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Efficient NIR-I fluorescence photoswitching based on a giant fluorescence quenching in photochromic nanoparticles

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A photoswitchable near-infrared (NIR) fluorescent nanoparticle (NP) was designed and prepared. The NP showed a characteristic AIE property and a high-contrast NIR fluorescence photoswitching with full reversibility. Such efficient NIR fluorescence photoswitching would be originated from amplified fluorescence quenching mechanism based on intermolecular energy transfer in a densely packed NP state.

Photoswitchable fluorescent nanostructured materials based on photochromic compounds have attracted much attention because of their potential in various optoelectronic applications including optical memories, bioimaging, and photoswitches with high-sensitivity.¹⁻⁴ Especially, recent progress in fluorescence-based imaging technology including super-resolution microscopy encourages the development of near-infrared (NIR) emissive molecules because such molecules have salient advantages in biological applications such as deep tissue penetration, low tissue autofluorescence, and minimum phototoxicity.⁵ However, as a matter of fact, photoswitchable NIR fluorescent molecular systems have been scarcely reported so far.⁶⁻⁸ This undesirable situation might be caused from the difficulty of molecular design. A convenient approach to prepare photoswitchable fluorescent molecules is to combine a fluorescent unit with a photochromic moiety in a molecule through a non-conjugated spacer unit and to modulate the fluorescence property by means of intramolecular fluorescence resonance energy transfer (FRET) or electron transfer mechanisms.⁹⁻¹¹ Among several photochromic compounds, diarylethene (DAE) derivatives have attracted particular attention because of their high fatigue resistance and excellent

photoreactivity even in the solid state.¹² In typical DAE-based photochromic FRET systems, only the closed-ring isomer of the DAE unit has an absorption band overlapping the fluorescence band of the fluorophore, and thus the fluorescence would be quenched due to the intramolecular energy transfer process from the fluorophore (donor) to the closed-ring DAE unit (acceptor). Based on this mechanism, a great number of highly fluorescent photoswitchable DAEs have been developed.^{3,11,12} Therefore, we can prepare a NIR fluorescence photoswitchable DAE molecule based on this well-established molecular design strategy in principle. However, unfortunately, most of DAE derivatives have a strong absorption band only in the visible wavelength region (<700 nm) in the closed-ring isomer and the absorption coefficient in NIR region is typically small, which results in an insufficient FRET efficiency due to the small spectral overlap between the fluorescence spectra of NIR fluorophore and the absorption spectra of the closed-ring isomer of DAE unit.

In this study, to overcome such unsatisfied situation, we focused on the amplified fluorescence quenching behaviour in condensed systems.¹³⁻¹⁵ Recently, we successfully demonstrated extremely rapid and high on/off fluorescence contrast with full reversibility in nanoparticles (NPs) consisting of a DAE linked to a benzothiadiazole (BTD) fluorophore.¹⁶ In the NPs, a large number of the excited BTD fluorophores are quenched by the closed-ring unit of a single DAE due to the efficient "intermolecular" energy transfer in densely packed NPs. This means the fluorescence quenching efficiency dramatically enhances in the NP state, and therefore it is anticipated that we have a chance to achieve an efficient NIR fluorescence photoswitching by preparing a photoswitchable NIR fluorescent NP even though its "intramolecular" FRET efficiency from the NIR fluorophore to the closed-ring DAE unit is not high in a molecularly isolated solution condition.

In order to prepare efficient NIR fluorescence photoswitchable NPs, we focused on an aggregation-induced emission luminogen (AIEgen). Typically, the fluorescence brightness (quantum yield) of organic fluorophores decreases

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with shifting its fluorescence to longer wavelength region due to the dominated non-radiative decay in low-bandgap materials. In addition, the fluorescence of conventional organic fluorophores in the condensed state such as NPs strongly suppresses by the aggregation-caused quenching (ACQ) effect. To overcome these drawbacks, some NIR AIEgens with strong electron donor-acceptor (D-A) structures have been developed recently.¹⁷ Referring to such excellent works, here we

synthesized a D-A type NIR fluorophore (DPAFTQ) by using diphenylamine (DPA) and thiadiazoloquinoxaline (TQ) as the donor and acceptor, respectively (Fig. 1). The fluorene (F) group is introduced between DPA and TQ groups as a π -conjugation unit, which facilitates the intramolecular charge transfer (ICT) from DPA to TQ, and therefore leading to a much lower electronic bandgap or a longer absorption band.

