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Reversible photoswitching of dye-doped core–shell nanoparticles†

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We present a simple and versatile mechanism for the reversible photoswitching of dye-doped core–shell nanoparticles. Photochromic dithienylethenes are incorporated into the outer shell, close enough to the dyes entrapped in the core to efficiently quench them by energy transfer when photoconverted with UV light. The emission can be switched back on by irradiation with $\lambda > 450$ nm.

The possibility of switching fluorescence signals on and off is the basis for most advanced imaging techniques based on fluorescence. Most super-resolution microscopy techniques (STORM, PALM, SOFI)[1–4] are based on a common idea: using information stored in several different images to create one upsized image. Algorithms thus extract details from every image of a sequence to reconstruct super-resolved images. If the object under investigation is identical in all frames, collecting more frames and/or with a longer acquisition time can only result in sharper images, but resolution will not be pushed beyond the diffraction limit. Such a limit can be overtaken only if an ensemble of emitters, though not moving within a certain number of frames, changes its global appearance by partially turning on and off intermittently, showing all its sub-diffraction features within the frame series. In this framework, switchable fluorescent probes are now a major issue, since their brightness and the signal difference between the on and off states determine the performance of super-resolution techniques.

We have recently reported on the synthesis and characterization of a new family of silica core/polyethylene glycol (PEG) shell nanoparticles,[5–7] featuring extremely interesting properties: high brightness, emission wavelength spanning all visible and near-infrared spectral regions, water solubility, low toxicity, and functional groups at the external shell for selective targeting of biomolecules.[6] Furthermore, we have also shown that the polymeric shell is able to reversibly host apolar molecules.[8] Hosted dyes experience a significant spatial confinement because of the small structure of the outer shell. DLS, TEM† and AFM[5] measurements assign to this shell a thickness of *ca.* 7 nm, even though the effective hosting region is probably thinner, since hydrophobic molecules are expected to stay far from the external water environment. Such guest molecules are thus hosted within a very small region, close to each other and to the core-embedded dyes so that energy transfer processes can occur.

We thought then to exploit this innovative architecture to realize a very versatile nanometric photoswitch of simple preparation. We have inserted into the outer shell of the nanoparticles a suitable photochromic compound, whose ability as an energy acceptor for the dyes buried in the silica core depends on its (open or closed) form.

Among the different families of photochromic compounds, dithienylethenes are widely studied because of their favourable properties such as high fatigue resistance and thermal stability.[9] The photocyclization reaction leading to the close-ring isomer is usually triggered by UV-light with wavelengths below 350 nm, while the ring opening reaction is carried out with visible light (>450 nm). In our experiments, we used the dithienylethene derivative **PS**,[10,11] in its open (**PS-o**) and closed (**PS-c**) forms (Fig. 1). The photoconversion from **PS-o** to **PS-c** leads to several changes in the UV bands and, more importantly, to a new broad absorption band in the visible region, centred at 630 nm, displaying an interesting molar extinction coefficient (ε = 11 800 M^{-1} cm^{-1}, Fig. 2). The two forms of **PS** are well soluble in acetonitrile and other organic solvents, but they are insoluble in water.

We prepared NPs covalently doped with **R** (a Rhodamine B derivative, Fig. 1) 0.25% mol/mol *vs.* TEOS (**R@NPs**), using direct micelles of Pluronic F127 in water as nanometric templates.[8] These nanoparticles are stable for months and no dye leakage is observed. **R@NPs** present absorption and emission spectra analogous to the ones of **R** in solution and a fluorescence quantum yield of 0.35.[8] **R** was chosen as the doping agent because of the large match between its emission and the absorption band of **PS** only in its close-ring form, as shown in Fig. 3. If **PS** is added to a suspension of **R@NPs** 10^{-7} M under physiological conditions (PBS 1 × buffer) from a concentrated acetonitrile solution, it is rapidly included into the NPs' shell, as demonstrated by ultrafiltration experiments.[8] In fact, if the suspension of NPs and **PS** is centrifuged through a 100 kD cut-off membrane, **PS** is found only in the retentate together with the NPs, and not in the filtrated solution (see Fig. S4 in the ESI†). Moreover, **PS** still undergoes photoconversion under irradiation with UV and visible light (Fig. 3) in the presence of NPs, although with a slightly

