EFFICIENT PHOTODYNAMIC THERAPY WITH UNMODIFIED ROSE BENGAL PHOTOSENSITIZER CONNECTED TO UPCONVERTING NANOPARTICLES

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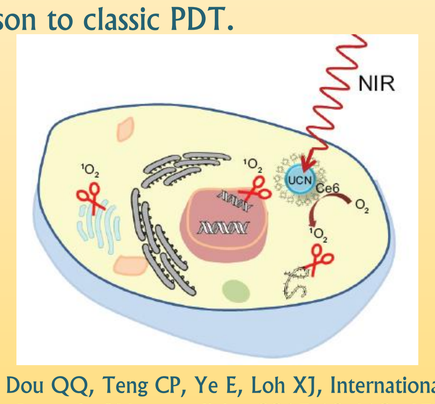
Introduction

Upconverting $NaYF_4$ nanoparticles (UCNPs) doped by rare earth ions are nowadays commonly studied for applications in photodynamic therapy (PDT). The therapeutic effect is obtained by production of reactive oxygen species (ROS), which are capable for destroying targeted cells. ROS are generated by energy transfer from the infrared-excited UCNP to photosensitive organic dye such as Rose Bengal (RB). Due to infrared excitation the therapeutical effects in upconverting PDT can be enchanced in comparison to classic PDT.

Our aim is to develope the multifunctional UCNPs-RB with efficient ROS generation for medical approaches.



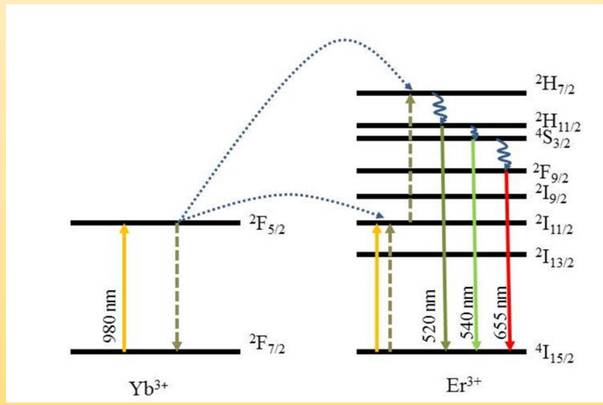
Dou QQ, Teng CP, Ye E, Loh XJ, International Journal of Nanomedicine 2015:10 419–432

Mechanism of upconversion

Upconversion is a process which consist of conversion long wavelength radiation into short wavelength radiation.

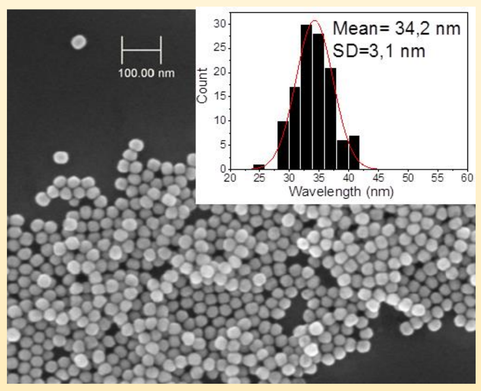
Energy transfer upconversion (ETU) occurs when first ion (sensitizer) absorbs a near infrared (NIR) photons and transfers the energy to the second ion (activator), which is capable of emitting a visible photon.

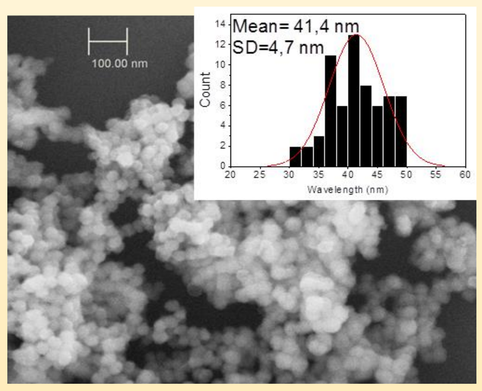
Yellow line – photonic excitation
Dashed line – energy transfer
Twisted line – multiphoton relaxation
Color arrows – emission
Thick lines – metastable energy states

