REVIEW

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of the materials submitted for participation in the competition for the occupation of the academic position “Professor” in the field of higher education 4. Natural sciences, mathematics and informatics; professional field 4.3. Biological Sciences; Genetics (molecular genetics, bioinformatics and synthetic biology)

Common part

The competition for "Professor" is in the field of higher education 4. Natural sciences, mathematics and informatics, professional field 4.3. Genetics (Molecular Genetics, Bioinformatics and Synthetic Biology) and He was announced for the needs of the Department of Genetics, BF of Sofia University "St. Cl. Ohridski ”in the State Gazette No. 93 / 26.11.2019 The only candidate is Assoc. Prof. Dr. Robert Dimitrov Penchovsky from the same department. Examination of the documents shows that the procedure for opening and announcing the competition has been followed. The presented set of materials in paper and electronic form have been prepared in accordance with the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Rules for its implementation and the internal regulations of the Sofia University “St. Kl. Ohridski ”, and they meet the criteria for acquiring the academic position of“ Professor ”.

Short biographical data

Dr. Robert Dimitrov Penchovky graduated with a degree in Biochemistry and Microbiology (specialization Genetics) from the Faculty of Science at Sofia University “St. Kliment Ohridski ”in 1995. He also completed a two-year advanced qualification course in applied informatics at the Free Faculty, Sofia University. In 1996 he joined the Institute of Molecular Biology at the Bulgarian Academy of Sciences. From 1998-1999 he worked at the Institute of Molecular Biotechnology in Jena, Federal Republic of Germany, and from 1999 to 2002 he worked as a researcher at Fraunhofer Gesellschaft, St. Augustine, Federal Republic of Germany. In 2003 he received his Doctorate degree from the University of Cologne, Federal Republic of Germany, recognized by the Higher Education Commission. From 2003 to 2006, he worked at the Department of Molecular, Cellular Biology and Developmental Biology, Yale University, New Haven, USA (Postdoctoral Fellow), and from 2007 to 2010 was a scientific consultant to foreign biotechnology companies. From 2010 to 2013 is Ch. Assistant Professor in the Department of Genetics, BF, Sofia University, and in 2013 he was elected Assoc. Professor in Genetics, Synthetic Biology and Molecular Evolution. From 2018 he is the Head of the Molecular Biology Laboratory „Clean Technologies and Environment“ at the Center of Competence at Sofia University.

General presentation of scientific works

Assoc. Prof. Penchowsky has published a total of 48 scientific papers in the field of competition. In 46 of them, he is the lead author. 27 of the publications are in prestigious journals with IF and have a total IF of 126.49. Scopus data shows that his works have been cited 349 times, with an h-index of 10. He has participated in 27 national and international scientific forums.

In this competition, apart from his dissertation and associate professor's competition, Dr. Penchowsky has presented 25 scientific papers (14 publications in peer-reviewed journals, 1 book, 4 book chapters in prestigious publishers such as Springer and Elsevier, 4 publications in scientific forums and 2 patents - 1 Bulgarian and 1 international). In all publications he is a leading author. The abstract list of publications (I. Publications in Scientific Journals - 3 and IV. Conference Publications - 20,21, 22 and 23) contains abstracts or posters from scientific forums and cannot be counted as scientific publications. The book presented under No. 15 is the doctoral dissertation of Assoc. Prof. Penchovski and is not subject to review, as it has already been peer-reviewed, but has been recognized by the NACID as legitimate according to the attached correspondence and will be reflected in the scoring system. The entries submitted for the competition have a total of IF 39 and have been cited 319 times according to Scopus data. Assoc. Prof. Penchowski participated in 17 scientific forums after occupying the academic position of Assistant Professor

Of the publications presented, 4 (numb. 1, 4, 6, 9) are distinguished as habilitation work. According to the quarters in which the Journal of Citation Reports (JCR) of Web of Science groups Impact Factors (IF) scientific journals all four have Q1, with which Assoc. Prof. Penchovsky covers the required 100 points according to indicator C of the Rules for Implementation of the LDASRB. Group D indicators are as follows: 3 publications with Q1 = 75 points .; 2 publications with Q4 = 24 pts.; published book based on dissertation = 20 pts.; 4 book chapters = 60 pts and 2 patents = 50 pts, collecting a total of 229 pts (minimum 200 pts required). According to the quotation indicator, there are 638 pojnts (minimum 100 points required).

Scientific contributions

The main contributions of Assoc. Prof. Penchovsky's research are the fields of synthetic biology, bioinformatics and molecular evolution and molecular genetics of bacteria..