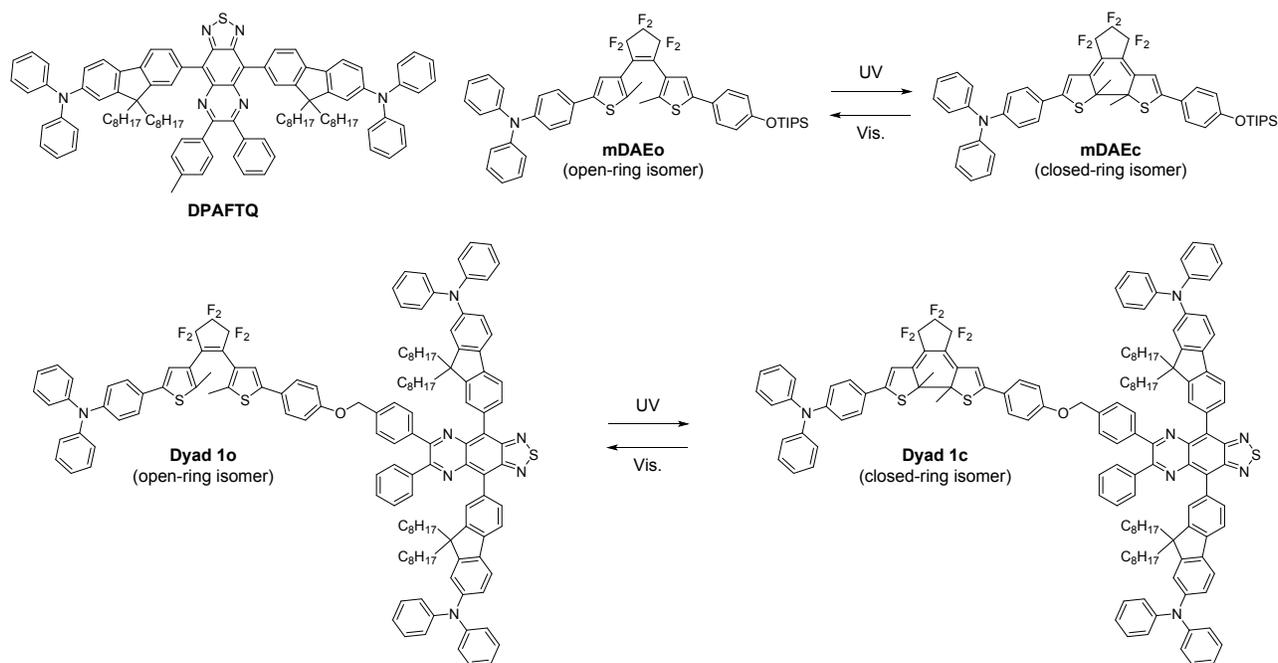


Fig. 1 Molecular structures and photochromism of DPAFTQ, mDAE, and dyad 1.

Before preparing a NIR fluorescence photoswitchable molecule, we studied the fluorescence properties of a model NIR fluorophore (DPAFTQ) in THF solution as well as in a suspension of NPs. NPs of DPAFTQ were prepared by a conventional reprecipitation method (the detailed procedure is described in Electronic Supplementary Information (ESI)). Absorption and fluorescence spectra of DPAFTQ in THF solution and in a suspension of NPs are shown in Fig. S2. DPAFTQ showed a strong absorption band in the spectral region of 500–700 nm with a peak at ~ 590 nm and emitted a NIR fluorescence in the first NIR (NIR-I) region with the fluorescence maximum at 785 nm in THF solution. The fluorescence quantum yield (Φ_f) was estimated to be 0.86% by comparing the fluorescence intensity with that of a reference NIR fluorophore.¹⁸ The absorption spectrum of DPAFTQ in the NP state slightly red-shifted (~ 10 nm) compared with that in THF solution. The fluorescence of DPAFTQ NPs was clearly observed in NIR-I region ($\lambda_{\text{max}} \sim 760$ nm) and the fluorescence intensity was dramatically enhanced (ca. 10-fold, $\Phi_f \sim 10.2\%$) compared to that in the THF solution. This result suggested that the freely rotating phenyl rings around the planar core would inhibit intermolecular π - π interaction, thus favouring an AIE signature and strong fluorescence in the NP state.

