Wrocław, 22 August 2025

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REVIEW OF THE DOCTORAL DISSERTATION

by

Sada Raza, MSc

Entitled

Inactivation of bacteriophages

Basis: *Resolution of the Scientific Council of the Institute of Physical Chemistry of the Polish Academy of Sciences, dated June 17, 2025, and the official letter dated June 23, 2025, issued by the Deputy Director for Scientific Affairs Jacek Gregorowicz, PhD, DSc, Institute Professor.*
Legal basis: *Compliance with the elements outlined in Article 187 of the Act of July 20, 2018, Law on Higher Education and Science.*

The dissertation submitted for review by Sada Raza, MSc, was conducted at the Institute of Physical Chemistry of the Polish Academy of Sciences, under the supervision of Jan Paczesny, PhD, DSc, Institute Professor, who is an internationally recognized authority in the field of nanoscience, directly relevant to the research area of the dissertation.

The dissertation will be evaluated in four areas explicitly defined in Article 187 of the Act of July 20, 2018, *Law on Higher Education and Science*:

1. The dissertation demonstrates the candidate's general theoretical knowledge in the discipline(s) and the ability to conduct independent scientific or artistic research.
2. The dissertation constitutes an original solution to a scientific problem, an original application of research findings in the economic or social sphere, or an original artistic achievement.
3. The dissertation may take the form of a written work, including a scientific monograph, a set of published and thematically related scientific articles, a

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design, construction, technological, implementation, or artistic work, as well as an independent and distinct part of a collective work.

4. An abstract in English must be attached to the dissertation; if the dissertation is prepared in a foreign language, a Polish abstract must also be attached. If the dissertation is not a written work, a description in both Polish and English must be provided.

Following the guidelines of the Council for Scientific Excellence, my opinion will concern only the aspects listed above.

Evaluation of the form of the doctoral dissertation with justification

The doctoral dissertation consists of an independent written work based on research presented in eight collaborative publications (one was presented as a preprint at *bioRxiv*, https://doi.org/10.1101/2025.03.13.643104 but at the time of the preparation of my assessment the paper was published in its final form in the *Applied Microbiology & Biotechnology* https://doi.org/10.1007/s00253-025-13526-x) related to the thesis, one book chapter and one patent. The candidate is a co-author of all these works, each of which has been published and is thematically interconnected, focusing on novel approaches in the field of antibacterial and antiviral agents. **Therefore, I conclude that the dissertation has the proper format and meets the requirements of Art. 187 of the Act of July 20, 2018, *Law on Higher Education and Science*:** *the dissertation may take the form of a written work, including a scientific monograph, a set of published and thematically related scientific articles, a design, construction, technological, implementation, or artistic work, as well as an independent and distinct part of a collective work.*

Evaluation with justification as to whether the doctoral dissertation demonstrates the general theoretical knowledge of the candidate applying for the doctoral degree in a given discipline or disciplines

Sada Raza, MSc, is applying for a doctoral degree in the discipline of *chemical sciences*. The candidate surveys a diverse set of phage inactivation strategies, classified into physical, chemical, and nanotechnological categories. Each approach is accompanied by relevant mechanisms, for example, thermal capsid deformation and DNA ejection are noted, as is ROS-mediated damage to nucleic acids and capsid proteins. Nanomaterial-based inactivation mechanisms, such as toxic ion release from silver nanoparticles (AgNPs), phage adsorption *via* charge-based interactions, and surface-specific filtration using CNTs or functionalized silica, are relevant to the topic and addressed well. In terms of the efficacy aspects of existing solutions, the theoretical background is prepared very well. However, the dissertation would

