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RESEARCH ARTICLE

Condensed state fluorescence switching of hexaarylbiimidazole-tetraphenylethene conjugate for super-resolution fluorescence nanolocalization

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Abstract This paper reported the synthesis of hexaarylbiimidazole-tetraphenylethene (HABI-TPE) conjugated photochromic fluorophore, which simultaneously exhibited photochromic property, condensed state enhanced emission and reversible fluorescence switching. Upon UV irradiation, a green species with a broad absorption band between 550 and 800 nm (the absorption maximum at 697 nm) was observed, which readily faded to colorless in the darkness. HABI-TPE launched strong fluorescence with the maximum emission wavelength at 520–580 nm under the excitation with 450–500 nm visible light in condensed state, which is in contrast to nonfluorescence in solution. The maximum emission wavelength in condensed state was dependent of excitation wavelength. More interestingly, HABI-TPE exhibited reversible fluorescence switching upon alternating irradiation with blue or near-UV light (wavelength less than 490 nm) and green light (more than 490 nm) in condensed state. Our evaluation demonstrated that HABI-TPE exhibited great photoswitchable fluorescence, which is a promising photoswitchable fluorophore for localization-based super-resolution microscopy, evidencing by resolving nanostructures with sub-100 nm resolution in polymethylmethacrylate films.

Keywords fluorescence switching, super-resolution imaging, nanolocalization, hexaarylbiimidazole (HABI), tetraphenylethene

1 Introduction

Recently, photochromic materials, which reversibly change their optical properties upon excitation of various wavelengths, have stimulated much interest due to their promising applications in optical waveguides and switches, erasable memory, and sunglasses [1]. Hexaarylbiimidazole (HABI), which was first found by Hayashi and Maeda in 1960, has subsequently attracted significant interest because of its unusual physical properties [2–5]. By oxidization of Lophine, Hayashi and Maeda obtained a photochromic species that turned purple under UV irradiation. This photochromic behavior is generally attributed to the photoinduced homolytic cleavage of the C-N bond between the imidazole rings. Further modified HABI derivatives mainly done by Jiro Abe, increased the responding speed of HABI from hour to second, and even millisecond [6–10]. Such intense photocoloration and fast thermal bleaching properties could be indispensible for possible fast light modulator applications. It has been found that the introduction of larger aromatic conjugated substituted groups is an effective method to modulate the photoswitching properties of HABI derivatives with an absorption band in the longer wavelength region. Because the photo-induced dissociation yield of HABI derivatives is usually high (close to the unity), the larger absorption coefficient in the long wavelength region is an important factor to increase the total sensitivity of the molecule [11].

Aggregation induced emission (AIE) was discovered in 2001 [12], and within the last few years a great deal of effort has been undertaken to study this phenomenon. AIE-active materials, such as silole [13,14] and tetraphenylethene [15,16], are chromophores that exhibit fluorescence quenching in the solution, contrary to it solid state, which shows enhanced luminescence. Tetraphenylethene (TPE) was selected as an ideal fluorophore due to the large

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Stokes' shifted fluorescence as well as enhanced fluorescence in the solid state than in solution [15]. Taking advantage of the attractive properties of TPE and since it can be easily modified as a molecular building block it would be desirable to design hexaarylbiimidazole-tetraphenylethene (HABI-TPE) conjugate enabling condensed state enhanced fluorescence behavior and condensed state photoswitching simultaneously.

Photoswitchable fluorophores possess many potential applications in optical memory and bioimaging [17–19]. Particularly in recently-developed super-resolution imaging, photoswitchable fluorophores become indispensible and more desirable [20–26]. In general, photoswitchable fluorophores are used for super-resolution bioimaging agents in cells [24]. Here we explore the potential opportunity of HABI-TPE as a novel photoswitchable emitter in condensed state for nanolocalization-based super-resolution imaging in polymers, nanomaterials and nanostructures. Thus, molecular design based on the photochromism of HABI and condensed state enhanced fluorescence of TPE can lead to the development of a new family of photoswitchable fluorophores with fast switching speeds and remarkable condensed state emission, which could eventually be promising in solid-state photonic materials and devices with unique fluorescence photoswitching character. We reported the synthesis of HABI-TPE conjugated photoswitchable fluorophore that is nonfluorescence in solution while exhibits bright green luminescence in the solid state, under the excitation with visible light. We investigated the efficiency of the photochromism and fluorescence photoswitching of HABI-TPE upon alternating short/long wavelength irradiation. Finally, solid state amorphous films in PMMA have been fabricated on a glass substrate that exhibit "on-off" fluorescence switching followed by algorithm processing allowing for super-resolution fluorescence nanolocalization.

2 Methods

The HABI-TPE was synthesized according to the method shown in Fig. 1. The detailed synthesis and the molecular structures identified with ^{1}H NMR, MS are provided in supporting information.

3 Results and discussion

HABI-TPE shows photochromic reaction changing its color from colorless to green on UV irradiation in both solid and solution. Figure 2 shows the absorption spectra of HABI-TPE in solution and solid film upon irradiation with 302 nm light. HABI-TPE underwent photochromic reactions in solution and films upon irradiation with 302 nm light for 5–60 s (Fig. 2(a)). The absorption band in the visible region ($\lambda_{\max}$ = 695 nm) was assigned to of bi-radical form of HABI-TPE (HABI-TPE-R). HABI-TPE in solution attained 98% of absorbance at the 302 nm photostationary state (PSS, the equilibrium state after 302 nm irradiation) upon irradiation with 302 nm light at 1.0 mW/cm^2 for 20 s (50% at 5 s) while HABI-TPE in films attained about 90% of PSS in 1 min (Figs. 2(b) and S1). Comparably, the photoconversion rate of HABI-TPE film becomes slower than solution. The slower switching speed of HABI-TPE in condensed state than HABI-TPE

