

REVIEW

on the procedure for acquiring the academic position “Associate Professor” in professional field 4.1. Physical Sciences (Biophysics and Medical Physics), published in the State Gazette, issue 99, November 20, 2020, for the needs of the Faculty of Medicine, Department of Physics, Biophysics and Radiology.

Candidate: Assistant Professor Dr. Dessislava Anri Lazarova

Reviewer: Assoc. Prof. Genoveva Antonova Zlateva, PhD – Department of Physics, Biophysics and Radiology, Faculty of Medicine, Sofia University “St. Kliment Ohridski”

The review was prepared based on the Law for Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for application of the ZRASRB and the Regulations for the conditions for acquiring scientific degrees and for obtaining academic positions at Sofia University “St. Kliment Ohridski” (PURPNSZADSU). It was written and presented, based on order № RD-38-28/18.01.2021 of the Rector of Sofia University “St. Kliment Ohridski” for appointment of the scientific jury for this procedure, as well as a decision of the scientific jury regarding the procedure (Protocol № 1/25.01.2021).

Documents for participation in the procedure, within the legally regulated period, are submitted electronically by the only candidate: **Assist. Prof. Dr. Dessislava Anri Lazarova**.

1. General description of the submitted materials

I was given access to the candidate's documents before the first meeting of the scientific jury by the secretary of the jury. The candidate has submitted all the required materials, in accordance with PURPNSZADSU: CV; copies of higher education diplomas; copy of the diploma for scientific degree "doctor"; certificate for professional experience in the academic position "assistant"; reference for educational workload; list of all publications; list of publications submitted for participation in the procedure; a list of publications, conferences, projects and scientific manuals generated by the "Authors" system; reference for fulfilment of the minimum national requirements, according to the Regulations for application of the ZRASRB; reference for citations; author's reference for the contributory impact of the scientific works; reference on the indicators under Art. 112, para. 2 of PURPNSZADSU, copies of scientific papers; a copy of the announcement in the State Gazette. In addition, Assist. Prof. Dr. Lazarova presented a copy of the diploma for the acquired specialty "Biophysics" in the health care system, under Ordinance № 1 of the Ministry of Health, from January 22, 2015, which is especially important for lecturers in medical schools in the country.

2. Brief biographical data

Assist. Prof. Dr. Dessislava Anri Lazarova was born on April 8, 1981. After graduating with a Bachelor degree in Molecular Biology, she also completed a Master's program in Biophysics at the Faculty of Biology, at Sofia University “St. Kliment Ohridski” in 2006 with full honours.

Since 2008 Dr. Lazarova is a lecturer in the Department of Physics, Biophysics and Radiology at the Medical Faculty (MF) of Sofia University “St. Kliment Ohridski”. She has

presented her doctoral dissertation in 2019, in professional field 4.1. Physical sciences (biophysics) on the topic: "Contrast-enhanced magnetic resonance techniques for visualization of pathologies, associated with disorders of cellular redox status." Some of the activities for the development of this scientific work were carried out at the National Institute of Radiological Research of Japan, where Dr. Lazarova specialized in 2014.

3. Lecturing activity

From the attached report on the lecturing workload of Dr. Lazarova it can be seen that it is over 600 hours per year, which significantly exceeds the required lecturing hours at Sofia University.

Assist. Prof. Dr. Dessislava Lazarova has been conducting exercises in Biophysics since 2008, and since 2015 she also conducts exercises in Medical physics for students at the Faculty of Medicine of Sofia University, in Bulgarian and English. Since 2020, Dr. Lazarova has been conducting exercises in Biomechanics for students majoring in "medical rehabilitation and occupational therapy" at the Faculty of Medicine, and is a co-author of the curriculum in this discipline. Dr. Lazarova also presents some of the lectures in Biophysics for nurse students.

As a lecturer, Assist. Prof. Dr. Lazarova is distinguished by excellent professional competence and skills for interesting and comprehensive presentation of the material in the disciplines led by her, which is highly appreciated by the students.

Dr. Lazarova has exceptional experience in the organization of lecturing, in the preparation and updating of seminars and practical classes, in the precise preparation of lecturing materials and exams.

From the reference under Art. 112, para. 2 of PURPNSZADSU is visible that Assist. Prof. Dr. Dessislava Lazarova has participated as an academic mentor to students in the project "Student Internships" of Ministry of Education and Science, which is aimed at improving the quality of professional education of students.

Given the above, I can conclude that Assist. Prof. Dr. Lazarova is a highly competent university lecturer who successfully copes with all the challenges in the educational process and works for its further improvement.

