Micellar carrier for triplet–triplet annihilation-assisted photon energy upconversion in a water environment

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Abstract. In this paper, we demonstrate energetically conjoined triplet–triplet annihilation-assisted photon energy upconversion (UC) operating in an aqueous environment. Obtained micellar structures show very efficient UC emission in a water environment under extremely low excitation light intensity, down to 10 mW cm\textsuperscript{-2}. The demonstrated sub-linear intensity dependence of the UC emission is of crucial importance for life science applications, allowing upconverted photons to be generated even at low intensity that then serve as a local, \textit{in situ}, optical excitation source for subsequent light-triggered processes.

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1. Introduction

The process of photon energy upconversion (UC), wherein photons strongly blue shifted relative to the excitation wavelength are generated, has been studied intensively. Different UC techniques, including two-photon absorption (TPA) [1] and energy transfer UC (ETU) [2], have been used. In these techniques, near-infrared (NIR) or infrared (IR) light sources are used. The importance of such UC optical processes is evident from the remarkable applications reported, such as biological imaging [3, 4], sensing [5, 6] and photodynamic therapy of cancer [7, 8].

However, energetically conjoined triplet–triplet annihilation-assisted photon energy UC (TTA-UC) has the essential advantage that extremely low excitation intensities are required. For instance, with excitation intensities of the order of 50 mW cm\(^{-2}\) it is possible to create locally blue-shifted photons [9–13] at a sufficient photon flux for bio-applications. This low excitation intensity keeps the radiation stress on the living tissue at an acceptable level. The TTA-UC also resolves another shortcoming of the conventional methods for UC (such as ETU [2] and all types of TPA [1])—the necessity to excite the samples with extremely bright optical sources (such as lasers). Excitation sources emitting light with extremely low spectral power density (down to 125 \(\mu\)W nm\(^{-1}\)) can generate efficient TTA-UC emission [14]. The sensitizer molecules have broad absorption spectra, in some cases even up to 80 nm (full-width at half-maximum (FWHM)). As a consequence, optical sources with flexible chosen emission wavelengths can be used, without any consequence on the UC emission spectra or UC efficiency. Another important feature of the TTA-UC is the sub-linear dependence of the UC emission on the excitation intensity [15]. This behavior of the UC emission will help to predict precisely the necessary radiation dose and to avoid unwanted optical stress of the bio-samples.

The process of TTA-UC was earlier demonstrated for metallated porphyrins and aromatic hydrocarbon dyes (serving, respectively, as sensitizer and emitter) in various organic media: volatile or non-volatile organic solvents [16–18] and styrene oligomers [19]. Replacing the organic solvent by an aqueous medium would enable a cluster of unique applications in the fields of material science and life science.

Practically, solubilization in water can be achieved by various methods including chemical modification of the sensitizer and emitter in order to enhance their solubility in highly polar...
solvents, and encapsulation (or embedding) of the hydrophobic dyes in colloidal carriers (such as micelles of an appropriate surfactant, or nanoparticles of polymeric or inorganic nature).

Of the mentioned techniques, solubilization by encapsulation in micelles of non-ionic surfactants is a powerful method, widely used for the delivery and controlled release of bioactive hydrophobic components [20, 21]. Recently, efficient solubilization of substances with low water solubility was demonstrated using amphiphilic block-copolymers from the family of polyoxyethanyl α-tocopheryl sebacate (PTS) as a surfactant [22]. The chemical structure of PTS is presented in figure 1(a). It has been shown that PTS forms spherical micelles with diameter of the order of 20–25 nm, which possess a high capacity for loading strongly hydrophobic materials; for instance, the highly water insoluble Q10 coenzyme has been successfully solubilized in PTS and delivered into the human body [23]. In a typical formulation the molar ratio between PTS and Q10 coenzyme was 2 : 1. Thus, a very high solubility is achieved. For instance, 5 wt% solution of PTS in water can solubilize nearly 1.8 wt% (or 21 mM) of the Q10 coenzyme. Therefore, we expected that concentrations of the sensitizer and the emitter ranging from 10 µM up to 10 mM can be obtained. It should be noted that the local concentration of the sensitizer and emitter dyes inside the PTS micelles is nearly one order of magnitude higher and therefore more than sufficient to observe effective TTA-UC [10, 14]. Furthermore, PTS micelles have been used also as nanoreactors: chemical transformations such as Heck and Suzuki coupling, olefin metathesis and ring-closing metathesis were conducted with good yield at ambient conditions [24–26]. These chemical processes are only possible if the core of the micelles has a liquid-like nature and the reactants diffuse freely. This suggests that the requirement of high local mobility of the active substances necessary to achieve efficient TTA-UC is also met in this system. Therefore, these micelles are ideal candidates for transferring an efficient TTA-UC process from an organic solvent environment to a biologically relevant aqueous environment.

