

Review

Considering the competition for the **Academic position "Professor"** in area of Higher education 4. Natural sciences, mathematics and informatics, **professional field 4.3 Biological Sciences, scientific specialty "Biophysics"** for the needs of Biomacromolecules and Biomolecular Interactions Department at the **Institute of Biophysics and Biomedical Engineering (IBPhBME)**, Bulgarian Academy of Sciences (BAS), published in the State Gazette, issue 94, pages 69-70/ 12 November 2021.

Reviewer: Prof. Galya Marcheva Staneva, PhD, IBPhBME - BAS

In the announced competition for "Professor", **the only candidate for the completion is Assoc. Prof. Svetla Zhelyazkova Todinova, PhD from IBPhBME-BAS.** The review of the submitted documents shows that the procedure for opening and conducting the competition has been followed as the documents have been prepared in accordance with the requirements of the Academic Staff Development Act in the Republic of Bulgaria, the Regulations for its implementation and the Regulations for Development of the Academic Staff (RDAS) at IBPhBME-BAS. The candidate has attached in a very diligent and logical form the full set of required documents.

Scientific profile of Assoc. Prof. Svetla Todinova, PhD:

Professional development: Svetla Todinova graduated with a master's degree in Radio Electronics Engineering from the Technical University, Sofia in 1982. Her PhD in Biophysics Svetla Todinova acquired in 2013 entitled "Thermodynamic profile of plasma proteome in malignant diseases". From 1982 to 1988, Svetla Todinova worked as a constructor and research associated in research and applied activities at the Institute of Electronics and NPP "PULMA" – BAS. In 1988, Svetla Todinova started working at the Institute of Biophysics, later renamed as IBPhBME – BAS. She pursued the positions of Research associate (1988-2018) and Associate Professor (2018- until now). The candidate stands out with years of experience in the field of research and applied activities with 33 years and 5 months of experience in the field.

Research Activity: The research activity of Assoc. Prof. Svetla Todinova, PhD is multidisciplinary and is at the interface of the fields of biophysics, biochemistry, cell biology and biomedicine. The candidate's scientific work is closely related to the topics developed in the Department of Biomacromolecules and Biomolecular Interactions" and is mostly in the field of temperature and conformational stability of native proteins in biological samples, studied mainly by differential scanning calorimetry with applications in biomedicine.

The total publishing activity of the candidate includes 57 publications, 54 of which have an impact factor (IF). After acquiring her PhD, the candidate published 47 scientific papers, of which 18

publications were used for the academic position "Associate Professor", 3 for scientific degree "Doctor of Biophysics" and an autoreferat.

Assoc. Prof. Svetla Todinova, PhD is participating in the competition for "Professor" with 26 scientific publications, 24 with IF (total IF 78,025), 1 with SJR, 1 without IF and impact rank (IR). 7 of the scientific publications are included in journals in category Q1 (quartile 1; the first 25 in the field), 11 in Q2, 7 in Q3, 1 without quartile.

The reference for fulfillment of the minimum requirements shows that according to indicators B (170 points), D (335 points), E (342 points) and E (239 points), Assoc. Prof. Svetla Todinova, PhD significantly exceeds the required number of points, as well as the minimum number of 15 publications in journals with IF, according to the specific requirements of IBPhBME-BAS for holding the academic position "Professor".

The candidate is the first or last author in 10 of the 26 publications presented in the competition for "Professor".

Extremely prestigious is the fact that Assoc. Prof. Todinova, PhD has published in international journals with high IF and quartiles such as *Molecules* (4,879), *Journal of Molecular Liquids* (5,056), *International Journal of Molecular Science* (5,924), *International Journal of Biological Macromolecules* (5.953) and others.

The overall IF of the presented publications is extremely high (78.025), which determines the scientific work of Assoc. Prof. Todinova, PhD as perspective and meets the high international standards to perform a research in the field of Biophysics.

