Electronic Supplementary Information

Achieving Long-lived thermally activated delayed fluorescence in the atmospheric aqueous environment by nanoencapsulation

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1.General Information

The experimental details.

Absorption spectra were obtained using a UV-Vis spectrophotometer (Cary Eclipse from Agilent Tech). Fluorescence spectroscopy were recorded on a fluorometer (Cary Eclipse from Agilent Tech). The data were obtained under the control of a Windows-based PC running the manufacturers' supplied software. Nanosecond fluorescence lifetimes were using the TCSPC technique on HORIBA Jobin Yvon IBN photo counting florescence system with Nano-LED excitation at 455 nm. Microsecond fluorescence lifetimes were using HORIBA Jobin Yvon IBN photo counting florescence system with spectra-LED excitation. HC-3018 high-speed centrifuge for the separation of fluorescent nanoparticles and liquid phase, two-dimensional linear ion trap-electrostatic field orbit Fourier transform combined mass spectrometer for mass spectrometry testing. Fluorescence imaging was performed using an Olympus FV-1000 inverted fluorescence microscope with a 60× objective lens. The fluorescence intensity and fluorescence lifetime of NP-2 in HeLa cells were observed under a Leica TCS-SP2 confocal fluorescence microscope and TCSPC FLIM equipment (SPC150).

All the reactions were carried out under a nitrogen atmosphere with dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Silica gel (200-300 mesh) was used for flash column chromatography, Ultrapure deionized water from a Milli-Q ultrapure system was used for all synthesis and storage steps.

Cell and Culture Conditions.

HeLa cell line was purchased from the Chenyu biological company (Dalian, China). The culture medium of HeLa cells was Dulbecco's modified Eagle medium (Kaiji, Nanjing, China) containing 10% fetal bovine serum (Sijiqing, Zhejiang, China). The culture conditions of HeLa cells were 37 °C in an atmosphere of 5% CO_2 in an incubator. HeLa cells were seeded in a 20 mm glass bottom dish from NEST company and incubated for 24 h.

In vitro cytotoxicity of fluorescent nanoparticles

HeLa cells were used to evaluate the cytotoxicity of nanoparticles using the methyl tetrazolium (MTT) method. Briefly, the cells were seeded in 96-well plates at a density of 5×10^4 per well and maintained in DMEM containing 10% fetal bovine serum, supplemented with 100 U mL^{-1} penicillin and 100 mg mL^{-1} streptomycin, and incubated at 37 ℃ in a humidified cell culture incubator (MCO-20AIC,Sanyo, Japan) with a 5% CO_2 atmosphere for 24 h. The cells were washed three times with PBS, followed by incubation with 100 uL of FSNPs solution at different concentrations of 0, 10, 30, 60, 100, 300,500, 700, 900 and 1000 mg mL^{-1}, which were added to each well. The cells were subjected to MTT assay after being incubated for another 24 h.

2.Details of composition

The DCF-BYT was synthesized according to the literature method.[1]

Synthesis of fluorescent nanoparticles NP-1.

Take TX-100 3.5 g in a 100 mL round bottom flask, stir in a water bath at room temperature, then add 4.4 mL of n-octanol until the mixed solution is clear, then add 20 mL of cyclohexane and continue to stir for 20 min. After 20 min, add 1 mL of deionized water to the system, then add 200 μL of TEOS to the system dropwise, and stir for 2 min before adding 100 μL of ammonium hydroxide. After 30 min reaction, the rotation speed was reduced. After 12 h, 20 μL of APTES was added dropwise to the system. After 12 h of reaction, an excess of acetone was added to the solution system to break the emulsion. After the precipitate was precipitated, the nanoparticles were centrifuged by a centrifuge, and washed three times with 10 mL of absolute ethanol in the process. After the end of the centrifugation, the nanoparticles were dried in an oven at 60 ° C, dried and taken out and stored in a ventilated and dry environment.