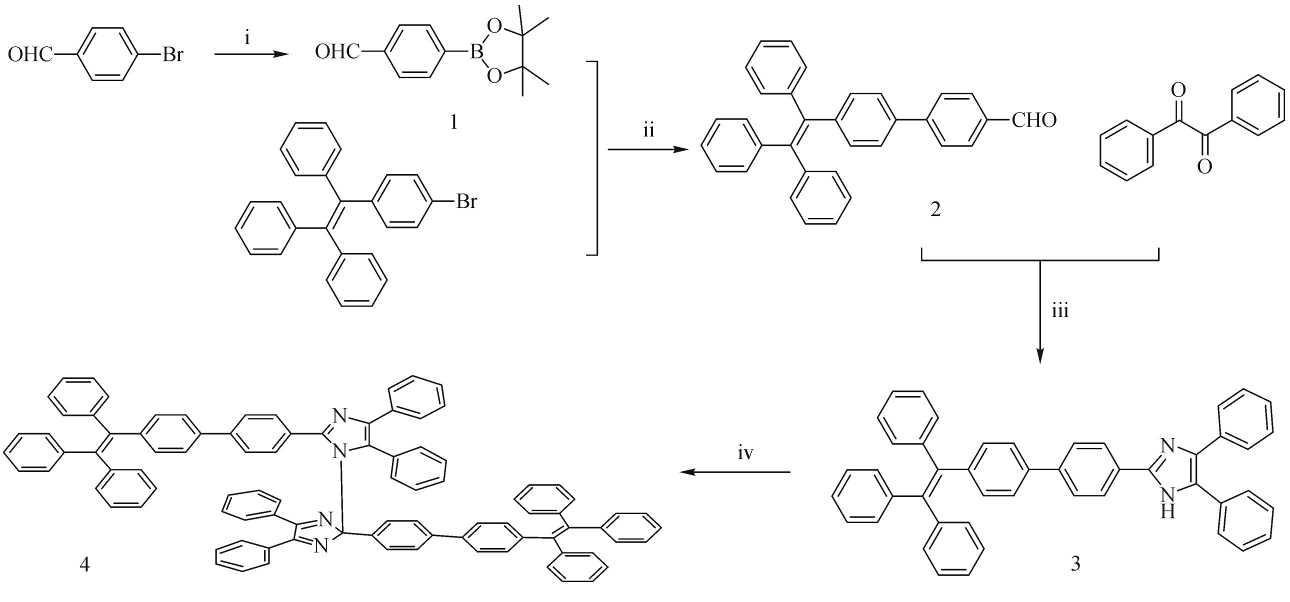

Fig. 1 Synthesis of HABI-TPE conjugate.Reaction conditions. i, bis(pinacolato)diboron, Pd(dppf)Cl$_2$, KOAc, in DMF at 90°C for 8 h; ii, PTC, K_2CO_3, Pd(PPh3)4, toluene/H_2O at 90°C for 1 day; iii, benzyl, ammonium acetate, acetic acid at 90°C for 12 h; iv, $K_3Fe(CN)_6$, KOH, benzene/H_2O at room temperature for 8 h

