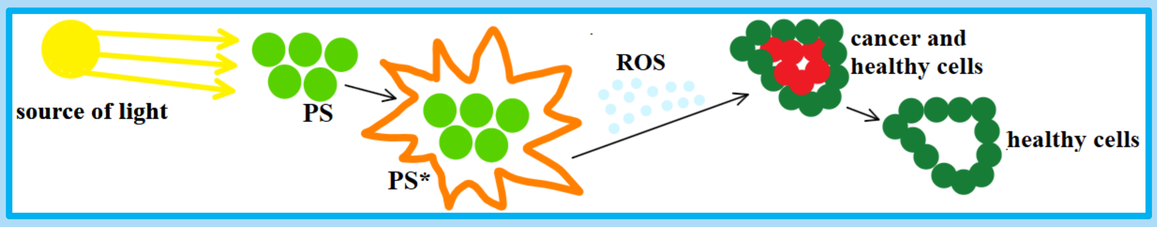
Up-converting nanoparticles with core-shell-shell structure for photodynamic therapy and bioimaging

Pasławska R.*[1,2], Wojciechowski T.[1], Sobczak K.[3], Borodziuk A.[1], Kowalik P.[1], Sikora B.*[1]

[1]*Institute of Physics, Polish Academy of Sciences, Al. Lotnikow 32/46, 02-668 Warsaw, Poland*
[2]*Faculty of Physics, University of Warsaw, Ludwika Pasteura 5, 02-093 Warsaw, Poland*
[3]*Biological and Chemical Research Centre, University of Warsaw, Zwirki i Wigury 101, 02-089 Warsaw, Poland*
r.paslawska@student.uw.edu.pl, rpaslawska@ifpan.edu.pl and sikorab@ifpan.edu.pl

NANOPARTICLES FOR PHOTODYNAMIC THERAPY

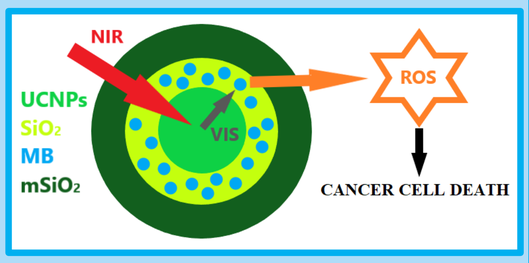
Photodynamic therapy (**PDT**) is a promising technique for cancer treatment. It involves the activation of photosensitizer (**PS**) molecules using visible (**VIS**) or ultraviolet (**UV**) light, leading to the production of Reactive Oxygen Species (**ROS**). These reactive species accumulate in cancer cells and cause their death. However, PDT has limitations, including the restricted penetration of human tissue by the applied radiation, which hinders the treatment of deep-seated tumors. In this study, the focus was on developing and characterizing core-shell-shell nanostructures with specialized properties. These structures enable the use of near-infrared (**NIR**) radiation and offer multifunctional capabilities for all-in-one applications in PDT. They facilitate the simultaneous release of therapeutic drugs, generation of ROS, and cellular imaging.



Scheme of the photodynamic therapy mechanism.

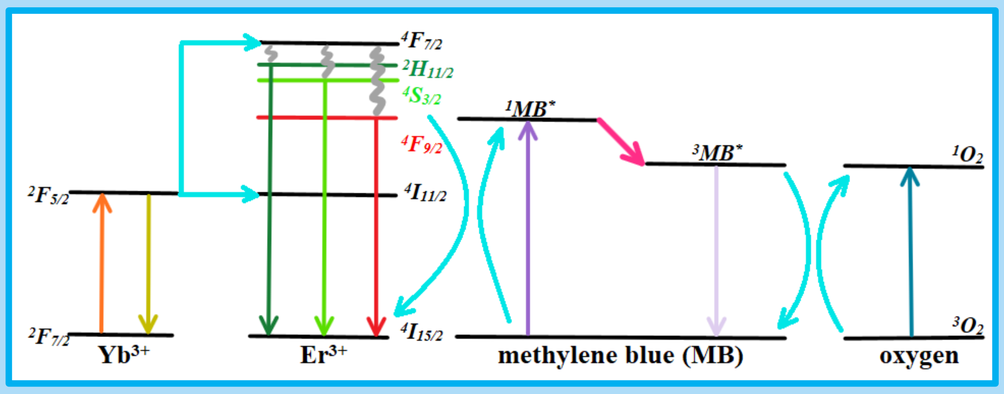
PHOTOSENSITIZER

Within the described structures, Methylene Blue (**MB**) plays the role of the photosensitizer. It was selected for its high efficiency in generating ROS. Upon excitation, MB transitions from the singlet state to the excited triplet state, where it interacts with molecular oxygen to produce ROS via the II mechanism. This mechanism involves direct energy transfer between the photosensitizer and oxygen, resulting in the formation of highly reactive singlet oxygen ($^1O_2$).



