

Gliwice, October 30<sup>th</sup> 2023.

**Review of the doctoral thesis by Mrs. Maria Cristina Nevarez Matrinez entitled “Gold Nanorod – Affibody Conjugates for Targeted Photothermal Therapy on HER2-Positive Cancer Cells”**

## 1. Introduction

The doctoral thesis entitled “Gold Nanorod – Affibody Conjugates for Targeted Photothermal Therapy on HER2-Positive Cancer Cells” by Mrs. Maria Cristina Nevarez Martinez, MSc was carried out and submitted at the Faculty of Chemistry of the University of Gdańsk under the supervision of Full Professor Adriana Zaleska-Medynska and co-supervision of Dr. Ewa Wiczerzak. Additionally, two other units contributed to the work, that is Faculty of Mathematics, Physics and Informatics of the University of Gdańsk and the Centre for Cancer Research in London.

## 2. Characterization of the dissertation

The doctoral thesis is written in simple, clear and understandable, but fully professional English. I really like this way of presenting scientific issues clearly and concisely. For me, this is proof of the PhD Student's great maturity, as well as her broad horizons and good preparation to carry out such an ambitious and interdisciplinary task. The work contains numerous photos, tables, diagrams, and illustrative schemes, and at the same time is almost completely devoid of chemical equations, which is no longer surprising in the field of synthesis of nanoparticles and their chemical modifications. I did not find any linguistic or editorial errors in the work. And although I am not a native speaker and I am not particularly sensitive to mistakes in the text, I can confidently confirm that my perception of the formal aspects of the work, places it in a unique, elite group of "elegant" works.

I found only two minor chemical inconsistencies: p. 33 I would call "gold(III) chloride trihydrate, ( $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ )" tetrachloroauric(III) acid trihydrate, and  $\text{Au}^{1+}$  (pp. 18, 35) I would write as  $\text{Au}^+$ . The layout of the work is classic, it consists of three main parts: introduction (state of art), materials and methods and the main sections is results and discussion.

At the beginning of the work there is a rich list of abbreviations, table of contents, graphics, and tables. The work ends with a very factual summary and a valuable outlook. These last two sections give the impression that the PhD Student had further visions, or even desires, to continue this research based on the results obtained and experience she gained. I assess this as a particularly positive effect of the young Researcher's development.



The author cited 215 professional literature items, mainly from 2010 to this year. I really appreciate the presence of the brief chapter "Aim of the PhD Study". The potential of Au NRs was identified and summarized in the introductory section. These characteristics were linked to the possibilities of the intensively developed immunotherapeutic field. The goals of the work were clearly defined. Such a presentation of a research hypothesis is a particularly important element of every scientific experiment (or project in general) based on the so-called scientific method.

### 3. General presentation of the topic

The studies carried out as part of the doctoral thesis concern the extremely important topic of fighting against cancer, which was indicated in the thesis as included in the United Nations Sustainable Development Goal 3 on Good Health and Well-Being. Therefore, the research is a response to burning issues of humanity and an aging society, and also contributes to a better understanding of the mechanisms of the emergence and development of oncological diseases. The subject of the research were rod-shaped gold nanoparticles (Au NR), which enable the transformation of energy emitted by a laser into heat in photothermal therapy. The purpose of decorating such nanoparticles with PEG was to make them more biocompatible and tunable. The targeting of these constructs (also sometimes called nanohybrids or bioconjugates) at HER2 receptors was achieved by affibodies.

A particularly clear feature of the research carried out is its outstanding interdisciplinarity. I have the impression that Mrs. Nevarez is already a specialist in the inorganic chemistry of gold nanoparticles, surface material chemistry, material characterization, molecular biology, and diagnostic and therapeutic oncological medicine. It is incredible what challenges the PhD Candidate faces and the number of skills she needed to acquire. Moreover, what enormous opportunities they had given to her to carry out such a research.

### 4. Methodology

Mrs. Nevarez Martinez thoroughly examined the method of obtaining gold nanorod particles using the seed-growth synthesis method. As she showed, the methods described in the literature do not describe the synthesis precisely enough to repeatedly obtain a dispersion of nanoparticles with the expected morphology. The main parameter studied was the stirring speed of the solutions during the nucleation and growth of the nanoparticles.

