



Aarhus, May 31, 2022

## Assessment and Recommendation

Dear committee,

Here I provide my evaluation of the PhD dissertation by Anna Graczyk (AG) entitled “Structural RNA conjugated with gold nanoparticles as a tool for gene expression regulation” describing her PhD studies done under the supervision of Prof. Arkadiusz Chworos and Dr. Roza Pawlowska at the Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences. The thesis describes the PhD project of AG that has focused on functionalization of gold nanoparticles with RNA nanostructures presenting small interfering RNAs (siRNAs) for delivery and knockdown of a target protein in human cells. The thesis consists of an introduction (35 pages), a result and discussion section (35 pages), a summary (1 page), materials and methods (21 pages), appendices (4 pages), and a reference list (20 pages).

The PhD thesis is very well presented, clearly written and demonstrates expert and interdisciplinary knowledge of both biology, biochemistry, nanotechnology, nanoparticle materials and medicine. The scientific level of the thesis is high and the research problem and motivation clearly stated. The introduction gives a thorough overview of RNA structure and function and of previous research performed in the fields of RNA nanotechnology and nanoparticle functionalization. The last section of the introduction highlights the relevance and unique opportunities of developing RNA medicine in the wake of the SARS-CoV-2 pandemic and the important development of mRNA vaccines and lipid nanoparticle delivery methods. The introduction in general shows command of the research field, knowledge and use of the literature.

The research builds on previous work performed in the laboratory of Prof. Arkadiusz Chworos, but represents a novel direction of using metal nanoparticles as delivery vehicles. The results involve RNA-nanoparticle functionalization, cytotoxicity studies, transfection studies, electron transmission microscopy studies and gene expression regulation studies. The work has clearly been driven by the PhD candidate and even though some experiments have been done in collaboration with other researchers the main research contribution is done by the candidate. New knowledge is obtained on the effects of attaching RNA nanostructures on metal nanoparticles and the effects when delivered to cells. The data obtained is consistently presented,

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analyzed and interpreted, and of high significance for the research field. The results are presented in figures and graphs that are of high quality, well annotated and with statistical evaluation where relevant.

AG shows a strong command of the general research process by clear scientific arguments, clear definitions of central concepts and careful documentation of the experiments performed. The design of experiments is well done with alternative strategies and control experiments. The experimental methods and characterization techniques are well chosen and suitable for the object under study and everything is carefully documented in the materials and methods section, which allows reproducibility of the research performed. The quality and carefulness of the research documentation supports the view of a strong candidate for the PhD degree and for leading future research projects.

The quality of the work is further supported the two peer-reviewed articles that AG has published as first author: A research article in 2020 in the ACS journal Bioconjugate Chemistry entitled “Gold Nanoparticles as Carriers for Functional RNA Nanostructures” and a review article in 2021 in the MPDI journal Molecules entitled “Gold Nanoparticles in Conjunction with Nucleic Acids as a Modern Molecular System for Cellular Delivery”. The papers contain core elements of the PhD thesis and are of high quality. Apart from these major research products, AG has been strongly involved in the scientific community by presenting her work at several conferences with award winning talks at e.g., the prestigious Gordon Research Seminar on RNA Nanotechnology in Ventura, California. The corona virus pandemic has likely affected AG’s ability to work and engage internationally, but she has clearly been able to continue her strong engagement through local lab work and online conference.

In conclusion, the PhD thesis of AG is of high scientific quality and demonstrates that AG has the necessary skills and knowledge in the research field. I can therefore recommend acceptance of the thesis for further stages of evaluation towards her final PhD degree. In fact, I think the thesis is of very high quality because of the difficulty of the interdisciplinary topic and the experimental procedures that I can also recommend to have it awarded a prize for excellent work.

Yours sincerely,

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