

REVIEW

from Prof. Tatyana Ivanova Vlaykova, PhD

Department of Medical Chemistry and Biochemistry,
Faculty of Medicine, Trakia University, Stara Zagora

Scientific specialty: "Biochemistry",

Professional field: 4.3. Biological sciences.

E-mail: tatyana.vlaykova@trakia-uni.bg

Tel: +359 888 002438

Regarding: the procedure for the Academic position of "**Associate Professor**" in the scientific specialty **Molecular biology**, professional field 4.3. Biological sciences, field of higher education 4. Natural sciences, mathematics and informatics

1. Information about the procedure

The procedure, announced for the needs of Section "Regulation of gene activity", Institute of Molecular Biology "Academician Rumen Tsanev", BAS (IMB-BAS), was published in the State Gazette, No. 52/18.06.2024

2. Brief information about the candidates in the competition

The only candidate in the competition is Head Assistant Professor Elena Bozhidarova Krachmarova, PhD. The candidate's documents are well organized and arranged and are in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations on the Development of the Academic Staff at the IMB-BAS.

In 2009, Head Ass. Prof. Krachmarova received a bachelor's degree in the specialty "Biotechnological Engineer", and in 2010 she received a master's degree in the same specialty from the University of Chemical Technology and Metallurgy, Sofia. In 2018, Elena Krachmarova successfully defended her PhD thesis on "Expression and properties of human interferon-gamma and its mutant analogs", prepared under the supervision of Acad. Ivan Ivanov and Prof. Genoveva Nacheva, and received a PhD in "Molecular Biology".

In the Institute of Molecular Biology "Acad. Rumen Tsanev" (IMB-BAS), in the Section "Regulation of gene activity", Elena Krachmanova started work, in 2010, first as a specialist chemist, from 2011 as an Assistant Professor, and from 2018 until now as the Head Assistant Professor. During the period 2015-2018, she was a part-time PhD student in the same section.

According to the report from the Department "Human Resources" of the IMB-BAS, Head Assistant Professor Krachmarova has over 14 years and 5 months of work experience in the specialty.

In addition to the main scientific engagements, Head Ass. Prof. Krachmarova also performs administrative and organizational activities. In 2012, she was the coordinator of the FEBS Advanced Lecture Course, and in 2024, she was the technical secretary of the National Scientific Conference "80 years of the Union of Scientists in Bulgaria - at the service of society", October 4 - 6, 2024, Complex "St. St. Constantine and Helena".

Elena Krachmarova has received three national awards: an award from the Union of Scientists in Bulgaria with a diploma from the competition for high scientific achievements, field "Scientific achievements in PhD thesis by scientists under the age of 35", while in 2018 and 2020 she receives the "Eureka" award twice for achievements in science.

There is no information presented about courses and specializations held to increase her qualifications, expand her scientific interests and acquire new practical skills.

3. Completion of the requirements for obtaining the academic position "Associate Professor".

In the competition for academic position "Associate Professor" Head Ass. Prof. Krachmarova participated with 14 publications, in journals referenced in Scopus and/or in Web of Science (11 with IF; Total IF=47,536), and with two patents, one European and one Bulgarian. Very correctly, Elena Krachmarova has also presented a list of three more publications in journals, referenced in WoS, included in the PhD thesis.

Elena Krachmarova's personal contribution to the conduct of research and the presentation of scientific results is proven by the fact that in four out of 14 articles and 2 patents (25%), with which Dr. Krachmarova participated in the competition, she is the leading author (first author), and in another 3 she is the second author (12%). In 2023, Elena was an invited speaker at the 47th FEBS Congress, Tours, France and she has given oral presentations at over 10 national and international conferences and with poster presentations in over 20 conferences.

All publications and patents of Elena Krachmarova fully correspond to the specialty of the current competition "Molecular Biology".

Elena Krachmarova has also included in the documents a list of participations in scientific projects: she is the head of one national scientific project and a member of the research teams of 2 international and 12 national projects.

When consulted in WoS, Elena Krachmarova's profile is associated with over 100 citations (probably also self-citations) and a citation index of **6** (h-index, according to WoS).