Based on the fluorescence property of the DPAFTQ fluorophore, we prepared a NIR fluorescence photoswitchable dyad 1, in which a photochromic DAE is linked to DPAFTQ fluorophore through a non-conjugated spacer. Detailed synthesis and characterizations of all molecules including DPAFTQ fluorophore are described in ESI. As a photoswitching unit, we selected a model DAE derivative (mDAE) having a DPA unit at one thiophene-ring and a phenol group protected with a triisopropylsilyl (TIPS) at the other thiophene-ring. The closed-ring isomer of mDAE has a strong absorption band in the long visible wavelength region and the absorption tail extends over the NIR-I region (~ 750 nm). Fig. 2a exhibits the spectral overlaps between the fluorescence spectrum of DPAFTQ and the absorption spectra of each isomer of mDAE. The fluorescence band of DPAFTQ slightly overlaps with the absorption tail of mDAE only in the closed-ring isomer, while the absorption band of the open-ring isomer of mDAE shows no overlap. From these spectral properties, it is expected that only the closed-ring isomer of mDAE works as fluorescence quencher for dyad 1.

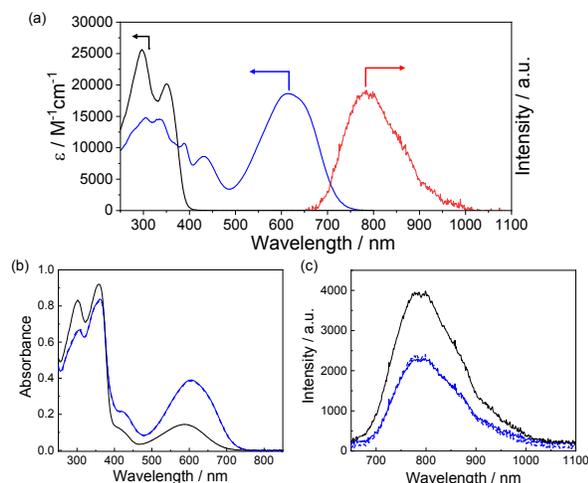


Fig. 2 (a) Spectral overlap between absorption and fluorescence of each component of dyad **1** in THF solution; Absorption spectra of the opening isomer (black-line) and the closed-ring isomer (blue-line) of mDAE, and the fluorescence spectrum of DPAFTQ (red-line). (b) Absorption and (c) fluorescence spectral changes of dyad **1** in THF solution upon alternate irradiation with 365 nm and >650 nm light; the open-ring isomer (solid black-line), the closed-ring isomer (solid blue-line), and PSS under irradiation with 365 nm light (dashed blue-line).

The absorption spectrum of **1o** in a THF solution, displayed in Fig. 2b, exhibits a main band in the visible range with a maximum at 588 nm, which corresponds to the DPAFTQ fluorophore. Upon UV (365 nm) light irradiation, an absorption band in the 500–750 nm range gradually rises (Fig. 2b), corresponding to the appearance of the closed-ring isomer of the DAE unit (**1c**). The absorption spectrum completely returned to the initial one upon irradiation with visible (>650 nm) light. **1o** is weakly emissive in solution with a wide fluorescence spectrum in the NIR-I region ($\lambda_{f,max} \sim 786$ nm, $\Phi_f \sim 0.89\%$) (Fig. 2c). Upon UV light irradiation, the fluorescence intensity gradually decreased and then fully restored under irradiation with visible light. Although the conversion yield at photostationary state (PSS) under irradiation of 365 nm UV light was estimated to be $\sim 99\%$ from the absorption spectra, the fluorescence quenching ratio was limited to 40% at PSS. The pure closed-ring isomer **1c**, isolated by HPLC, was found to be still emissive with the same intensity level of that at the PSS (Fig. 2c). These results suggest that the intramolecular FRET efficiency of **1c** is not sufficient in a molecularly isolated solution condition. Förster radius (R_0) and FRET efficiency (E_{FRET}) of dyad **1** in THF were theoretically calculated to be 16.6 Å and 38%, respectively (the details are described in ESI). Therefore, these theoretical results are well-compatible with the experimental observation described above.