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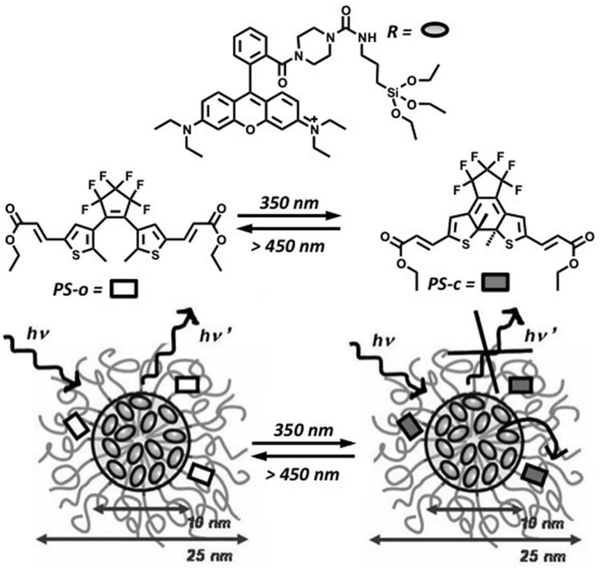

Fig. 1 Molecular structures of the rhodamine B derivative (**R**) and the dithienylethene in the two forms (**PS-o** and **PS-c**) obtained by VIS and UV irradiation, respectively. The cartoon represents the photoswitchable energy transfer process, and consequent emission quenching, within the proposed core–shell nanostructure.

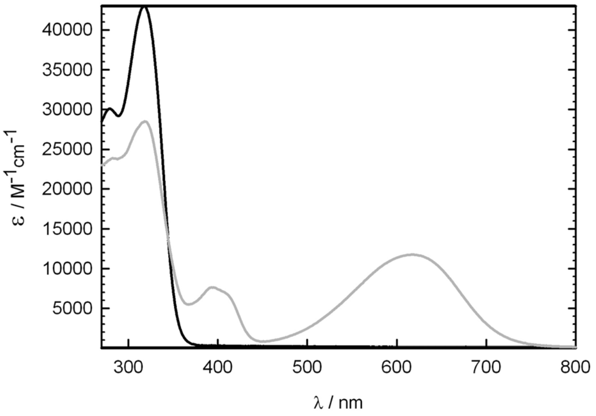

Fig. 2 Absorption spectra of **PS-o** (dark line) and **PS-c** (gray line) in acetonitrile.

reduced efficiency and photostability compared to the ideal behaviour observed in acetonitrile solutions (Fig. 2).

In a first experiment we wanted to prove that irradiation with UV light (320 nm) could lead to the photoconversion of **PS** in its close-ring form and consequently to the quenching of the emission of **R**, while visible light irradiation ($\lambda > 450$ nm) could reset the system back to the initial fluorescent configuration. We thus irradiated and monitored through absorption and emission spectroscopy a volume of 2.5 mL of a water suspension of 1.6×10^{-7} M **R@NPs** upon addition of 10 μl of 6.4×10^{-4} M acetonitrile solution of **PS** added in the dark, corresponding to an average of *ca.* 13 **PS** per NP. The resulting absorption spectrum, before irradiation, is the sum of the spectra of **R@NPs** and **PS-o**. The emission spectrum is identical in shape and magnitude to that of **R@NPs** before addition of **PS-o**, confirming that FRET does not occur at this stage.

After 1 min irradiation at 320 nm, the appearance of an absorption band at 630 nm indicates the photoconversion of **PS** into its close-ring form. The emission spectrum does not change in shape, but its intensity is drastically reduced by roughly 95% (Fig. 3, gray dashed line), proving that an efficient energy transfer process has been triggered on. The inverse process takes place if the same solution is irradiated with visible light ($\lambda > 450$ nm). The absorption band centred at 630 nm decreases, and **R@NPs** emission is fully recovered to its initial intensity. This process could be repeated many times (>20) with only minor changes in the initial and final states (Fig. 4). These cycles were performed in a low volume cuvette (50 μL), which ensured that the incident excitation beam irradiated the whole sample. It has to be noted here that the observed emission intensity decrease cannot be attributed to inner filter effects if not for a negligible amount (<5%),[12] because of the low absorbance of **PS-c** under our experimental conditions (Fig. 3). The microscopy experiments (see below), in which the optical path is very short, further support this conclusion.

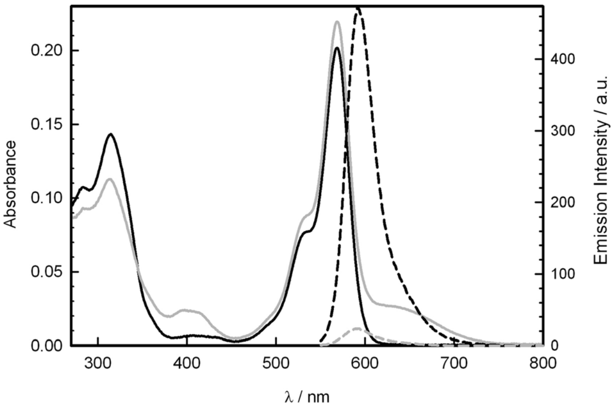

Fig. 3 Absorption and emission spectra of a suspension containing **R@NPs** and **PS-o** (dark lines, solid and dashed respectively), or **R@NPs** and **PS-c**, *i.e.* after UV irradiation (gray lines, solid and dashed respectively).

