



Giant Amplification of Photoswitching by a Few Photons in Fluorescent Photochromic Organic Nanoparticles

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Abstract: Controlling or switching the optical signal from a large collection of molecules with the minimum of photons represents an extremely attractive concept. Promising fundamental and practical applications may be derived from such a photon-saving principle. With this aim in mind, we have prepared fluorescent photochromic organic nanoparticles (NPs), showing bright red emission, complete ON–OFF contrast with full reversibility, and excellent fatigue resistance. Most interestingly, upon successive UV and visible light irradiation, the NPs exhibit a complete fluorescence quenching and recovery at very low photochromic conversion levels (< 5 %), leading to the fluorescence photoswitching of 420 ± 20 molecules for only one converted photochromic molecule. This “giant amplification of fluorescence photoswitching” originates from efficient intermolecular energy-transfer processes within the NPs.

Triggering property changes of a large number of molecules by only a few photons is a challenge, one which when solved would represent a significant energy- and time-saving added value in the field of molecular fluorescent photoswitches,^[1] which are applied in ultrahigh density all-optical data storage, tunable biological sensing, or super-resolution fluorescence imaging.^[2] With this aim in mind, fluorescence-quenching based on the long-range intermolecular Förster Resonance Energy Transfer (FRET), which enables a single molecular acceptor to quench the fluorescence of surrounding multiple donor fluorophores is of particular interest. Such an “ampli-

fied quenching” effect has been often observed in condensed solid states or well-ordered supramolecular assemblies.^[3] However, most of these systems suffer from a lack of reversibility. Only very few examples exploit this effect in photochromic fluorescent materials in order to achieve “amplified photoswitching”.^[4]

Hybridization of a photochromic acceptor unit and a fluorescent donor unit in materials, such as nanoparticles (NPs), quantum dots, polymer dots, or up-converting particles, is a typical approach to prepare fluorescent photoswitchable systems,^[4a,c,d,5] because the hybridization is a convenient way to prevent formation of non-fluorescent aggregates. However, in these systems, it is often difficult to achieve efficient photoswitching and good fatigue resistance because of the heterogeneous distribution of molecular arrangements or photodegradation issues. Otherwise, the preparation of photoswitchable fluorescent NPs from a single fluorescent photochromic molecular component appears to be a particularly promising approach to reach excellent fluorescence photoswitching properties.^[4a] In such NPs, a quite high density of chromophores can be obtained, providing a way to enhance the brightness and maximize the intermolecular FRET efficiency, leading to a groundbreaking amplification of fluorescence photoswitching. In this approach, the aggregation induced emission (AIE)^[6] is often utilized to overcome concentration (aggregation) fluorescence quenching. However, the fluorescence quantum yields of photoswitchable fluorescent NPs based on AIE systems are unfortunately not sufficient (a few percent) compared to the original AIE unit. Indeed, the photoswitching unit disrupts the ideal packing of the AIE moiety and the fluorescence process competes with the photoreaction process.^[4a,6d]

Herein we developed photoswitchable fluorescent NPs based on a diarylethene (DAE) benzothiadiazole (BTD) dyad **PF** (Figure 1a) with excellent fluorescence and photoswitching properties. The DAE unit undergoes reversible cyclization and cycloreversion reactions between its open-form (OF) and closed-form (CF) under UV and visible irradiation even in the solid state.^[7] The BTD fluorophore has been selected for its strong fluorescence properties in both solution and solid states, its large Stokes shift, and high photostability.^[8] BTD derivatives maintain their bright red fluorescence emission even when additional functional units are connected to the BTD backbone. Therefore, excellent photochromic and fluorescence properties are anticipated by combining covalently DAE and BTD moieties into **PF** dyads and then gathering the **PF** molecules into NPs, providing

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Supporting information and ORCID(s) from the author(s) for this article are available on the WWW under <http://dx.doi.org/10.1002/anie.201510600>.

