# Electronic Supplementary Information (ESI)

## Single-Wavelength-Controlled in situ Dynamic Super-Resolution Fluorescent

## Imaging for Block Copolymer Nanostructures via Blue-Light-Switchable FRAP

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### 1. General

All commercially available starting materials, reagents and solvents were used as supplied, unless otherwise stated, and were purchased from Energy, alfa aesar, J&K and Zhengzhou Huawen Chemical Co. Ltd. All reactions were carried out under a dry nitrogen atmosphere and the temperatures were measured externally. Reported yields are isolated yields. Purification of all final products was accomplished by gravity column chromatography, using silica gel. For qualitative purity tests of all intermediates and final products, a single spot (visualised using UV-light at 254 nm and 365 nm) was obtained.

The NMR spectra were recorded on a 600 M Bruker AV600 using CDCl<sub>3</sub> or CD<sub>2</sub>Cl<sub>2</sub> as the solvents. Elemental analysis (Elementar Vario Micro-cube) was used for quantitative purity analysis of all final products. The mass spectra were recorded at an ESI Ion-Trap mass spectrometer (Agilent 1100 series SL LC/MSD (Trap) CA, USA).MALDI TOF mass spectra were recorded with a MALDI-TOF(Bruker ultrafleXtreme). UV absorbance spectra were recorded with an UV-3600UV-VIS-NIR spectrophotometer (Shimadzu) at a wavelength scan rate of 4nm/s. The absorbance changes from photochromism of HABI into 2, 4, 5-triphenylimidazolyl radicals (TPIR) were obtained after subtraction of the absorbance spectra between final TPIR and original HABI. Fluorescence spectra of HABIs were recorded with a FLS 920 spectrophotometer (Edinburg) at a wavelength scan rate of 2 nm/s. The emission intensity changes from photochromism of HABIs into TPIRs were obtained upon continuous irradiation (excitation). The 302 nm UV irradiation for the photochromic reaction was performed by using an ultraviolet transmission platform (with 4 ultraviolet tubes, each 8W and 220 V, the measured power is about 0.85 mW/cm<sup>2</sup>).

### 2. Synthesis

Synthetic procedure of 1a

The synthetic procedure of **1a** could be found in our former work.<sup>[1]</sup>

Synthetic procedure of 1b



**1a** (0.39 g, 1 mmol), diethanolamine (1.05 g, 10 mmol) and potassium carbonate (0.41 g, 3 mmol) were dispersed in 2-methoxyethanol (30 ml) in a 100 ml flask. The mixture was heated to reflux for 5 h under N<sub>2</sub> atmosphere. The reaction was detected by TLC silica gel plate (eluent: DCM-MeOH = 4.5 - 0.5, v - v) and a strong green fluorescent spot was observed. After the starting material is fully converted, the solvent 2-methoxyethanol was evaporated under reduced pressure. The residue was further purified by column chromatography on silica gel (200 - 300) using DCM and Methanol as eluent. The polarity of the eluent was increased from pure DCM, DCM-Methanol (500 - 5, v - v), DCM- Methanol (500 - 10, v - v) ...... And 0.37 g sticky brown liquid was obtained (yield 90%).

<sup>1</sup>**H** NMR  $\delta_{\rm H}$  (CDCl<sub>3</sub>, 600 MHz): 8.86 (d, 1H, J = 12.6 Hz), 8.45 (d, 1H, J = 10.8 Hz), 8.39 (d, 1H, J = 12 Hz), 7.62 (t, 1H, J = 11.4 Hz), 7.34 (d, 1H, J = 12 Hz), 4.07 (m, 2H, imide position, N-CH<sub>2</sub>-), 3.87 (t, 4H, J = 7.8 Hz, N-CH<sub>2</sub>-CH<sub>2</sub>-OH), 3.61 (d, 4H, J = 7.8 Hz, N-CH<sub>2</sub>-CH<sub>2</sub>-OH), 3.17 (2H, br, -OH), 1.89 (m, 1H), 1.60-1.10 (m, 8H), 0.96 (t, 3H, J = 12 Hz) 0.91 (t, 3H, J = 7.8 Hz); LC-MS (APCI): cald 412.24, found [M+H<sup>+</sup>] 413.31;

