СОФИЙСКИ УНИВЕРСИТЕТ
„СВ. КЛИМЕНТ ОХРИДСКИ"
БИОЛОГИЧЕСКИ ФАКУЛТЕТ

SOFIA UNIVERSITY
ST. KLIMENT OHRIDSKI
FACULTY OF BIOLOGY

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SOFIA UNIVERSITY

EXPERT OPINION

By: Professor Svetla Dimitrova Petrova, PhD
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Member of the Scientific Jury, appointed by order No. № 23-ОБ/28.01.2025 of the Director of the Institute of Molecular biology – Bulgarian Academy of Sciences "Acad. Roumen Tsanev" (IMB-BAS) – **Assoc. prof. Anastas Gospodinov,** PhD

Re: Materials submitted for participation in a competition for the academic position of **Associate Professor** in Higher Education area 4. Natural sciences, mathematics and informatics, Professional area 4.3. Biological Sciences (Molecular biology) launched for the needs of "Genomic stability" Laboratory at the IMB-BAS, "Acad. Roumen Tsanev" and announced in State Gazette, issue No. 104/10.12.2024, p.113, according to the Law for the Development of Academic Staff in the Republic of Bulgaria (LDASRB) and the Regulations for Implementation of LDASRB at the IMB - BAS.

Candidate: Chief asst. prof. Radoslav Aleksandrov Aleksandrov PhD, Laboratory of "Genomic stability", IMB-BAS.

1. General presentation of the procedure and the applicant

The competition procedure was strictly followed as all necessary documents, accurately prepared and completed in accordance with legal requirements, were submitted on time. They meet the recommended scientific criteria required for the academic position of "*Associate Professor*" in Professional area 4.3. Biological sciences, as well as the additional information on candidate's teaching and project activities in terms of qualitative and quantitative indicators.

Chief asst.prof. Dr. Radoslav Aleksandrov graduated the National High School of Natural Sciences and Mathematics "Acad. L. Chakalov", with honors and awards for high achievements and participation in the International Biology Olympiads. Immediately after that, he was admitted to specialty Molecular Biology at the Faculty of Biology, Sofia University "St. Kliment Ohridski", where in 2012 graduated with a Bachelor's degree in Molecular Biology as the top student in the course with full honors and a gold medal. Radoslav Aleksandrov obtained his Master's degree in Biochemistry (scientific field Molecular Biology) at Sofia University "St. Kliment Ohridski" with an excellent defense of his MSc thesis on the topic: "Study of proliferation curves, sugar signaling pathways and sugar absorption in cell cultures of *Vitis spp.*", prepared through an Erasmus exchange program in the PHYMOTS laboratory (Laboratoire Physiologie Moléculaire du Transport des Sucres chez les Végétaux) at the University of Poitiers (France) under the supervision of prof. Dr Rositsa Atanasova.

Radoslav Aleksandrov began a doctoral program in "Molecular Biology" at the IMB-BAS under the supervision of assoc. prof. Dr Stoyno Stoynov, which he completed in 2018 with a brilliantly defended PhD thesis entitled: "Dynamics and sequence of binding of proteins responsible for DNA repair". The results of his research (published in *Molecular Cell* (PMID: 29547717), demonstrated an innovative study of the kinetic mechanisms and dynamics of intermolecular interactions, providing high coordination between many individual DNA repair pathways. Creating an unique kinetic model Radoslav Aleksandrov et al., tracked the sequential accumulation and removal of 70 proteins involved in DNA repair mechanisms in living cells at the sites of specifically induced DNA damage. This research showed also new aspects in the processes of inhibition of the PARP1/2 enzyme (Poly [ADP-ribose] polymerase), which change the chronology of the entire reparative process, raising the scientific

questions to a higher biomedical level with opportunities for in-depth study of the action of various anticancer agents on DNA repair processes.

Dr. Aleksandrov has been working in the Laboratory of Genomic Stability at the IMB (BAS) since 2016, successively holding the positions of biologist, asst. prof. (until 2021) and chief ass. prof. (from 2022). His scientific interests encompass various aspects of establishing the molecular mechanisms of PARP inhibitors (PARPi) in living cells, development of an experimental approach to prove the activity of various drugs targeting DNA repair systems and regulatory mechanisms ensuring genomic stability. Since these extremely complex, integral and interdependent processes of continuous exchange of biochemical information between cellular metabolic activity, regulation of gene expression and genomic stability are absolutely necessary for the maintenance of cellular integrity, specialization and proliferation, their understanding will require more than a large-scale study. I believe that the participation of Dr. Radoslav Aleksandrov in the competition for the academic position of "*Associate professor*" is a completely logical and necessary step in his career as a scientist and future head of a research group.