Figure 1. Structure of (a) polyoxyethanyl α-tocopheryl sebacate (PTS), (b) meso-tetraphenyl-tetrabenzoporphine palladium (PdTBP) and (c) dibenz[de,kl]anthracene (perylene). (d) A photograph of the obtained water solutions.
In this work, we experimentally demonstrate effective TTA-UC in a water environment by incorporating the upconverting dyes in micelles of a biocompatible optically inert matrix. The parameter TTA-UC quantum yield (QY) was defined in classical terms as the ratio of the emitted to the absorbed photons [10]. It is important to note, that the QY of the process of energetically conjoined TTA-UC is an integral value, depending on series of material and experimental parameters of the multicomponent organic systems, such as local and relative molecular concentration of the optically active species, nature and viscosity of the optically inactive UC-matrix, the molecular structure of the studied UC-dyes. Therefore, in order to increase the experimental compatibility of the results, in the following study the model compound system used is based on the well-studied UC couple, namely PdTBP/perylene. The experimental results can be transferred to each of the other UC couples already demonstrated.

2. Results and discussion

The molecular structure of the surfactant (PTS), the model compound system comprising a sensitizer (PdTBP) and an emitter (perylene) used in this work, is presented in figure 1. The spectral properties of the sensitizer or emitter dissolved in PTS/water mixture (designated as ‘water solution’ hereafter) or an organic solvent (toluene) are compared in figure 2.

At the presented concentration of the dye, the absorption and fluorescence spectra of perylene in the water solution are almost identical to those in toluene. Similarity of spectra demonstrates that neither dye aggregation in ground state nor confinement-induced effects occur. Also, the strong Q-band (around $\lambda = 630$ nm) absorption of PdTBP is not modified. In the water solution, we observed a decrease in the oscillator strength of the dyes compared to that in toluene. The size of the PTS micelles loaded with the UC dyes (figure 3) ranges from about 30 to 35 nm and stays almost constant for a wide range of concentrations of PTS. Consequently, the obtained solutions are highly transparent (figure 1(d)). Decreasing the amount of PTS below 2.5 wt% induces dramatic size alteration: the micelle size reaches 100 nm at a PTS concentration of 0.5 wt% and the solution becomes turbid. An additional consequence of the micelle growth is the strong decrease of the amount of solubilized hydrophobic dyes. This conclusion is supported by the decrease of the optical absorption of the water solution demonstrated in figure 3. Thus, in further investigations, PTS concentrations higher than 2.5 wt% were used.

2.1. Fluorescence lifetime of perylene

The decay of perylene fluorescence does not show a deviation from mono-exponential behavior, both for the toluene solution and for the water solution (figure 4). In the water phase the lifetime is significantly longer (table 1). This fact can be explained by the obtained high local concentration of perylene. Taking into account that perylene is soluble only in the hydrophobic part of the micelles and, additionally, the water solution contains only 5 wt% of PTS, the calculated local concentration of the dye is about 20 times higher than the average concentration.