Synthesis procedure of 1c



Solutions of sodium hydroxide (2.0 g, 50 mmol) in water (20 ml) and **1b** (0.41 g, 1 mmol) in THF (10 ml) were combined and cooled to 0 °C in an ice bath. To the resulting mixture solution, was added a solution of *p*-toluenesulfonyl chloride (0.48 g, 2.5 mmol) in THF (10 ml) over a period of 20 min, keeping the reaction temperature below 5 °C. The reaction mixture was stirred at 0 °C for 1 day. Then the sodium hydroxide solution was separated and to the organic phase DCM (40 ml) was added. This organic solution was washed with water (3 × 30 ml) and then dried under magnesium sulfate. The solvent was then evaporated under reduced pressure and the residue was further purified by column chromatography using DCM and Methanol as eluent on silica gel (200 - 300). The polarity of the eluent is increased from pure DCM, 500ml DCM – 5 ml Methanol, 500 ml DCM -10 ml Methanol ...... And 0.45 g yellow solid with strong green fluorescence was obtained (yield 63%). <sup>1</sup>**H NMR \delta\_{\text{H}} (CDCl<sub>3</sub>, <b>600MHz):** 8.58 (d, 1H, J = 10.8 Hz), 8.35 (d, 1H, J = 7.2 Hz), 8.33 (d, 1H, J = 6.6 Hz), 7.69 (t, 1H, J = 11.4 Hz), 7.62 (d, 4H, J = 12 Hz), 7.20 (d, 4H, J = 12 Hz), 7.15 (d, 1H, J = 12 Hz), 4.15 (m, 2H), 4.05 (t, 4H, J = 7.8 Hz), 3.70 (d, 4H, J = 7.8 Hz), 2.38 (s, 6H), 1.94 (m, 1H), 1.60-1.10 (m, 8H), 0.96 (t, 3H, J = 10.8 Hz), 0.91 (t, 3H, J = 10.2 Hz); LC-MS (APCI): cald 720.25, found [M+H<sup>+</sup>] 721.18;

Synthesis procedure of 1d



Into a 50 ml two-neck flask, **1c** (0.72 g, 1 mmol), *p*-hydroxybenzaldehyde (0.49 g, 4 mmol) and potassium carbonate (0.83 g, 6 mmol) were dispersed in 30 ml N,N-dimethylformamide (DMF). The mixture was heated to 100 °C and stirred for 1 day under N<sub>2</sub> atmosphere. After the reaction was finished, the mixture was poured into 50 ml dichloromethane (DCM) and washed with water (3 × 50 ml). The organic phase was then dried under magnesium sulfate, filtered and the solvent was evaporated under reduced pressure. The residue was further purified by column chromatography using DCM and Methanol as eluent on silica gel (200 - 300). Finally, 0.52 g yellow solid was obtained, yield 81%. **<sup>1</sup>H NMR**  $\delta_{\rm H}$  (CDCl<sub>3</sub>, 600MHz): 9.86 (s, 2H), 8.61 (d, 1H, J = 4.8 Hz), 8.59 (d, 1H, J = 1.8 Hz), 8.55 (d, 1H, J = 12 Hz), 7.79 (d, 4H, J = 13.2 Hz), 7.72 (t, 1H, J = 12 Hz), 7.52 (d, 1H, J = 12 Hz), 6.90 (d, 4H, J = 12.6 Hz), 4.23 (t, 4H, J = 7.8 Hz), 4.15 (m, 1H), 3.98 (t, 4H, J = 7.8 Hz), 1.96 (m, 1H), 1.60-1.10 (m, 8H), 0.96 (t, 3H, J = 10.8 Hz) 0.91 (t, 3H, J = 10.2 Hz); LC-MS (APCI): cald 620.29, found [M+H<sup>+</sup>] 621.40;