Представената Академична справка за изпълнение на минималните национални изисквания показва, че гл.ас. Елена Кръчмарова покрива изискваните точки по показатели А, В, Г, Д и допълнителните изисквания по показател Е

The submitted Academic report for the fulfillment of the minimum national requirements shows that the Head Ass. Prof. Elena Krachmarova covers the required points under indicators A, B, G, D and the additional requirements under indicator E

<i>Indicators' groups</i>	<i>Indicators</i>	<i>Required points</i>	<i>Head Ass. Prof. Elena Krachmarova</i>
A	1. PhD thesis	50	50
B	4. Habilitation thesis – scientific publications in Web of Science and Scopus*	100	4 x 25(Q1) = 100
G		220	230
	Г7. Scientific publications WoS/Scopus		4 x25 (Q1) = 100 1 x 20(Q2) = 20 2 x15 (Q3) = 30

			3 x10 (SJR) = 30
	G9. Invention, patent or utility models		2 x 25 = 50
D	11. Citations (WoS/Scopus) (x2T)	60	91x2=182
		no	171.9
E	14. Participation in a national scientific or educational project		12x10=120
	15. Participation in an international scientific or educational project		2X20=40
	16. Management of a national scientific or educational project		1x20=20
	18. Funds raised for projects managed by the applicant	9400/5000	1.9

4. Assessment of the candidate's educational and teaching activities

Head Ass. Prof. Krachmarova is involved in the educational process of students from bachelor's and master's programs of the Faculty of Biology of Sofia University "St. Kliment Ohridski" and Chemical Technology and Metallurgical University, Sofia. She is a consultant in the development of one diploma thesis for the degree "Bachelor" and two for the degree "Master" (one of a student from Egypt under the Erasmus program).

5. Brief description of the presented scientific papers

In her research work, Head Asst. Prof. Krachmarova continues and deepens the direction developed in her dissertation work - *Investigation of factors influencing the biological activity of human gamma-interferon (hIFN γ) and its production as a recombinant protein.*

Two other main directions in Elena Krachmarova's scientific interests are 1) *Study of the molecular mechanisms of action of the ORF6 and Nsp13 proteins of the SARS-CoV-2 virus in infected cells. Approaches to mastering the cytokine storm* and 2) *Thermodynamics of interaction of ionic liquids with the transport protein serum albumin.*

The studies conducted by the Head Ass Prof. Krachmarova are implemented with the financial support of a significant number of Research projects. As mentioned above, she is the head of one national and a member of the scientific collectives of 12 national and 2 international projects.

6. Brief evaluation of the candidate's main scientific and scientific-applied contributions

The scientific works, including the results of the research in the first field ("*Investigation of factors affecting the biological activity of human gamma-interferon (hIFN γ) and its production as a recombinant protein*"), are 7 (5 articles from indicator G7.4, G7.5, G7.6, G7.7, G7.9 and the two patents G9.1 and G9.2). A major focus of research is the post-translational modifications by glycosylation of native hIFN γ .

An original contribution is the model structures developed for the first time by molecular dynamics (MD) simulations of glycosylated homodimers of wild-type hIFN γ in their full length. Furthermore, these model structures are one of the few MD simulations of glycoproteins described so far in the literature (G7.7). Through molecular dynamics simulations, it was found that the glycans mainly interact with the globular part of the protein, but also make sporadic contacts with the C-termini of the hIFN γ molecule (G7.7). Other results having contribution character, which are obtained using these MD simulations, are those proving the reasons for the proteolytic resistance of the N-terminal FLAG peptide of hIFN (G7.9).

Other studies aiming at clarifying the composition of the so-called "inclusion bodies" (IBs) obtained in the purification of recombinant hIFN demonstrate that nucleic acids are a typical component of the IBs and not contaminants resulting from co-precipitation. These results are original with a scientific-applied contribution character, because they open perspectives for the development of new methods for the purification of recombinant proteins from included bodies (G7.4).

Another contribution of an applied nature is the developed new method for the expression and purification of hIFN γ and its mutant analogs in a biologically active form as a soluble protein (from the cytosolic fraction) (G7.6). The method is inexpensive, easy to implement, and can be used to produce other recombinant proteins with therapeutic applications.

To find suitable storage stabilization conditions after purification of recombinant hIFN γ and its mutant analogues, Elena Krachmarova and colleagues from the research group conducted a broad screening study including 61 buffers and additives (G7.5). The most suitable storage buffer identified (Tris buffer, pH 8.0, supplemented with NaCl and 0.3 M trehalose) is an original result of an applied nature, with potential application for the formulation of hIFN γ -based pharmaceuticals.

The results of research on the second direction of scientific interests of Elena Krachmarova ("*Investigation of the molecular mechanisms of action of ORF6 and Nsp13 proteins of the SARS-CoV-2 virus in infected cells. Approaches to control the cytokine storm*"), are presented in 6 of the publications (B4.1-B4.4, G7.8, G7.10). The main object of research involving computer simulations and experimental studies are the most toxic protein of SARS-CoV-2, ORF6, and the helicase enzyme Nsp13. The created 3D computer model of ORF6, molecular dynamic simulations of its interactions with proteins of infected cells and experimental confirmation of the simulation data prove that one of the important interaction targets of ORF6 is RAE1, one of the main participants in the transport of mRNA in the cytoplasm (B4.1). Binding of RAE1 by ORF6 results in the immobilization of RAE1 on cytoplasmic membranes and blocking of important cellular functions, including the cell's immune response required to inhibit viral replication. These results are original and for the first time they propose a mechanism by which ORF6 of SARS-CoV-2 leads to genome instability.

