

REFERY REPORT

by Prof. Elena Ivanova Georgieva, Member of the Scientific Jury appointed by order of the Rector of Sofia University "St. Kliment Ohridski" № ПД38-58 / 20.01.2020, on holding a competition for occupation of the academic position "Professor", professional field 4.3. Biological Sciences (Genetics - molecular genetics, bioinformatics and synthetic biology) 93 of November 26, 2019, for the needs of the Department of Genetics at the Faculty of Biology of Sofia University "St. Kliment Ohridski".

The procedure for opening and announcing the competition has been followed and the documents have been prepared in accordance with the requirements of the Law for the development of the academic staff in the Republic of Bulgaria and the Rules for its implementation.

1. General data on the career and thematic development of the applicant.

The only candidate in the competition is Assistant Professor, Dr. Robert Dimitrov Penchovski, Head of Molecular Biology Laboratory, Center for Competence "Clean Technologies and Environment", Sofia University "St. Kliment Ohridski". The candidate was born in 1971 in Sofia. After a thesis entitled: "Expression of DFRA and DJRA proto-oncogenes in tumor suppressor mutation 1 (2) gd", in 1994 he holds a Master's degree in Biochemistry and Microbiology, with a specialization in Genetics from the University of Sofia. In 1995 he completed a two-year course in Molecular and Functional Biology and after a well-defended diploma thesis on "Computer Analysis of Genetic Texts" at the Free Faculty at Sofia University "St. Kliment Ohridski" has acquired additional qualification in Applied Informatics. After completing compulsory military service, in 1996 at the Penitentiary in Pleven, Assoc. Prof. Penchovski entered the service of a researcher at IMB-BAS. Since 1997 until 1998 works as a programmer for foreign companies. One year later (1998/1999) he was appointed as a researcher at the Molecular Biotechnology Institute (now the Institute for Aging Research), Jena, Germany, and from 1999 to 2002 he was a researcher at Fraunhofer Gesellschaft, Birlinghoven Castle, St. Augustine, Germany. In the period 2000 - 2003 is a doctoral student at the Institute of Genetics at the University of Cologne, Germany, where in 2003 defended his thesis on Integrated DNA Selection in Micro-Reactors as a Method of Molecular Calculus and Diagnosis and obtained his Doctorate degree and diploma, recognized with certificate # 1847 / 21.12.2009 by the Higher Attestation Committee for the scientific specialty Genetics (code 01.06.06). After successfully defending her doctoral thesis, for a period of three years (2003-2006) Assoc. Prof. Penchovski worked as a postdoctoral fellow at Yale University, Department of Molecular, Cellular Biology and Developmental Biology, New Haven/USA, and from 2007-2010 is a scientific consultant to foreign biotechnology companies. Since 2010 is a General Assistant in Genetics at the Department of Genetics at BF, Sofia University "St. Kliment Ohridski". After successfully completing the competition in 2013, the candidate acquires the academic position of Assistant Professor in Genetics, Synthetic Biology and Molecular Evolution at the Department of Genetics, BF, Sofia University "St. Kliment Ohridski".

For over 14 years of total work experience, of which 13 years and 3 three months in the specialty, Assoc. Prof. Penchovski presents with remarkably high scientometric indicators. The scientific production of the applicant is fully consistent with the direction of the announced competition. Assoc. Prof. Robert Penchovski presents a list of 48 scientific papers,

