The ROS-generating bio-functionalized $NaYF_4$:Yb,Tm@SiO_2 upconverting nanoparticles for photodynamic therapy application

Przemysław Kowalik*[1], I. Kamińska[1], K. Fronc[1], A. Borodziuk[1], M. Duda[1], T. Wojciechowski[1], K. Sobczak[2], D. Kalinowska[1], M. T. Klepka[1], B. Sikora[1]

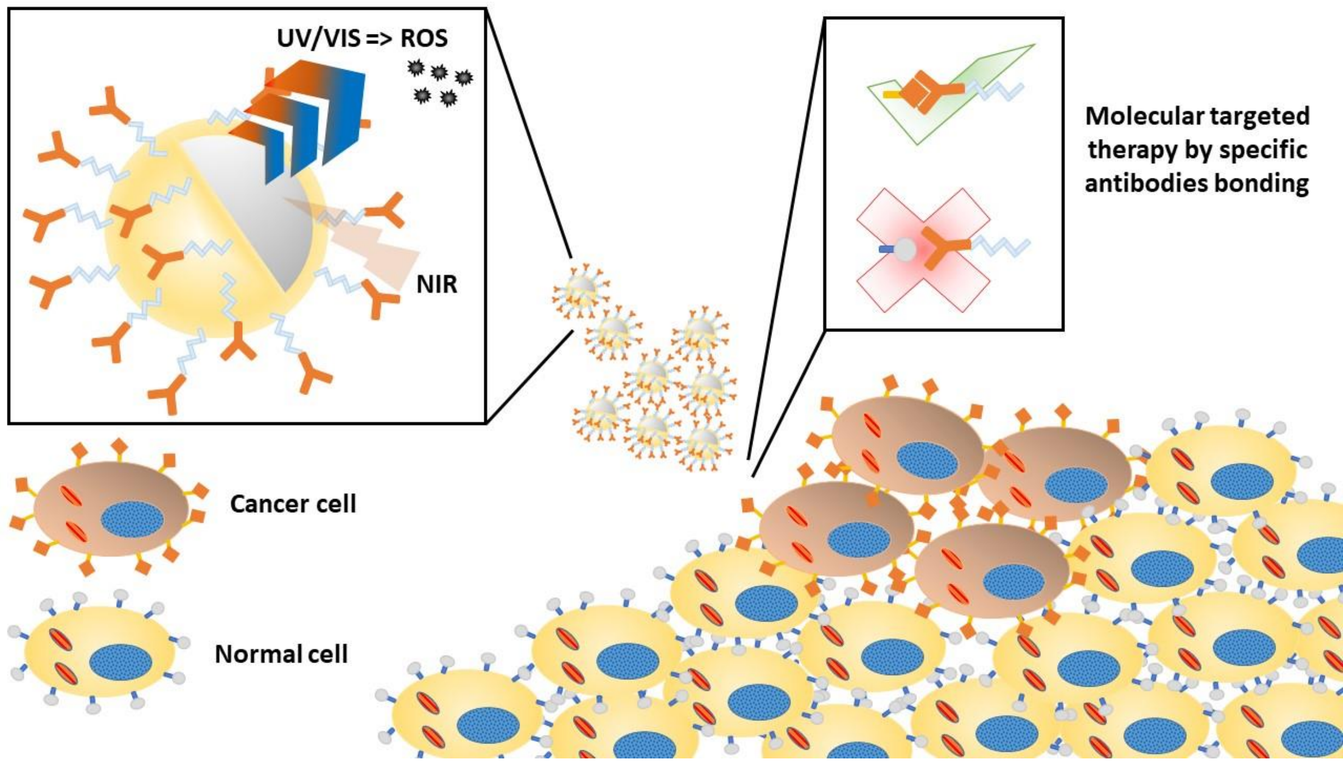
*contact e-mail: pkowalik@ifpan.edu.pl

1 Institute of Physics, Polish Academy of Sciences, Warsaw, Poland
2 Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Warsaw, Poland

