OPINION

by competition for the academic position "Associate Professor" in the direction "Structural Crystallography and Materials Science" at the Institute of Mineralogy and Crystallography at the Bulgarian Academy of Sciences (BAS)

Applicant: Principal assistant Hristina Ilieva Dimitrova, Ph.D

Opinion by: Associate professor Yordan Svetoslavov Handzhiyski IMB-BAN

By order No. 27 RD 09/10.01.2024 of the Director of IMC-BAN, I was appointed as a member of a scientific jury in a competition for the academic position "Associate Professor" in the professional direction "4.3 Biological Sciences" (Crystallization and structural analysis of macromolecules) at the department "Structural Crystallography and Materials Science" at the Institute of Mineralogy and Crystallography. For participation in the announced competition has submitted documents a single candidate - P. assistant Hristina Ilieva Dimitrova, PhD. The candidate has submitted all the documents required by the Academic Staff Development Act in the Republic of Bulgaria (ASDARB), the Regulations for its implementation and the relevant regulations of the IMC-BAS, which makes her legitimate to participate in the competition. The enclosed documentation is complete, detailed and extremely well presented.

PhD. Hristina Dimitrova completed her higher education in 2005 at Sofia University "St. Kliment Ohridski" as a master's degree in "Ecology and environmental protection". In the period after her graduation she held successively the following positions at IMC-BAS: assistant, research assistant and principal assistant from 2019 until now. He obtained her PhD in 2018 at the Institute of Mineralogy and Crystallography. In the period from 2015 to 2018, she had a specialization at the Stanford Synchrotron Radiation Lightsource (SSRL), California, USA. with the topic "4G-quadruplexes - ligand interactions". She received an award from the Bulgarian Crystallographic Society (BCS) for the best poster presentation at the 7th National Crystallographic Symposium, October 3-5, 2018, Sofia. These brief CV data testify to a dynamic, focused and successful career development of PhD. Dimitrova in the field of crystallization and structural analysis of macromolecules, which is in full accordance with the specialty of the announced competition.

Principal assistant Dimitrova participates in this competition with a total of 20 publications in international scientific journals with a total JCR-IF of 23,372. It is noteworthy that the articles with the highest IF are from recent years. The paper with the highest IF (8.457) was published in the prestigious journal "Chemico-Biological Interactions", Elsevier, in 2023. The publications are divided by quartiles as follows: Q1- 7 pcs; Q2 – 5 pcs.; Q3 – 2 pcs. and Q4 – 6 pcs. Fifteen of the publications are refereed in Web of Science and Scopus, and 5 only in Scopus. Her scientific works have been cited 85 times in publications, referenced and indexed in world-famous databases with scientific information (Web of Science and Scopus). She has participated in a total of 15 international scientific forums, has been a speaker at one of them and co-author of 14 posters. P. assistant PhD Hr. Dimitrova has participated in national and international projects related to: "The crystallographic deciphering (structural determination) of DNA and protein structures", "Newly synthesized mementin derivatives with potential preventive effects against dementia of the Alzheimer's type" and "Co-crystallization of Alzheimer's DNA promoter sequences of the amyloid precursor (APP) gene with Thioflavin T and other fluorescent markers", and she was also the leader of the last project. All this testifies to PhD Dimitrova's active research activity and her ability to participate in and lead scientific research.

The main contributions of PhD Dimitrova of fundamental and applied nature refer to the study of various palindromic DNA sequences related to the mechanisms of cellular regulation, as well as those that successfully bind to many medicinal and biologically active substances. In summary, these contributions can be summarized as follow: a) as a result of her research, the crystal structure of the sequence 5'-GCCCACCACGGC-3', which was reported for the first time in the PDB under the number 8ASK, was determined at a resolution of 2.96 Å.This DNA has a conformation that is typical of B-DNA; b) the conditions for crystallization and co-crystallization of selected DNA sequences from the promoter region of the APP gene were optimized, as well as their

subsequent co-crystallization with Thioflavin T and other fluorescent markers and ligands; c) 2-((4-(dimethylamino)benzylidene)amino)-3,6-dimethylbenzo[d]- thiazol -3-ium iodide (compound 3 or monomer XRB in PDB) was synthesized, which is a new homolog of Thioflavin T .The interaction of this compound (XRB) with DNA was confirmed by FID and mono crystal X-ray structural analysis; d) the structure of the tetradecamer oligonucleotide sequence d(CCGGGGTACCCCGG)2 with XRB was established at a resolution of 1.84 Å. The interaction of XRB with the A-DNA form is based on strong hydrophobic interactions; e) The crystal structure of co-crystallization forms of the oligonucleotide sequences 5'-CGTGAATTCACG-3' and 5'- CGCGAATTCGCG-3' with the fluorescent markers DAPI, Berenil, AK3-4, AK3-9, DL72, DL89 and EtBr was studied using synthetic obtained DNA for experimental single crystal growth; f) The structure of d(CGTGAATTCACG)2 was solved by single crystal X-ray diffraction at 2.0 Å resolution. Coordinates and structure factors have been deposited in the PDB database under accession number 5JU4;(g) A crystal of d(CGTGAATTCACG)2 grown under conditions including the fluorescent marker Berenil is imaged for the first time.The structure was taken in the same P212121 space group as 5T4W and 5JU4.The structure clarification revealed the presence of a cacodylate ion instead of the expected ligand (Berenyl); h) The crystallization conditions of the heterologously expressed recombinant protein Ts-PCHTP were optimized. The protein was expressed and purified from the soluble protein fraction in its native form; g) The genetic polymorphism of kappa casein was investigated and the correlation between κ-CN genotypes and casein mycelium size in milk samples was found, and the studies were performed on 16 milk samples with different CSN3 genotypes (AA / AB / BB). it was also found that the protein and fat content of milk could not be related to the size of the casein micelles. **The publication of all these results in peer-reviewed publications referenced in world-renowned databases is evidence of their international recognition.**

The following table shows the compliance of PhD Dimitriva indicators A to D with the minimum national requirements according to Annex 1 of the Regulations for the implementation of the ASDARB (adjusted for BAS for indicators G and D):

A group of indicators	**Minimum points**	**Candidate's Points**
A	50	50
B	100	105
C	200 (220 for BAS)	290
D	50 (60 for BAS)	170
Total	**430**	**615**

From the data, it can be seen that **PhD Dimitrova fully meets the minimum national requirements, including the increased BAS criteria, for the academic position of Associate Professor.** Although the assets under indicator E of the above table are not required for "Associate Professor". I will only note that PhD Dimitrova has significant project activity. She has been a leader/participant in three research projects, one of which she is also the leader of the project. The accumulated extensive experience in the implementation of research projects is a good prerequisite for the successful implementation of the candidate in the new academic position.

CONCLUSION:

From the analysis of the documents submitted by PhD Dimitrova, it is evident that she possesses the required competences in the field of crystallography and structural analysis of macromolecules for holding the academic position of Associate Professor under the present competition. Her scientific metrics fully meet the requirements of the ASDARB, the Regulations for its implementation and the Regulations of BAS and and IMC-BAS for holding this position. The candidate's scientific works contain original contributions of significant scientific and applied character. These studies have been published in reputable international journals and have been cited many times. PhD. Dimitrova also has good skills in providing financial support for her scientific research and has been awarded

for her scientific and project activities. All this gives me a reason to give a positive assessment of the application of P. Assistant Hristina Ilieva Dimitrova, PhD and to recommend to the Scientific Council of IMC-BAS her election to the academic position of Associate Professor.

February 23, 2024

associate professor Y. Handzhiyski