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Supplementary Information

Optically-Manipulated Multiaddressable all-ESIPT Fluorescence Nanomicelles Prepared with a Single Fluorophore

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1. Experimental details

Materials. Unless otherwise noted, all reagents were purchased from Energy Chemical (Shanghai, China). And all solvents were purchased from Sinopharm Chemical Reagent Beijing Co., and solvents were dried according to established procedures. Deionized water was purified by Milli-Q system. Pluronic P123 (PEO_{20}–PPO_{70}–PEO_{20}; 5800 g / mol) was purchased from Macklin (Shanghai, China). Poly(styrene-co-maleic anhydride), cumene terminated (PSMA, average Mn ~1,900 g / mol) was purchased from Sigma-Aldrich.

Instruments. Absorption spectra were measured using a Shimadzu UV-2550 PC double-beam spectrophotometer. Steady state fluorescence spectra were measured using a Shimadzu RF-5301 PC spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (101 and 151 MHz) spectra were recorded on a Bruker AVANCE500 at room temperature. LC-HRMS analysis was performed on an Agilent 1290-micro TOF-Q II mass spectrometer. The fluorescence quantum yields (Φ_f) and fluorescence lifetimes were measured on Edinburgh FLS 920 steady state spectrometer. The photographs of fluorescence images were acquired by a Nikon camera. A portable hand-held ultra-violet lamp (range from 348 to 395 nm, maximum at 365 nm, 6 W) for UV light irradiation, its light intensity (light power density) irradiated on sample is 0.3 mW/cm^2. A Wota F25 fishing blue light lamp (range from 416 to 490 nm, maximum at 448 nm, 20 W) for visible/blue light irradiation, its light intensity irradiated on sample is 12 mW/cm^2. Cell imaging was taken with Confocal laser scanning microscope (Carl Zeiss Microscopy LLC, Jena, Germany).

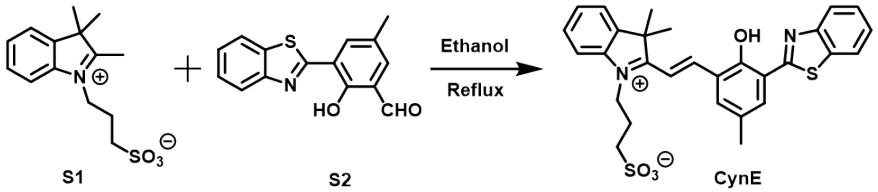
Methods

Cytotoxicity assays. The viabilities of cells treated with **CynE** and nanomicelles were measured by a well-established 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Generally, HeLa cells were seeded in 96-well plates harboring 2 mL of 10% FBS-containing DMEM at a density of 8000 cells per well. The cells were incubated with drugs at concentrations from 0 to 200 μg/mL, and cells without treatment were used as a control. After the incubation at 37 ^{o}C for 24 h, the standard MTT assay was used to determine the cell viability. Three repeats were conducted for each sample.

Bioimaging experiments of **CynE-P123** were conducted by confocal laser scanning microscope (CLSM). HeLa cells were seeded into 6-well plates at a density of 2.5×10^5 cells/well and cultured with a sterilized coverslip overnight. Then the cells were treated with **CynE** and nanomicelles at the concentration of 10 μg/mL for 6 h, respectively. Then the cells were washed with PBS twice, followed by fixed with 4% paraformaldehyde for 30 min. Finally, the coverslips were taken from the wells and observed with a confocal laser scanning microscope.

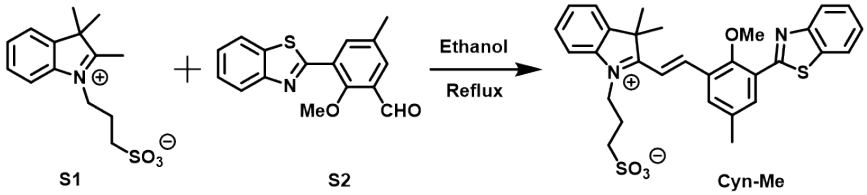
Synthetic procedures and characterization

Procedures for the synthesis of **CynE**



A mixture of Indole (S1[1], 1.75 g, 6.5 mmol) and HBT (S2[2], 1.4 g, 5 mmol) was stirred in ethanol (30 ml) and the reaction mixture was refluxed for 9h. After cooling to room temperature, the reaction mixture was filtered. The resulting solid was recrystallization ethanol and washed with ether three times and dried to obtain the product as orange color solid (2.32g, yield: 87%). ^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.89 (d, *J* = 16.3 Hz, 1H), 8.60 (s, 1H), 8.25 (d, *J* = 16.3 Hz, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 7.98 (d, *J* = 8.0 Hz, 1H), 7.80 (m, 1H), 7.72 – 7.70 (m, 1H), 7.66 – 7.61 (m, 3H), 7.59 – 7.55 (m, 1H), 7.50 – 7.46 (m, 1H), 5.02 – 4.89 (m, 2H), 3.03 – 3.00 (m, 2H), 2.50 (s, 3H), 2.47 – 2.43 (m, 2H), 1.87 (s, 6H). ^{13}C NMR (151 MHz, CD_2Cl_2) δ 182.97, 169.30, 157.66, 151.86, 150.55, 143.96, 141.20, 135.86, 134.29, 133.24, 131.30, 130.29, 127.65, 126.68, 123.63, 123.41, 122.63, 122.40, 118.00, 115.00, 112.78, 52.82, 47.34, 46.50, 27.85, 25.61, 20.64. LC-HRMS (ESI): m/z: calcd for $C_{29}H_{29}N_2O_4S_2$ $[M+H]^+$: 533.1563; found: 533.1562. LC-HRMS (ESI): m/z: calcd for $C_{29}H_{29}N_2O_4S_2$ $[M+H]^+$: 533.1563; found: 533.1562.

Procedures for the synthesis of **Cyn-Me**



The synthesis procedure is similar to **CynE**. The product is orange solid (yield: 65%).^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (s, 1H), 8.49 – 8.45 (m, 2H), 8.21 (d, *J* = 7.9 Hz, 1H), 8.14 – 8.06 (m, 3H), 7.94 – 7.92 (m, 1H), 7.68 – 7.66 (m, 2H), 7.59 (t, *J* = 7.6 Hz, 1H), 7.52 (d, *J* = 7.4 Hz, 1H), 4.97 – 4.93 (m, 2H), 3.96 (s, 3H), 2.71 – 2.68 (m, 2H), 2.52 (s, 3H), 2.29 – 2.24 (m, 2H), 1.86 (s, 6H). ^{13}C NMR (151 MHz, DMSO) δ 181.77, 161.14, 156.27, 151.68, 146.00, 143.88, 140.83, 135.52, 135.40, 134.08, 132.61, 129.81, 129.28, 128.46, 126.63, 126.50, 125.62, 123.13, 122.85, 122.14, 115.59, 114.98, 64.24, 52.32, 47.14, 45.93, 25.97, 24.88, 20.24. LC-HRMS (ESI): m/z: calcd for $C_{30}H_{31}N_2O_4S_2$ $[M+H]^+$: 547.1720; found: 547.1726.

