REVIEW

on the application for **Associate Professor position**
in Mathematics (Mathematical modeling and Applied Mathematics – applications in Computational Physics and Biology)
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by Prof. Dr. **Anela Nikolova Ivanova**
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There is a single applicant – Dr. Elena Boyanova Lilkova – for the position open at the Laboratory for Scientific Computing, 3D digitalization and microstructural analysis of the Institute of Information and Communication Technologies (IICT), Bulgarian Academy of Sciences (BAS). All required documents are available, together with information on additional criteria related to the selection procedure.

The applicant has graduated Sofia University as a M. Sc. in Medicinal Physics in 2011 г. Since 2015 she holds PhD in Physics of atoms and molecules after successful defense of her PhD thesis „Study of the human interferon-γ by molecular dynamics simulations". The research on the thesis was performed at the Faculty of Physics of Sofia University "St. Kliment Ohridski". From 2015 until 2017 Dr. Lilkova has been employed as an Assistant Professor and then as software developer at IICT, BAS. In the last 4.5 years she has been appointed as an Associate Professor in Computer modeling of biological molecules at the same institute. Dr. Lilkova takes active part in the development of various software instruments at the National Centre of Excellence in HPC applications and in the European network PRACE.

Dr. Lilkova is a co-author of 18 scientific publications in specialized journals, 15 of which published in peer-reviewed journals with impact factor or SJR. Five works, focused on method development or implementation, are published in the information bulletin PRACE whitepapers. She has submitted for the selection procedure 21 publications (14 of them indexed in Web of Science and Scopus). One of the articles (from group D, D.1) has been used for obtaining the PhD degree. Hence, in line with Art. 29, Sec. 1, P. 3, 4 of the Law for promotions in academia in Bulgaria (LPAB), all submitted publications are used to evaluate the scientific contributions of the candidate. Dr. Lilkova is first author in 6 of the publications and corresponding author in 7 of them. This signifies her substantial share in the performed research. Part of the publications are in international journals specialized in the field of molecular modeling: Journal of Molecular Modeling (1 paper), Journal of Biomolecular

Structure and Dynamics (1 paper), International Journal of Molecular Sciences (1 paper) and the remaining ones are in more general computer-oriented, mathematical or physical journals. The publications submitted for assessment have been cited 30 times (Source: Scopus) in international peer-reviewed journals. The current total number of independent citations of the publications of Dr. Lilkova is 32 (Source: Scopus). The applicant has coordinated two institutional research projects for young scientists and participated in one national Centre of Excellence, one national Research Programme and in the European network PRACE. She was also a member of the research teams of 4 bilateral research projects, 1 COST Action and 5 national research projects. Results from the research of Dr. Lilkova have been reported at 66 scientific conferences (7 abroad and the rest in Bulgaria, 21 of the Bulgarian events with international participants). The applicant has taken part in the organization of 5 international and 2 national scientific fora. She is part of the Editorial Boards of 4 Springer series.

Dr. Lilkova presents the following achievements to fulfill the minimum national requirements and the additional conditions of BAS for occupying the Associate Professor position (the scores are listed according to the national/institutional calculation procedure):

- indicators group A - defended PhD thesis – 50 points out of minimum required 50;
- indicators group C - 4 publications (1 in Q2 journal, 1 in Q3 one and 2 in journals with SJR only) – 165/110 points out of minimum required 100;
- indicators group D - 17 publications, different from those in group C (2 in Q1 journals, 10 – in journals with SJR only and 5 not indexed in the required databases) – 450/300 points out of minimum required 220;
- indicators group E - 26 citations of the publications submitted for evaluation at the time of applying – 208/156 points out of minimum required 60;

It is evident from the above summary that the applicant either fulfills or goes beyond the minimum national requirements and the additional criteria in all groups of indicators. The overall scientific metrics is in compliance with the general requirements of LPAB, the statutes for its application, and the additional recommendations of IICT, BAS.

The research of Dr. Lilkova is focused mainly on application of the method of classical molecular dynamics to study the structure and interactions of biologically active molecules.

The publications from group C are devoted to investigation of fragments of the human interferon-γ protein (hIFN-γ). Two of the works (C.1 and C.4) report the results from modeling of the folding of part of the C-terminus of the protein and of tracking the binding of hIFN-γ to heparin and its derivatives. Force field parameters for a residue from the heparin molecule are also derived. Key structural characteristics of the obtained intermolecular complexes are determined and a hypothesis for the mechanism of therapeutic action of heparin is formulated. Another study (C.2) reveals the influence of glycosylation on the behavior of a His6-FLAG peptide attached to the N-terminus of hIFN-γ. In the last work of

group C (C.3), it is shown how glycosylation stabilizes the structure of the cytokine. No habilitation thesis is submitted by the applicant but there is an extended synopsis of the research contributions. There, a concise and clear summary of the findings is provided.

