

R E V I E W

in connection with the competition for a professor in professional field 4.3. „Biological sciences“, scientific specialty „Biophysics“, announced by the Institute of Biophysics and Biomedical Engineering (IBPhBME) – BAS (Newspaper of State 41/21.05.2019 г.), for the needs of the Department „Biomacromolecules and Biomolecular Interactions“.

by: corresponding member, professor Andon Radev Kossev, DSc – IBPhBME - BAS

Just one candidate had applied for the open competition - Assoc. Prof. Dr. Sashka B. Krumova from the Institute of Biophysics and Biomedical Engineering (IBPhBME) – BAS. In 2001 She completed her university education at Faculty of Biology of Sofia University “St. Kl. Ohridsky” with MS degree. The same year She started her career in science in IBPhBME – BAS and the Institute of Plant Biology of Hungarian Academy of Sciences. In 2006 Sashka Krumova completed successfully her PhD thesis entitled “Thermal stability of pigment-protein complexes in thylakoid membranes of higher plants. Thermo-optic effect” at IBPhBME – BAS. From 2008 to 2009 Dr. Krumova was postdoc in the Wageningen University, the Nederland. Since 2009 she continued her research in IBPhBME – BAS as a specialist and in 2010 she became an assistant professor. Since 2013 Dr. Krumova became an associate professor. Since March 2019 Dr. Krumova is a scientific secretary of the Institute.

Publication activity of Dr. Krumova

The publications of Dr. Krumova are arranged up to the requirement – in three separate lists. The first two of them are the publications that had been used in the previous procedures – in relation with PhD thesis and the position of associate professor. These publications have been already assessed positively and will be no assessed further.

For the current competition the candidate has presented in total 21 papers, 18 of which are with impact factor (total impact factor of 51.4), and 10 of which are published in journals with Q1 rang. One of the papers is with SJR and 2 papers are published in international journals without impact factor, nor SJR. The papers are published in the period 2013–2018. The majority of the papers have been published in prestige international journals as: *Analytical Chemistry* (IF:5.636), *Langmuir* (IF:3.993), *Biochimica et Biophysica Acta - General Subjects* (ISI IF:3.679), *International Journal of Biological Macromolecules* (IF:3.671), *Biophysical Journal*

(IF:3.632), *Photosynthesis Research* (IF:3.091), *Journal of Photochemistry and Photobiology B* (IF:2.96), *Journal of Bioenergetics and Biomembranes* (IF:2.914), *Colloids and Surfaces A: Physicochemical and Engineering Aspects* (IF:2.752), *Plant Physiology and Biochemistry* (IF:2.756), *Thermochimica Acta* (five publications with IF:2.181), *Journal of Plant Growth Regulation* (IF:2.073), *Journal of Molecular Recognition* (IF:1.868), *European Biophysics Journal with Biophysics Letters* (IF:1.472).

Dr. Krumova has presented list with 137 citations of her papers. These citations are from “Web of Science” and “Scopus”. “Google Scholar” have been not used as a source of citations and thus citations in books, proceedings, dissertations, patents and so on are missing. It is worth to underline that the publications of the candidate in relation to the present completion have been cited 46 times. That is excellent indicator, especially having in mind that 6 of papers were published in 2018 and it is early to expect citations.

Scientific contributions in the papers of Dr. Krumova

In general I accept the statements of scientific contributions based on the publications with which Dr. Krumova participates in the competition and I will analyze them in two separate parts, as she did.

1. Contributions related to the collection of publications equivalent to habilitation work:

The habilitation work consists of eight publications joint under the common topic: „Calorimetric markers for detection and monitoring of patients diagnosed with multiple myeloma”. An analysis of the calorimetric characteristics of blood serum derived from patients with different types of multiple myeloma and Waldenström macroglobulinemia is presented. Common calorimetric markers for the different secretory and non-secretory forms of the disease are established. The observed changes in the calorimetric profiles of blood serum are most probably related to myeloma-specific ligands that interact with the major serum proteins and stabilize their conformation. Specific calorimetric markers for multiple myeloma are defined, that distinguish it from other hematological and oncological diseases. (publications № 1, 3, 4, 5, 6, 7 and 8 from the list of publications with which the candidate applies for professorship).