The candidate presents a total of 395 citations, mainly from foreign authors, for which evidence was provided. The scientific activity of Assoc. Prof. Svetla Todinova, PhD measured by the Hirsch Index so far amounts to 12, which determines high recognition and acknowledgment from the international scientific community through positive citations of the work of the research group, in which she works.

Since 2018 until now, Assoc. Prof. Todinova, PhD has participated in 10 national and international projects. International partnerships are within bilateral projects with France, Hungary, Austria and Italy. The candidate is currently leading a research project from the National Research Fund (NSF) entitled "New biophysical markers for the diagnosis of coagulation disorders in high-risk pregnancies".

Assoc. Prof. Todinova, PhD has successfully supervised one PhD student and currently supervises other one and two interns from the Faculty of Biology at Sofia University, and has worked with a large number of young scientists from the Institute.

Assoc. Prof. Todinova, PhD is a member of authoritative professional organizations such as the Union of Scientists in Bulgaria, the Bulgarian Society of Biomedical Physics and Engineering and the Society "International Society for Biological Calorimetry". The candidate has reviewed dissertations of doctoral students, research projects, articles and monographs in prestigious scientific journals, etc.

Based on the scientific profile of the candidate and the above-mentioned scientometric indicators, Assoc. Prof. Todinova, PhD is formed as a highly responsible scientist capable of leading a research team and research activities by setting the scientific task, precise implementation of methods, analysis, description and discussion of the obtained results, as well as their successful defense in front of international reviewers.

Scientific contributions of the candidate: The main scientific contributions of Assoc. Prof. Svetla Todinova, PhD are formulated in 5 directions, cited below, which definitely have a fundamental and applied research character, raise and prove new hypotheses, new facts are obtained, new theories and approaches are created and with the potential to be used for the development of diagnostic methods in medicine:

1: Thermal and conformational stability of native proteins in biological samples. Application of the calorimetric approach in biomedicine.

2: Thermal stability and properties of native and chemically modified therapeutic proteins. Effect of ionic liquids on their stability and activity.

3: Calorimetric characteristics of cancer cells treated with conventional and plant based secondary metabolites. Objects of study: normal (MCF-10A) and tumor cell lines (MCF-7, MDA-MB-231), Therapeutics: miltefosine, cisplatin, herbal anticancer therapeutics; *Cotinus coggygria Scop.*

4: Morphometric and nanomechanical characteristics of platelets and erythrocytes in normal and pathological conditions.

5: Structural stability and macroorganization of the pigment-protein complexes that make up the photosynthetic apparatus of higher plants and cyanobacteria.

The main scientific contributions of Assoc. Prof. Svetla Todinova, PhD in the competition for "Professor" are organized as scientific contributions in habilitation work (1) and outside habilitation work (2):

1. Scientific contributions to the habilitation thesis are presented through the publications in indicator B4 of the detailed report on the implementation of the minimum national requirements for holding the academic position "Professor".

- 1.1 The research group, in which Assoc. Prof. Todinova, PhD works, Department of “Biomacromolecules and Biomolecular Interactions” at the IBPhBME - BAS has a significant contribution to the validation of the thermodynamic approach for establishing calorimetric markers for various diseases and the application of method in medicine. Evidence of this are the studies conducted over the past 10 years, published in prestigious international journals on the thermodynamic characterization of blood plasma / serum in multiple myeloma and colorectal cancer caused a wide international response, reflected in numerous citations of Assoc. Prof. Todinova. These studies were subsequently extended to serum of patients with multiple myeloma type IgM, IgA and Waldenström disease, where the heterogeneity of DSC profiles determined by the genetic nature of these diseases was demonstrated. DSC screening of patients with multiple myeloma in conventional treatment and after autologous hematopoietic stem cell transplantation was performed, as well as in patients with schizophrenia during antipsychotic treatment. The large-scale study, conducted by the research group, provides an opportunity to prove that the method can distinguish these diseases and provides information about the effect of the applied treatment.
- 1.2 The thermodynamic characteristics derived from the DSC profiles of rat blood plasma clearly distinguish the effect of persistent inflammation. The established similarity of the thermograms of experimental models (mouse models) with those of soft tissue inflammation in humans gives reason to believe that the calorimetric approach is suitable to be introduced into clinical practice as a non-invasive and inexpensive complementary method for detecting inflammatory processes in soft tissues.
- 1.3 Differences in the thermodynamic behavior of blood plasma related to sex and age of animal models have been also demonstrated. A connection between the thermodynamic behavior of the blood plasma proteome and the development of pre- and neoplastic lesions in the colon of rats treated with dimethylhydrazyl (DMH) was established. It has been demonstrated that the formation of precancerous lesions in female and male animals leads to significant changes in calorimetric parameters, and therefore these parameters can be considered as indicators of malignant transformation of intestinal cells and progression of tumor formation. Evidence has been presented that the calorimetric method is highly sensitive to the occurrence of premalignant lesions and carcinomas in experimental rat models.
- 1.4 DSC was applied for thermodynamically characterization of the aging process of red blood cells isolated from women with miscarriages and patients with neurodegenerative (ND) diseases (Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis).