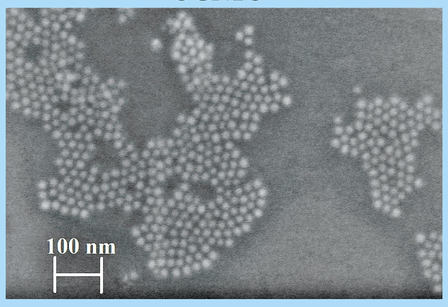
PROPERTIES

The role of the core is fulfilled by up-converting nanoparticles (**UCNPs**) of $NaYF_4$ doped with Yb^{3+} and Er^{3+} ions. Dopings are responsible for the emmision of visible (green and red) radiation upon NIR (980 nm) laser excitation by energy transfer upconversion (**ETU)**. The red light is absorbed by MB, which, in turn, upon intersystem crossing, is excited to the triplet excited state. Subsequently, another energy transfer occurs, resulting in the generation of ROS.

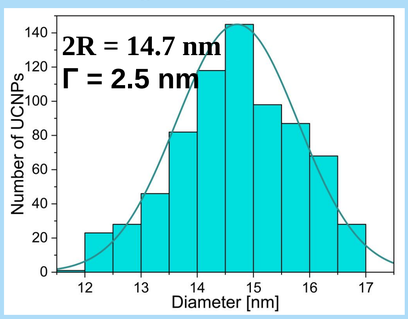


SEM IMAGING

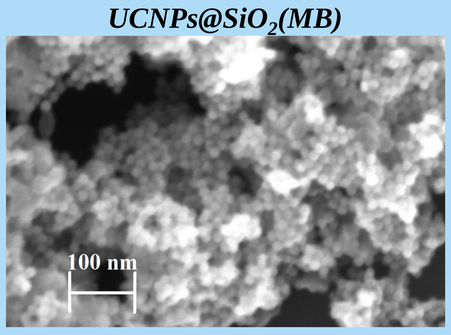
UCNPs



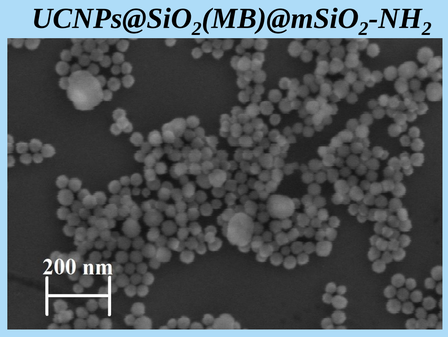
Histogram of UCNPs



UCNPs@SiO$_2$(MB)