Furthermore:

- A unique calorimetric characteristic assigned to monoclonal free light chains is established, namely an endothermic transition at 57 °C or 47 °C, the presence of which is associated with the formation of amorphous aggregates of those proteins. (publications № 1 and 4);

- It is demonstrated that the changes in the electrophoretic mobility of *IgA* paraproteins are due to altered molecular interactions, related to the formation of IgA oligomers and/or complexes of this type of paraproteins with albumin in multiple myeloma with secretion of IgA. (publication № 8);

- It is established that for patients with multiple myeloma and Waldenström macroglobulinemia the observed changes in the thermograms of blood serum correlate to a large extent with the clinical status of the patients and the applied treatment. Hence, calorimetry is suitable for clinical practice utilization as a fast, noninvasive and cheap method, complementary to the routinely used so far traditional immunological tests. This is particularly true for patients with non-secretory myeloma, for who the diagnostics and monitoring largely rely on invasive techniques. (publications № 1, 3, 4, 5, 6, 7 and 8);

- For the first time the potential of differential scanning calorimetry for the discrimination of multiple myeloma is directly compared to a fluorescence method based on the emission of NADP(H), and the higher sensitivity of the calorimetric approach for detection of IgM MM cases is demonstrated. (publication № 2)

2. Contributions related to publications outside the habilitation work (13 publications):

Slightly more than 2/3 of those publications (9 publications) are in the field of Biophysics of photoexcitable membranes:

1. A number of factors that regulate the structural organization and stability of the major light-harvesting pigment-protein complexes in higher plants and cyanobacteria are characterized. Isolated photosynthetic membranes and their fragments, intact cyanobacterial cells and monomolecular layers of isolated light-harvesting complexes are studied (publications № 12, 13, 14, 19 and 20). These investigations have contributed to the deeper understanding of the mechanisms of adaptation of higher plants and cyanobacteria to the environmental conditions:

1.1. Specific structural changes in the light-harvesting complexes of photosystem 1 and 2 (LHCI and LHCII), related to the photoprotection processes of higher plants, are established:

- The switch of LHCII from light-harvesting to photoprotective state, induced via protonation of the complex, changes its conformation, stimulates the clustering of the complex and reduces its thermal stability in both Langmuir monomolecular layers and in isolated granal fragments. The monolayer studies reveal that the registered structural changes are reversible and probably associated with a higher flexibility of LHCII molecules in the protonated state, needed for the accomplishment of the photoprotective function of the complex. A plausible prerequisite for the trigger of the structural reorganization in the studied model systems, as well as in native photosynthetic membranes of higher plants, is the weakening of the molecular interactions between the trimers and monomers upon protonation, established for LHCII monolayers. (publications № 12, 13 and 14);
- The structural stability of LHCII is higher upon its interaction with photosystem 1 than with photosystem 2, which is a strong indicator for a different conformation of the complex in these two structural states that strongly differ in their ability to distribute the excitation energy between the two photosystems. (publication № 19);
- The detachment of Lhca1/4 dimer of LHCI from the core complex of photosystem 1 can be induced either by a temperature jump or by illumination with high light intensity - a thermo-optic mechanism that regulates the light harvesting function of photosystem 1. (publication № 11).

1.2. A newly developed calorimetric approach for the investigation of the structural stability of phycobilisomes (the light-harvesting complexes in cyanobacteria) in intact cells of *Synechocystis* sp. PCC 6803, i.e. in the native cellular environment of the complexes, is presented. It is demonstrated that the denaturation temperature of phycobilisomes can be used as a measure of their structural integrity when characterizing different cyanobacterial mutants with altered composition and structural organization of the photosynthetic apparatus. (publication № 20).

2. The role of exogenous growth regulators for the structural organization and functionality of the photosynthetic apparatus in higher plants is demonstrated. The treatment with

different concentrations of 24-epibrassinolide (for pea), indol-3-butyric acid, IBA, and N6-bezyladenine, BA (for *Artemisia*), as well as different combinations of IBA+BA, results in changes in the structural organization of thylakoid membranes and their photosynthetic activity. The optimal protocols for treatment of plants that lead to high photosynthetic activity and accumulation of important secondary metabolites such as polyphenols and flavonoids (in the case of *Artemisia*) are determined. (publications № 10, 16 and 21).

Furthermore:

The diagnostic potential of the method differential scanning calorimetry is explored on the basis of the thermodynamic properties of:

- *the plasma proteome in schizophrenia*. A classification of the registered blood plasma thermograms of schizophrenia patients is presented, that reflects the different extent of stabilization of the major plasma proteins. The established correlation between the changes in the thermograms (resembling or deviating from the standard “healthy” thermograms) and the effect of the applied treatment strongly suggest that the applied method could enhance the objective evaluation of patients condition. (publication № 9);
- *normal and cancer cell lines*. The comparison of the calorimetric profiles of normal human fibroblasts and human cancer cell lines (HeLa, JEG-3, Hep G2, SSC-9, PC-3, HT-29, MCF7), as well as isolated nuclei, allowed for the establishment of specific thermodynamic characteristics of the investigated cancer cell lines – strong reduction of the enthalpy of the thermal transitions associated with denaturation of the cytoplasmic proteins, the proteins of the nuclear matrix and RNA; destabilization or lack of the endothermic transition with the lowest transition temperature; reduction in the difference between the excess heat capacities of the denatured and the native state of cancer cells in comparison with that of normal fibroblasts. (publication № 17).