50 mg of amino-functionalized nanoparticles were placed in a 100 mL round bottom flask and first placed in an oven at 60 ° C for activation. After activation, 5 mL of DMF was added to the round bottom flask and the flask was placed

in ultrasound. After DMAP 200 mg and EDCI 60 mg were stirred uniformly in the flask, a pre-configured DMF solution of DCF-BYT (0.02 mmol/L) was added dropwise. After 12 h, the reaction was stopped, centrifuged, washed three times with 10 mL of DMF, washed three times with 10 mL of absolute ethanol, and dried under vacuum to obtain fluorescent nanoparticles NP-1

Synthesis of fluorescent nanoparticles NP-2.

Compound DCF-BYT 6.6 mg, EDCI 20 mg, DMAP 5 mg and DCF 4.01 mg, EDCI 20 mg, and DMAP 5 mg were weighed into two round bottom flasks, respectively, and evacuated. 10 mL of anhydrous DMF was added to the flask, and the reaction was carried out under N_2. After stirring at room temperature for 1 h, 40 μL of APTES was added, and after 12 h of reaction, the reaction was stopped. A large amount of diethyl ether was added to the reaction, and the mixture was filtered with a sand funnel. The obtained solid was dissolved in methanol and dried to give compound DCF-BYT-Si.

Take TX-100 3.5 g in a 100 mL round bottom flask and stir in a water bath at room temperature, then add 4.4 mL of n-octanol to the clear solution, then add 20 mL of cyclohexane and continue to stir for 20 min, 20 After the minute, pre-configured DCF-BYT-Si deionized water was added dropwise to the system, then 200 μL of TEOS was added dropwise to the system, and after stirring for 2 min, 100 μL of ammonia water was added. After 30 min reaction, the rotation speed was reduced. After 24 h of reaction, an excess of acetone was added to the solution system to break the emulsion. After 5 minutes, the mixture was stirred. After the precipitate was precipitated, the nanoparticles were centrifuged by a centrifuge, and washed three times with 10 mL of absolute ethanol. After the end of centrifugation, fluorescent nanoparticles NP-2 were obtained. The nanoparticles were dried in an oven at 60 ° C, dried and taken out and stored in a ventilated and dry environment.

Luminescence lifetime test of NP-1 and NP-2 under solution conditions

(1) Instantaneous fluorescence lifetime of fluorescent nanoparticles NP-1 and NP-2

The prepared fluorescent nanoparticles NP-1 and NP-2 mother liquor were transferred to a quartz dish equipped with a deaerator at a concentration of 0.5 mg/mL. Firstly, test the instantaneous fluorescence lifetime of the sample without deoxidation; then seal the quartz dish, fill it with nitrogen gas with a flow rate of 20 mL/min, and seal the quartz dish quickly after 20 minutes to test the oxygen removal state. The instantaneous fluorescence lifetime of the sample.

(2) Delayed fluorescence lifetime of fluorescent nanoparticles NP-1 and NP-2

The prepared fluorescent nanoparticles NP-1 and NP-2 mother liquor were transferred to a quartz dish equipped with a deaerator at a concentration of 0.5 mg/mL. The quartz dish was sealed and filled with nitrogen gas using a deaerator with a flow rate of 20 mL/min. After 20 minutes, the quartz dish was quickly sealed and tested for delayed fluorescence lifetime.

3. Supplementary figures and tables.

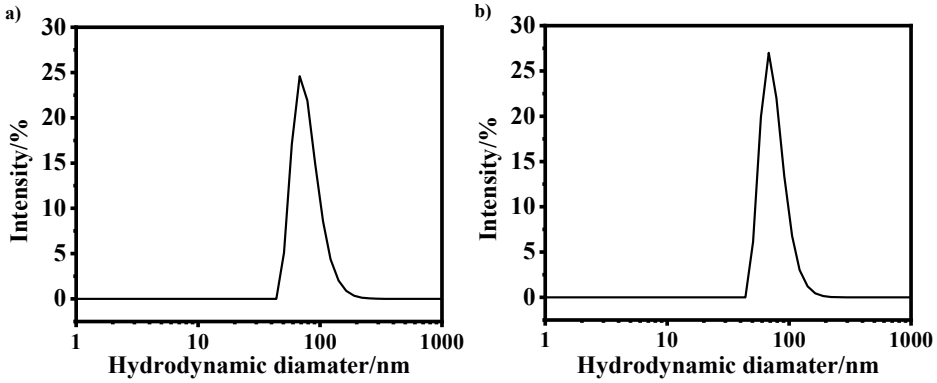


Figure S1. DLS results of (a) NP-1 and (b) NP-2 in PBS.