#### Synthetic procedure of 1e



1d (0.62 g, 1 mmol), benzil (0.84 g, 4 mmol) and ammonium acetate (1.54 g, 20 mmol) were dispersed in acetic acid (40 ml) into a 100 ml one-neck flask. The reaction mixture was stirred and heated to 90°C for 2 days. After the reaction was finished, the solution was poured into water (250 ml) and neutralized to pH 7 with aqueous ammonia. Then the yellow-green solid was filtered and dried under vacuum at 80°C for 12 hours. The residue was further purified by column chromatography on silica gel (200 - 300) using DCM-Methanol as eluent. 0.95 g yellow solid was obtained, yield 95%. <sup>1</sup>H NMR  $\delta_{\rm H}$  (<sup>d</sup>-DMSO, 600 MHz): 12.48 (s, 2H), 8.70 (d, 1H, J = 12.6 Hz), 8.49 (d, 1H, J = 10.8Hz), 8.43 (d, 1H, J = 12Hz), 7.95 (d, 4H, J = 12.6Hz), 7.83 (t, 1H, J = 12 Hz), 7.64 (d, 1H, J = 12.6 Hz), 7.53 (m, 4H), 7.48 (m, 4H), 7.44 (m, 4H), 7.37 (m, 2H), 7.32-7.10 (m, 6H), 6.95 (d, 4H, J = 13.2 Hz), 4.30 (t, 4H, J = 12.6 Hz, -N-CH<sub>2</sub>-CH<sub>2</sub>-O-TPI), 4.00 (m, 6H, the peak of imide N-CH<sub>2</sub> (2H) overlap with -N-CH<sub>2</sub>-CH<sub>2</sub>-O-TPI (4H)), 1.86 (m, 1H, N-CH<sub>2</sub>-CH(CH<sub>2</sub>)<sub>2</sub>), 1.45 - 1.10 (m, 8H), 0.88 (t, 3H,J = 10.8 Hz), 0.84 (t, 3H, J = 10.2 Hz); MALDI-TOF: cald 1000.4676 (100.0%), 1001.4710 (71.4%), 1002.4743 (25.1%), 1003.4777 (5.8%); Found 1002.4700, 1003.4801; Elemental Analysis: Found: C, 79.21; H, 6.05; N, 8.34; O, 6.52; C<sub>66</sub>H<sub>60</sub>N<sub>6</sub>O<sub>4</sub>, requires C, 79.17; H, 6.04; N, 8.39; O, 6.39;

#### Synthetic procedure of NI-N-HABI (1)



K<sub>3</sub>Fe(CN)<sub>6</sub> (0.66 g, 2 mmol) and KOH (0.28 g, 5 mmol) were dissolved in water (20 ml). This solution was added into 1e (0.10 g, 0.1 mmol), which was dissolved in DCM (40 ml). The mixture was vigorously stirred and covered from light by aluminum foil. The reaction was detected by TLC plate until the starting materials were totally converted to the products. After the reaction was finished, the organic layer was collected and washed with water (3  $\times$  50 ml). The organic phase was then dried under magnesium sulfate, filtered and the solvent was evaporated under reduced pressure. The residue was further purified by column chromatography using DCM and Methanol as eluent (4.9 ml - 0.1 ml, v - v) on basic Al<sub>2</sub>O<sub>3</sub> (200 - 300). 60 mg yellow green solid was obtained, yield 60%. <sup>1</sup>H NMR  $\delta_{\rm H}$  (<sup>d</sup>-**DMSO, 600 MHz):** 8.84 (d, 1H, J = 12.6 Hz), 8.69 (d, 1H, J = 10.8Hz), 8.55 (d, 1H, J = 12.6 Hz), 8.43 (d, 1H, J = 12Hz), 7.95 (d, 4H, J = 12.6Hz), 7.83 (t, 1H, J = 12 Hz), 7.64 (d, 1H, J = 12.6 Hz), 7.53 (m, 4H), 7.48 (m, 4H), 7.44 (m, 4H), 7.37 (m, 2H), 7.32-7.10 (m, 6H), 6.95 (d, 4H, J = 13.2 Hz), 4.30 (t, 4H, J = 12.6 Hz, -N-CH<sub>2</sub>-CH<sub>2</sub>-O-TPI), 4.00 (m, 6H, the peaks of imide N-CH<sub>2</sub> (2H) overlap with -N-**CH<sub>2</sub>-CH<sub>2</sub>-O-TPI** (4H)), 1.86 (m, 1H, imide N-CH<sub>2</sub>-**CH**(CH<sub>2</sub>)<sub>2</sub>), 1.45-1.10 (m, 8H), 0.88 (t, 3H, J = 10.8 Hz), 0.84 (t, 3H, J = 10.2 Hz); MALDI-TOF: cald 998.4520 (100.0%), 999.4553 (71.4%), 1000.4587 (25.1%), 1001.4620 (5.8%); found 999.3581, 1000.3116, 1001.3272; Elemental Analysis: Found C, 79.36; H, 5.84; N, 8.39; O, 6.43; C<sub>66</sub>H<sub>58</sub>N<sub>6</sub>O<sub>4</sub>, requires C, 79.33; H, 5.85; N, 8.41; O, 6.40.