with an impressive total impact factor (**IF**) of **126.49**, of which 46 are the lead author (first or last). His publishing activity has found international expression in over 349 citations (without self-citations; according to Scopus). According to Scopus data, **Hirsch's index** of Assoc. Prof. Dr. Penchovski is **10**. Of these 48 papers, 27 are publications in highly specialized and indexed scientific journals, 1 is a book, 7 are book chapters, 8 are publications from scientific conferences, 4 are patents, 1 is a doctoral dissertation and 27 are papers and posters presented at national and international congresses. In this competition, according to the list presented, Assoc. Prof. Robert Penchovski presents himself with **25 publications with IF 39** (according to Scimago Journal Rank (SJR); of these with quartile **Q1-7, and of Q4-3**), published after acquisition of the academic position of Associate Professor and are distributed as follows: 14 scientific articles (two accepted for printing with editorial documents), 4 chapters of books (two accepted for printing and one under printing with editorial documents), 4 publications from scientific conferences, 2 patents accepted - one published in Bulgaria (112506-17.05.2017r) and one international (WO 2018/197926). All works related to the competition have been published in refereed journals, and 10 of them are in highly prestigious publications with IF such as: Biotechnology Advances, Biosensors and Bioelectronics, Expert Opinion on Therapeutic Expert Opinion on Drug Discovery and others. Also included in the list is one book, published in full text on the applicant's website, which will not be reviewed, as it is essentially the dissertation of Prof. Dr. Penchovski for the degree of Doctor of Science and has already passed review. The four publications from scientific conferences will not be reviewed, but will be taken into account in my overall assessment.

For the period 2012-2014 in the field of synthetic biology, bioinformatics, molecular evolution, molecular genetics and the discovery of new antibiotics, Assoc. Prof. Penchovski was awarded with a prestigious award for outstanding scientific achievements by the Union of Scientists in Bulgaria.

The reference for the fulfillment of the minimum national requirements for participation in a competition for the occupation of the academic position of "professor" in the respective field of higher education shows that Assoc. Prof. Penchovski meets the criteria for all groups, namely: in group A (Dissertation thesis for ONS "Doctor" - 50t.), In group B - 100t., In group D of required 200t. - 229 t., In group D of required 200 t. an impressive 638t. and in Group E of the required 150t. - 265t.

Assoc. Prof. Dr. Penchovski is fluent in seven computer programming languages C / C ++, HTML, PHP, Perl, SQL, JavaScript, CSS.

2. Main directions of the candidate's research work and the most important scientific contributions

The review of the research activity of Assoc. Prof. Dr. Robert Penchovski shows that until his habilitation as Assistant Professor, and then he develops and refines in new, difficult, priority and strategically important fields of science and country. According to the information provided and on the basis of the publication activity, the applicant groups his / her scientific achievements in 4 interrelated research areas, which are completely in line with the announced competition, namely:

- Synthetic biology
- Bioinformatics and molecular evolution
- Molecular genetics of bacteria

- Microreactors and their application in genomics

I accept and approve this qualification for his research achievements, most of them pioneering, of original character and adding new facts to his previous research.

Synthetic biology (№3, №4, №7, №8, №10, №12, №13, №14, №19) is the most dynamically researched candidate field. Much of the research in these articles focuses on the creation of high-performance biochemical tests for the detection of new antibiotics that overcome antibacterial resistance by specifically binding to riboswitches in many human pathogenic bacteria resistant to a large number of antibiotics. A new strategy and promising approach for the design and administration of antisense oligonucleotides (ACOs) is presented, as novel antibacterial agents attached to cell-penetrating peptides that target specific bacterial mRNAs and inhibit bacterial growth of many pathogenic bacterial species. Extending her studies, Assoc. Penchovski tested the antibacterial effect of the 10 AACs against various human pathogenic bacteria by monitoring cell growth and, as a result, determined the minimum concentration of ASOs that inhibited the growth of the tested bacteria. Tests have also been conducted to evaluate the overall toxicity of ASOs using human cell lines - HEK293 (human embryonic kidney cells) and Internet-based bioinformatics software has been developed. The latter includes 15 free access programs that open a database of bacterial riboswitches such as antibacterial drug targets called RSwitch. ACOs are associated with cell-permeable oligopeptides that transport them into the cell and inhibit 100% bacterial growth, including *Staphylococcus aureus*, *Listeria monocytogenes* and *Escherichia coli*. The engineering of new synthetic antibacterial agents based on ACOI is applicable to the rapid development of new classes of antibiotics.

This section discusses various applications of nanotechnology based on nucleic acids used in biomedical and pharmaceutical research. Areas include the construction of nanostructures using DNA oligonucleotides, the self-binding of integrated RNA-based nanocarriers for molecular calculation and diagnosis, the detection of antibacterial drugs, exogenous control of gene expression and gene silencing.

