REFEREE REPORT

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Evaluation of candidates for the position of "Professor" in the field of higher education: 4. Natural sciences, mathematics and informatics; professional field 4.5. Mathematics; scientific specialty "Mathematical Modeling and Application of Mathematics (Applications in Computational Physics and Biology)" at the Institute of Information and Communication Technologies (IICT), BAS

1. General comments

The Full Professor position in the field of higher education 4. Natural sciences, mathematics and informatics; professional field 4.5. Mathematics; scientific specialty "Mathematical Modeling and Application of Mathematics (Applications in Computational Physics and Biology)" at the IICT was announced in State Gazette, issue. 41/21. 05. 2019. The only candidate is Assoc. Prof. Dr. Nevena Petrova Ilieva-Litova from the same Institute. The overview of the presented documents shows that the procedure for opening and announcing of the position has been complied with the requirements of the Law for Development of the Academic Staff of the Republic of Bulgaria (LDASRB) and the Rules for its implementation as well as with the internal regulations of the Bulgarian Academy of Sciences (BAS).

2. Biography

Assoc. Prof. Dr. Nevena Ilieva-Litova was born on December 27, 1957. She is a graduate of the Department of Physics, Sofia University "St. Kl. Ohridski ", graduated with a master's degree in Physics (Atomic Physics). After graduation, she joined the Joint Institute for Nuclear Research in Dubna, Russia (1982), where, until 1990, she held the positions of Associate Fellow, Researcher and Senior Fellow and defended her PhD dissertation (1988) in the field of Theoretical and Mathematical Physics. From 1988 to 2015 she was an employee of the Institute for Nuclear Research and Nuclear Energy, BAS, where she was promoted Associate professor in 2003. During 1992-1993, 1994-1995, 1996-2001 she worked at the Institute of Theoretical Physics at the Vienna University, and in 2001-2003 at the Erwin Schrodinger Institute for Mathematical Physics, Vienna. In 2003-2007 she worked at CERN, and from April 2015 moved to the IICT at BAS. She has 29 years of service, of which 18 years as a senior researcher.

3. Research activity and scientific achievements

3.1. Overview of the candidate's scientific publications

According to the presented list of papers, Assoc. Prof. Ilieva-Litova has 104 scientific papers, of which 39 are published in IF journals, 12 are articles in SJR journals, 9 in other indexed journals, 14 are published in various books and periodic, 16 - in international editions, 3 are manuals and textbooks and 11 refers to as "indexed summaries". To my understanding, the summaries, even if published in indexed scientific journals, are not equivalent to full-text publications. That is why I will exclude them from reviewing. The total IF of the scientific production of Assoc. Prof. Ilieva is 55.277. Her papers are cited so far 170 times and her h-index is higher than 7.

Dr. Ilieva applies for Professor with 23 scientific papers published in the period 2009-2019, of which 15 are in IF journals (total IF 37.878). They are divided into four quadriles as follows: in quadriles Q1, Q2 and Q3 - 5 papers each and in Q4 – 2 papers. According to the new regulation for the implementation of the LDASRB, these articles carry 748 merit points. In 9 articles she is

analogue, which have a similar primary structure but different biological activity. She demonstrates the great importance of the side chains at positions 5 and 16 in maintaining their conformation.

Part of Dr. Ilieva's research is related to the development of criteria and methods for assessing the quality and reliability of the results generated by MD (P4, P7 and P13). In this regard, the so-called lagged RMSD analysis method has been developed to estimate the required durations of MD simulations. The method is based on RMSD values for configuration pairs separated by variable time intervals. Another useful method is the SMCC (spatio-temporal multistage consensus clustering) method designed to minimize the dependence of the results on the reference structure. It is based on minimizing the variation of the distances between Cα atom pairs as a target function. The model allows reliable identification of the basic structural subunits of biomolecular complexes and differentiation of the conformational influence of point mutations.

N. Ilieva has also developed a three-dimensional visualization method, using the intrinsic protein geometry defined by the peptide planes and the side chains (P20, P22) rather than the extrinsic one. The method is based on the construction of appropriate coordinate frames for determining atoms' positions with a subsequent projection on a unit sphere. It was probated on the human myoglobin as a model, where all histidyl structures were analyzed at a ultra-high resolution level in search of evidence for a possible pH dependence of the ligand-gate mechanism.

b) In silico studies of immunoreactive molecules and complexes (P2, P3, P8, P9, P11, P14, P16, P18, P21 and P23).

