Energy transfer in upconversion nanoparticles –
phthalocyanine hybrid complexes

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Abstract. In this work, we have studied the interaction between upconversion nanoparticles, and aluminum octacarboxyphthalocyanine in water solutions. It was shown that the self-assembled hybrid complexes are stable in water and NaCl solutions. The efficiency of nonradiative energy transfer from nanoparticles to the aluminum phthalocyanines increases with the number of phthalocyanine molecules in solution, but phthalocyanine sensitized fluorescence decreases due to phthalocyanine dimerization process. Also, singlet oxygen was generated by the phthalocyanine in the hybrid complex under infrared laser irradiation. The detected effects are of interest from the point of view of the directional search of components for a hybrid, highly efficient photosensitizers.

Introduction

Recently, lanthanide-doped upconversion nanoparticles (UCNPs) have attracted considerable attention in biological applications [1]. A feature of these particles is that they luminesce in the visible spectrum region when excited in the near-infrared (NIR) region. Utilization of NIR excitation light not only allows for deeper light penetration and reduced photodamage effects, but also offers lower autofluorescence, reduced light scattering, and phototoxicity. UCNPs are promising light labels for the biodetection of various types of analytes and fluorescence bioimaging.

In addition, nanoparticles can also potentially serve as a donor of energy to other molecules, for example, photosensitizers (Ps). Photosensitizers used in photodynamic therapy absorbed light in the region of 600-750 nm, which is the lower boundary of the so-called “window of transmission” of the biological tissues. Next, Ps goes to triplet excited state and can produce reactive oxygen species when interacting with molecular oxygen. Upconversion nanoparticles have one of the luminescence band in the area of 660 nm. Hence, the excitation of UCNPs using an NIR light renders it possible to activate a photosensitizer through a Forster resonance energy transfer (FRET) mechanism, provided that the emission of UCNPs could well match with the absorption of the photosensitizer, resulting in the generation of \( ^1 \text{O}_2 \) under NIR irradiation without recourse to new fluorophores, that absorbing NIR light by themselves.

Previously, it has been demonstrated that energy transfer occurs between UCNP and different fluorophores [2, 3]. In this work, we studied the transfer of energy from the upconversion nanoparticle to the photosensitizer molecules, that forming a hybrid complex in an aqueous solution based on electrostatic interactions. We used Na\( \text{YF}_4(\text{Er/Tm,Yb})/\text{NaYF}_4 \) core-shell structure UCNPs with an absorption maximum at 980 nm (Mesolight, USA), which luminescence spectra are shown in figure 1, a. The particles had a polymer PEG shell with amine groups, creating a positive charge on the surface of the particle. We used aluminum octacarboxyphthalocyanine (NIPIK, Russia) as photosensitizer and acceptor of the energy (Pc(-8)). We choose Ps with a maximal charge for strongest electrostatic
interaction; also, we prefer Al and not Zn due to the higher probability of ZnPc(-8) to aggregate in aqueous solutions.

Figure 1(a, b). (a) UCNP luminescence spectrum in water (control) and with Pc(-8) of different concentrations. After addition of Pc(-8) in solution UCNP luminescence in red band (with a maximum in 654 nm) decreases, and sensitized Pc fluorescence near 700 nm appears. Excitation by 980 nm diode laser in continuous mode. Inset: Pc(-8) fluorescence spectrum as a result of energy transfer from UCNP - (1), under 980 nm laser irradiation) and (2), under 390 nm excitation of UCNP-Pc(-8) hybrid complex. (b) UCNP at 654 nm (red) and Pc(-8) at 700 nm (blue) luminescence decay curves under 980 nm diode laser excitation in pulse mode. Inset – Pc(-8) fluorescence decay curve under 390 nm excitation.

Experimental

Absorption spectra were recorded on USB2000 spectrometer with a DT-MINI-2-GS deuterium tungsten halogen light source (Ocean Optics, USA), in standard 10 mm quartz cuvette.

