dr Bożena Sikora-Dobrowolska
Institute of Physics PAS

Summary of Professional Accomplishments

"Design, production and characterization of multifunctional opto-magnetic nanoparticles with improved physicochemical properties in the context of their potential biological and medical applications"

"Mechanism of interaction between a solid (UCNPs nanoparticles) and biological structures (cells and tissues)"

Warsaw 2024

Table of Contents

Summary of Professional Accomplishments

1. Name

Full Name	Bożena Sikora-Dobrowolska
Contact	Institute of Physics, Polish Academy of Sciences Al. Lotników 32/46, 02-668 Warsaw, Poland e-mail: sikorab@ifpan.edu.pl phone: +48 785 549 062

2. Information about diplomas and academic degrees

2014	**Doctoral degree in physics** (with honors) Doctoral thesis title *"Design and characterization of biosensors based on colloidal nanoparticles for applications in biology and medicine"* Entity granting the degree: Scientific Council of the Institute of Physics PAS Supervisor: prof. Danek Elbaum
2012	**Postgraduate studies in Forensic Sciences** and related disciplines in the Center for Forensic Science, University of Warsaw
2008	**Master of Science in Chemistry** Master's thesis title: *„Kinetic study of antioxidant activity of selected neurotransmitters"* Entity granting the degree: Faculty of Chemistry, University of Warsaw Supervisor: prof. dr hab. Grzegorz Litwinienko

3. Information on employment in research institutes

2014 - now	Assistant Professor in the Laboratory of Biological Physics in the Institute of Physics, Polish Academy of Sciences. (employment form: employment contract)
2017 - now	Lecturer in Wyższa Szkola Inzynierii i Zdrowia (University of Engineering and Health) – Warsaw (employment form: mandate contract)
2008 - 2014	PhD student at the Laboratory of Biological Physics in the Institute of Physics, Polish Academy of Sciences

4. Description of the achievements, set out in art. 219 para 1 point 2 of the Act

4.1. The title of the first scientific achievement

"Design, production and characterization of multifunctional opto-magnetic nanoparticles with improved physicochemical properties in the context of their potential biological and medical applications"

A series of publications forming the first scientific achievement

No	Authors, publication title, journal, year of publication, volume, pages	Impact Factor[1]	Number of Citations[2]
A1	A. Borodziuk*, P. Kowalik, M. Duda, T. Wojciechowski, R. Minikayev, D. Kalinowska, M. Klepka, K. Sobczak, Ł. Kłopotowski, **B. Sikora*** *„Unmodified Rose Bengal photosensitizer conjugated with $NaYF_4$:Yb,Er upconverting nanoparticles for efficient photodynamic therapy"* **Nanotechnology** 2020, 31, 465101	3.874	20
A2	P. Kowalik*, D. Elbaum, J. Mikulski, K. Fronc, I. Kamińska, P. C. Morais, P. E. de Souza, R. B. Nunes, F. H. Veiga-Souza, G. Gruzeł, R. Minikayev, T. Wojciechowski, E. Mosiniewicz-Szablewska, M. Szewczyk, M. Pawlyta, A. Sienkiewicz, M. Łapiński, K. Zajdel, P. Stępień, J. Szczepkowski, W. Jastrzębski, M. Frontczak-Baniewicz, W. Paszkowicz, **B. Sikora** „*Upconversion fluorescence imaging of HeLa cells using ROS generating SiO_2-coated lanthanide-doped $NaYF_4$ nanoconstructs"* **RSC Adv** 2017, 7, 30262-30273	2.936	27
A3	P. Kowalik*, I. Kamińska, K. Fronc, A. Borodziuk, M. Duda, T. Wojciechowski, K. Sobczak, D. Kalinowska, M. T. Klepka, **B. Sikora*** *„The ROS-generating photosensitizer - free $NaYF_4$:Yb,Tm@SiO_2 upconverting nanoparticles for photodynamic therapy application"* **Nanotechnology** 2021, 32, 475101	3.953	10
A4	P. Kowalik, J. Mikulski, A. Borodziuk, M. Duda, I. Kamińska, K. Zajdel, J. Rybusinski, J. Szczytko, T. Wojciechowski, K. Sobczak, R. Minikayev, M. Kulpa-Greszta, R. Pazik, P. Grzaczkowska, K. Fronc, M. Lapinski, M. Frontczak-Baniewicz, **B. Sikora*** *„Yttrium-Doped Iron Oxide Nanoparticles for Magnetic Hyperthermia Applications"* **J Phys Chem C** 2020, 124, 12, 6871-6883	4.126	40
A5	**B. Sikora-Dobrowolska*,** A. Borodziuk, M. Kulpa-Greszta, R. Pazik, T. Wojciechowski, K. Sobczak, J. Rybusinski, J. Szczytko, L. Klopotowski *„Opto-magnetic nanoparticles with upconverting properties for optical imaging and photothermal therapies"* **J Magn Mag Mater** 2024, 591, 171714	2.700	0

*corresponding author

[1]according to the year of publication

[2]according to Web of Science on 16.04.2024

4.2. The title of the second scientific achievement

"Mechanism of interaction between a solid (UCNPs nanoparticles) and biological structures (cells and tissues)"

A series of publications forming the second scientific achievement

No	Authors, publication title, journal, year of publication, volume, pages	Impact Factor[1]	Number of Citations[2]
B1	**B. Sikora**[*], P. Kowalik, J. Mikulski, K. Fronc, I. Kamińska, M. Szewczyk, A. Konopka, K. Zajdel, R. Minikayev, K. Sobczak, W. Zaleszczyk, A. Borodziuk, J. Rybusiński, J. Szczytko, A. Sienkiewicz, T. Wojciechowski, P. Stępień, M. Frontczak-Baniewicz, M. Łapiński, G. Wilczyński, W. Paszkowicz, A. Twardowski, D. Elbaum *„Mammalian cell defence mechanisms against the cytotoxicity of $NaYF_4$:(Er,Yb,Gd) nanoparticles"* **Nanoscale** 2017, 9, 14259-14271	7.233	17
B2	K. Zajdel[*], D. Bartczak, M. Frontczak-Baniewicz, D. A. Ramsay, P. Kowalik, K. Sobczak, I. Kamińska, T. Wojciechowski, R. Minikayev, H. Goenaga-Infante, **B. Sikora**[*] *„Nano–bio interactions of upconversion nanoparticles at subcellular level: biodistribution and cytotoxicity"* **Nanomedicine** 2023, 18(3), 233-258	5.500	1
B3	K. Zajdel[*], J. Janowska, M. Frontczak-Baniewicz, J. Sypecka, **B. Sikora**[*] *„Upconverting Nanoparticles as a New Bio-Imaging Strategy—Investigating Intracellular Trafficking of Endogenous Processes in Neural Tissue"* **Int J Mol Sci** 2023, 24(2), 1122	6.208	3

*corresponding author

[1]according to the year of publication

[2]according to Web of Science on 09.02.2024

Additional data:

ResearcherID	S-7493-2016
ORCID	0000-0001-5902-9682
Number of publications indexed in Web of Science	26
Number of all citations (excluding self-citations)*	339 (299)
Hirsch index*	12

* data according to the Web of Science portal, as of 16.04.2024

4.3. Statement of individual contribution to publications

Paper [A1] – My contribution to this work included planning and managing research. I coordinated the work of the team while performing research tasks. I presented research problems and work concepts. I received funding for the research (work was carried out as part of the SONATA project, of which I was the leader). I created the concept of surface modification of nanoparticles with the Rose Bengal photosensitizer (without the addition of hexanoic acid) to increase the efficiency of energy transfer between them and the detection of

reactive oxygen species from this nanoconstruct. I participated in cell experiments, their planning, preparation and interpretation. I made cell measurements with confocal microscopy. I participated in the analysis and interpretation of all results obtained during this work. I participated in the preparation of the draft and final version of the article. The work was part of a doctoral thesis for which I was an auxiliary supervisor.

Paper [A2] – My contribution to this work included planning and managing research. I coordinated the work of the team while performing research tasks. I presented research problems and work concepts. I received funds for the research (the work was carried out as part of the SONATA project, of which I was the leader). I was responsible for the concept of reactive oxygen species production by nanoparticles upconverting 980 nm light to UV and blue light (without the use of photosensitizers or other surface additives). I participated in the development of the synthesis of the obtained nanostructures and their modifications. I developed methods for the introduction of nanomaterials into HeLa cells. I participated in measuring the luminescence of nanoparticles and developing a method for measuring reactive oxygen species generated from nanoparticles using the Electron Paramagnetic Resonance (EPR) method. I conducted EPR measurements in collaboration with the group of Prof. Paulo Eduardo Narcizo de Souza at the University of Brasilia. I participated in the analysis and interpretation of all the results obtained in this work. I conducted confocal microscopy measurements of HeLa cells. I prepared a draft and the final version of the article.

Paper [A3] – My contribution to this work included planning and managing research. I coordinated the work of the team while performing research tasks. I presented research problems and work concepts. I received funds for the research (the work was carried out as part of the SONATA project, of which I was the leader). I participated in the development of a method to measure the cytotoxicity of 4T1 cancer cells with upconverting nanoparticles excited by NIR radiation (without the use of photosensitizers on the surface of the nanoparticles). I developed a method for functionalization and biofunctionalization of nanoparticles and a method for their characterization. I performed confocal microscopy measurements of 4T1 cells with nanoparticles. I participated in the analysis and interpretation of all the results obtained as part of this work. I prepared a draft and the final version of the article.

Paper [A4] – My contribution to this work included planning and managing research. I coordinated the work of the team during the research tasks. I presented research problems and work concepts. I received funds for the research (the work was carried out as part of the SONATA project, of which I was the leader). I was responsible for the concept of doping Fe_3O_4 nanoparticles with different amounts of Y^{3+} ions. I participated in hyperthermia measurements of the nanoparticles. I have developed a concept and plan of nanoparticles in cell hyperthermia experiments. I analyzed and interpreted the results of this work. I was the author of a concept that explains the effect of Y^{3+} dopants on the magnetism of nanoparticles. I prepared a draft and the final version of the article.

Paper [A5] – My contribution to this work included planning and managing research. I coordinated the work of the team during research tasks. I presented the research problems and

concepts of the work. I developed a method for connecting optical and magnetic nanoparticles. I analyzed and interpreted the results obtained in this work. I calculated the specific absorption rate (SAR), interpreted the results of irradiation of nanoparticles with 808 nm laser, and compared them with 880nm laser. I prepared a draft and the final version of the article.

Paper [B1] – My contribution to this work included planning and conducting most of the research and experiments. I presented the research problem, the work concept and planned the research needed to solve the problem. I performed all nanomaterial syntheses and their luminescence characteristics. I planned all experiments involving cells. I performed measurements of the cells using confocal microscopy. I am the author of a method for the colocalization of nanoparticles with cell organelles based on luminescence spectra recorded in a confocal microscope. I analyzed and interpreted the results obtained in this work. I proposed a mechanism for the internalization and excretion of nanoparticles using Hela, HEK cells and astrocytes. I prepared a draft and the final version of the article.

Paper [B2] – My contribution to this work included planning and managing research. I coordinated the work of the team during research tasks. I presented research problems and work concepts. I participated in the analysis and interpretation of the obtained results. I proved the mechanism of clathrin- and caveola-dependent endocytosis of nanoparticle internalization by HeLa cells, which I proposed in my previous work. I performed confocal microscopy measurements of cell samples. I participated in the analysis and interpretation of all results obtained in this work. I participated in the preparation of the draft and final version of the article. The results were part of a doctoral thesis for which I was an auxiliary supervisor.

Praca [B3] – My contribution to the work was to plan and coordinate part of the research. I measured samples of hippocampal slice using confocal microscopy. I have demonstrated the mechanism of clathrin- and caveolae-mediated endocytosis by neurons located in the hippocampal slices. I participated in the analysis and interpretation of all results obtained as part of this work. I participated in the preparation of the draft and final version of the article. The results were part of a doctoral thesis for which I was an auxiliary supervisor.

Co-authors' declarations about their contribution to the publications are included in Appendix no. 8 "Statements of Contributors".

4.4. Discussion of the first scientific achievement

- **Introduction**

My research aimed to design and produce nanoparticles with specific physical properties suitable for direct application in diagnostics or anticancer therapy. The physical properties of these nanoparticles were crucial for achieving the intended goals.

This property can be used in imaging cancer cells or to excite a photosensitizer attached to the nanoparticle's surface. The photosensitizer excited in this way generates reactive oxygen species (ROS) that are toxic to cells.

The nanoparticles I designed exhibited upconverting (UC) properties, which involve the conversion of lower-energy radiation (e.g., near-infrared, NIR radiation) to higher-energy radiation (e.g., visible or ultraviolet radiation). This property can be used for imaging cancer cells or for exciting a photosensitizer attached to the nanoparticle's surface. Then, the photosensitizer generates reactive oxygen species (ROS) that are toxic to cells. These kinds of nanoparticles can be used in photodynamic therapy (PDT). These strategies are described in papers A1, A2 and A3.

The magnetic properties of the nanoparticles I design and produce can be used for magnetic targeting and as contrast agents in Magnetic Resonance Imaging (MRI). The magnetism of these types of nanomaterials can also induce tissue heating by raising the local temperature when exposed to an alternating magnetic field (magnetic hyperthermia) or under near-infrared laser irradiation (photothermal therapy). These applications are discussed in papers A4 and A5.

Additionally, it is crucial to combine multiple therapeutic modalities into a single nanoparticle with both optical and magnetic properties. This enables the development of multifunctional nanoparticles. Such a nanoparticle was designed, created, and examined in paper A5.

After attaching proteins specific to cancer cells onto nanoparticles, these nanoparticles can selectively bind to cancer cells. This approach is called targeted therapy. The concept of creating multifunctional nanoparticles that can be used in cancer diagnostics (due to their upconverting properties), in PDT therapy and targeted therapy was investigated and described in paper A3.

The research I present are interdisciplinary, combining chemistry, physics, and biology. The core focus lies in exploring the physical properties of nanoparticles - such as upconversion, energy transfer, and magnetism - in relation to their applications in biology. Additionally, various physical methods were employed to characterize the nanomaterials, including optical spectroscopy, Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), Superconducting Quantum Interference Device (SQUID), and confocal microscopy.

My significant scientific achievement, which has greatly contributed to the understanding of nanomaterials for applications in biology and medicine, involves developing nanoparticles with enhanced physical properties tailored for biological use. This achievement is detailed across a series of five publications [A1-A5], published in 2017 to 2024.

- **Detailed description**

One of the methods that can be used in anticancer treatment is photodynamic therapy (PDT). This method requires the presence of a photosensitive organic dye called a photosensitizer (PS). A photosensitizer excited by light is capable of producing reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), hydroxyl radicals ($^{\bullet}OH$), or superoxide radicals ($O_2^{\bullet-}$).[1,2] The presence of large amounts of ROS is toxic to cells and consequently leads to their death.[3,4]

PDT is a non-invasive cancer therapy with minimal side effects, as only a localized area containing the PS is irradiated. Moreover, PDT is a cost-effective treatment compared to chemotherapy or radiotherapy.[1]

However, PDT also has some disadvantages. Organic PS molecules require excitation with visible (VIS) or ultraviolet (UV) light to generate ROS. This light is strongly absorbed by tissues, limiting the therapy's effectiveness due to shallow penetration depth.[5] Additionally, organic compounds can undergo photobleaching when exposed to high-intensity light. Patients receiving a photosensitizer must remain in darkness until the compound accumulates in the affected tissue.[6,7]

One solution to enhance PDT efficacy is to use near-infrared (NIR) light for ROS generation. Unlike traditional PDT limited to superficial layers of the skin, NIR-PDT can penetrate tissue depths of up to 10 cm.[8]

An effective approach for using NIR light to generate ROS involves nanoparticles doped with rare earth ions, known as upconverting nanoparticles (UCNPs). By carefully selecting doping ions, it is possible to achieve UV or visible light emission following NIR excitation.[9,10] UCNPs that exhibit relatively high quantum efficiency of upconversion include hexagonal $NaYF_4$ nanoparticles doped with erbium and ytterbium ions ($NaYF_4$:Yb^{3+},Er^{3+}) or thulium and ytterbium ions ($NaYF_4$:Yb^{3+},Tm^{3+}).[11,12] The Yb^{3+} ions absorb NIR light, while Er^{3+} or Tm^{3+} ions are light emitters.

The notable efficiency of upconversion in rare-earth ion-doped $NaYF_4$ nanoparticles can be attributed to the alignment of the $NaYF_4$ crystal lattice with the ionic radii of the doping ions, which inhibits defect formation. Additionally, the hexagonal crystal structure is characterized by low phonon energy, minimizing non-radiative energy relaxation.[11]

To obtain $NaYF_4$ nanoparticles water-soluble and facilitate surface functionalization, they are often coated with a SiO_2 layer.[13,14,15] This modification enables the conjugation of therapeutic molecules onto the nanoparticle surface for applications such as PDT therapy.[16,17]

For effective energy transfer between UCNPs and PS molecules, which ultimately leads to the generation of ROS, it is crucial that the emission spectrum of UCNPs overlaps with the absorption spectrum of PS.