Procedures for the synthesis of **CynE1**

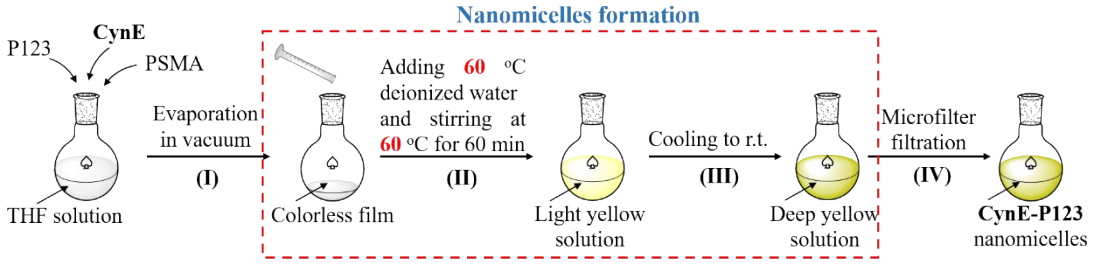
The synthesis procedure is similar to **CynE**. The product is orange solid (yield: 58%). ^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.80 (d, *J* = 16.3 Hz, 1H), 8.68 (s, 1H), 8.23 (d, *J* = 16.4 Hz, 1H), 8.06 (s, 1H), 7.99 (d, *J* = 8.0 Hz, 1H), 7.83 (s, 1H), 7.70 – 7.56 (m, 5H), 7.50 (t, *J* = 7.6 Hz, 1H), 5.00 (t, *J* = 7.3 Hz, 2H), 2.52 (s, 3H), 2.04 – 1.97 (m, 2H), 1.89 (s, 6H), 1.66 – 1.57 (m, 2H), 1.03 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, CD_2Cl_2) δ 183.04, 169.19, 157.58, 151.64, 150.19, 144.08, 141.33, 135.40, 135.18, 133.13, 130.92, 130.27, 130.06, 127.64, 126.67, 123.39, 123.30, 122.51, 122.37, 117.92, 115.41, 113.54, 52.97, 48.58, 31.53, 27.70, 20.61, 20.48, 14.15. LC-HRMS (ESI): m/z: calcd for $C_{30}H_{31}N_2OS$ $[M]^+$: 467.2152; found: 467.2152.

Procedures for the synthesis of **CynE2.** The synthesis procedure can refer to the reported

literature.[3]

General procedure for Preparation of nanomicelles.

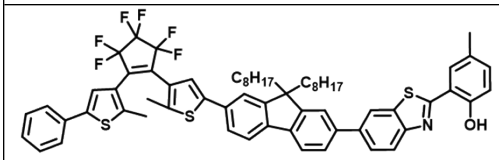
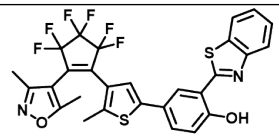
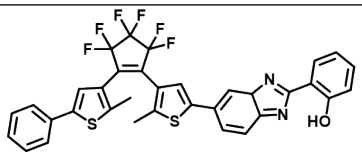
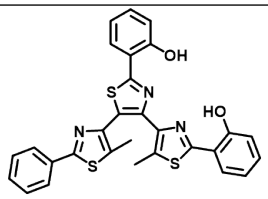
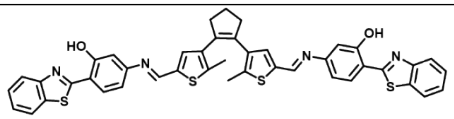
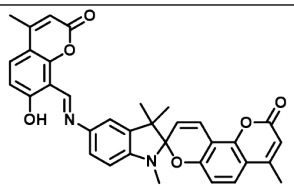
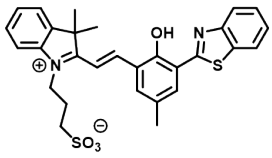
The nanomicelles mentioned in this work were generally prepared by using the film rehydration method. A mixture of P123 (0.1 g), fluorophore (0.5%), and PSMA (5%) were first dissolved in 3 ml THF. And the THF was evaporated in a rotary vacuum evaporator at 40°C and the residue in vacuum to form a film on the inside of round-bottomed flasks (**Step I**). Then, 10 ml 60 °C deionized water was added into this flask and stirred at 60 °C for 60 min (**Step II**). After cooling to room temperature (**Step III**), the solution was filtered through 0.45 μm microfilter to obtain the yellow color nanomicelles aqueous solution (**Step IV**).



Scheme S1 Procedure for preparation of **CynE-P123** nanomicelles by "Thin-film hydration method" in this work.

2. Supplementary Figures and Table

Table S1 ESIPT-based photoswitchable fluorophores.

Structure Formula	Reference	Regulation Method
	*Res. Chem. Intermed.* **2017**, *43*, 5321-5336	Energy transfer following Photoisomerization
	*Tetrahedron* **2016**, *72*, 2935-2942	
	*Beilstein J. Org. Chem.* **2019**, *15*, 2204-2212	
	*Chem. Eur. J.* **2014**, *20*, 12279-12288	
	*RSC Adv.* **2019**, *9*, 4812-4815	
	*Photochem. Photobiol. Sci.* **2018**, *17*, 1365-1375	
	This work	Photoisomerization

