

OPINION

on the dissertation for the acquisition of the scientific and educational degree "doctor" in the professional field "4.3. Biological Sciences", Doctoral Program Biophysics

of Diana Slavchova Benkova, doctoral student in independent training at the Section "Lipid-Protein Interactions" at the Institute of Biophysics and Biomedical Engineering at the Bulgarian Academy of Sciences

on the topic:

"BIOLOGY ACTIVITY OF CHITOSAN-BASED NANOMATERIALS"

With scientific supervisors Prof. Dr. Galya Staneva and Assoc. Prof. Dr. Anelia Kostadinova, Section "Lipid-Protein Interactions" at the Institute of Biophysics and Biomedical Engineering at the Bulgarian Academy of Sciences

Prepared by: Prof. Dr. Tanya Ivanova Topuzova-Hristova, Department of Cellular and Developmental Biology, Faculty of Biology, Sofia University "St. Kliment Ohridski"

Data about the doctoral student and doctoral studies.

Diana Slavchova Benkova graduated with a Bachelor's degree in Molecular Biology from the Faculty of Biology of Sofia University "St. Kliment Ohridski" in 2020 and a Master's degree in Biochemistry (module Molecular Biology) at the same university in 2022. In 2023, she was enrolled as a doctoral student in independent training in the professional field 4.3. Biological Sciences, Doctoral Program in Biophysics at the Section "Lipid-Protein Interactions" at the Institute of Biophysics and Biomedical Engineering at the Bulgarian Academy of Sciences on the basis of Art. 67 of the Act on the Development of Academic Staff in the Republic of Bulgaria, Art. 11 of the Regulations for the Implementation of the Act on the Development of the Academic Staff in the Republic of Bulgaria, and the Regulations on the Terms and Procedure for Acquiring Scientific Degrees and Holding Academic Positions at the Institute of Biophysics and Biomedical Engineering at the Bulgarian Academy of Sciences For the term of the doctoral studies, Diana Benkova has successfully passed all the necessary exams and completed the tasks, according to the approved individual plan. On 01.08.2024, an open sectional meeting of the section "Lipid-Protein Interactions" was held for graduation with the right to defend a dissertation, and on 07.04.2026, an approbation was held for preliminary discussion of the dissertation and a decision was made to direct it to an official defense. All deadlines and minimum national requirements have been met, as the submitted documentation fully meets the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria, and the dissertation and the abstract have successfully passed the plagiarism prevention check. No violations were identified in the implementation of the procedures for the preparation, assessment and preliminary discussion of the dissertation.

Dissertation data.

The topic of the dissertation work "BIOLOGY ACTIVITY OF CHITOSAN-BASED NANOMATERIALS" fully reflects the essence of the work, which is in a rapidly developing area of biomedical sciences. The topic is relevant in terms of clarifying the biological activity of nanomaterials based on natural biopolymers. These studies logically fit into the general work plan of the team of the Section "Lipid-Protein Interactions", as a complex study, including methods from the field of physicochemical and biophysical studies of membrane organization, which leads to results of both fundamental and applied importance.

The main parts of the dissertation follow the generally accepted plan for such work and include: Introduction – 2 pages, Literature review – 42 pages, Aim and objectives – 2 pages, Materials and methods – 22 pages, Results – 31 pages, Discussion – 14 pages, Conclusion – 3 pages, Conclusions – 2 pages and Contributions – 1 page. A total of 59 figures and two tables were used for illustration. The dissertation is well balanced, and the discussion of the results obtained is well done in view of the physicochemical parameters affecting the formation of membrane domains and their importance in interpreting the data in the various model systems. In total, the dissertation contains 148 pages.

The literature review contains a total of four sections, examining the classification of nanomaterials, the structure of biological membranes, oxidative stress and its influence on membrane components, as well as features of the phytopathogenic fungi *Fusarium solani* and *Alternaria solani*, used to assess the antifungal effect.

The dissertation has two goals:

- 1) To reveal the principles of interaction between chitosan-based and PEG-based hybrid nanocomposites with membrane models that mimic the lipid phase heterogeneity of eukaryotic membranes.
- 2) To investigate the antifungal activity of chitosan-based hybrid nanocomposites containing ZnO, CuO, SiO₂ against some of the most important phytopathogenic fungi – *Alternaria solani* and *Fusarium solani*.

Each of the goals is supported by three logically and systematically formulated tasks, outlining the logic and plan according to which the experimental part of the dissertation was conducted. The tasks include the creation of comprehensive model systems (large unilamellar vesicles (LUVs) composed of one-, two- and three-component lipid mixtures by the extrusion method and giant unilamellar vesicles (GUVs) from one- and three-component lipid mixtures by the electroforming method) and the study of changes in lipid ordering in Ld, Lo and Ld/Lo phase coexistence induced by nanoparticles using Laurdan fluorescence spectroscopy, DLS, ELS and phase-contrast and fluorescence microscopy. The antifungal activity was studied by a classical approach (by agar-diffusion method), combined with the assessment of pro-oxidant activity and quantitative changes in lipid ordering of fungal cell membranes.

