EXPERT OPINION

By: Prof. Ivanka Georgieva Tsacheva, PhD, Department of Biochemistry, Sofia University “St. Kliment Ohridski”, member of the scientific jury appointed by order No 169-ОБ / 01.10.2025 of the Director of Institute of Molecular biology "Rumen Tsanev", Bulgarian Academy of Sciences, Assoc.Prof. Anastas Gospodinov, PhD.

Re: The materials submitted for participation in a competition for the academic position „Associate Professor" at the Department of Gene Regulation, Institute of Molecular biology "Rumen Tsanev", Bulgarian Academy of Sciences in Professional area 4.3. *Biological sciences*, scientific specialty "*Molecular biology*".

The competition for the academic position “Associate Professor” in Professional area 4.3. *Biological sciences*, scientific specialty "*Molecular biology"* has been launched for the needs of the Department of Gene Regulation, Institute of Molecular biology "Rumen Tsanev", Bulgarian Academy of Sciences in SG no. 66/12.08.2025. Assist. Prof. ROSITSA GEORGIEVA TSEKOVSKA, PhD, is the only applicant who has submitted documents for this competition within the deadline regulated by the law. She currently works on a permanent position at the same department.

General presentation of the procedure and the applicant

The presented materials are in compliance with the requirements of the Act for the Development of the Academic Staff in Republic of Bulgaria, the Regulations for its implementation, and the Regulations for the conditions and the order for acquiring scientific degrees and holding academic positions in the Institute of Molecular biology "Rumen Tsanev", Bulgarian Academy of Sciences. They also meet the recommended criteria for holding the academic position of “Associate Professor” in Professional area 4.3. Biological sciences. The documentation for the competition is structured in a way that fully reflects the educational, scientific and applied research activities of the applicant in both qualitative and quantitative aspects.

Assist. Prof. Dr. Rositsa Tsekovska graduated from Sofia University of Chemical Technology and Metallurgy in 1999 with a Master degree in Chemical engineering. She defended her PhD thesis "Non-enzymatic glycosylation of proteins in *Escherichia coli*" in 2005 at the Department of Gene Regulation, Institute of Molecular biology "Rumen Tsanev", Bulgarian Academy of Sciences. She began her academic career at the same department in 1999 as a specialist chemist. From 2005 to 2011 she was a research associate, second degree, and from 2011 till now she is a chief Assistant Professor at the Department " Gene Regulation".

Assist. Prof. Dr. Rositsa Tsekovska has authored 27 articles in peer-reviewed and indexed journals including 16 scientific articles with a total **IF of 53,138**, and total **SJR 17.656**, 3 articles in journals without IF and 3 chapters in books published by a foreign publishing house. According to Scopus/Web of science research databases, the presented papers are cited 168 times. Twenty-two publications are presented in the competition for the academic position "Associate Professor". Among them, distributed in quartiles as follows: Q1 - 7 papers, Q3 - 8 papers, Q4 - 1 paper and 3 book chapters. Her H-index is 4. The results of the research activity were reported at 23 international and national scientific forums. The intensive scientific and applied research, and educational activity of Assist. Prof. Dr. Rositsa Tsekovska and her professional skills in the field are reflected in her participation in 4 projects. She was the principal investigator in two of the projects. In 2020, she was the supervisor of the thesis of a student at the Bachelor's Degree Program in Biotechnology, on the topic: "Comparative analysis of glycation end products in cultured and wild Atlantic salmon."

Assessment of academic achievements of the applicant

Scientific papers

The reference for compliance with the minimal state requirements in accordance with Art. 2b of the Act for the Development of the Academic Staff in the Republic of Bulgaria for Professional area 4.3. *Biological sciences,* scientific specialty "*Immunology"*, indicates that the applicant research achievements fully fit the stipulated criteria, as follows:

✓ Indicators of group A: PhD thesis	**50 p.** (min 50)
✓ Indicators of group B: monograph	**107 p.** (min 100)

✓ Indicators of group C: research articles	**225 p.** (min 200, *institutional requirement of min 220)*
✓ Indicators of group D: citations	**335 p.** (min 50, *institutional requirement of min 60*)
✓ Indicators of group E: projects	**120 p.** (*not required*)

Clearly, the applicant's academic achievements exceed the minimal state requirements. Assist. Prof. Dr. Rositsa Tsekovska also exceeds the additional requirements set by the Institute of Molecular biology "Rumen Tsanev", Bulgarian Academy of Sciences.

