

REVIEW

of the materials submitted for participation in the competition for occupation of academic position "ASSOCIATE PROFESSOR" in the professional field **4.3. Biological Sciences** (*Crystallization and Structural Analysis of DNA and Proteins*) for the needs of the Department "Structural Crystallography and Materials Science" at the Institute of Mineralogy and Crystallography "Acad. Ivan Kostov" (IMC) - BAS, announced in the *State Gazette*, No. 95/ 14.11.2023

Candidate: Chief Assist. Prof. Hristina Ilieva Dimitrova, PhD, IMC - BAS

Reviewer: Assoc. Prof. Maya Georgieva Chochkova, PhD
*South-West University "Neofit Rilski", member of
the scientific jury under Order No. 27 RD-09/10.01.2024*

Chief Assist. Prof. Hristina Ilieva Dimitrova, PhD from the Department "Structural Crystallography and Materials Science" at the Institute of Mineralogy and Crystallography "Acad. Iv. Kostov" (IMC) - BAS is the only candidate in the announced competition for the academic position (AP) "Associate Professor", in the professional field **4.3. Biological Sciences** (*Crystallization and structural analysis of DNA and proteins*).

1. Brief biographical details of the applicant

Hristina Dimitrova completed her higher education in 2004 at Sofia University "St. Kliment Ohridski" with a Bachelor's degree in Biology. After that, in 2005, she obtained her Master's degree in "Ecology and Environmental Protection" at the same University.

In 2018, she defended PhD thesis at the Institute of Mineralogy and Crystallography, "Acad. Ivan Kostov", Bulgarian Academy of Sciences with the topic: "Crystallization and structural analysis of two palindromic DNA sequences with fluorescent markers" under the supervision of Prof. Dr. Boris Shivachev.

From 2015 to 2019, she successively held positions at the IMC "Acad. Ivan Kostov" – BAS as: Assist. Prof. (2015-2018), Researcher (2018-2019) and Chief Assist. Prof. (from 2019- until now).

For the period 2015 - 2018, the candidate specialized at *Stanford Synchrotron Radiation Lightsource (SSRL), California, USA*, Grant 4B60 with the topic: 4G-quadruplexes - ligand interactions.

Chief Assist. Prof. Hristina Dimitrova has been a member of the Bulgarian Crystallographic Society (BCS), the European Crystallographic Association (ECA) and the International Union of Crystallographers (IUCr).

2. General description of the presented materials

Dr. Hristina Dimitrova presented all documents in compliance with the requirements of the Academic Staff Development in the Republic of Bulgaria Act (ASDRBA), the Rules for its implementation, the Rules for the implementation of the ASDRBA of the Bulgarian Academy of Sciences and covers the Rules for the Development of the Academic Staff of IMC - BAS for taking up the AP "Associate Professor".

In the current competition, Dr. Dimitrova presented a total number of **23** scientific papers, however I have accepted for evaluation only **20** of them, since three of publications have not been published in the largest worldwide databases such as *Web of Science* and/or *SCOPUS*. Therefore, the points awarded for those 3 articles (3 x 6 points) will be valid only if they are in Professional fields (PF 4.5 or PF 4.6).

The reference concerning the fulfillment of the minimum national and additional requirements shows that Dr. Hr. Dimitrova covers the quantitative indicators, and by some of the indicators exceeds the required minimum points.

A more detailed analysis of the scientific-metric indicators is presented below, in Section 4.

3. General characteristics of the scientific, scientific- applied and pedagogical activity of the candidate

The scientific activity of Dr. Hr. Dimitrova after the acquired educational and scientific degree "Doctor" is connected mainly with the continuation of research in the field of structural biology.

In the current competition, Chief Assist. Prof. Dr. Hr. Dimitrova has presented data for participation in three national and international projects, being a project leader of one of them.

Her participation in the development and implementation of scientific projects is related to the crystallographic studies of *DNA* and *protein structures*, of *AA-/phenolic derivatives of*

the NMDA-antagonist-memantine, as well as the crystallization of Alzheimer DNA Promoter Sequences from Amyloid Precursor Gene with Thioflavin T and other fluorescent markers.

Data for the pedagogical activity of the candidate were not provided in the competition for the academic positions "Associate Professor".

My recommendation to the future Associate professor Dimitrova is to share the knowledge and experience of X-ray structural analysis with students by designing a course in this field, as well as to be an advisor/consultant of diploma or doctoral students.

4. Reflection of the candidate's scientific publications in Bulgarian and foreign literature

The analysis of the 20 accepted articles for review in the competition for AP "Assoc. Prof." includes publications, that are indexed in WoS and/or SCOPUS, and the remaining 3 articles, which are not awarded points, will be taken into account when forming my final assessment of the candidate.

According to the SJR metric, the publications can be grouped as follows: 7 articles belong to first quartile (Q1), 5 - in Q2, 2 - in Q3 and 6 articles in Q4.

According to *indicator „B4“*- 5 publications are equivalent to a thesis, achieving 105 points, which exceeds the required minimum of 100 points.

According to *indicator "G 7"* - the candidate has co-authored 18 articles, achieving 290 points.

The reduction of points is due to the fact that since 2018, *Bulgarian Chemical Communications* has not been indexed in WoS yet (holding impact rank only). As 2 articles were published in that year, so the earned points should be 2 x 10 points (20 points). An additional reduction of 18 points is at the expense of 3 articles x 6 points (for PF 4.5 and PF 4.6 only), so the final number of points achieved under this indicator is 268. According to this criterion, Dr. Dimitrova exceeds the required 220 points.