2. Fulfillment of the state requirements for the academic position "*associate professor*"

Chief Asst. Prof. Dr. Radoslav Aleksandrov has presented his research work through 15 impressive scientific publications in peer-reviewed and indexed journals (world-known databases with scientific information Scopus and Web of Science) with a total IF of 140.065 (13 of them for the actual competition with a total IF of 113.227), which have been cited over 530 times (until 8.1.2025) are the real proof that Dr. Radoslav Aleksandrov is already an established scientist with interdisciplinary knowledge in various fields of modern biology.

Some of the publications in which Radoslav Aleksandrov is the first/last author have been selected as the best scientific publications of IMB (2018 and 2021). In 2019, Dr. Aleksandrov received 3 awards: "Marin Drinov" for young scientist of the Bulgarian Academy of Sciences; award for the best scientific publication by an young scientist in the competition for the 150 anniversary of BAS; and an individual "Acad.Roumen Tsanev" award of IMB-BAS. In 2016, Dr. Aleksandrov was awarded also a scholarship from the World Federation of Scientists.

All presented achievements significantly exceed the minimum national recommended criteria for holding the academic position of "*Associate professor*" in Professional area 4.3. Biological sciences:

✓ indicator A1 (50 pts) – PhD Thesis - "Dynamics and sequence of binding of proteins responsible for DNA repair" (Diploma No. 001105/21.01.2019)

✓ indicator B4 (100 pts) – *4 scientific publications in journals with rank Q1* (***Cell Reports***, 2024, 43(5) IF = 7.5; ***Cell***, 2024, 187(4), IF = 64.5; ***Biomedicines***, 2021, 9(9), IF = 6.081; and ***Int. J. Mol. Sci***., 2023, 24(23), IF = 4.9)

✓ indicator G7 (220 pts) - *10 scientific publications*: - 7 publications in Q1; 1 publication - Q2; 1 publication - Q3; 1 publication without IF.

✓ indicator D11 (1060 pts) – *530 citations* of the presented scientific publications (until 01.2025)

✓ indicators E14-E18 (488 pts) – *participation* in 11 national scientific projects and 1 international scientific project (International Centre for Genetic Engineering and Biotechnology (ICGEB) - CRP/BGR16-03); *project leader* of 2 national scientific projects and a *leadership* of the Bulgarian team of the international scientific project – "Promotion of Young Scientists in Central and Eastern Europe (PROMYS)" granted by the Swiss National Science Foundation (SNSF) - IZPYZ0_228842 until 2029. Dr. Alexandrov has presented his scientific results at numerous international and national forums.

✓ The funding for the projects led by Dr. Aleksandrov reaches 1,340,000 BGN.

I accept the certificate of chief asst. prof. Dr Radoslav Aleksandrov for fulfillment of the minimum national requirements under Art. 2b of the LDASRB, for the Higher Education area 4. Natural sciences, mathematics and informatics, Professional area 4.3 Biological sciences (Molecular biology).

3. Assessment of candidate's scientific and applied research activities

The main scientific interests of Dr. Radoslav Aleksandrov are focused on the study of the molecular mechanisms for maintaining genomic stability and DNA repair - a set of the most controlled and integrated processes, which provide remarkable coordination in time and space of hundreds of proteins and enzymes necessary for the dynamic functioning of all metabolic and signaling networks in the cell. Dr. Aleksandrov has presented his scientific achievements according to the scientific pursuits of the entire team:

A. Dynamics of DNA repair in living cells

Dr. Aleksandrov's PhD thesis is only the beginning of his scientific "dedication" into the complexity of the interconnected and coordinated processes of DNA repair, studied in the Laboratory of Genome Stability, by applying state-of-the-art methods for tracking the dynamics of association of fluorescently labeled proteins at the sites of complex DNA damage, induced with exactness in a precisely defined chromatin region. Repair process kinetics is analyzed by time-lapse microscopy and mathematical modeling to calculate the rate constants and half-lives of association and dissociation of the studied DNA Damage Response (DDR proteins) proteins, participating in the integral process of DNA repair.