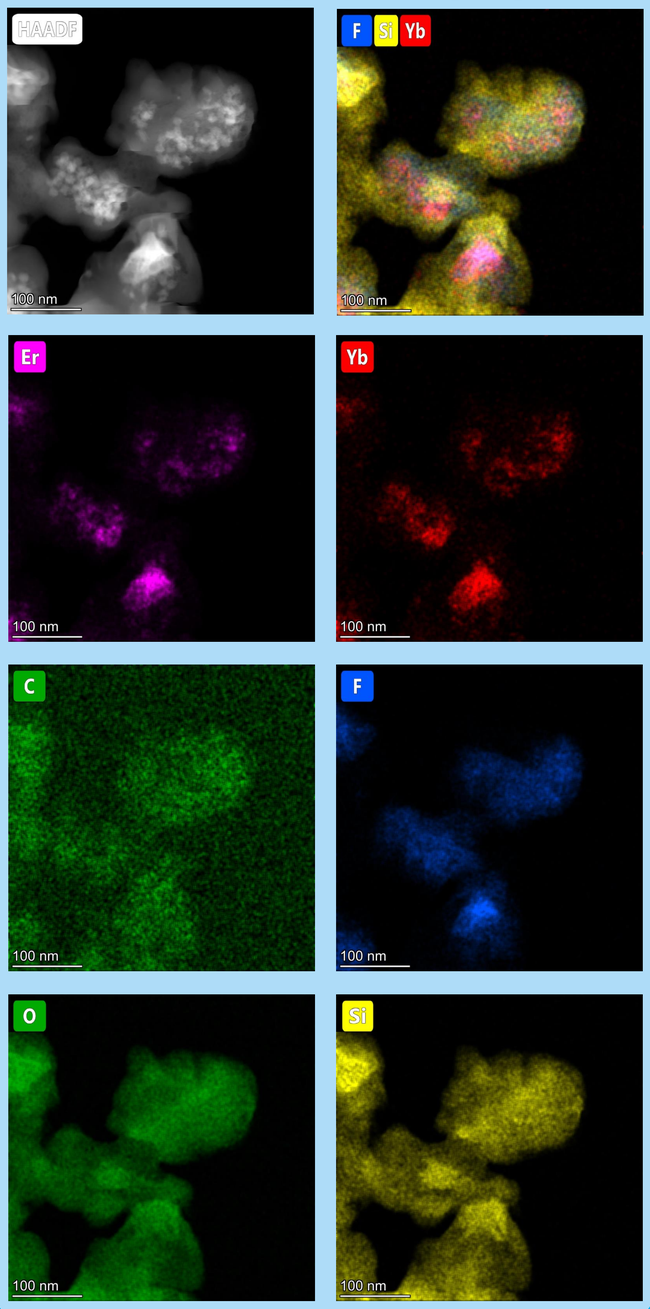


UCNPs@SiO$_2$(MB)@mSiO$_2$-NH$_2$

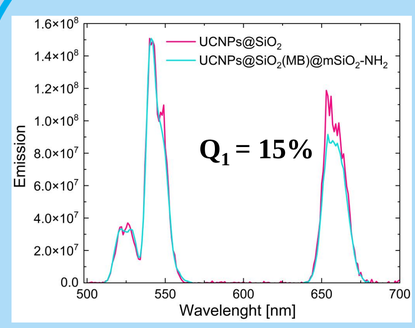


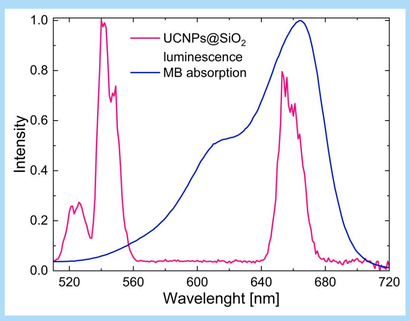
TEM IMAGING

UCNPs@SiO$_2$(MB)@mSiO$_2$-NH$_2$



SPECTRAS

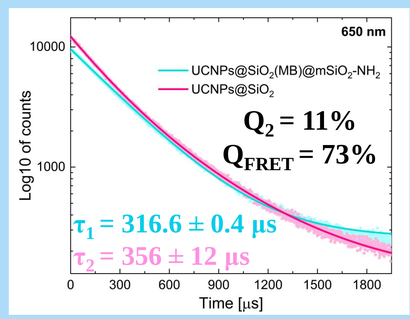


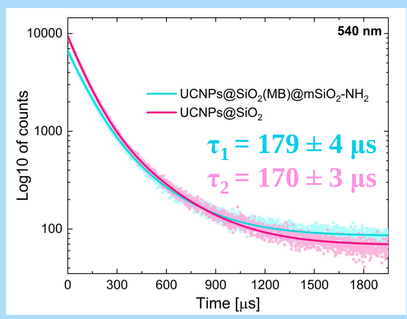


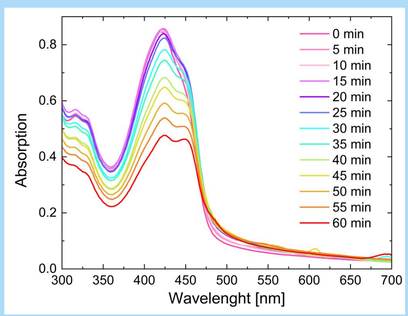
Upconvertion emission
excitation: 980 nm
power density: 3 W/cm^2