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benefit from a more detailed in-depth critical assessment of the safety (environmental and human) of the application of existing solutions, especially regarding the practical use of silver nanoparticles and indigo carmine, and therefore the challenges that it brings. These aspects are very important for the development of new disinfection technologies (which is one of the aims of the candidate's work), which would be non-trivial as silver nanoparticles have been under microscope for some time (please see Hansen, S. F., & Baun, A. (2012). When enough is enough. *Nature Nanotechnology*, 7(7), 409-411. https://doi.org/10.1038/nnano.2012.115), Ag-, Au-, and Cu-NPs have been recently banned in cosmetics (please see Commission Regulation (EU) 2024/858 of 14 March 2024 on the use of some nanomaterials, the law has taken effect since the beginning of 2025), and the authorization for indigo carmine (dye Blue No. 2) will be revoked for the food industry in the US by the end of 2026 (please see FDA News Release from April 2025: https://www.fda.gov/news-events/press-announcements/hhs-fda-phase-out-petroleum-based-synthetic-dyes-nations-food-supply). The candidate mentioned briefly the environmental risks of NPs but in my opinion this aspect should be more developed. The above issues are more relevant to stages beyond the fundamental research that constitutes the focus of the candidate's work, and there is no doubt that the candidate has identified a research gap in understudied approaches to novel antibacterials and antivirals. She referred to previous literature reports and critically evaluated them in terms of their variable efficacy and proposed possible significant improvements beyond the state-of-the-art. **Therefore, I state that the doctoral dissertation meets the requirements of Art. 187 of the Act of July 20, 2018, *Law on Higher Education and Science*: *the dissertation demonstrates the candidate's general theoretical knowledge in the discipline(s) and the ability to conduct independent scientific research.***

Evaluation with justification as to whether the doctoral dissertation demonstrates the ability of the candidate to conduct independent scientific research

The doctoral dissertation of Sada Raza demonstrates her ability to conduct independent scientific research. The candidate's contributions go beyond routine laboratory work and encompass critical intellectual and organizational roles. These traits are explicitly shown in the published manuscripts related to the doctoral thesis, and the input of co-authors in most of the papers and the thesis is precisely described. In *Nanoscale Advances*, she shared responsibility for conceptualization, conducted extensive experiments and data analysis, and was directly involved in the preparation of a scientific manuscript. In *Viruses*, she played a central role in methodology design, formal analysis, data curation, and even aspects of supervision and project administration. In *Applied Microbiology and Biotechnology*, she is the first author,

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contributed to methodology development, formal analysis, and acted as lead author in the original draft preparation, while also coordinating project administration. The contribution of the candidate to the work in Chapter 6 would require some more detail. Although the article published in *Nanoscale* does not provide explicit author contribution details, the candidate is listed as the first and co-corresponding author, and as the co-first author of the manuscript submitted to *Materials & Design* (on targeted inactivation of bacteriophages by polypyrrole nanoparticles). These authorship positions clearly indicate that the candidate played a leading role in the research concerning engineered selectivity through charge-based interactions.

The candidate had a leading role in the research described in the doctoral dissertation. Therefore, I conclude that **the doctoral dissertation meets the requirements of Art. 187 of the Act:** ***the dissertation demonstrates the candidate's*** *general theoretical knowledge in the discipline(s) and the* ***ability to conduct independent scientific research.***

Evaluation with justification as to whether the doctoral dissertation constitutes an original solution to a scientific problem, an original application of the candidate's research results in the economic or social sphere, or an original artistic achievement

In her doctoral dissertation, the candidate clearly and precisely presents the scientific problem that forms the basis of the research. The primary objective was to develop scalable, selective, and biocompatible strategies for inactivating bacteriophages in biotechnological settings, with minimal impact on bacterial populations and mammalian cells. This problem is of increasing importance given the growing prevalence of phage contamination in industrial microbial processes, where infections can lead to substantial economic losses and production disruptions. The main objective was divided into detailed research goals, whose achievement enabled verification of the well-formulated research hypothesis, namely: that it is possible to design and develop selective materials capable of inactivating bacteriophages without adversely affecting host bacteria or mammalian cells, and that such materials can be implemented in scalable processes. To achieve the research objectives, the candidate employed advanced methods, including the green synthesis of nanoparticles, a wide range of analytical techniques, and various assays examining nanoparticle and small-molecule interactions with microorganisms, bacteriophages, and cell lines. The candidate demonstrated that while green-synthesized silver nanoparticles possess strong antibacterial and antifungal activity, they lack specificity against phages. The second approach, involving indigo carmine, showed high efficacy against phages without negative effects on bacterial growth or productivity. The selective mechanism provided a proof-of-concept solution for

