iScience*, *Seminars in Oncology*, *Cells*, *Journal of Clinical Medicine*, *FEBS Open Bio* and *Toxicon*.

3.2. Compliance with the requirements for the academic position of Associate Professor

The analysis of the brief and detailed reports submitted by Dr. Radoslav Alexandrov, along with the accompanying documentation, clearly demonstrates that his scientific activity fully meets, and in some indicator groups (D and E) substantially exceeds the minimum requirements for occupying the academic position of Associate Professor, in accordance with Article 26, Paragraph 1 of the Act for the Development of the Academic Staff in the Republic of Bulgaria (ADASRB) and the Regulations of IMB–BAS.

Under **Group A**, a defended doctoral dissertation is presented, awarding the Candidate 50 points and thereby meeting the required 50 points. The indicators under **Group B** are covered through four publications in Q1 journals, earning 100 points against the required 100 points. Regarding **Group C**, Dr. Alexandrov has submitted 10 publications: seven in Q1 (175 points), one in Q2 (20 points), one in Q3 (15 points) and one in a journal indexed by SJR without an Impact Factor (10 points). Thus, the total score in this group is 220, meeting the required 220 points. The Candidate also fulfills the requirements under **Group D**, with a total of 530 citations in the WoS/Scopus databases (as of January 8, 2025), corresponding to 1060 points, greatly exceeding the minimum requirement of 60 points. For Indicators 12–20 under **Group E**, which are not mandatory for this position, Dr. Alexandrov has accumulated an additional 488 points through participation in 11 national projects, 1 international project, and the leadership of 2 national and 1 international project, attracting funding of over 1 340 000 BGN. These indicators demonstrate high scientific productivity, sustained citation impact and active engagement in research projects. Furthermore, the volume and quality of the scientific output of the Candidate, his leadership role in research projects and his contributions to the development of open-access tools such as the *DNARepairK* database and the *CellTool* software underline his contribution to the scientific community and his established capacity for independent research activity.

According to the data presented in Table 1 (Brief report) and Table 2 (Detailed report), Dr. Alexandrov has accumulated a total of **1918 points**, vastly exceeding the minimum requirement of 430 points.

3.3. *Research achievements*

The achievements of Dr. Radoslav Alexandrov can be summarized into five main thematic areas:

3.3.1. Investigation of DNA repair dynamics in living cells

The Candidate has developed and applied an innovative experimental approach for studying the dynamics of proteins involved in DNA repair through confocal microscopy of living cells combined with

mathematical modeling. Using this approach, he uncovered key aspects of the mechanism of action of PARP inhibitors and introduced new kinetic parameters (PRC, PTC, PIC) for assessing their effectiveness [*Publication B4.1*]. Additionally, he demonstrated that PARP1 acts as a physical barrier at DNA damage sites, a finding with implications for the clinical activity of these molecules. Dr. Alexandrov also identified a novel function of PARP1 in stabilizing broken DNA ends through the formation of PARP1-DNA condensates, a process dependent on the FUS protein, studied in collaboration with an international research team [*Publication B4.2*]. Furthermore, he showed that the spread of phosphorylated histones (γH2AX) in chromatin occurs via diffusion of activated ATM kinase beyond the primary damage site [*Publication G7.2*]. The data from these studies have been integrated into the open-access software *CellTool*, developed in his laboratory, which includes specialized algorithms for the registration, segmentation, tracking and analysis of the dynamics of DNA repair proteins [*Publication B4.4*]. The findings were also incorporated into the *DNARepairK* database, containing kinetic profiles of 70 DNA Damage Response (DDR) proteins [*Publication B4.3*]. Both platforms are already being utilized by external research groups. These results expand the understanding of DNA repair mechanisms and provide innovative tools for the evaluation of therapeutic agents targeting genome stability.

3.3.2. Investigation of DNA replication dynamics in living cells

In this research topic, Dr. Alexandrov explores the molecular mechanisms by which cells respond to replication stress, a condition that threatens genome stability through the slowing or stalling of replication forks. By the development of an experimental approach for tracking the dynamics of replication foci, he analyzed the behavior of key replication complex proteins, including PCNA and RPA1, under normal conditions and stress induced by hydroxyurea [*Publication G7.1*]. His studies show that inhibition of the ATR kinase does not prevent the removal of PCNA from replication sites but leads to an increased accumulation of single-stranded DNA, highlighting the role of ATR in coordinating DNA unwinding and synthesis under stress conditions. Additional investigations in *Saccharomyces cerevisiae* revealed a novel function of the Dia2 protein in the regulation of cell cycle progression, control of cell size, and adaptation to replication stress [*Publication G7.5*]. These findings contribute to a deeper fundamental understanding of the cellular response to replication stress and have applications in studying the mechanisms of action of chemotherapeutic agents targeting DNA replication processes.