According to *indicator „D“*, related to the citability (without self-citations) of the candidate's scientific works, the information provided by her includes 83 citations, At the time of writing this review, according to WoS & Scopus, there are 67 citations (134 points).

The minimum requirements for this indicator are 50 points, so the points are twice the requirements.

As can be seen, at the time of this review, Dr. Hr. Dimitrova has Hirsch index of 5, based on Scopus, which provides the relevance of her scientific work.

Part of Dimitrova's research is disseminated among the scientific community with 15 presentations in international and national scientific forums. She won the Best Poster Award for "Crystallization and crystal structure of lysozyme with nanosized titanium oxide" at the "VIIth National Crystallographic Symposium NCS2018" Sofia, Bulgaria (03 - 05 October 2018) with international participation.

5. Main scientific and/or scientific - applied contributions

Dr. Hristina Dimitrova's main scientific contributions are related with the finding of new scientific facts of various palindromic DNA sequences linked with the mechanisms of cellular regulation. The topic on crystallographic determination of DNA using X-ray diffraction data obtained from co-crystallized fluorescent ligands with DNA sequences represents the continuation of the candidate's dissertation work.

For the first time the crystal structure of the sequence 5'-GCCCACCACGGC-3' (in the PDB database, under the number **8ASK**), at a resolution of 2.96 Å was estimated. The established DNA conformation is typical for the B-conformation form with structural parameters that closely match the theoretical values [publ. No. 4].

An important point in Dimitrova's research work is the obtaining of new analogue of Thioflavin T (**ThT**): 2-((4-(dimethylamino)benzylidene)amino)-3,6-dimethylbenzo[d]thiazol-3-ium iodide (monomer **XRБ** in the PDB database). The interaction of compound **XRБ** with DNA was confirmed by Fluorescence Intercalation Displacement (FID) method and single crystal analysis [publ. No. 4].

Crystallization and co-crystallization conditions of selected DNA sequences were optimized. Alzheimer DNA Promoter Sequences from the Amyloid Precursor Gene, as well as their further co-crystallization with **ThT** or with **XRБ** were studied. Crystallographic analysis of the two DNA sequences: 5'-GCCCACCACGGC-3' (**8ASK** in PDB) and d(CCGGGGTACCCCGG)₂ (PDB **8ASH**) in the presence of **ThT** and **XRБ** were solved by the molecular replacement method. The structure of the tetradecamer oligonucleotide sequence d(CCGGGGTACCCCGG)₂ with **XRБ** was solved at a resolution of 1.84 Å. The **XRБ** interaction with A-DNA form is driven by hydrophobic interactions. [publ. № 4].

Another significant contribution of the applicant is the X-ray analysis of new squaramides as potential deoxyribonuclease I (DNase I) inhibitors *in vitro*. It has been shown that the target compounds inhibited DNase I with IC₅₀ values below 100 µM, being at the same time more potent DNase I inhibitors than crystal violet (positive control) [publ. № 1, 3].

The crystallographic conditions of the heterologally expressed recombinant protein from *Trichinella spiralis* (rTs-PCHTP) were optimized and a variety of suitable crystallization conditions for Ts-PCHTP was defined by using vapor-diffusion method - hanging drop.

The metalloprotein (rTs-PCHTP) with poly-histidine moiety at the C-term (histidine-tailed protein) is expressed and purified by Ni - affinity chromatography from soluble protein fraction. According to DLS analysis the protein forms dimer formations [publ. № 22].

The contribution for the comparative DLS - analysis of the size of the casein micelle in a cow's milk sample as a function of the *kappa*-casein (CSN3) genetic polymorphism of the breed - *Bulgarian Rhodope cattle* (genotype (AA/ AB/ BB)) is quite interesting.

The correlation between κ -CN genotypes and casein micelles size in milk samples was revealed for the first time, which could be extremely valuable, in order to improve the technological properties of milk [publ. No. 19]

In addition, the protein database (PDB) is enriched with the coordinates and structure factors of modified drugs and with biologically active compounds.

Novel molecular complexes of 4-halophenylboronic acid with acyclovir, caffeine, nitrofurazone, and proline were produced by co-crystallization experiments. The single crystal analyses of the complexes revealed a variety in the hydrogen bonding network interactions that can be produced by the $-B(OH)_2$ motif [publ. № 2].

The lysozyme- nanosized particles TiO_2 interaction was also studied using single crystal XRD, LA-ICP-MS and cyclic voltammetry. The X-ray structure solution revealed that lysozyme crystallizes in the P43212 space group. It has been found that Ti^{4+} binds successfully with the enzyme and the complex formation is irreversible at pH 6.5 [publ. № 14].

6. Critical remarks of the reviewer on the submitted materials, incl. and on the literary awareness of the candidate

I have no critical remarks to the materials submitted for participation in this competition. By the way, I did not find a plan for the candidate's future research work.

CONCLUSIONS

After thoroughly reviewing the submitted materials for the competition and the scientific achievements of the candidate, I have no doubts regarding Chief Assistant Prof. Dr. Dimitrova's personal contribution to the obtained results.

In my opinion she can be defined as highly qualified specialist in the field of "Structural Crystallography and Materials Science" and I will definitely give my positive assessment.

I recommend to the esteemed Scientific Council of the Institute of Mineralogy and Crystallography "Acad. Iv. Kostov" (IMC) - BAS to award Dr Hristina Ilieva Dimitrova the academic position "ASSOCIATE PROFESSOR" in professional field 4.3. Biological Sciences (Crystallization and structural analysis of DNA and proteins).

25.03.2024

Reviewer:

/Assoc. Prof. Maya Chochkova, PhD/