Research in this new to Dr. Ilieva field, has been conducted over the last decade in collaboration with research teams from the Institute of Molecular Biology "Acad. Rumen Tsanev", BAS and the Center for Medical Statistics, Informatics and Intelligent Systems at the Medical University of Vienna. Together with the first team she examines the spatial structure of human gamma interferon (hIFNγ) and its interaction with its receptor, and with the second team she has studied the major histocompatibility complex (MHC) known to man as HLA.

hIFNγ is a key cytokine secreted by proinflammatory T cells that plays an important role in triggering and modulating of the human immune response. In addition, it has a strong antiviral activity and due to this it is a part of the natural antiviral protection system. Research over the past decades has also revealed the relationship of hIFNγ to over 80 autoimmune diseases, which has additionally heightened researchers' interest in it.

hIFNγ is composed of 143 amino acids and naturally forms a stable homodimer. hIFNγ exerts its biological action by binding to a specific receptor, thus forming a hIFNγ-receptor complex. Because the tertiary structure of hIFNγ is determined (by X-ray diffraction) with low resolution, we have a very poor knowledge on the hIFNγ-receptor interaction. The latter justifies its computer modeling which is important for both basic science and drug development.

It is well known that the X-ray diffraction analysis does not provide any information on unstructured domains in the protein molecule. Due to this the organization of nearly 20% of the hIFNγ amino acids is unclear. These are the ones located in the unstructured C-terminus. By applying MD simulations and other approaches (see above), Dr. Ilieva manages to model the C-terminal part of hIFNγ and demonstrated its role in receptor recognition and establishment of the first contacts between the two molecules. She also revealed the role of heparan sulfates (sulphated

leading (first / last) author. In my review I will refer to the papers with their original numbering according to the attached list.

The list of Dr. Ilieva's activities does not provide information about her participation in scientific fora, however, judging by her self-evaluation report she has been repeatedly invited as a lecturer at many international scientific events held in at least 15 different countries.

3.2. Evaluation of the candidate's scientific achievements

The papers of Assoc. Prof. Ilieva-Litova can be divided into four thematic groups: *a) Methods for modeling, examining and visualization of the structure and dynamics of proteins* (P4, P7, P10, P12, P13, P15, P19, P20, P22); *b) In silico studies of immunoreactive molecules and complexes* (P2, P3, P8, P9, P11, P14, P16, P18, P21, P23); *c) Modeling of physical processes* (P1, P5); *d) Tools and techniques for high performance calculations* (P6, P17).

My scientific background and qualifications allow me to evaluate the scientific achievements and contributions of the works belonging to the first two groups.

a) Methods for modeling, examining and visualization of the structure and dynamics of proteins (P4, P7, P10, P12, P13, P15, P19, P20 and P22).

By protein structure here we should mean secondary and tertiary structures, which are undoubtedly derivatives of the primary protein structure. It should be noted that the advent of computer technology combined with biomath has made a real revolution in molecular biology. The development of rapid and high-throughput genomic technologies over the past decades has made it easy to determine the primary structure of a gene and therefore of the protein encoded by it, but not the higher structures of the protein determining its biological functions.

Biomathematics and application of computer technologies in biology and medicine is a relatively new field for Bulgaria and Dr. Ilieva is one of the pioneers in this field. Thanks to her experience and knowledge in topological field theory and topological properties of gauge theories she has been involved in studying the structure and organization of a number of human proteins. She combined successfully the mean-field approach with molecular dynamics (MD) in a multi-step algorithm to model at atomic-level precision the folding of complex polypeptide chains over long time intervals. To this end she developed a number of topological techniques based on the concept of integrable models and the nonlinear Schrödinger equation.

In her studies Dr. Ilieva choosed to use proteins not only with important biological functions but also with potential application in medicine and pharmacy such as the gp41 glycoprotein (component of the HIV virus envelope) and the Myc oncoprotein (P10, P12, P15, P19). For their modeling, she uses three force fields, one of which has united atoms and the other two are all-atom. She proved that the formation of alpha-helices resembled the formation of a topological Bloch domain wall along a virtual spin chain, and the individual domain walls can be modeled with an accuracy of several angstroms to tens of angstroms. Her studies show that at room temperature, the monomeric Myc oscillates between different conformations. By varying the temperature and applying Glauber dynamics to determine the behavior of the multisoliton describing the Cα protein profile, she managed to identify a stable subset in which the two helical segments of the original multisolitons are oriented parallel to each other.

Using *in silico* protocols, MD simulations and well-tempered metadynamics, Dr. Ilieva examines the conformational dynamics in solution of the antimicrobial peptide MG2 and its MG2m

sugars on the cell surface) and of the hIFNγ glycosidic residues in stabilizing the ligand-receptor complex.