Fluorescence spectra and lifetimes were measured at room temperature using fluorolog-3 fluorimeter (Horiba-Jobin-Yvon, France) with kinetic console Fluoro-Hub (Horiba Scientific). For registration of fluorescence spectra led laser with a wavelength of 980 nm (1.6 W, 12 VDC, Laserlands, China), operating in stationary mode was used as a light source. The 200 µl sample was placed in a 5x5 mm quartz cell and was constantly stirred throughout the experiment. To increase the energy of the exciting light and the intensity of the irradiation short throw lens were mounted between the laser and the cuvette with the sample, and the cuvette was placed in a special holder with two reflecting spherical mirrors, which allowed to increase sensitivity almost three times.

Measurement of the UCNP luminescence lifetimes was performed at a wavelength of 540 nm and 654 nm, and the Pc(-8) fluorescence lifetime at a wavelength of 700 nm. In this case, the laser radiation was modulated by an external TTL signal (5V) with a frequency of 50 Hz. Duration of laser n-shaped light pulse was determined by the time of the incoming TTL signal and we set 15 µs. The second TTL trigger pulse (5V, 1mks) was used to start the scan read of the emitted photons and it followed through 10 µs after the beginning of the laser flash. Detection of luminescence lifetimes was carried out by using DataStation v2.6 (Horiba Scientific) in mode Multi Channel Scaling (MCS). The time scale was set to 1 ms (1000 channels with step 1 µs) when UCNP emission at the wavelength of 540 nm had been recorded, and the accumulation was carried out for 10000 single pulses. In the case of luminescence lifetime registration at the wavelength of 655 nm (UCNP) or 700 nm (sensitized Pc(-8) fluorescence), the timeline was 2 ms (2000 channels in increments of 1 µs), and accumulation was carried out during 20000 and 100000 pulses, respectively.

To measure the Pc(-8) fluorescence lifetime on nanosecond time scale Fuoro-Hub was transferred to Time Correlated Single Photon Counting (TCSPC). In this case, the source of pulsed
excitation light was NanoLED-390 (390 nm, ∼ 1 ns, 11 pJ, Horiba Scientific), operating at a frequency of 1 MHz. Analysis of kinetic curves was carried out using the program DAC - 6, which included in the software of the fluorimeter.

For the detection of singlet oxygen generated by the photosensitizer in the complex of the hybrid complex with the nanoparticle, we used the method proposed in [4]. Briefly, imidazole was used as singlet oxygen scavenger, and we can detect decreasing of an optical density of p-nitrosodimethylaniline (RNO) upon its interaction with imidazole derivatives. As a control, a solution of Pc(-8), RNO and imidazole without UCNP was used. Irradiation was carried out by 980 nm laser in continuous mode. Concentrations of RNO and imidazole were the same as recommended in [4]. The experiment was performed in a thermostated cell Qpod 2e (Quantum Northwest, United States) at 30°C under magnetic stirring.

**Results and discussion**

It was shown that the hybrid complex Pc-UCNP self-assembled in aqueous solution probably due to electrostatic interactions. After adding phthalocyanine to the water solution of nanoparticles, we observe a quenching of UCNP luminescence intensity and enhance of Pc sensitized fluorescence with excitation by infrared laser with a wavelength of 980 nm (fig. 1, a). Simultaneously, the lifetime of the UCNP luminescence is also reduced by ∼20% (from 84 to 64 microseconds). UCNP luminescence decay curve was monoexponential in a control without Pc(-8) and in hybrid complex, which indicates that a) UCNP sample is highly monodispersed and b) all nanoparticles in the solution containing Pc(-8) are in complex with phthalocyanine molecules. Also, one should note (fig. 2) that after interaction of UCNP with Pc(-8) only UCNP luminescence red band at 654 nm but not a green band at 540 nm was quenched as the result of energy transfer by Forster mechanism.

According to FRET theory, an efficiency of energy transfer from donor to acceptor molecule depends on integral overlap $S$ between fluorescence spectrum of the donor and absorption spectrum of the acceptor:

$$S = \int F_d(\lambda)\varepsilon_a(\lambda)\lambda^4 d\lambda$$

(1)

where $F_d(\lambda)$ is the normalized fluorescence spectrum of the donor, $\varepsilon_a(\lambda)$ denotes the absorption spectrum of the acceptor, $\lambda$ is the light wavelength. The rate constant $k$ of FRET is calculated by the relation

$$k = (R_0/R)^6/\tau_0$$

(2)

where $R$ – the distance between energy donor and acceptor molecules, and $R_0$ is Forster radius, which determined as

