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Referee's Report on the PhD thesis

by ing. Kseniya Popovich
entitled Biofunctionalization of luminescent nanocomposites

The reviewed dissertation thesis of Kseniya Popovich, entitled "Biofunctionalization of luminescent nanocomposites", is 102 pages long and consists of 11 introductory pages (Title, Acknowledgements, Bibliographic entry, Abstracts, Table of contents and List of Abbreviations), 5 numbered main chapters (Introduction, Theory, Experimental part, Results and Discussion, Conclusions) followed by References and List of publications. The document comprises 13 pages of bibliography (157 references), 53 figures and 5 tables. PhD student has been supervised by doc. ing. Václav Čuba, Ph.D. from the Department of Nuclear Chemistry, Faculty of Nuclear Sciences and Physical Engineering, Czech Technical University in Prague.

The presented thesis deals with multimodal luminescent nanocomposite materials. Development of nanocomposite materials is extremely fast growing field of material research with an unprecedented number of possible applications. In medicine, nanocomposite materials can be applied as multimodal drugs combining different functions, such as simultaneous imaging, drug delivery and therapy. The aims of the thesis defined by PhD student were design, synthesis, and evaluation of properties of multimodal luminescent nanocomposite materials.

In the Introduction, candidate clearly formulates aims of the PhD study and defines own contribution to the work and the contribution of students that she supervised. In the chapter Theory, basic categories of nanocomposite materials are introduced and described. Particular attention is devoted to luminescent materials and their multimodality. I consider this part well organized and explanatory. In particular, I appreciate the final chapter of this section explaining rationale for choice of materials for nanocomposite design.

Third chapter deals with instruments and methods applied in experimental work. Synthesis of YSO:Ce^{3+} and LSO:Ce^{3+}, LuAG:Pr^{3+} and LuAG:Ce^{3+} is described together with subsequent coating of nanoparticles by amorphous silica shell and functionalization by protoporhyrin IX or anti-FasL antibody. Obtained results are presented in the most extensive part of the thesis Results and discussion.

First group of nanocomposites, YSO:Ce^{3+} and LSO:Ce^{3+}, were shown to have higher luminescent intensity compared to BGO standard. However, these nanocomposites cannot be applied *in vivo* due

to their big size. Studies involving biofunctionalization of second group of luminescent nanoparticles, LuAG:Pr^{3+} and LuAG:Ce^{3+}, led to a design of a promising drug for X-ray induced photodynamic therapy and another for cathodoluminescence bioimaging.

LuAG:Pr^{3+}@SiO2-Protoporphyrin IX was successfully prepared as a drug for application in X-ray induced photodynamic cancer therapy. Photodynamic therapy is based on an excitation of a photosensitizer by absorption of light or X-rays. Deexcitation of photosensitizer triplet state leads to production of highly cytotoxic singlet oxygen. I consider this part of the thesis the most interesting and valuable. Besides nanocomposite drug synthesis, its properties related to X-ray induced photodynamic therapy have been followed and confirmed. Radiative energy transfer from the LuAG:Pr^{3+} core to Protoporphyrin IX outer layer was found by luminescence spectral characteristics measurements. Subsequent production of singlet oxygen was monitored by measuring of photoluminescence emission spectra with addition of Aminophenyl Fluorescein probe, sensitive to $^1O_2$, and NaN_3, an inhibitor of OH radicals.

Functionalization of LuAG:Ce^{3+}@SiO2 by anti-FasL antibody was chosen for application in cathodoluminescence bioimaging. Proof-of-concept of bioimaging in cathodoluminescence beam was developed and tested on yeast cells *Saccharomyces cerevisiae* and T-lymphocytes. It was found, that luminescent nanomaterials could attach tested cells via sorption or by targeting transmembrane protein.

PhD thesis is written in English, which means that it can serve as a source of scientific information not only for students and for researchers at national level but also abroad. I appreciate nice colored figures in the thesis, which increase educational quality of the manuscript. Overall, formal quality of the thesis and the English language is good with minimum of typos. I would only recommend modifying some graphs, mainly spaces in legends or between numbers and axes. However, all presented graphs are legible.

I have several questions to the submitted thesis:

1. Chapter 3.7: Samples containing LuAG:Pr^{3+}@SiO2-PpIX have been irradiated by 40 kV X-ray tube. What was the reason for selection of this X-ray source? Can you expect variations of radiation chemical yield of singlet oxygen as a function of incident energy of X-rays? Can you evaluate the yield of singlet oxygen formed in the system? Is there any possibility how to estimate the yield of singlet oxygen formed by one nanoparticle located in tumor tissue when irradiated by typical dose of 2 Gy? Do you envisage to study the impact of X-ray induced photodynamic therapy using LuAG:Pr^{3+}@SiO2-PpIX on cell viability?
2. Chapter 3.8: Why T-lymphocytes were incubated with LuAG:Ce^{3+}@SiO_2/anti-FasL at 4 °C and not physiological conditions 37 °C, 5 % CO_2? Why phosphate buffer has been used and not lymphocyte cultivation media?

Specific comments:

1. Acknowledgement, page 46, etc.: The correct name is the Czech Academy of sciences with abbreviation CAS, not AS CR.

2. Page 16: "... nanocoposite materials can be divided into two basic categories: nanolayered materials, nanocomposites with nanoparticles dispersed in bulk matrix and core-shell nanocomposites." Two categories are mentioned and three listed.
3. Figure 26, 35, 36, 38, 40: Decimal points instead of commas should be used.
4. Page 87: "... YSO:Ce^{3+}(1%) and YSO:Ce^{3+}(1%) ..." I suppose it should be "... YSO:Ce^{3+}(1%) and LSO:Ce^{3+}(1%) ..."

Kseniya Popovich published as a principal author four peer-reviewed papers directly related to the topic of the thesis and another 2 scientific papers as a member of research team of the Department of Nuclear Chemistry, FNSPE, CTU. Two papers were published in Radiation Measurements (IF=1,512), one in the Journal of Photochemistry and Photobiology (IF=3.261) and the most recent in IEEE Transactions on Nuclear Science (IF=1.740). PhD student is a member of author team for several functional samples. Unfortunately, her contribution to the work is not clearly declared and therefore it is difficult to include these applied results in the evaluation of the thesis.

In summary, the reviewed thesis and the number of published papers in reputable scientific journals allows me to assess research competences of ing. Kseniya Popovich. The performed work is original, with further research perspectives and possible practical applications in X-ray induced photodynamic therapy of cancer. I propose that the submitted dissertation thesis is accepted within the formal procedure of awarding the PhD title to Ing. Kseniya Popovich by the Czech Technical University in Prague,
Faculty of Nuclear Sciences and Physical Engineering.

In Prague, 8th September 2020

ing. Marie Davídková, CSc.
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