Results of a contributing applied nature have been obtained from *in silico* and *in vitro* studies showing that hIFN γ , and more specifically peptides based on its C-termini, effectively inhibit ORF6 by forming stable, non-covalent complexes with its C-terminus (B4.2). These results present for the first time an inhibitor of ORF6 of the SARS-CoV-2 virus, and have the potential to be applied to the development of potential therapeutics for the treatment of COVID-19.

Another important focus of Elena Krachmarova's research is the NSP13 helicase enzyme of the SARS-CoV-2 virus. A comprehensive analysis of the structure, physicochemical and biodynamic properties of two pockets of Nsp13 that could be used as binding targets for potential inhibitors was performed. Based on this information, dockings were performed with databases containing a wide range of natural and synthetic compounds and their binding affinities for the two pockets. One of the existing substances found to have the best binding and inhibitory effect is the active ingredient Retonavir (G7.10), which is marketed by Pfizer as a treatment for HIV and hepatitis C under the trade name Norvir.

Other research in this direction are aimed at finding potential inhibitors of the pro-inflammatory cytokines IFN γ and interleukin-6 (IL-6), involved in the cytokine release (CRS), called cytokine storm, characteristic of SARS-CoV-2 infection. Using

MD simulations, studies of the hIFN γ -hIFN γ R1 complex formation process were conducted under different conditions (B4.3, G7.8). A result with contribution nature is the established role of heparan sulfate (HS) and its proteoglycans in all processes involving the signal transduction pathways driven by hIFN γ .

The results of Elena Krachmarova's *in silico* and *in vitro* studies prove that the fractionated form of heparin with low molecular weight (LMWH) blocks the binding of hIFN γ to its extracellular receptor and, as a result, interrupts the signaling pathways induced by it. Similarly, LMWH modulates the biological function of IL-6 by blocking the formation of the IL-6/IL-6R α /gp130 signaling complex (B4.4). The results of these studies have a scientific-theoretical and scientific-applied contribution character, shedding light at the anti-inflammatory effect of heparin and its significance for the prevention and suppression of the cytokine storm characteristic of SARS-CoV-2 infection.

Another essential part of Elena Krachmarova's publications includes results of studies of *the thermodynamics of interaction of ionic liquids with the transport protein serum albumin*. In a series of studies (G7.1-3), in which the interaction of medicinal preparations in the form of ionic liquids with the model protein bovine serum albumin (BSA) was analyzed, proved that:

1) The nine ibuprofen-based ionic liquids are characterized by a pharmacokinetic profile close to that of ibuprofen (G7.1), indicating the potential of these ionic liquids to improve drug delivery by increasing the stability and solubility of ibuprofen.

2) Eight ionic liquids based on salicylic acid (G7.2) exhibit low cytotoxicity to human fibroblasts and keratinocytes and have the ability to inhibit the cytokine IL-6 in lipopolysaccharide-stimulated keratinocytes.

3) The synthesized amino acid ester salts of the anti-inflammatory drug **naproxen** are characterized by a high binding affinity to the BSA molecule (G7.3).

The results of these studies have a scientific-applied contribution character and could be used in the pharmaceutical industry in the development of new medicinal forms and improvement of existing ones.

CONCLUSIONS:

In the procedure for the academic position " Associate Professor" in the field of higher education 4. Natural sciences, mathematics, and informatics, professional direction 4.3. Biological Sciences, and scientific specialty "Molecular biology", documents has submitted **Head Assistant Professor Elena Bozhidarova Krachmarova, PhD** .

According to the materials and documents presented to me for review, I believe that the scientific interests and contributions of Elena Krachmarova are in accordance with the profile of the announced competition. All the mandatory requirements for scientometric indicators being in the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for the Development of the Academic Staff at IMB-BAS have been met, and for some criteria are exceeded.

The candidate's works contain original scientific contributions that have received international recognition, proven by the importance of the journals in which they were published and by the large number of their citations by scientists from international scientific groups and institutions. Head Ass. Prof. Krachmarova's scientific and professional qualification is unquestionable.

Based on the fulfillment of the mandatory scientometric indicators, as well as my general assessment of her scientific activity, I consider that Head Ass. Prof. Elena

Krachmarova meets all the requirements for holding the academic position "Associate Professor".

All this gives me the reason to announce my positive assessment, and in my capacity as a member of the Scientific Jury for the announced competition, I recommend the respected members of the Scientific Jury and the Scientific Committee of IMB-BAS to vote positively for the awarding of **Head Assistant Professor Elena Bozhidarova Krachmarova, PhD** of the academic position "Associate Professor" in the scientific specialty "Molecular biology",

21.10.2024, Stara Zagora

Member of the Scientific Jury:
/Prof. Tatyana Vlaykova, PhD/