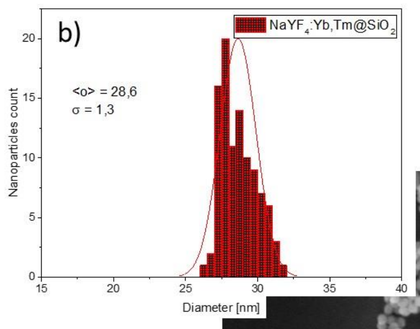
Introduction

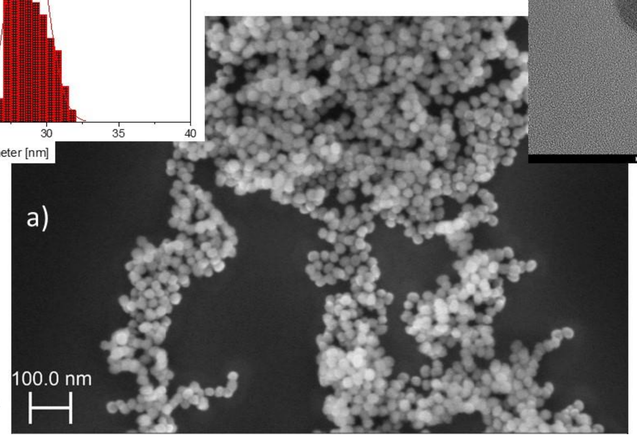
In our studies we optimize synthesis process, characterization of physical properties and biological application of modified optical nanoparticles. The basic material is yttrium sodium fluoride nanoparticles – $NaYF_4$ – doped by rare earth ions: Yb/Tm, with upconverting properties (UCNPs). The nanoparticles are capable to convert near-infrared (NIR) to visible (VIS) and ultra-violet (UV) light. We functionalized the surface of UCNPs by silicon oxide shell and biological molecules for targeting theranostic (diagnosis and therapy by one nanosystem).
Thanks to use thulium ions for doping the presented $NaYF_4$:20%Yb,0.2%Tm@SiO_2, nanoparticles are capable to reactive oxygen species (ROS) generation ater excitation by 980nm laser light.
The $NaYF_4$:20%Yb,0.2%Tm@SiO_2, nanoparticles were bio-functionalizatized by polimer and antibody molecules. Bio-functionalized nanoparticles are able to recognize specific antygen (i.e. cancer cells) what makes tchem promising factor for diagnosis and molecular targeting cancer therapy.

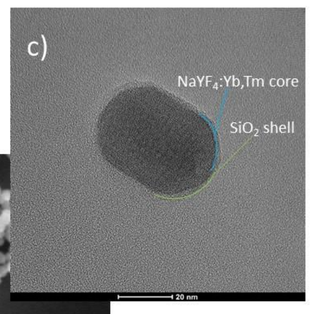
Molecular targeted photodynamic therapy by bio-functionalized $NaYF_4$: 20%Yb,0.2%Tm@SiO_2 nanoparticles