For such high perylene molar concentrations ($4 \times 10^{-4} - 4.7 \times 10^{-3}$ M), a similar increase in the perylene lifetime was observed in [27]. The significantly longer fluorescence lifetime is explained by the increased fluorescence re-absorption taking place at high molar concentrations.
Table 1. Fluorescence lifetime of perylene

|               | Toluene |                 | C$_{PTS}$ = 5 wt% |
|---------------|---------|-----------------|------------------|
| $C_{perylene}$ | 1 × 10$^{-6}$ M | 5 × 10$^{-4}$ M | 1 × 10$^{-6}$ M | 5 × 10$^{-4}$ M |
| $\tau_{(perylene)}$ (ns) | 4.27    | 5.15            | 6.17            | 6.59            |

2.2. Quantum yield of upconversion

In figure 5, the typical luminescence spectrum of the UC under investigation is shown. The cw-luminescence spectrum clearly shows the fluorescence of perylene with a maximum at $\lambda = 450$ nm and the phosphorescence of PdTBP with a maximum at $\lambda = 800$ nm. The existence
Figure 3. Dependence of the optical absorption of perylene dissolved in PTS/water mixture (hollow circles) and the size of micelles (filled circles) on the PTS concentration. The perylene molar concentration was fixed at $5 \times 10^{-5}$ M.

Figure 4. Decay of perylene fluorescence (observed at $\lambda = 460$ nm) for perylene dissolved in PTS/water mixture ($C_{\text{perylene}} = 10^{-6}$ M, $C_{\text{PTS}} = 5$ wt%).

of sensitizer phosphorescence comparable in intensity with the emitter fluorescence is a direct proof for less effective use of the stored optical energy. For instance, the cw-luminescence spectra of the same UC dyes, dissolved in toluene, contain only a residual amount of sensitizer phosphorescence. The UC photon flux in the water environment can be estimated to be a factor of 4 less than that of the same couple of dyes in the organic solvent, keeping all the other experimental conditions identical (such as the excitation intensity, sample temperature, excitation spot diameter and sample thickness). For comparison, the only previous literature example of TTA-UC in a water environment [28] shows four orders of magnitude lower UC
Figure 5. The UC luminescence spectrum for the PdTBP/perylene pair ($C_{\text{perylene}} = 4 \times 10^{-4}$ M, $C_{\text{PdTBP}} = 2 \times 10^{-5}$ M) dissolved in PTS/water ($C_{\text{PTS}} = 5$ wt%) mixture (red line) and in toluene ($C_{\text{perylene}} = 4 \times 10^{-4}$ M, $C_{\text{PdTBP}} = 2 \times 10^{-5}$ M) (black line). The excitation wavelength was suppressed more than $10^6$ times by using a notch filter ($\lambda = 633$ nm). Inset: a photograph of the studied water solution, daylight conditions.

The optimization of the QY of the TTA-UC with respect to two environmental parameters, namely the local molar concentration of the optically active species and their molar ratio, is shown in figure 6. The term ‘local molar concentration’ is understood as the concentration of the upconverting dyes inside the PTSs micelles.

As shown in figure 6 (red and blue hollow circles), an increase of the emitter molar concentration leads to an increase of UC QY (at a constant sensitizer molar concentration, $C_{\text{PdTBP}} = 1 \times 10^{-6}$ M). For each concentration of the PTS there is critical concentration ($C_c$) of the embedded hydrophobic dyes above which the PTS micelles become unstable. For instance, for $C_{\text{PTS}} = 2.5$ wt% in the PTS/water mixture, the critical concentration is $C_c = 5 \times 10^{-5}$ M, whereas for $C_{\text{PTS}} = 5$ wt%, $C_c = 5 \times 10^{-4}$ M. The sensitizer/emitter molar concentration ratio was increased monotonically from 1 : 5 up to 1 : 500. In both cases, a local maximum of the parameter UC QY was not reached, because higher loading of the hydrophobic...
Figure 6. Dependence of the UC QY for the PdTBP/perylene pair dissolved in PTS/water mixture at different PTS concentrations on the emitter molar concentration. Molar concentration of the sensitizer; see the text. The experimental error is less than 1%.