Determining thermodynamic stability during red blood cell aging may serve as an additional criterion for assessing the risk of miscarriage. The established differences in the calorimetric enthalpy of the DSC curves in aging cells between the studied ND pathologies suggest differences in the energy required to disrupt the interactions stabilizing the structure of Hb and cytoskeletal proteins. These differences in thermodynamic parameters between normal and pathological conditions reflect significant modification in the conformation of cellular proteins and may serve as promising biomarkers for ND diseases.

- 1.5 For the first time, by using DSC and circular dichroism, fundamental research was conducted on the thermal denaturation of hemocyanins (oxygen-carrying protein in invertebrates) and its isoforms isolated from the hemolymph of *Helix lucorum* (HIH) and *Helix aspersa maxima*. The determined temperature and conformational stability of hemocyanins from these organisms and their isoforms makes them potential candidates for the production of immunostimulatory drugs.

2. Scientific contributions outside habilitation work (publications in indicators G7 and 8):

- 2.1 For the first time, haemocyanins from *Rapana thomasiana* and *Helix lucorum* have been conjugated to ferulic, rosemary (RA) and folic (FA) acids. In vitro experiments reveal the inhibitory effect of the tested conjugates on the viability of MCF-7 and MDA-MB-231 breast cancer cells. Temperature and conformational changes resulting from the reorganization of the hemocyanin protein molecule are characterized. The results obtained could serve as a basis for the development of new pharmaceutical products.
- 2.2 The effect of biocompatible ionic liquids (IT) on the structure, stability and antitumor properties of hemocyanins isolated from the haemolymph of *Rapana thomasiana* (RtH) and *Helix pomatia* (HpH) was evaluated. The structural changes of the RtH protein induced by IT ([Chol] [Arg] and [Chol] [Lys]) increase its selectivity and cytotoxicity to breast cancer cells. It was found that at the highest applied concentration of β -HpH-[Chol] [Trp], the complex produced the same cytotoxic effect on MDA-MB-231 cells as that reported for doxorubicin.
- 2.3 The potential of a series of ionic liquids containing the biocompatible choline cation [Chol] and a negatively charged amino acid to stabilize insulin dissolved in an acidic medium - pH 2.0 - was evaluated. It has been found that in the presence of [Chol] [Glu] and [Chol] 2 [Asp], the temperature stability of insulin increases. The results have the potential to develop new low molecular weight insulin-stabilizing compounds.