Scanning and Transmission Microscopy of $NaYF_4$: 20%Yb,0.2%Tm@SiO_2 NPs





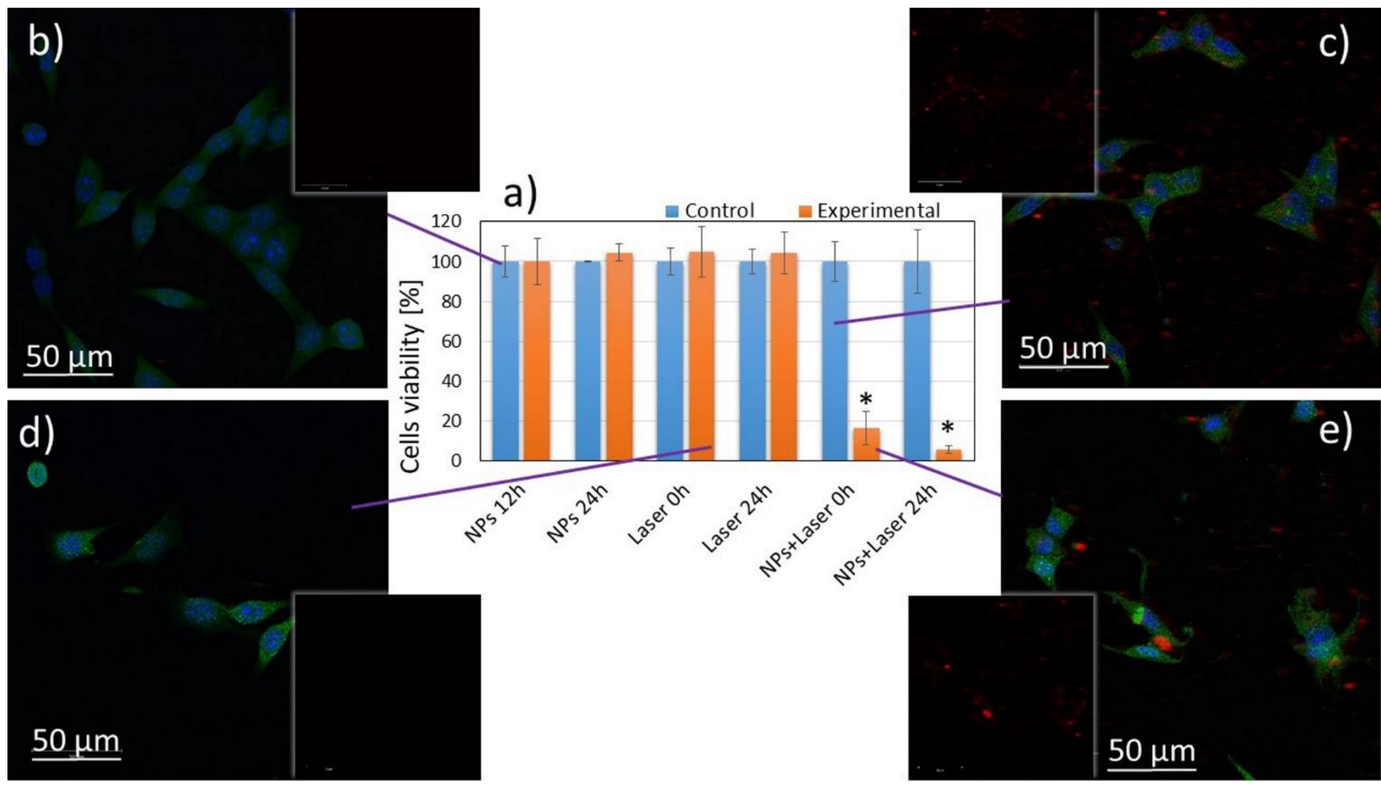


a) Scanning electron microscopy image of the $NaYF_4$:20%Yb,0.2%Tm@SiO_2 nanoparticles

b) Histogram of the $NaYF_4$: 20%Yb,0.2%Tm@SiO_2 nanoparticles size

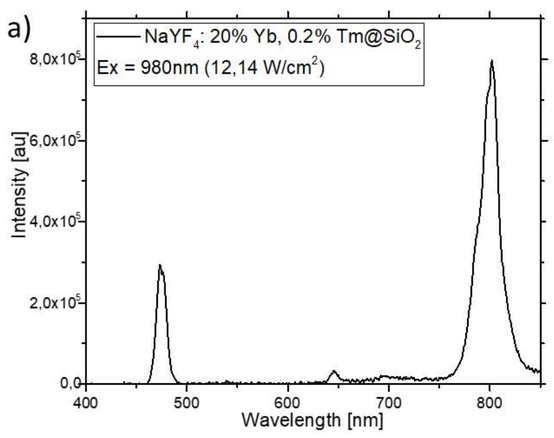
c) Transmission electron microscopy image of $NaYF_4$:20%Yb,0.2%Tm@SiO_2 nanoparticle showing thin silicon oxide shell