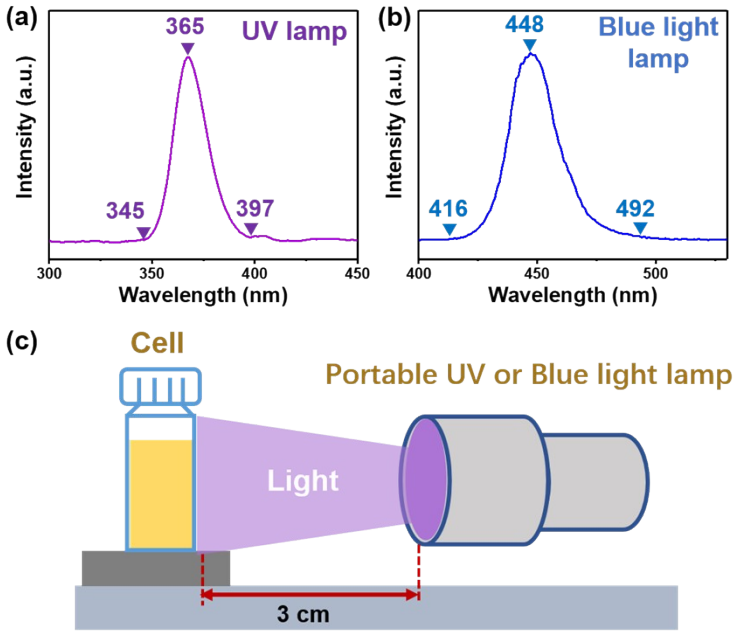
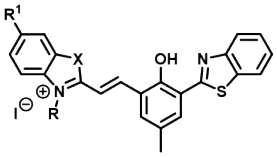
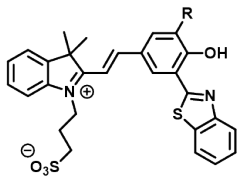


Fig. S1 Emission spectra of (a) UV and (b) blue light for photoswitching. (c) Illustration of irradiation setup.

Table S2 Cyanine derivatives with HBT as ESIPT units.

Structure Formula	Reference	Application
	*Chem. Commun.* **2017**, *53*, 3697-3700	lysosome-targeting probe
	Chem. Sci. **2017**, *8*, 6257-6265	biothiol probe
	New J. Chem. **2015**, *39*, 8940-8947	
	*Anal. Chem.* **2016**, *88*, 4426−4431	Bisulfite probe
	Anal. Chim. Acta. **2016**, *920*, 72-79	
	This work	Optically-manipulated ESIPT switch

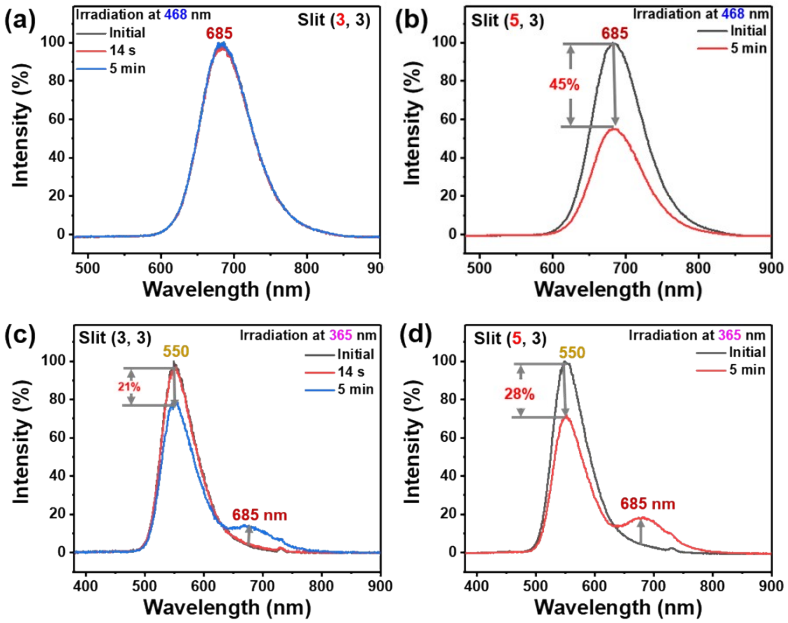


Fig. S2 Fluorescence spectrum for **CynE-P123** before and after irradiation upon (a) excitation light at 468 nm and (c) excitation light of 365 nm for 14 s and 5 min with excitation slit width are 3; Fluorescence spectra of **CynE-P123** before and after irradiation upon (b) excitation light at 468 nm and (d) excitation light of 365 nm for 5 min with excitation slit width are 5.

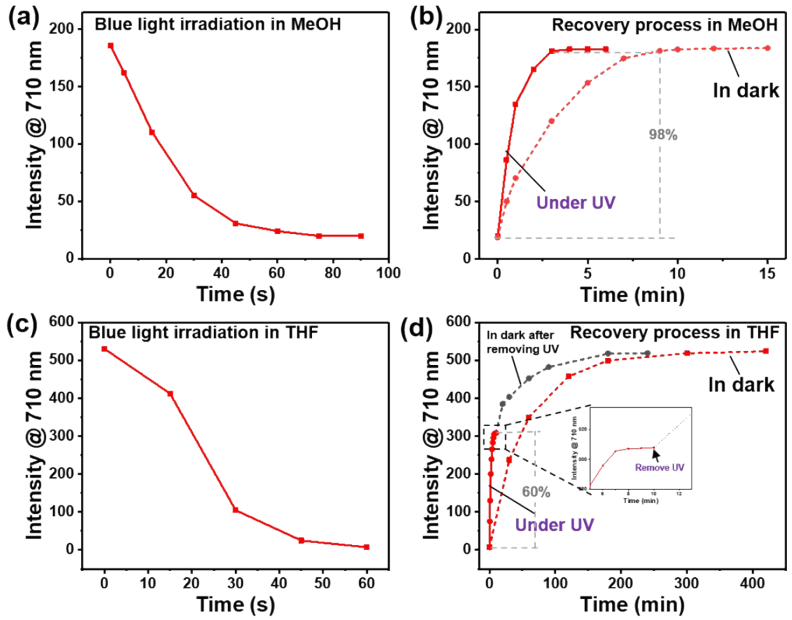


Fig. S3 Plot of emission peaks of **CynE** in (a) MeOH and (c) THF under blue light irradiation for fluorescence switching. Reverted plot of emission peaks of blue light-irradiated **CynE** in dark (dash line) and under UV irradiation (red solid line) (b) in MeOH and (d) in THF, black dash line represents reverted plot of emission peaks of blue light-irradiated **CynE** in dark after remove of UV (red solid line). ($C = 5 \times 10^{-5}$ M, excitation at 492 nm, slit (3,3))