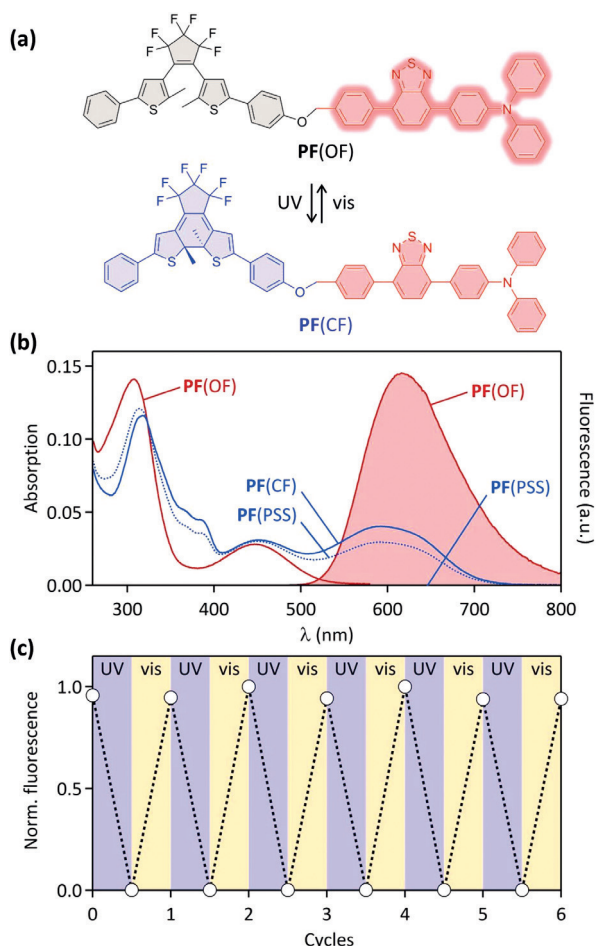


Figure 1. a) Chemical structure and photochromism of the **PF** molecular dyad (DAE unit gray/blue, BTB unit red). b) Absorption (empty curves) and emission spectra (shaded curves) of a suspension of **PF** NPs (10^{-5} M in H₂O/THF 80:20) in its open-form (OF, red curves), closed-form (CF, blue curves), and at the photostationary state (PSS, dotted blue curves). c) Fluorescence intensity of a suspension of **PF** NPs (10^{-5} M in H₂O/THF 80:20) for several UV (313 nm, 0.28 mW cm⁻²)-visible (575 nm, 3.25 mW cm⁻²) irradiation cycles.

a promising design strategy to maximize the performance of photoswitchable fluorescent NPs.

PF was synthesized from an appropriate hydroxy-substituted photochromic fragment and a benzyl bromide substituted fluorescent unit, in one step with almost quantitative yield, as shown in Scheme S1 in Supporting Information Section 1. Typical absorption and fluorescence characterization (spectra, conversion yields, photochromic and fluorescence quantum yields, lifetimes) of **PF**(OF) and **PF**(CF) in THF solution are given in the Supporting Information Sections 2.2, 2.4, and 2.9. NPs of **PF** were prepared by the reprecipitation method in a H₂O/THF 80:20 mixture.^[9] Atomic force microscopy (AFM) and electron microscopy on NPs deposited on glass or silicon substrates from the suspension provided an average diameter of 25 ± 10 nm (see Supporting Information, Section 2.5). Powder X-ray diffraction (PXRD) on the NPs provided hallow patterns as shown in the Supporting Information Section 2.5, indicating that NPs are amorphous. The absorption spectrum of the initial