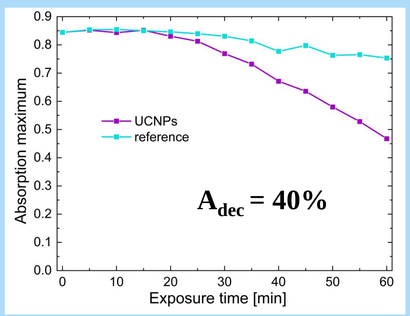
Overlap between UCNPs emission and MB absorption (normalised)

Decay Times
excitation: 980 nm
power density: 2.5 W/cm^2
frequency: 250 Hz





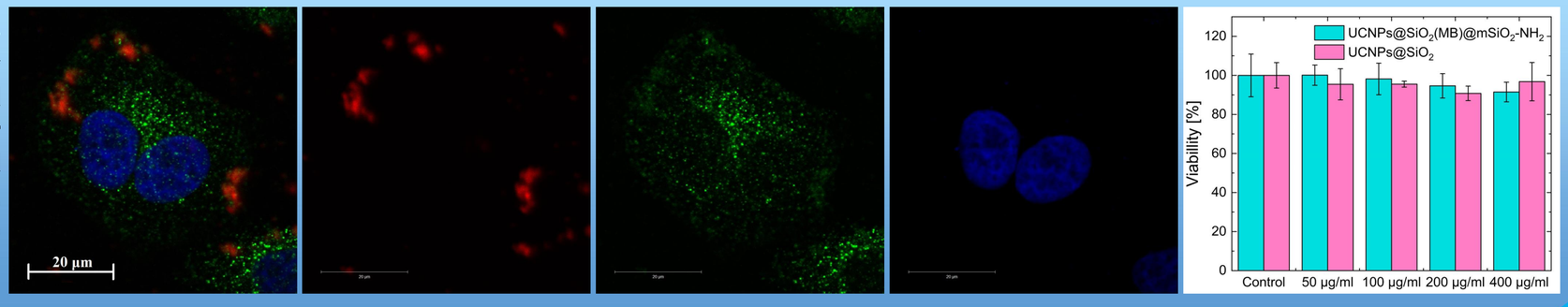




ROS Measurement
excitation: 980 nm
power density: 3 W/cm^2

IN VITRO STUDIES

In the absence of 980 nm radiation, UCNPs@SiO_2(MB)@mSiO_2-NH_2 and UCNPs@SiO_2 nanoparticles do not exhibit cytotoxicity against HeLa cancer cells. Confocal microscopy imaging confirmed the effective internalization of the nanoparticles into HeLa cancer cells. Upon excitation with NIR radiation, no background autofluorescence or additional scattering effects were observed, confirming that NIR light is a viable alternative to UV or VIS radiation in PDT.



CONCLUSIONS

- Core-shell-shell UCNPs@SiO_2(MB)@mSiO_2-NH_2 were obtained.
- The role of the photosensitizer is fulfilled by Methylene Blue.
- The final structures, upon excitation with 980 nm NIR radiation, emit visible radiation in the green (520 nm, 540 nm) and in the red (650 nm) range.
- Based on the comparison of emissions of UCNPs@SiO_2 and UCNPs@SiO_2(MB), the energy transfer occurs with an efficiency of 15%. Analysis of luminescence decay times reveals an 11% efficiency for energy transfer. Overall, it was determined that FRET contributes to 73% of the total transfer.
- An hour-long exposure of the structures to NIR light leads to a decrease in the absorption by approximately 40%.
- UCNPs@SiO_2(MB)@mSiO_2-NH_2 and UCNPs@SiO_2 nanoparticles show no cytotoxicity on HeLa cancer cells in the absence of exposure to 980 nm radiation.
- Confocal microscopy imaging confirmed the effective ntroduction of nanoparticles into HeLa cancer cells.
- Upon excitation with NIR radiation, no background autofluorescence or additional scattering effects were observed from UCNPs@SiO_2(MB)@mSiO_2-NH_2 and UCNPs@SiO_2 particles inside the cells, confirming that NIR light is a good alternative to UV or visible radiation in photodynamic therapy.
- The mesoporous SiO_2 layer allows for further functionalization.