Energy transfer can occur through either a radiative or non-radiative process. Radiative transfer involves the reabsorption of photons emitted from UCNPs by PS molecules. Although weakly dependent on the distance between light-emitting Er^{3+} ions and light-absorbing PS molecules, this process is inherently inefficient. In contrast, the non-radiative process occurs via Förster Resonance Energy Transfer (FRET), which strongly depends on the distance between the UCNPs and PS molecules. FRET efficiency scales inversely with the sixth power of the distance.[18]

In paper A1, Rose Bengal (RB) was used as a PS. RB is a well-known organic dye extensively studied for various biological and medical applications, ranging from cell staining

to non-invasive PDT.[19] The absorption spectrum of RB overlaps with the emission spectrum of Er^{3+} ions in $NaYF_4$:2%Er^{3+},20%Yb^{3+} nanoparticles. Among hydrophilic PSs, RB demonstrates the highest efficiency in producing ROS, reaching up to 75%.[20,21] The most commonly described method for attaching RB to UCNPs in literature involves using hexanoic acid (HA) as a linker between these two compounds. [18,22,23] However, the use of HA complicates the production process and increases the distance between the donor and acceptor, thereby limiting the efficiency of the energy transfer process and, consequently, the efficiency of ROS production.

The $NaYF_4$:2%Er^{3+},20%Yb^{3+} and $NaYF_4$:0,2%Tm^{3+},20%Yb^{3+} nanoparticles used in the research papers constituting the habilitation achievement cycle were prepared using the thermal decomposition method. Subsequently, their physical properties were examined, including luminescence spectra, size distribution, crystal structure, etc., Fig. 1.

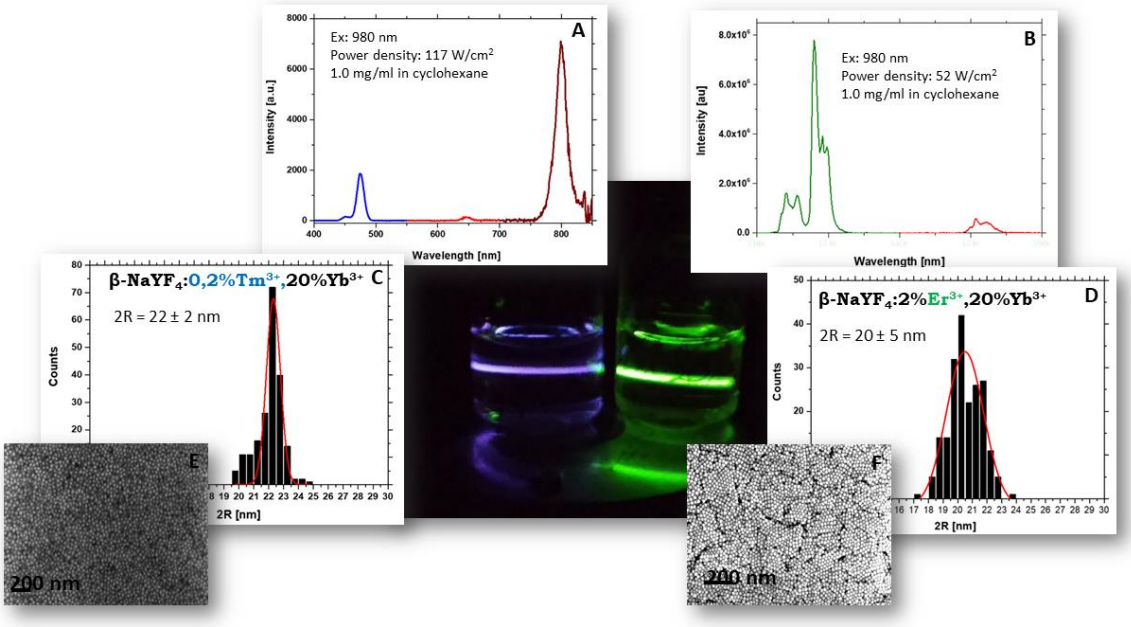


Fig. 1. Luminescence spectra of (A) $NaYF_4$:0,2%Tm^{3+},20%Yb^{3+}, (B) $NaYF_4$:2%Er^{3+},20%Yb^{3+} nanoparticle solutions excited by a 980 nm laser. Histograms of (C) $NaYF_4$:0,2%Tm^{3+},20%Yb^{3+}, (D) $NaYF_4$:2%Er^{3+},20%Yb^{3+} nanoparticle sizes obtained from SEM results. SEM pictures of (E) $NaYF_4$:0,2%Tm^{3+},20%Yb^{3+}, (F) $NaYF_4$:2%Er^{3+},20%Yb^{3+} nanoparticles.

The upconversion (UC) luminescence mechanism of $NaYF_4$ nanoparticles doped with Er^{3+} and Yb^{3+}, as well as Tm^{3+} and Yb^{3+} ions, is well understood.[24] In the case of $NaYF_4$:2%Er^{3+},20%Yb^{3+}, excitation energy is transferred from the $^2F_{5/2}$ excited state of Yb^{3+} ions to the $^4I_{11/2}$ state of Er^{3+} ions. Subsequently, a second energy transfer from Yb^{3+} ions can populate the $^4F_{7/2}$ state of Er^{3+} ions. Er^{3+} ions can undergo nonradiative relaxation processes to the $^2H_{11/2}$ and $^4S_{3/2}$ levels, and emit green light through radiative transitions such as $^2H_{11/2} \rightarrow {}^4I_{15/2}$ i $^4S_{3/2} \rightarrow {}^4I_{15/2}$. Alternatively, Er^{3+} ions can nonradiatively relax to the $^4F_{9/2}$ state, resulting in red emission via the radiative transition $^4F_{9/2} \rightarrow {}^4I_{15/2}$.[25]

On the other hand, nanoparticles doped with Yb^{3+} and Tm^{3+} ions exhibit four main emission bands at wavelengths of 474 nm, 645 nm, 697 nm, and 800 nm. Upon absorption of 980 nm photons by Yb^{3+} ions, energy is transferred from the $^2F_{5/2}$ state of Yb^{3+} to the $^3H_6$, $^3H_5$,

$^3F_2$, and $^1G_4$ states in Tm^{3+} ions, respectively. This energy transfer and subsequent nonradiative transitions between levels lead to emission through radiative transitions, including: $^1G4 \rightarrow {}^3H_6$, $^1G4 \rightarrow {}^3F_4$, $^3F_3 \rightarrow {}^3H_6$ and $^3H_4 \rightarrow {}^3H_6$.[25]

The nanoparticles obtained as part of the habilitation achievement were coated with a layer of SiO_2 or SiO_2 containing an amine functional group, depending on the intended application of the study.

Subsequently, Rose Bengal (RB) molecules were attached to the $NaYF_4$:2%Er^{3+},20%Yb^{3+}@SiO_2-NH_2 UCNPs described in paper A1 by forming an amide bond. The existence of this bond was confirmed through FTIR measurements. It is noteworthy that complete coverage of the UCNPs@SiO_2 surface with RB molecules was achieved without the need to functionalize RB with hexanoic acid (HA). This finding indicates that RB functionalization is unnecessary for effective coating of UCNP@SiO_2. This is significant because it was previously believed that RB functionalization was required to prevent steric hindrance during amide bonding to SiO_2 surfaces.[22]

To investigate the mechanism of energy transfer between UCNPs and RB, luminescence spectra of the nanoparticles and luminescence decay times were measured for each luminescence wavelength of the UCNPs (Fig. 2). The energy transfer efficiency was calculated in both cases: for luminescence ($E_1 = 29\%$) and for decay times ($E_2 = 19\%$ and $E_2 = 17\%$ for the 520 nm and 540 nm bands, respectively). A comparison of E_1 and E_2 values indicates that both reabsorption and FRET processes occur within the nanomaterial, with FRET accounting for approximately 62% of the total energy transfer.

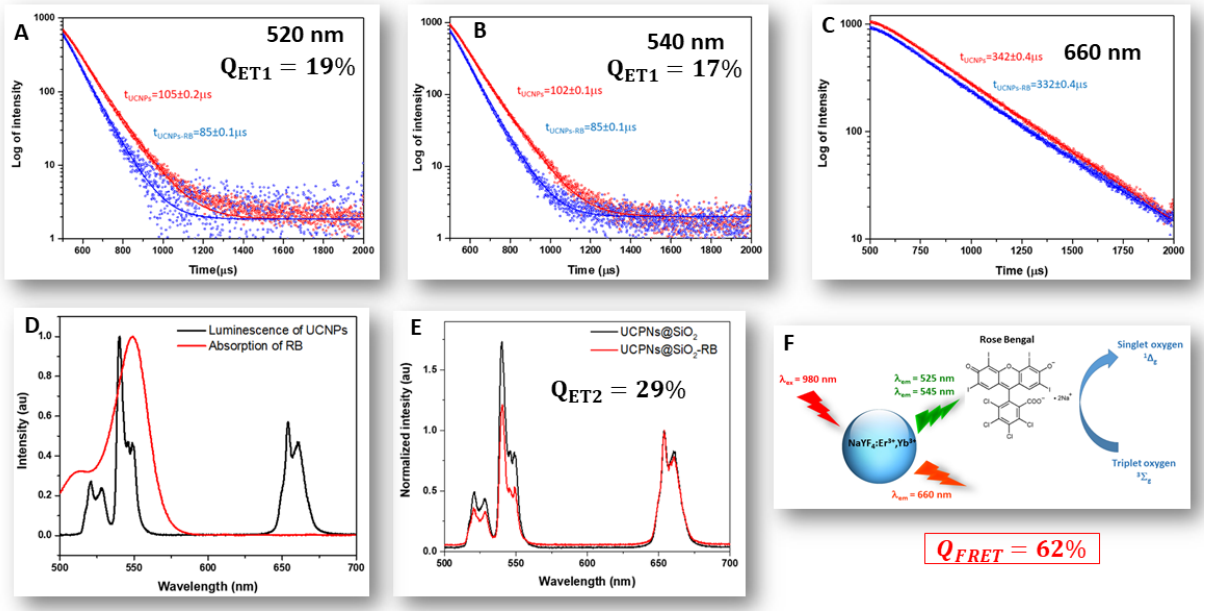


Fig. 2. Luminescence decay curves for UCNPs@SiO_2 (red points) and UCNPs@SiO_2-RB (blue points) recorded at wavelengths of 520 nm (A), 540 nm (B), and 660 nm (C). Overlay of the absorption spectra of RB and luminescence of UCNPs (D). Luminescence spectra of UCNPs@SiO_2-RB and UCNPs@SiO_2 normalized to a 660 nm wavelength after irradiation with a 980 nm laser with a power of 19 W cm^{-2} (E). Scheme of energy transfer from UCNPs to RB (F).

The FRET efficiency, represented by E_2 values, decreases significantly with increasing distance between the light-emitting Er^{3+} ions and the absorbing RB molecules. Therefore, it is

expected that E_2 will be much lower when the surface of UCNPs@SiO_2 is functionalized with hexanoic acid (HA), as the six-carbon chain of HA adds approximately 0.6 nm to the distance between RB and the SiO_2 surface on UCNPs. To estimate the expected decrease in E_2 resulting from the increased distance, we referred to the work of Melle et al., who calculated the dependence of FRET efficiency on the distance between UCNP@SiO_2 and CdTe nanocrystals.[26] Since CdTe nanocrystals and RB molecules have similar molar absorption coefficients in the spectral range of 500-550 nm, the calculated FRET efficiency between UCNPs@SiO_2 and CdTe nanocrystals is expected to be comparable to that in the UCNPs@SiO_2-RB system. Analysis of the results allowed us to estimate that E_2 would decrease by approximately 30% in the case of RB molecule conjugation with HA. This reduction significantly contributes to improving the FRET efficiency between UCNPs and the photosensitizer (RB), which directly impacts the efficiency of ROS generation and, consequently, the efficiency of PDT therapy.

The efficiency of ROS generation was determined by measuring the decrease in absorption of the singlet oxygen ($^1O_2$) indicator, 1,3-Diphenyl-2-benzofuran (DPBF), in an aqueous solution of UCNPs@SiO_2-RB nanoparticles (64 μg ml^{-1}) after 980 nm laser irradiation at a power density of 3 W cm^{-2}. After 35 minutes of irradiation, a 40% decrease in DPBF absorbance was observed (Fig. 3). In contrast, the mixture of UCNPs with RB showed insignificant decrease in DPBF absorption. These results demonstrate that only RB molecules bound to UCNP@SiO_2 can effectively generate $^1O_2$ upon NIR irradiation.

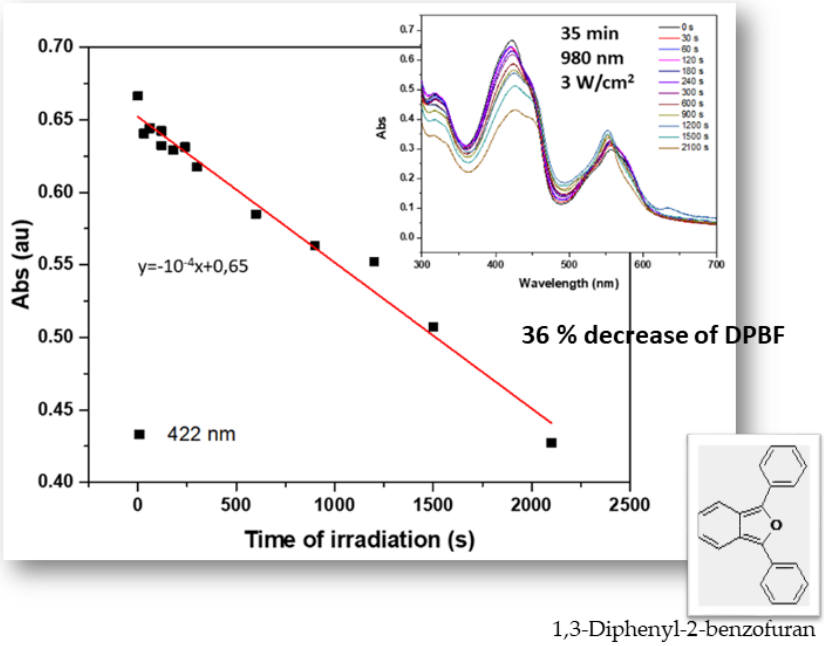


Fig. 3. (A) Time dependence of the normalized DPBF absorbance at 422 nm for the aqueous solution of UCNPs@SiO_2-RB after irradiation with a 980 nm laser. The inset shows the absorbance spectra of 64 μg ml^{-1} UCNPs@SiO_2-RB and 50 μM DPBF nanoparticles.

To demonstrate the efficiency $^1O_2$ production, the absorbance of DPBF was measured in a solution containing free RB molecules at the same concentration as that used for UCNPs@SiO_2-RB, irradiated with visible light at a wavelength of 549 nm and an estimated power density of 13 mW cm^{-2}. The dynamics of the decrease in absorbance were similar in both

cases, indicating that approximately the same amount of singlet oxygen was produced in both experiments. This confirms our achievement that the nanomaterials we produce generate a comparable amount of ROS through efficient energy transfer upon NIR light excitation (which is more beneficial in biological applications) compared to the amount produced from photosensitizers alone when excited by visible light.

In the next stage, we examined whether the amount of ROS generated from UCNPs@SiO_2-RB was sufficient to kill cancer cells. For this purpose, mouse mammary carcinoma cells (4T1) were incubated with UCNPs@SiO_2-RB for 2.5 hours at a concentration of 250 µg ml^{-1}. The cells were then irradiated with a 980 nm laser at a power density of 2 W cm^{-2} for 10 minutes. Subsequently, the Presto Blue (PB) Assay was performed. Cell viability decreased to 60% after this treatment. Twenty-four hours later, the PB Assay was repeated on the same cells, revealing a further decrease in viability to 33% (Fig. 4).