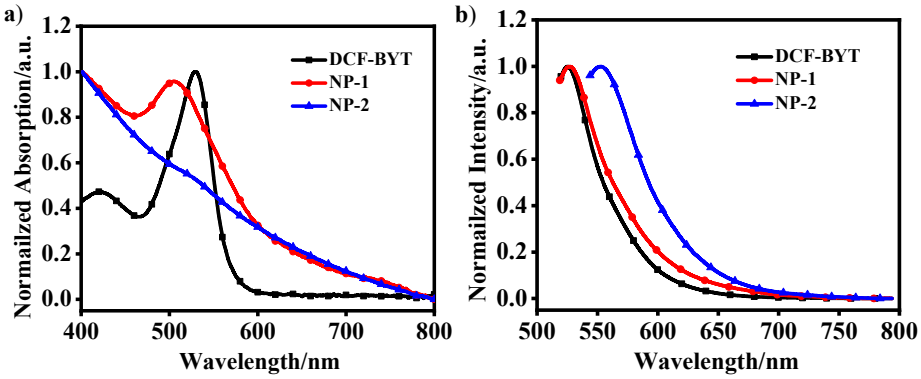
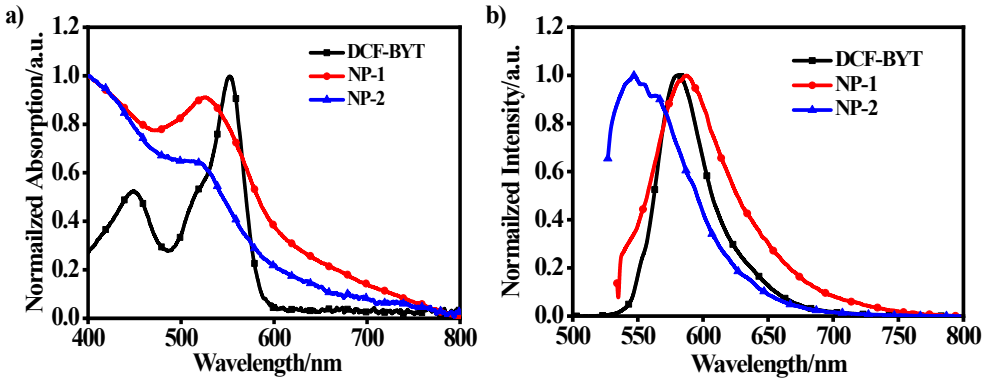


Figure S2. (a) The normalized absorption spectra and (b) normalized emission spectra of NP-1 (0.5 mg/mL, λ_{ex} = 505 nm), fluorescent nanoparticles NP-2 (0.5 mg/mL, λ_{ex} = 502 nm) and compound DCF-BYT(10 μM, λ_{ex} = 530 nm) , PBS (pH = 7.4).