3.3.3. Investigation of the etiology of chronic rhinosinusitis

In this research area, Dr. Alexandrov investigates the role of bacterial biofilms and mucin glycoproteins in the development of chronic rhinosinusitis (CRS), a condition affecting over 10% of the adult population in developed countries. By combining confocal microscopy and quantitative PCR, he analyzed tissue samples from 85 patients to establish relationships between the presence of biofilm and the expression of the main mucins MUC5AC and MUC5B in the nasal mucosa [*Publication G7.4*]. The results show a higher frequency of biofilms and increased expression of MUC5B in patients with CRS, but no statistically significant difference was found for MUC5AC. These data point to the potential role of MUC5B in the pathogenesis of the disease. At the same time, the lack of direct correlation between mucin expression and the presence of biofilm emphasizes the complex and multifactorial nature of CRS. The study is interdisciplinary in nature and demonstrates the Candidate ability to effectively apply cellular and molecular approaches to expand knowledge about common clinical conditions.

3.3.4. Investigation of metabolic differences between embryogenic and non-embryogenic plant cells

Dr. Alexandrov examines the physiological and metabolic differences between embryogenic and non-embryogenic cell cultures of *Vitis vinifera* (grapevine), despite their identical genetic origin [*Publication G7.7*]. Using a combination of cytological and biochemical approaches, he established that embryogenic cultures are characterized by moderate and organized growth in the form of proembryogenic aggregates, active aerobic metabolism, low glycolytic activity and high oxygen consumption. On the other hand, non-embryogenic cells exhibit disorganized, rapid growth and a metabolic profile dominated by intensive glycolysis and fermentation, combined with reduced oxygen consumption. These contrasting metabolic

characteristics reflect the differences in cellular differentiation and the potential for involvement in the somatic embryogenesis pathway. The results contribute to a better understanding of the metabolic prerequisites for embryogenic capacity in plant cultures and have implications for improving methods of in vitro vegetative propagation.

3.3.5. Investigation of the mechanism and properties of the neurotoxin vipoxin, isolated from Vipera ammodytes meridionalis

Dr. Alexandrov participated in research on the primary neurotoxin from the venom of the Bulgarian subspecies of the nose-horned viper, *Vipera ammodytes meridionalis*. Vipoxin is a heterodimer composed of a toxic secretory phospholipase A2 (sPLA2) and a non-catalytic acidic subunit (VAC) [*Publication G7.10*]. Through biochemical analyses, amino acid residues responsible for catalytic activity, substrate binding and enzyme stability were characterized. The cytotoxic effects of the toxic subunit were confirmed in various cell lines (RPE-1, MDCK, A-549), which exhibit increased membrane permeability, deformation and cell death [*Publication G7.8*]. Given the limitations of conventional immunotherapy for snakebites, Dr. Alexandrov applied phage display technology and selected over 30 scFv antibody fragments targeting vipoxin and its components [*Publication G7.9*]. Some of these fragments effectively neutralized the enzymatic and hemolytic activity of the toxic subunit. The obtained results open opportunities for the development of more targeted and safer antidotes against snake venoms and represent a contribution to modern biomedicine and toxicology.

3.3.6. Evaluation of the presented research perspectives

The research priorities outlined by Dr. Alexandrov are distinguished by high scientific relevance, strategic consistency and a clear vision based on his accumulated experience and substantial research achievements. The core directions of the international project he leads, "Deciphering DNA damage response dynamics in living cells", funded by the Swiss National Science Foundation (SNSF) under the PROMYS (Promotion of Young Scientists in Central and Eastern Europe) program, demonstrate his capacity to formulate original ideas with strong potential for scientific breakthroughs and clinical application. The planned investigation into the mechanisms of action of emerging classes of inhibitors, such as PARGi and ATMi, demonstrate active engagement with contemporary challenges in molecular medicine. His research focusing on ubiquitin-dependent chromatin organization in response to DNA damage, along with the development of a kinetic atlas of DNA damage response (DDR) proteins, illustrates visionary thinking and a commitment to creating valuable long-term research resources. Collectively, these research plans reveal a high degree of professional maturity, scientific originality and international competitiveness. In this context, the research perspectives of Dr. Alexandrov represent a compelling argument in favor of his habilitation.