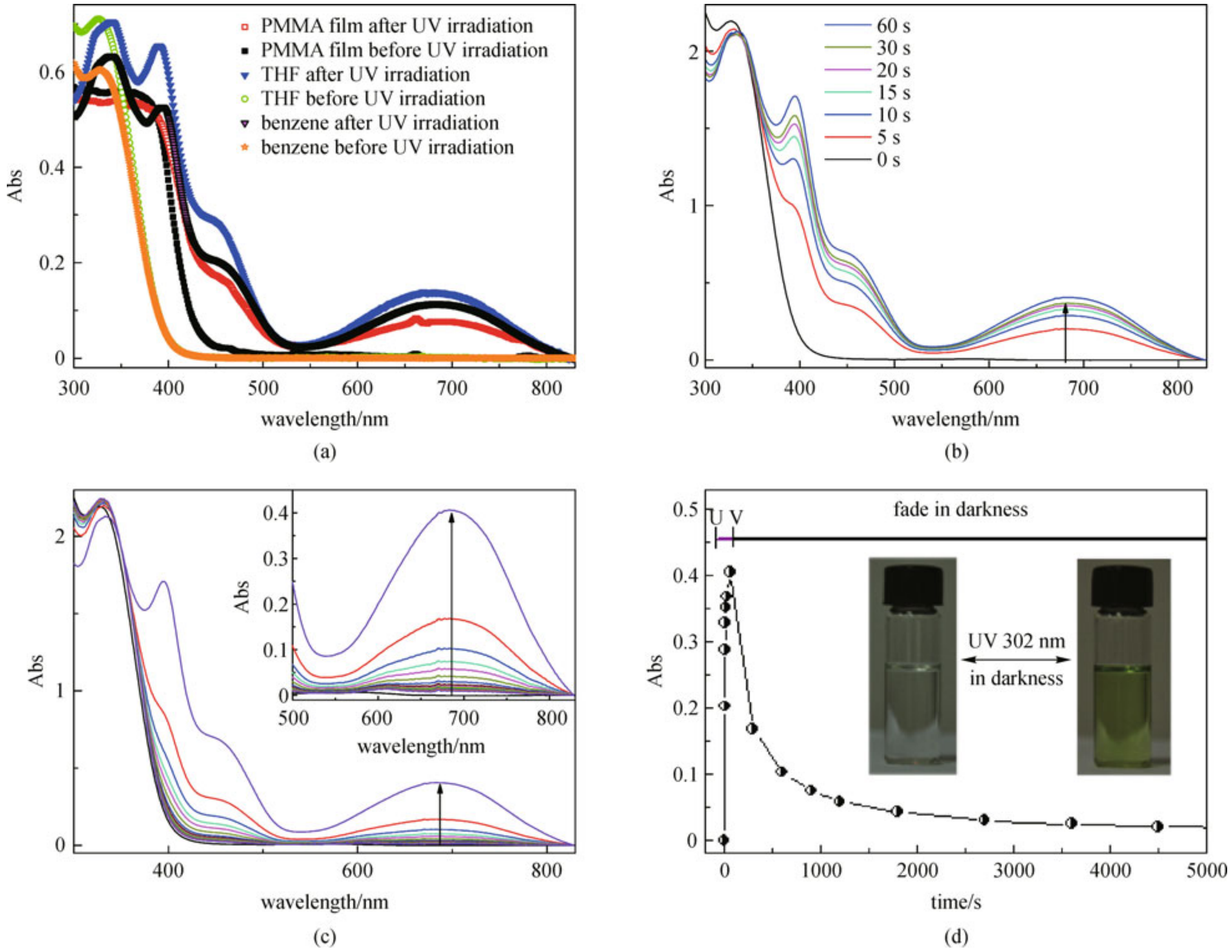

Fig. 2 Absorption properties of HABI-TPE. (a) Absorption spectra of HABI-TPE in benzene, THF and solid state PMMA film before and after UV irradiation; (b) absorbance spectrum change of HABI-TPE in solution (2.5×10^{-4} M$^{1)}$) at different 302 nm light irradiation time; (c) absorption spectrum change of HABI-TPE in solution (2.5×10^{-4} M) in darkness after 302 nm irradiation at different time (from top to bottom: 0 min, 5 min, 10 min, 15 min, 20 min, 30 min, 45 min, 60 min, 75 min, 90 min, 2 h, 3 h, 6 h; (d) absorbance change of HABI-TPE with 302 nm irradiation and heat fade of HABI-TPE in darkness. Inset: pictures of HABI-TPE before and after 302 nm irradiation

solution probably reflects the restricted molecule motion in localized sticky solid-like environment in condensed state, slowing down the photochromic reaction. The reverse fading reaction from HABI-TPE-R to HABI-TPE is much slower than photocoloration (Fig. 2(c)). The absorbance of HABI-TPE went up and down reversibly with alternating UV and visible light irradiation, demonstrating the highly efficient photochromism of HABI-TPE (Figs. 2(d) and S2).

TPEs generally launch enhanced fluorescence in solid state, which is desirable in optoelectronic devices. With conjugation with HABI, emission of HABI-TPE in condensed state can be tuned with light stimuli. The fluorescence spectra of HABI-TPE in solution and condensed state were measured (Fig. 3(a)). It was discovered that HABI-TPE possesses various large Stokes' shift (λ_{abs} = 340 nm, λ_{em} = 490–580 nm). The fluorescence of HABI-TPE in solution was poor and negligible while strongly enhanced fluorescence in the solid state was discovered, almost 10 times stronger than in solution. It was found that HABI-TPE emits fluorescence with emission wavelength arranging from 490 to 580 nm, depending on the excitation wavelength (Figs. 3(b) and 3(c)). The emission wavelength is 490 nm when HABI-TPE is excited at 365 nm. However, the emission wavelength shifts to 580 nm upon 500 nm excitation.

The emission spectra of HABI-TPE show different evolution behavior upon repeated excitation of different wavelength. The emission intensity decreases by one third after 450 nm excitation for 9 min (Fig. 3(d)). It was found that HABI-TPE emission intensity decreases at continuous