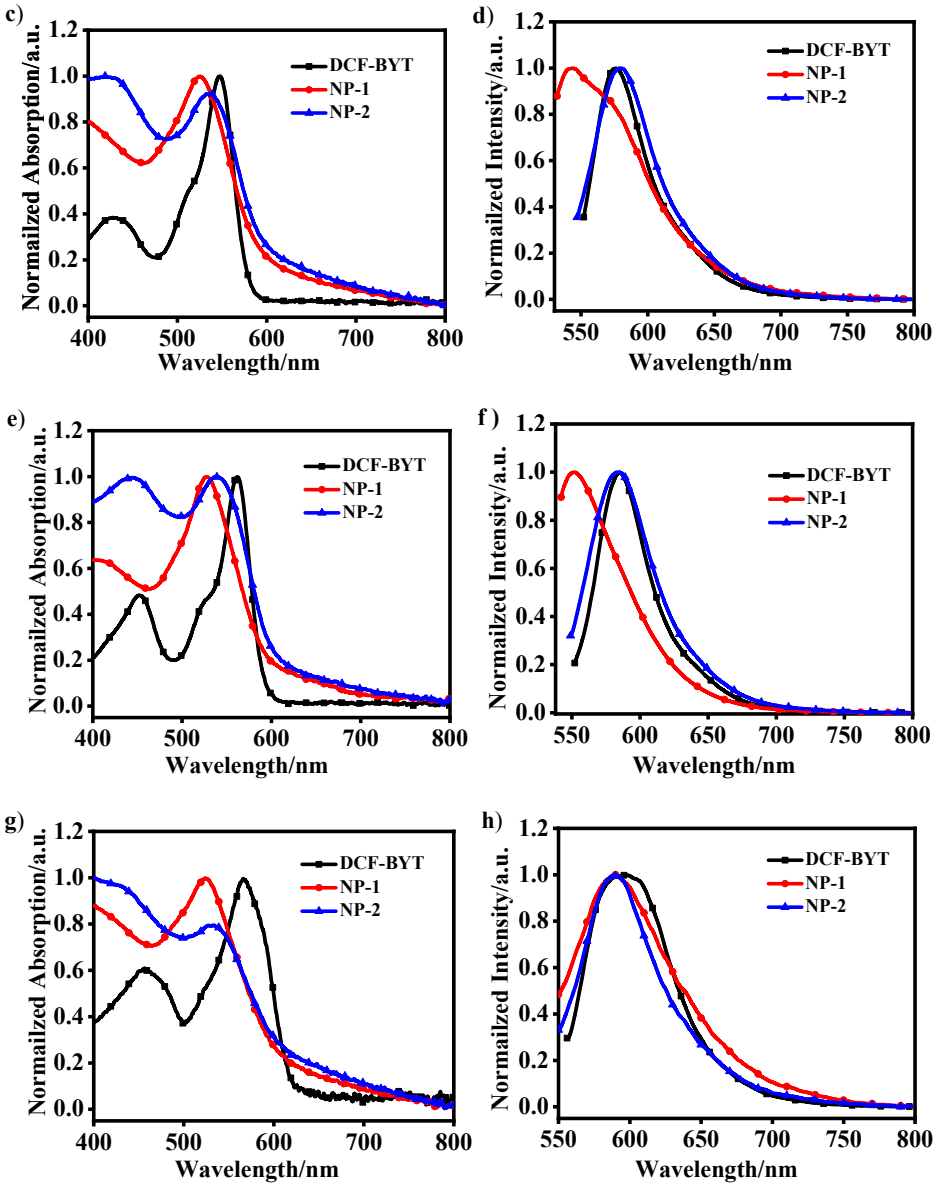


Figure S3. The normalized absorption spectra and normalized emission spectra. (a) and (b) in acetonitrile; (c) and (d) in ethyl alcohol; (e) and (f) in dimethylformamide; (g) and (h) in ethyl acetate , compound DCF-BYT (3.3 μM), fluorescence nanoparticles NP-1 (0.5 mg/mL), fluorescent nanoparticles NP-2 (0.5 mg/mL). The excitation wavelength is the maximum absorption wavelength.

Table S1. The photophysical properties of fluorescent nanoparticles.

	DCF-BYT		NP-1		NP-2	
	λ_{abs} [nm]	λ_{em} [nm]	λ_{abs} [nm]	λ_{em} [nm]	λ_{abs} [nm]	λ_{em} [nm]
PBS	530	551	505	525	502	527
DMF	562	585	526	550	542	583
EtOH	547	575	523	542	540	578
MeCN	552	581	525	587	515	547
EtOAc	567	607	524	590	532	590