It is important to stress that, although the absorption coefficient of **PS** is not very high, the efficiency of the energy transfer process is close to unity, indicating that a very high electronic communication can be established among the dyes buried in the silica core and the species present in the outer PEG shell, allowing the design of systems suitable for many research needs.

The adduct of **R@NPs** and **PS** was also tested using a wide-field fluorescence microscope. We used a continuous

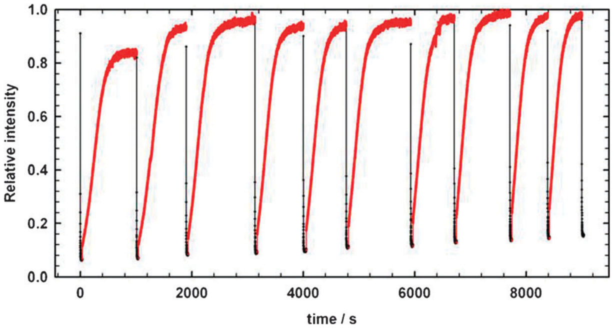

Fig. 4 Real time **R@NPs** fluorescence ($\lambda_{\text{emission}}$ = 590 nm) monitored during several photoconversion cycles. Data collected on a quartz cuvette for low volume samples (optical path length 0.3 cm, cuvette volume 50 μL). Irradiation at 320 nm (black dots and lines) and at 580 nm with a Xenon lamp (red dots and lines).

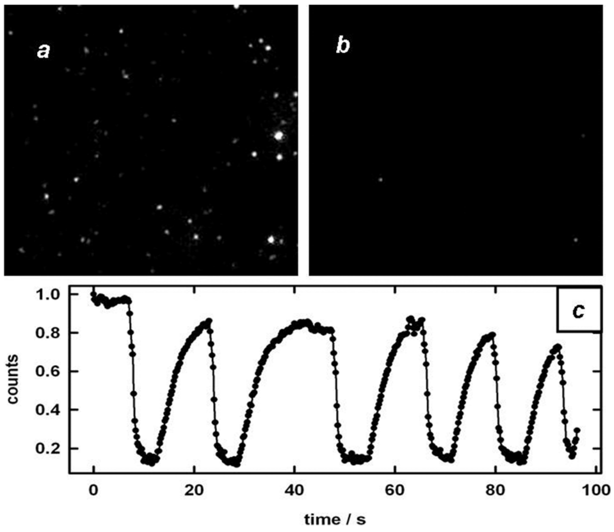

Fig. 5 Wide-field image of **R@NPs** and **PS** deposited on a glass coverslip before (a) and after (b) UV irradiation. (c) Normalized fluorescence intensity of all particles in the field of view upon turning on and off the UV irradiation, under constant 488 nm laser excitation. For single particles, see Fig. S5 (ESI†).

laser excitation at 488 nm (filtered to obtain a 10 mW power) and a cooled CCD camera to image the nanoparticles dispersed on a glass coverslip from a drop of water suspension containing **R@NPs** of 5×10^{-9} M and **PS** of 1×10^{-4} M. The irradiation from the top of the glass slide with a UV lamp resulted in a strong emission quenching, as clearly visible in Fig. 5b. Once the UV irradiation was turned off, the continuously running 488 nm excitation reset the system back to the initial fluorescent configuration by photoconverting **PS-c** in its uncoloured open-ring form **PS-o**. Fig. 5 displays five cycles of these quenching steps followed by fluorescence recovery.

In conclusion, we proved by means of fluorescence spectroscopy and wide-field fluorescence microscopy that the luminescence of dyes embedded in the core of silica-core/PEG-shell nanoparticles is switched on and off upon UV light irradiation. The quenching mechanism is based on an energy transfer process from the dyes in the core to a dithienylethene derivative **PS** hosted in the PEG shell. The absorption spectrum of **PS** changes dramatically upon irradiation, resulting in strong variations in the energy transfer efficiency from the donor dyes embedded in the core. This makes the emission quenching strongly dependent on the state of **PS**, thus switchable with light. In this context, a further improvement of the system is possible, in particular enhancing the absorption coefficient (to obtain a more favourable Förster radius), the efficiency and robustness of the photoswitch in a water-based environment, a research that is in progress in our laboratories.

Worth noticing is the nature of the nanoparticles, which are water soluble, non-toxic and membrane-permeable, and thus they respond to the most stringent criteria for *in vivo* and *in vitro* investigations. Moreover, the photoswitching mechanism that we propose is highly versatile, and many different fluorescent and photochromic dyes can be advantageously used, allowing fine tuning of the spectral and photophysical properties of the nanostructure. This strategy can thus be able to produce structures having the features required to meet different research needs.

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