4. Administrative, expert and organizational activities

Throughout the years, as a university lecturer, Assist. Prof. Dr. Dessislava Lazarova has been actively involved in the activities of the academic community. Dr. Lazarova is extremely familiar with the regulations related to higher education, as well as the specific normative documents for Sofia University. Therefore, she is a sought-after member of the team, able to assist in many activities within the Faculty of Medicine.

Assist. Prof. Dr. Lazarova is a member of the Faculty Quality Commission, actively organizing its work and in multiple occasions has presented the necessary information for the quality management process in the Faculty of Medicine for self-assessment reports or to expert groups of National Accreditation Agency in the accreditation procedures at the faculty. She is a member of the General Assembly of Sofia University "St. Kliment Ohridski" from the quota of MF in 2014, taking an active part in its meetings. Currently, Dr. Lazarova is also a member of the Faculty Council of the Faculty of Medicine and its secretary. Assist. Prof. Dr.

Lazarova also performs a number of activities related to the functional organization of the Department of Physics, Biophysics and Radiology.

In addition to the administrative and expert experience of the candidate, I would like to point out that she was part of the management team – coordinator of project BG051PO001-3.3.06-0040 "Building interdisciplinary teams of young scientists in the field of fundamental and applied researches, relevant to medical practice" under the Operational Program "Human Resources Development", under a grant scheme from the ESF "Support for the development of doctoral and postdoctoral students, postgraduates and young scientists" – a two-year project that supported the scientific development of the target group – over 40 young scientists, postdoctoral and doctoral students.

5. Research activity

5.1. Science-metric indicators and implementation of minimum national requirements (MNR)

Assist. Prof. Dr. Dessislava Lazarova has presented for participation in the procedure (apart from the publications on the dissertation) 20 publications in journals, referenced and indexed in the world-famous databases with scientific information Web of Science and Scopus, 5 publications in non-refereed journals with scientific review and 23 participations in scientific forums.

I would like to point out the significant number of publications on her dissertation for "Doctor" degree – 4 articles in journals with impact factor and 2 articles in non-refereed journals with scientific review. The total number of points from the publications, according to the Regulations for application of the ZRASRB, is 70, which significantly exceeds the minimum national requirements – 30 points, for this degree in professional field 4.1. Physical sciences.

From the reference for the completion of the MNR under art. 2b of ZRASRB for scientific field 4. Natural sciences, mathematics and informatics, professional field 4.1. Physical Sciences, presented by Dr. Lazarova, it is evident that the candidate fully meets and significantly exceeds in some groups of indicators, the minimum national requirements for obtaining the academic degree "Associate Professor".

| Group of indicators | MNI | Candidate's reference |
|----------------------------|--|------------------------------|
| A | 50 | 50 |
| B | 100 | 100 |
| Г | 200 | 261 |
| Д | 50 | 214 |
| E | Not required for Assoc. Prof. 150 for "Professor" | 150 |

The total impact factor of the publications submitted for participation in the procedure is 34,881, the SJR index of the publications without impact factor is 0.383.

The total number of citations of the scientific works of Assist. Prof. Dr. Dessislava Lazarova is 144, of which in journals referred to in Web of Science and Scopus – 107, which is several times higher than the MNI in the group of indicators D.

Assist. Dr. Lazarova has been a participant in a significant number of research and training projects.

5.2. Contributions of the scientific works of the candidate

The scientific contributions of Assist. Prof. Dr. Dessislava Lazarova are very well described and summarized in the report presented by her. In view of the reference and scientific publications, the scientific contributions can be divided into following scientific directions:

I. Contrast-enhanced magnetic resonance techniques for visualization of pathologies related to disorders in cellular redox status – *in relation with the dissertation of the candidate.*

The relationship between endogenous oxidants and reducing agents – tissue redox status, determines the regulation of cellular signalling, maintaining cellular homeostasis within normal limits. The redox balance in cells, tissues and body fluids is closely related to the pathogenesis of many diseases: carcinogenesis, neurodegenerative and autoimmune diseases, atherosclerosis, inflammatory processes and others.

Therefore, the creation and implementation in clinical practice of new methodological approaches for assessment of cellular and tissue redox status *in vivo* is an extremely relevant area in scientific imaging. Contrast-enhanced magnetic resonance imaging methods for imaging pathologies associated with cellular redox status disorders using non-toxic cyclic nitroxide radicals can be applied both *in vitro* and *in vivo* and are characterized by high sensitivity and high resolution.