suspension of **PF**(OF) NPs, as shown in Figure 1b, exhibits a main band in the UV range with a maximum at 308 nm, which corresponds to the overlay of two bands originating from both fluorescent and photochromic dyes, whereas the second absorption band at 447 nm matches the fluorescent moiety only (see Supporting Information, Section 2.1). **PF**(OF) NPs are emissive in the red region ($\lambda_{\text{max}} = 615$ nm), with a high fluorescence quantum yield $\Phi_F = 0.65$ and a lifetime of 9.6 ns (see Supporting Information, Section 2.9), that are comparable to the corresponding values in THF solution ($\Phi_F = 0.67$ and a lifetime of 7.8 ns). Upon irradiation in the UV (up to 380 nm), the typical absorption band of the **PF**(CF) appears in the 500–700 nm range of the spectrum, as shown in Figure 1b. When the photostationary state (PSS) is reached, the conversion yield was estimated by high-performance liquid chromatography (HPLC) to be 0.73 for 313 nm illumination, which is lower than **PF** in THF solution (0.93). Under alternate UV-visible irradiation cycles, the fluorescence of the NPs suspension can be completely switched OFF and ON again, many times, without any alteration of the fluorescence levels of both states (Figure 1c and SI Section 2.7). Additionally, the contrast between the ON and OFF states reaches an extremely high value of 10000:1, and the fluorescence levels of the system can be probed continuously over 1 h without any change of the signal (see SI Section 2.8) under excitation at 447 nm corresponding to the absorption band of BTB unit, in which the open-form (respectively, closed-form) of the photochromic unit has no absorbance (respectively, very weak absorbance). Furthermore, the 25 nm-NPs contain about 7000 **PF** molecules, yielding a brightness of $n \times \epsilon \times \Phi_F \approx 7 \times 10^7$ L mol⁻¹ cm⁻¹ at 450 nm, that is more than 100 times brighter than most common quantum dots.^[10]

Most interestingly, the fluorescence switching behavior of the **PF** molecular system appears rather different in solution and in NPs suspension, in terms of both efficiency and rate of switching. In THF solution, the normalized fluorescence intensity versus conversion yield (I_F vs. CY, see SI Section 2.10) correlation plot displayed in Figure 2a shows a linear behavior for the forward reaction (under UV light, blue markers) and the backward reaction (under visible light, red markers) between the **PF**(OF) state (high fluorescence level) and the PSS (low fluorescence level). This linear response is compatible with a simple intramolecular FRET process.^[11] It is clearly visualized on a series of cuvette pictures containing the **PF** molecule dissolved in THF, illuminated at increasing UV irradiation times, together with the corresponding conversion yield determined by absorption control (Figure 2b). The minimum level of fluorescence depends on the PSS composition and cannot reach zero (Figure 2a). In contrast, the I_F versus CY correlation plot presented in Figure 2c, corresponding to the NPs suspension of **PF**, shows a different behavior. The initial fluorescence of the **PF**(OF) NPs decreases dramatically at very low conversion yield. More than 90% of the whole fluorescence is quenched for only 1% of **PF**(CF), and the fluorescence can be considered to reach almost zero (more than 99% fluorescence quenching) for only 5% of **PF**(CF). This trend is highlighted for a series of fluorescence cuvette