The independent synthesis of Affi, as well as its analyzes and characterization, allowed it to be attached to the modified nanoparticles. The direct introduction of Affi onto the surface of the nanoparticles did not ensure their sufficient stability. Therefore, additional steps were taken to change the nature of the surface (confirmed by Z-potential measurements) by covering the nanoparticles with PEG containing thiol residues. In this way, stable dispersions of



nanoparticles were obtained, on which the targeting Affi was secondarily anchored. In vitro tests carried out at the Institute of Cancer Research in London under the supervision of Prof. G. Kramer-Marek contributed a lot of cognitive value to the specificity of action against HER2 cells and the cytotoxicity of new constructs. I think this chapter is very interesting and shows how complex the mechanisms of cell behavior towards nanoparticles are. Additionally, I would like to express great appreciation for the PhD Student's inquisitiveness in this topic, while not succumbing to the temptation of unfair selection of the results in order to strongly confirm her hypothesis. I believe that the researcher's task is to testify the truth - to observe the effects of experiments, and this attitude was demonstrated by the PhD Student.

The study of activity in photothermal therapy was the culmination of the task. First, the activity of nanoparticles in the emission of thermal energy was demonstrated, and then research on cells was undertaken. Initially, mild conditions were used, which did not directly demonstrate the expected effect. However, the tests at 37 °C turned out to be a success, in which attention was paid to many technical details that affect the reliable evaluation of the results. The observation of agglomeration in SKOV-3 cells confirms the validity of the hypothesis assumed at the beginning and opens the way to the development of research on theranostic applications of targeted Au NR constructs in immunotherapy.

## 5. Scientific achievements

According to the supplementary list of scientific achievements, Mrs. Nevarez Martinez is the main author of 3 articles on nanoparticles ( $\text{TiO}_2$ ,  $\text{AgInS}_2$ ,  $\text{ZnO}$ ) and their application in catalysis. I believe that these studies enabled the development of crucial skills for the investigation of Au NR within the doctoral thesis. The internship at the Institute of Cancer Research gave measurable results for the dissertation and supported important studies on the cells. Additionally, I appreciate 3 lectures given at national conferences which exposed the PhD Candidate to an open discussion.

Mrs. Nevarez Martinez was the principal investigator in 3 projects – 2 within the university and 1 led by the National Science Centre (Preludium18). She also participated in the large Opus grants.

Although there is no solid publication directly related to the results of the Ph.D., I believe that the scientific activity and achievements presented are satisfactory and confirm the PhD Candidate's involvement in scientific research in the area of synthesis and research on the properties and applications of nanoparticles.

## 6. Discussion

Although I consider the work to be very well written, the interesting reading left me with room for curiosity. Therefore, taking advantage of the opportunity to discuss with the PhD Candidate, I would like to present a few issues below:

- Despite great efforts by the part of the PhD Student to balance all the elements of nanoparticles as carriers, passive and active therapeutics, and diagnostic media in the literature part of the work, I believe that this last issue is worth special attention. Perhaps, this is due to my subjective interest in molecular imaging, but I would like to ask the PhD Candidate to elaborate on the perspectives of using gold nanoparticles in tomographic techniques.
- I am also very interested in the historical reference on page 15: “The produced heat can be utilized in the treatment of cancer since heat has been one of the main tumor therapy methods since its ancient use from 1700 BC.” Please expand on this story a bit.
- Mrs. Maria Nevarez devoted a lot of time to selecting the right conditions for obtaining Au NR. One of the elements that was particularly closely studied was the dynamics of ingredients when creating and growing seeds. This process was described by stirring speed. According to the information contained in the Materials and Methods section, the stirring bar and the volume of component solutions are precisely characterized. I wonder whether the optimal stirring speed selected on a small scale allows one to obtain satisfactory nanoparticles on a 20-fold larger scale. Perhaps, the dumbbell shape is (apart from the arguments presented in this paper) the result of too heterogeneous dynamics on a larger scale. Therefore, I propose to consider whether there are more general parameters of the liquid that would eliminate the influence of the size and shape of the stirring bar and vessel (reactor) and which could help describe this difficult issue of selecting reaction conditions to use the best possible nanoparticles.
- On p. 77 the PhD Student wrote: “antibodies or affibody molecules directly over Au NPs should stabilize their surface by a series of interactions”. Initially, the statement was confusing to me. Does it mean expectations to be fulfilled or a result that the proteins endow to the nanoparticle upon decoration? Further reading made it more clear – the second possibility. Nevertheless, I would like the PhD Candidate to elaborate and specify what surface stabilization she is talking about. What is the result of this stabilization?
- On p. 78 it is written: “The CTAB bilayer packing area is  $22 \text{ \AA}^2$ .” Please, make me familiar where this calculation comes from and what evidence there is for bilayer character of CTAB.
- I noticed that all the photos at work add a lot and give a picture of the behavior of the dispersions. However, p. 80, Figure 29 - this photo has little value for me, because I only see dispersions with a very similar color. Dispersion stability is very good information, however, perhaps the PhD Student has another photo that would better highlight the differences or other characteristics of these dispersions. Her subjective assessment would also be reliable.
- Pp. 53 and 59 – Photos of the photothermal conversion system. They give some vision of the reality, nevertheless a scheme would give a better imagination and understanding of the