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1) 1 M = 1 mol/L

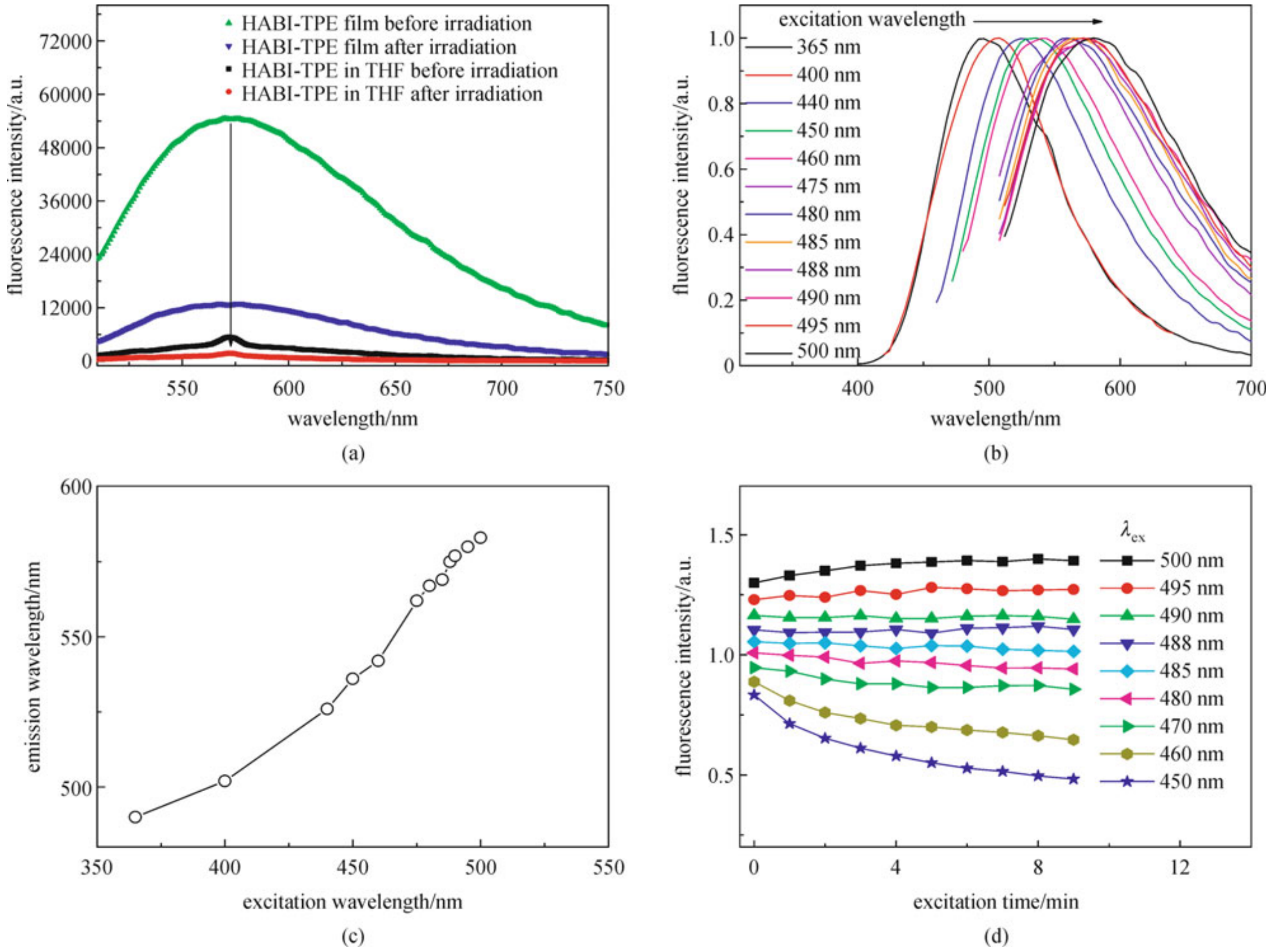

Fig. 3 Fluorescence properties of HABI-TPE. (a) Fluorescence spectra of HABI-TPE in THF and solid state PMMA film under excitation with 490 nm before and after UV irradiation; (b) excitation wavelength-dependent emission spectra of HABI-TPE in condensed state films; (c) change of emission wavelength versus excitation wavelength; (d) identification of excitation wavelength for photoswitching by fluorescence spectra. The change of fluorescent intensity at 550 nm of HABI-TPE in condensed state with excitation time upon excitation of different wavelength was determined

sub-490 nm excitation, indicating that more HABI-TPE transformed to HABI-TPE-R radicals. On the other hand, longer-wavelength light above 490 nm made more HABI-TPE-R radicals back to HABI-TPEs. Upon excitation with wavelength lower than 490 nm, the emission spectra shows exponential decay tendency while excitation above 490 nm causes slightly-increased emission. Therefore, optimal critical excitation wavelength was screened by monitoring the change of fluorescence spectra and emission intensity under continuous excitation with different wavelengths. It is found that the critical excitation wavelength is 490 nm (Fig. 3(d)). For both HABI-TPE and HABI-TPE-R radicals, here, the fluorescence excitation was carried out at 490 nm, at which the photoinduced interconversion rates were found to achieve the equilibrium.

The dynamic change of fluorescent spectra of HABI under UV irradiation and the reverse fade process are shown in Fig. 4. The maximum emission intensity decreases by one third after 450 nm excitation for 9 min (Fig. 4(a)). The emission intensity of HABI-TPE even decreases to half upon 400 nm excitation for 5 min (Fig. S3). Comparably, the emission intensity of HABI-TPE basically does not change upon continuous 490 nm excitation for 9 min (Fig. 4(b)). Under the current condition, about 5 s of 302 nm irradiation at 1.0 mW/cm^2 induced 50% decrease of emission intensity in the condensed state (Fig. 4(c)). The reverse fading process was accomplished in darkness, though consuming longer time (Fig. 4(d)). The fluorescence photoswitching of HABI-TPE is reversible, which could be repeated reversibly (Fig. S4).

The exploration of HABI-TPE absorption and emission properties demonstrates that HABI-TPE in condensed state launches a novel potential photoswitchable fluorophore for imaging applications. The basic photochromic and fluorescence switching process is described in Fig. 5. When