4. PROJECT AND TRAINING ACTIVITIES

Dr. Radoslav Alexandrov demonstrates organizational and leadership skills through his participation in 11 national and 1 international project, as well as being the principal investigator of two national projects and the international project "*Deciphering DNA damage response dynamics in living cells*", funded by SNSF with a budget of 1.3 million BGN and duration of five years. This project represents a logical continuation of his previous work and focuses on key aspects of genome stability, a critical factor for the prevention and treatment of oncological, neurodegenerative and hereditary diseases. Project management requires strategic planning, coordination of scientific activities and effective allocation of resources, which in this case emphasizes his scientific competence and his administrative capacity for successful implementation of large-scale research initiatives. His scientific activity is complemented by participation in 14 national and international forums, where he has presented 8 oral and 6 poster presentations, including by special invitation from the organizers, a testament to the high recognition of his expertise. Alongside his impressive research achievements, Dr. Alexandrov actively participates in the training of young

researchers. He has supervised four graduates - three Bachelor students from the Faculty of Biology at SU and one Master student from Maastricht University (Netherlands). Since 2009, Dr. Alexandrov has also been teaching biology at the National High School of Natural Sciences and Mathematics, thereby contributing to the education of young talents and fostering continuity between the scientific community and the next generation of researchers. The overall project and teaching activities of the Candidate represent a substantial addition to his scientific biography and confirm his role as a researcher with active contributions to the scientific and educational fields.

4. CRITICAL REMARKS AND RECOMMENDATIONS

I have no critical remarks regarding the Candidate research activities or the materials submitted for the competition. A minor inconsistency is noted in the Extended habilitation report, where the Candidate indicates participation in the competition with 13 publications, whereas the presented list includes 14 publications (4 under Group B indicators and 10 under Group C). Similarly, the total number of publications is cited as 15, whereas the list contains 16. These discrepancies do not affect the scientific merit of the application and, if anything, are favorable to the Candidate, but this could be clarified for the sake of documentation precision.

I would recommend that Dr. Alexandrov build upon his expertise in live-cell imaging and kinetic analysis by expanding his research on the spatial organization of DNA repair sites, focusing on the role of biomolecular condensates and the principles of liquid-liquid phase separation. The successful validation of the dynamics of PARP1-DNA condensates in live cells, achieved in collaboration with the group of Prof. Simon Alberti, provides a strong foundation for such studies. Integration of CRISPR-based genome screening and single-cell analyses would enable a comprehensive characterization of the regulatory networks governing chromatin compartmentalization during the DNA damage response. This research direction would complement his current work on the dynamics of DNA repair and further advance his scientific contributions to the field of modern molecular and cellular biology.

CONCLUSION

The materials submitted by Assistant Professor Dr. Radoslav Alexandrov demonstrate his established scientific expertise and consistent professional development in the field of molecular biology. His research on DNA repair and replication processes in living cells, the action of antitumor agents and the creation of specialized research databases represents a considerable scientific contribution, recognized in the academic community. His leadership of a prestigious international project further highlights his research competence, strategic planning ability and capacity to develop competitive scientific initiatives. Dr. Alexandrov scientific interests are fully aligned with the thematic profile of the Laboratory "Genome Stability" at IMB-BAS, and reveal strong potential for innovative, interdisciplinary and internationally impactful scientific activity. The submitted habilitation materials fully meet, and in some aspects exceed, the requirements of ADASRB and the corresponding Regulations for appointment to the academic position of Associate Professor.

Based on the above, I would recommend that the esteemed Scientific Jury propose to the members of the Scientific Council of IMB-BAS that **Assistant Professor Dr. Radoslav Alexandrov Alexandrov be elected to the academic position of ASSOCIATE PROFESSOR in Professional field 4.3. Biological Sciences, Scientific specialty "Molecular biology".**

April 28, 2025

Sofia

Prepared by:

Prof. Dr.