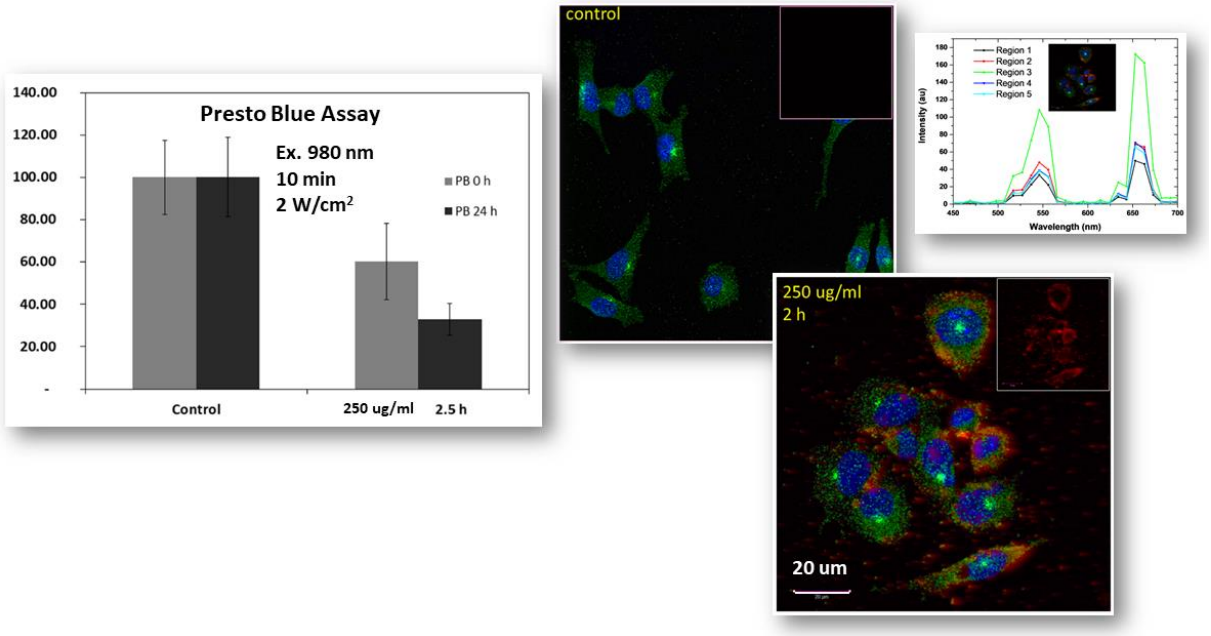


Fig. 4. Results of the Presto Blue Assay of viability on 4T1 cells incubated with 250 µg ml^{-1} UCNPs@SiO_2-RB after 10 minutes of irradiation at 980 nm (2 W cm^{-2}, 10 minutes) at 1 hour and 24 hours after irradiation. Confocal microscopy images of UCNPs@SiO_2-RB nanoparticles inside 4T1 cells. Green color - lysosomes, blue color - cell nuclei, red color - nanoparticles.

In summary, in paper A1, we demonstrated effective *in vitro* PDT using RB-conjugated upconverting $NaYF_4$:2%Er^{3+},20%Yb^{3+} nanoparticles upon NIR light excitation at 980 nm. Following 10 minutes of irradiation of 4T1 cancer cells incubated with 250 µg ml^{-1} of nanoparticles using a 980 nm laser at a power of 2 W cm^{-2}, cell viability decreased to approximately 30% compared to the control group (cells incubated with nanoparticles but without NIR irradiation). Importantly, this high therapeutic efficacy was achieved without the need for hexanoic acid (HA) functionalization. The procedure employed in this study enabled complete coverage of nanoparticle surfaces with RB molecules, demonstrating that functionalization with HA is unnecessary, contrary to previous assumptions.[23] Omitting HA functionalization not only simplified the synthesis process but also reduced the distance between UCNPs and RB molecules. Time-resolved fluorescence measurements confirmed that

energy transfer between UCNPs and RB mainly occurs via FRET, resulting in efficient ROS production

Although PDT using PS attached to UCNPs offers several advantages, there are still unresolved issues related to the photobleaching of PS.[27] Additionally, patients treated with such nanoconstructs must remain in the dark until the material accumulates in the diseased tissue due to ongoing ROS production from the PS upon visible light excitation.

An alternative approach involves using $NaYF_4$ nanoparticles doped with ytterbium (Yb) and thulium (Tm) ions to ROS without relying on unstable photosensitizer molecules.[28] The emission spectrum resulting from the Yb^{3+}-Tm^{3+} ion pair, excited by a 980 nm laser, enables the upconversion system to generate ROS without the need for additional organic compounds, such as PS molecules. The emitted high-energy UV light can decompose water molecules into toxic radicals.

In work A2, UCNPs doped with Yb^{3+} and Tm^{3+} ions and coated with SiO_2 were synthesized. The $NaYF_4$:0.2%Tm^{3+},20%Yb^{3+} nanoparticles exhibit intense luminescence in the UV, blue, red, and NIR ranges upon 980 nm laser irradiation. The UV emission range enables the generation of ROS in an aqueous environment, as confirmed by electron paramagnetic resonance (EPR) spectroscopy studies following 980 nm laser irradiation.

To assess ROS generation, an aqueous solution of nanoparticles at a concentration of 15 mg ml^{-1} was mixed with the spin trap 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (CMH), which yields an EPR signal upon reaction with free radicals. The EPR signal was measured every ten minutes during exposure to the laser. A 70% increase in the EPR signal was observed after 20 minutes of exposure (Fig. 5). This represents a significant advancement in the development of new materials capable of producing ROS without the need for photosensitizers or additional inorganic layers described in previous studies.[29,30,31,32,33,34]

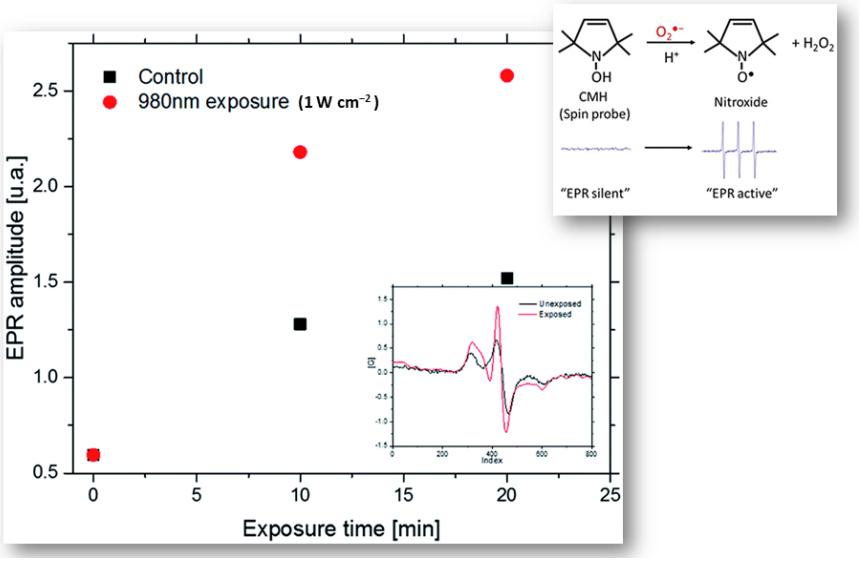


Fig. 5. The EPR plot of the CMH spin trap signal. CMH was activated by reactive oxygen species formed as a result of the decomposition of water by nanoparticles emitting high-energy radiation. Control group - non-irradiated solution. The inset shows an example EPR spectrum (after 20 minutes of exposure).

Cytotoxicity tests demonstrated that all prepared nanomaterials are non-toxic even at relatively high concentrations (50 µg ml^{-1}) after a 48-hour incubation period (MTT assay performed on HeLa cervical cancer cells). It is noteworthy that confocal microscopy measurements of nanoparticles inside HeLa cells did not show any measurable autofluorescence signal from biological structures following 980 nm excitation in the nanoparticle channel.

In paper A3, we evaluated the aforementioned UCNPs on live 4T1 cancer cells as an alternative to photosensitizer (PS)-modified UCNPs in PDT. For the first time in this publication, an experiment was conducted using PDT based on nanoparticles doped with Tm^{3+} and Yb^{3+} ions, without the addition of PS. *In vitro* PDT was performed on 4T1 cancer cells incubated with $NaYF_4$:0,2%Tm^{3+},20%Yb^{3+}@SiO_2 nanoparticles (100 µg ml^{-1}) and irradiated with NIR light at 980 nm (2 W cm^{-2}; 10-minute cycle: 1.5 minutes of exposure followed by a 0.5-minute break). After 24 hours post-irradiation, cell viability assessed using the Presto Blue assay and microscopic measurements decreased to below 10%, demonstrating the excellent effectiveness of the therapy (Fig. 6). This study exemplifies the application of the physical properties of UCNPs in improving PDT therapy on living cancer cells, making a significant contribution to the field of physics and its biological applications.

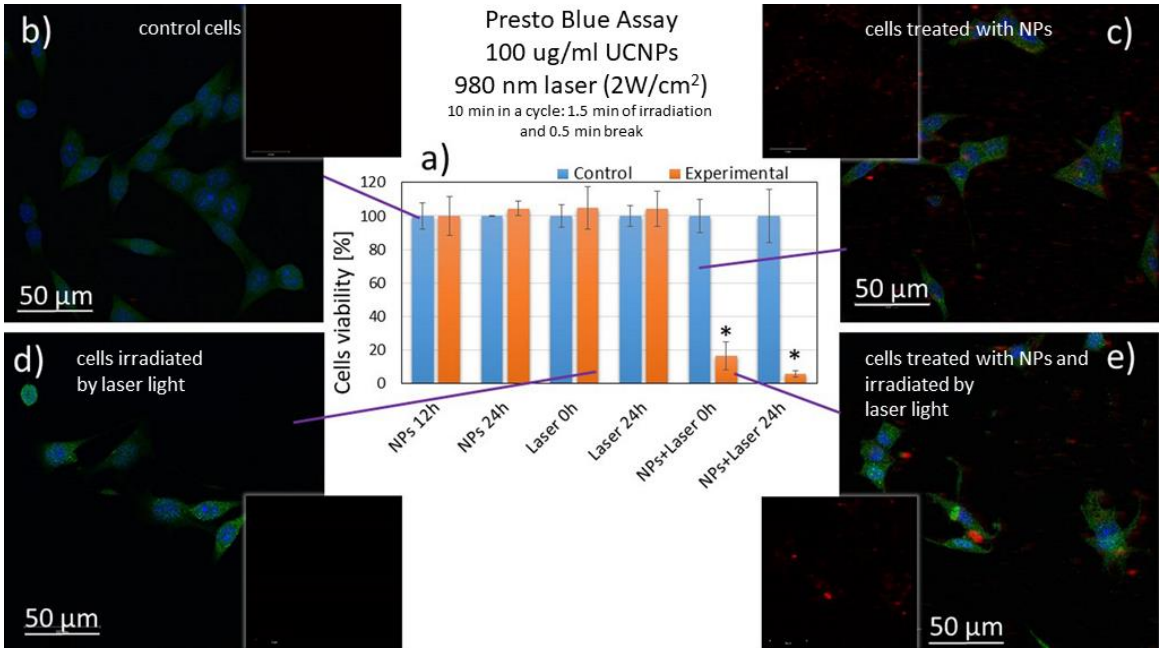


Fig. 6. PrestoBlue viability assay results of 4T1 cancer cells incubated with UCNPs@SiO_2 (100 µg ml^{-1}) and irradiated with 980 nm laser light (a). Confocal microscope images: control cells (b), cells with nanoparticles (c), cells irradiated with a 980 nm laser (d), and cells with nanoparticles after irradiation with a 980 nm laser (e). Green color - early endosomes, blue color - nuclei, and red color - UCNPs@SiO_2.

The availability of numerous methods for modifying the silicon oxide shell, coupled with the continuously expanding knowledge of changes in cancer cells, enables the development of individually targeted anticancer therapy. Cancer tissues can be identified using biofunctionalized nanoparticles. The presence of specific receptors on the surface of cancer cells allows them to be targeted with nanoparticles containing specific antibodies on their

surface. With the ability to generate ROS, it becomes possible to selectively kill the cells to which the nanoparticles have been attached.[35,36,37]

In paper A3, an anti-human IgG antibody (anti-h-IgG) was attached to $NaYF_4$:0,2%Tm^{3+},20%Yb^{3+}@SiO_2 nanoparticles modified with silane-PEG-NHS 2000. Polyethylene glycol (PEG) prevents aggregation, reduces nonspecific binding, and improves the solubility of nanoparticles in water. NHS ester groups are highly reactive towards proteins.[38]

This attachment was confirmed by an experiment using a model membrane covered with h-IgG. The antibodies on the nanoparticles specifically attached to h-IgG on the membrane. The UCNPs@SiO_2-PEG-anti-h-IgG nanoparticles successfully bound to the h-IgG protein covering the membrane at all tested concentrations (Fig. 7). This achievement is significant because UCNPs@SiO_2 can be used not only for PDT but also for specific cellular biolabeling.

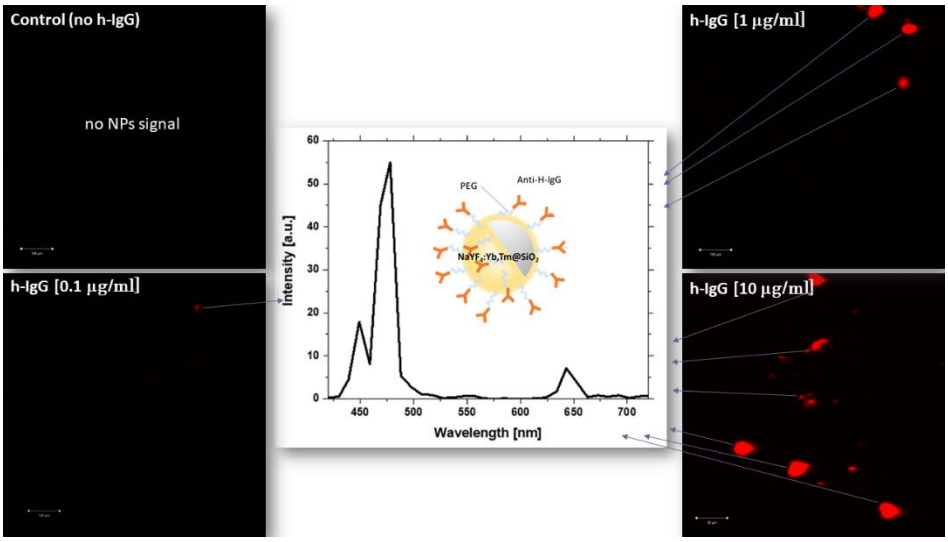


Fig. 7. Surfaces of nitrocellulose membranes with attached UCNPs@SiO_2-PEG-NHS-anti-h-IgG analyzed using a confocal microscope at 980 nm excitation. The characteristic spectrum of nanoparticles measured at 980 nm excitation.

As mentioned earlier, in addition to nanoparticles with optical properties, Fe_3O_4 nanoparticles with magnetic properties were also designed and produced as part of the habilitation achievement. The magnetism of these nanomaterials can be used for MRI imaging, as well as for tissue heating through exposure to an alternating magnetic field (magnetic hyperthermia) and near-infrared laser irradiation (photothermal therapy). These topics are described in works A4 and A5.

In magnetic hyperthermia, the heating effect is achieved by exposing the nanoparticles to an alternating magnetic field (AMF). When the particles are in the superparamagnetic state, meaning their sizes are below a critical value (about 30 nm for Fe_3O_4), the Néel-Brown relaxation mechanism dominates the heat generation under the AMF.[39,40] The Néel relaxation mechanism involves the rotation of magnetic moments within each particle, while the Brownian relaxation mechanism involves the rotation of the entire nanoparticle, aligning its magnetic moments with the field.[41,42]

The primary objective of the research presented in paper A4 was to synthesize magnetic Fe_3O_4 nanoparticles (magnetite structure) doped with Y^{3+} ions at varying concentrations (0%,

0.1%, 1%, and 10%) to achieve maximum heating efficiency in magnetic hyperthermia for cancer therapy applications.

The crystal structures of the obtained nanoparticles examined by X-ray diffraction (XRD) confirmed a single-phase structure of reverse spinel ($Fe^{2+}Fe_2^{3+}O_4^{2-}$) for all samples. The nanoparticle size increased with increasing Y^{3+} content in the structure (18 nm for Fe_3O_4 nanoparticles doped with 0.1% Y^{3+}; 23 nm for nanoparticles with 1% Y^{3+}; and 168 nm for 10% Y^{3+}). The Fe_3O_4 nanoparticles prepared in this manner exhibited ferromagnetic properties at room temperature, as illustrated by the hysteresis (gap) between the zero-field-cooled (ZFC) and field-cooled (FC) magnetization curves. The maximum magnetization was achieved for the sample doped with 1% Y^{3+}, confirming that magnetization increases with dopant concentration up to a maximum, followed by a decrease with further dopant percentage (Fig. 8).