NPs of dyad **1** were prepared by the same reprecipitation methods mentioned above. Spherical NPs with the size of 100 nm were clearly confirmed from SEM images (Fig. S1). The absorption spectrum of the initial suspension of **1o** NPs, as shown in Fig. 3a, is similar to that in THF solution. Upon irradiation with 365 nm light, the absorption band around 600 nm remarkably increased, which was originated from the

photogeneration of **1c** in the NPs. The conversion yield at PSS under irradiation with 365 nm light was estimated to be $\sim 99\%$, which indicates the efficient photoswitching of dyad **1** in the NP state. The fluorescence of **1o** NPs extends over a wide wavelength region from 650 nm to 1000 nm ($\lambda_{f,max} = 765$ nm) with a dramatically enhanced fluorescence intensity ($\Phi_f \sim 9.8\%$), which is similar to DPAFTQ NPs. These results suggested that the DAE unit in dyad **1** did not disturb the fluorescence and the AIE properties of the fluorescence unit even in the NP state.

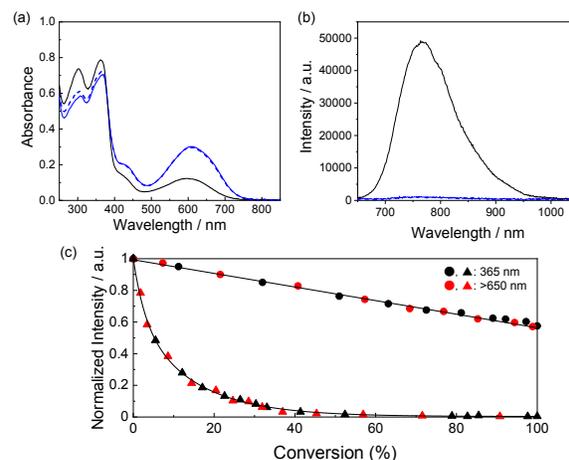


Fig. 3 (a) Absorption and (b) fluorescence spectral changes in a suspension of dyad **1** nanoparticles upon alternate irradiation with UV (365 nm) and visible (>650 nm) light; the open-ring isomer (solid black-line), the closed-ring isomer (solid blue-line), and PSS under irradiation with 365 nm light (dashed blue-line). (c) Plots of fluorescence intensity as a function of the conversion yield from **1o** to **1c** in THF (circle) and in a suspension of nanoparticles (triangle).

Most interestingly, the fluorescence switching behaviour of dyad **1** appears rather different between in solution and in NPs suspension, in terms of both efficiency and rate of switching. Upon irradiation with 365 nm UV light to a suspension of **1o** NPs, the fluorescence signal was dramatically quenched to almost zero, as shown in Fig. 3b. Of course, the fluorescence signal completely recovered to the original level upon irradiation with visible (>650 nm) light and such efficient fluorescence photoswitching can be repeated for many cycles (Fig. S2). R_0 and E_{FRET} in the NPs were also theoretically calculated to be 24.6 Å and 87%, respectively (see ESI). These parameters remarkably increased compared to those in THF solution. These results can be attributed to the enhancement of fluorescence quantum yield in the NP state originated from the AIE property. Fig. 3c shows the normalized fluorescence intensity as a function of the conversion yield in THF solution and in the NP state. In the THF solution, a linear fluorescence photoswitching behaviour was observed and this phenomenon is well-expressed by a simple intramolecular FRET process depending on the population of closed-ring isomers. In contrast, the correlation plot presented in Fig. 3c, corresponding to the NPs suspension of dyad **1**, shows a distinct non-linear fluorescence photoswitching behaviour, where the fluorescence of dyad **1** NPs is mostly (>90%) quenched when