parameters and results of the experiments, especially that the discussion on pp. 89 refers in details to the experiments.

- P. 112 referring to the results presented on the Figure 54, there is a statement: “Affi-Au NRs aggregates”. It is very promising and intriguing, thus raising many questions. Is there any information on the resulting local temperature? What aggregates in fact – the nanoparticles, proteins? Is it possible to have some more details on these aggregates?

## 7. Summary

In summary, I would like to express that the doctoral thesis presented to me for the review touches on extremely interdisciplinary issues at the intersection of the fields of chemistry, physics, biology, and medicine. The topic of the work is in line with the most current trends and needs of the modern world. The work is based on the hypothesis that gold nanoparticles combined with an element targeting receptors specific to cancer cells are able to "recognize" such cells and constitute a useful medium for photothermal therapy. This hypothesis contains a number of scientific novelties. The implementation of this work demonstrated the validity of this hypothesis. At the same time, the research carried out has a very valuable cognitive approach. Mrs. Nevarez Martinez demonstrated broad horizons, flexibility in work, inquisitiveness, and reasonable presentation and discussion of the results. In connection with the implementation of this research, the PhD Candidate participated in 5 research projects and published 3 works and gave 3 conference presentations.

Moreover, the work is presented clearly, the results are of trustworthy scientific value, and are complemented by a broad discussion. I read the work with great pleasure and interest.

## 8. Recommendation

It should be said that the PhD Candidate has demonstrated an exceptional ability to combine the research from various areas of chemistry. She confirmed the ability to conduct scientific work independently. As a result of such diverse activities, Mrs. Nevarez Martinez prepared a valuable dissertation, which is additionally accompanied by publications, conference presentations, and contributions for grants. The work contains important elements of scientific novelty. **Therefore, the presented dissertation meets the requirements arising from the applicable Act on academic degrees and titles (art. 187 para. 1-3 of the Law on Higher Education and Science of 2018, as amended).**

**According to the above, I am appealing to the Discipline Council of Chemical Sciences at the University of Gdańsk to admit Mrs. Maria Cristina Nevarez Martinez to the next stages of the doctoral procedure.**



Należy stwierdzić, że Pani mgr inż. Maria Cristina Nevarez Martinez wykazała się wyjątkową zdolnością do łączenia badań z różnych obszarów nauk ścisłych i przyrodniczych. Potwierdziła umiejętność samodzielnego prowadzenia pracy naukowej. W wyniku tak różnorodnych działań Doktorantka przygotowała cenną rozprawę, której dodatkowo towarzyszą publikacje, prezentacje konferencyjne i potwierdzony udział w projektach badawczych. Praca zawiera ważne elementy nowości naukowej. **Dlatego w mojej ocenie przedstawiona mi do recenzji rozprawa spełnia warunki określone w art. 187 ust. 1-3 Ustawy Prawo o szkolnictwie wyższym i nauce z 2018 r., z późniejszymi zmianami.**

**Wnoszę zatem do Rady Dyscypliny Nauki Chemiczne na Uniwersytecie Gdańskim, aby dopuścić Panią Marię Cristinę Nevarez Martinez do kolejnych etapów postępowania o nadanie stopnia doktora.**