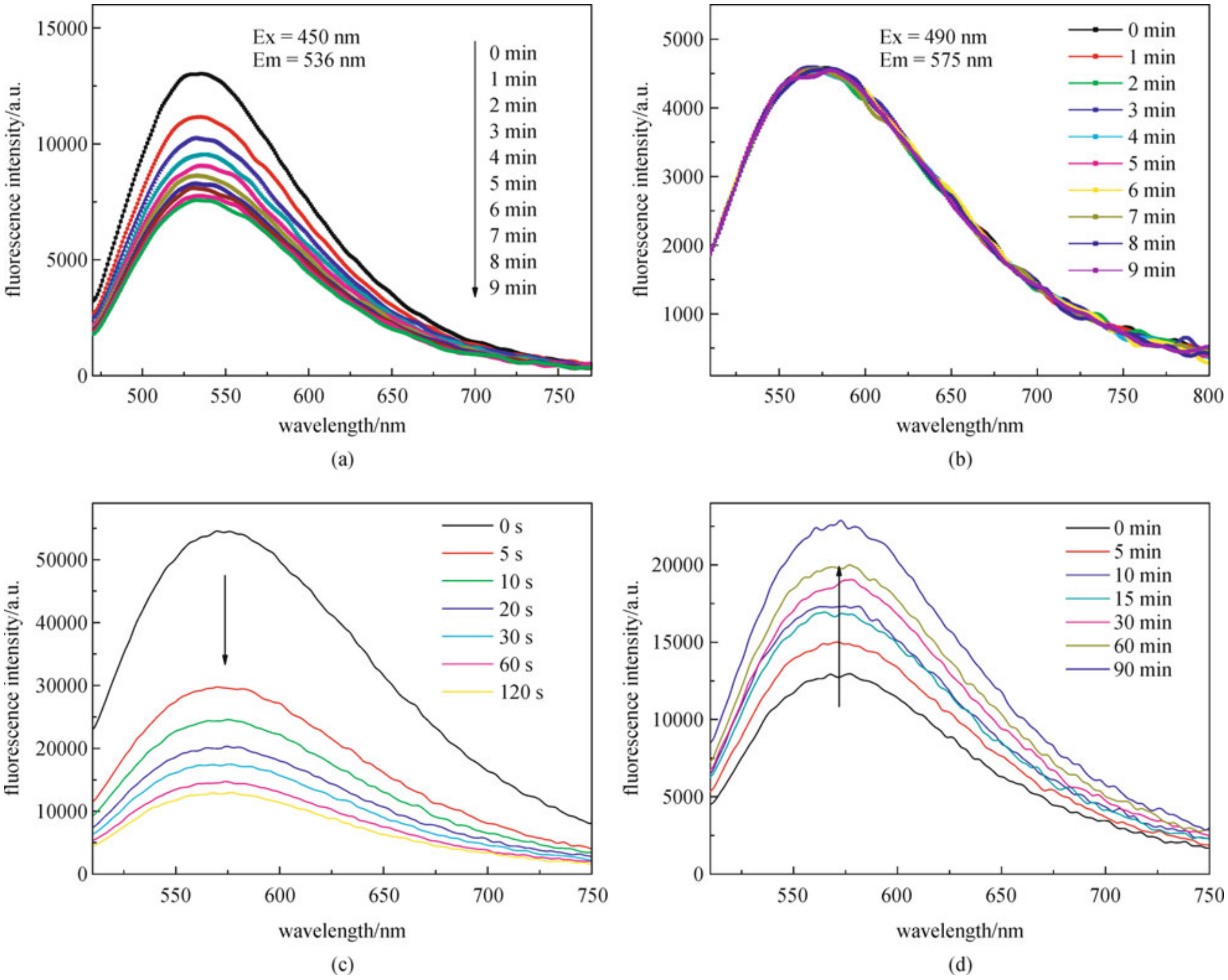

Fig. 4 Fluorescence switching of HABI-TPE. (a) Fluorescence spectra change of HABI-TPE in solid state at excitation of 450 nm with scanning time; (b) fluorescence spectra changes of HABI-TPE in solid state at excitation of 490 nm with scanning time; (c) fluorescence spectra changes of HABI-TPE in solid state upon 302 nm irradiation for 5, 10, 20, 30, 60 and 120 s, excitation wavelength: 490 nm; (d) fluorescence spectra changes of HABI-TPE in solid state at excitation of 490 nm with fading time for 5, 10, 15, 30, 60, and 90 min in darkness after 302 nm irradiation for 120 s

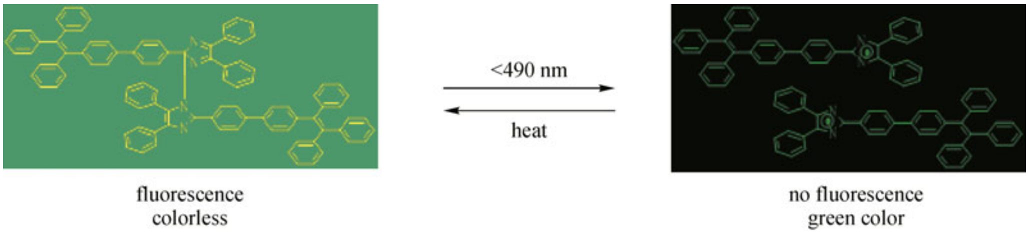

Fig. 5 Photoswitching of HABI-TPE with color and fluorescence changes

HABI-TPE in condensed state is irradiated under blue or UV light (less than 490 nm), the colorless film would become green and the fluorescence of HABI-TPE film decreases. The photochromic and fluorescence switching phenomena of HABI-TPE is much different from previous reports on spiropyran nanoparticles [17]. In the latter case, UV irradiation induces and increases the intrinsic fluorescence of molecular switches in color form. For HABI-TPE fluorescence molecular switch, evidently the short wavelength (blue or near-UV less than 490 nm) causes the photochromism while accompanied with the decrease of fluorescence of HABI-TPE-R in color form.

Super-resolution imaging based on the nanoscale localization of photoswitchable fluorophores allows the reconstruction of images with a higher resolution than the diffraction limit and thus enables the observation of many sub-micron structures [21–24]. The photophysical and photochemical properties of fluorophores, particularly the photoswitching capability of fluorophores, are critical for localization-based super-resolution fluorescence imaging. Here, we demonstrate the capabilities of HABI-TPE in localization-based super-resolution microscopy for sub-wavelength nanostructure. Our fluorescence images are based on high-precision localization of individual fluorophores that are switched on and off at different wavelength excitation. Since fluorescence switching is indispensible and desirable for super-resolution fluorescence imaging, we prepared the HABI-TPE film and quantitatively examined the imaging resolution. We identified the nanolocalization of vicinal SP-PCL nanoparticles and their distribution in solid film with a home-built localization-based super-resolution microscopy (Fig. 6). In comparison to the conventional fluorescent image (Fig. 6(a)), the super-resolution image (Fig. 6(b)) is much clearer and more distinguishable. It seems that the magnified conventional fluorescent imaging is not competent to discriminate the vicinal HABI-TPE emitters (Figs. 6(c) and 6(f)). However, super-resolution imaging mode provided much distinct images with sub-100 nm resolution (Figs. 6(d) and 6(g)). The lowest distance between HABI-TPE emitters, which can be separated from the super-resolution image was determined (Figs. 6(e) and 6(h)). The distances of two HABI-TPE pairs are measured with 110 and 90 nm apart (Figs. 6(i) and 6(j)). The narrowest full width in half maximum (FWHM) among the as-measured emitters was measured to about 60 nm. The