The different factors of stabilization and destabilization of the tetrameric soluble proteins thymidylate synthase and hemoglobin are characterized:

- The mechanism and energetics of binding of the cofactor flavin adenine dinucleotide and the substrate deoxyuridine monophosphate by thymidylate synthase are different for the thermophilic (*Thermotoga maritima*) and mesophilic (*Paramecium bursaria*)

chlorella virus-1) organisms. The denaturation temperature of the mesophilic thymidylate synthase is significantly lower than the one of the thermophilic protein, but the stabilization of the enzyme upon binding of flavin adenine dinucleotide is stronger for *Paramecium bursaria* chlorella virus-1, than for *Thermotoga maritima*. The key role of serine 88 amino acid for the binding of deoxyuridine monophosphate is confirmed for thermophilic organisms. (publication № 15);

- A relation between the changes in the morphology of red blood cells and the structural stability of their basic protein components (hemoglobin, proteins of the cytoskeleton and the plasma membrane) related to cells ageing is established. More specifically, a correlation between the extent of cell deformation, the decrease in cell roughness and strong reduction of the cells thermodynamic stability is observed. It is established that the denaturation of the cytoplasmic domain of band 3 protein affects (directly or indirectly) the structural stability of hemoglobin, probably via the enzymes of glycolysis that regulate the hemoglobin oxidation. (publication № 18).

The contributions described above have the character of gaining new knowledge (new facts and new mechanisms), and it should be noted that a considerable part of them are undoubtedly applicable in the clinical practice. For me, the relevance of the research topics of Assoc. Prof. Krumova does not raise any doubt. Soluble and membrane embedded proteins and pigment-protein complexes are investigated in their isolated state as well as in an environment close to their native one. The results from the experimental work contribute to the understanding of the relation between structure and function at different levels of the structural organization of the biomolecules. The studies conducted are interdisciplinary and fall within the scope of the following disciplines: Biophysics, Biochemistry, Cell Biology, Analytical Chemistry, Plant Sciences and Hematology. In this regard, Dr. Krumova's extensive collaboration with a number of Bulgarian and foreign research organizations is noteworthy. The Bulgarian ones include National Specialized Hospital for Active Treatment of Hematologic Diseases – Sofia, The Medical University – Sofia, The Sofia University “St. K. Ohridski” and 7 institutes of BAS. With France and Italy, this cooperation includes 2 scientific organizations from each of the two countries. Some of the experiments are performed with partners from Spain, Hungary and Czech

Republic. All this shows the scientific authority of both Assoc. Prof. Krumova and the department in which she works as a sought-after partner in the country and abroad.

Teaching and Supervising activity in IBPhBME - BAS

Assoc. Prof. Krumova has served as supervisors of regular post-graduate student that has successfully completed PhD thesis in IBPhBME – BAS. Additionally she is supervisor of an other post-graduate student that is working successfully and is on the final stage of the work.

Participation in project research with external financial support

Dr. Krumova is very active in project research with external financial support. She has participated in 13 national projects. Six of them were projects in the frame of exchange agreements of BAS with Hungary and Italy, 5 and 1 project respectively. She has worked in 3 projects in frames between Bulgaria and France, Germany and Switzerland.

Dr. Krumova has served as supervisor in two national projects - DMU02-7/18.12.2009 and project BAS – Hungarian Academy of Sciences. She has also participated in 4 international projects.

Personal impression

As a director of the institute I am under excellent impression for the work of the candidate. Ten years ago when the new department „Biomacromolecules and Biomolecular Interactions“ was established, together with the head of the department Prof. Taneva, Dr. Krumova joined successfully and contributed essentially for the powerful start of the research. Her active research is continuing up to now.

She is well build scientist with responsibility and critical attitude towards colleagues and herself. A half year ago Dr. Krumova joined the administrative executive body of the institute as Scientific Secretary. Nevertheless the short term, at this stage, I may declare with confidence my excellent impression.

Conclusion

The publications that have been presented in the present competition are on high scientific level. The scientific indicators fully responded to the law and requirements for the occupation of

the academic position “Professor” in IBPhBME – BAS. All these facts are giving me confidence to recommend to the members of Scientific Jury to recommend to Scientific Counsel of the Institute of Biophysics and Biomedical Engineering – BAS to elect Assoc. Prof. Dr. Sashka B. Krumova on an academic position “Professor”

10.09.2019 / Sofia

Signature:

/Prof. Andon R. Kossev/