### 3. Super-resolution fluorescent imaging

The optical setup for imaging was based on a home-built microscope setup consisted of an Olympus IX71 inverted optical microscope, a 100×/NA1.49 oil immersion TIRF objective (UAPON 100XOTIRF, Olympus), three solid-state lasers (405, 473, and 561 nm, all from CNILaser, China), and an AndoriXon 897 EMCCD camera. During super-resolution imaging, two electronic shutters (UNIBLITZ VS14, Vincent Associates) were used to control the duration of laser irradiance, and a dichroic mirror (Di01-R488/561, Semrock) and a long pass filter (BLP01-561R-25, Semrock) were used to separate the collected fluorescence from scattering laser and impurity fluorescence. The Image J plugins written in Java was used to analyze the images. An ultrafast and high-precision image unit (GPU)), was established. <sup>[3, 4]</sup> Briefly, Gaussian fitting with the following equation was used to localize the single molecules in the conventional fluorescence microscopy images.

$$I = I_{sig} \exp\left(-\frac{(i - x_0)^2 + (j - y_0)^2}{2s^2}\right) + I_{bkg}$$

Here  $(x_0, y_0)$  was the position of fluorescent molecule, s was the width of Gaussian kernel, and Isig and Ibkg denoted the peak value of signal and the intensity of background photon (including background fluorescence, remnant laser scattering, and average readout noise), respectively. The localization precision for individual fluorescent molecule imaged by EMCCD camera could be calculated by

$$\langle \Delta x^2 \rangle = \frac{2s^2 + a^2/12}{\phi N} + \frac{16\pi s^4 (\phi I_{\rm b})}{a^2 (\phi N)^2}$$

where s is the width of Gaussian kernel, a is the pixel size,  $I_b$  is the background photon ( $I_b = I_{bkg} - Nr^2$ ), Nr is the readout noise,  $\Phi$  is the quantum efficiency, and N is the number of the photons collected.

# 4. Additional characteristic data

	$\lambda_{abs, max} (nm)$	$\lambda_{em}(nm)$	FLQY (%)
Benzene	395	485	17.8
CHCl <sub>3</sub>	399	496	22.3
DCM	398	498	21.3
1, 4-dioxane	393	493	20.1
DMF	406	525	10.1
DMSO	411	534	4.2
Ethyl acetate	396	504	24.1
Hexane	385	468	7.1
THF	398	503	21.5
toluene	393	482	15.7

Table S1  $\lambda_{abs,\,max}, \lambda_{em,\,max}$  and FLQY of NI-N-HABI in various solvents.