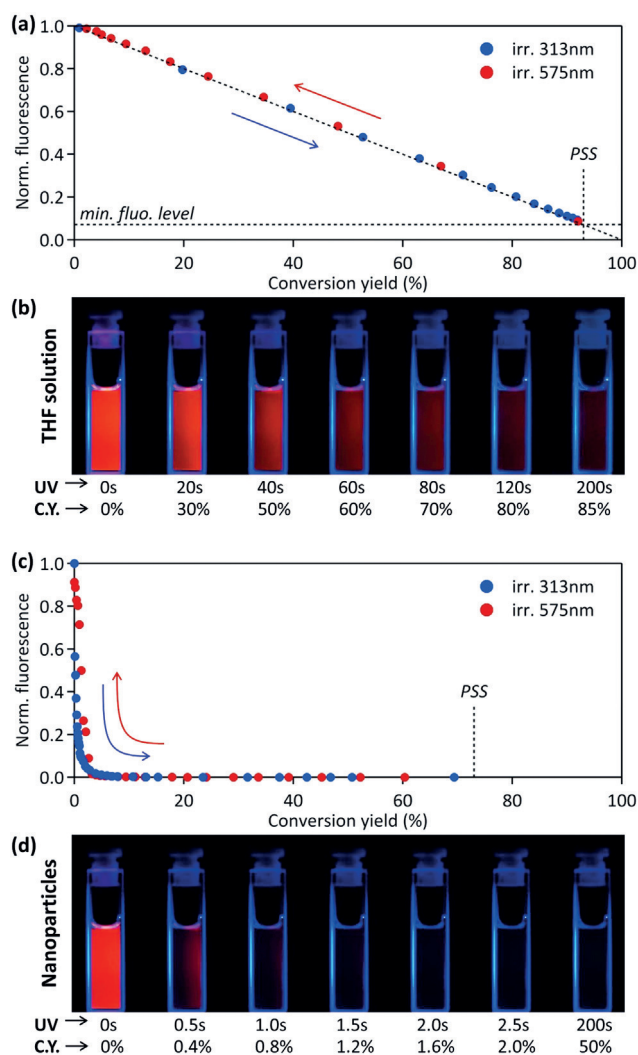


Figure 2. a), c) Fluorescence intensity versus conversion yield (CY) correlation plots and b), d) photographs of sample cuvettes for **PF**. a), b) in solution (2×10^{-6} M in THF) and c), d) in NPs suspension (10^{-5} M in $\text{H}_2\text{O}/\text{THF}$ 80:20), under increasing UV (blue dots) and visible (red dots) exposure times. Irradiation conditions: for UV, 313 nm light (a: $45 \mu\text{Wcm}^{-2}$, b, d: $50 \mu\text{Wcm}^{-2}$, c: $41 \mu\text{Wcm}^{-2}$), and for visible, 575 nm light (a: 9.45 mWcm^{-2} , c: 3.25 mWcm^{-2}) were employed.

images shown in Figure 2d, where the fluorescence is entirely switched OFF in a few seconds of 313 nm irradiation ($50 \mu\text{Wcm}^{-2}$) (see SI Movie S1). This non-linear fluorescence photoswitching behavior can be compared to other photo-switchable fluorescent multichromophoric systems mentioned above,^[4a–c] but in the present case, the quenching efficiency has been greatly enhanced by several orders of magnitude (see below).

Such a “giant amplification of fluorescence photoswitching” can actually be ascribed by a very efficient intermolecular FRET process between the fluorescent units and the photochromic moieties in their closed form within the NPs prepared exclusively from the **PF** molecular dyads. Indeed, the Förster radius R_0 was calculated to be 4.9 nm, with $\kappa^2 = 0.496$ for fixed molecules with random orientations. The

packing of **PF** molecules being quite dense in the solid matrix of the NPs, a **PF**(CF) molecule can actually play the role of energy acceptor for many neighboring **PF**(OF) molecules located in its Förster volume of interaction (the detailed discussion is described in SI Sections 2.3 and 2.11).^[12] As a first qualitative picture, the number of **PF** molecules included in the Förster sphere of a single **PF**(CF) acceptor (with diameter $2 \times R_0 = 9.8$ nm) reaches 420, over 7000 **PF** molecules are estimated to be contained in a NP of 25 nm diameter. The validity of a FRET-induced giant amplification of fluorescence photoswitching observed in **PF** NPs suspensions was further examined by means of a complete numerical simulation based on a Förster energy-transfer model. The I_F versus CY profile in NPs was calculated using straightforward assumptions, considering spherical NPs composed of evenly space-distributed and randomly oriented **PF** molecules. Our simplified model assumes that a given **PF**(OF) molecule is not quenched when the neighboring **PF** molecules, located at a distance shorter than the Förster radius R_0 , are all in the **PF**(OF) state, and fully quenched in all other cases (see SI Section 2.11). The calculated number of emitting molecules versus the number of converted molecules is in agreement with the experimental data, as shown in Figure 3a. Consequently, the principle of FRET-induced giant amplification of fluorescence photoswitching, based on intermolecular dipole–dipole interactions at long distances between **PF**(OF) and **PF**(CF), is identified to be the main photophysical process at the origin of the strong nonlinear behavior of the fluorescence quenching in **PF** NPs. Note that, in the present case, exciton diffusion process^[3e, 4d] plays a minor role in the photophysics of such NPs, since the spectral overlap between emission and absorption of the BTD fluorophores is negligible (Figure 1b and SI Section 2.1–2.3). The general Scheme of the FRET-induced giant amplification of fluorescence photoswitching is illustrated in Figure 3b. The number of quenched **PF** molecules per converted **PF**(CF) unit can be deduced from the initial slope of the curves plotted in Figure 3a, providing a huge amplification factor: around 420 ± 20 **PF** molecules are estimated experimentally (310 from the numerical simulation, see SI) to be quenched by intermolecular energy transfer to a single **PF**(CF) acceptor molecule. Another way to figure out this amplification is to express it in terms of photons needed to switch OFF the whole fluorescence of the NPs. From this point of view, around 40 **PF** molecules are switched OFF per photon absorbed by the photochromic unit (see detailed calculation in SI Section 2.11), leading to a groundbreaking improvement of practical efficiency in photoswitchable materials.

