

## ASSESSMENT

from Prof. Dr. Ivanka Georgieva Stankova, SWU "Neofit Rilski", Department of Chemistry, a member of the scientific jury in the contest for academic position "ASSOCIATE-PROFESSOR", in professional field 4.3. Biological Sciences" (Crystallization and structural analysis of macromolecules) for the needs of the department "Structural Crystallography and Materials Science" at the Institute of Mineralogy and Crystallography - BAS

announced in the "State Gazette", no. 95 /14.11.2023

**Candidate:** Hristina Ilieva Dimitrova, Ph.D. - Institute of Mineralogy and Crystallography "Acad. Ivan Kostov" at the BAS - Sofia

In the announced competition, Dr. Hristina Dimitrova participated with 23 scientific publications of which indexed in WoS, Scopus – 20. In Q1 – 7, Q2 – 5, Q3 – 2, Q4 – 6 and indexed in WoS or Scopus, but without IF and SJR – 3 .

Lead author is in 25%, second or subsequent author is in 75% of publications, and H-index is 5. A reference with 83 citations is presented.

The candidate is a participant in a total of 3 scientific research projects funded by the National Institute of Scientific Research and one has a participation in OP - BG051PO001-3.3.06-00 ADB "Support for the development and realization of doctoral students, postdoctoral students and young scientists in the field of materials science, mineralogy and crystallography".

Dr. Dimitrova's scientific research activity is mainly focused on research in the field of structural biology - the study of various palindromic DNA sequences related to the mechanisms of cellular regulation, as well as those that are successfully associated with many medicinal forms and biologically active substances .

The main contributions are:

The crystal structure of the sequence 5'-GCCACCCACGGC-3' was reported for the first time in the PDB under accession number 8ASK at 2.96 Å resolution. The DNA exhibits a conformation that is typical of B-DNA with structural parameters that exactly match the theoretical values.

Conditions for crystallization and co-crystallization of selected DNA sequences from the promoter region of the APP gene, as well as subsequent co-crystallization with Thioflavin T and other fluorescent markers and ligands, were optimized. A new homolog of Thioflavin T - 2-((4-(dimethylamino)benzylidene)amino)-3,6-dimethylbenzo[d]-thiazol -3-ium iodide was

synthesized. The interaction of the compound (XRB) with DNA was confirmed by FID and single crystal X-ray structural analysis.

The structure of the tetradecamer oligonucleotide sequence d(CCGGGGTACCCCGG)<sub>2</sub> was investigated with XRB at a resolution of 1.84 Å. Coordinates and structure factors have been deposited in the PDB database under accession number 8ASH. The interaction of XRB with the A-DNA form is based on strong hydrophobic interactions.

Three novel monosquaramides (3a–c) were synthesized and structurally determined, characterized by IR, NMR and X-ray structural analysis and evaluated for inhibitory activity against deoxyribonuclease I (DNase I) and xanthine oxidase (XO) in vitro. The target compounds inhibited DNase I with IC<sub>50</sub> values below 100 μM, while being more potent DNase I inhibitors than crystal violet, used as a positive control.

Experiments were carried out on the co-crystallization of biologically active substances - acyclovir, caffeine, nitrofurazone and proline with 4-halophenylboronic acid to obtain new molecular complexes. Single crystal analyzes of the new complexes have revealed an unexpected diversity in the interactions of the hydrogen bond network that can be produced by the –B(OH)<sub>2</sub> motif.

Single-crystal XRD, LA-ICP-MS and cyclic voltammetry studies of the interaction of the protein Lysozyme with nanosized TiO<sub>2</sub> particles (JRC NM-101) were observed, and the crystal structure of co-crystallization forms of the oligonucleotide sequences 5'-CGTGAATTCACG-3' and 5 was studied. '-CGCGAATTCGCG-3' with the fluorescent markers DAPI, Berenil, AK3-4, AK3-9, DL72, DL89 and EtBr using synthetically produced DNA for experimental single crystal growth.

Single crystals of DNA (5'-CGTGAATTCACG-3') were studied for the first time in the presence of DAPI and Berenil and the coordinates and structure factors have been deposited in the PDB database under the number 5T4W. As well as for the first time, an experiment from a crystal of d(CGTGAATTCACG)<sub>2</sub> grown under conditions including the fluorescent marker Berenil has been recorded and the coordinates and structure factors have been deposited in the PDB database under the number 5NT5.

The crystallization conditions of the heterologously expressed recombinant protein Ts-PCHTP were optimized and crystallization experiments were performed using the "hanging drop" diffusion method.

The genetic polymorphism of kappa casein was investigated, focusing on the correlation between κ-CN genotypes and casein mycelium size in individual milk samples, and it was found that milk protein and fat content could not be related to mycelium size of casein.

From the presented publications it is clear that Dr. Hristina Dimitrova has extensive experience in the crystallization of various types of crystalline substances, in the selection of conditions for conducting an experiment, reading single crystal data and powder samples.

**Conclusion**

On the basis of everything stated above in this opinion, I believe that Dr. Hristina Dimitrova, meets the requirements of the Regulations of IMC-BAS, PPZRAS of the Republic of Bulgaria for occupying the academic position "associate professor" and I vote with a positive assessment for the overall and scientific work.

25.03.2024

Prof. Ivanka Stankova, PhD