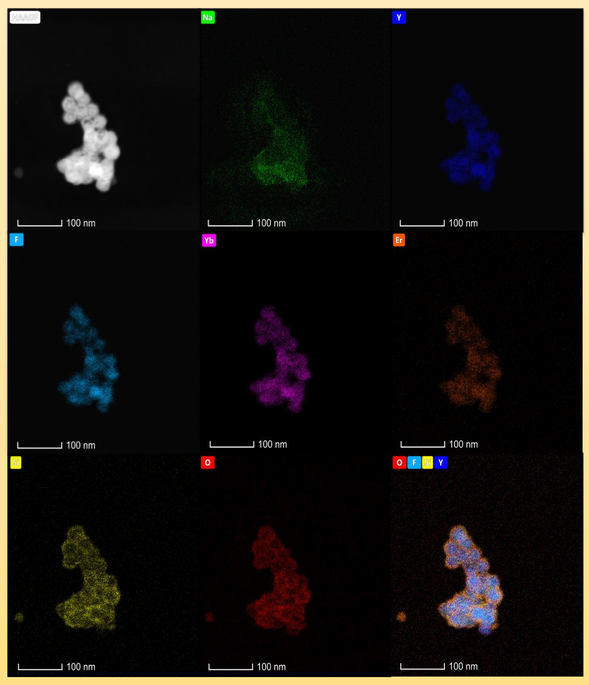


F. Wang, X. Liu, Chem. Soc. Rev, 2009, 38, 976-989

Morphology of UCNPs

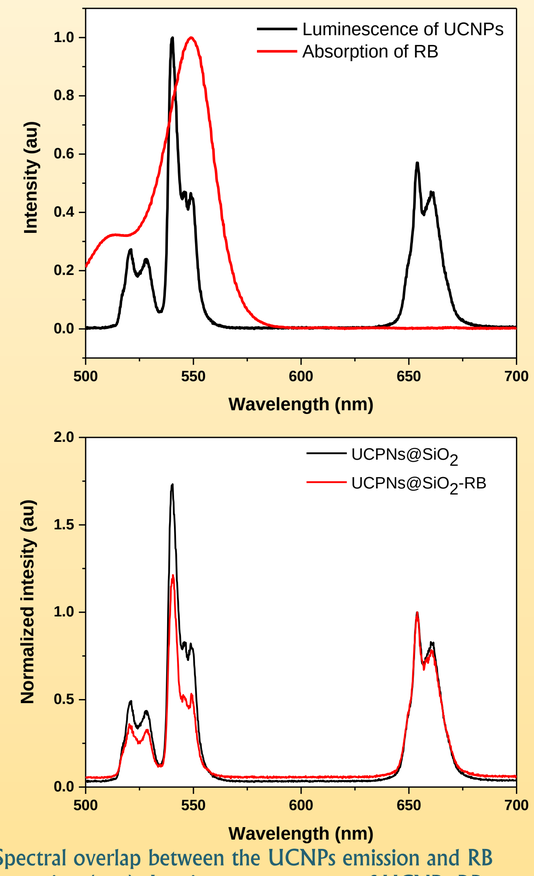


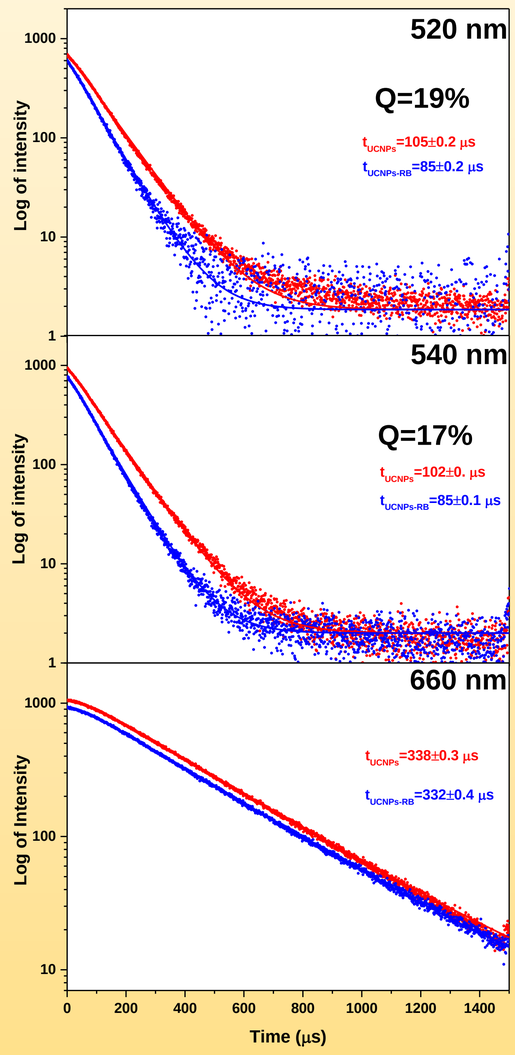




Top: SEM image and size distribution of UCNPs without (left) and with (right) SiO_2 shell. Bottom: TEM image and EDX mapping analysis of composites after encapsulation of $NaYF_4$:2%Er,20%Yb nanoparticles in SiO_2 shell.

Confirmation of energy transfer

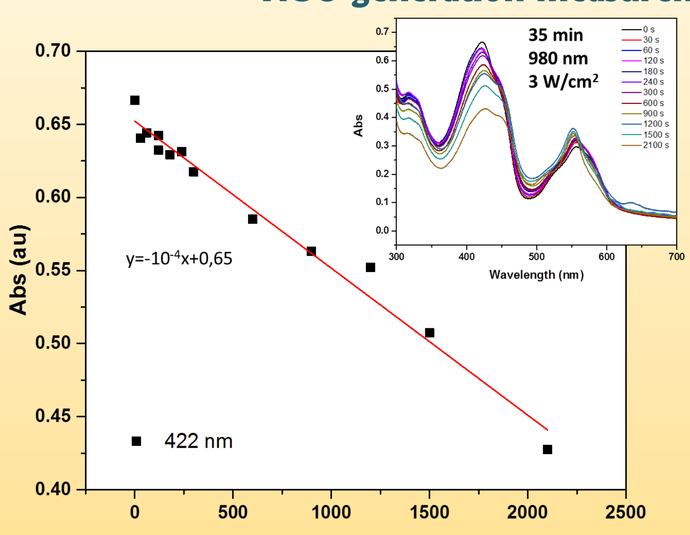




Left: Spectral overlap between the UCNPs emission and RB dye absorption (top). Luminescence spectra of UCNPs-RB and UCNPs normalized to the red band. Samples were suspended in water and irradiated with a 980 nm laser with power density 19 W/cm² (bottom).

