REVIEW

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Regarding:: The application of Assist. Prof. Rositsa Georgieva Tsekovska, PhD in the competition for the academic position of "Associate Professor" in the scientific specialty "Molecular Biology" from Professional Direction 4.3 "Biological Sciences", announced in the State Gazette No. 66/12.08.2025 for the needs of the "Regulation of Gene Activity" section of the Institute of Molecular Biology "Acad. Rumen Tsanev" - Bulgarian Academy of Sciences.

General information

The review was prepared in connection with a decision of the first meeting of the Scientific Jury, appointed by Precept No. 169-OB/01.10.2025 of the Director of IMB, held on.10/16/2025. The only candidate in the competition, Assist. Prof. Rositsa Tsekovska has submitted proper documentation, in accordance with the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the Implementation of the LDASRB and the Regulations for the Development of the Academic Staff in IMB. The selection procedure is carried out in full accordance with the stages and deadlines set out in the Law.

Biographical note

Rositsa Tsekovska graduated in 1999 from the University of Chemical Technology and Metallurgy (HTMU) – Sofia with a Master's degree in Chemical Engineering. Her thesis is in the field of molecular biology and was prepared under the supervision of distinguished scientists from IMB. In the same year, she joined IMB as a specialist chemist, and in 2000 she was enrolled as a PhD student in the section "Regulation of Gene Activity" with scientific supervisors Prof. Ivanov and Assoc. Prof. Mironova. In 2004, she defended her thesis for the acquisition of the PhD degree on the topic: "Non-enzymatic glycosylation of proteins in *Escherichia coli*". In 2005, she was appointed as a research assistant, and in 2011, as an assist. prof. in the section "Regulation of Gene Activity" of IMB, where she works until now. Her experience in the specialty is nearly 26 years. Dr. Cekovska's main interests are related to the study of spontaneous non-enzymatic glycosylation of proteins (bacterial and human), the loss of activity of the corresponding proteins, the resulting pathological processes, as well as the finding of suitable agents with aglycating properties to suppress the unwanted transformation.

Fulfillment of the requirements for holding the academic position of "associate professor"

In fulfillment of the minimum requirements for acquiring the academic title of "associate professor", set forth in the Regulations for the Application of the LDASRB and the Regulations for the Development of the Academic Staff of the Institute of Molecular Biology of the Bulgarian Academy of Sciences, Dr. Tsekovska has presented a detailed report on indicators from A to E, as follows:

Indicator	Minimum number of points	Points
A: PhD thesis	**50**	**50**
B: Publications in journals included in WoS or Scopus, equivalent to a habilitation thesis - **6 publications**	**100**	**107**
Г: Publications in journals included in WoS or Scopus. Chapter of a book or monograph. **13 scientific publications and 2 book chapters from international publishers**	**220**	**225**
Д: Citations **168 citations in WoS or Scopus publications, presented in a separate appendix**	**60**	**336**
E: Participation in projects:		
E15: International – **2**	**-**	**40**
E16: National Project Manager - **2**	**-**	**40**
E18: Funds raised for projects – **200 000 lv.**	**-**	**40**
Total	**430**	**836**

The table shows that the candidate significantly exceeds the minimum requirements set out in the IMB Regulations for acquiring scientific degrees and holding academic positions. Her active publication activity is impressive, mainly in journals, refereed and indexed in international databases. This fact, as well as the significant number of citations noted, are an excellent certificate of the quality of her scientific developments. Also, in the absence of a special requirement for participation in projects, Dr. Tsekovska has not only taken part in 2 international projects, but has also led 2 projects at the Scientific Research Fund of the Ministry of Education and Science, thereby contributing to the quality of scientific research in IMB. In addition to the indicators above, the candidate has participated with reports and posters in 23 scientific forums - 10 international and 13 national, including with international participation. The data on her educational activities shows that she was a consultant to one graduate student from the Faculty of Biology of the Sofia University.