Synthetic biology

In the last decade, antibacterial drug resistance has been a major challenge in modern medicine due to the increase in bacterial pathogenic strains that are resistant to many antibiotics. Assoc. Prof. Penchovsky has focused on creating high-performance biochemical tests to detect new antibiotics that bind specifically to fish switches in many human pathogenic bacteria. Assoc. Prof. Penchovsky presents a new strategy for the design and application of antisense oligonucleotides (ASOs) as novel antibacterial agents that target specific bacterial mRNAs responsible for the function of various biosynthetic pathways that synthesize essential metabolites in bacteria. Growth

inhibition in a number of pathogenic bacteria, including *Staphylococcus aureus*, *Listeria monocytogenes* and *Escherichia col*i, has been demonstrated. (3)

6 web based platforms have been created including DNA / RNA translation, AminoCODE transformer, virtual PCR analyzer, generating fragments with or without DNA protruding ends, hydrophobicity of protein sequences, reverse protein translator, and eukaryotic open reading frame finder.(4). While synthetic DNAs are commonly used to self-associate nanostructures and devices in vitro, functional RNAs, such as ribozymes, are used both *in vitro* and *in vivo.* Allosteric ribozymes have applications in molecular calculations, biosensors, high-throughput screening arrays, exogenous control of gene expression, and more. They switch on and off their catalytic function as a result of a conformational change induced by ligand binding. Computational methods are proposed for the design of allosteric ribozymes to respond to different effectors through in vitro selection. These methods give the desired ribozyme sequences in minutes, unlike in vitro selection methods that require weeks. (13)

Various applications of design ribozymes and RNA based approaches for molecular monitoring and diagnosis, detection of antibacterial drugs, exogenous control of gene expression and gene silencing have been discussed (14). Specific features have been considered in clinical trials of antisense oligonucleotides, aptamers, small interfering si-RNAs, and ribozymes. The current state of play has been discussed and perspective options for using antisense oligonucleotides and aptamers as drugs are listed (8). Specific RNA molecules with antibacterial activity, mechanisms of antibiotic resistance, and mechanisms for its prevention have also been considered (11, 12, 18).

Nanobiotechnology and synthetic biology can integrate the necessary advances and technologies to create organisms with new desired properties. Assoc. Prof. Panchovsky presents new revolutionary methods in synthetic biology for the creation of new genomes, discussing the technical aspects of their creation and their present limitations. Genome editing technologies based on the CRISPR-Cas system are discussed, as well as the use of large RNA-based methods for designing genetic control networks, both in prokaryotes and eukaryotes, including humans (19)

An interesting approach is the identification of NC genes in the genome of *Arabidopsis thalian*a (Arabidopsis) by homology to human Lecithin-retinal acyl transferase (LRAT) and picornavirus 2A protein. Arabidopsis proteins contain two motifs (H-Box and NC), identified in a huge variety of organisms. Related proteins include *C. elegans* EGL-26, a regulatory protein of cellular morphogenesis in the vulva, and human proteins that may be associated with cell proliferation or development. Human homologs include HRAS-like tumor suppressors induced by Tazarotene gene 3 (TIG3) and 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 not only be indicative of the importance of these domains for plant protein function, but may also to open a new group for the design and development of tumors targeted drugs. (7)

Bioinformatics and molecular evolution

Prof. Penchovsky's main focus is the design, synthesis and biochemical testing of allosteric ribozymes that are activated or deactivated by specific binding of certain DNA, RNA oligomers or small molecules.

In silico analyzes riboswitches are presented, which may be suitable as antibacterial drug targets. A complete and comprehensive genome-wide bioinformatic analysis is presented for the use of eight riboswithes as antibacterial drug targets in various pathogenic bacteria. Based on *in silico* analyzes, riboswitches are classified into four different groups based on their ability to be used as antibacterial drug targets. FMN, SAM-I, glmS, TPP, and lysine fish switches have been shown to be promising sites for the detection of antibacterial drugs, as their inhibition leads to inhibition of the growth of certain pathogenic bacteria (5). These achievements are recognized by one international patent (25) and a decision granting a Bulgarian patent (24).

Basic Bioinformatics Web Services (EBWS) has been implemented on a new PHP-based server that provides useful tools for analyzing DNA, RNA and protein sequences by implementing a user-friendly interface. There are currently nine web based applets available on the web server. These include reverse complementary DNA and arbitrary DNA / RNA / peptide oligomer generators, a motif sequence finder, DNA restriction cut, a prokaryotic ORF (open reading frame) search engine, and any DNA / RNA mutation generator. It also includes a DNA / DNA melting point (Tm) calculator, RNA / RNA and DNA / RNA hybrids, a lead RNA (gRNA) generator for the CRISPR / Cas9 system, and a binding temperature calculator for multiplex PCR. The model search applet has no limit on the number of motive inputs and implements a box of Regex tools that can be used to define complex RNA, DNA, and protein sequences. The DNA Enzyme Assimilation Program uses a large database of 1502 restriction enzymes. The lead RNA generator has a database of 25 bacterial genomes that can be searched for gRNA target sequences, and there is an option to search in any genome sequence specified by the user. All programs are permanently available online at http://penchovsky.atwebpages.com/ without restrictions. (9)

Assoc. Prof. Penchovsky is the author of methodological developments concerning the design and creation of microreactors that work as DNA-based computers. It demonstrates the automated transfer of DNA hybridization into a microflow reactor by moving paramagnetic beads between two spatially separated solutions with different pH values. This development may find application in the production of DNA microarrays, functional screening of nucleic acids, new methods for DNA sequencing, molecular diagnostics. (2).