Dyes was not possible. The dependence of the UC QY on the sensitizer concentration is more complex: an increase of the sensitizer concentration leads to a decrease of UC QY by almost 1.9 times (figure 6, point 2, where \( C_{\text{PdTBP}} = 1 \times 10^{-5} \text{ M} \); \( C_{\text{perylene}} = 5 \times 10^{-4} \text{ M} \)). It is important to note that the UC photon flux is increased more than five times, because of an increased number of absorbed photons. It is possible to find optimal material parameters for which both the UC photon flux and the UC QY have a local optimum (figure 6, point 3, where \( C_{\text{PdTBP}} = 1 \times 10^{-5} \text{ M} \); \( C_{\text{perylene}} = 4 \times 10^{-4} \text{ M} \)).

The dependences of integral UC fluorescence in the organic solvent and the PTS/water mixture on the excitation intensity are shown in figure 7. The solid lines are power law fits:

\[
I_{\text{UpConv}} = a \times I_{\text{exc}}^b
\]

with \( b = 1.11 \) for the TTA-UC in toluene solution (figure 7, black line) and \( b = 1.18-1.22 \) for the TTA-UC in micellar systems (figure 7, blue lines). It is important to note that the region of excitation intensities, where the UC-molecular systems show an intensity dependence that is well approximated with a sub-linear function, is more than three orders of magnitude broader.

At first glance, this behavior is contrary to the classical representation [29] of the TTA process. We agree with the classical view that the probability for TTA depends quadratically on the concentration of the excited emitter triplets, but only under certain conditions: namely, when the concentration of excited triplet states is very low. This low density is realized in the classical experiments for TTA, where emitter triplets were created through direct absorption of a UV photon in the emitter singlet, followed by the strongly prohibited process of intersystem crossing in the emitter molecule. Thus, there is linearity between the concentration of the created triplet states and the excitation intensity. As a consequence, quadratic dependence between the excitation intensity and the intensity of the TTA signal is observed. In the classical TTA-UC schema [29], delayed fluorescence is observed at excitation intensities, comparable with those for the nonlinear optical processes (MW cm\(^{-2}\) [1]).
Figure 7. Dependence of the integral TTA-UC fluorescence on the excitation light intensity for the UC couple PdTBP/perylene in different solvents and various molar concentration ratios \(C_{\text{sensitizer}}/C_{\text{emitter}}\) as listed below: (1) in toluene \((2 \times 10^{-5} \text{ M}/4 \times 10^{-4} \text{ M}, 1 : 20)\); (2) in 5 wt% PTS/water mixture \((2 \times 10^{-5} \text{ M}/4 \times 10^{-4} \text{ M}, 1 : 20)\); (3) in 5 wt% PTS/water mixture \((1 \times 10^{-6} \text{ M}/5 \times 10^{-4} \text{ M}, 1 : 500)\); (4) in 2.5 wt% PTS/water mixture \((1 \times 10^{-6} \text{ M}/5 \times 10^{-5} \text{ M}, 1 : 50)\).

However, in the currently reported energetically conjoined TTA-UC, realized in a diffusion-controlled environment, the concentration of excited emitter triplets is orders of magnitude higher than that in the classical experiments. In this regime, the relationship is weaker than a quadratic one. In fact, even in the classical experiments at high excitation intensities (and hence high concentrations of the excited triplet states), linear or sub-linear dependences between the excitation intensity and the intensity of the generated UC-fluorescence are observed [30]. The crucial benefit of the energetically conjoined TTA-UC studied in this paper is that a high concentration of the excited triplet states is obtained at a very low excitation intensity (of the order of mW cm\(^{-2}\)).