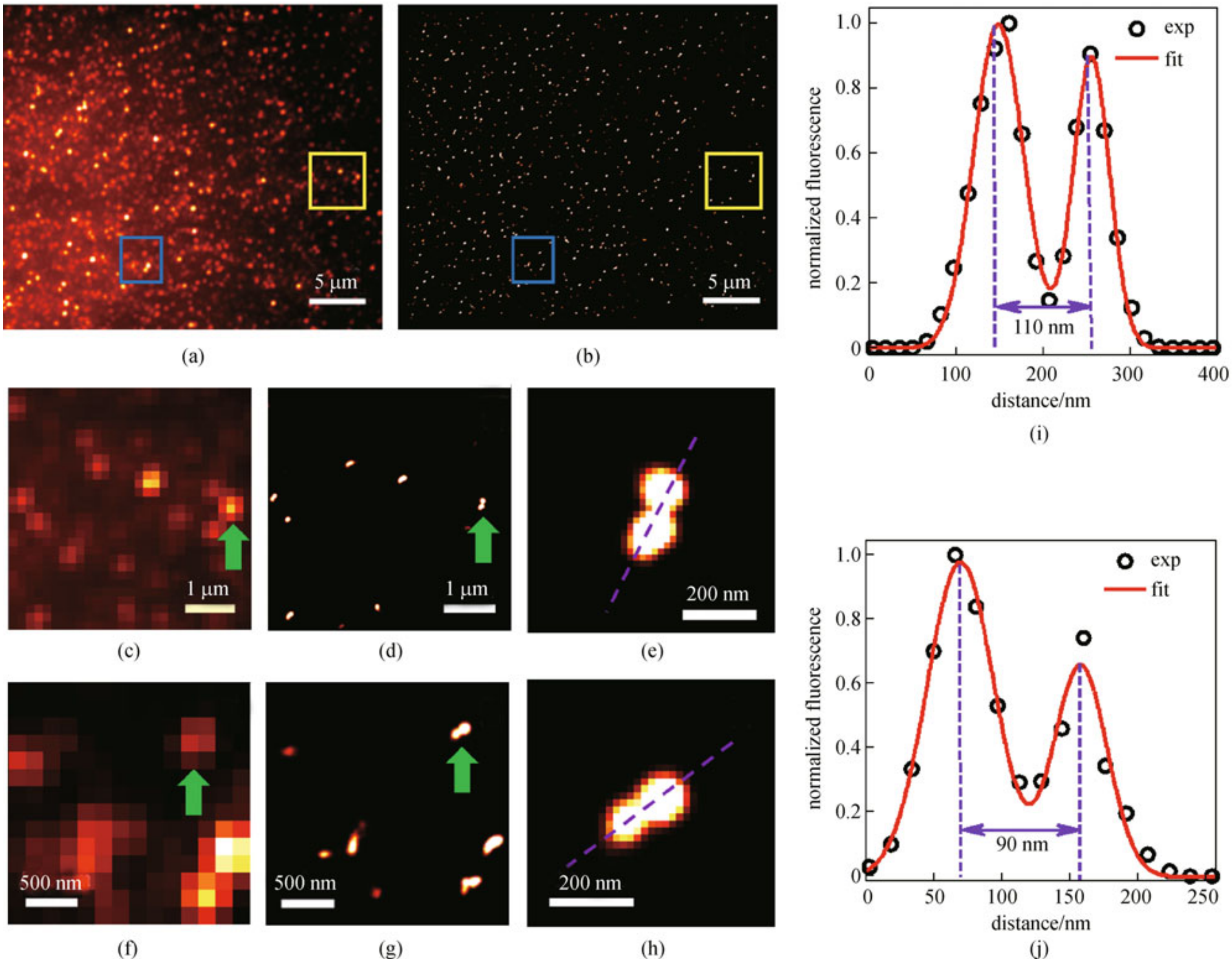

Fig. 6 Super-resolution imaging of spin-coated HABI-TPE-loaded PMMA film. (a) Conventional fluorescent image displaying the distribution of HABI-TPE fluorophores in solid film; (b) super-resolution fluorescent image for (a); (c) and (f) expanded view of the marked regions in (a); (d) and (g) expanded super-resolution fluorescent images of the marked regions in (b), corresponding to (c) and (f); (e) and (h) expanded super-resolution fluorescent images of two pairs of vicinal HABI-TPE emitters in (d) and (g), respectively; (i) and (j) fluorescence cross-sectional profiles of two pairs of vicinal HABI-TPE emitters along the dashed lines in (e) and (h), indicating the spatial resolution of 110 and 90 nm, respectively

results indicate that super-resolution imaging can surround the optical limitation of diffraction enabling two closed nanoparticles in sub-100 nm region to become well differentiated in the super-resolution images.

4 Conclusions

In conclusion, we have synthesized HABI-TPE conjugate capable of undergoing reversible fluorescence switching in condensed state and demonstrated that reversible photochromism and fluorescence switching can be elicited with alternating blue or near-UV light (< 490 nm) and green light (> 490 nm). HABI-TPE conjugate in condensed state has been localized in super-resolution imaging. The super-resolution fluorescence nanolocalization indicates that vicinal HABI-TPE emitters are discernible with sub-100 nm resolution higher than conventional fluorescent imaging. As novel fluorescence-switchable materials, HABI-TPE conjugate has potential utility in localization-based super-resolution imaging as an alternative nanoimaging agent.