Fluorescence images of individual **PF** NPs deposited on a glass cover slide, recorded under continuous excitation at 488 nm and displayed in Figures 4a–c, show that individual **PF** NPs can be efficiently and rapidly switched OFF and ON under UV light (365 nm, 80 mWcm^{-2}) and visible light (488 nm, $150 \mu\text{Wcm}^{-2}$) excitation: the ON→OFF (resp. OFF→ON) transition requires 150–200 ms (resp. 1.0–1.5 sec) illumination time. The fluorescence signal of an individual NP under UV–visible irradiation sequences, shown in Figure 4d, clearly demonstrates the perfect reversibility and excellent fatigue resistance at the single NP level. A

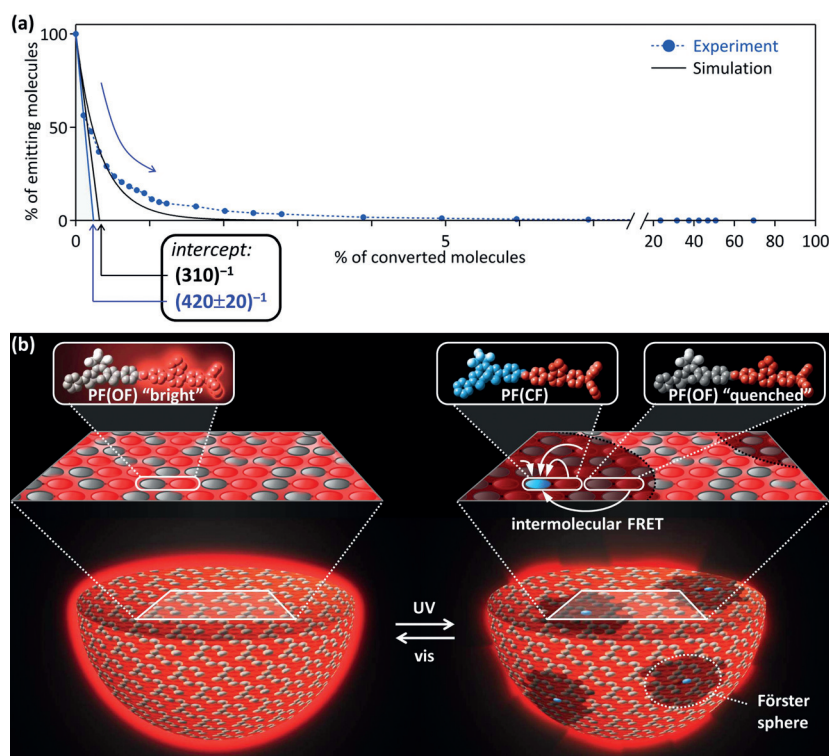


Figure 3. a) Percentage of emitting PF molecules versus percentage of converted PF(CF) molecules in NPs, plotted from experimental (blue dots) and simulated data (black curve). The slopes at the origin are shown, and the intercept with the x-axis quantifies the amplified switching effect reached in this system. b) Illustration of the FRET-induced "giant amplification of fluorescence photoswitching" in NPs. Left: every NP is initially composed of emissive PF(OF) molecules densely packed together (depicted in gray/bright red molecular dyads) subjected to a limited number of excitation hopping steps by energy migration. Right: when a few molecules are promoted to the non-fluorescent PF(CF) (blue/dark red dyads) at very low UV irradiation conditions, a large number of PF(OF) are quenched (gray/dark red dyads) by long-range intermolecular FRET process PF(OF) \rightarrow PF(CF) within each Förster sphere.

