

## OPINION

**From Prof. Dr. Rumiana Dimitrova Tzoneva, Institute of Biophysics and Biomedical Engineering - BAS**

regarding a competition for “Associate Professor” in a professional field 4.3. Biological sciences, scientific specialty "Biochemistry", announced in the state gazette, issue 63 from 30. 07. 2021 (correction in SG бр. 65 from 06.08.2021), for the needs of the Medical Faculty, SU "St. Kliment Ohridski”.

Assistant Professor Asya Svilenova Tsanova, is the only candidate in the announced competition. The documents provided by the candidate for holding the academic position "Associate Professor" are prepared in accordance with the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its implementation.

Asya Svilenova Tsanova graduated from Sofia University "St. Kliment Ohridski”, Faculty of Biology in 2005 with a master’s degree in “ Molecular Biology ”, specialization “ Plant Physiology”. In 2014 he successfully defended his doctoral dissertation in the professional field 4.3. Biological sciences, scientific specialty "Molecular Biology", on the topic „*Properties and mechanisms of action of neuropeptides with model membranes with a view to their application in pharmacology* », with a head corr. mem. Prof. Zdravko Lalchev at Sofia University "St. Kliment Ohridski ”, Faculty of Biology.

Dr. Asya Tsanova works as an assistant in biochemistry in the Department of Chemistry and Biochemistry, Physiology and Pathophysiology of the Faculty of Biology, Sofia University "St. Kl. Ohridski ”from 2007 to 2015. From 2015 until now she is an assistant professor at the Faculty of Medicine, Sofia University "St. Kliment Ohridski“.

In the current competition, Assistant Professor Dr. Asya Tsanova participated with 41 publications outside the publications included in the doctoral dissertation, 21 of which were published in international journals with an impact factor. Asya Tsanova is the first author in six of the articles with an impact factor. The above papers have 34 citations in international journals, and the results of scientific research have been reported at 73 international and national scientific forums. So far, Dr. Tsanova has a total of 21 publications in SCOPUS, which are cited 31 times without auto-citations of all authors (h-index = 4- according to SCOPUS). Dr. Asya Tsanova is a co-author in the creation of 4 textbooks.

**The research activity of** Assistant Professor Dr. Asya Tsanova is directed in two main directions. Most of the publication of the inclusion in the competition is related to the study of alveolar surfactant moisture. The research is fundamental and applied to clinical practice in finding and applying adequate methods for diagnosis and treatment of lung diseases. The second direction in the research of the candidate is related to the study of the interaction of different biological molecules with model membranes. Research in this area is fundamental and is related to the elucidation of the molecular mechanisms of action of the tested molecules on biological membranes.

# **I. ANALYSIS OF THE COMPOSITION, PROPERTIES AND CHARACTERISTICS OF THE ALVEOLAR SURFACTANT IN NORM AND PATHOLOGY**

## **A. *Investigations of the composition and properties of alveolar surfactant in Neonatal Respiratory Distress Syndrome***

1. It has been found that the administration of the exogenous surfactant Curosurf in neonates improves the composition and properties of the pulmonary surfactant in neonates (publ. **I.14, I.15, II.11**).
2. For the first time, the phospholipid composition and biophysical equilibrium and dynamic surface characteristics of clinical samples of gastric (GA) and tracheal (TA) aspirates from newborns were studied and differences in the composition of phospholipids in premature infants of the two types of aspirates were found ( publ. **II.1, II.3**).
3. A new non-invasive method for determining the pulmonary maturity of newborns has been established, which consists in the collection and analysis of gastric aspirates. For the first time, the fatty acid profile in gastric aspirates from preterm infants was analyzed and the saturated / unsaturated fatty acid ratio was found to be highest in full-term infants, with saturated palmitic acid (C16: 0) being the most common and it is a major component of lipids in the alveolar surfactant (publ.**I.9, I.11, II.7, II.8**).
4. For the first time the method of the hanging drop for determination of the surface characteristics of gastric aspirates as a non-invasive and sparing model for diagnosing lung maturity was introduced and applied (publ.**I.9, I.11, II.8**).
5. For the first time in clinical practice, a combination of two analyzes based on axisymmetric analysis of the drop shape (ADSA) and Brewster-angle microscopy (BAM) was used to assess the risk of developing Neonatal Respiratory Distress Syndrome in premature infants (publ. **I.5**).
6. The method of thin liquid films for examination of gastric aspirates from premature infants with primary surfactant deficiency as a result of NRDS as a reliable method for diagnosis (publ.. **I.1**).
7. The effectiveness of the application of corticosteroid therapy has been analyzed and proven in pregnant women after *in vitro* fertilization and with multiple pregnancies to activate the biosynthesis of alveolar surfactant (publ.**I.1, I.4**).
8. Specific surfactant proteins have been identified and analyzed протеини (SPs) SP-A, SP-B and SP-C in gastric aspirates from premature infants born after antenatal corticosteroid therapy of the mothers and a correlation was found with the stage of maturation (publ **I.2**).