- 2.4 The interactions of ionic liquids (IL) composed of cations of alkyl esters of L-amino acids and the anion ketoprofen (KETO) with human serum albumin (HSA) were studied for the first time. All HSAs in the presence of IL were found to cause a change in the secondary structure of the HSA molecule, but no aggregation was observed. This study of ketoprofen-based HSA complexes with IL is of interest for the development of new drug formulations.
- 2.5 For the first time, an inhibited interaction between serum albumin HSA and graphene oxide GO in blood plasma has been demonstrated, suggesting that the protein is protected from possible toxic effects of GO under natural conditions.
- 2.6 The potential of the differential scanning calorimetry method for selection of the most effective drugs against tumor cell lines was studied. By DSC for the first time the effect of two anticancer drugs is analyzed. They differ in their main action, miltefosine and cisplatin (cis-Pt), on breast tumors, the less metastatic — MCF-7 and the highly metastatic — MDA-MB -231) and a normal breast epithelial (MCF-10A) cell line.
- 2.7 The potential of extracts from the medicinal plant *Cotinus coggygria Scop* to inhibit the viability of MCF7 cancer cells has been studied. *C. coggygria* has been shown to block the S-phase cell cycle in MCF7 cells and trigger apoptosis by inducing DNA damage. DSC analysis clearly showed that *C. coggygria* extract significantly modifies nuclear and cytoplasmic proteins and RNA. The established sensitivity of the DSC approach confirms that the method has a high biomedical potential for selection of the most effective drugs on cell cultures of different origins.
- 2.8 The mechanical properties and topography of platelets isolated from patients with various pathologies have been established, as well as for defining the specific effect of polymorphisms in thrombophilia genes on nanomechanics and the state of platelet activation.
- 2.9 For the first time with atomic force microscopy the effect of carrying the PIA1 / A2 polymorphism in the glycoprotein IIb / IIIa (GPIIb / IIIa) gene on the morphometric and mechanical characteristics of platelets isolated from healthy individuals and patients with deep vein thrombosis (DVT) was assessed. Carrying the PIA2 allele has been shown to modulate the state of activation, morphology and elasticity of the platelet membrane. The findings contribute to the understanding of the factors responsible for hemostasis and thrombosis.
- 2.10 Platelets from patients with early pregnancy loss have been shown to be significantly more active than controls (women without established pregnancies and women with normal pregnancies), with pronounced cytoskeletal rearrangements, which include:

reduced membrane roughness and platelet height; strong change in the Young's modulus of the plasma membrane; increased platelet micro-particle production and higher expression of pro-coagulant surface markers. Morphometric and nano-mechanical characteristics serve as a new criterion for assessing the cause of miscarriage and offer the prospect of an innovative approach for diagnostic purposes.

The scientific product generated by the work of Assoc. Prof. Todinova, PhD has proven contributions, whose originality lies in the priority areas for the development of diagnostic methods for detecting various types of pathologies worldwide, which outlines a very positive outlook for future research.

Personal impressions: Over the years, Assoc. Prof. Svetla Todinova, PhD has proven to be an extremely responsible, hardworking and fair colleague who is always willing to participate in joint research projects with colleagues from the Institute and beyond. Assoc. Prof. Todinova, DSC and the department, in which she works, have successful collaborations with the National Specialized Hospital for Active Treatment of Hematological Diseases, the University Specialized Hospital for Active Treatment in Oncology and the University Multidisciplinary Hospital for Active Treatment in Neurology and Psychiatry "St. Naum", Sofia, etc., which proves the interest of hospitals in quality research conducted by the research group.

Conclusion: Based on what has been said so far, I conclude that the significant contributions of the scientific papers presented in the competition, their repercussions in the scientific literature and clearly defined scientific profile identify Prof. Svetla Todinova, PhD as a dedicated and highly qualified scientist in the field of thermal and conformity stability of proteins in biological samples studied by differential scanning calorimetry with applications in biomedicine. The scientometric indicators of Assoc. Prof. Svetla Todinova, PhD meet all the requirements for holding the academic position "Professor", in professional field 4.3 Biological Sciences, scientific specialty "Biophysics" for the needs of the Department "Biomacromolecules and Biomolecular Interactions" at IBPhBME-BAS. Therefore, I recommend to the respectful members of the Scientific Jury to recommend to the respectful members of the Scientific Council of IBPhBME-BAS to vote in favor for awarding Assoc. Prof. Svetla Zhelyazkova Todinova, PhD the academic position "Professor".