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targeted phage inactivation by small molecules, which was patented (Patent No. P.441746). In the third approach, the candidate explored the rational design of selective nanomaterials through charge- and ligand-based engineering. Mixed-ligand gold nanoparticles and polypyrrole nanoparticles were shown to have very high inactivation potential of phages while maintaining over 90% viability in bacterial and mammalian cell models. These results demonstrate the feasibility of nanotechnological approaches for phage deactivation. Analyzing the results described in the dissertation, I conclude that the candidate achieved the main scientific objective, positively verified the research hypotheses, and built a rational design strategy of targeted antiphage systems. Importantly, the new findings presented in the thesis were disseminated through a series of peer-reviewed publications in reputable international journals like *Nanoscale*, *Nanoscale Advances*, *Langmuir*, *Applied Microbiology and Biotechnology*.

Therefore, I conclude that the doctoral dissertation meets the requirements of Art. 187 of the Act: *the subject of the dissertation is an original solution to a scientific problem*, *an original solution in the field of application of research results in the economic or social sphere, or an original artistic achievement*.

Evaluation of the aspect of Art. 187, section 4, of the Act of July 20, 2018, *Law on Higher Education and Science*:

The submitted doctoral dissertation is a written work. The dissertation includes abstracts in both Polish and English. **I state that the condition set out in Art. 187, section 4, of the Act of July 20, 2018, *Law on Higher Education and Science* has been met.**

List of questions and issues that will constitute the starting point for the discussion during the public defense of the doctoral dissertation

1. Have you made attempts to quantify key constituents of tea extracts used for NP synthesis?
2. Please explain the title of Chapter 5 (Naturally selective antiviral compounds), presented background on naturals (5.1.), with the fact that the chapter focuses on indigo carmine (food colour Blue No. 2), one of the classic synthetic colours.
3. It is not clear why the paragraph on the screening of FDA-approved food dyes, including indigo carmine, appears at the end of the chapter (page 85). You describe it as the next part of your studies, yet at the end of the paragraph, you state that this was the starting point. Why did you not place it at the beginning of your narrative in Chapter 5? Moreover, if you initially tested

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only indigo carmine, what was the rationale for choosing this particular synthetic food dye?

4. Please describe your contribution to the work presented in Chapter 6 of the thesis.
5. Please clarify the magnification levels used to capture Fig. 29a,f and Fig. 29b,c,e, as I was not able to find this information in the thesis.

Final Conclusion

Based on my evaluation of the dissertation by Sada Raza, MSc, entitled *Inactivation of Bacteriophages*, **I conclude that it fulfills the requirements of Article 187, sections 1–4 of the Act of July 20, 2018, *Law on Higher Education and Science* (Journal of Laws 2024, item 1571, as amended).**

The candidate demonstrates not only a profound understanding of the subject matter but also the ability to critically evaluate existing fundamental knowledge, identify research gaps, and design approaches to address them. The presented work reflects strong methodological competence, as advanced experimental techniques and analytical strategies have been applied to solve complex scientific problems. Importantly, the candidate's contributions to the published works show intellectual independence. The dissertation presents scientifically significant results that advance the discipline of chemical sciences, particularly by providing, for the first time, proof of the feasibility of using a blue food dye for bacteriophage inactivation. **Therefore, I conclude that the dissertation exceeds the standards expected for a doctoral degree. In recognition of the scientific excellence, originality, and the demonstrated independence of the candidate, I strongly recommend the distinction of the doctoral dissertation.**

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