Right: Comparison of luminescence decay times for UCNP with (blue circles) and without photosensitizer (red circles) at 520 nm (top), 540 nm (middle) and at 660 nm (bottom). We calculate the efficiency of FRET as $Q = 1 - t_{UCNPs\text{-}RB}/t_{UCNPs}$ where $t_{UCNPs\text{-}RB}$ is donor (UCNPs) decay time in presence of acceptor (RB) and t_{UCNPs} is donor decay time. To calculate Q we take $t_{UCNPs/UCNPs\text{-}RB}$ from the fitting at 520, 540, 660 nm, respectively.

ROS generation measurements

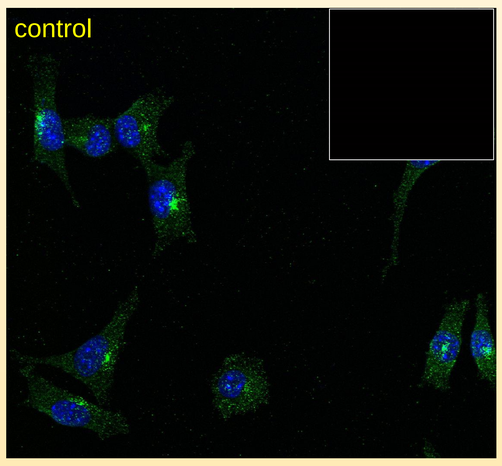


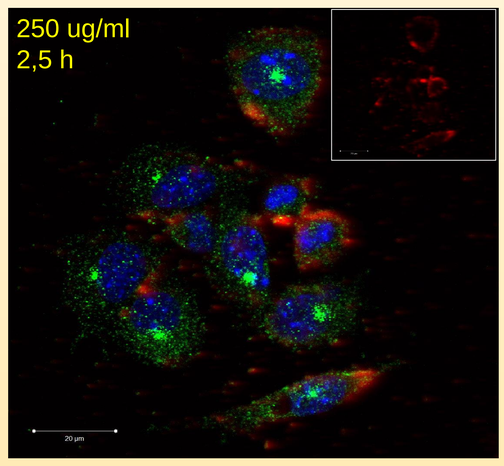
$$Q_{ROS} = \frac{a_{sample}}{a_{ref}} Q_{ref}$$