Scientific and applied research contribution

The applicant's research contribution is in the field of Molecular Biology.
Major scientific contribution:

✓ *Non-enzymatic glycosylation.*

The process of non-enzymatic glycosylation (glycation) is the subject of research in a large part of the publications in connection with this competition. It is presented as possible not only in higher eukaryotes, but also in prokaryotes, with the subject of the experiments being *Escherichia coli*. It was found that recombinant proteins synthesized in transformed bacteria are also glycated and the effect on human and bacterial physiology was monitored.

- A relationship has been established between glycation end products during storage of recombinant human IFN-γ and structural and functional changes through proteolysis in the cytokine, resulting in loss of biological activity and the no less dangerous occurrence of immunogenicity.
- Approaches to suppress glycation in E. coli using recombinant human IFN-γ through the use of various organic substances have been analyzed. Seven have been identified - acetylsalicylic acid, aminoguanidine, arginine, vitamin B1, the vitamin B6 vitamers pyridoxine, pyridoxal 5'-phosphate and pyridoxamine - which lead to an increase in the biological activity of recombinant human IFN-γ (rhIFN-γ).

- Arginine has been shown to be an inhibitor of protein aggregation of rhIFN-γ, of the accumulation of one of the major glycation end products (N^{ε}-carboxymethyl(lysine)), causing a change in the protein structure of the cytokine.
- A correlation has been established between carboxymethyllysine (CML) and two diseases – diabetes and chronic kidney disease.
- Fructosamine levels have been shown to be elevated in diabetics with diabetic nephropathy (DN) compared to diabetics without DN, which identifies fructosamine as a potential biomarker for glycemic control in diabetic patients and in particular in patients with DN.
- It has been shown that glycation in *E. coli* is initiated by endogenously formed metabolites, rather than by exogenously entering the cells substances with glycating potential.
- Oxidative stress has been studied as a key factor in modulating glycation. Resveratrol, characterized by its antioxidant properties, has been found to strongly inhibit the formation of advanced glycation end products, while glucosamine has a minor effect.
- A new function of the *E. coli* enzyme phosphoglucose isomerase (Pgi) has been discovered - to catalyze the deglycation of proteins.

✓ *Non-enzymatic DNA degradation.*

- Deoxyribonuclease activity of 2-methyl-4-phenylhexa-2,3-dienoic acid, S-2-methyl-4-phenylbuta-2,3-dienoic acid and R-2-methyl-4-phenylbuta-2,3-dienoic acid has been identified, which catalyzes the cleavage of the phosphodiester bond in DNA.

✓ *Nanotechnology*.

- Several publications have presented developed reliable strategies for grouping and risk assessment of nanomaterials (NM), their applicability in EU regulatory frameworks and their integration with the "Safety by Design" concept.

A significant part of Dr. Tsekovska's main contributions are original and show for the first time that:

1. Recombinant protein (rhIFN-γ), produced by the intestinal bacterium E. coli, is glycated during its synthesis in bacterial cells, which causes structural changes, loss of biological activity and immunogenicity of interferons.
2. Glycation contributes to the “aging” of bacteria in the phases of active division and dormancy.
3. Chronic kidney disease correlates with significantly higher serum levels of N□-carboxymethyl(lysine) than type 2 diabetes mellitus.
4. Glycating agents in *E. coli* have an endogenous origin.
5. The glycolytic enzyme phosphoglucose isomerase of E. coli exhibits deglycating activity.
6. Allenoic acids exhibit DNA nuclease activity.

Conclusion

All formal requirements specified in the Act for the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its implementation, and the Regulations for the conditions and the order for acquiring scientific degrees and holding academic positions in the Institute of Molecular biology "Rumen Tsanev", Bulgarian Academy of Sciences, have been fulfilled. Convincing evidence for scientific and applied research, and educational activity of high quality are presented. The assessment of their significance on my part allows me to **strongly recommend** to the esteemed scientific jury, appointed by order No 169-ОБ/ 01.10.2025 of the Director of Institute of Molecular biology "Rumen Tsanev", Bulgarian Academy of Sciences, Asoc. Prof. Anastas Gospodinov to issue a report-proposal to the Scientific Council of the Institute of Molecular biology "Rumen Tsanev" for the election of Assist. Prof. Dr. ROSITSA GEORGIEVA TSEKOVSKA for the academic position “Associate Professor” in Professional area 4.3. *Biological sciences*, scientific specialty "*Molecular Biology"*.

19.11.2025 Expert opinion author:

Sofia Prof. Ivanka Tsacheva, PhD