For **CynE**, considering its fluorescence switching is related with solvent environment, its time-course fluorescence spectra in MeOH and THF upon blue light irradiation (ring-closing isomerization) and reverse processes (upon UV irradiation and in dark) have been investigated via monitoring the changes of its representative NIR ESIPT emission at 710 nm (This emission is red shifted by 25 nm compared with that in nanomicelles) with time. In MeOH, upon blue light irradiation, emission at 710 nm decreased about 80% in MeOH in 45 s and to lowest value in 75 s (**Fig. S3a**). This photoinduced isomerization could be reversed by UV irradiation or keeping in dark. Upon UV irradiation, the emission intensity recovered about 98% in 3 min (**Fig. S3b**, **red solid line**), and it would take 9 min to recover to same extent in dark (**Fig. S3b**, **red dash line**). In THF, upon blue light irradiation, emission at 710 nm decreased about 80% in 30 s and to lowest value within 60 s (**Fig. S3a**). This photoinduced isomerization could be reversed in dark and recovered 99% in 300 min (**Fig. S3b**, **red dash line**). But upon UV irradiation, it recovered about 60% for 7 min without further increase (**Fig. S3b** and **inset**, **red solid line**), which was faster than that recovery to same extent in dark. And after remove of the UV light, it further recovered to 99% in 240 min in dark (**Fig. S3b**, **black solid line**). These results indicate that there is a photostationary state under UV irradiation in the recover process, which is determined by solvents, UV irradiation is a faster way for recovery. In addition, we found that the

recovery rate of **CynE** in MeOH is faster than that in THF (Fig. S3). This may be due to the fact that relative to aprotic solvent THF, protonic solvent MeOH can easier transfer proton from the solvent environment to SP to promote the ring-opening reaction.

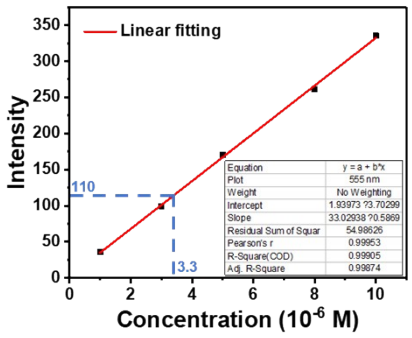


Fig. S4 Plot of emission of HBT at 555 nm against concentration in MeOH. Blue mark represents the emission intensity and concentration of the freeze-dried residue of 2 ml **CynE-P123** aqueous solution in 10 ml MeOH.

The amount of HBT generated from hydrolysis of CynE has been estimated by fluorescence spectrometry. The details are as follows: 2 ml (one fifth) aqueous solution of **CynE-P123** NMs was freeze-dried, and the residue was dissolved in 10 ml methanol. It's found that in a certain concentration range, the fluorescence intensity of CynE is linear with the concentration, according to this, the concentration of HBT in methanol was determined as 3.3×10^{-6} M (Fig. S4, blue mark). Using this result, we estimated that there was 18% **CynE** degraded to HBT.

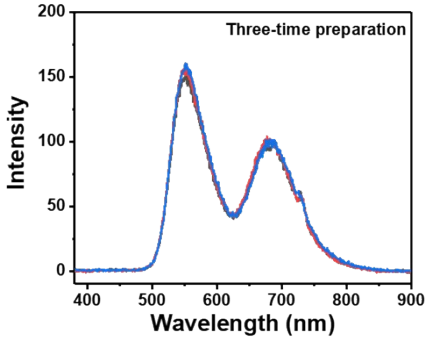


Fig. S5 Fluorescence spectra of three time-prepared **CynE-P123** (hydration at 60 ºC for 1 h). Excitation at 365 nm. Slit (3,3).

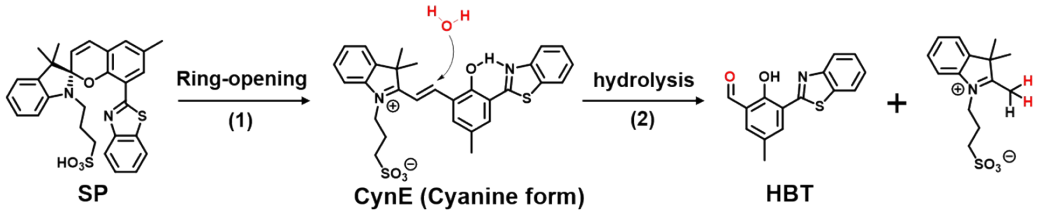


Fig. S6 Proposed mechanism for generation of HBT in nanomicelles by thin-film hydration method.

As shown in **Fig. S6**, it contains two processes, 1) ring-opening transformation from ring-closed SP form; 2) hydrolysis to HBT. At high temperature (i.e., 60 °C), the hydrolysis rate of CynE in nanomicelles is faster than ring-opening of SP ($k_1 < k_2$), and at room temperature, ring-opening of SP is dominant ($k_1 > k_2$). Thus, it's beneficial for in-situ hydrolysis of CynE by using thin-film hydration method.

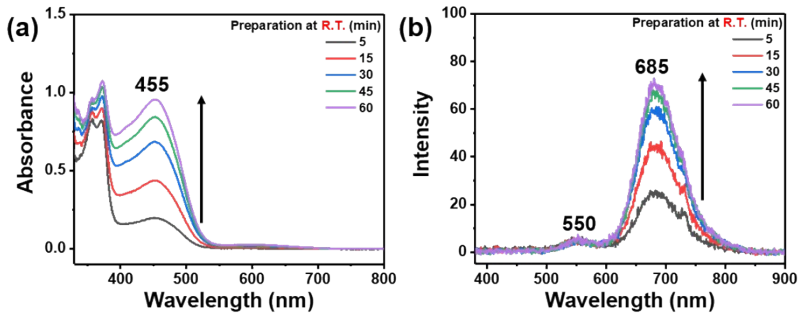


Fig. S7 The time variable (a) absorption and (b) fluorescence spectra of **CynE-P123** for preparation at room temperature. Excitation light is 365 nm, slit (3, 3).

For preparation at room temperature, both the absorbance at 455 nm and the emission intensity at 685 nm kept rising gradually, and there was a very small emission peak at 550 nm observed (**Fig. S7**), indicating that instead of hydrolysis, transformation from ring-closed SP to ring-open cyanine form of **CynE** is the main process at room temperature.