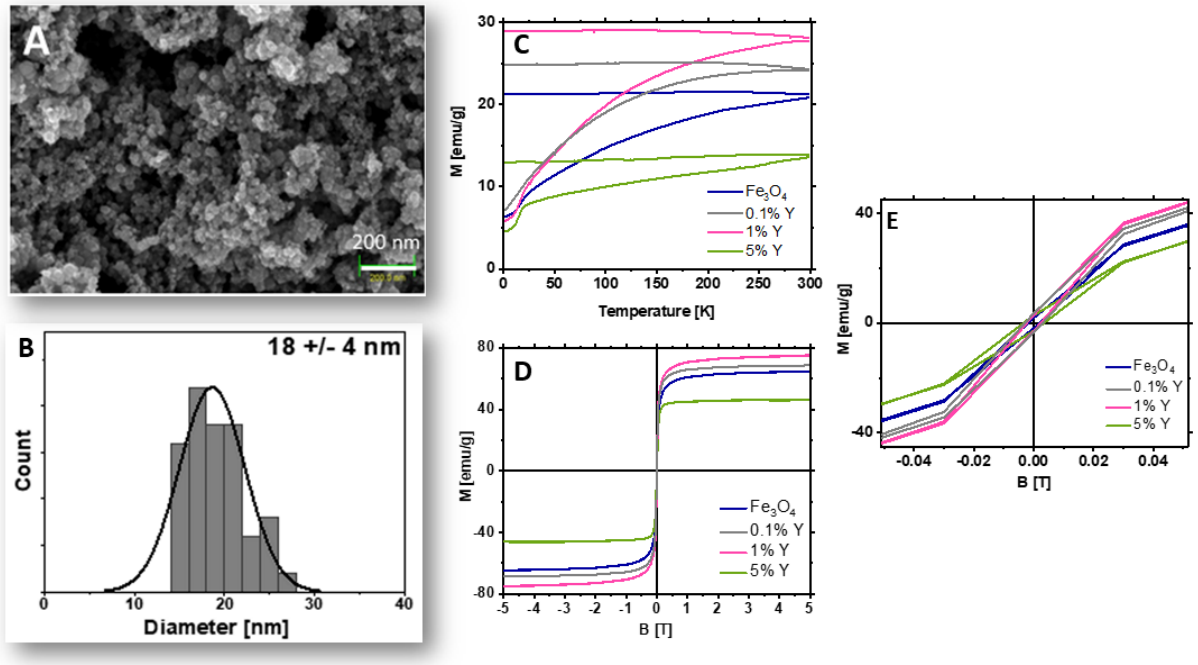


Fig. 8. SEM image (A) and histogram (B) of Fe_3O_4 nanoparticles. ZFC/FC graph of nanoparticle (C), and hysteresis loops recorded at 310 K (D, E).

The magnetization of Fe_3O_4 nanoparticles results from differences in the net magnetic moment of ions occupying tetrahedral and octahedral sites within the lattice. Octahedral gaps are filled with a combination of Fe^{2+} and Fe^{3+} ions, while tetrahedral gaps contain only Fe^{3+} ions. The dominant interactions are AB super-exchange interactions over AA and BB intra-sublattice interactions (according to the Néel model).[43] Néel's model considers three types of exchange interactions between unpaired electrons of two ions: (i) both ions located in A sites (AA interaction), (ii) both ions located in B sites (BB interaction), and (iii) one ion in site A and the other in site B (AB interaction). The AB interaction predominantly aligns magnetic spins in site A in one direction and those in site B in the opposite direction, resulting in a net magnetic moment difference between sublattices A and B.[44,45,46]

The magnetization directly reflects the distribution of Fe^{3+} ions between the two sublattices. If Fe^{3+} ions occupy both octahedral and tetrahedral sites, ferrimagnetic ordering is observed. Magnetization is typically higher in magnetic nanoparticles than in bulk materials due to the

formation of a partially reversed spinel. The presence of Fe^{3+} ions in tetrahedral sites facilitates superexchange (Fe^{3+}_A–O–Fe^{3+}_B), resulting in increased magnetization.

Replacing Fe^{3+} ions with non-magnetic Y^{3+} ions generally decreases octahedral coordination magnetization, consequently reducing overall magnetization. However, with small amounts of Y^{3+} added (up to 1%), the opposite trend is observed. At low Y^{3+} concentrations, magnetization values increase. This effect may be explained by non-magnetic Y^{3+} ions entering tetrahedral sites within the spinel, leaving Fe^{3+} in octahedral sites, thereby increasing magnetization. Alternatively, the presence of Y^{3+} ions increases nanoparticle size, elevating blocking temperature and saturation magnetization at low dopant concentrations. Further increasing Y^{3+} doping results in larger nanoparticles, eventually leading to decreased magnetization as non-magnetic yttrium replaces magnetic iron in octahedral sites.[47,48]

The magnetic hyperthermia of the magnetic nanoparticles in water was measured (Fig. 9). The specific absorption rate (SAR) and intrinsic loss power (ILP) values were determined.[49,50] An important factor in hyperthermia is the exposure time. Therefore, the ability of magnetic nanoparticles to produce sufficient heat in AMF in a shorter time makes them more useful for magnetic hyperthermia.

The best results were obtained with Fe_3O_4 nanoparticles doped with 0.1% Y^{3+} (SAR = 194 W g^{-1} and ILP = 1.85 nHm^2 kg^{-1} for a magnetic field of 16 kA m^{-1} and a frequency of 413 kHz).

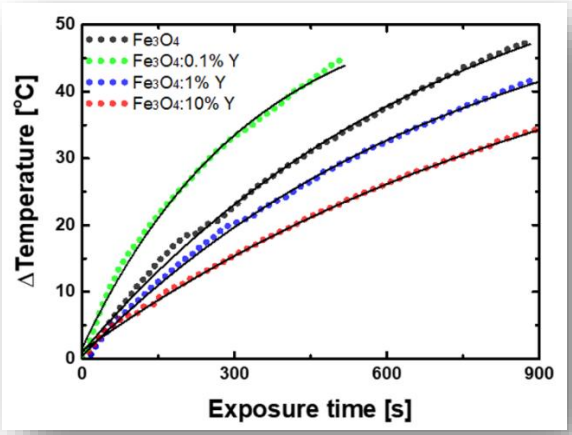


Fig. 9. Measurements of temperature increase in an alternating magnetic field depending on time (413 kHz; 200 Gs; CNPs=3 mg ml^{-1}).

The Fe_3O_4:Y nanoparticles exhibited low cellular cytotoxicity, as demonstrated in four independent cytotoxicity tests (MTT, CyQuant, PrestoBlue, and Live/Dead assays) conducted on 4T1 cells, even at relatively high concentrations (35 μg ml^{-1}). The different stages of nanoparticle internalization and their intracellular localization were studied using TEM and confocal microscopy. Research results indicated that magnetic nanoparticles are internalized into 4T1 cells via active transport (endocytosis).

Magnetic hyperthermia experiments conducted on 4T1 cells using Fe_3O_4:Y magnetic nanoparticles showed that Fe_3O_4 nanoparticles doped with 0.1% Y^{3+} significantly reduced cell viability compared to undoped nanoparticles. Specifically, Fe_3O_4:0.1% Y nanoparticles at a concentration of 35 μg ml^{-1} reduced cell viability to 77%, and at a concentration of 100 μg ml^{-}

[1] to 68% after application of AMF for 30 minutes. In comparison, incubation with Fe_3O_4 nanoparticles reduced cell viability to 85% at a concentration of 35 μg ml^{-1} and to 72% at 100 μg ml^{-1} (Fig. 10).

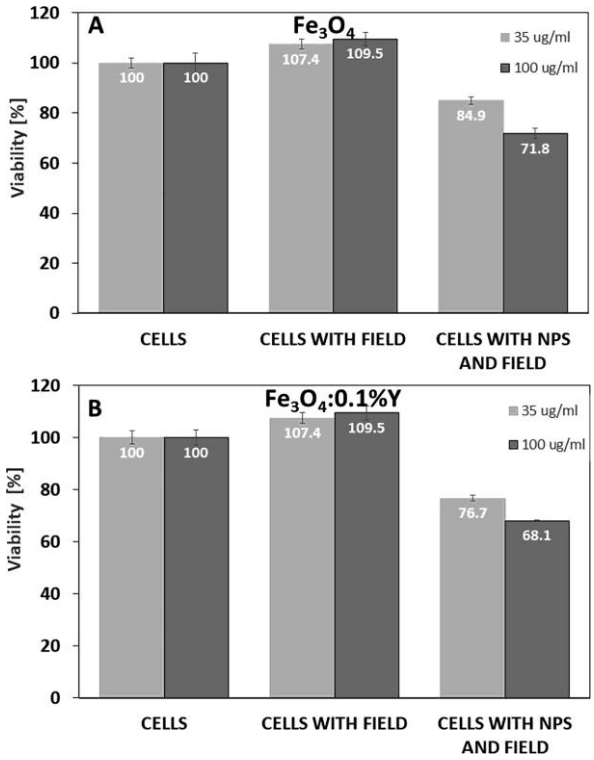


Fig. 10. Magnetic hyperthermia results performed on 4T1 cells with (A) Fe_3O_4 and (B) Fe_3O_4:0.1%Y nanoparticles (35 μg ml^{-1} and 100 μg ml^{-1}). The MTT cytotoxicity test was performed immediately after the application of a magnetic field (30 min, 20 mT, 423 kHz).

These results suggest that Fe_3O_4 nanoparticles doped with Y^{3+} ions, owing to their magnetic properties, are suitable for biomedical applications, particularly in magnetic hyperthermia treatments.

Scientists are developing magnetic nanoparticles with the highest magnetization and the best temperature response in an alternating magnetic field, aiming for sizes as small as possible for hyperthermia applications. In work A4, I demonstrated one approach to enhancing magnetic properties beyond those of Fe_3O_4 by doping with Y^{3+} ions. Additionally, I proposed a mechanism for increasing Fe_3O_4 nanoparticle magnetization through this type of doping. This represents a significant achievement in the fields of physics, chemistry, and biological applications.

The Fe_3O_4 nanoparticles are not only useful for magnetic hyperthermia but also promising candidates for photothermal therapy (PTT). The primary mechanism involves the conversion of near-infrared (NIR) light into heat through photon absorption and subsequent energy dissipation in non-radiative processes, leading to phonon activation within the crystal lattice and material temperature increase.[51,52,53,54]

In work A5, we developed multifunctional nanoconstructs comprising SiO_2-coated upconverting nanoparticles (UCNPs: $NaYF_4$:2%Er^{3+},20%Yb^{3+}) with covalently attached functionalized Fe_3O_4 nanoparticles (UCNPs@SiO_2@Fe_3O_4). Upon NIR laser irradiation, these

nanoconstructs can be used simultaneously for photothermal therapy and upconversion luminescence imaging. Both PTT and imaging exploit NIR light within the biological window.

The biological window encompasses wavelengths of 650-900 nm, corresponding to deep red and near-infrared light. In this range, absorption and autofluorescence are minimal, facilitating maximum light transmission through tissues. Wavelengths beyond 900 nm exhibit increased water absorption.[55]

Upconversion of UCNPs occurs upon excitation with 980 nm light. Short exposure times result in negligible tissue heating due to water absorption. However, prolonged exposure can lead to tissue damage.[56] To confine heat generation from Fe_3O_4 nanoparticles to specific locations and prevent tissue-wide water excitation, 808 nm and 880 nm lasers were used.

In work A5, UCNPs and Fe_3O_4 nanoparticles were covalently linked (Fig. 11). Initially, the Fe_3O_4 nanoparticle surface stabilized by oleic acid was modified with dibromodimethylpropionic acid (BMPA) via ligand exchange (Fe_3O_4-BMPA). The bromine groups of BMPA undergo nucleophilic substitution reactions with amino groups on the SiO_2 surface of the UCNPs.[57]

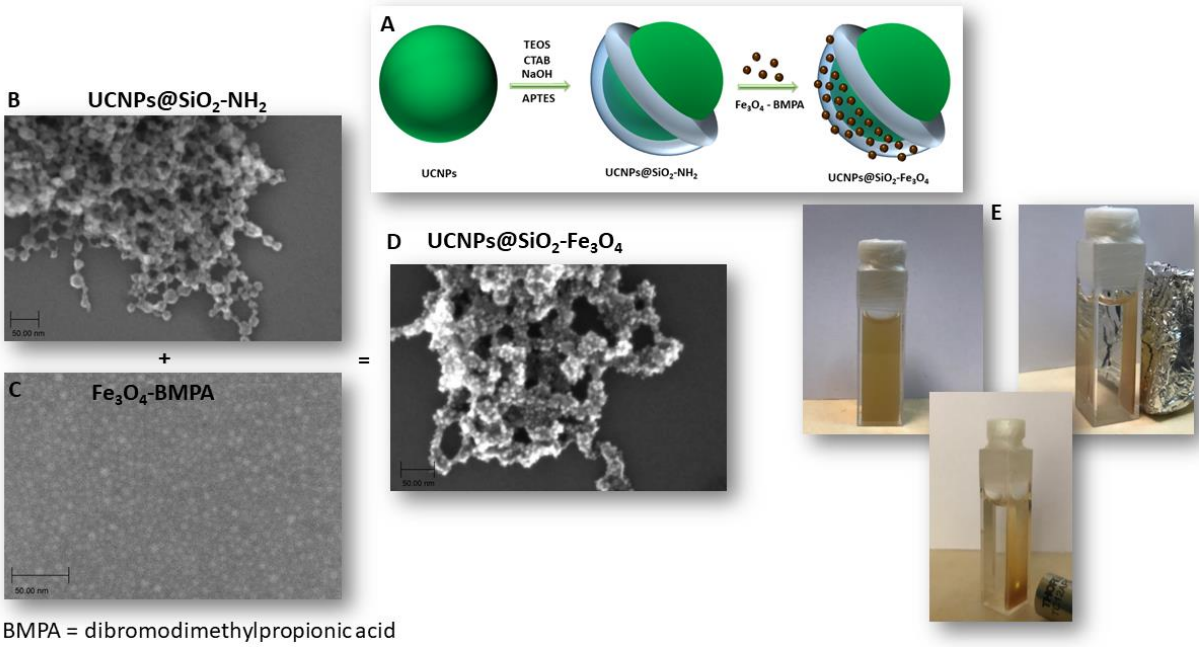


Fig. 11. (A) Steps of UCNPs@SiO_2-Fe_3O_4 nanoparticles synthesis. TEOS – tetraethoxysilane, CTAB – n-hexadecyltrimethylammonium bromide, BMPA – dibromodimethylpropionic acid. SEM images of UCNPs@SiO_2-NH_2 (B), Fe_3O_4-BMPA (C), UCNPs@SiO_2-Fe_3O_4 (D) nanoparticles. (E) A photo of the UCNPs@SiO_2-Fe_3O_4 nanoparticle solution before and after applying a magnetic field and a photo of the luminescence of the nanoparticles excited by 980 nm laser light.

To confirm the connection of Fe_3O_4 nanoparticles with UCNPs@SiO_2, luminescence under 980 nm excitation and absorption measurements of the UCNPs@SiO_2-Fe_3O_4 nanoparticle solution were conducted. Subsequently, the UCNPs@SiO_2-Fe_3O_4 nanoparticle solution was subjected to magnetic separation for 24 hours. Following magnetic separation, luminescence and absorption measurements were repeated. Luminescence measurements revealed characteristic radiation in the visible range indicative of upconversion of UCNPs following 980 nm laser excitation. Absorption measurements demonstrated absorption corresponding to Fe_3O_4. The observed decrease in luminescence and absorption intensity post-magnetic field

application suggested the absence of nanoparticles in the solution, providing indirect evidence of Fe_3O_4 attachment to the UCNP@SiO_2 surface (Fig. 12 A, B). SQUID magnetization measurements confirmed the magnetic properties of UCNPs@SiO_2-Fe_3O_4 (Fig. 12D). After introducing nanoparticles into MDA-MB-231 cells, their presence was verified via confocal microscopy (Fig. 12C)

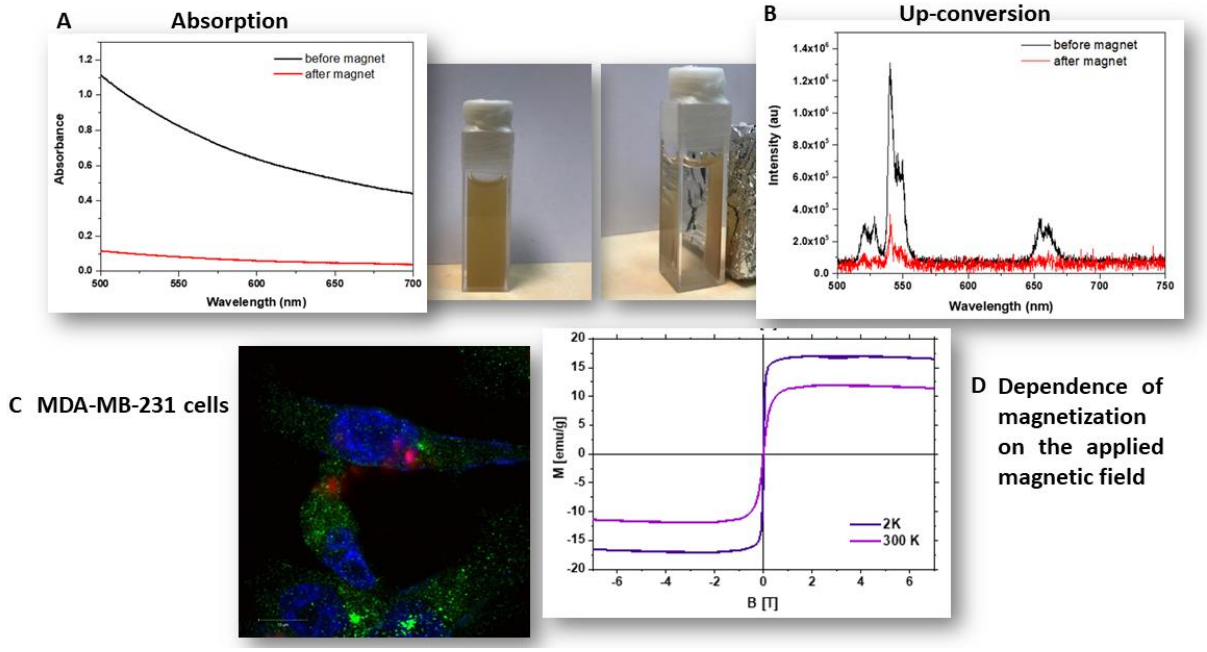
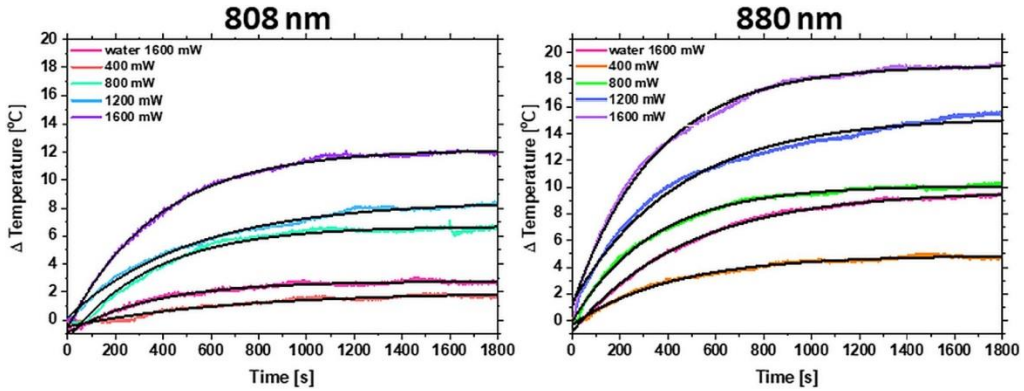