3. Conclusion

In summary, energetically conjoined TTA-UC was performed in a PTS/water mixture with a high QY. The main benefit of embedding the hydrophobic dyes in micellar structures is that the local molar concentration of the UC dyes is significantly increased, which enables UC in an aqueous environment with an efficiency similar to that in a volatile organic solvent. Extremely low light excitation intensity (as low as 10 mW cm\(^{-2}\)) was sufficient to generate effective UC fluorescence. The surfactant used for solubilizing the UC species has outstanding biocompatibility. The sub-linear intensity dependence of the UC emission demonstrated enables sensitive life science applications wherein the generated UC photons serve as a local, in situ, optical excitation source for subsequent light-triggered processes.
4. Experimental

4.1. Materials and preparation of solutions

Surfactant PTS (15% (w/w) solution in water), perylene (99+%) and PdTBP complex were purchased from Sigma-Aldrich. THF (spectroscopic grade) and deionized water were used for solubilization. First, perylene (1 mM) and PdTBP (0.1 mM) stock solutions in THF were prepared; then certain amounts of the stock solutions were mixed with 15% (w/w) solution of PTS and finally an additional volume of water was slowly added while stirring. The THF was removed by rotor evaporation (overnight, at 200 mbar and $T = 45^\circ C$). An additional amount of water was added to compensate for the water lost during evaporation. A control experiment was performed to prove the efficiency of THF evaporation. Briefly, 100 µl of THF was added to 6 ml of D$_2$O. Then, THF was evaporated, generally by the same procedure as solutions for UC were prepared. After evaporation of THF, a fixed amount of tert-butanol (5 ppm) was added and H-NMR spectra were recorded ($^1$H NMR 300 MHz). The signal from 9H of methyl groups (1.24 ppm) was clearly observed. At the same time, signals at 1.88 and 3.74 ppm (protons of THF) were not detected. Therefore, we expect a level of THF concentration < 0.5 ppm (or < 5 × 10$^{-3}$ mol.%).

4.2. Spectroscopic measurements

Obtained solutions for UC were transparent and did not show any precipitate. UV/vis and fluorescence spectra were recorded with a PerkinElmer Lambda 25 spectrophotometer. A Spex Fluorolog 3 spectrometer was used for the measurement of fluorescence spectra. The size of the micelles was estimated by photon crosscorrelation spectroscopy (PCCS) using a Nanophox (SimpaTec). PCCS is based on dynamic light scattering and allows the problem of multiple scattering to be overcome in the investigation of concentrated emulsions and dispersions.

Decay of fluorescence was recorded with the time correlation single-photon counting (TCSPC) technique (FluoTime 200, PicoQuant GmbH). A cuvette (thickness 1 mm) with a solution was excited by the laser diode LDH-P-C-375 ($\lambda = 375$ nm). Right angle geometry of the sample was chosen for fluorescence collection. Glen Thompson polarizers (for excitation and detection) were arranged under magic angle condition. An additional long-pass filter, HQ455LP (Chroma Technology Corp.), with a cut wavelength of 455 nm, was placed in front of a Sciencetech Model 9030 monochromator for better elimination of scattered light. A photon-counting photomultiplier, PMA 165, was used as the detector.

4.3. Triplet–triplet annihilation-assisted photon energy upconversion measurements

All measurements were made in oxygen-free conditions. Samples were kept in a glovebox (Uni Lab, mBraun, Germany) with the concentration of oxygen below 4 ppm. Before measurement the samples were placed in specially designed glass tubes and sealed to prevent oxygen influence. As the excitation source for the UC measurements, a temperature stabilized, continuous wave, single-mode diode laser operating at $\lambda = 635$ nm was used. For the focusing of the excitation light and the collection of the sample luminescence an apochromatic lens with NA = 0.8 was used, so the excitation spot and the sample emission region would completely overlap. The excitation spot has a diameter of 120 µm; the sample thickness is 400 µm. The excitation spot diameter was constantly monitored by a beam profiler (Thorlabs BP104-VIS).
The emission spectra were registered by an optical multichannel analyzer (Hamamatsu Photonics, C7223) with absolute wavelength calibration and corrected spectral response.