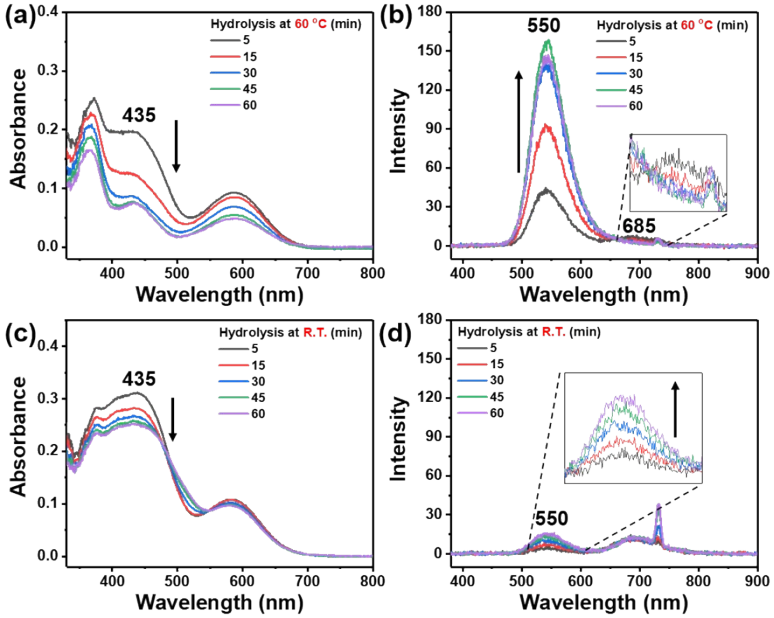


Fig. S8 (a) Absorption and (b) fluorescence spectra of **CynE** in 10% methanol aqueous solution were monitored at 60 °C for 60 min. (c) Absorption and (d) fluorescence spectra of **CynE** in 10% methanol aqueous solution were monitored at room temperature for 60 min. C = 2 × 10^{-5} M, slit (3, 3), excitation light is 365 nm.

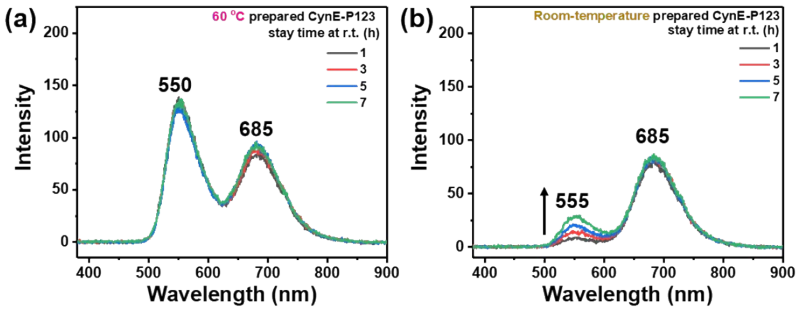


Fig. S9 Fluorescence spectra for (a) 60 °C and (b) room-temperature prepared **CynE-P123** staying at room temperature for hours. Excitation light is 365 nm, slit (3, 3).

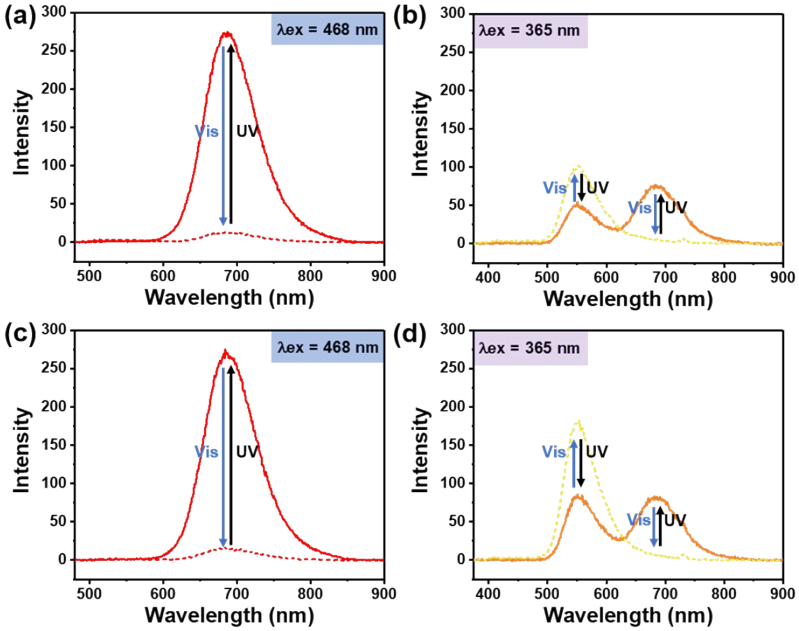


Fig. S10. Fluorescence spectra of **CynE-P123-1** prepared by hydration for 10 min upon irradiation, (a) excitation at 468 nm and (b) excitation at 365 nm. Fluorescence spectra of **CynE-P123-2** prepared by hydration for 30 min upon irradiation, (c) excitation at 468 nm and (d) excitation at 365 nm. Slit (3,3).

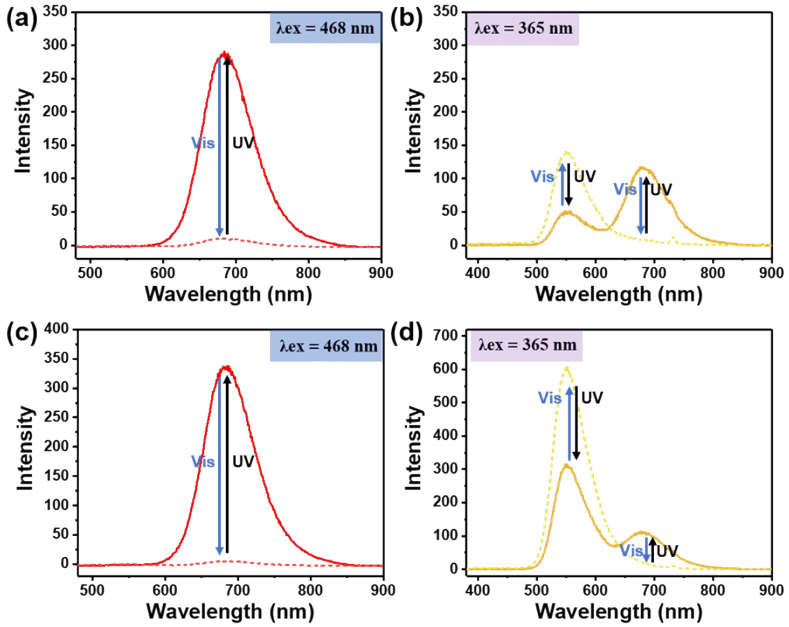


Fig. S11. Fluorescence spectra of **CynE-P123** prepared by hydration at 50 °C for 1 h upon irradiation, (a) excitation at 468 nm and (b) excitation at 365 nm. Fluorescence spectra of **CynE-P123** prepared by hydration at 70 °C for 1 h upon irradiation, (c) excitation at 468 nm and (d) excitation at 365 nm. Slit (3,3).