Fig. 12. (A) Absorption spectrum of the UCNPs@SiO_2-Fe_3O_4 nanoparticle solution before and after applying a magnet. (B) Upconversion spectrum of UCNPs@SiO_2-Fe_3O_4 before and after applying a magnet (980 nm, power density: 15 W cm^{-2}). (C) Confocal microscopy image of MDA-MB-231 cells after 16 h of incubation with UCNPs@SiO_2-Fe_3O_4 (500 μg ml^{-1}). Nanoparticles were excited with a 980 nm laser (red), stained lysosomes were excited with a 488 laser (green), and stained nuclei were excited with a 690 nm laser (blue). (D) Dependence of magnetization on the applied magnetic field for UCNPs@SiO_2-Fe_3O_4 nanoparticles at 2 K and 300 K.

Our studies have demonstrated that aqueous suspensions UCNPs@SiO_2@Fe_3O_4 can be effectively heated by both lasers (808 nm and 880 nm). The temperature response was measured as a function of laser power (Fig. 13), showing a significant increase in temperature with increasing laser power for both wavelengths in aqueous nanoparticle solutions at a concentration of 2.5 mg ml^{-1}. These results were compared with measurements for water under the highest laser power.

A notable difference in temperature increase was observed between the 880 nm and 808 nm lasers for all powers, attributed to the greater heating effect of water after 880 nm laser irradiation, owing to its higher absorption in this wavelength range.[58] However, when comparing the results with water exposed to the highest laser power, the most significant differences were observed with the 808 nm laser (which falls within the biological window wavelength range).

Due to the optical properties of the nanoconstructs, we were able to localize our materials inside MDA-MB-231 cancer cells using confocal imaging under 980 nm laser excitation. Furthermore, we presented the results of cytotoxicity tests, which showed that concentrations of nanoconstructs up to 500 μg ml^{-1} are non-toxic to MDA-MB-231 cells.



Ryc. 13. Temperature increase curves of the nanoparticles after 808 nm and 880 nm laser irradiation depending on the laser power.

In summary, in paper A5, we demonstrated the effectiveness of phototherapy using UCNPs connected with Fe_3O_4 in heat induction. Combining these photothermal properties with upconversion capabilities, we developed a multifunctional nanoconstruct suitable for imaging (tumor detection) and phototherapy within the optical biological window. This represents a significant novelty and a major interdisciplinary achievement.

Summary

My scientific achievement involves the creation of multifunctional nanoparticles with enhanced physicochemical properties that hold significant practical importance, particularly in the fields of biology and medicine. The key physical properties pivotal to this achievement include upconversion [A1, A2, A3], magnetic [A4], and opto-magnetic [A5] properties, with potential applications in these domains.

This scientific achievement encompasses a series of five articles published between 2017 and 2024. The most significant results contributing to the understanding of nanomaterial physicochemical properties in the context of their applications in biology and medicine can be summarized as follows:

- I developed a method to connect UCNPs with the photosensitizer, Rose Bengal, effectively shortening the distance between the nanoparticle and the dye [A1]. This led to an increased FRET efficiency between UCNPs and the photosensitizer, resulting in ROS generation under NIR light. This significantly enhanced the efficacy of cancer cell elimination when irradiated with 980 nm radiation.

- Leveraging the upconversion property of UCNPs doped with Tm^{3+} and Yb^{3+} ions, I demonstrated the conversion of NIR light to UV and blue light, enabling ROS generation directly from UV interaction in an aqueous environment [A2, A3]. This efficient ROS generation after NIR excitation contributed to cancer cell elimination. Furthermore, by attaching proteins to the nanoparticle surface, I achieved specific targeting to membrane surface sites.

- I created Fe_3O_4 nanoparticles doped with Y^{3+} ions to enhance magnetic properties compared to undoped nanoparticles [A5]. The slight Y^{3+} doping increased nanoparticle

magnetization by occupying tetrahedral sites in the spinel structure, thus locating Fe^{3+} ions in octahedral sites. This enhancement significantly increased the temperature rise rate of aqueous solutions of Fe_3O_4 nanoparticles doped with 0.1% Y^{3+} under alternating magnetic fields, resulting in more effective cancer cell elimination.

- I developed a nanoconstruct by connecting UCNPs with upconverting properties, coated with a SiO_2-NH_2 layer, with Fe_3O_4 nanoparticles coated with BMPA to combine magnetic and upconversion capabilities [A6]. These nanoparticles exhibited increased temperature upon irradiation with 808 nm and 880 nm lasers, suitable for photothermal therapy. Additionally, their upconversion properties allowed imaging without autofluorescence of biological structures after 980 nm excitation.

4.5. Discussion of the second scientific achievement

- **Introduction**

A crucial aspect to consider at every stage of designing new materials for biological and medical applications is their toxicity, accumulation, biodistribution, and interaction with target biological materials.

In the context of using nanoparticles in biomedical research, their interaction with cellular components like the cell membrane and organelles is paramount. Information regarding the mechanisms of nanoparticle internalization and intracellular pathways activated by nanomaterials is critical for understanding these interactions. These interactions are influenced by the size, shape, charge, and surface properties of nanoparticles.[59]

The primary process through which cells internalize nanoparticles is endocytosis, a necessary process for nutrient uptake. This process can occur with or without the participation of cell surface receptors and is vital for regulating metabolism and information/molecule transfer within cells or to distant tissues.[60,61] Nanoparticles efficiently internalized via endocytosis can potentially deliver imaging agents for diagnostics and drugs for therapeutics.

Understanding the physical and chemical interactions between nanoparticles and biological structures is key to answering fundamental questions such as: 1) What happens to nanoparticles upon introduction into biological systems? 2) Do individual biological components react with nanoparticles, and if so, how? 3) Can nanoparticles modulate biological processes, and if yes, how? Comprehensive research providing answers to these questions has been performed and described in papers B1, B2, and B3.

Endocytosis is traditionally classified into phagocytosis and pinocytosis depending on the type of material collected. Phagocytosis involves cells like macrophages, monocytes, neutrophils, and dendritic cells, whereas pinocytosis occurs in all non-phagocytic eukaryotic cells. Endocytosis encompasses four main mechanisms: clathrin-mediated, caveolae (caveolin protein)-mediated, clathrin- and caveolae-independent, and macropinocytosis. The preferred endocytosis pathway depends on the type and size of nanoparticles.

The cellular uptake of nanoparticles is a two-step process: nanoparticle binding to the cell membrane followed by internalization. The first stage is greatly influenced by the physicochemical properties of nanoparticles, particularly their surface charge.[62]

The mechanism of UCNPs' internalization directly influences their intracellular localization. Therefore, understanding the distribution and localization of UCNPs within cellular organelles like endosomes and lysosomes is essential for developing effective drug delivery systems, leveraging drug release triggered by environmental pH changes.[63]

Existing data indicate insufficient information on detailed endocytic mechanisms involved with certain nanoparticles, often reflecting complex interactions between nanomaterials and biological systems, alongside varying physicochemical properties across nanoparticle types.[64,65,66,67,68,69,70] Selecting optimal experiments to evaluate these interactions is crucial. Factors such as cell line choice, selective endocytosis inhibitors, accurate quantification of intracellular nanoparticle numbers, and subcellular localization are considerations. Combining various physicochemical techniques to assess nanoparticle uptake and specific endocytosis pathway activation is highly justified in such experimental setups.[71]

- **Detailed description**

In paper B1, the internalization mechanism and cytotoxicity of UCNPs were investigated on three cell lines (HeLa, HEK293, and astrocytes). No cytotoxic effects of UCNPs were observed even at relatively high concentrations (up to 50 µg ml^{-1}). The mechanism of UCNPs internalization and their intracellular localization were studied using confocal microscopy to colocalize the emission spectra of UCNPs with spectra from stained organelles. These findings were corroborated by TEM measurements, revealing that UCNPs primarily entered cells through clathrin-mediated endocytosis and were excreted via lysosomal exocytosis. This study represents a significant achievement, as it demonstrates for the first time the colocalization of UCNPs within distinct cellular organelles, showcasing the potential applications of their physicochemical properties in biology and medicine.

In paper B2, the interactions between UCNPs and HeLa cells were investigated, including:

- Assessment of UCNPs uptake by cells and the mechanisms of cellular internalization
- Determination of the subcellular localization of UCNPs within HeLa cells
- Evaluation of the cytotoxicity profile of UCNPs and ultrastructural changes in HeLa cells post-exposure
- Analysis of oxidative stress in cells following UCNPs exposure

Various methods are described in the literature for studying nanoparticle transport into cells and intracellular regions. Classic approaches involve blocking specific entry pathways to assess their contribution to nanoparticle internalization, using methods such as selective pharmacological inhibitors, RNA interference, or manipulating the expression of essential proteins involved in endocytosis.[72]

Chemical inhibitors of endocytosis are commonly used to study pathways involved in nanomaterial internalization. These inhibitors act rapidly and are often reversible upon removal, although they may not completely block specific pathways and could induce cellular toxicity with prolonged exposure or high concentrations.[73,74]

In paper B2, the following chemical inhibitors were employed:

- Amiloride: inhibits macropinocytosis
- Sodium azide (NaN_3): inhibits almost all energy-dependent endocytosis pathways
- Chlorpromazine: inhibits clathrin-dependent endocytosis
- Cytochalasin D: inhibits phagocytosis and macropinocytosis
- Dansylcadaverine: inhibits clathrin-dependent endocytosis
- Methyl-β-cyclodextrin (MβcD): inhibits caveolin-dependent endocytosis/lipid rafts
- Hypertonic sucrose solution: inhibits clathrin and caveolin-dependent endocytosis
- Nystatin: inhibits caveolin-dependent endocytosis
- Cold temperature (4°C): inhibits almost all energy-dependent endocytosis pathways

HeLa cells were exposed to these inhibitors or maintained at 4°C for 30 minutes, followed by a 2-hour incubation with UCNPs (1 µg ml^{-1}) or exposure to UCNPs in the presence of low temperature for 1 hour. TEM results demonstrated that each inhibitor reduced the amount of internalized UCNPs compared to control cells without inhibitors (Fig. 14).

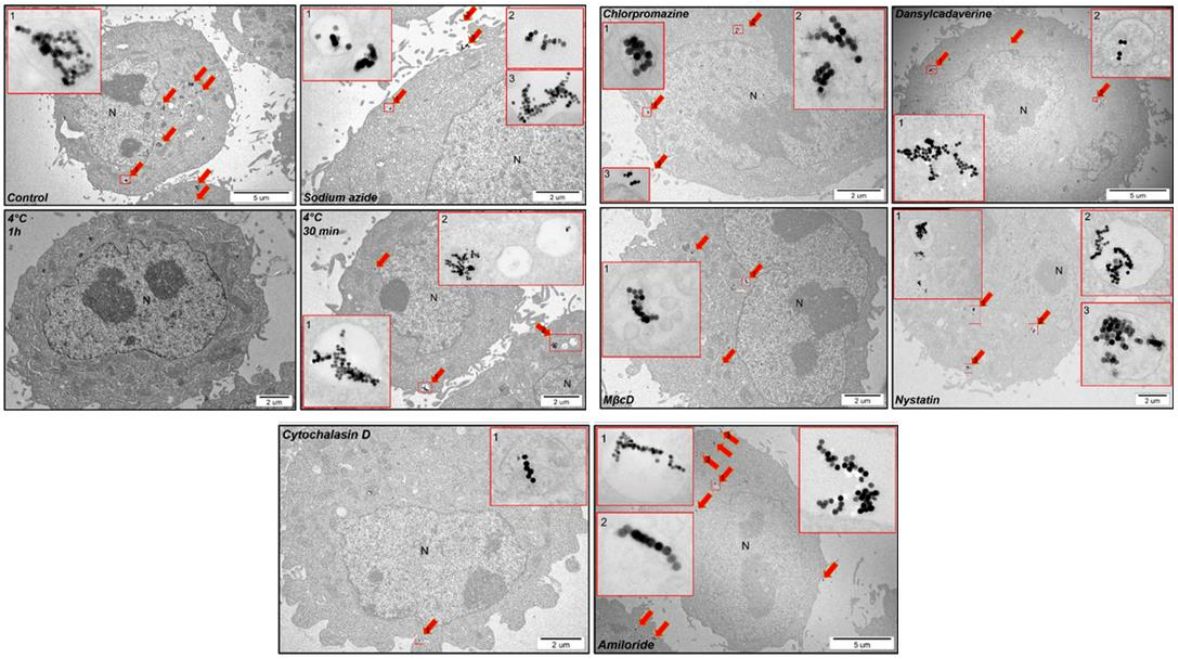


Fig. 14. TEM images of HeLa cells with endocytosis inhibitors and UCNPs. Red arrows indicate UCNPs. MβcD: methyl-β-cyclodextrin; N: cell nucleus.

To quantitatively determine the effect of inhibitors on specific endocytosis pathways, we employed inductively coupled plasma-mass spectrometry (ICP-MS)

For this purpose, cells were incubated with inhibitors at 37°C (under normal conditions) or at a low temperature of 4°C (to inhibit active uptake) for 30-60 minutes prior to the addition of UCNPs. The percentage of internalized UCNPs (%) was determined based on the amount of Y detected in HeLa cells after 2 hours of incubation with 1 μg ml^{-1} UCNPs.

Results obtained after 30 minutes of incubation with selected inhibitors demonstrated that the internalization of UCNPs was most effectively inhibited by exposing cells to a hypertonic sucrose solution, which inhibits clathrin- and caveolae-dependent endocytosis. Dansylcadaverine, known for inhibiting clathrin-mediated endocytosis, also significantly reduced UCNPs internalization. Additionally, incubation with sodium azide (an inhibitor of energy-dependent processes) decreased UCNPs internalization by approximately 20% (Fig. 15A).

In a subsequent experiment, cells were exposed to selected inhibitors for 60 minutes prior to nanoparticle administration to assess their impact on internalization rates. After 60 minutes, the most effective inhibition of endocytosis was observed in the presence of sodium azide and under low-temperature conditions. Moreover, incubating cells with UCNPs in the presence of a sucrose solution for 1 hour reduced internalization by approximately 30% (Fig. 15B).

We have proposed two main mechanisms of endocytosis involving clathrin and caveolae. Nanoparticles were observed within membrane cavities, early endosomes, late endosomes, and lysosomes, which are characteristic of the intracellular endocytic transport of nanoparticles enclosed in vesicles. We proposed and demonstrated the endocytic mechanism of UCNPs internalization and intracellular transport inside HeLa cells.

