REVIEW

From: **Prof. Iva Ugrinova**, PhD - Institute of Molecular Biology, BAS

Subject: Competition for Associate Professor, published in the State Gazette, issue 52/18.06.2024

General Information:

The only candidate who submitted documents for the competition is **Chief Assistant Dr. Elena Bozhidarova Kruchmarova** from the "Regulation of Gene Activity" section at the Institute of Molecular Biology "Acad. Rumen Tsanev" (IMB) – BAS.

The document review shows that the procedure for announcing the competition complies with the requirements of the Bulgarian law on the Development of the Academic Staff. The detailed report on meeting the minimum requirements under indicator B presents four publications with Q1 rank, ensuring the required 100 points. For indicator G, ten publications and two patents are presented, providing 230 points (with the required 220). For indicator D, 182 points are calculated from citations (with a requirement of 60). For indicator E, the candidate accumulates nearly 172 points, although this indicator is not considered for the competition for Associate Professor.

Biographical Information:

Chief Assistant Dr. Elena Kruchmarova started her work as a graduate student in the "Regulation of Gene Activity" section at the Institute of Molecular Biology "Acad. Rumen Tsanev" (IMB-BAS) in 2010, under the supervision of Acad. Ivan Ivanov and Prof. Genoveva Nacheva. In 2011, she successfully completed her master's degree in "Biotechnology" at the University of Chemical Technology and Metallurgy (UCTM) – Sofia and immediately joined IMB as an assistant. She started a part-time PhD at IMB, which she successfully defended in 2018 (specialty 04.02.01). The same year, she won the competition for Chief Assistant in the "Regulation of Gene Activity" section at IMB, where she continues to work. Dr. Kruchmarova has been recognized multiple times with prestigious national awards, including an award from the Union of Scientists in Bulgaria for high scientific achievements in defending a doctoral dissertation by scientists under 35. She also received the "Eureka" award for achievements in science in 2018 and 2020.

Scientometric Indicators:

The candidate is applying for the competition with 14 publications and two patents. Her works describe original scientific results published in reputable international journals with impact factors. Under group indicator B, Elena presents four publications, all ranked Q1, with a total IF of 22.308. For group indicator G, she presents ten publications, 4 of which are ranked Q1, one is Q2, two are Q3, and two are Q4. Three publications are without IF but are in journals with SJR. From the 11 works with IF, the candidate accumulates an IF of 24.582. It's also worth mentioning the two patents, one European and one registered in the Bulgarian Patent Office. With these scientific achievements, Dr. Kruchmarova fully meets and exceeds the

minimum IMB (BAS) requirements for an associate ɪrofessor. Fifteen of her papers are published in impact factor journals, with a total IF of 48.89 according to ISI Web of Knowledge Journal Citation Reports for the respective years of publication. The high value of the published results is evidenced by the international response, with 92 citations (excluding self-citations), according to Web of Knowledge. Her h-index is 6. In 4 out of 18 articles (23%), Dr. Kruchmarova is the leading (first) researcher, reflecting her personal contribution to the research.

Evaluation of the Main Scientific Contributions:

The main original results in Dr. Kruchmarova's works, based on the provided materials (using the numbering from the list of scientific publications), can be grouped into three main thematic areas:

1. **Investigation of the Molecular Mechanisms of Action of the ORF6 and Nsp13 Proteins of SARS-CoV-2 in Infected Cells and Approaches to Controlling the Cytokine Storm**:

- SARS-CoV-2 blocks the innate immune response by interrupting mRNA export from the nucleus to the cytoplasm and blocking interferon signaling pathways. Elena's research focuses on the molecular mechanisms of action of the ORF6 and Nsp13 proteins of SARS-CoV-2 and ways to control the cytokine storm. ORF6 has been identified as the most toxic protein of SARS-CoV-2. In **silico** (computer simulations) and **in vitro** (experimental) studies, it was shown that ORF6 blocks the export of newly synthesized mRNA from the nucleus to the cytoplasm by binding to the cellular protein RAE1, a participant in this process. This leads to the immobilization of RAE1 on intracellular membranes (ER, Golgi, etc.), blocking essential cellular functions, including the immune response. This blockade causes the accumulation of DNA-RNA hybrids (R-loops), leading to replication stress and impaired cell cycle progression.
- A 3D model of ORF6 showed that **hIFNγ-based peptides** effectively inhibit its function, forming stable complexes with it, restoring interferon signaling and cell cycle progression. These results, for the first time, reveal the mechanism of action of ORF6 and offer **hIFNγ** as an inhibitor for future therapeutic applications.
- **Nsp13** is a helicase essential for unwinding double-stranded RNA and plays a key role in the replication and transcription of the viral genome. Structural and biophysical analyses of Nsp13 identified two binding pockets that can serve as targets for inhibitors. Docking tests revealed that **Retonavir**, a component of the drug **Norvir**, showed high affinity for these pockets, suggesting its potential use as an inhibitor of Nsp13 in the fight against COVID-19.

Main Contributions:

- Discovery of the ORF6 mechanism leading to replication stress.
- Identification of **hIFNγ** as the first reported inhibitor of ORF6.
- Mechanism of Nsp13 inhibition by **Retonavir**.
- Strategy for using **heparin** as an inhibitor of the cytokine storm.