*B. Interaction of alveolar surfactant with hydrophilic polymers in order to increase the therapeutic efficacy of exogenous surfactants*

1. The role of various hydrophilic polymers in improving the surface properties of exogenous surfactants and inhibiting the adsorption of albumin has been studied (publ. **I.10, I.17, II.13, II.14, II.16**).
2. The role of the direct addition of the hydrophilic polymers polyvinylpyrrolidone and polyethylene glycol to tracheal aspirates from premature infants with NRDS and the positive role they have on the biophysical parameters of the studied clinical samples (publ. **II.9**).
3. It has been found that the effect of hydrophilic polymers polyvinylpyrrolidone and polyethylene glycol is much stronger when added to exogenous surfactants used in clinical practice than their application directly to the tested tracheal aspirates (publ. **II.9**).

*C. In vitro analyzes of bronchoalveolar lavage by a patient with pulmonary alveolar proteinosis*

1. Conducted biochemical and biophysical analyzes of bronchoalveolar lavage from a patient with NRDS during complete pulmonary lavage, collected after each stage of the procedure, shows a gradual decrease in the content of proteins and phospholipids and increases the equilibrium surface tension (publ. **I.8, II.6**).
2. The use in clinical practice of the quantification of proteins and lipids in patients with NRDS contributes to the diagnosis and treatment of NRDS (publ. **I.8, II.6**).

*D. In vitro analyzes of broncho-alveolar lavage in patients with non-small cell lung cancer*

1. Based on biochemical and biophysical analyzes, it has been found that there is a change in the composition of the alveolar surfactant in bronchoalveolar lavage from non-ventilated (operated) and ventilated lungs in patients with non-small cell carcinoma. Hypoxia and inhalation anesthesia have been shown to alter the biochemical and biophysical properties of the alveolar surfactant (publ. **I.7**).
2. The effectiveness of the hanging drop method in the clinical diagnosis of the functionality of the alveolar surfactant and the application of adequate therapy (publ. **II.2, II.5**).

## **II. INTERACTION OF MOLECULES OF BIOLOGICAL SIGNIFICANCE WITH MODEL MEMBRANES**

1. It has been found that the interaction between neuropeptides (Met-enk) and monolayers of zwitterionic dimyristoyl phosphatidylcholine is determined mainly by the amphiphilicity of

the peptides, while electrostatic forces play a significant role in the insertion of cationic Met-enk-NH<sub>2</sub> in -monolayers, especially at high package density (publ.**I.19**, **I.21**).

2. Different degrees of penetration of methionine enkephalins to monolayers of lipids included in the membrane shelves was established, and a different association of enkephalins to the liquid-ordered phase of the monolayers depending on their final functional groups was shown (publ.**I.20**, **I.22**, **II.12**, **II.23**).
3. When studying the interactions of leucine-enkephalin (Leu-enk) and its amidated form with lipids has been shown that both leucine enkephalins affect the surface characteristics of lipid monolayers and lead to an increase in the surface density of mixed surface lipid / enkephalin films in loose lipid packaging (publ.**I.6**, **II.19**).
4. Differences in the surface morphology of monolayers consisted of POPC, raft-forming lipids and their mixtures in the presence of enkephalins have been registered (Met-enk and Leu-enk, and their amides) at different monolayer densities. It has been shown that the surface morphology of the films is most altered in loose packaging of lipid molecules with the participation of the amidated forms of enkephalins in cholesterol-containing mixtures (publ.**I.6**, **I.20**).

#### *B. Investigation of the mechanisms of antimicrobial action of newly synthesized benzantrone on model membranes*

1. Penetration of newly synthesized water-soluble quaternary ammonium benzantrone (Compound B) into monolayers composed of lipids (as a model of bacterial membranes) was found. Electrostatic interactions, hydrogen bonding and hydrophobic interactions between Compound B and membrane components have been reported as the most noticeable effects in the penetration of Compound B (publ.**I.3**).