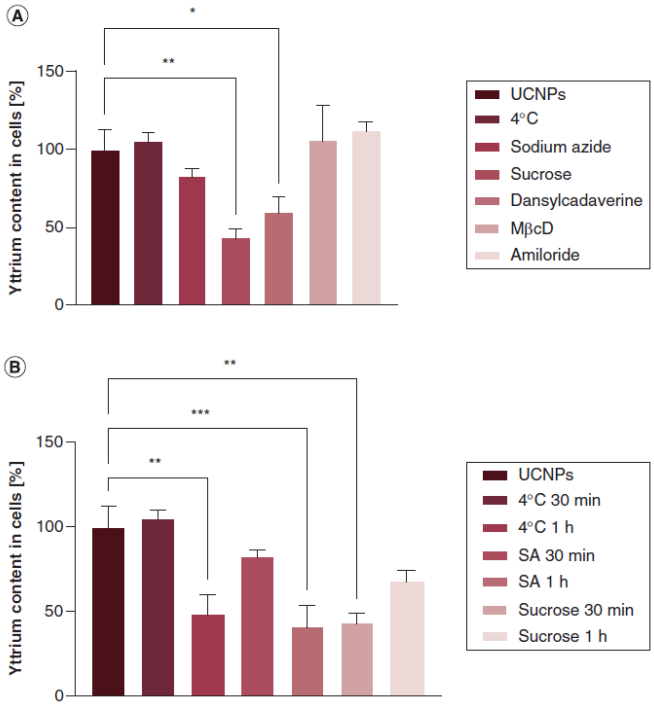


Fig. 15. The ICP-MS results of UCNPs introduced into cells after the use of inhibitors. (A) Percentage of Y in cells exposed to selected endocytosis inhibitors for 30 min before incubation with UCNPs. * ($p < 0.05$); ** ($p < 0.01$). (B) Percentage of Y in cells exposed to selected endocytosis inhibitors for 30 min before incubation with UCNPs or for 1 h with the inhibitor and UCNPs. ** ($p < 0.01$); ***($p < 0.001$).

Upon addition of UCNPs to cells, numerous membrane invaginations and cavities formed at the points of contact between UCNPs and the plasma membrane. These membrane fragments then invaginated into the cytoplasm, forming intracellular (endocytic) vesicles containing UCNPs. Within the cell, these vesicles fused with early endosomes located near the cell membrane. Early endosomes transported UCNPs deep into the cytoplasm toward the perinuclear region. In this region, early endosomes gradually transformed into late endosomes or fused with existing late endosomes. Subsequently, late endosomes fused with lysosomes, transferring UCNPs to them. Finally, UCNPs were recycled back to the cell membrane and removed from HeLa cells by exocytosis (Fig. 16). The absence of toxicity indicates that UCNPs were dynamically taken up and eliminated from the cells. Furthermore, the absence of nanoparticle accumulation in other cellular organelles, such as mitochondria, suggests minimal impact on biological functions related to cellular metabolism. These findings are crucial for the future use of UCNPs as potential theranostic agents.

Additionally, the obtained results suggest that UCNPs do not accumulate in the cytoplasm of cells during intracellular transport or removal, excluding passive transport through the cell membrane, which is important information for controlled delivery. To date, only a few studies have been published on the mechanisms of UCNPs endocytosis and their intracellular presence.[75]

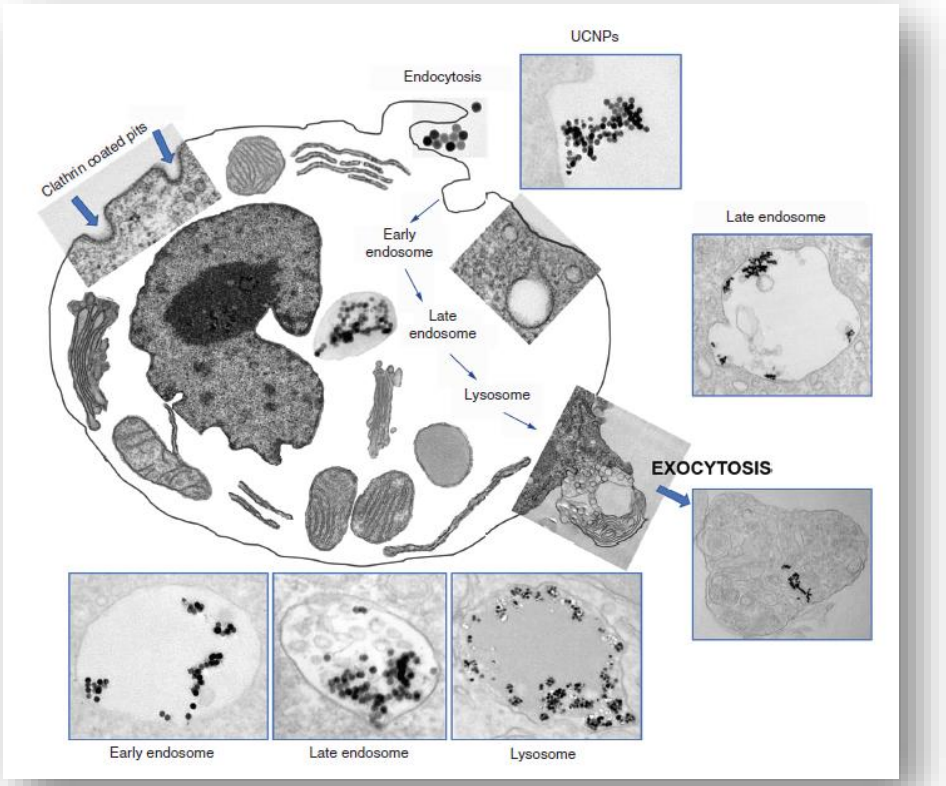


Fig. 16. Diagram of the interaction of UCNPs with HeLa cells.

At the current state of knowledge, papers B1 and B2 represent the first comprehensive studies on UCNPs transport inside living cells and their subcellular visualization using the TEM method. Previously, UCNPs localization was primarily documented using fluorescence or confocal microscopy techniques.[76,77,78,79]

This research could significantly contribute to the development of new, highly functional UCNPs nanomaterials with excellent optical properties and low cytotoxicity, paving the way for their potential use in biomedicine. The ability to design and utilize multifunctional, safe nanosystems for biological imaging holds promise for introducing revolutionary solutions in diagnosis and treatment, particularly in diseases related to the central nervous system

Research into the use of nanomaterials for treating diseases of the nervous system is addressing an increasing array of issues.[80,81] Key applications of nanotechnology in neurodevelopmental, neurological, and neuropsychiatric disorders include utilizing nanoparticles or nanocarriers for drug delivery or gene therapy, employing nanotechnology to reconstruct, strengthen, and stabilize the cytoskeletal matrix, developing biohybrid devices for compound transport, and coating electrodes with nanoparticles.[82]

Organotypic cultures of brain slices represent a valuable tool for studying physiological and pharmacological processes in tissues, as well as the cellular and molecular mechanisms underlying central nervous system (CNS) disorders, and evaluating potential CNS disease treatments.[83] Tissue models serve as an excellent intermediary step between cell culture and live organism testing. Notably, brain slice models preserve tissue architecture and microenvironment, enabling observation of nanomaterial-induced tissue changes and testing of

new therapeutic strategies. The hippocampus is the brain region most commonly cultured,[83] with the Organotypic Hippocampal Slice Cultures (OHSC) model frequently used to study neuronal damage, synaptic plasticity, cell proliferation, and maturation. Understanding nanoparticle internalization mechanisms and their impact on tissue morphology is critical for developing new therapies that can be modeled using hippocampal tissue. Designing and preparing nanoparticles for controlled drug delivery to the brain could enhance the effectiveness of brain therapies.[83,84]

The aim of research paper B3 was to assess biological interactions between UCNPs and the *ex vivo* OHSC model, including:

- Determining the potential internalization of UCNPs by neurons.
- Assessing cytotoxicity and analyzing ultrastructural tissue changes after exposure to UCNPs.

To confirm the internalization of UCNPs by neuronal cells, TEM analysis of *ex vivo* organotypic hippocampal slice cultures was performed. The hippocampi used in the experiments were isolated from 7-day-old rats. The isolation process was approved by the 4th Local Ethics Committee for Animal Care (decision no. 39/2015) and conducted in accordance with Ministry of Science and Higher Education guidelines (Fig. 17).

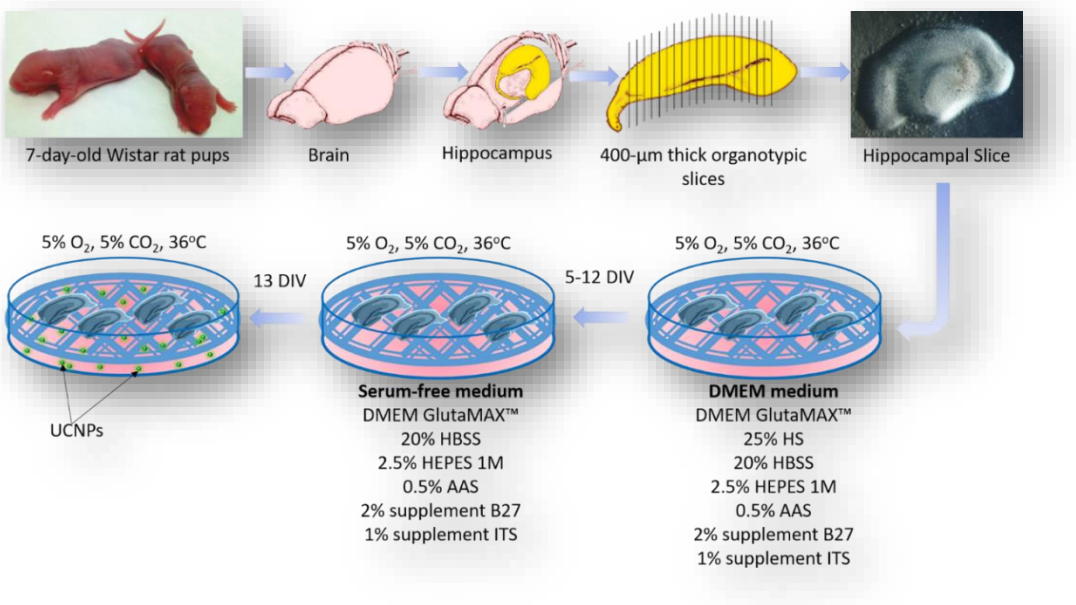


Fig. 17. Experimental design using UCNPs and OHSC slices.

TEM results confirmed the presence of large clusters of UCNPs enclosed within vesicular structures in neurons. UCNPs were located in early and late endosomes, inside lysosomes, and within large autophagolysosomes. Similar to the previously studied *in vitro* model, no UCNPs were observed in the cell cytoplasm or within other cellular organelles, indicating that UCNPs were transported into the cells via endocytosis.

These results suggest that OHSCs may serve as an effective model for testing various therapeutic strategies for neurological diseases using nanostructures. The high expectations regarding brain imaging capabilities using UCNPs are attributed to their unique upconverting properties, potentially enabling imaging of deeper layers in living brain tissue. Additionally,

the tested material (a slice fragment embedded in epoxy resin) was trimmed several times to confirm the presence and distribution of UCNPs in deeper areas of the slices. This allowed us to conclude that UCNPs are not only dynamically internalized by cells but also transported to deeper regions within the section. Moreover, large agglomerates of non-internalized UCNPs accumulated within the intercellular spaces around the cells. Electron microscopic data enabling an in-depth assessment of OHSC morphology exposed to nanomaterials were not available in the literature, nor was there information on the intracellular localization of nanoparticles in the slices. Few studies confirming the presence of nanoparticles in OHSCs using TEM focused on quantum dots biofunctionalized with the Palm1 peptide (CL4 QD-Palm1),[85] while others examined the neuroprotective properties of CeO_2 nanoparticles[86] or the localization of Ag nanoparticles within neuronal cytoplasm.[87]

We proposed and verified an endocytic mechanism of UCNP internalization and intracellular transport within OHSC slices as a model for neural tissues.

Based on the presented results, it can be concluded that UCNPs are readily internalized by hippocampal cells. The biodistribution of UCNPs inside cells closely correlates with intracellular processes and the tendency of UCNPs to localize specifically within intracellular organelles, such as endosomes and lysosomes. This model may prove useful for studying UCNP biodistribution in tissues and assessing morphological and ultrastructural changes caused by nanomaterial presence.

The results presented in papers B1, B2, and B3 underscore the importance of conducting detailed studies at every stage of nanoparticle analysis to mitigate the risk of nanomaterial toxicity, particularly in clinical applications. Nanoparticles must undergo thorough characterization and detailed study within biological systems throughout the experimental design process. These findings also highlight the crucial role of *ex vivo* biological imaging of tissue fragments using UCNP's unique upconverting properties.

Summary

My scientific achievement involves elucidating the mechanism of interaction between inorganic upconversion nanoparticles (UCNPs) and biological materials, such as cells and tissues.

This achievement comprises a series of three articles published between 2017 and 2024. The most significant results, which substantially contribute to understanding the physicochemical properties of nanomaterials in the context of their potential applications in biology and medicine, can be summarized as follows:

- Using transmission electron microscopy (TEM), inductively coupled plasma mass spectrometry (ICP-MS), and confocal microscopy, I determined the mechanism of UCNP internalization by HeLa cells and rat hippocampal tissues [B1, B2, B3]. The proposed mechanism involves clathrin- and caveolae-mediated endocytosis.
- I identified the intracellular localization of these nanostructures within endosomes and lysosomes across various cell types, including HeLa cells [B1, B2] and neuronal cells [B1, B3], demonstrating no cytotoxicity even at relatively high concentrations (up to 500 μg/ml) and long incubation times (up to 48 hours).

5. Presentation of significant scientific or artistic activity carried out at more than one university, scientific or cultural institution, especially at foreign institutions

5.1. University of Warsaw, Faculty of Chemistry

As part of my master's thesis conducted at the Faculty of Chemistry, University of Warsaw, I investigated the antioxidant activity of seven neurotransmitters (dopamine, adrenaline, norepinephrine, L-DOPA, γ-aminobutyric acid, glutamic acid, and acetylcholine) in both homogeneous and heterogeneous solutions. The study involved measuring reaction rate constants with the model 2,2-diphenyl-1-picrylhydrazyl radical (dpph$^{\bullet}$) and assessing the autoxidation rate of lipid emulsions.

The results revealed that the kinetics of the reaction between catecholamine neurotransmitters and radicals strongly depend on pH. Notably, the reaction rate constants of dpph$^{\bullet}$ with catecholamine neurotransmitters (dopamine, adrenaline, norepinephrine, L-DOPA) in water-methanol systems increased approximately one hundredfold with a change in pH from 5.5 to 7.4. This acceleration is attributed to an increase in the concentration of phenolate anions involved in the reaction with dpph$^{\bullet}$, a phenomenon explained by the Sequential Proton-Loss Electron-Transfer (SPLET) mechanism involving phenol dissociation and electron transfer from the phenolate anion to the radical.

In emulsion systems, catecholamines act as moderate antioxidants (retardants) when autoxidation is initiated by an azo compound. However, a strong synergistic effect was observed between catecholamines and 2,2,5,7,8-pentamethyl-6-hydroxychroman (PMHC), an α-tocopherol analog, particularly at pH 6-8. This effect is explained by the regeneration of the lipid-phase antioxidant (PMHC) by water-soluble co-antioxidants (catecholamines).

Oxygen uptake rate measurements were also conducted for several non-catecholamine neurotransmitters (acetylcholine, glutamic acid, GABA), but they did not exhibit antioxidant properties or show a strong synergistic effect with PMHC.

In conclusion, the study proposes an antioxidant mechanism involving catecholamine neurotransmitters.

These findings were published in two papers, one in 2009 [P16 - listed in section 7.1 of publications] and the other in 2022 [P2].

5.2. Ecole Polytechnique Fédérale de Lausanne (EPFL), Laboratory of Physics of Complex Matter (LPMC), Institute of Condensed Matter Physics (ICMP), Faculty of Basic Sciences (FBS), Lausanne, Switzerland

As part of two week-long training sessions under the "LLP-Erasmus Program Individual Training: Program for Staff Training Mobility" during the academic years 2012/2013 and 2013/2014 at EPFL in Switzerland, I conducted experiments aimed at characterizing $NaYF_4:Er^{3+},Yb^{3+},Gd^{3+}$ nanoparticles. Specifically, I used Electron Paramagnetic Resonance (EPR) spectroscopy to study the photodynamic efficiency and magnetic properties of $NaYF_4:Er^{3+},Yb^{3+}$ and $NaGd_xY_{1-x}:Er^{3+},Yb^{3+}$ nanoparticles. My stay in Switzerland fostered long-term collaboration with Prof. Andrzej Sienkiewicz, and the results obtained during this period were published in 2017 and is a part of the habilitation achievement [B1].

During my stay at EPFL, I also conducted experiments on the generation of reactive oxygen species by $NaYF_4:Er^{3+},Yb^{3+}$ nanoparticles in the presence of photosensitizers (including Rose Bengal) following near-infrared (NIR) radiation, using the EPR technique. These experiments formed the basis of my subsequent work at IP PAS, resulting in a publication included in the habilitation achievement [A1].

Additionally, I investigated the absorption of $NaGd_xY_{1-x}:Er^{3+},Yb^{3+}$ nanoparticles by aquatic plants, specifically Vallisneria spiralis. This study aimed to detect the presence of $NaGd_xY_{1-x}:Er^{3+},Yb^{3+}$ nanoparticles in the leaves of these plants after introducing their roots into a nanoparticle solution. Both EPR technique (at EPFL) and confocal microscopy (at IP PAS) were employed for this research. Furthermore, I examined the photodynamic efficiency in model aquatic plants using the EPR technique.