Evaluation of scientific contributions

Studies with the model protein recombinant human interferon gamma (rhIFNγ)

An important part of Dr. Tsekovska's contributions are the result of scientific research conducted in continuation of the achievements reflected in her PhD thesis. In her thesis, the author proves that glycation also occurs in bacteria (*Escherichia coli*) and it affects not only native proteins, but also recombinant ones (on the example of rhIFNγ), which affects the quality of biotherapeutics produced with this technology.

In continuation of these studies, the main structural changes in proteins as a consequence of the Maillard reaction and the Amadori transformation have been clarified. It has been proven that the storage of proteins leads to the formation of glycation end products, which are the basis of drastic changes in the structure, such as proteolysis and covalent dimerization. Proteolysis leads to partial, and dimerization to a complete loss of activity of rhIFNγ. This result is of essential importance for the application of recombinant biotherapeutics in clinical practice.

In the search for ways to suppress these undesirable processes, Dr. Cekovska has studied a number of potential anti-glycating agents applied to the bacterial culture medium. Of particular importance in these experiments is that the activity of the protein was measured not only immediately after its preparation, but also over time, which is a useful indicator of the degree of stabilization of the protein structure. Aspirin was chosen as the most suitable for this type of treatment, as it gives the most durable preservation of the activity of rhIFNγ. These studies provide valuable guidance on how to obtain quality products with recombinant technology.

Dr. Cekovska has investigated another problem for the quality of the produced protein, namely non-covalent aggregation, which can occur during the isolation and purification processes. Attempts have been made to add arginine to the culture medium and during the purification processes, as a known inhibitor of the formation of Amadori products and glycation end products from the pharmaceutical industry. It has been shown that arginine in the renaturation step during the purification significantly reduces the levels of carboxymethyl lysine (a major glycation end product) in rhIFNγ and significantly stabilizes the protein, which is also useful as an indication for the method of purification.

It has also been shown that protein glycation is related to the aging processes in bacteria, which opens new opportunities for future research on the mechanisms of aging in other organisms.

Serum levels of advanced glycation end products in patients with various diseases

Of particular interest are the studies of serum levels of carboxymethyl lysine (CML) in patients with chronic kidney disease (CKD), those with type 2 diabetes mellitus (T2DM) and patients with both diseases (CKD+T2DM). Contrary to expectations that elevated glucose levels in diabetic patients would lead to high levels of CML, it turns out that serum levels of CML are highest in CKD, much lower in CKD+T2DM and lowest in T2DM. This leads the authors of the publication to the hypothesis that in diabetic patients there is a mechanism for cleansing the blood of CML, which is not present in CKD. The subject of future research by Dr. Cekovska, as she declares, will be the verification of this hypothesis. The results make it clear that elevated CML can serve as a marker for the development of CKD.

In other experiments with diabetic patients, it has been shown that the levels of one of Amadori's products, namely fructosamine, are particularly high in patients with established diabetic nephropathy, making it suitable as a biomarker for glycemic control in these patients.

Medicinal products with a high content of human interferons alpha and beta, which are widely used to treat various diseases, have been studied. Glycation end products of hIFNβ have been found in a preparation for the treatment of patients with multiple sclerosis. The examination of the patients' serum shows the definite presence of antibodies against the glycation end product, which raises questions about the efficiency of this drug. This work proves the direct connection of Dr. Tsekovska's scientific research with clinical practice and the useful advice that can be given to manufacturers and doctors.

Studies with model animal proteins. Approaches to suppress glycation

Rat histone H1 and bovine serum albumin BSA were used in the experiments. Glycation of H1 was studied in lysates of E. coli cultured in two types of media – rich in nutrients and minimal (containing no carbonyl compounds). It was found that differences in the media did not affect the degree of glycation of the protein. This indicates that glycation is the result of the action of endogenous factors for the bacteria and has no connection with the cultivation medium.

Based on the results obtained, a study was conducted on the potential of toxic carbonyl compounds to penetrate the bacteria from the culture medium. Dicarbonyl 3-deoxyglucazone was used as a target substance. According to the results, the compound does not penetrate, but is released from the bacteria into the medium. This proves the claim of glycation under the influence of internal factors, but also raises the question of the mechanisms that limit the penetration of toxic substances in *E. coli*.