The publications from group D may be classified as follows:

- study of other aspects of the behavior of hIFN-γ (D.1, D.4, D.9-D.11)

A large number of protein mutations are modeled and their influence on the length of formed α-helices is traced. Secondary structure of the C-terminus of the macromolecule is proposed and an energy-supported confirmation of its preferred conformation is provided.

Additional factors related to the changes in the biological activity of hIFN-γ as a result of glycosylation or tagging are clarified. Strong binding of heparin to two cytokines - hIFN-γ and IL-6, is established and used to explain the inhibitory capacity of this ligand and its corresponding anti-inflammatory activity. Oligosaccharide sequences similar to heparin are designed, which bear potential for yet more efficient inhibition of hIFN-γ.

- modeling of the structure of peptides with antimicrobial activity in search of relation to their action in vivo (D.5-D.8, D.12)

Both the primary structure and the spatial conformation of antimicrobial peptides in aqueous solution are analyzed. The differences in the antimicrobial activity of the wild type magainin 2 and its synthetic analogue are rationalized. The aggregation of indolicidin and bombinin in aqueous solution is described and evidence is presented for a mechanism of antimicrobial activity, which requires pre-association of the peptides influencing their secondary structure. Profiles of the free energy variation are used to determine the preference of a series of ten antimicrobial peptides to locate in a model bacterial cell membrane. It is shown that seven of them could be trapped therein.

- development and implementation of computational methods (D.2, D.14-D.17)

A method for unbiased identification of collective variables for metadynamics simulations of proteins is suggested, which relies on the most invariant part of the protein structure.

A library is programmed containing an implicit model of the solvent effect, which may be implemented in molecular dynamics packages and is included in the code DL_POLY_4. A library for DL_POLY_4 is developed, offering an optimized and flexible computation of electrostatic interactions combined with low-dimensional periodic boundary conditions. A parallelization algorithm for the method is also devised that can be applied efficiently on phi coprocessors.

- estimation of the efficiency of software packages on HPC systems (D.3, D.13)

The performance of the software package GEANT4 on a platform with phi coprocessors is quantified. A solution for scalability problems of Gromacs when modeling large biosystems is suggested (which is successful in NAMD), which is based on the variable

time step approach.

The molecular models in the above studies include the target biomolecules solvated in explicit water, described with a modified TIP3P model, with applied periodic boundary conditions. It leaves a good impression that upon construction of the models special attention is paid to the specifics of the biomolecular structure related to their physiological activity. The appropriate ionic strength of the solutions is maintained, too. Standard biomolecular force fields are employed, which are appropriate for the description of the studied systems. Where needed, new force field parameters are derived by automated procedures. All-atom level of treatment is exclusively preferred, which enables taking into account all specific interactions. There is also one study with a coarse-grain force field.

The computational protocols used to perform the simulations are based on the method of classical molecular dynamics. They are suitably supplemented by application of enhanced sampling techniques (methadynamics and the free energy perturbation method) allowing evaluation of free energy changes in the studied systems. The models are adequate, the computational toolbox is appropriate and in general applied very well. The chronologic refinement of the protocols is positive, e.g., switching to simulations at body instead of room temperature, since this could influence significantly the behavior of flexible biomolecules such as proteins and saccharides. Another example is fixing only the lengths of the hydrogen-containing bonds and not of all bonds in order to prevent bias on the interactions, for instance on the formation of hydrogen bonds.

The outcome of the performed research is diverse. The knowledge on the structure of several bioactive molecules is supplemented with data that were not available experimentally, molecular-level explanation is given of experimentally observed bioactivity or of modulations thereof, experiments are directed by molecular design carried out in advance, new compounds with potential for well-expressed bioactivity are suggested, hypotheses about the mechanism of certain steps of bioprocesses are outlined. Useful computational methods and algorithms are implemented, too. All this adds to the existing knowledge in the respective scientific areas.

I have the following question to the applicant:

Over a period of several years, a number of molecular dynamics simulations with increasing length and exhaustiveness are carried out to model the folding of the C-terminus of hIFN-γ. Are there marked differences in the middle structure obtained from the various simulations and if yes, what are they?

The personal contribution of Dr. Lilkova to the research is singled out explicitly only in a few of the publications but it becomes evident from the submitted documents that she masters the employed computational methodology, performs in-depth analysis of the problems in the respective research area and has undoubted capacity to pursue the career of

an independent habilitated scientist. She works successfully both at the Laboratory for Scientific Computing, 3D digitalization and microstructural analysis and within broader national and international teams. Dr. Lilkova extends and maintains successfully her skills in several directions of science and technology – biology, physics, informatics and information technologies. All this delineates her as a prospective member of the academic community of IICT.

In summary, the materials submitted for the evaluation comply with all requirements of the law and with the additional recommendations of IICT, BAS for an Associate Professor position. This motivates me to assess positively the applicant Dr. Elena Lilkova and to vote for her appointment as an Associate Professor.

July 6, 2022 Member of the scien

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