5.3. Instituto de Fisica, Nfflcleo de Fsica Aplicada Universidade de Brasilia, oraz Depto de Genética e Morfologia CNANO - Instituto de Ciências Biológicas, Universidade de Brasília

In 2016, I completed two internships in Brazil under the EU Research Project grant FP7-People-2012-IRSES-BRASINOEU (Project Number: PIRSES-GA-2012-318916).

During these internships, I conducted the initial magnetic hyperthermia measurements of Fe_3O_4 nanoparticles and nanohybrids composed of upconverting nanoparticles (UCNPs) and magnetic Fe_3O_4 nanoparticles encapsulated in SiO_2, which I had previously synthesized at IP PAS. The measurements were performed using hyperthermia equipment from Nan Thericts. Additionally, I participated in constructing a system for simultaneous measurement of temperature changes under an alternating magnetic field (AMF) and optical properties. The knowledge gained from these experiments was instrumental in planning and executing measurements published in a work that is part of my habilitation achievement [A4].

While at the University of Brazil, collaborating with Prof. Paulo Eduardo Narcizo de Souza's group, I measured the ROS generated from $NaYF_4:0.2\%Tm^{3+},20\%Yb^{3+}$ nanoparticles. These nanoparticles exhibited intense luminescence across UV, blue, red, and near-infrared (NIR) ranges under 980 nm laser irradiation. To quantify ROS, I mixed an aqueous solution of nanoparticles with the spin trap 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (CMH), which produces an electron paramagnetic resonance (EPR) signal upon reacting with free radicals. I measured the EPR signal every ten minutes during exposure and observed a 70% increase in the EPR signal after 20 minutes. These results were published in paper A2, part of my habilitation achievement.

At the Institute of Biology at UNB, I participated in pilot experiments using nanoparticles with a SPCD photosensitizer attached to their surface for anticancer therapy against 4T1 breast cancer cells and in BALB/c mice (8 weeks old) with induced breast cancer (4T1). Tumor growth was monitored using bioluminescence in the IVIS® Lumina LT In Vivo Imaging System. After 17 days following the application of nanoparticles and irradiation with a 980 nm laser, a reduction in tumor growth rate was observed in mice treated with UCNPs compared to those without UCNPs.

5.4. University of Warsaw, Faculty of Physics.

In collaboration with the Faculty of Physics at the University of Warsaw, I supervised numerous bachelor's, master's, and engineering theses.

In 2016, a bachelor's thesis titled *"Energy transfer between organic dyes ('antenna') attached to the surface of upconverting nanoparticles to improve the efficiency of up-conversion for use in biology and medicine"* was conducted. The aim was to optimize the quantum efficiency of upconversion of UCNPs using energy transfer. Organic dyes served as 'antennas' for NIR light and were covalently bonded to $NaYF_4$:2%Er^{3+},20%Yb^{3+} nanoparticles encapsulated in SiO_2. Luminescence spectra were analyzed using 808 nm and 980 nm lasers to verify the transfer between the dye and nanoparticles.

That same year, a master's thesis titled *"Study of the energy transfer from up-converting NaYF4 nanoparticles doped with rare earth metal ions to selected photosensitizers and the generation of reactive oxygen species for biology and medicine applications"* was completed. This work involved attaching two photosensitizers, SPCD and HP, to UCNPs, studying energy transfer from nanoparticles to photosensitizers, and investigating ROS generation upon 980 nm laser irradiation. Spectrophotometric nanothermometers made of UCNPs were also developed as a result of these experiments.

In 2017, a bachelor's thesis titled "*Synthesis and characterization of Fe_3O_4 nanoparticles with applications in magnetic hyperthermia"* was carried out. Iron oxide nanoparticles doped with yttrium ions were synthesized and characterized, followed by magnetic hyperthermia measurements. The best results were observed for samples containing 0.1% Y^{3+}, which contributed to publication A4 as part of the habilitation achievement.

Also in 2017, a master's thesis titled *"Optimization of opto-magnetic UCNPs&SPIONs@SiO_2 nanoparticles with upconverting properties for applications in biology and medicine"* was written. This research focused on optimizing multifunctional nanoconstructs with opto-magnetic properties, combining $NaYF_4$:20%Yb^{3+},2%Er^{3+} and Fe_3O_4 in a SiO2 shell. Absorption and luminescence analysis allowed for the examination of Fe_3O_4's influence on the upconverting properties of the nanoconstructs.

In 2018, I supervised a master's thesis titled *"Multifunctional nanoparticles upconverting infrared light to visible light and downconverting to infrared light after 808 nm laser excitation - synthesis, characterization, and application."* The goal was to design, synthesize, and characterize $NaYF_4$ or $NaGdF_4$ nanoparticles doped with rare earth ions (Nd^{3+}, Yb^{3+}, Er^{3+}) for upconversion and downconversion properties. Various nanoparticle samples were created to optimize upconversion efficiency of 808 nm light, and the best sample was used for imaging 4T1 mouse breast cancer cells. Organic dyes were also investigated as 'antennas' of infrared light to enhance UCNPs' upconversion efficiency.

In 2023, I supervised an engineering thesis titled "*Synthesis and characterization of upconverting nanoparticles for biology and medicine."* This work focused on core-shell-shell nanoparticles with UCNPs at the core, a SiO_2 shell containing a photosensitizer (methylene blue), and a second SiO_2 shell for further functionalization. Morphological and elemental analysis, optical properties examination, ROS generation studies, and *in vitro* tests on HeLa cancer cells were conducted, with publication of results forthcoming.

5.5. Warsaw University of Technology, Faculty of Physics

In 2019, in collaboration with the Faculty of Physics at the Warsaw University of Technology, I supervised the master's thesis titled *"Opto-magnetic properties and synthesis optimization of multifunctional nanoconstructs for applications in cancer theranostics."* The objective was to design, synthesize, and characterize multifunctional nanoconstructs that combine the optical properties of $NaYF_4$ nanoparticles doped with rare earth ions (ytterbium and erbium or ytterbium and thulium) with the magnetic properties of Fe_3O_4 nanoparticles for applications in cancer diagnostics and therapy. For this purpose, several samples of $NaYF_4$:Yb^{3+},Er^{3+}/Tm^{3+} (UCNPs) and magnetic Fe_3O_4 (SPIONs) nanoparticles were independently prepared and then combined in three different configurations. The first approach involved creating a magnetic shell around the optical core (UCNPs@SPIONs), the second involved creating an optical shell around the magnetic core (SPIONs@UCNPs), and the third method entailed coating the $NaYF_4$ nanoparticle with a SiO_2 shell and covalently attaching the Fe_3O_4 nanoparticles (UCNPs-SPIONs). All types of nanostructures produced were characterized using TEM, SEM, XRD, SQUID, and optical property measurements. Subsequently, the study determined which method of core-shell nanoparticle synthesis yields nanoparticles with the best upconversion efficiency and magnetization.

5.6. University of Engineering and Health

In the academic year 2023/2024, I supervised six engineering theses in the field of cosmetic chemistry at the University of Engineering and Health (WSIiZ). All of this work was carried out under my supervision at WSIiZ.

One notable work is titled *"The Use of Silver Nanoparticles in Cosmetics Intended for Daily Skin Care and Their Use in Alleviating the Symptoms of Dermatological Diseases."* This study focused on examining the impact of silver nanoparticles (AgNPs) on the skin microbiome and general skin condition. During the research, it was demonstrated that an emulsion containing AgNPs significantly inhibited the growth and development of bacteria collected from the skin surface of test subjects in microbiological cultures, confirming the antibacterial properties of AgNPs. Additionally, emulsions with AgNPs were found to increase skin hydration. This work has significant implications for the development of using silver nanoparticles in cosmetic products for moisturizing and antibacterial applications. The results of this study will be published upon completion.

Another thesis to be highlighted is titled *"Development and Analysis of the Properties of Emulsions with Liposomal Curcumin."* This study aimed to develop a cosmetic emulsion containing liposomal curcumin and investigate its effects on the skin. Two types of emulsions were developed: one without the active ingredient and the other containing liposomal curcumin. Physicochemical properties of the emulsions (pH, density, etc.) were examined, along with skin hydration levels and the degree of redness. The results confirmed the efficacy of liposomal curcumin in increasing skin hydration and reducing skin redness in the tested subjects. This work provides a solid foundation for further exploration of liposomes and their potential as carriers of active substances in cosmetics.

6. Presentation of teaching and organizational achievements as well as achievements in the popularization of science

6.1. Teaching achievements

2022	**Assistant supervisor of the Ph.D. thesis** *„Properties of upconverting nanoparticles and their application in photodynamic therapy*" at the Institute of Physics, Polish Academy of Sciences
2022	**Assistant supervisor of the Ph.D. *thesis*** *„Assessment of functionality and cytotoxicity of luminescent $NaYF_4$:Yb^{3+},Er^{3+} nanoparticles for bio-medical applications"* at the Mossakowski Medical Research Institute Polish Academy of Sciences, partially implemented at the Institute of Physics PAS
From 2014	Supervision 2 more doctoral students who are currently working on their doctoral theses (**auxiliary supervision**)
2023	**Supervisor of 6 engineering theses** in the field of Cosmetic Chemistry completed and defended at the University of Engineering and Health: *„The use of silver nanoparticles in cosmetics intended for daily skin care and their use in alleviating the symptoms of dermatological diseases"* *„Development and analysis of the properties of emulsions with liposomal curcumin"* *„The use of optical and antibacterial properties of nanoparticles in hair dyes"* *„Analysis of the properties of Brahmi oil and its exemplary use in hair cosmetics"* *„Natural Cosmetics in the Consumer's Opinion"* *„Nanotechnological modifications of Centella asiatica (L.) extract in the context of the cosmetics industry"*
2023	**Co-supervisor of engineering thesis** *„Synthesis and characterization of upconverting nanoparticles for biology and medicine"* realized at IP PAS and defended at the Faculty of Physics of the University of Warsaw
2019	**Co-supervisor of master's thesis** *"Opto-magnetic properties and synthesis optimization of multifunctional nanoconstructs for applications in cancer theranostic"* realized at IP PAS and defended at the Faculty of Physics of the Warsaw University of Technology
2018	**Co-supervisor of master's thesis „***The multifunctional nanoparticles upconverting infrared light to visible light and downconverting to infrared light after 808 nm laser excitation- synthesis, characterization and*

	application" realized at IP PAS and defended at the Faculty of Physics of the University of Warsaw
2017	**Co-supervisor of master's thesis** *„Optimization of opto-magnetic UCNPs&SPIONs@SiO_2 nanoparticles with upconverting properties for applications in biology and medicine"* realized at IP PAS and defended at the Faculty of Physics of the University of Warsaw
2017	**Co-supervisor of bachelor's thesis** *„Synthesis and characterization of Fe_3O_4 nanoparticles with applications in magnetic hyperthermia"* realized at IP PAS and defended at the Faculty of Physics of the University of Warsaw
2016	**Co-supervisor of master's thesis** *„Study of the energy transfer from the up-converting $NaYF_4$ nanoparticles doped with the rare earth metal ions to selected photosensitizers and the generation of reactive oxygen species for biology and medicine applications"* realized at IP PAS and defended at the Faculty of Physics of the University of Warsaw
2016	**Co-supervisor of bachelor's thesis** *"Energy transfer between organic dyes ("antenna") attached to the surface of upconverting nanoparticles to improve the efficiency of up-conversion for use in biology and medicine"* realized at IP PAS and defended at the Faculty of Physics of the University of Warsaw
2023	**Conducting *"Nanobiotechnology"* lectures** in English for PhD students at the Institute of Physics, Polish Academy of Sciences
From 2017	**Conducting lectures and exercises:** *"Modeling and design of technological processes"*, *"Physics"*, *"Quantum Chemistry"*, and *"Physical Chemistry"* for students of Cosmetic Chemistry, General Chemistry, and Cosmetic Technology at the University of Engineering and Health in Warsaw
From 2009	Conducting student internships (two weeks, one month, and two months) at IP PAS during the summer period
2012	**Organizing and conducting two-day workshops** for third-year students of Biophysics at Adam Mickiewicz University as part of the promotion of the Operational Programme Innovative Economy "*Quantum semiconductor nanostructures for applications in biology and medicine - development and commercialization of a new generation devices for molecular diagnostic on the basis of new Polish semiconductor devices"* regarding the synthesis of nanoparticles and imaging of stained cells in a confocal microscope
2009-2010	**Conducting workshops** (National Children's Fund) at the IP PAS

6.2. Organizational achievements

From 2023	**Member of the Scientific Council of the IP PAS** for the **2023-2026**, member of the Committee for Adjuncts and Assistants as well as the Committee for the Institute's Development Strategy.
From 2017	**Statutory topic Leader** at the IP PAS *"Synthesis of passivated biosensor nanostructures"*
2016	**Organization of two sessions at the EMRS conference** (19-22.09.2016): Nanomaterials, nanostructures and nano-devices (B) Bioinspired and biointegrated materials as frontiers nanomaterials VI: Session 3.1 "Nanostructures at Surfaces, in Films, and Nanoparticles Fundamentals and Functions" Session 3.2 "Nanotechnology, Fundamentals, and Functions Nanoparticles and Nanostructures"

6.3. Achievements in the popularization of science

2015	I wrote a popular science article "Nanoparticles and infrared light will help fight cancer" in cooperation with A. Kowalik, P. Kowalik, D. Elbaum, S. Góźdź, published in the magazine for oncology patients at the Holy Cross Cancer Center (no. 2/2015, May 2015 AMICUS).
2015	Conducting classes during an open day at the Institute of Physics, Polish Academy of Sciences
2013	Conducting demonstration lessons at the Institute of Physics, Polish Academy of Sciences as part of the Physics Popularization campaign for teachers and high school students

7. Other scientific achievements

7.1. List of publications, not included in the discussed scientific achievement

No	Authors, publication title, journal, year of publication, volume, pages	Impact Factor[1]	Number of Citations[2]
P1	A. Borodziuk, K. Sulowska, Ł. Zinkiewicz, M. Szymura, A. Reszka, A. Bogucki, **B. Sikora**, S. Maćkowski, Ł. Kłopotowski "*Interaction with Silver Nanowires Disrupts the Excitation Pathways in Upconverting Nanoparticles"* **J Phys Chem. C** 2022, 126(45), 19219-19228	3.700	2
P2	K. Jodko-Piórecka, **B. Sikora**, M. Kluzek, P. Przybylski, G. Litwinienko, "*Antiradical Activity of Dopamine, L-DOPA, Adrenaline, and Noradrenaline in Water/Methanol and in Liposomal Systems"* **J Org Chem** 2022, 87(3), 1791-1804	3.600	12
P3	I. Kamińska, A. Wosztyl, P. Kowalik, **B. Sikora**, T. Wojciechowski, K. Sobczak, R. Minikayev, K. Zajdel, M. Chojnacki, W. Zaleszczyk, K. Łysiak, W. Paszkowicz, J. Szczytko, M. Baniewicz, W. Stryczniewicz and Krzysztof Fronc "*Synthesis and characterization of Gd_2O_3: Er^{3+}, Yb^{3+} doped with Mg^{2+}, Li^{+} ions—effect on the photoluminescence and biological applications"* **Nanotechnology** 2021, 32, 245705	3.953	6
P4	A. Borodziuk, M. Baranowski, T. Wojciechowski, R. Minikayev, **B. Sikora**, D. K. Maude, P. Plochocka, and Łukasz Kłopotowski "*Excitation efficiency determines the upconversion luminescence intensity of β-$NaYF_4$:Er^{3+},Yb^{3+} nanoparticles in magnetic fields up to 70 T"* **Nanoscale** 2020, 12, 20300-20307	7.790	12
P5	I. Kamińska, D. Jankowski, **B. Sikora**, P. Kowalik, R. Minikayev, T. Wojciechowski, M. Chojnacki, K. Sobczak, J. Rybusiński, J. Szczytko, K. Zajdel, A. Suchocki, W. Paszkowicz, M. Frontczak-Baniewicz, K. Fronc *"Structural, optical and magnetic properties of $Y_{3-0.02-x}Er_{0.02}Yb_xAl_5O_{12}$(0<x<0.20) nanocrystals: effect of Yb content"* **Nanotechnology** 2020, 31, 225711	3.874	7
P6	I. Kamińska, D. Elbaum, **B. Sikora**, P. Kowalik, J. Mikulski, Z. Felcyn, P. Samol, T. Wojciechowski, R. Minikayev, W. Paszkowicz, W. Zaleszczyk, M. Szewczyk, A. Konopka, G. Gruzeł, M. Pawlyta, M. Donten, K. Ciszak, K. Zajdel, M. Frontczak-Baniewicz, P. Stępień, M. Łapiński, G. Wilczyński, K. Fronc *„Single-step synthesis of Er^{3+} and Yb^{3+} ions doped molybdate/Gd_2O_3 core–shell nanoparticles for biomedical imaging"* **Nanotechnology** 2018, 29, 025702	3.399	12
P7	J. Mikulski, **B. Sikora**, K. Fronc, P. Aleshkevych, S. Kret, J. Suffczyński, J. Papierska, Ł. Kłopotowski and J. Kossut "*Synthesis and magnetooptic characterization of Cu-doped ZnO/MgO and ZnO/oleic acid core/shell nanoparticles"* **RSC Adv** 2016, 6, 44820-44825	3.108	8