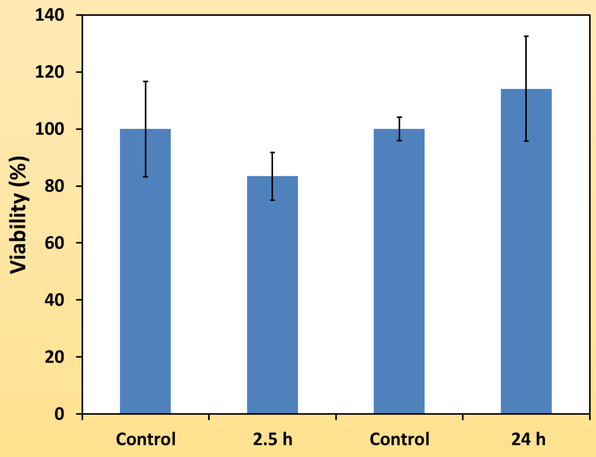
$$\mathbf{Q_{ROS} = 50\%}$$

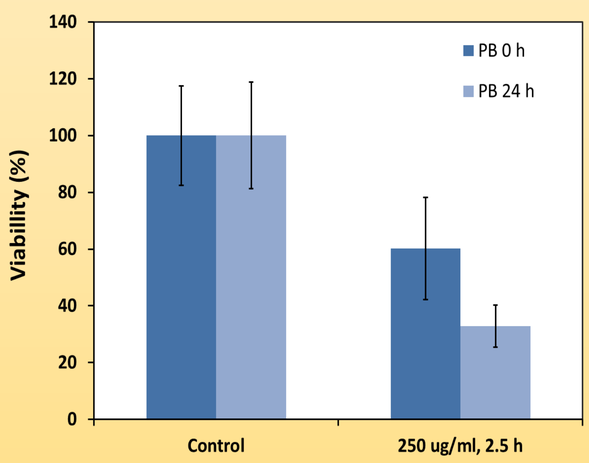
Inset: Absorbance spectrum of 64 µg/ml $NaYF_4$:2%Er,20%Yb@SiO_2-RB nanoparticles and 50 µM DPBF (ROS indicator) in water after 980 nm laser irradiation with power density 3 W/cm².
Time dependence of maximum DPBF absorbance (at 422 nm) for above solution after 980 nm laser irradiation. The decrease of DPBF absorbance is caused by increasing of ROS concentration in the sample.

Cytotoxicity and *in-vitro* theraphy with UCNPs-RB









Top: Confocal imagining of 4T1 cells after incubation 2.5 h with 250 µg/ml of UCNPs-RB. Red channel: UCNPs (ex. 980 nm, em. 500-730 nm), green channel: lysosomes (ex. 488 nm, em. 495-572 nm), blue channel: nucleus (ex. 705 nm, em. 425-475 nm).
bottom: Results of Presto Blue Viability Assay on 4T1 cells. Assay was made for concentration 250 µg/ml of UCNPs-RB nanoparticles in the darkness for 2.5 h and 24 h incubation the cells (left). The same concentration of UCNPs-RB with 4T1 cells were irradiated by 980 nm laser with power density 2 W/cm² for 10 min. Directly after therapeutic session and after 24 h Presto Blue Assay was made (right).

Conclusions

We have observed a decrease of luminescence intensity for the UCNPs with the RB sensitizer compared to the encapsulated UNCPs. It is caused by an energy transfer from the UCNPs to the RB dye. From the lifetime measurements we estimated about 20% efficiency of the transfer process.

The ROS generation by UCNPs-RB was determined by spectroscopy method. We performed 50% efficiency of ROS generation.

Confocal imaging showed that 250 µg/ml concentration of UCNPs-RB do not influence of the cell morphology. Furthermore, viability tests on 4T1 cancer cells showed that in the darkness this concentration of UCNPs-RB is nontoxic, but after irradiation UCNPs-RB by laser light with 2 W/cm² power density cell viability was reduced to 30%.

Our results showed that our UCNPs could be use in photothermal therapy and in theranostic applications.

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