Other experiments on the glycation of the two proteins in different media, including in a suspension medium from the plant Dactylis glomerata L., were conducted in order to determine the influence of L-lysine and oxidative stress on the process of non-enzymatic glycosylation. The results obtained were somewhat unexpected, but are important for identifying environmental factors influencing the undesirable process. It was shown that the role of L-lysine is reduced to suppression in the early stages, but acceleration of the formation of glycation end products, while oxidative stress triggers protective mechanisms that inhibit the process.

In attempts to find substances with pronounced aglycation properties, the effect of resveratrol, glucosamine and a number of purine derivatives on the glycation of BSA was studied. The results highlighted resveratrol (a plant polyphenol) and the purines theophylline and hypoxanthine as moderate inhibitors of glycation. An interesting future study would be related to the chemical basis of this effect of purine derivatives.

As a particularly distinctive contribution in this group, I would like to point out the newly discovered function of the enzyme phosphoglucose isomerase (Fgi) from *E. coli* to catalyze the deglycation of proteins. This property of the enzyme opens a wide field for future research. Since Fgi is highly conservative, the authors hypothesize that it may act as a glycation inhibitor in other living organisms as well.

Non-enzymatic DNA degradation

Given the fact that substances with DNA-degrading function have a recognized potential for application as antibiotics and chemotherapeutics, Dr. Cekovska has conducted an extensive study with a series of allenic acids and a substrate supercoiled plasmid DNA. Promising results were obtained for irreversible cleavage of the phosphodiester bond and the absence of restoration of the supercoiled structure under the action of enzymes. The mechanism of non-enzymatic degradation remains to be investigated.

Nanotechnology

Dr. Tsekovska's research in the field of nanotechnology is related to the implementation of tasks under an international project funded by the EU - Horizon 2020 program. Her contribution is dedicated to developing a strategy for grouping and risk assessment of nanomaterials (NM) with the aim of their safe application in various fields. The problem to be solved is the wide variety of NM with unique properties, which makes it difficult to individually assess their toxicity to humans and the environment. As a result of her work, an electronic platform "Template Wizard"

was developed and presented, which provides a reliable tool for building templates and targeted collection and reporting of data from scientific experiments. This tool is of great importance for the unification and widespread use of experimental data generated by different researchers, in order to ensure compliance with European legislation related to the environment and human health and the development of nanotechnology.

Other contributions

Dr. Tsekovska also presents some of her review publications dedicated to the glycation of proteins and the accompanying changes in their biological activity. They also include results from her own research. One of the reviews is distinguished by its wide interest among the scientific community and high citation rate.

In the process of work, a unique collection of monoclonal antibodies against advanced glycation products has been created with applications in both scientific research and biotechnology.

Conclusion: The documents presented by Assist. Prof. Dr. Rositsa Tsekovska reveal a rich scientific research activity with excellent scientometric indicators, which not only cover, but also significantly exceed the minimum criteria for holding the academic position of "associate professor", enshrined in the ZRASRB and the Regulations for the implementation of the Law. It is striking how purposeful and consistent the research conducted by the candidate is. The results obtained enrich the knowledge of the processes occurring with proteins after their synthesis and outline some risks for the quality of biotechnology products. At the same time, they also have a wide potential for application in a number of areas such as therapy with products from recombinant technologies, identification of new markers for the development of pathological processes, etc. A strong impetus in the development of Dr. Tsekovska's scientific career is her participation in a project under the Horizon -2020 program. Her work on the project has led to a program platform closely related to the safety of nanomaterials, which expands and enriches the sphere of her scientific interests. Undoubtedly, the candidate is a scientist with great opportunities for future research and excellent prospects.

Based on the above, I strongly recommend that the esteemed Scientific Jury award Dr. Rositsa Georgieva Tsekovska the academic title of "Associate Professor" in the specialty "Molecular Biology" from Professional Field 4.3. "Biological Sciences".

11/12/2025 Reviewer:

/prof. M. Dimitrova/