II. Modulation of redox status and increased sensitivity of tumour cells to conventional chemotherapeutics.

The main problem of conventionally applied antitumor therapy is the lack of selectivity and the induction of harmful side effects on normal (non-cancerous) cells and tissues. Therefore, efforts in this area are aimed at finding an appropriate approach to selectively reduce the viability of cancer cells. The applicant's scientific publications with N^oN^o 2, 4, 6, 8 include studies of combinations of antitumor drugs with different substances that selectively modulate the redox status of cancer cells or affect the cytostatic/cytotoxic effect of the antitumor drug. The results of these studies could be of practical importance for the development of new protocols for therapy in oncology, as their use would allow reducing the therapeutic doses of chemotherapy, respectively the side effects.

1. The increased cytotoxic effect of conventional and new generation antitumor drugs when combined with docosahexaenoic acid (DHA) was researched for the first time. A strong synergistic cytotoxic effect has been demonstrated after treatment of leukemic lymphocytes with DHA in selected combinations with chemotherapeutics, and in leukemic lymphocytes the cytotoxicity of the drug combinations is accompanied by strong induction of apoptosis and ROS production, which is not seen in non-cancer cells.

2. The effect of the combination of vitamin C and provitamin K₃ (menadion) on the redox status of leukemic and normal lymphocytes, as well as their sensitizing effect on various anticancer drugs was studied. A synergistic effect was found in all combinations of antitumor drug with vitamin C and provitamin K₃, which allows modulation of redox homeostasis with the combination of Vitamin C and provitamin K₃, as an additional component in antitumor chemotherapy, which would reduce therapeutic doses of chemotherapy, respectively of side effects.
3. The effect of menadione/ascorbate (M/A; also called Apatone®) on the viability of tumour cells with and without combination with a conventional chemotherapeutic was studied. It was found that low/moderate doses of M/A do not cause irreversible cytotoxicity in tumour cells, but cause irreversible metabolic changes. Possible explanations for the origin of the observed cytostatic/cytotoxic effect of M/A have been investigated.
4. Survival studies of tumour cells using flavonoids (quercetin hydrate and catechin hydrate) have been performed to investigate the possibility of inclusion in conventional antitumor therapy. Quercetin hydrate has been shown to have good anti-proliferative activity at low/moderate concentrations.

The reduction of the therapeutic doses of chemotherapy, respectively of the side effects, could be realized by looking for ways to facilitate the penetration of the antitumor drug into the cancer cells, thus increasing its local concentration.

In Article N^o 3 of Dr. Lazarova's list of publications, the combination of SN38 chemotherapy with electroporation (EP) on colon cancer cells was studied and it was found that the anticancer effect of SN38 combination therapy with EP was associated with changes in redox regulation, leading to higher levels of apoptosis and ferroptosis.

Article N^o 14 is devoted to the study of the influence of more than 20 antitumor drugs on changes in redox status and viability of isolated cancer cell lines, which may subsequently provide information on possible combinations of these substances with selective redox status modulators.

III. Nitroxide-enhanced EPR for visualization and assessment of cellular redox status.

The possibilities for visualization of the cell redox status with nitroxide-enhanced electronic paramagnetic resonance (EPR) were studied, using three different paramagnetic probes – cyclic nitroxide radicals – mito-TEMPO and methoxy-TEMPO and CPx. EPR experiments were performed on cell lines with different proliferative index and redox status – cancerous and non-cancerous, and the results were confirmed by conventional analytical tests. A methodology has been proposed that allows the detection of superoxide overproduction in living cells and their differentiation (cancerous from non-cancerous) based on intracellular redox status.

IV. Optical image with the help of polymersomes.

Optical imaging, based on the fluorescence of quantum dots encapsulated in polymer particles, water-soluble, capable of selectively binding to functional groups of a polymer microparticle of biological molecules (antibodies, peptides, DNA) is currently the subject of intensive research.

Articles N^oN^o 9, 10, 13 in the list of publications, submitted by the candidate, describe *in vivo* experiments using chitosan-modified quantum dot-labeled polymersomes (QD⁷⁰⁵-polymersomes) on colorectal cancer models. The registration of fluorescence in the area of the tumour and the excellent visualization of the angiogenic network of the whole body immediately after the injection of polymersomes, outline significant prospects for the application of polymersomes in the diagnosis and treatment of cancer. The high selectivity of accumulation of QD⁷⁰⁵-labeled polymersomes has been demonstrated. The application of electroporation facilitates the penetration of nanoparticles into the tumour.