Table S3 Fluorescence lifetimes of **CynE-P123**, **CynE** and **HBT**

No.	Sample	solution / solid	Excitation Wavelength (nm)	Emission (nm)	τ_i (ns)[a]	A_i (%)	<τ> (ns)	χ^2 [b]
1	CynE-P123	H_2O	366	550	2.35 4.41	25 75	3.89	1.10
2		H_2O Upon blue light	366	550	4.70	100	4.70	1.16
3		H_2O	400	685	1.29 2.87	42 58	2. 21	1.33
4	CynE	THF	400	710	2.41	100	2.41	1.31
5		powder	400	674	1.40 3.59	66 34	2.14	1.11
6	HBT	THF	366	555	3.04	100	3.04	1.25
7		powder	366	558	2.91 5.65	36 64	4.61	1.09

[a] τ_i (i = 1, 2) is the fitted fluorescence lifetime. A_i is the percentage of τ_i. In the bi-exponential case, $\langle\tau\rangle = A_1\tau_1 + A_2\tau_2$; $A_1 + A_2 = 1$.

[b] The goodness-of-fit is indicated by the value of χ^2.

Table S4 Fluorescence quantum yields (Φ) and the corresponding radiative (k_r) and non-radiative decay (k_{nr}) constants of **CynE-P123, CynE** and **HBT**

	Emission (nm)	Φ	<τ> (ns)	k_r (10^8 s^{-1})[a]	k_{nr} (10^8 s^{-1})[b]
CynE-P123	685	0.20	2.21	0.91	3.62
	550	0.03	3.89	0.08	2.49
CynE[c]	710	0.42	2.41	1.74	2.41
HBT[c]	550	0.16	3.04	0.53	2.76

[a] $k_r = \Phi / \tau$. [b] $k_{nr} = (1 - \Phi) / \tau$. [c] In THF.

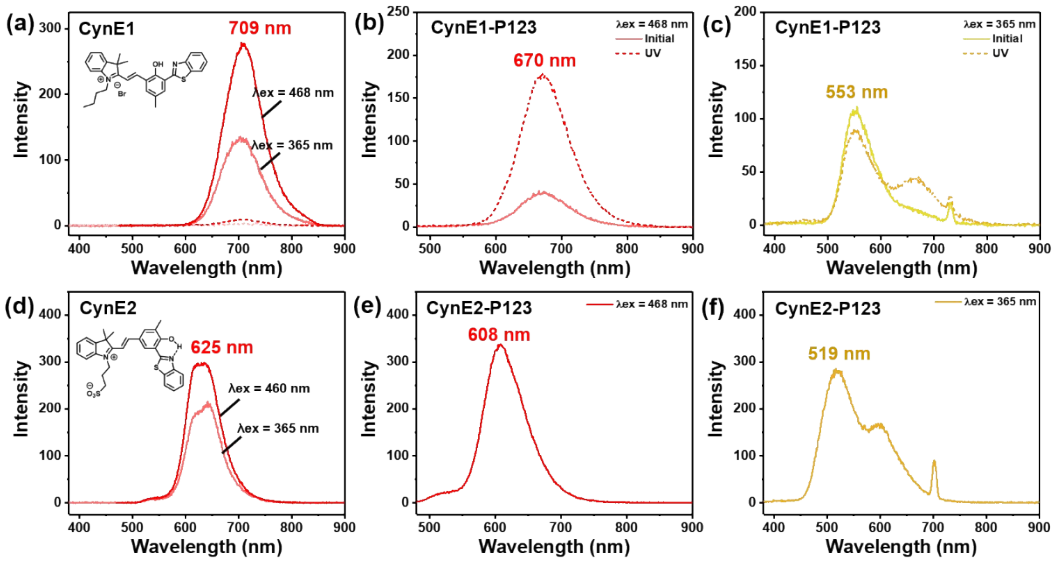


Fig. S12 (a) Fluorescence spectra of **CynE1 in ethanol** before (solid lines) and after (dashed lines) UV irradiation (λex = 365, 468 nm, C = 5 × 10^{-5} M). Fluorescence spectra of nanomicelles **CynE1-P123** before and after UV irradiation ((b) λex = 468 nm and (c) λex = 365 nm, slit (3, 3)). (d) Fluorescence spectra of **CynE2** in dichloromethane (λex = 365, 468 nm, C = 5 × 10^{-5} M). Fluorescence spectra of nanomicelles **CynE2-P123** ((e) λex = 468 nm and (f) λex = 365 nm, slit (3, 3)).

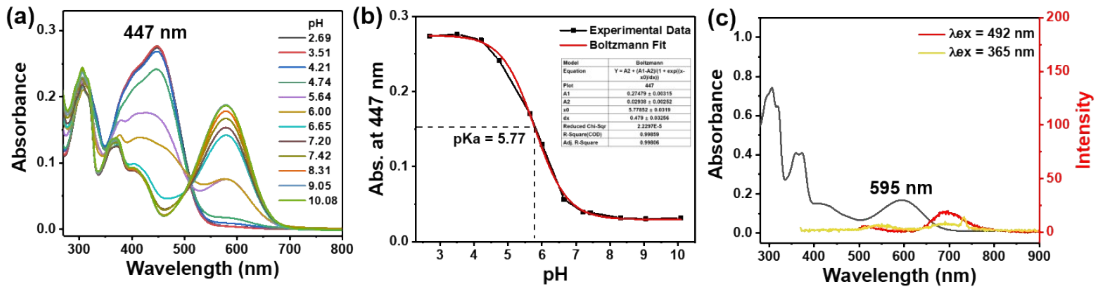


Fig. S13 (a) UV-vis absorption spectra of **CynE** (C = 1.5×10^{-5} M) in buffer solution at different pH. (b) Boltzmann's fitting for the plot of absorbance at 447 nm versus pH value for the compound **CynE**. The pKa from the plot is 5.77. (c) Absorption and fluorescence spectra of **CynE** in THF / H_2O (10 / 90) (C = 5 × 10^{-5} M, slit (3, 3)).

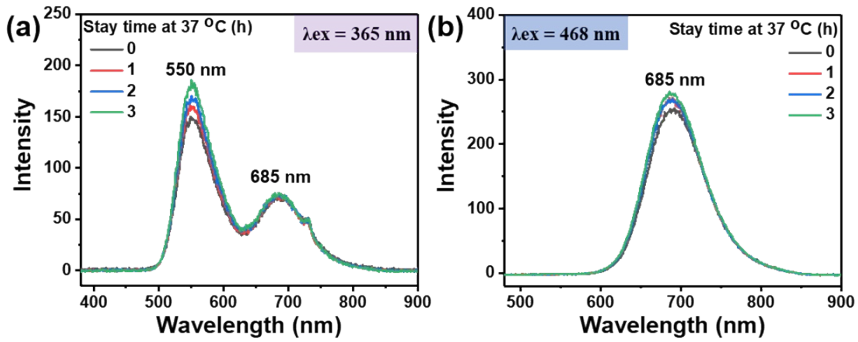


Fig. S14 Fluorescence spectra for prepared **CynE-P123** staying at 37 °C for hours. Excitation light is 365 nm, slit (3, 3).