The scientific results, published in *Molecular cell and Nature Communications* (IF 14.7 and 12,124, respectively), are very impressive not only for establishing specific molecular mechanisms, kinetic dynamics, efficiency and biological significance of the studied repair proteins, but Dr. Aleksandrov demonstrated also a new mechanism of action of a class of PARP inhibitors (PARPi), which raises the idea of using this research approach to study the effects of anticancer agents on DNA repair processes (*high applied potential*). In this field - the large-scale study using specific inhibitors PARPi and designed specific kinetic method, Dr. Aleksandrov et al. (last author, *Cell Reports* (2024)) established integral mechanism of PARP1 enzyme activity (DNA damage sensor) at the sites of DNA breaks in living cells. The innovative scientific approach includes introduction and calculation of different coefficients for the precise tracking of the *kinetics of accumulation and removal of PARP1* (Retention Coefficient, PRC); *rate of capture/retention and exchange* of PARP1 in chromatin (Trapping Coefficient, PTC); and enzyme PARP1 activity *inhibition* (PARP1 Inhibition Coefficient, PIC) at the very sites of complex DNA damage in living cells. The effects of 7 different PARPi, used in different concentrations, were monitored and analyzed, which is a laborious experimental process. Dr. Aleksandrov et al. also proved the manifestation of a *specific regulatory allosteric mechanism (reverse allostery)* for both steps - association to the complex and catalytic activity of PARP1, through which they determined the dependence of PARPi cytotoxicity on the retention time (PRC) of PARP1 enzyme at the sites of breaks in DNA. Thus, the measurements of the PRC coeff. values become a valuable biomarker for determining the effectiveness of different PARPi and studying the mechanisms of action of other molecules targeting DNA repair systems.

The biological role of formed PARP1-DNA complexes/condensates (increasing the catalytic activity of PARP1) during repair has also been established. They accelerate the assembly with additional PAR-dependent proteins and stabilize the formed complexes (the PAR-dependent protein FUS, XRCC1 and DNA ligase 3, e.g.), which in turn can quickly and efficiently ligate damaged DNA molecules at the binding site (paper in *Cell*, 2024). This large-scale study (using different enzyme forms - wild-type PARP1 and a series of PARP1 mutants) as well as many molecular biological and enzyme-kinetics methods) raises new scientific questions about PARPi clinical effectiveness.

Another parallel study (*iScience*, 2024) demonstrates *the role of a heterotrimeric MRN complex (MRE11-RAD50-NBS1) for binding and activating the apical DDR protein kinase ATM*, which triggers a cascade for chromatin polyubiquitination in the damaged chromatin region by phosphorylating a number of nuclear proteins (histone variant H2AX, e.g.). A *specialized biological role of chromatin polyubiquitination* is suggested - creating a scaffold around the damaged DNA region for the association and regulation of ubiquitin-binding proteins involved in DNA repair, i.e. *a temporary non-membrane ubiquitin-dependent compartment around the damaged chromatin region.*

Here, the methodological part is also impressive - combination of live cell microscopy, microirradiation, kinetics, mathematical modeling, and simulations, for which a *unique open-access software* - **CellTool** (https://dnarepair.bas.bg/software/CellTool), Int. J. Mol. Sci., 2023) has been *designed* in the Laboratory of "Genomic stability", where also extremely valuable and with high applied potential (*Biomedicines*, 2021) is the created unique DNARepairK database (http://dnarepair.bas.bg/index.php/dnarepairk/, freely available on the laboratory's website), which makes visible the kinetics of 70 DDR proteins through representative movies with experiments for each of the proteins, both in untreated and in talazoparib (PARP inhibitor) treated cells.

B. Dynamics of DNA replication in living cells

Dr. Aleksandrov's research in this area is focused on ATR kinase (Ataxia telangiectasia and Rad3-related), which phosphorylates various components of the replisome, certain proteins of the DNA repair systems and cell cycle regulation, integrating these processes. The methodological model here includes studying the dynamics of the accumulation and removal of fluorescently labeled proteins PCNA and RPA1 within replication foci in living cells during the S-phase of the cell cycle (i.e., by stopping and restarting replication forks) (*Int. J. Mol. Sci.*, 2025). It has been found that inhibition of ATR uncouples the processes of unwinding and separating DNA strands (catalyzed by helicases) from the synthesis of a new DNA strand (polymerases), allowing experimental investigation of the effects and mechanisms of anticancer drugs that induce replication stress.