V. Contrasting multimodal samples.

Article N^o 1 presents an extremely innovative approach for visualization of the redox status of cells and tissues, developing a highly sensitive molecular probe – biosensor, representing a two-component sensor system: sensor for total antioxidant (reducing) capacity: TRC–QD@CD-TEMPO and oxidative stress sensor – OxiStress–QD@CD-TEMPOH. The structure of both redox sensors is made up of small quantum dots coated with nitroxide-functionalized cyclodextrin (paramagnetic CD-TEMPO or diamagnetic CD-TEMPOH), conjugated with triphenylphosphonium groups (TPP) to achieve intracellular penetration and mitochondrial localization. Nitroxides interact simultaneously with different oxidants and reducers, and the sensors are transformed from a paramagnetic radical form (QD@CD-TEMPO) to a diamagnetic hydroxylamine form (QD@CD-TEMPOH) and vice versa, according to the redox cycle of nitroxides. These transformations are accompanied by dynamics of their contrast characteristics due to the quenching of the fluorescence of QD by the nitroxide radical. The TRC sensor was used for EPR analysis of *in vitro* cell redox status of isolated cells with different proliferative index, as well as for non-invasive MRI of redox imbalance and severe oxidative stress *in vivo* in mice with renal dysfunction.

VI. Image processing for extraction of contrast-enhanced signals from MRI.

Contrast-enhanced MRI tomography requires additional software processing of the obtained images to extract the intensity of the contrast-enhanced signal and to track its change over time after injecting. The software processing of contrast-enhanced MRI images makes it possible to minimize the artefacts. Publication N^o 7 in the list of Assist. Prof. Dr. Dessislava Lazarova deals with the description of an algorithm for extraction of contrast-enhanced signals from magnetic resonance imaging (MRI) images, using ImageJ script – an open source program, publicly available for researchers and specialists in the field of image diagnostics.

VII. Abiotic stress in plants and active forms of oxygen.

Part of the research activity of Assoc. Prof. Dessislava Lazarova includes researches, related to the inactivation of the photosynthetic apparatus of plants under abiotic stress and the role of reactive oxygen species – articles N^oN^o 11, 18, 19, 20.

The impact of prolonged exposure to high light intensities such as abiotic stress on electronic transport through FSII and photobleaching of FSI particles has been studied. The mechanisms of this impact are described as the influence of different active forms of oxygen (articles N^oN^o 18, 19).

Publication N^o 20 describes a study of the thermal sensitivity of the two photosystems to changes in the fluidity of isolated thylakoid membranes and the influence of the

thermotropic characteristics of the pigment-protein complexes by the inclusion of fluidity-reducing substances.

The changes on the structure and function of the photosynthetic apparatus when irradiated with UV-B radiation at different temperatures and the impact of different scavengers of reactive oxygen species on these changes have been studied.

VIII. Mechanisms of resistance to extreme drought of the resurrected plant *Haberlea rhodopensis*.

Interesting studies on the effect of high temperature and dehydration in the resurrection plant *Haberlea rhodopensis* on the function of photosynthetic apparatus of the plant are presented in articles №№ 15, 16, 26 and 29 of the list of publications presented by Dr. Lazarova. *In vitro* and *in vivo* experiments and analyzes were performed, on the basis of which conclusions were made, about the changes in the photosynthetic apparatus of the plant under different conditions.

In view of everything described in this section, I can say that all scientific publications of Assist. Prof. Dr. Dessislava Lazarova are in the field of biophysics and medical physics, based on current and high quality researches and analysis with serious scientific and applicable contributions.

6. Personal impressions

I have known Assistant Professor Dr. Dessislava Lazarova since she joined the Faculty of Medicine at Sofia University “St. Kliment Ohridski”. I have been impressed many times by her professional qualities and abilities. In addition to being a fully established and competent university lecturer and scientist, Dr. Lazarova has remarkable abilities to solve problems, related to the organization and administration of various activities in the faculty, for which she always contributes with a great sense of responsibility and motivation.

CONCLUSION

Based on the materials and scientific papers submitted for the competition, the above analysis of their importance and the scientific contributions contained in them, as well as my personal opinion about the candidate, as an excellent lecturer and highly erudite scientist, I confidently give my positive assessment and recommend to the Scientific Jury to prepare a report-proposal to the Faculty Council of the Faculty of Medicine at Sofia University “St. Kliment Ohridski” for awarding the academic position “Associate Professor”, in professional field 4.1. Physical Sciences (Biophysics and Medical Physics) to Assistant Professor Dr. Dessislava Anri Lazarova.

Assoc. Prof. Dr. Genoveva Antonova Zlateva

February 14, 2021

Sofia