* - corresponding author

[1]according to the year of publication

[2]according to Web of Science of 16.04.2024

No	Authors, publication title, journal, year of publication, volume, pages	Impact Factor[1]	Number of Citations[2]
P8	I. Kaminska, K. Fronc, **B. Sikora**, M. Mouawad, A. Siemiarczuk, M. Szewczyk, K. Sobczak, T. Wojciechowski, W. Zaleszczyk, R. Minikayev, W. Paszkowicz, P. Stępien, P. Dziawa, K. Ciszak, D. Piątkowski, S. Mackowski, M. Kaliszewski, M. Włodarski, J. Młynczak, K. Kopczynski, M. Łapinski and D. Elbaum *"Upconverting/magnetic: Gd_2O_3:(Er^{3+},Yb^{3+},Zn^{2+}) nanoparticles for biological applications: effect of Zn^{2+} doping"* **RSC Adv** 2015, 5, 78361-78373	3.289	32
P9	**B. Sikora***, K. Fronc, I. Kamińska, K. Koper, M. Chwastyk, P. Stępień**,** W. Paszkowicz, T. Wojciechowski, K. Sobczak, D. Elbaum *"Fluorescence resonance energy transfer between ZnO/MgO/carboxymethyl-b-cyclodextrin and Nile Red in HeLa cells – biosensing applications"* **RSC Adv** 2015, 5, 1323-1330	3.289	3
P10	I. Kamińska, K. Fronc, **B. Sikora**, K. Koper, R. Minikayev, W. Paszkowicz, K. Sobczak, T. Wojciechowski, M. Chwastyk, A. Reszka, B. J. Kowalski, P. Stępień, D. Elbaum *"Synthesis of $ZnAl_2O_4$:(Er^{3+},Yb^{3+}) spinel type nanocrystalline upconverting luminescent marker in HeLa carcinoma cells, using a combustion aerosol method route"* **RSC Adv** 2014, 4, 56596-56604	3.840	25
P11	**B. Sikora*,** K. Fronc, I. Kaminska, K. Koper, S. Szewczyk, B. Paterczyk, T. Wojciechowski, K. Sobczak, R. Minikayev, W. Paszkowicz, P. Stępień, D. Elbaum *"Transport of $NaYF_4$:Er^{3+}, Yb^{3+} up-converting nanoparticles into HeLa cells"* **Nanotechnology** 2013, 24, 235702	3.672	25
P12	**B. Sikora*,** K. Fronc, I. Kamińska, K. Koper, P. Stępień, D. Elbaum "*Luminescence of colloidal ZnO nanoparticles synthesized in alcohols and biological application of ZnO passivated by MgO"* **J Phys: Condens Matter** 2013, 25, 194104	2.223	12
P13	I. Kamińska, **B. Sikora**, K. Fronc, P. Dziawa, K. Sobczak, R. Minikayev, W. Paszkowicz, D. Elbaum *"Novel ZnO/MgO/Fe_2O_3 composite optomagnetic nanoparticles"* **J Phys: Condens Matter** 2013, 25, 194104	2.223	7
P14	**B. Sikora***, K. Fronc, I. Kaminska, A. Baranowska-Korczyc, K. Sobczak, P. Dłużewski, D. Elbaum *„The growth kinetics of colloidal ZnO nanoparticles in alcohols"* **Journal of Sol-Gel Science and Technology** 2012, 61, 197-205	1.660	19
P15	A. Baranowska-Korczyc, A. Reszka, K. Sobczak, **B. Sikora**, P. Dziawa, J. Bujak, M. Aleszkiewicz, Ł. Kłopotowski, W. Paszkowicz, P. Dłużewski, B. J. Kowalski, T. A. Kowalewski, M. Sawicki, D. Elbaum, K. Fronc *„Magnetic Fe doped ZnO nanofibers obtained by electrospinning"* **Journal of Sol-Gel Science and Technology** 2012, 61, 494-500	1.660	28
P16	K. Jodko, E. Kowalewska, **B. Sikora**, G. Litwinienko, "*Studies on the synergistic effects of PMHC (an analogue of vitamin E) and selected catecholamines in the context of neurodegenerative"* **Free Radical Research** 2009, 43, 58-58.	2.215	0

* - corresponding author

[1]according to the year of publication

[2]according to Web of Science of 16.04.2024

7.2. List of patents, not included in the discussed scientific achievement

Lp	Autorzy, tytuł
D1	Patent no. P.401873 *"Method for producing nanopowders with luminescent-magnetic properties and nano-vapours produced this way"* by Izabela Kamińska, Krzysztof Fronc, **Bożena Sikora**, Danek Elbaum, granted in 2014

7.3. Reviews of scientific articles

- Tygiel Publishers (2019)
- Acta Physica Polonica (2019)
- Advanced Science (2019)
- Nanotechnology (2015, 2018)
- Journal of Alloys and Compounds (2010, 2011)

7.4. Grants

Leader (Principal Investigator) in the SONATA 8 project of the National Science Center *"Synthesis of multifunctional up-converting nanoparticles and studying the mechanisms of generating reactive oxygen species from nanoparticles in the presence of photosensitizers and their interaction with biological materials"* (project number: DEC-2014/15/D/ST5/02604)

PostDoc in the project MAESTRO 4 of the National Science Center *„Copper-containing magnetic semiconductor quantum dots"* (project number: UMO-2013/08/A/ST3/00297)

Participant in the project "*Development of the Biomedical Engineering Center Cluster"* co-financed by the European Union under the Innovative Economy Operational Program (project number: UDA-POIG.05.01.00-00)

Participant in the project OPUS 4 of the National Science Center *„(Zn,Mg)Al_2O_4 spinels as up-converting markers of tumor cells: production, properties and physical mechanisms of fluorescence energy transfer processes and their biological functionalization"* (project number: DEC-2012/07/B/ST5/02080)

Participant in the project "*Quantum semiconductor nanostructures for applications in biology and medicine - development and commercialization of a new generation devices for molecular diagnostic on the basis of new Polish semiconductor devices"* realized in the framework of the Operational Programme Innovative Economy in 2007-2013 (project number: POIG.01.01.02- 00-008/08)

7.5. Foreign internships

10-12.2016	**Two-month internship in Brazil** at Depto de Genética e Morfologia CNANO - Instituto de Ciências Biológicas, Universidade de Brasília. Type of stay: Scientific and research work (nanotechnology and biomedical research) under the EU Research Project grant FP7-People-2012-IRSES-BRASINOEU (project number: PIRSES-GA-2012-318916)
03-05.2016	**Two-month internship in Brazil** at the Instituto de Fisica, Nfflcleo de Fsica Aplicada Universidade de Brasilia. Type of stay: Scientific and research work (nanotechnology and biomedical research) under the EU Research Project grant FP7-People-2012-IRSES-BRASINOEU (project number: PIRSES-GA-2012-318916)
04-05.2015	**One-month internship in Wuhan (China)** at the School of Automation, Huazhong University of Science and Technology. Type of stay: Scientific research work (nanotechnology and magnetic research) as part of the EU Research Project EP7-People-2012-IRSES-BRASINOEU (project number: PIRSES-GA-2012-318916)
10-15.08.2014	**One-week internship in Lausanne (Switzerland)** at Ecole Polytechnique Fédérale de Lausanne (EPFL), Laboratory of Physics of Complex Matter (LPMC), Institute of Condensed Matter Physics (ICMP), Faculty of Basic Sciences (FBS). Type of stay: Scientific and research work (ROS measurements using EPR) as part of the "LLP-Erasmus Program Individual training. Program for Staff training mobility. Academic year 2013/2014."
09-12.12.2012	**One-week internship in Lausanne (Switzerland)** at Ecole Polytechnique Fédérale de Lausanne (EPFL), Laboratory of Physics of Complex Matter (LPMC), Institute of Condensed Matter Physics (ICMP), Faculty of Basic Sciences (FBS). Type of stay: Scientific and research work (EPR measurements on particles doped with gadolinium ions) as part of the "LLP-Erasmus Program Individual training. Program for Staff training mobility. Academic year 2012/2013."

7.6. Invited lectures

25-28.09.2019	**Lithuania - Poland Workshop on Physics and Technology in Vilnius, Lithuania** Lecture title: *"Biofunctionalized multifunctional nanoconstructs based upconverting $NaYF_4$ doped rare earth and magnetic Fe_3O_4 nanoparticles for theranostic applications."*
06-07.12.2016	**II Simpósio do Programa de Pós-Graduação em Patologia Molecular, Universidade de Brasília in Brazil** Lecture title: *"Multifunctional opto-magnetic $NaYF_4:Er^{3+},Yb^{3+},Gd^{3+}\&Fe_3O_4@SiO_2$ nanoconstructs - towards biomedical applications"*
19-22.09.2016	**E-MRS Fall Meeting Conference in Warsaw, Poland** Lecture title: *„Multifunctional opto-magnetic $NaYF_4:Er^{3+},Yb^{3+},Gd^{3+}\&Fe_3O_4@SiO_2$ nanoconstructs – towards biomedical applications"*
06-10.12.2015	**Surface Modification for Chemical and Biochemical Sensing SMCBS 2015 Conference in Pultusk, Poland** Lecture title: *„Opto-magnetic nanoparticles for biomedical applications"*
25-27.06.2015	**7th National Nanotechnology Conference in Poznan, Poland** Lecture title: *„Multifunctional opto-magnetic nanoconstructs based on up-conversion rare ions – doped $NaYF_4$ and superparamagnetic Fe_3O_4 nanoparticles – towards novel theranostic anticancer agents"*
11-15.05.2015	**E-MRS Spring Meeting 2015 Conference in Lille, France** Lecture title: *„Multifunctional magnetic and photo-luminescent $NaYF_4:Er^{3+},Yb^{3+},Gd^{3+}$ - based nanoconstructs – towards novel theranostic anticancer agents"*
04-05.03.2015	**Science for Industry Conference** Lecture title: *"Wielofunkcyjne nanocząstki – nadzieja współczesnej teranostyki antynowotworowej"*

7.7. Oral conference communications

29.11.2023	**Nomaten Winterschool 2023 in Otwock, Poland** Lecture title: *"Multifunctional opto-magnetic nanoparticles with upconverting properties - designing, synthesis and applications in cancer diagnostic"*
12-14.04.2023	**11th International Congress Nanotechnology in Biology & Medicine, Graz, Austria** Lecture title: *„The $NaYF_4$:Yb,Tm@SiO_2 upconverting Nanoparticles for Photodynamic Therapy Application"*
26.09.2022	**2nd International Research and Practice Conference Nanoobjects & Nanostructuring, Lviv, Ukraine, online** Lecture title: *„The NaYF4:Yb,Tm@SiO_2 upconverting nanoparticles for photodynamic therapy application"*
15-17.04.2019	**1st World Nanotechnology Conference 2019 in Dubai, United Arab Emirates** Lecture title: „*Multifunctional nanoconstructs based up-converting $NaYF_4$ doped rare earth"*
05-10.09.2016	**YOUCOMAT 2016 Conference in Herceg Novi, Montenegro** Lecture title: *"Multifunctional opto-magnetic $NaYF_4$:Er^{3+},Yb^{3+},Gd^{3+}&Fe_3O_4@SiO_2 nanoconstructs - towards biomedical applications"*
13-16.09.2016	**COST Action MP1302 Nanospectroscopy Topical Meeting on Nanoparticles Synthesis, Assembly, Characterization and Applications Conference in Warsaw, Poland** Lecture title: *"Multifunctional opto-magnetic nanoparticles for theranostic applications"*
23-25.05.2016	**1st Conference and Spring School on Properties, Design and Applications of Upconverting Nanomaterials in Wroclaw, Poland** Lecture title: „*Multifunctional opto-magnetic nanoparticles for theranostic applications"*
09-11.12.2015	**The Fourteenth Young Researchers' Conference Materials Sciences and Engineering in Belgrade, Serbia** Lecture title: *„Multifunctional opto-magnetic nanoparticles for theranostic applications"*

07-08.05.2014	**6th Conference on Quantum Semiconductor Nanostructures for Applications in Biology and Medicine in Warsaw, Poland** Lecture title: *"Zaprojektowanie i scharakteryzowanie biosensorów opartych na koloidalnych nanocząstkach do zastosowań w biologii i medycynie"*
16-20.09.2013	**E-MRS Fall Meeting Conference in Warsaw, Poland** Lecture title: *„Multifunctional $NaYF_4:Er^{3+},Yb^{3+},Gd^{3+}$ nanoparticles in cancer imaging and photodynamic cancer therapy”*
10-16.02.2013	**5th Winter Biophysics Workshops of the Department of Molecular Biophysics, Faculty of Physics, Adam Mickiewicz University in Sienna - Czarna Gora, Poland** Lecture title: *"Colloid nanoparticles and their applications in biology and medicine"*
18-19.04.2012	**4th Conference on Quantum Semiconductor Nanostructures for Applications in Biology and Medicine in Warsaw, Poland** Lecture title: *„Design of semiconductor biosensors for applications in biology and medicine”*
29-04.02.2012	**4th Winter Biophysics Workshops of the Department of Molecular Biophysics, Faculty of Physics, Adam Mickiewicz University in Sienna - Czarna Gora, Poland** Lecture title: *„Design strategies for biosensors based on semiconductor nanoparticles"*
09-13.05.2011	**7th National Student Science and Technology Seminar BIOMEDITECH in Spot, Poland** Lecture title: *„Properties and applications of ZnO and ZnO/MgO core/shell nanoparticles in biology and medicine”*
12-17.04.2010	**6th National Student Science and Technology Seminar BIOMEDITECH in Poznan, Poland** Lecture title*: "Properties and applications of ZnO and ZnO/MgO core/shell nanoparticles obtained by the sol-gel method"*
13-14.04.2010	**2nd Conference on Quantum Semiconductor Nanostructures for Applications in Biology and Medicine in Warsaw, Poland** Lecture title: *"Biosensory properties of ZnO"*

Additionally, since 2010, I have presented the results in the form of posters at numerous international conferences. I also gave **11 invited seminars in Poland and abroad**.

7.8. Certificates

2024	**Certificate in LumiScan multispectral skin analysis** Issuing institution: Synergy Medical Company sp. z o.o. A certificate authorizing the ability to perform skin analysis using the LumiScan gen 3.0 device
2024	**Certificate „Good Clinical Practice"** Issuing institution: NIDA Clinical Trials Network Certificate of completion of a 6-hour course on good clinical practice.
2023	**Certificate of completion of training in the use of skin examination devices manufactured by Courage + Khazaka Electronic GmbH** Issuing institution: Eprus Sp. z o.o. A certificate authorizing the ability to perform skin analysis using the Courage + Khazaka electronic GmbH device
2022	**Certificate Google Data Analytics Specialization** Issuing institution: Coursera A certificate confirming completion of eight Google-developed courses at an introductory level in Data Analytics. Confirms competence in using spreadsheets, SQL, Tableau, and R. Confirms knowledge of how to prepare, process, analyze, and share data to take informed actions.

7.9. Awards

- Award for the best presentation *"Multifunctional opto-magnetic nanoparticles for theranostic applications"* at The Fourteenth Young Researchers' Conference Materials Sciences and Engineering in Belgrade (Serbia) **09-11/12/2015**

- Honorable mention for presenting the results in the form of a poster *„β-$NaYF_4$:Er^{3+},Yb^{3+},Gd^{3+} up-converting nanoparticles: potential diagnosis and treatment tools for cancer disease"* at The International Conference EMRS Fall Meeting 2013 in Warsaw, **15-18.09.2014**

- Honorable mention for presenting the results in the form of a poster *„Multifunctional up-converting $NaYF_4$:Er^{3+},Yb^{3+},Gd^{3+} nanoparticles for applications in biology and medicine"* at the 17th International Conference on Luminescence and Optical Spectroscopy of condensed matter in Wroclaw, **13-18.07.2014**

- Doctoral thesis *"Design and characterization of biosensors based on colloidal nanoparticles for applications in biology and medicine"* was awarded by the Scientific Council of the Institute of Physics, Polish Academy of Sciences, and by director in **2014**.

- Gold medal in the INNOVATIONS 2011 competition for "Development of technology for obtaining $ZnAl_2O_4$ upconverting spinels doped with rare earth elements, i.e. Er^{3+}, Yb^{3+} by aerosol synthesis for biomedical applications" by I. Kamińska, K. Fronc, B. Sikora, A. Baranowska-Korczyc, K . Sobczak, A. Reszka, T. Wojciechowski, W. Paszkowicz, K. Koper, G. Wilczyński, J. Włodarczyk, A. Szczepankiewicz, P. Stępień, B. J. Kowalski, D. Elbaum at the 7th Industrial Technology, Science and Technology Fair Innovation "Technicon Innovations" in Gdańsk

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