Photodynamic therapy at living mice breast cancer cells

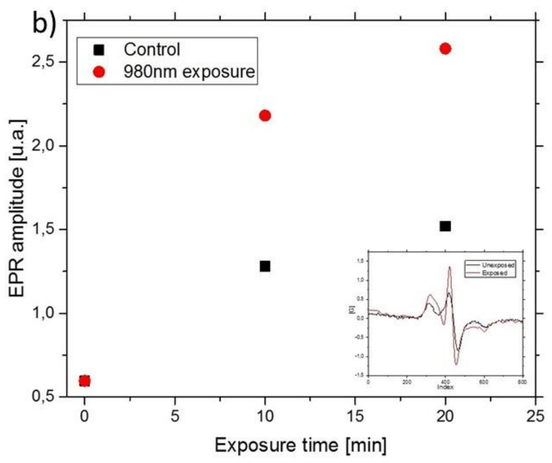


The 4T1 cells viability assay (Presto Blue) results after photodynamic therapy. Cells were treated by $NaYF_4$: 20%Yb,0.2%Tm@SiO_2 nanoparticles (100 µg/ml) and irradiated by 980 nm laser light (2 W/cm2, 10 minutes in a cycle: 1.5 minute of irradiation and 0.5 minute break) *p<0.05 (Student's t-test) (a);
The confocal microscope image of: control group (untreated and unexposed cells) (b), cells only treated with NPs (c), cells irradiated by laser light (irradiation parameters the same as for cells viability test) (d) and cells treated with NPs and irradiated by laser light (e). At the confocal microscopy images (b, c, d and e) green colour indicate early endosomes, blue colour shows nucleus area and red colour indicate $NaYF_4$: 20%Yb,0.2%Tm@SiO_2 nanoparticles (the inserts shows images only from nanoparticles emission channel).

Luminescence spectrum and ROS generation by $NaYF_4$: 20%Yb,0.2%Tm@SiO_2 NPs under 980nm laser light



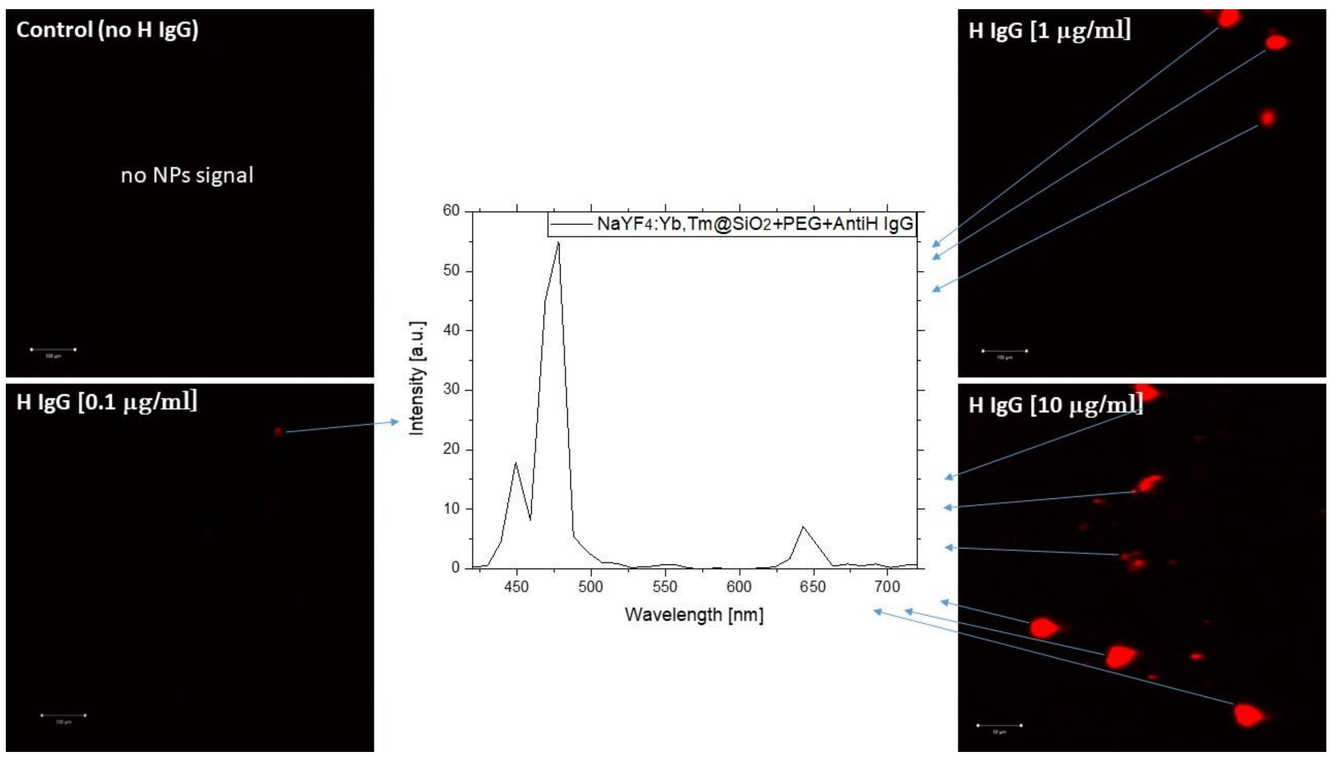
a) Luminescence spectrum of $NaYF_4$:20%Yb,0.2%Tm@SiO_2 nanocparticles water solution under 980nm laser light excitation (power density=12.14W/cm^2).



b) EPR measurement of spin trap (CMH) and $NaYF_4$:20%Yb,0.2%Tm@SiO_2 NPs solution excited by 980nm laser (power density: 1W/cm^2). Recorded signal came from CMH.