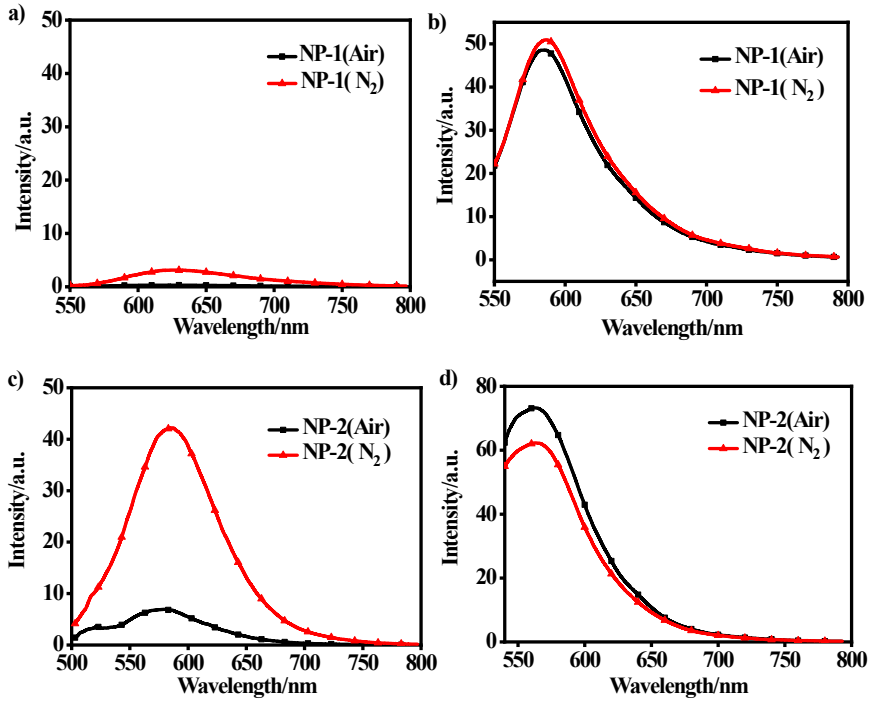


Figure S4. (a) Time-resolved emission spectra and (b) steady-state fluorescence spectrum of fluorescence nanoparticles NP-1 (0.5 mg/mL in acetonitrile, λ_{ex} = 505 nm); (c) Time-resolved emission spectra and (d) steady-state fluorescence spectrum of fluorescence nanoparticles NP-2 (0.5 mg/mL in acetonitrile, λ_{ex} = 502 nm). Time-resolved emission spectra: fluorescence under air atmosphere; fluorescence under N_2 atmosphere. All conditions were carried out in phosphorescence mode (total decay time, 5 ms; delay time, 0.1 ms; gate time, 1 ms). Steady-state fluorescence spectrum: fluorescence under air atmosphere; fluorescence under N_2 atmosphere. All conditions were carried out in fluorescence mode (delay time, 0 ms).

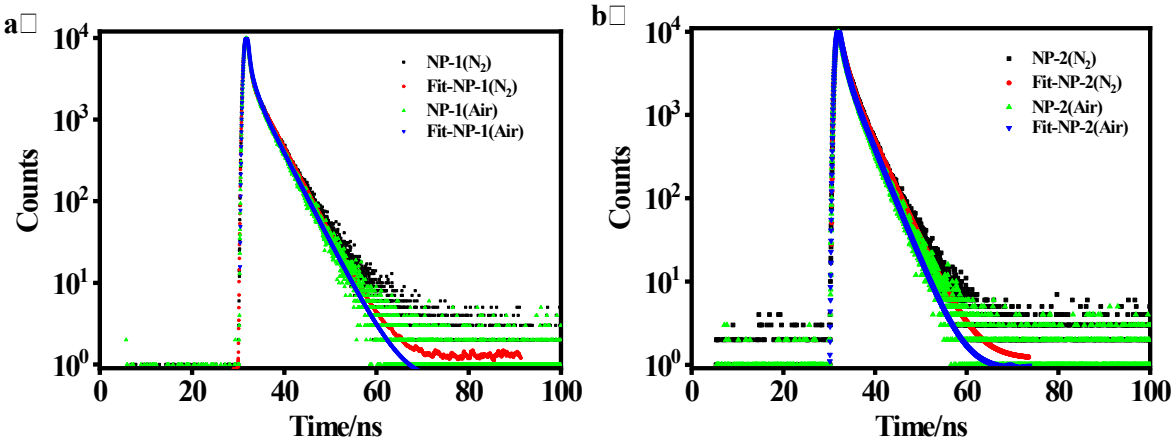


Figure S5. The fluorescence life attenuation curve of fluorescent nanoparticles (a) NP-1 (0.5 mg/mL) and (b) NP-2 (0.5 mg/mL) in acetonitrile at room temperature. The light source is NanoLED 456 nm laser and monitored at 587 nm and 547 nm.