Molecular evolution and molecular genetics of bacteria.

Part of the work of Assoc. Prof. Penchovsky is focused on the study of riboswitches that are promising for the development of new antibacterial drugs. The aim is to facilitate the rapid detection and development of antibacterial drugs based on compounds that attack bacterial riboswitches using modern techniques such as high-throughput screening. Therefore, Dr. Penchovskyi, PhD, deals with the overall distribution, structure and function of 28 different classes of riboswitches that regulate gene expression through four different mechanisms. In addition, it

tests their role through a new approach in molecular genetics, namely antisense technology. It involves the use of designer antisense oligonucleotides, which he develops and subsequently tests for bacterial cells that are pathogenic to the human body. The invention 112506-17.05.2017 published on 02.01 / 15.02.2019 and approved on 22.01.2020 (24) is directed to the development of antibacterial agents through the use of chimeric antisense oligonucleotides, together with peptides penetrating the cell that are bind to specific bacterial RNAs and inhibit their functions.

The development of new antibiotics is extremely important due to the growing number of resistant pathogenic bacteria. This can be achieved by using new targets to detect antibacterial drugs. In a review article (11), several different types of RNA molecules used as antibacterial medicinal purposes are discussed. RNA is the most ambiguous biopolymer in a cell that has many different functions. For example, tRNA, rRNA and mRNA are essential for gene expression in both pro- and eukaryotes. However, all these types of RNAs have sequences and 3D structures that are bacterial-specific and 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 measures to treat bacterial strains with multiple resistance have been discussed (16, 17, 18).

As an indicator of the scientific value of the activity of Assoc. Prof. Penchovski, besides the high IF of the published articles and the citation of his works, he received the prize from the Bulgarian National Competition for Excellence for Scientists in all fields over 35 years, organized by the Union of scientists in Bulgaria from 2012 to 2014 for his work in the field of synthetic biology, bioinformatics, molecular evolution, molecular genetics and the discovery of new antibiotics.

Project activity

According to the completed report on the implementation of minimum national requirements, Assoc. Prof. Penchovski is a participant in the development of 4 scientific projects funded by the Ministry of Education and Science and is the leader of 3 projects funded by the Ministry of Education and Science. The total value of the attracted funds from the projects under his leadership amounts to BGN 326,000 BGL which according to indicator E brings him a total of 165.2 points.

Educational activity

Assoc. Prof. Penchovsky is the holder and author of programs of a number of lecture courses in Bachelor and Master programs for full-time and part-time students at the Sofia University: Bioinformatics and Molecular Evolution - Compulsory Courses for Genetics and Genomics and Genetic and Cellular Engineering; Synthetic Biology - compulsory Masters Degree in Genetics and Cell Engineering and freely elective in Genetics and Genomics; Genomics - Compulsory Masters Degree in Genetics and Cell Engineering, Genetics and Genomics; Molecular Genetics - compulsory course for undergraduate students in the field of Molecular Biology. Bioinformatics - compulsory course for undergraduate students in the fourth course in the field of

Agrobiotechnology. He was the head of 16 successfully defended Masters in Genetics and Genomics and Genetic and Cell Engineering and 4 students at the Modern Science in Biology: Bioinformatics, Genomics and Synthetic Biology. Under his supervision, two doctoral theses were defended, two doctoral students were awarded with the right of defense and three are current ones, which according to indicator E brings him 100 points.

Expertise

Assoc. Prof. Penchovsky is a member of the editorial board of the international scientific journal EC Microbiology. He was a reviewer of scientific articles and project proposals. He has been a member of the Scientific Jury for Assistant Professor and Associate Professor.

Conclusion:

The documents and materials presented by Dr. Penchovsky meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for its implementation and the internal regulations of the Sofia University "St. Kl. Ohridski ", and go beyond the criteria for acquiring the academic position of" Professor ". The presented materials and personal impressions give me reason to express my positive opinion regarding the application of Assoc. Prof. Dr. Robert Dimitrov Penchovki for the academic position of "Professor". He is an established specialist and lecturer, with authority in the scientific community and in the student community. As a member of the Scientific Jury of the announced competition, I give a positive assessment and recommend that the members of the Honorable Faculty Council of the Faculty of Biology vote positively for the election of Assoc. Prof. Dr. Robert Dimitrov Penchovky in the academic position "Professor" in the professional field 4.3. Biological sciences, specialty Genetics (molecular genetics, bioinformatics and synthetic biology).

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