An important practical achievement established by Assoc. Prof. Dr. Penchovski is that while synthetic DNAs are commonly used for the self-binding of nanostructures and devices *in vitro*, functional RNAs, such as ribozymes, are used both *in vitro* and *in vivo*. The programmed design of allosteric ribozymes, such as molecular biosensors, have found application in molecular calculations, biosensors, exogenous control of gene expression, and more. Assoc. Prof. Penchovski presents applicable computational methods for the design of allosteric ribozymes with different logical functions that determine oligonucleotides or small molecules. The advantage of these methods is that they give the desired ribozyme sequences in minutes, unlike *in vitro* selection methods that require much longer time. Methods for the synthesis and biochemical study of ribozymes are also discussed. Various applications of design ribozymes and different approaches based on RNA in medical genomics are presented. Areas under consideration include RNA-based approaches for molecular monitoring and diagnosis, detection of antibacterial drugs, exogenous control of gene expression, and gene silencing. These approaches were made possible by the refinement of various methods for engineering functional RNAs, as well as the discoveries made in the biology of RNA. Assoc. Prof. Penchovski discusses the new revolutionary methods in synthetic biology that allow the engineering of genetic control networks, genome editing, and whole genome creation, thus creating new species with pre-refined combinations of properties other than those observed in

nature (No. 19). The edited genomic technologies that have been developed over the last few years based on the CRISPR-Cas system have also been discussed. RNA-based methods for the design of genetic control in prokaryotes and eukaryotes, including humans, are also presented.

Of particular interest are the data described in Article No. 7 showing that the NC gene family identified in the genome of *Arabidopsis thaliana*, through homology to human lecithin-retinal acyl transferase (LRAT) and picornavirus 2A protein, is a possible substrate for the development of novel cancer drugs. *Arabidopsis* proteins contain two motifs, H-Box and NC, identified in a huge variety of organisms. Human homologues include HRAS-like tumor suppressors induced by Tazarotene gene 3 (TIG3) and desumoylating isopeptidase (PNAS-4), which induces apoptosis in lung cancer cells. Preserving the two observed motifs in *Arabidopsis* proteins in homology with tumor suppressors and retaining residues important for LRAT function among *Arabidopsis* homologs may unveil a new group for the design of drug-targeted herbal tumors.

The results of the research of Assoc. Prof. Robert Penchovski are promising and of practical importance for the diagnosis and treatment of serious diseases. This information is presented in depth in a chapter in book (# 8) describing clinical testing of functional nucleic acids: antisense oligonucleotides and aptamers, and discusses the current state and prospective uses for AOs and aptamers as drugs. The information provided and discussed thoroughly demonstrates how functional nucleic acids such as aptamers, antisense oligonucleotides, small interfering (si) RNAs and ribozymes are considered by some researchers as an opportunity for the development of therapeutic agents.

Particularly important are the studies of Assoc. Prof. Penchovski, who aim to identify compounds derived from genetically modified plant cells and their potential role in drug development (No. 10). They make it possible to trace the evolution of plant biotechnology as a relatively inexpensive source for the creation of recombinant pharmaceuticals used for the treatment of various diseases tested for the treatment of several types of cancer, diseases of the central nervous system, such as enhancers during chemotherapy, etc. Plant-based miRNAs have been shown to have the ability to inhibit mammalian tumors. A method for integrated DNA selection in microflow reactors has been applied as an approach for molecular calculation and diagnosis.

In the next section, Bioinformatics and Molecular Evolution (No. 1, No. 5 and No. 9), Assoc. Prof. Penchovski presented a comprehensive bioinformatic analysis of eight riboswitches as antibacterial drug targets throughout the genome in various pathogenic bacteria. Data are shown on which of the 8 riboswitches are suitable for antibiotic targets. Their sequences were discovered and investigated. The presence of active transport proteins, the expression of which is controlled by some of the eight fish switches, was examined. Based on in silico analyzes, riboswitches are classified into four groups based on their suitability for use as antibacterial medicinal purposes. FMN, SAM-I, glmS, TPP and lysine riboswitches have been found to be promising targets for the detection of antibacterial drugs. These data are an opportunity for future studies to focus on the detection of antibacterial drugs on fish switches, whose inhibition will inhibit the growth of certain pathogenic bacteria. A new database of fish switch bioinformatics (RSwitch) has been created that also provides various useful tools for DNA, RNA and protein sequence analysis using a user-friendly interface. Currently, RSwitch contains information and explanations for 215 bacterial

