REVIEW

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About the competition for the scientific position "Associate Professor" announced in State Gazette issue 30 / 15.04.2022 in professional field 4.3

"Biological Sciences" (Biochemistry) for the needs of the Department of Biochemistry,Faculty of Biology, Sofia University "St. Kliment Ohridski"

At the announced competition for the academic posission of “Associate Professor” at the Department of Biochemistry, Faculty of Biology, Sofia University, only one candidate submitted documents, who was admitted to the competition, Assistant Professor. Dr. Kirilka Stefanova Mladenova. Dr. Kirilka Mladenova was born in 1987 in Sofia. In 2011she graduated bachelor's degree in Molecular Biology, and in 2013 a master degree in Cell Biology and Pathology. Since 2017, she is a PhD in Biological Sciences. According to the attached materials , the candidate fulfil the minimum requirments of the Low for the Development of the Academic Staff of the Republic of Bulgaria and the relevant regulations for its implementations of Sofia University necessary for the academic position “Associate Professor”

The scientific papers with which Dr. Kirilka Mladenova applied for the competition are: 16 papers with IF, with a total IF 40.383 and one book chapter. A number of participations in scientific events have been attached also . 37 citations of her scientific papers have been noted.

Dr. Mladenova was the leader of 2 scientific contracts funded by Sofia University and a participant in a number of contracts funded by Sofia University , National Science Fund and the European Social Fund. Under her supervision, two diploma theses at the Department of Biochemistry were successfully defended. The teaching activity of the candidate consists of more than 1000 teaching hours per

year. It mainly includes exercises in Biochemistry in several faculties of Sofia University – Faculty of Biology, Faculty of Chemistry and Pharmacy, Faculty of Physics. Dr. Mladenova also conducts lecture courses in Biochemistry for students for different specialisations such as Agrobiotechnology and Biology in the Faculty of Biology. From these data, it can be concluded that Dr. Mladenova's teaching activity per year exceeds the teaching hours required for the scientific position "Associate Professor".

The main scientific fields in which Dr. Mladenova works are in the field of biochemistry, biocatalysis, biophysicochemistry, molecular and cellular biology. Her main scientific contributions are related to the elucidation of the molecular basis of socially significant retinal pathologies.

The main scientific field in her work are related to: the physicochemical characterization of the transmembrane protein bestrophin-1 (hBest1); characterization of the biological properties of nanoparticles, as well as study of the mechanism of action of some biologically active substances on cell lines.

On my opinion the main scientific contributions of the candidate can be divided into several groups:

1. **Investigations related to the structure , lipid interactions and localization of hBest1 in the cell membrane.**

The first group of her scientific contributions are related to the study of the properties and behavior of human bestrophin-1, to which her PhD thesis was also dedicated. This protein is known to be a calcium-dependent chloride channel that is expressed on the basolateral surface of the retinal pigment epithelium. Mutations in the gene lead to the development of pathological conditions that are accompanied by progressive vision loss and are currently incurable. Recently, hBest1 has been suggested to be related to neurodegenerative diseases (such as Alzheimer's disease, Parkinson's disease, epilepsy, etc.) .

Langmuir monolayer model was use to characterize the morphology and some physicochemical characteristics of pure hBest1 or mixed hBest1/lipid monolayers with or without the presence of Ca2+, Glu and GABA .

Analysis of the π/A isotherms, hysteresis curves, and module of compression demonstrated that the addition of Ca2+, Glu, and GABA to subphase of pure hBest1 monolayers resulted in a change in the areas of the isotherms at the same surface pressure. The largest changes in molecule area was found at the presence of Ca ++ , and least of all in the presence of GABA. In mixed monolayers of protein and unsaturated phospholipid, POPC "masked" the effect of Ca, Glu, and GABA on the surface dynamics, and a phase separation was observed between the protein and the lipid, indicating that phospholipids can influence the organization and activity of the protein in the membrane. In mixed hBest1/SM monolayers, the addition of Ca2+, Glu, and GABA leads to changes in the surface conformation, structure, self-organization, and surface dynamics of hBest1. The determination of the changes in the Gibb's free energy of these monolayers, indicate that this process is thermodynamically favorable and spontaneous mixing occure. The addition of cholesterol in both types of mixed monolayers exhibits its condensing effect. Brewster Angle Microscopy was used to characterize the morphology of pure and mixed monolayers of the protein with Ca2+, Glu, and GABA.

The films of hBest1 and hBest1GABA were shown to be homogeneous, while those of hBest1Ca and hBest1Glu contained condensed and gas phases in a different ratio with respect to the applied surface pressure. For the first time in the scientific literature, the morphology of hBest1 molecules was determined by Atomic Force Microscopy applied to Langmuirblodget films. The obtained results of the pure protein were such as : lateral dimensions of about 100 × 160 Å and height of about ∼75 Å, as well as formation of dimeric structures in the presence of Ca2+ ions with lateral dimensions of ∼200 × 670 Å and height of 220 Å and trimeric structures of dimensions : lateral 200 × 990 Å and height of 220 Å.