Dr. Ilieva developed a metadynamics approach (molecular dynamics, complemented by metadynamics investigation in the space of selected torsion angles along the protein backbone) to determine the free energy in small sections of the protein molecule that has been successfully applied to characterize more than 100 experimentally derived hIFNγ mutants, intended to be tested as agents for treatment of autoimmune diseases (P2, P3, P14, P16, P18, and P21).

MD simulations were also used to study three different MHC complexes. They present on the cell surface fragments of foreign proteins (epitopes) in the form of an MHC complex. They are recognized by T cells, forming a TCR-pMHC complex that elicits an immune response. Dr. Ilieva has investigate by MD simulations the dynamics of the alpha helices forming the MHC binding pocket. Judging by the geometrical characteristics of the spirals (the area of the surface on the axes of the two helices and the distance between them), she found that the geometrical characteristics of the inactive complex remain stable whereas those of the two aloreactive complexes change on varying the interpolating function. The mutual orientation and movements of key regions in bound and free TCR and pMHC proteins were also investigated. It was found that under coupled conditions the motions between α1- and α2-helices in the MHC and the variable regions of the T cell receptor (TCR Vα and TCR Vβ) are limited. Increased conformational flexibility of the α2-helix and CDR loops in the T-cell receptor has been observed in unbound compared to bound states. The MD method has also been applied for studying of large complex protein structures such as that of the MHC class I, epitope, T-cell receptor and CD8 coreceptor complex. It contains more than 300,000 atoms. To characterize the relative orientation and motions of its domains Dr. Ilieva has generated a local coordinate systems based on its major components. By determining the directional cosines and Euler angles, she has found that the co-receptor influences the dynamics of the whole protein complex as well as the relative motions of the helices Gα1 and Gα2. The results of these studies are presented in publications P8, P9, P11 and P23.

The list of Dr. Ilieva's papers contains a few publications dedicated to the modeling of physical processes with application in PET and MRI imaging (P1 and P5), as well as to new tools for high performance calculations (P6 and P17). As they are far from my research field I'll skip their reviewing, but I'll take them into account when making my overall assessment.

4. Teaching

Assoc. Prof. N. Ilieva was giving lectures for PhD students at the Institute of experimental morphology and pathology, BAS. She has also supervised two PhD students from the Beijing Institute of Technology.

5. Research projects

Dr. Ilieva has been a project leader of two bilateral cooperation projects with Austria and the People's Republic of China and participant in a bilateral project with Russia. She was deputy head of the Bulgarian team in three international projects under the Horizon 2020 European programs – PRACE 4IP, 5IP and 6IP and is head of the Bulgarian team in one project under the COST program. She has participated in three national scientific projects funded by the NSF at the Ministry of Education and Science, and is currently the leader of one such project, as well as of a work package in the National Research Program "BioActiveMed" funded by the Ministry of Education and Science. For many years she maintains stable collaboration with scientists from

Austria, England, Belarus, Bulgaria, Vietnam, Georgia, Italy, China, Poland, Russia, Slovakia, Slovenia, Uzbekistan, Finland, Czech Republic, Sweden, Switzerland and South Africa.

6. Organizational and representative activity

Dr. Ilieva has been a member of the organizing committees of many national and international scientific forums such as BIOMATH'14, BIOMATH'15, BIOMATH'18; large scale scientific computations (LSSC'17, LSSC'19); Numerical methods for scientific computing and advanced applications (NMSCAA'16, NMSCAA'18); High performance computing 2019. She is a member of the Board of Directors of the National Association for the Center for Supercomputing Applications (NSCP) and is a representative of Bulgaria in the Scientific board of Cost Action 17139 European Interdisciplinary Topology.

Conclusion: Assoc. Prof. Nevena Ilieva-Litova is an established researcher in the field of mathematical modeling and the application of mathematics and supercomputers in computational physics and biology. During the last decade she has demonstrated an impressive activity in this modern, but rare for our country field. She has published more than 20 new scientific papers in peer reviewed journals. Her name is well known and respected in Bulgaria and abroad. She satisfies (even exceeds) the formal national minimum criteria and the internal requirements of ICCT, BAS for the academic position "Professor" in the scientific field *4.5. Mathematics*, scientific specialty *Mathematical Modeling and Application of Mathematics (Applications in Computational Physics and Biology)*. Taking into consideration all this, I am highly recommending the distinguished Scientific Jury and the Scientific Council of ICCT to award Dr. Nevena Ilieva-Litova the academic position "Professor".

Sofia, 12.09.2019

Signature:

/Prof. Ivan G. Ivanov/