riboswitches of 16 different types found in 50 human pathogens. Classes of riboswitches include FMN, glmS, cobalamin, lysine, SAH, SAM, purine, TPP, c-di-GMP, c-di-GMP II, Moco, PreQ1, fluoride, glycine, Mg²⁺ and Mn²⁺ type bacterial fish switches. This bioinformatics database provides all the information necessary to evaluate the suitability of presented riboswitches as antibacterial drug targets.

A manifestation of Prof. Penchovski's high-level computing skills are his introduction to the new Essential Bioinformatic Services (EBWS) server, nine web-based applets that provide useful tools for analyzing DNA, RNA and protein sequences. This user-friendly computer interface provides the user with a quick and inexpensive approach to create allosteric ribozymes responsive to specific oligonucleotides and synthetic elements to control gene expression in cells. All unrestricted programs are constantly available online at <http://penchovsky.atwebpages.com/>.

Molecular Genetics of Bacteria is the next well-grounded scientific field in the research of Assoc. Prof. Dr. Penchovski (**№6, №11, №16, №17, №18 and invention supported with a patent #112506-17.05.2017**). The candidate discusses the overall distribution, structure, and function of 28 different classes of fish switches, focusing his attention on riboswitches in bacteria that regulate the expression of a limited number of genes, most of which are responsible for the synthesis of major metabolites without which the cell cannot function. Therefore, the distribution of riboswitches is also important for the development of antibacterial drugs. In a review article, Dr. Penchovski focuses his attention on RNA as an effective target for the detection of antibacterial drugs. RNA is the most ambiguous biopolymer in a cell that has many different functions. For example, mRNA, rRNA and tRNA are essential for gene expression in both pro- and eukaryotes. However, all these types of RNAs have sequences and 3D structures that can be used to stop major biochemical processes in bacteria only. All these characteristics make RNA a very powerful target for the development of antibacterial drugs. Key mechanisms of antibacterial drug action, the development and spread of antibacterial resistance, and the most urgent multidrug resistant bacterial strains are also discussed. Particularly successful is the generalized discussion of all important strategies for the prevention and containment of antimicrobial resistance, which is a growing problem for the world health system, as well as the risk factors and mechanisms for the development and spread of antimicrobial resistance. A thorough analysis has been made on the various strategies for preventing the emergence of antimicrobial resistance by reducing the selective pressure of pathogenic bacteria. Various strategies for containment of widespread antibiotic-resistant bacteria are presented, including internationally recognized guidelines for antibiotic administration. The broad scientific field of prevention presented to limit antimicrobial resistance would be of interest to both scientists and physicians working in or interested in these fields.

Another group of studies is devoted to "Microreactors and their application in genomics" (# 2, # 8). Automated transfer of DNA hybridization with mobile superparamagnetic microbeads in a microflow reactor has been established. The presented microfluidic platform is marketable and applicable in many areas of modern biotechnology, such as microarrays for DNA hybridization, molecular calculation, selection on functional nucleic acid chips, screening of chemical libraries for drug discovery and DNA amplification and sequencing. Clinical tests of functional nucleic acids have been described: antisense oligonucleotides and aptamers, and functional nucleic acids such as aptamers, antisense

oligonucleotides (ACOs), small interfering (si) RNAs and ribozymes have been considered by some researchers as valuable tools treatment.