The effect of hBest1 expression in cells was investigated and it was shown that their morphology, growth rate and mitotic index did not change. It was established that the protein is preferentially localised in the liquid-disordered phase of the cell membrane and its expression leads to a change in the physicochemical characteristics of the membranes in the direction of an increase in the liquid-disordered membrane domains. A change in cell polarization was observed in bestrophin-transfected cell lines.

2. Contributions to research on newly synthesized nanoparticales

Another part of Dr. Maldenova's research is devoted to nanoparticles as carriers of biologically active substances. Creating carriers for pharmaceuticals and genes as well as DNA molecules biocompatible, degradable and non-toxic is essential for the treatment of various pathologies. Dr. Mladenova has studied cytotoxicity, the mechanism of inclusion of nanoparticles in cells, as well as their stability . Several types of nanoparticles have been investigated. For polyethyleneimine nanoparticles, it has been shown that the cell entry routes and the delivery efficiency of an intact and functionally active DNA molecules depend on the topology and shape of the polymer chain, Polyplexes with a more solid structure were "promising" systems for gene transfection in eukaryotic cells .

Nanoparticles based on a diblock copolymer of poly(ethylene glycol) methacrylate (POEGMA) and poly(L-lysine) and plasmid DNA have been shown to be incorporated into the cell directly through the plasma membrane rather than via the endosomal pathway and can release intact and a functionally active plasmid.

The study of triblock copolymer poly(2-(dimethylamino) ethyl methacrylate) – block-poly (ε-caprolactone) – block – poly(2-(dimethylamino) ethyl methacrylate) nanoparticles containing DNA proves that their cytotoxicity depends on their structure. Conjugates between DNA and a synthetic polymer have been shown to be biocompatible, nontoxic, easily incorporated by cells, and stable in the presence of nucleases .

3. Contributions to research of natural biological active substances .

Secretory enzymes isolated and purified from *Vipera ammodytes meridionalis* snake venom were investigated. The main toxic component of vipoxin is a heterodimer composed of secretory phospholipase A2 and a non-enzymatic subunit. These two subunits have been shown to have different pharmacological activity on cell lines. Phospholipase causes cytotoxicity, affects the structure of the cytoskeleton and leads to early apoptosis. Vipoxin and the non-enzymatic subunit exhibited a high degree of genotoxicity, while the DNA damage induced by the PLA2 subunit could be defined as moderate and unrelated to its catalytic activity. These results are promising for their use as anticancer agents.

Part of the research is aimed at studying plant extracts for use in various pathological conditions. Extracts from *Haberlea rhodopensis*, a medicinal plant, has been proven affect the cell periphery, the permeability of the membrane and lead to the disruption of the tight contacts of keratinocytes, which makes them successful for the complex treatment of pathological dermatological conditions. .

Lamium album L. extracts are well known for their therapeutic effects and are used in folk medicine. Extracts obtained with two different solvents have been shown to alter cell polarity and actin filaments independently of the solvent used and block cell polarization. These extracts strongly affect cell membrane permeability, cell adhesion properties, and cancer cell morphology, making them promising anticancer agents.

Dr. Kirilka Mladenova's research is systematically aimed for clarifying the structure and behavior of the protein hBest1, one of the main cause of significant social pathologies. She started these studies while she was working on her PhD thesis. Significant results have been obtained on the localization of the protein in the cell and the importance of the structural elements of the cell membranes for its localization and its activity. Research on nanoparticles as carriers of various agents that can be used as pharmaceuticals for various types of pathologies is also noteworthy.

In addition to her teaching and scientific activities, Dr. Mladenova also has some exsecutive duties as a member of the Mandate Committee and a member of the Faculty Council of the Faculty of Biology.

On my opinion all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria have been met in the announced competition.

Conclusion: Dr. Kirilka Mladenova has presented for the competition scientific production and citations that meet the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria for the academic position of Associate Professor. Her teaching activities also fulfills the

requirements for this position. The present scientific contributions are with original character. For the first time, the molecular organization of hBest1 protein and the importance of the membrane bilayer structure for the protein's activity have been demonstrated. The obtained results have found a wide response among the world scientific community. All this gives me ground strongly recommend to the members of the honorable Scientific Jury and to Faculty Council of the Faculty of Biology at Sofia University "St. Kliment Ohridski" Dr.. Kirilka Mladenova to be elected for the administrative and academic position of ASSOCIATE PROFESSOR in the professional field 4.3 Biological Sciences (Biochemistry) for the needs of the Faculty of Biology, Sofia University " St. Kliment Ohridski".

4.08.2022 Reviewer:

/ Prof. Diana Petkova/