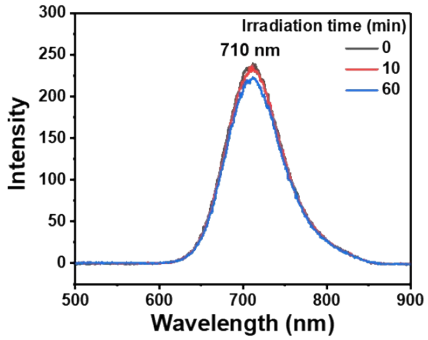


Fig. S15 Fluorescence spectra of **CynE** in MeOH upon irradiation with portable ultraviolet lamp (365 nm). The excitation wavelength was 492 nm, slit (3,3).

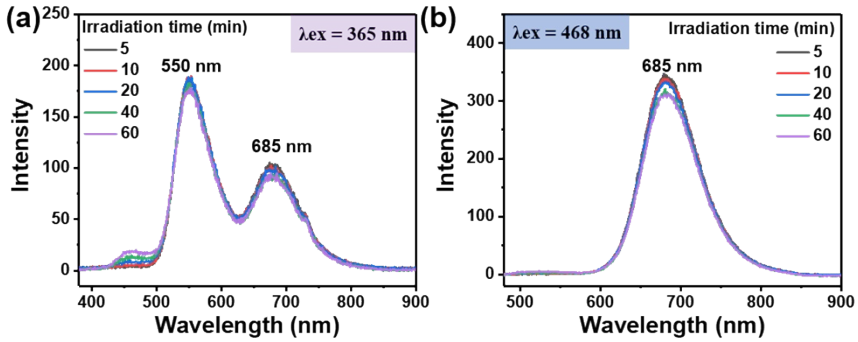


Fig. S16 Fluorescence spectra of **CynE-P123** upon irradiation with Portable ultraviolet lamp (365 nm), slit (3,3).

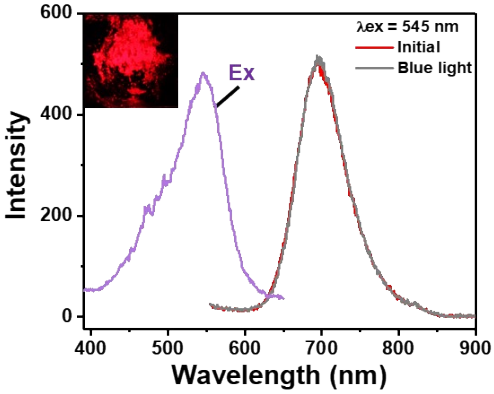


Fig. S17 Excitation (Ex) and emission spectra of **CynE** in solid state. The excitation spectrum for emission at 692 nm, slit (1.5, 3).

Table S5 Photochromic parameters of **CynE** and **CynE-P123**

	Open-ring isomers		Closed-ring isomers		Φ^a		PR (%)
	absorption λmax (nm)	ε (10^4 cm^{-1} M^{-1})	absorption λmax (nm)	ε (10^4 cm^{-1} M^{-1})	Φ_{O-C}	Φ_{C-O}	
CynE[b]	458	3.2	355	1.0	0.32[c]	0.29[c]	95 (99[d])
CynE-P123[e]	455	-	372	-	0.36[f]	0.28[f]	99

[a] Quantum yields of cyclization reaction (Φ_{o-c}) and cycloreversion reaction (Φ_{c-o}). [b] In MeOH. [c] Calculated quantum yields according to reported method (Tetrahedron 2006, 62, 5855–5861). [d] In THF. [e] in water. [f] determination of quantum yields is shown in Fig. S18 and Fig. S19.

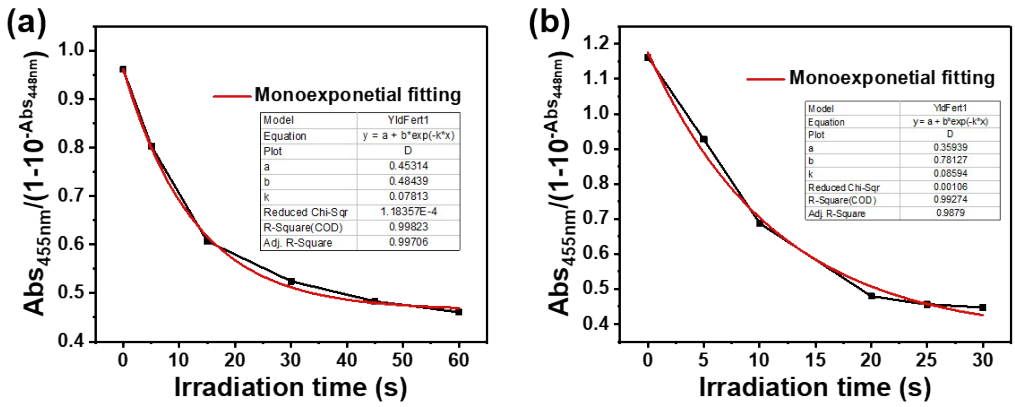


Fig. S18. Photocyclization quantum yield measurement. (a) Corrected absorption of **CynE** at 455 nm in MeOH upon continuous irradiation of blue light (448 nm). (b) Corrected absorption of **CynE** at 455 nm in nanomicelles upon continuous irradiation of blue light (448 nm). From the comparison of two rate constants (k) in (a) and (b), the photocyclization quantum yield of **CynE** in nanomicelles is calculated from the reference value (0.32) in MeOH, resulting in a value of 0.36.