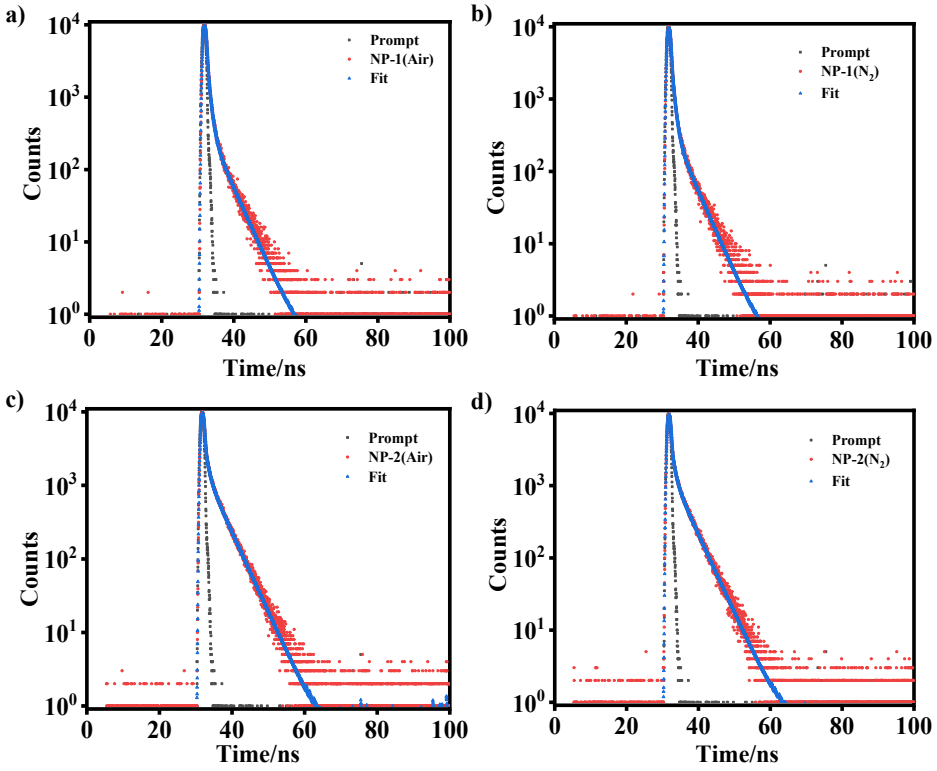


Figure S6. The fluorescence life attenuation curve of fluorescent nanoparticles (a) NP-1 (0.5 mg/mL) and (b) NP-2 (0.5 mg/mL) in PBS at room temperature. The light source is NanoLED 456 nm laser and monitored at 525 nm and 527 nm.

Table S2. Fluorescence lifetime compositions of prompt components of fluorescent nanoparticles NP-1 and NP-2 in acetonitrile after deoxygenating and under air atmosphere, and decay of the emission are monitored at 587 nm and 547 nm. The light source is NanoLED 456 nm laser.

	Conditions	τ_1 (ns)	n_1%	τ_2 (ns)	n_2%	τ_3 (ns)	n_3%	τ_p (ns)
NP-1	Atmospheric	1.09	19.94	3.97	51.15	0.25	28.92	2.32
NP-1	Oxygen-free	0.42	41.39	4.09	58.61	--	--	2.57
NP-2	Atmospheric	0.89	41.21	3.23	58.79	--	--	2.27
NP-2	Oxygen-free	1.21	42.18	3.70	57.82	--	--	2.38

Table S3. Fluorescence lifetime compositions of prompt components of fluorescent nanoparticles NP-1 and NP-2 in PBS after deoxygenating and under air atmosphere, and decay of the emission are monitored at 525 nm and 527 nm. The light source is NanoLED 456 nm laser.