2. **Investigation of Factors Affecting the Biological Activity of Human Gamma-Interferon (hIFNγ) and its Production as a Recombinant Protein. Approaches for Inhibiting the Overexpression of hIFNγ in Some Diseases**:

- **hIFNγ** is a secreted glycoprotein critical for immune response. Glycosylation of its two centers (Asn25 and Asn97) is essential for its stability and secretion but does not affect its activity. Molecular dynamics simulations showed that glycans interact with the C-termini of the protein, increasing its stability and aiding the formation of biologically active truncated forms in the bloodstream.
- It was found that **hIFNγ** produced in **E. coli** forms inclusion bodies (IBs) containing nucleic acids, likely participating in the aggregation of the protein via electrostatic interactions. This discovery is important for improving recombinant protein purification methods.
- A method for expressing and purifying **hIFNγ** and its mutants in soluble form using **RTX protease** has been developed. This method ensures high purity and yield and is applicable for therapeutic protein production.
- A systematic study of buffers and additives for stabilizing **hIFNγ** and its mutants was conducted. The study identified an optimal buffer for long-term storage, which is significant for pharmaceutical formulation.

Main Contributions:

- First published models of glycosylated hIFNγ homodimers.
- Discovery of the proteolytic resistance mechanism of the N-terminal FLAG peptide.
- Clarification of the role of nucleic acids in inclusion body formation.
- Developed a method for expressing and purifying soluble **hIFNγ**.
- Optimized buffer for stabilizing **hIFNγ** and its mutants.

3. **Thermodynamics of the Interaction of Ionic Liquids with the Transport Protein Serum Albumin**:

- Ionic liquids are “tunable” solvents whose properties can be modified to improve drug solubility, administration routes, and pharmacokinetics. Elena’s research analyzed the interaction of drug-based ionic liquids with **bovine serum albumin (BSA)**, a model for human serum albumin.
- **Ionic liquids based on ibuprofen** showed a pharmacokinetic profile similar to ibuprofen but with improved stability and solubility.
- **Ionic liquids based on salicylic acid** exhibited low cytotoxicity and the ability to inhibit **IL-6** in keratinocytes, with higher affinity to BSA, suggesting improved treatment of skin diseases.
- **Ester salts of naproxen** showed high binding affinity to BSA, and thermodynamic analysis demonstrated how esterification could affect drug binding.

Main Contributions:

- Demonstrated potential of ionic liquids to improve the solubility and stability of ibuprofen.

- Created **salicylic acid-based ionic liquids** with improved efficiency for treating skin diseases.
- Showed how **naproxen esterification** can influence its pharmacokinetic properties, opening new avenues for drug formulation.

In addition to original scientific results, Dr. Kruchmarova includes **two patents** in her application, one European (EP 3381935) and one Bulgarian (BG 67190 B1). These patents protect mutant analogs of **hIFNγ**, designed to target its overexpression in autoimmune and cardiovascular diseases. These **antagonists** maintain an affinity for **hIFNγ's receptor** but with significantly reduced or no biological activity. These antagonists compete with endogenous hIFNγ for its receptor without activating the intracellular signaling pathways, reducing the activity of endogenous hIFNγ. The advantage of this approach is that patients will not be subjected to immunosuppression or additional cytokines, preserving essential immune functions. As far as we know, **antagonists of hIFNγ** have not been developed to date.

The two patents protect mutant analogs of hIFNγ that are shortened by five amino acid residues at the C-terminus (producing molecules consisting of 138 amino acids) and include the following specific amino acid substitutions: **K88Q**, **K88Q_T27Y,** and **K88Q_D41N**. These mutations improve the stability of the protein and either preserve or even enhance the affinity for the receptor while significantly reducing biological activity (by 2 to 4 orders of magnitude). These mutants have strong competitive potential with wild-type hIFNγ and may serve as potential **candidate therapeutic agents** for treating diseases associated with hIFNγ overexpression.

Main Contributions:

- Development of an **innovative approach** for targeted therapy using **hIFNγ antagonists**.
- **Patented mutant analogs of hIFNγ** with reduced activity and preserved receptor affinity.
- The mutants demonstrate potential as **candidate drugs** for autoimmune and cardiovascular diseases.

Evaluation of Additional Indicators:

Project Activity and Financial Support:

Dr. Kruchmarova has participated in **nine national** and two **international research projects**. She has led one scientific project funded by the **BAS program** for supporting young scientists and PhD students. Currently, she is involved in three research projects funded by the **National Science Fund** and the **Ministry of Finance**.

Her project activity is more than satisfactory, contributing significantly to the overall success of the IMB.

Participation in International and National Forums:

Dr. Kruchmarova has given **over ten presentations** at various national and international scientific forums and **20+ poster presentations**. In 2023, she was invited to speak at the **47th FEBS Congress** in Tours, France, where she gave an excellent presentation.

Teaching Activities:

Dr. Kruchmarova has been a consultant for preparing two master's theses, one of which was through the **Erasmus** program at UCTM-Sofia.

Conclusion:

In summary, the brief analysis of Dr. Elena Kruchmarova's publications convincingly demonstrates the results' high scientific value. Eight out of 14 of her publications are ranked in **Q1** journals, which is a sufficient argument for a positive evaluation. The candidate undoubtedly possesses the qualities of a well-established researcher characterized by analytical thinking, enthusiasm, and dedication.

Based on my personal impressions from our joint work on the national program "BioActiveMed" of the Ministry of Education and Science, I can conclude that **Dr. Kruchmarova** is an excellent scientist who combines the ability to work in a team, mentor younger colleagues, and advise more experienced peers. She is a valuable scientist and a respected colleague who fully deserves the position of Associate Professor at the **Institute of Molecular Biology – BAS**.

I consider Dr. Kruchmarova **fully deserving of the academic title of Associate Professor** at IMB.

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Prof. Iva Ugrinova,PhD