O P I N I O N

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About the competition for the academic position “Associated Professor“ in the professional field 4.3. "Biological Sciences" (Molecular Biology) from the Department "Regulation of gene activity,”, Institute of Molecular Biology announced in the State Gazette issue 52/18.06.2024

At the announced competition for the academic position “ Associated Professor”, only one candidate submitted documents, -Assistant Professor. Elena Bojidarova Krachmarova. PhD. According to the attached materials, the candidate exceeds the minimum requirements of the Law for the Development of the Academic Staff of the Republic of Bulgaria and the relevant regulations for its implementation of Bulgarian Academy of Sciences as well as of the Institute of Molecular Biology. for the academic position “Associated Professor” From the summary report, it can be seen that with the required minimum for an Associate Professor of 400 points, she collects 733.9 points.

Assistant Professor Elena Krachmarova has over 14 years of scientific experience at the Institute of Molecular Biology at the Bulgarian Academy of Sciences. In 2018, she defended her PhD thesis in professional field: 4.3. Biological Sciences, Molecular Biology, at the Institute of Molecular Biology, Bulgarian Academy of Sciences. Her PhD thesis received an award from the Union of Scientists in Bulgaria with a diploma from the competition for high scientific achievements, direction "Scientific achievements in defended PhD theses by scientists under the age of 35". In 2019, she was awarded the 2018 Evrika Faundation Award for Achievement in Science, and in 2020 she received the Evrika Faundation Award for Young Inventor, making her the only two-time Evrika Faundation Award winner. Since 2010, she has been working at the Institute of Molecular Biology as a biologist.

Since 2018 she is an Assistant Professor at the same Institute. During the period from 2020 to 2024, she published 16 articles in international journals with IF. Until

now, her papers have been cited 91 times in the world scientific literature. Some of the scientific achievements are registered as one patent in Bulgaria and European one . She has participated in several international and Bulgarian scientific forums. In 2023, she was invited speaker at the FEBS congress, which took place in Tours, France. She is the head of 2 scientific contracts and a participant in 11 with the Scientific Research Fund and other scientific organizations. She was a member as well as and of international scientific contracts with Germany and France. All this is an indication of the candidate's scientific activity and significant scientific achievements. The candidate participated in a number of courses related to the subject of her scientific field, which had a favorable effect on her growth as a researcher.

The main scientific achievements of Dr. Elena Krachmarova can be systematized mainly in 3 directions:

1. **Study of the molecular mechanisms of action of the ORF6 and Nsp13 proteins of the SARS-CoV-2 virus in infected cells. Approaches for treating cytokine storm.**

   In order to elucidate the molecular mechanisms of blocking the immune response through the interferon signaling pathways, the molecular mechanism of interaction between two proteins of the SARS-CoV-2 virus – ORF6 and the helicase Nsp13 was investigated by computer simulations and experimental studies. In order to study the mechanism of the toxicity of the ORF6 protein – the most toxic protein of the virus, a 3D model of the protein was created and the mechanism of interaction with infected cells was investigated by molecular dynamic simulations, which was confirmed experimentally. Thus, it has been proven that the protein interacts with one of the main regions in transport RNA thus immobilized the protein on different cytoplasmic membranes thus inhibites the immune response of the cell. These studies describe for the first time one of the mechanisms of genome instability due to the ORF6 protein

   In her investigations, the author proves that hIFNγ restores the normal functioning of the interferon pathways. For the first time, an inhibitor of ORF6 was reported in the literature. This research could serve as a basis for creating therapeutics for treatment of COVID-19.

The structure, physicochemical behavior and biodynamics of the two pockets of the NSP13 helicase, which can bind to various inhibitors, have been elucidated and it has been shown that the active ingredient of Retonavir, which is used to treat HIV and hepatitis C, binds most actively to them. Since the severe form of development of COVID-19 is associated with the development of a cytokine storm, research has been conducted to find inhibitors of this process. The mechanisms of binding of hIFNγ to its specific receptor hIFNGR1 and the formation of a complex have been investigated. The key role of heparin sulfate and its proteoglycans in signal transduction pathways stimulated by hIFNγ has been demonstrated more recently for the treatment of COVID-19.