	Conditions	τ_1 (ns)	n_1%	τ_2 (ns)	n_2%	τ_3 (ns)	n_3%
NP-1	Atmospheric	0.79	28.25	0.17	60.06	3.54	11.69
NP-1	Oxygen-free	0.15	52.28	0.76	28.96	3.87	11.76
NP-2	Atmospheric	0.88	17.55	0.11	47.84	3.90	34.61
NP-2	Oxygen-free	0.91	16.69	0.11	46.93	4.01	36.38

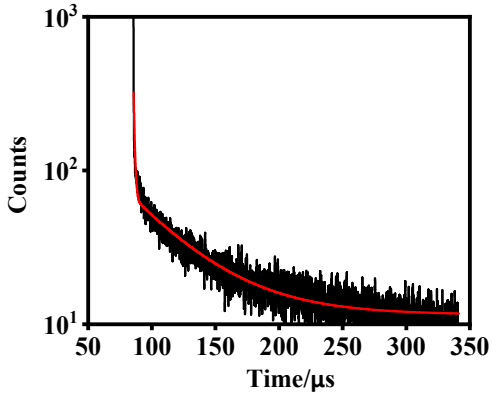


Figure S7. The fluorescence life attenuation curve of fluorescence nanoparticles NP-1 (0.5 mg/mL) in acetonitrile at room temperature under nitrogen condition. Red denotes the fitting curve of luminescence lifetime. The light source is SpectrumLED 450 nm laser and monitored at 587 nm.

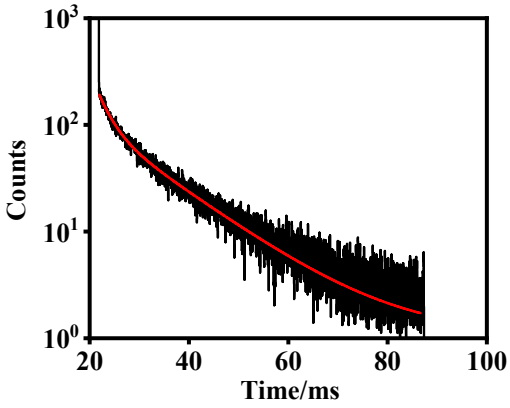


Figure S8. The fluorescence life attenuation curve of fluorescence nanoparticles NP-2 (0.5 mg/mL) in acetonitrile at room temperature under nitrogen atmosphere. Red denotes the fitting curve of luminescence lifetime. The light source is SpectrumLED 450 nm laser and monitored at 547 nm.

Table S4. The delayed fluorescence life attenuation and components of fluorescent nanoparticles NP-1 and NP-2 in acetonitrile. The light source is SpectrumLED 450 nm laser. The detection wavelength are 587 nm and 547 nm respectively.

Conditions	τ_1	n_1%	τ_2	n_2%	τ_d
NP-1, N_2	0.23 μs	10.31	21.37 μs	89.69	19.12 μs
NP-2, Air	2.35 ms	23.43	9.30 ms	76.57	7.67 ms
NP-2, N_2	2.57 ms	18.24	13.04 ms	81.76	11.13 ms

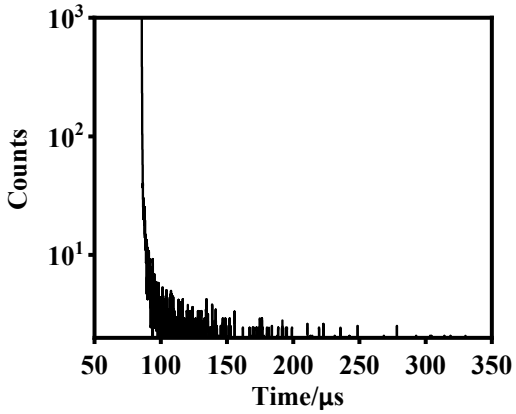


Figure S9. The fluorescence life attenuation curve of fluorescence nanoparticles NP-1 (0.5 mg/mL) in acetonitrile at room temperature under an air atmosphere. The light source is SpectrumLED 450 nm laser and monitored at 587 nm.