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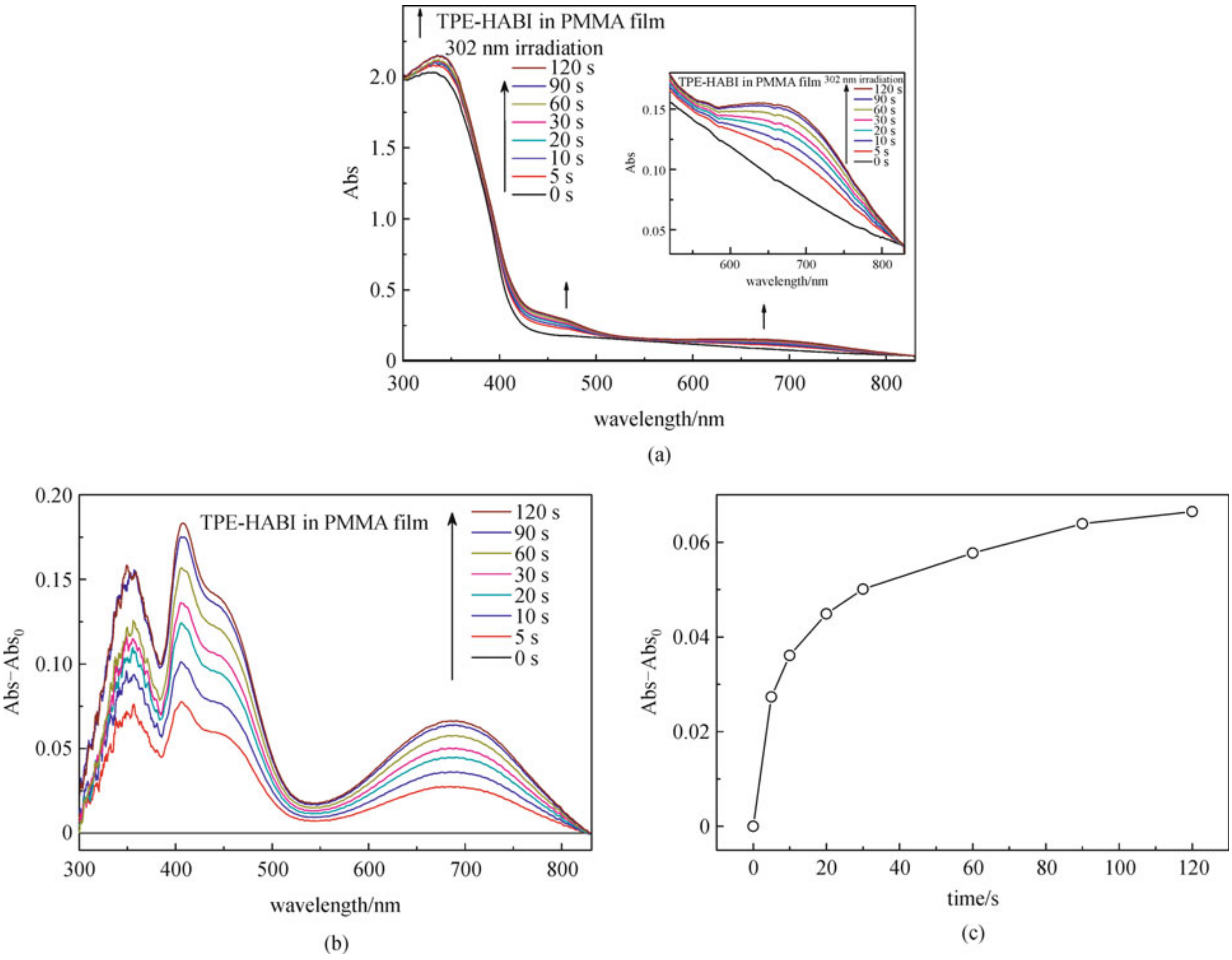
Appendix

1 UV-VIS absorption spectra



(a)

(b)

(c)

Fig. S1 Absorbance spectrum change of HABI-TPE in PMMA film at different 302 nm light irradiation time. (a) Original spectra; (b) Abs(t)−Abs(0); (c) time-dependent Abs at maximum absorption wavelength (691 nm)

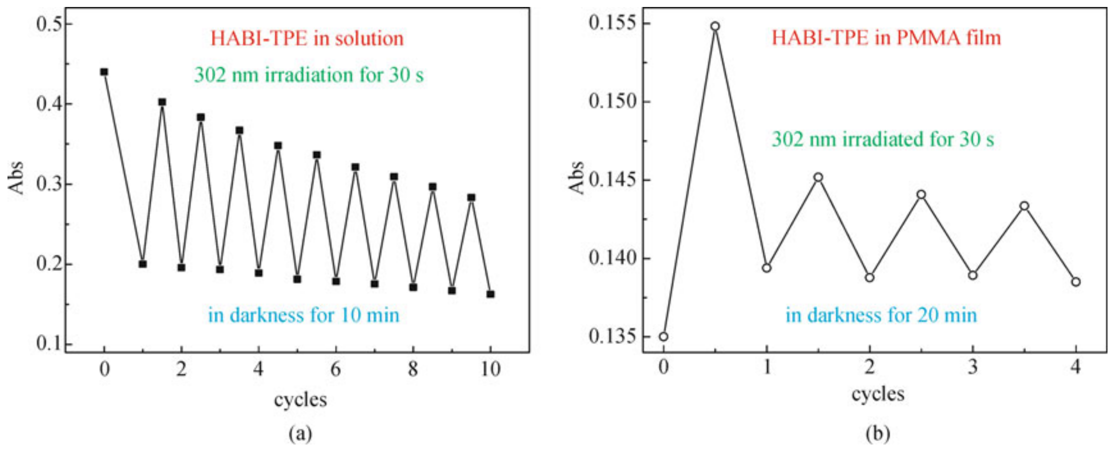


Fig. S2 Photoswitching cycles of HABI-TPE in solutions (a) and PMMA film (b), upon UV 302 nm irradiation, then put in darkness as 1 cycle. In PMMA film, the cycle may take much long time than in solution