The mechanisms of interaction of hIFNγ with heparin were investigated and new pathways for its inhibitory effect on cytokine storm processes have been demonstrated. It has been shown that this action is mainly based on the positions of the sulfate groups.

Thus, new facts about the anti-inflammatory effect of heparin and its importance in suppressing the cytokine storm have been proven.

2. **Investigation of factors influence the biological activity of human gamma-interferon (hIFNγ) and its production as a recombinant protein**

The role of the two glycosylation centers for interferon stability has been elucidated. By creating model structures of glycosylated homodimers of the wild-type protein, it has been proven that the stabilizing effects of glycosylation on hIFNγ occurs through two different mechanisms that are based on partial hydrolysis of certain regions of the molecule, which leads to the generation of shortened forms of the cytokine with high biological activities.

Model structures of full-length glycosylated homodimers of wild-type hIFNγ are published for the first time in the literature, and they are one of the few glycoprotein simulations described so far in the literature. Based on this, the reasons for the proteolytic resistance of the N-terminal FLAG peptide have been elucidated.

The proteolytic resistance of this peptide has been shown to be due to its interaction with the entire interferon globule.

The investigation of the expression of the interferon in *E.coli* in order to obtain mutant forms, demonstrated that inactive proteins aggregated into protein bodies. The examination of the composition of these bodies, showed that in addition to the protein, they also contain nucleic acids and, most likely, whole ribosomes, which stimulate the aggregation of the protein, thus leads to its deactivation. To prevent this process steps have been taken to obtain the protein in the cytosolic fraction of the cell, which resulted in obtaining a highly purified, stable protein with high biological activity. This method can be used to store other types of proteins in an active form.

3. **Thermodynamics of interaction of ionic liquids with the transport protein serum albumin**

   By thermodynamic approach, the binding affinity, enthalpy and entropy of bovine serum albumin with ibuprofen-based ionic liquids, which are characterized by a pharmacokinetic profile close to ibuprofen, were determined. It was shown that they are good providers and stabilizers of ibuprofen

   Silicic acid-based ionic liquids have been synthesized and characterized. Their cytotoxicity was studied and they were shown to exhibit low toxicity to human fibroblasts and keratinocytes and to inhibit cytokine. These liquids have a higher affinity for BSA than salicylic acid. It can be assumed that they will be more effective in the treatment of chronic skin diseases due to the more efficient delivery of drugs and their longer release.

   The binding affinity of the anti-inflammatory drug naproxen to BSA modified into ester salts was also investigated. The results of these studies can be used to develop ionic liquids with successful application in pharmacy.

   From the registered patents, it can be concluded that the scientific developments of Dr Elena Krachmarova can be used both in biotechnology and in the treatment of some irremediable pathologies.

Everything that has been said so far is an indication that Dr Elena Krachmarova has a clearly defined scientific field, which she develops successfully considering the number of publications in significant scientific international journals. The citations of her publications in the international database show of the great interest of the world scientific community for the obtained results.

Conclusion:

Assistant Professor Elena Krachmarova PhD, has conducted her scientific research in a very important scientific field - the mechanisms of inhibition and the development of COVID-19 as well as on the mechanisms and factors for inhibition of the cytokine storm and the action of various ionic liquid analogues of some drugs with the aim of their application to create more effective pharmaceutical forms. Her scientific activity exceeds the requirements of the Law for the Development of the Academic Staff of the Republic of Bulgaria and the relevant regulations for its implementation of Bulgarian Academy of Sciences as well as of the Institute of Molecular Biology. for the academic position “Associated Professor " in professional direction 4.3. "Biological Sciences"(Molecular Biology). Therefore I strongly recommend to the Scientific jury and the Scientific Council of the Institute of Molecular Biology Assistant Professor . Dr. Elena Krachmarova to be elected for the academic position "Assosiate Profesor" in profesional field 4.3. "Biological Sciences" (Molecular Biology.)