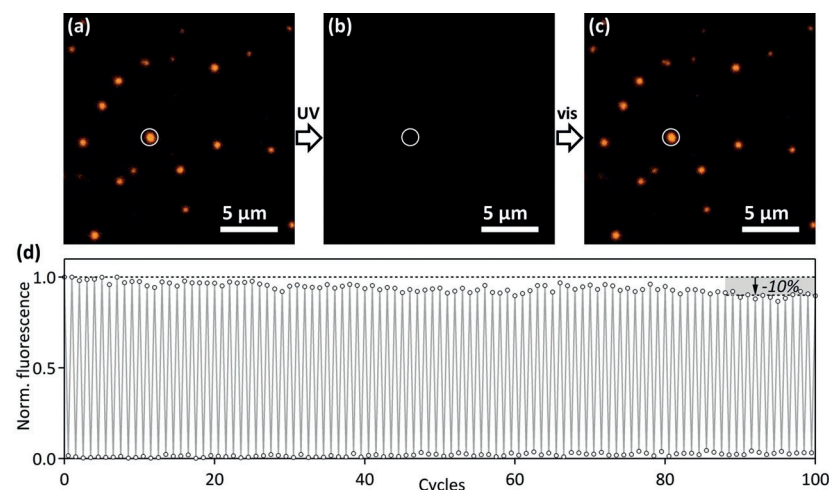


Figure 4. a)–c) Fluorescence microscopy images of PF NPs directly deposited on glass coverslips, under alternate UV–visible irradiation sequences. d) Normalized emission of one single PF NP (encircled in the images (a)–(c)), recorded for 100 cycles of UV–visible irradiation. Experimental conditions: 365 nm light (80 mW cm^{-2} , 1 s) is used to switch OFF the fluorescence of the PF NPs, and a 488 nm illumination ($150 \mu\text{W cm}^{-2}$, continuous) is employed for both excitation of the fluorescence and as a visible light source to induce the PF(CF) \rightarrow PF(OF) photochromic reaction.

decrease of only 10% of the individual NP fluorescence was observed after 100 cycles, and a very high ON/OFF contrast up to 600:1 was estimated in the first 20 cycles.

In conclusion, the PF NPs show outstanding state-of-the-art properties in the field of photochromic-fluorescent nanomaterials: easy preparation method, small size ($25 \pm 10 \text{ nm}$), bright red emission, high photostability, and excellent performances in terms of photoswitching, with very high contrast (10000:1) and fatigue resistance. These outstanding properties are based on a "giant amplification of fluorescence photoswitching", which is due to a very efficient intermolecular Förster energy transfer at the origin of the collective quenching of more than 400 molecules per switching event. Such strong nonlinear photoswitchability is highly beneficial to many applications which require sensitive fluorescent photoswitches working with the minimum number of photons, such as optical recording or subdiffraction fluorescence microscopy.

Acknowledgements

We thank Agence Nationale de la Recherche (France) for funding through Nano-PhotoSwitch and AZUR projects (K.N. and R.M.). This work was supported by JSPS KAKENHI, the Ministry of Education, Culture, Sports, Science and Technology (MEXT) Grant-in-Aid for Young Scientist (A) (No. 24685021) and a Grant-in-Aid for Scientific Research on Innovative Areas "Photosynergetics" (no. 26107008). PHEN-ICS network (CNRS GDRI), CNRS–JSPS program, SAKURA program (JSPS–Campus France), and ENS Cachan (invited professorship to T.F.) are acknowledged for supporting the collaboration.

Keywords: amplification · FRET · nanoparticles · photochromism · photoswitching

How to cite: *Angew. Chem. Int. Ed.* **2016**, *55*, 3726–3730
Angew. Chem. **2016**, *128*, 3726–3730

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Received: November 16, 2015

Published online: January 28, 2016