2 Fluorescent spectra of HABI-TPE

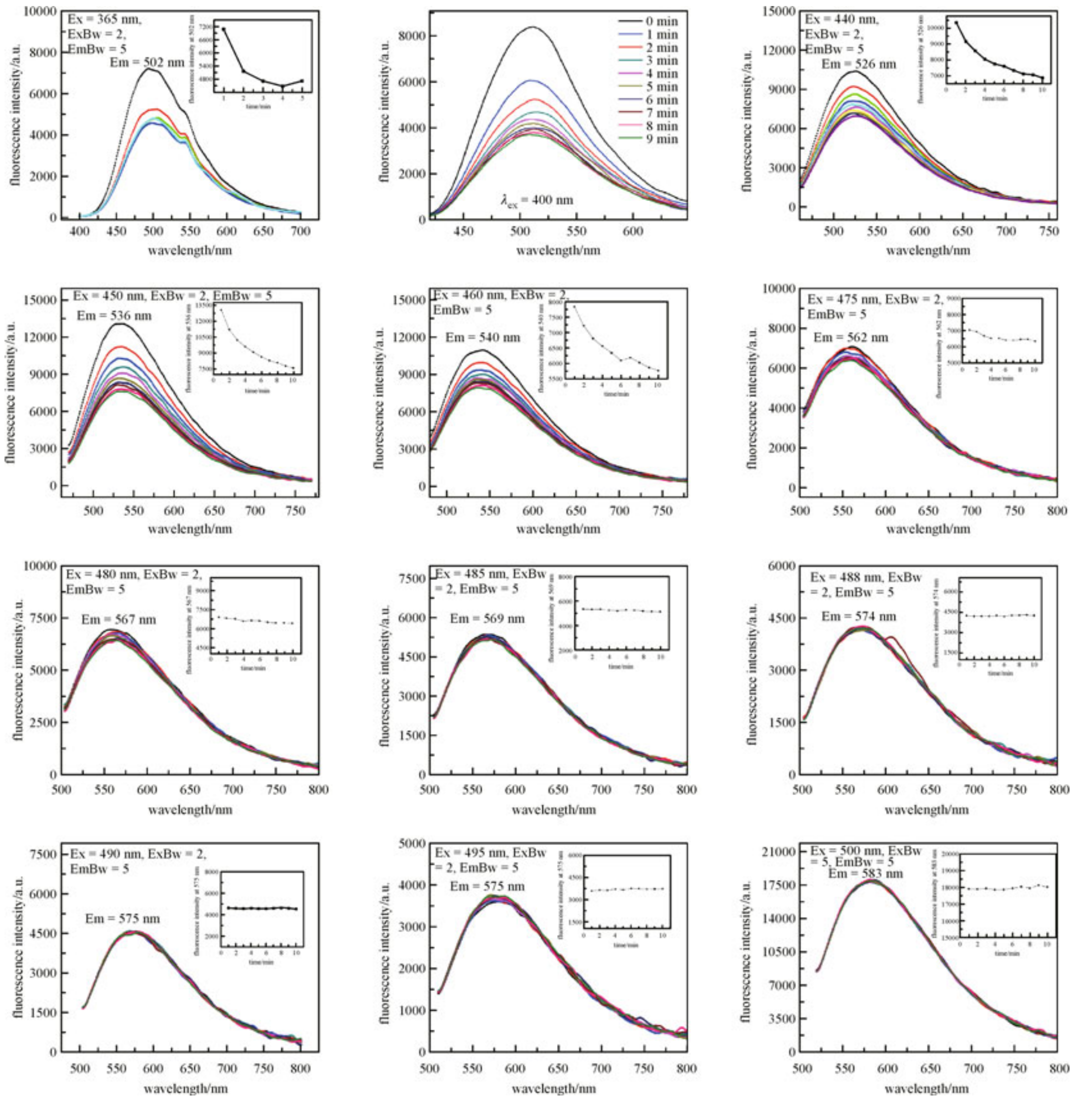


Fig. S3 Fluorescence spectra change of HABI-TPE in solid state at excitation of 365–500 nm with scanning time

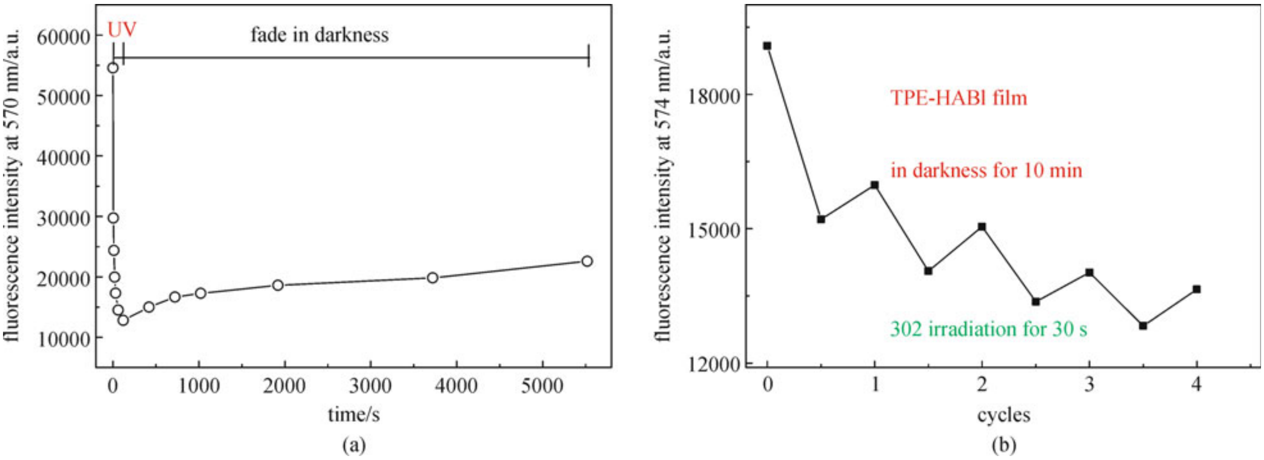


Fig. S4 (a) Fluorescence intensity change of HABI-TPE at 570 nm emission wavelength in condensed state with 302 nm irradiation and heat fade of HABI-TPE in darkness; (b) fluorescent photoswitching cycles of HABI-TPE in condensed state, upon UV 302 nm irradiation for 30 s, then put in darkness for 10 min as 1 cycle. In condensed state, the fluorescence intensity decay of HABI-TPE along with the cycling times is attributed to the restoration time in darkness for 10 min not enough to recover fluorescence to original strength level

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