Figure S1 (a) UV-vis sbsorption and of (b) FL spectra of NI-N-HABI in various organic solvents. The excitation wavelength is  $\lambda_{em} = 380$  nm. c, FL spectra of NI-N-HABI in DMSO-water mixture with  $f_w$  varies from 0 to 99% (10<sup>-6</sup> mol/L). d, Excitation and emission spectra of NI-N-HABI in DMSO.



**Figure S2** UV-vis spectra of NI-N-HABI in PS film under UV (302 nm, 0.85 mW/cm<sup>-2</sup>) irradiation with time varies from 0, 5, 10, 20, 30, 60 to 90 s. The film is prepared by dissolving NI-N-HABI in PS solution (5.0 g PS was dissolved in 50 mL distilled DCM and stirred overnight), and spin-coating (1000 rad/s, 1 min); the scan speed is 1200 nm/s.



**Figure S3.** A comparison of the relative conversion yield upon various time of UV irradiation at (**a**) 394 nm, (**b**) 611 nm and (**c**) normalized optical density changes of NI-N-HABI at photostationary state in PS film (black line + square) and pure film (red line + sphere)

In our system, the fluorophore shares no  $\pi$ -conjugation with HABI or the radicals after UV irradiation. Thus the optical changes could be easily attributed to the colored species and we could use the absorbance changes at maximum wavelength (394 nm or 611 nm) of radicals to represent the content of colored species after certain time of UV irradiation. And we use the absorbance of NI-N-HABI at its maximum wavelength (400 nm) as an internal standard to represent initial content of NI-N-HABI. Thus, the relative conversion yield ( $\Phi_c$ ) of NI-N-HABI to NI-N-TPIR could be roughly compared by using the equation below:

$$\Phi_{C} = \frac{A_{\lambda_{max,R'}t} - A_{\lambda_{max,R'}t}}{A_{\lambda_{max'},NI}}$$

Where  $A_{\lambda max,Rt}$  is the absorbance at  $\lambda_{max}$  (maximum absorption wavelength of radicals, ie, 394 or 611 nm) at a given time (t) of UV irradiation;  $A_{\lambda max,R0}$  is the absorbance of the film at  $\lambda_{max}$  before UV irradiation;  $A_{\lambda max,NI}$  is the absorbance of the fluorophore NI at  $\lambda_{max}$  (400 nm)

These results indicate that the conversion yields of NI-N-HABI at photostationary state in PS and pure film are the same. The lower absorption efficiency around 600 nm was due to the weaker radical-radical interaction in PS film.



**Figure S4**. EPR spectra of NI-N-HABI upon irradiation of a 405 nm laser (**a**) and the fading kinetics after certain time of irradiation in darkness (**b**) in solution (benzene, 298 K, 10<sup>-3</sup> M)



Figure S5 | Single-wavelength-controlled super-resolution fluorescent imaging based on NI-N-HABI for block copolymer micelles. a, Bright field of micelles. b, Fluorescence imaging of a. c, Super-resolution fluorescent imaging of a, d, Enlarged image from box in c. e, fluorescence cross sectional profiles of single cylindrical micelle.



Figure S6 | Nanoscale *in situ* optical visualization of block copolymer self-assembled into micelles.
Panel I: Early stage (0 h). a, Fluorescence imaging. b, Super-resolution fluorescent imaging of a.
Panel II: Collapsing stage occurs at 1.5 h (during time 10-15 seconds). c, Fluorescence imaging. d, Super-resolution fluorescent imaging of c.

## 5. <sup>1</sup>H NMR, <sup>13</sup>C NMR and MS Spectra



Figure S8 <sup>1</sup>H NMR spectrum of 1c in CDCl<sub>3</sub>



Figure S10 <sup>1</sup>H NMR spectrum of 1e in <sup>d</sup>-DMSO





## MALTI-TOF of NI-N-2TPI ([M+H]<sup>+</sup>)



Figure S12 MALDI-TOF spectrum of 1e



Figure S13 MALDI-TOF spectrum of 1 (NI-N-HABI)

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