![Figure 2(a, b).](image-url)
where $\varphi_d$ is the quantum yield of the donor in absence of the acceptor, $n$ is the refractive index of the surrounding medium, $\chi^2$ denotes the orientation factor between the transition dipole moments of the donor and acceptor (usually $\chi^2 = 2/3$ in solutions due to random orientation of molecules). The value of the Förster radius was calculated to be about 25 Å. This value is much smaller than the upconversion particle size (about 30 nm). Therefore, the energy transfer to the molecules of phthalocyanine involves primarily those emitting centers in UCNP that are closer to the surface of the particle. However, in the case of quenched emitting centers near the surface of the nanoparticle and unquenched emitting centers closer to the particle core, two time components would be observed in the kinetics of UCNP luminescence decay. Analysis of the kinetics of nanoparticles luminescence decay in the presence of Pc(-8) showed that the curve is monoexponential. Thus, the emitting centers in the core of the nanoparticle also participate in the interaction with the phthalocyanine, apparently transferring energy to the peripheral centers.

Of great importance is the number of Pc molecules per nanoparticle. It is known that phthalocyanine dimers are not fluorescent and cannot generate singlet oxygen, so formation of Pc dimers is highly unfavorable for PDT application. However, after adding an excess of Pc(-8) to UCNP solution, we observed the dimerization of molecules of the phthalocyanine, which leads to the quenching of sensitized Pc fluorescence. Moreover, dimerization of the Pc leads to a change of the shape of the absorption spectrum of Pc monomeric form, which causes the quenching of the nanoparticles luminescence due to the increase of the overlap integral of the donor fluorescence spectrum and an absorption spectrum of the acceptor molecule. The detergent (LDAO or Na-cholate, 0.5%) prevents the formation of the Pc aggregates, however, the interaction between the Pc and UCNP in the complex is also impaired. Also, we found that presence of the NaCl up to 2 mM cannot affect interaction of Pc and UCNP, as well as a Pc dimerization process. Surprisingly, UCNPs tend to precipitate in PBS buffer solution, perhaps, due to phosphate anions impact.

Further, we performed an experiment to determine the rate of singlet oxygen generation by phthalocyanine in complex with an upconversion nanoparticle (Pc:UCNP was 100:1) when irradiated with NIR light. We have recorded RNO absorption spectra and normalized the optical density value to its value before sample irradiation. The results are presented in fig. 3. In the absence of UCNP, we did not observe RNO bleaching because the Pc(-8) does not absorb light in the NIR region. In the absence of imidazole, RNO bleaching does not occur, which means that RNO does not degrade in direct contact with the excited state of phthalocyanine. Also, RNO does not bleach in a system that contains all the necessary components but in the absence of light irradiation. Thus, the bleaching of the RNO occurs due to the transfer of energy of absorbed NIR photon from the UCNP to the Pc(-8) and the resulting generation of $^1$O$_2$. 

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R_o = \frac{6}{\sqrt{8,8 \times 10^{-25}}} (\chi^2 n^{-4} \varphi_d S)
\]
Conclusions

We demonstrated the energy transfer from upconversion NaYF₄(Er/Tm,Yb)/NaYF₄ nanoparticle to aluminum octacarboxyphthalocyanine molecules in water solution, that leads to singlet oxygen generation by Pc in complex with UCNP under NIR irradiation. UCNP luminescence quenched and Pc sensitized fluorescence appears upon complexation, that reveals Forster mechanism of energy transfer. We estimate the distance between energy donors of UCNP and Pc(-8) molecules in 2.5 nm, that means close contact of components in the hybrid complex.

The main problem of such hybrid complex formation was to found the optimal ratio of [Pc]/[UCNP] because of phthalocyanine dimerization on the polymer shell of nanoparticles was occurred. The detergent (LDAO, Na-cholate) or buffer (PBS) molecules, which were used to prevent Pc aggregation, unfortunately, worsen the interaction between UCNP and Pc in the complex. We also found that the high ionic strength of the solution was not affected in any way by the degree of Pc aggregation in the presence of nanoparticles.

Finally, we can say that such photosensitizer-upconversion nanoparticle water-soluble conjugates as transducers of low energy light to toxic oxygen species may have several advantages for photodynamic therapy treatment of cancer cells.

Acknowledgments

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