3. Training and teaching activities

Prof. Dr. Penchovski's high qualification, extensive experience and excellent training is evident from his serious responsibilities as a scientist and teacher. Since 2012 he is the leader, author and holder of Bachelor's and Master's courses in Bioinformatics and Molecular Evolution, Synthetic Biology, Genomics, Genetics and Genomics, Molecular Genetics and Biology, Bioinformatics and Agrobiotechnology in Bulgarian and English, for Specialists , full-time and part-time training. For the last three years he has been the Head of the Molecular Biology Laboratory, the Center of Competence "Clean Technologies and the Environment", Sofia University "St. Kliment Ohridski "and Head of Postgraduate Qualification (DSS) in“ Contemporary Aspects of Biology: Bioinformatics, Genomics and Synthetic Biology ”for specialists and non-specialists in Bulgarian and English. Under his leadership, he successfully defended 2 PhD students, 2 PhD students were dismissed with the right of defense and is currently the head of 3 full-time PhD students. He is the head of 4 protected undergraduate and graduate students and 16 masters from 5 different nationalities (Bulgaria, Iraq, Macedonia and Latvia) at the Ministry of Genetics and Genomics and Genetic and Cell Engineering. The administrative record shows that Assoc. Prof. Penchovski also conducts intensive educational and pedagogical activities in practical and seminar classes with undergraduate and master's students. Over the years, the average annual workload of Assoc. Prof. Penchovski at the Sofia University as a lecturer is high and varied - over 500 hours of annual study and classroom employment. These data define Assoc. Prof. Penchovski as a well-established lecturer, well-established and internationally recognized scholar.

4. Participation in scientific projects and other activities of the applicant

The successful research work of Assoc. Prof. Robert Penchovski is evident from the impressive number of national and international collaborative projects, a total of 14, of which 8 is a leader and 6 is a member of the team. It is an indicator of the high scientific capacity, abilities and skills of Assoc. Prof. Dr. Penchovski to lead and work with groups of national and international researchers and specialists. He is a member of 4 prestigious scientific organizations: Union of Scientists in Bulgaria, Yale Alumni Association - Yale University, American Chemical Society, Cologne Alumni - Cologne University. He is a reviewer of 21 project proposals from the NSF at the Ministry of Education and Science; 15 scientific publications submitted for publication in scientific journals such as Biomacromolecules, ACS Synthetic Biology, Lab on a Chip, EC Microbiology; has participated in the jury in 2 competitions for assistant professor and 2 for associate professor; Member of the editorial board of the journal EC Microbiology.

5. Conclusion

The works of Assoc. Prof. Dr. Robert Penchovski extend to basic and applied research in bioinformatics and molecular evolution, synthetic biology and molecular genetics. They are pioneering, innovative and can undoubtedly be referred to the most sophisticated and importantly up-to-date perspectives on life, offering another approach for the evaluation and creation of medicines, potential options for the treatment of malignancies, the creation of new strategies for overcoming and retaining antimicrobial resistance to antibiotics, etc. The problems of his research fall completely within the scope of the announced competition. As a

scientist and university professor Assoc. Prof. Dr. Robert Penchovski is the initiator and one of the first researchers in Bulgaria in the field of synthetic biology and bioinformatics in scientific research and in student education. Proof of high quality, great importance and indisputable successes of his scientific achievements are winning numerous highly competitive and successfully developed projects, his publications in journals with prestigious impact index, high citation, intensive pedagogical activity, participation in scientific organizations, leadership of successful protected graduates and doctoral students, etc. Proficiency in a large number of computer languages demonstrates in-depth scientific knowledge allowing the applicant to publish original scientific results. For the first time, he has created algorithms for the computer design of allosteric ribozymes that are backed by accepted patents. Assoc. Prof. Penchovski manages to create and implement web based bioinformatics software including 15 free access programs. Applicant's excellence in science fills a gap in synthetic biology and bioinformatics. They are highly appreciated by publications dedicated to his scholarly articles and original contributions identified by well-known scholars as “ground-breaking”, ie. significant and posing new, positive ideas to science and society. All these activities make Dr. Penchovsky an established and promising scholar at national and international levels and deserve the highest praise.

The high scientometric indicators of Assoc. Prof. Robert Dimitrov Penchovski completely satisfy and even exceed the recommended criteria of the Law on Academic Development in the Republic of Bulgaria, the Regulations for its implementation and the internal Regulations of the Faculty of Biology of Sofia University “St. Kliment Ohridski ”for the academic position of Professor. From the overall analysis I make, I am convinced to give a high positive assessment and convinced to recommend to the Honorable Scientific Jury and the Scientific Council of BF of Sofia University to award Professor Robert Dimitrov Penchovsky the academic position "Professor".

Sofia, March 27, 2020

Reviewer:

/Prof. Elena Georgieva, Ph.D./