Dear Reviewer,

We would like to express our sincere appreciation for your careful attention to our manuscript and for the suggested improvements and valuable comments. We have revised the manuscript according to your remarks. Please find below the detailed description of the revision with our point-by-point answers.

Reviewer: The manuscript describes the investigation of novel photosensitizer (PS) as anticancer agents. The authors discuss the possibility to enrich these PS in the cells, investigate cellular death upon light irradiation and dark states and investigate also photostability. The paper is very interesting and meaningful, but requires a revision prior to publication.

Point 1: Please write also full word and abbrevi ation upon first use of photodynamic therapy.

Response 1: We added the acronym PDT at first mention of photodynamic therapy in the Introduction section (Line 33 in the revised version of the manuscript).

Quotation: *Photodynamic therapy (PDT) was developed over forty years ago and is now widely is used to treat of superficial tumors or tumors accessible with an endoscope.*

Point 2: Novel particulate (adsorbed dyes) [1], dispersed [2,3], or remote steerable containers [4] could more precisely deliver the PS to the target. The authors should mention such extended future uses. This could reduce common side effects like blindness if the PS migrates to the eyes. The μm size makes investigations of their fate easier and easier steerable.

References

[1] S. Rutkowski, L. Mu, T. Si, M. Gai, M. Sun, J. Frueh, Q. He, Magnetically-propelled hydrogel particle motors produced by ultrasound assisted hydrodynamic electrospray ionization jetting, Colloids Surfaces B Biointerfaces. 175 (2019) 44–55. https://doi.org/10.1016/j.colsurfb.2018.11.068.

[2] K. Köhler, H. Möhwald, G.B. Sukhorukov, Thermal behavior of polyelectrolyte multilayer microcapsules: 2. Insight into molecular mechanisms for the PDADMAC/PSS system, J. Phys. Chem. B. 110 (2006) 24002–10. https://doi.org/10.1021/jp062907a.

[3] K. Köhler, G.B. Sukhorukov, Heat treatment of polyelectrolyte multilayer capsules: A versatile method for encapsulation, Adv Funct Mater. 17 (2007) 2053–2061. https://doi.org/10.1002/adfm.200600593.

[4] S. Rutkowski, T. Si, M. Gai, M. Sun, J. Frueh, Q. He, Magnetically-guided Hydrogel Capsule Motors produced via Ultrasound assisted Hydrodynamic Electrospray

Ionization Jetting, J. Colloid Interface Sci. 25 (2019) 752–774. https://doi.org/10.1016/j.jcis.2019.01.103.

Response 2: We added the discussion on the topic drug delivery systems for photosensitizers to the Introduction section with links to the recommended articles as well as some additional relevant sources (Lines 80-93 in the revised version of the manuscript).

Quotation: *Another approach to increasing the effectiveness of PDT is the development of delivery systems for photosensitizers [32]. Nanoscale carriers are of particular interest as delivery systems. They can provide an enhancement in PS solubility and prolonged blood circulation of PS, facilitate PS targeted delivery and controlled release in the pathological site [33-37]. Targeted accumulation of photosensitizers in the tumor helps to reduce the serious side effects of PDT, such as prolonged (over several months) photosensitivity of the skin and eyes . Polymer particles and micelles [38], liposomes [39], gold, calcium and silica nanoparticles [40-42] are especially popular among the nanoparticles used for the delivery of PSs. Additionally, nanoparticles can be modified with stimulus-sensitive moieties. Stimulus-sensitive carriers are able to respond to specific factors of tumor microenvironment which leads to the disintegration of the carrier in the tumor site and controlled release of the drug. The influencing factors can be of exogenous (magnetic field, heating, exposure to UV and IR radiation or ultrasound) [32] and endogenous nature – changes in the pH level [41], enzymes and redox potential [43].*

Point 3: Equation 1-3 is unreasonably large, please reduce size to match the text size for the symbols.

Response 3: We reduced the size of Equations 1-3 as recommended.

Point 4: Page 1 line 143 symbol error.

Response 4: Corrected

Point 5: Where did the authors get the cells from?

Response 5: The origin of the cell cultures is added to the manuscript (Lines 154-157 in the revised version of the manuscript).

Quotation: *The experiments were performed using human epidermoid carcinoma cells A431 (All-Russian Collection of Cell Cultures, Institute of Cytology of the Russian Academy of Sciences, Saint-Petersburg, Russia), human keratinocyte cells HaCat and human adenocarcinoma ovarian cells SKOV-3.ip (provided from cell collection of the Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russia).*

Point 6: Did the authors synthesize their PS themselves? They mention it, where are the source chemicals for this synthesis from? Where is the reference to this synthesis route or a detailed description?

Response 6: The synthesis of PS had been performed by the authors of this paper. We have added a detailed description of the synthesis of **PzNPh** and **FePzNPh** and

indicated the sources of chemicals for their synthesis (Lines 129-139 in the revised version of the manuscript).

Point 7: What was the purity of the PS?

Response 7: The product of **PzNPh** synthesis was carefully purified by chromatography (Silica gel 60, 40-μm, Merk, THF eluent). Purification was repeated at least four to six times.

We don't subject the prepared **FePzNPh** to the purification with silica since (i) we used carefully purified free base for its preparation; (ii) only undesirable impurity (AcOH) was removed by washing of **FePzNPh** precipitate with pure ethanol (5 ml, 5 portions) that had been removed under vacuum afterwards.

We added information about **PzNPh** and **FePzNPh** purification to the manuscript (Lines 133-139 in the revised version of the manuscript).

Point 8: Optimum window of biological tissue is as far as I know in the range of 650-1100. It is better than shorter wavelengths, so the authors should not exaggerate their claim. Suitable window is enough.

Response 8: We agree with the reviewer that in most literature sources the range of the optical transparency window is indicated as 650-1100 nm. However, there are number of works where, according to the authors, the range of the optical transparency window is 600-1000 nm [1-3]. Nevertheless, in order to avoid ambiguity, we have removed the term "optical transparency window of biological tissues" from the description of the photophysical properties of the porphyrazines under study.

References:

1. Richards-Kortum R, Sevick-Muraca E. Quantitative optical spectroscopy for tissue diagnosis. Annu Rev Phys Chem. 1996; 47:555-606. doi: 10.1146/annurev.physchem.47.1.555.

2. Valery V. Tuchin Tissue Optics and Photonics: Light-Tissue Interaction II / doi: 10.18287/JBPE16.02.030201; Rocıo del Pilar Soto Astorga Haemoglobin sensing with optical spectroscopy during minimally invasive procedures// 2014, book

3. Valentina Bello, Elisabetta Bodo, Sara Pizzurro and Sabina Merlo In Vivo Recognition of Vascular Structures by Near-Infrared Transillumination // doi:10.3390/ecsa-6-06639

Quotation: *The analysis of photophysical properties of PSs has revealed that both porphyrazines absorb and fluoresce in red and far-red regions of the spectrum 500-650 nm and 600-800 nm, respectively (Line 259 in the revised version of the manuscript).*

PzNPh *and* ***FePzNPh*** *absorb and fluoresce in red and far-red regions of the spectrum 500-650 nm and 600-800 nm, respectively. (Line 421 in the revised version of the manuscript).*

In particular, they are characterized by absorption and fluorescence in red and far-red regions of the spectrum of tissues and are characterized by the sensitivity of fluorescent properties to the viscosity of the medium. (Line 458 in the revised version of the manuscript).