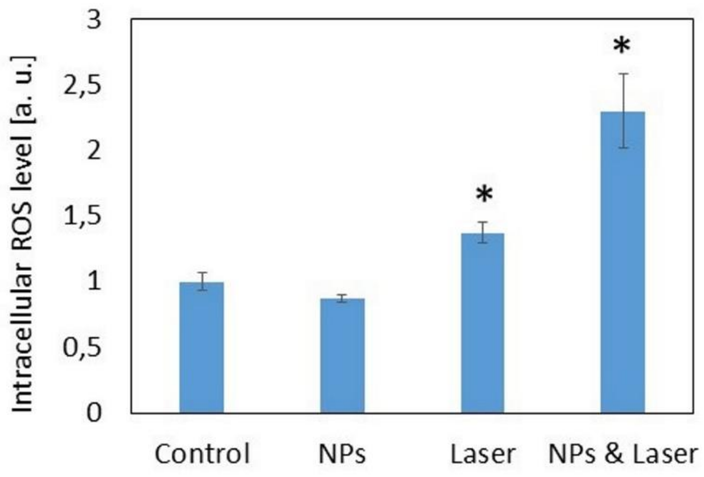
P. Kowalik et al., RSC Adv., 2017,7, 30262-30273.

Bio-functionalization test of $NaYF_4$: 20%Yb,0.2%Tm@SiO_2-PEG-AntiH:IgG



Confocal microscopy image (observe under 980nm light) of nitrocelulose membrane covered by human-IgG as a antygen in increasing concentrations (control area – uncovered). Membrane was incubated on bio-functionalized nanoparticles conjugated with antihuman-IgG antibody
($NaYF_4$: 20%Yb,0.2%Tm@SiO_2-PEG-AntiH:IgG). Recorded signal came from up-convertng core of nanoparticles.

The ROS-generation inside living cancer cells by $NaYF_4$: 20%Yb,0.2%Tm@SiO_2 NPs under NIR exposure



The intercellular ROS detector signal (DCF) registered at 4T1 cells treated by $NaYF_4$: 20%Yb,0.2%Tm@SiO_2 nanoparticles under 980 nm laser light exposure (2 W/cm2, 10 minutes in a cycle: 1.5 minute of irradiation to 0.5 minute break). The untreated and unexposed cells were as a control group; 'NPs' bar – cell treated by nanoparticles without NIR exposure; 'Laser' – cells irradiated by 980 nm laser light without nanoparticles; 'NPs & Laser' – cells treated by nanoparticles and exposed by 980 nm laser light. The signal value has been normalized to a control group (cells untreated by NPs and cultured in the dark) *p<0.05 (Student's t-test).

Conclusions

1. Yttrium sodium fluoride nanoparticles doped by rear-earth ions were synthetized with narrow size distribution (~20nm) and wide luminescence emission range (uv-blue-red).
2. The main advantage of using UCNPs is excitation light wavelength – near-infrered. The NIR light can be used for imaging of biological tissues without biological background (autofluorescence) and relatively low level of cytotoxicity.
3. Therapeutic potential of thulium doped nanoparticles was proved by experiment on living cancer cells and EPR system.
4. Upconverting nanoparticles were coated by SiO_2 shell and modified by PEG chains and specific antibody molecules. Used modification allows to prepare nanosystem for molecular targeting therapy.

Acknowledgements: The research was partially supported by the projects 2017/01/X/ST3/01380, NN DEC-2014/15/D/ST5/02604. This work has been done in the NanoFun laboratories co-financed by the European Regional Development Fund within the Innovation Economy Operational Program, the Project No. POIG.02.02.00-00-025/09/.