The molecular mechanisms and the relationship between DNA replication and cell life cycle have been studied by monitoring the behavior of a specialized Dia2 protein in yeast *(S. cerevisiae)*, responsible for maintaining genomic stability (*Molecules*, 2021). Increased cell cycle duration has been demonstrated due to a significant prolongation of S and G2/M-phases in yeast lacking *dia2 (dia2Δ)*, suggesting a definite Dia2 role in DNA replication and regulation of the S-phase checkpoint. Using this approach, the influence of various factors on replicative stress can be monitored, investigated and analyzed since the *dia2Δ* defect is phenotypically manifested by larger cell size.

C. Molecular mechanisms and etiology of chronic rhinosinusitis (CRS)

Dr. Aleksandrov participated in a clinical case project (85 patients) (*J. Clin.Med.*, 2023) by studying the expression levels of mucin glycoproteins (confocal microscopy and qRT-PCR). Increased expression levels of mucins MUC5B, but not MUC5AC, were found in the group of patients with CRS. No direct correlation was found between the presence of formed biofilm and the levels of mucin expression, which imposes the proposal of a new mechanistic model for the relationship between the formed biofilm and the expression of different classes of mucins in chronic RS.

D. The earlier stages of Dr. Aleksandrov's scientific progress were related to biochemical topics in the studies of metabolic differences between embryogenic and non-embryogenic plant cells, as well as studies on the mechanisms of catalytic and non-catalytic ("pharmacological") functional activities and properties of the neurotoxin vipoxin (GIIA-sPLA2), isolated from *Vipera ammodytes meridionalis*. Immunological manifestations of vipoxin were analyzed by the design of specific recombinant monoclonal antibodies (phage display) with high toxicity-inhibiting and applied potential.

The scientific and methodological activities are proof of Dr. Aleksandrov's abilities as a researcher and the need to move to a new stage in career development, when the leading of an independent research group and teaching of specialists and doctoral students, become essential.

As a researcher, Dr. Aleksandrov is an active participant in 11 national and 1 international scientific project, as well as the leader of 2 national and 1 international projects (the Bulgarian leadership).

4. Contribution to academic education

In addition to his scientific and research passion and activities, Dr. Aleksandrov has another exceptional talent related to his *excellent teaching skills*. I have been observing and admiring for years the ease and aptitude with which Dr. Aleksandrov teaches students from the NPMG and Faculty of Biology at Sofia University, presenting the most complex biological processes in a fascinating, understandable and at the same time scientifically sound manner. Radoslav Aleksandrov has been the

supervisor of students from the Faculty of Biology (SU), both in the Bachelor's and Master's degree programs, as well as of one graduate student from Maastricht University (Faculty of Health, Medicine and Life Sciences). The high grades they achieve in Biology Olympiads and at University, the passion for biology and scientific thinking that they will carry throughout their lives, are among the most valuable things and are definitely an extremely important contribution to holding the academic position of "associate professor".

5. Conclusion

As a member of the Scientific Jury for the announced competition, I entirely support the candidacy of chief assistant professor Dr Radoslav Aleksandrov for academic growth and habilitation.

Radoslav Aleksandrov's research achievements are in an extremely complex, integral and interdisciplinary scientific field, which requires the application of the most modern methodological approaches in order to shed light on some of the most unclear and controlled cellular processes, to propose new regulatory mechanisms for multilayer cell life and ultimately to improve and present future thinking in the field of modern biology. This is a scientific field whose applied contribution lies in the future and which will gain increasing biomedical importance, which places the candidacy of Dr. Radoslav Aleksandrov at a very high level.

The scientific and applied contributions of Dr Aleksandrov's research and highly rated publications exceed the requirements for awarding the academic position of "*Associate Professor*", referred to in the normative documents. Participation in various scientific projects and active teaching activities show that Dr. Aleksandrov possesses competencies and skills to carry out and lead both research group with individual scientific expertise and capacity, and tuition of doctoral students, which is absolutely essential requirement after habilitation.

The critical analysis of the significance of Dr Aleksandrov's achievements allows me to confirm the positive assessment presented above and to strongly recommend to the esteemed Scientific Jury to elect chief asst. prof. Dr. Radoslav Aleksandrov for "*Associate Professor*" in the professional area 4.3. Biological Sciences (Molecular Biology) for the needs of the Laboratory "Genomic Stability" at IMB-BAS.

Prof. Svetla Petrova, PhD
Member of the Scientific Jury

28th of April 2024
Sofia