around 30% of **1o** converted to **1c**. This efficient fluorescence quenching cannot be explained by a simple intramolecular FRET mechanism and such a fascinating “amplification of fluorescence quenching” can be ascribed by an efficient intermolecular energy transfer such as energy migration upon dye aggregation in the NPs. With the packing of dyads being quite dense in the NP state, every single **1c** molecule can play the role of energy acceptor for many neighboring fluorescent **1o** molecules.

Efficient NIR fluorescence photoswitching of dyad **1** NPs could be observed even at the single NP level under a fluorescence microscope. Fluorescence images of individual NPs of dyad **1** deposited on a glass cover slide were recorded under continuous excitation with 632 nm light. In the fluorescence microscopic images, the fluorescence signal from individual NPs was clearly observed, as shown in Fig. 5a. The fluorescence intensity sufficiently decreased to almost dark-level upon irradiation with UV (390 nm) light (Fig. 4b). After stopping UV light irradiation, the fluorescence intensity perfectly recovered under continuous excitation with 632 nm light (Fig. 4c). The fluorescence photoswitching could be repeated for many cycles without any remarkable loss of fluorescence signal (Fig. 4d). These results obviously indicated that the NPs of dyad **1** have an excellent NIR fluorescence photoswitching property in a solution as well as in the solid state.

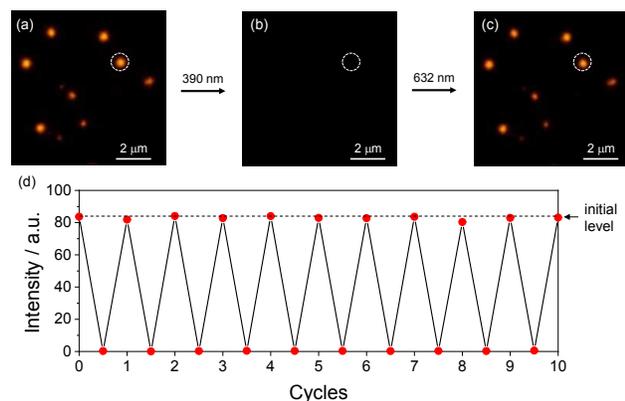


Fig. 4 (a)–(c) Fluorescence microscopy images of dyad **1** NPs directly deposited on glass coverslips under alternate UV-visible irradiation sequences. (d) Fluorescence intensity from one single dyad **1** NP (encircled in the images), recorded for 10 cycles of UV-visible irradiation; Experimental conditions: 390 nm light ($800 \mu\text{W}/\text{cm}^2$, 2 sec) was used to switch OFF the fluorescence of dyad **1** NPs, and a 632 nm illumination ($300 \mu\text{W}/\text{cm}^2$) was employed for both the fluorescence excitation and the induction of photocycloreversion reaction.

In summary, we designed and prepared NIR fluorescence photoswitchable dyad **1** by combining a DAE photoswitch with a NIR AIEgen. The intramolecular FRET efficiency of **1** is not high in a molecularly isolated state due to small spectral overlap between the fluorescence spectrum of NIR fluorophore and the absorption spectrum of photochromic acceptor unit. However, thanks to the amplification of fluorescence quenching efficiency originated from the intermolecular energy transfer in the

densely packed aggregated state, we successfully demonstrated the complete NIR fluorescence ON-OFF photoswitching. Although the fluorescence emission wavelength should be shifted up to the second near-infrared (NIR-II) region eventually, we believe that the molecular design strategy in this work pave the road to the development of novel photoswitchable NIR fluorescent nanomaterials toward future biological applications.

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Conflicts of interest

There are no conflicts to declare.

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