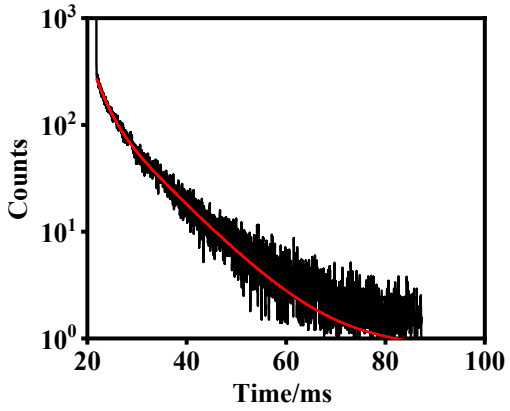


Figure S10. The fluorescence life attenuation curve of fluorescence nanoparticles NP-2 (0.5 mg/mL) in acetonitrile at room temperature under an air atmosphere. Red denotes the fitting curve of luminescence lifetime under air atmosphere. The light source is SpectrumLED 450 nm laser and monitored at 547 nm.

Table S5. The delayed fluorescence life attenuation and components of fluorescent nanoparticles NP-2 in PBS buffered solution after deoxygenating and under air atmosphere. The light source is SpectrumLED 450 nm laser and the detection wavelength is 574 nm.

Conditions	τ_1 (ms)	n_1%	τ_2 (ms)	n_2%	τ_d (ms)
Oxygen-free	11.21	79.92	2.52	20.08	9.47
Atmospheric	11.11	79.28	2.52	20.72	9.33

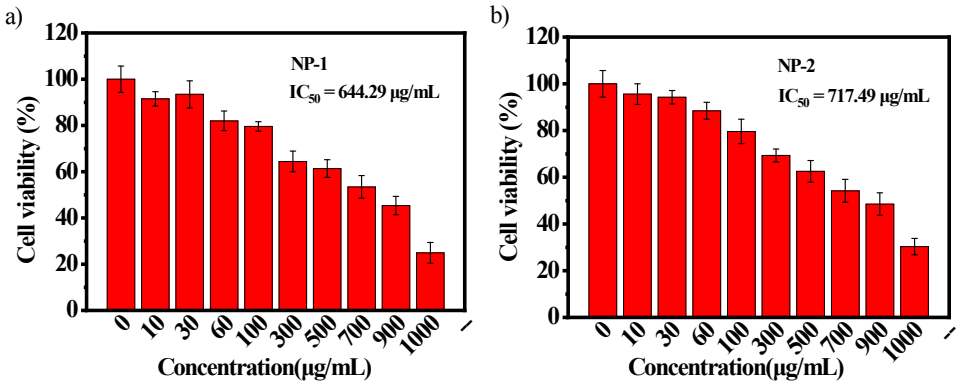


Figure S11. MTT assays of fluorescent nanoparticles in HeLa cells. (a) NP-1, (b) NP-2.

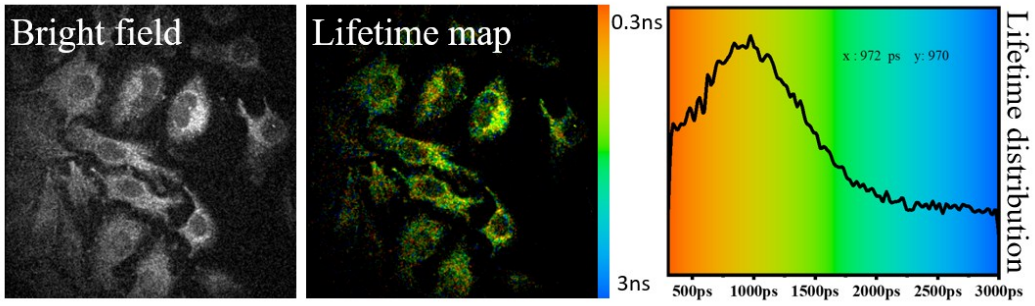


Figure S12. In vitro FLIM of HeLa cells stained with NP-2 (20 μg/mL). The excitation wavelength was femtosecond 800 nm, and fluorescence emissions were recorded at 575-630 nm.

4.References

1. Y. Wu, F. Song, W. Luo, Z. Liu, B. Song and X. Peng, *ChemPhotoChem*, 2017, **1**, 79-83.