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References

[1] Shen Y R 2002 The Principles of Nonlinear Optics (Berlin: Wiley-VCH)
[2] Auzel F 2004 Chem. Rev. 104 139–73
[3] Zipfel W R, Williams R M, Clark S W, Bruchez M P, Wise F W, Webb W W and Larson D R 2003 Science 300 1434–6
[4] Zako T, Nagata H, Terada N, Utsumi A, Sakono M, Yohda M, Ueda H, Soga K and Maeda M 2009 Biochem. Biophys. Res. Commun. 381 54–8
[5] Wang L, Yan R, Huo Z, Wang L, Zeng J, Bao J, Wang X, Peng Q and Li Y 2005 Angew. Chem., Int. Ed. 44 6054–7
[6] Rantanen T, Jaervenpaeae M-L, Vuojola J, Kuningas K and Soukka T 2008 Angew. Chem., Int. Ed. 47 3811–13
[7] Shan J, Qin X, Yao N and Ju Y 2007 Nanotechnology 18 445607
[8] Karotki A, Khurana M, Lepock J R and Wilson B C 2006 Photochem. Photobiol. 82 443–52
[9] Baluschev S, Miteva T, Yakutkin V, Nelles G, Yasuda A and Wegner G 2006 Phys. Rev. Lett. 97 143903
[10] Baluschev S, Yakutkin V, Miteva T, Wegner G, Roberts A, Nelles G, Yasuda A, Chernov S, Aleshchenkov S and Cheprakov A 2008 New J. Phys. 10 013007
[11] Singh-Rachford T N, Islangulov R R and Castellano F N 2008 J. Phys. Chem. A 112 3906
[12] Singh-Rachford T N and Castellano F N 2010 J. Phys. Chem. Lett. 1 195
[13] Monguzzi A, Mezyk J, Scotognella F, Tubino R and Meinardi F 2008 Phys. Rev. B 78 195112
[14] Baluschev S, Miteva T, Yakutkin V, Nelles G, Chernov S, Aleshchenkov S, Cheprakov A, Yasuda A and Wegner G 2007 Appl. Phys. Lett. 90 181103
[15] Baluschev S, Yakutkin V, Miteva T, Wegner G, Roberts T, Nelles G, Yasuda A, Chernov S, Aleshchenkov S and Cheprakov A 2008 New J. Phys. 10 013007
[16] Baluschev S et al 2007 Angew. Chem., Int. Ed. 46 7693–6
[17] Kozlov D V and Castellano F N 2004 Chem. Commun. 24 2860–61
[18] Yakutkin V, Aleshchenkov S, Chernov S, Miteva T, Nelles G, Cheprakov A and Baluschev S 2008 Chem. Eur. J. 14 9846–50
[19] Miteva T, Yakutkin V, Nelles G and Baluschev S 2008 New J. Phys. 10 103002
[20] Branco M C and Schneider J P 2009 Acta Biomater. 5 817–31
[21] Batrakova E V and Kabanov A V 2008 J. Control. Release 130 98–106
[22] Borowy-Borowski H, Sodja C, Docherty J, Walker P R and Sikorska M 2004 J. Drug Targeting 12 415–24
[23] Naderi J, Somayajulu-Nitu M, Mukerji A, Sharda P, Sikorska M, Borowy-Borowski H, Antonsson B and Pandey S 2006 Apoptosys 11 1359–9
[24] Lipshutz B H, Ghorai S and Aguihaldoa G T 2008 Adv. Synth. Catal. 350 953–6
[25] Lipshutz B H and Taft B R 2008 Org. Lett. 10 1329–32
[26] Lipshutz B H, Aguihaldoa G T, Ghorai S and Voigtritter K 2008 Org. Lett. 10 1325–8
[27] Katoh R, Sinha S, Murata S and Tachiya M 2001 J. Photochem. Photobiol. A: Chem. 145 23–4
[28] Tanaka K, Inafuku K and Chujo Y 2010 Chem. Commun. 46 4378–80
[29] Pope M and Swenberg C 1982 Electronic Processes in Organic Crystals (Oxford: Clarendon)
[30] Birks J B 1970 Photophysics of Aromatic Molecules (New York: Wiley-Interscience)