The used nanomaterials, lipids, fluorescent probes and fungal strains are listed in the **Materials and Methods section**. The methods are described in detail, with a full composition of the buffers and solutions used, as well as a sufficiently clear theoretical justification for their adequacy. In total, this part is illustrated with 12 figures and the necessary formulas for calculation when generating data. The methods are described well enough to be repeatable.

The results are well explained and illustrated, strictly following the tasks set. After the explanation of the results, a discussion follows on each of them. With the help of model unilamellar vesicles, it was

established that the newly synthesized nanoparticles studied induce changes in the lipid organization, having a pronounced ordering effect. All chitosan-based nanoparticles demonstrate efficient and long-lasting antifungal and strong prooxidant activity. Ten conclusions were drawn from the results obtained, which correspond to the presented data and analyses.

Scientific apparatus.

A total of 182 sources were cited. All sources are directly related to the research topic, which shows the excellent awareness of the doctoral student. The citations were made in compliance with the established standards for citing scientific literature.

The abstract contains 53 pages and correctly reflects the content of the dissertation. The main results are correctly presented, illustrated with a total of 22 figures. The Introduction and Materials and Methods parts are presented in the most abbreviated form to allow for a more complete presentation of Results, Conclusions and Contributions. A list of publications and participation in scientific forums related to the dissertation is also presented.

Publications.

The results of the dissertation have been presented at 6 national and international scientific forums and have been included in three articles published in journals with an impact factor and quartiles (two publications in Q1 and one in Q3, respectively). In all three articles, the doctoral student is the first author - independently or jointly. These publications fully meet and exceed the minimum national requirements for defending a dissertation for the scientific and educational degree of Doctor in scientific field 4.3. Biological Sciences, according to Appendix 1 of the ZRASRB. Part of the results of the dissertation work have been presented at a total of 6 scientific forums - 3 international and 3 national, with Diana Benkova receiving a Diploma for an excellently presented report by a young scientist at the Fourth National Congress of Physical Sciences, Sofia, Bulgaria, October 7-9, 2024. In addition, she is also the winner of the "Ivan Evstratiev Geshov" Award for the Youngest Scientists of the Bulgarian Academy of Sciences for 2025.

In addition to her work on her dissertation, Diana Benkova has increased her scientific capacity by participating in a total of 10 scientific projects (6 ongoing and 4 successfully completed) related to her professional field and the field of her dissertation topic, which are funded by the National Science Foundation of the Ministry of Education and Science and under the COST action program and a bilateral agreement with Egypt. Outside of her dissertation, doctoral student Diana Benkova is a co-author in a total of 12 scientific publications in the field of biomedical research and biophysics.

Scientific and applied scientific contributions.

The contributions from the dissertation are six in total, but could be combined into three more generalized and are of a scientifically applied and fundamental nature. The fundamental contributions are related to a proposed model of interaction of hybrid nanocomposites with cell membranes based on the results obtained from model unilamellar vesicles. This contribution is logically related to the quantitative characteristics of homogeneous and heterogeneous model membranes, derived as a third contribution. I recommend combining the two contributions into one. Another contribution is the establishment of a combined fungicidal mechanism of action of chitosan-based hybrid nanocomposites simultaneously through oxidative stress and changes in the membrane organization of fungal cells. The established correlation between the physicochemical parameters of the nanocomposites and their fungicidal action has practical application in the future development of commercial preparations. The last contribution is formulated more as a summary of the possible

applications of the established dependencies, and not as a specific result or conclusion of a contributory nature.

Conclusion.

The work presented to me for review is a study of the effect of oxidative stress on membrane lipid organization. The dissertation work is focused on the effect of chitosan-based hybrid nanoparticles on biological membranes and the antifungal and pro-oxidant effect of their nanocomposites with metal oxides. The obtained results emphasize the significant potential of chitosan-based hybrid nanocomposites as a new generation of antifungal materials with a combined membrane-damaging and pro-oxidant mechanism of action. Doctoral student Diana Benkova has fulfilled the goals and objectives set in the dissertation and fully meets the requirements of Appendix 1 of the Law on Biological Sciences of the Republic of Bulgaria in terms of the number of publications.

In conclusion, I believe that the doctoral student fully meets the requirements of the Law on Biological Sciences of the Republic of Bulgaria for the award of the scientific and educational degree "doctor" and I give my positive assessment to award the scientific and educational degree "doctor" in the scientific field 4.3. Biological Sciences to Diana Slavchova Benkova.

29.06.2026

City of Sofia

Prepared the opinion:


/prof. Dr. Tanya Topuzova-Hristova/