#### *C. Investigation of potential drug delivery systems on model membrane systems*

1. It was found that the interaction between poloxamers and phosphatidylcholine molecules in monolayers and foam films depends on both the size of the copolymer and the length of the acyl chain of phosphatidylcholines (publ.**I.18**).

#### *D. Investigation of the mechanism of interaction between the photosynthetic cytochrome b<sub>6</sub>f complex and the thylakoid membranes*

1. The study of the interaction between cytochrome b<sub>6</sub>f complex and monolayers composed of different charge membrane phospholipids demonstrates the importance of the combination of hydrophobic and electrostatic intermolecular forces in lipid-protein interaction (publ.**I.16**, **II.15**).

2. The mechanism of interaction of cytochrome b6f complex with the main compound of thylakoid membranes, uncharged lipid monogalactosyldiacylglycerol, is shown, assuming the hypothesis, this is due to a specific interaction between and cyt b6f and monogalactosyldiacylglycerol, which leads to depletion of lipid molecules from surfaces (publ. **I.12**).

### **III. OTHER SCIENTIFIC CONTRIBUTIONS**

1. It has been found that the combined application of  $Cd^{2+}$  and the herbicide paraquat increases the toxicity of cadmium in terms of growth, biomass accumulation, photosynthesis and plastid pigment content due to increased plasmalemma permeability to  $Cd^{2+}$  (publ. **II.21**, **II.22**).

### **IV. CONTRIBUTIONS TO EDUCATIONAL ACTIVITIES**

1. The candidate participates in the development and publication of textbooks prepared in accordance with the approved program for training in biochemistry of medical students at the Ministry of Finance of Sofia University "St. Kliment Ohridski "(publ. **III.1-4**)
2. The candidate participates in the development of various innovative educational methods such as small group discussions, didactic and role-playing games, multimedia training, etc. to optimize the learning process in the course of biochemistry for medical students (publ. **II.17**, **II.18**).
3. The candidate participates in the implementation and evaluation of the so-called "Hybrid learning" in which traditional learning is complemented by web-based learning, allowing for easy communication, sharing of learning materials, solving web-based tasks, conducting tests, creating a glossary, etc. (publ. **II.4**).
4. The candidate participates in a discussion among medical students at the Medical Faculty of Sofia University "St. Kliment Ohridski " on the need to create a terminology guide in Bulgarian and English, containing terms and phrases common in the literature such as human biology, cytology and histology, physics and biophysics, chemistry and biochemistry, and human anatomy (publ. **II.10**).

Dr. Asya Tsanova has participated in the implementation of 18 research projects (6 - funded by FNI, 12 - under contracts with higher education institutions in the country), 2 project under OP „Human Resources“ and 1 project under OP "Science and Education for Smart Growth".

As a Assistant Professor in biochemistry at the Medical Faculty of Sofia University "St. Kliment Ohridski " Dr. Tsanova has so far conducted over 1300 hours of exercises in“ Biochemistry ”for students majoring in Medicine, about 900 hours of exercises in“ Biochemistry ”for students majoring in“ Medicine in English ”, exercises in“ Peculiarities of metabolism in norm and

pathology ”of students majoring in“ Medicine ”and exercises on“ Peculiarities of metabolism in norm and pathology ”in English for students majoring in“ Medicine in English ”at the Medical Faculty of Sofia University.

**Conclusion:** From the presented scientific works of Assistant Professor Dr. Asya Tsanova shows that the overall scientific output of the candidate fully meets the requirements of the Law on Academic Development in the Republic of Bulgaria and the criteria for acquiring the scientific title "Associate Professor" at the Medical Faculty of Sofia University for professional field "Biological Sciences", scientific specialty "Biochemistry".

The scientific contributions mentioned by Dr. Asya Tsanova have both fundamental and scientific-applied and clinical significance.

My personal impressions of Asya Tsanova are of a very diligent and conscientious scientist and teacher.

Taking into account all the above, I will confidently vote positively in the scientific jury for the award of Dr. Asya Tsanova to the scientific title "Associate Professor”.

Question: How do hypoxia and inhalation anesthesia affect the biochemical and biophysical properties of lung surfactant in patients with non-small cell lung cancer?

26.11.2021 г.

Prof. Dr. Rumiana Tzoneva

София