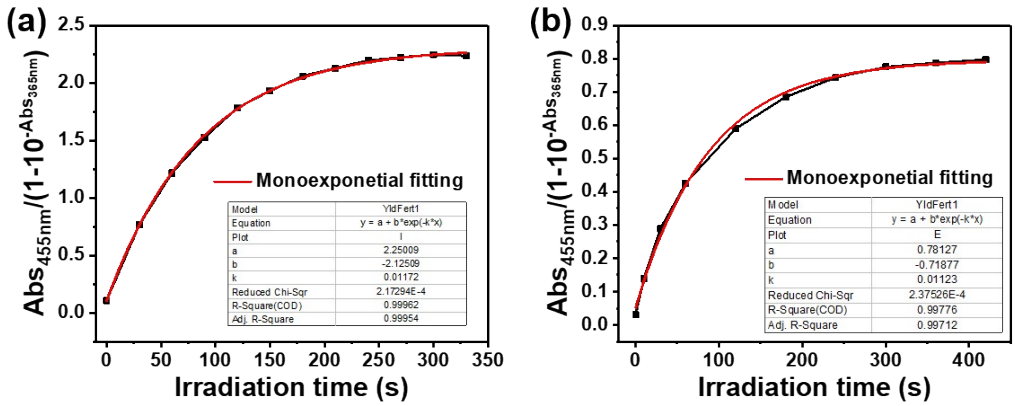


Fig. S19. Photocycloreversion quantum yield measurement. (a) Corrected absorption of **CynE** at 455 nm in MeOH upon continuous irradiation of UV light (365 nm). (b) Corrected absorption of **CynE** at 455 nm in nanomicelles upon continuous irradiation of UV light (365 nm). From the comparison of two rate constants (k) in (a) and (b), the photocyclization quantum yield of **CynE** in nanomicelles is calculated from the reference value (0.29) in MeOH, resulting in a value of 0.28.

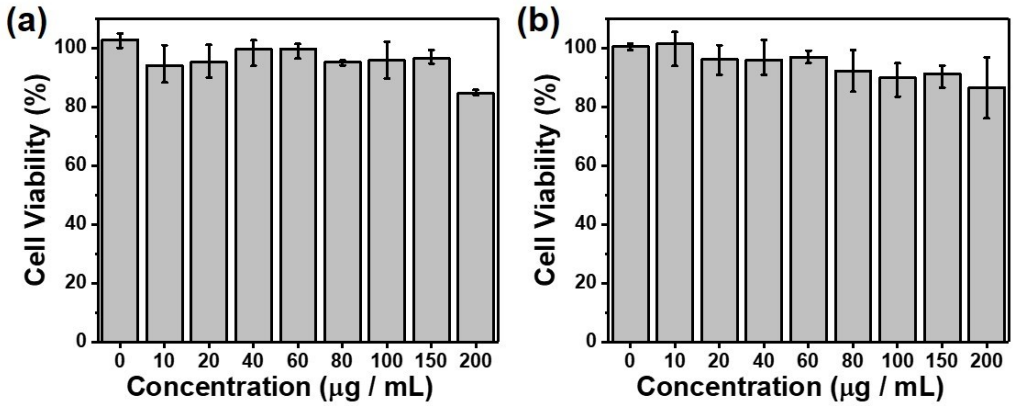


Fig. S20 Cell viability of HeLa cells incubated with different concentration of (a) **CynE** and (b) **CynE-P123** NMs for 24 hours.

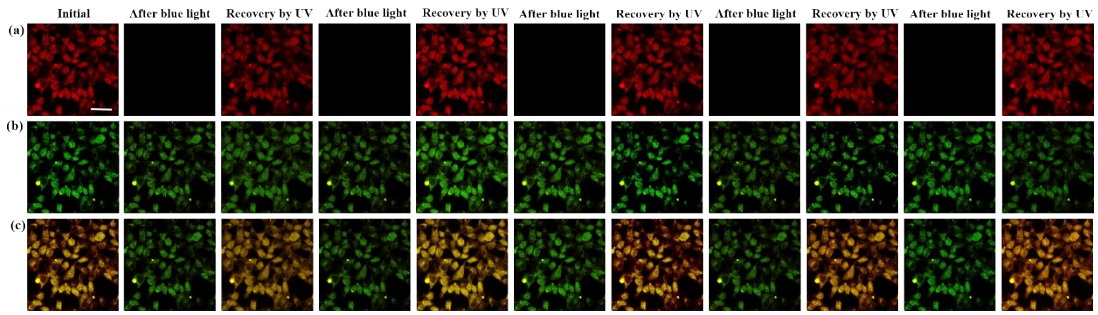
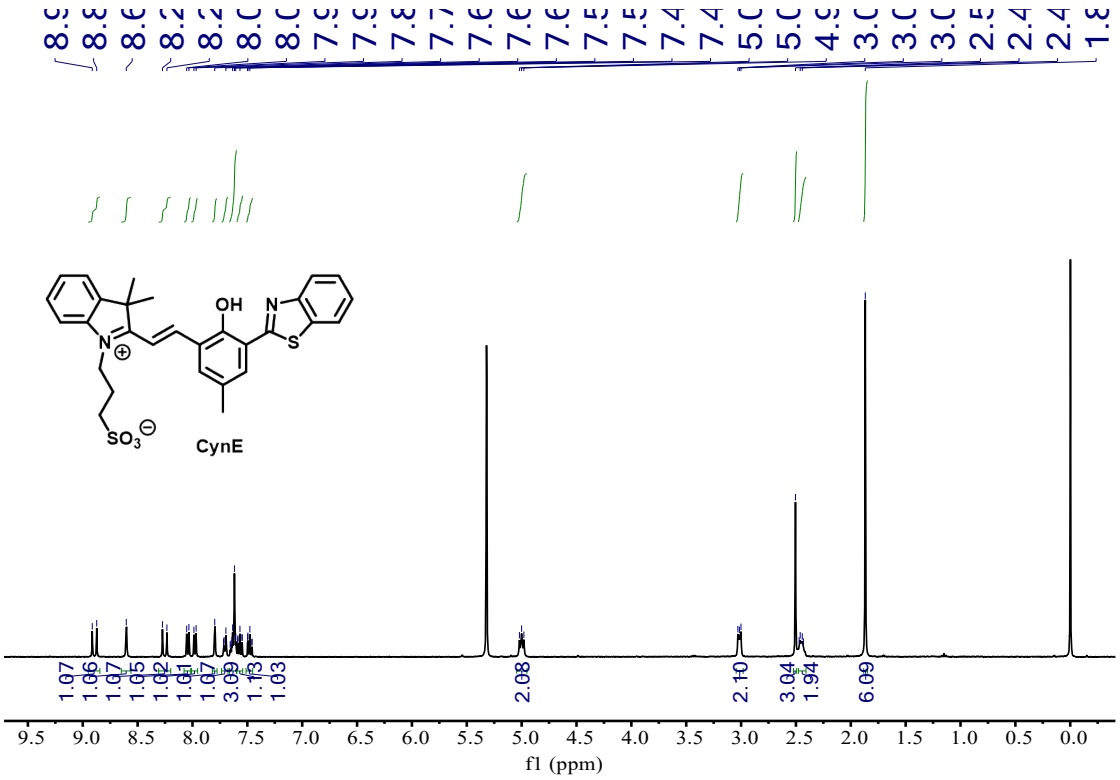
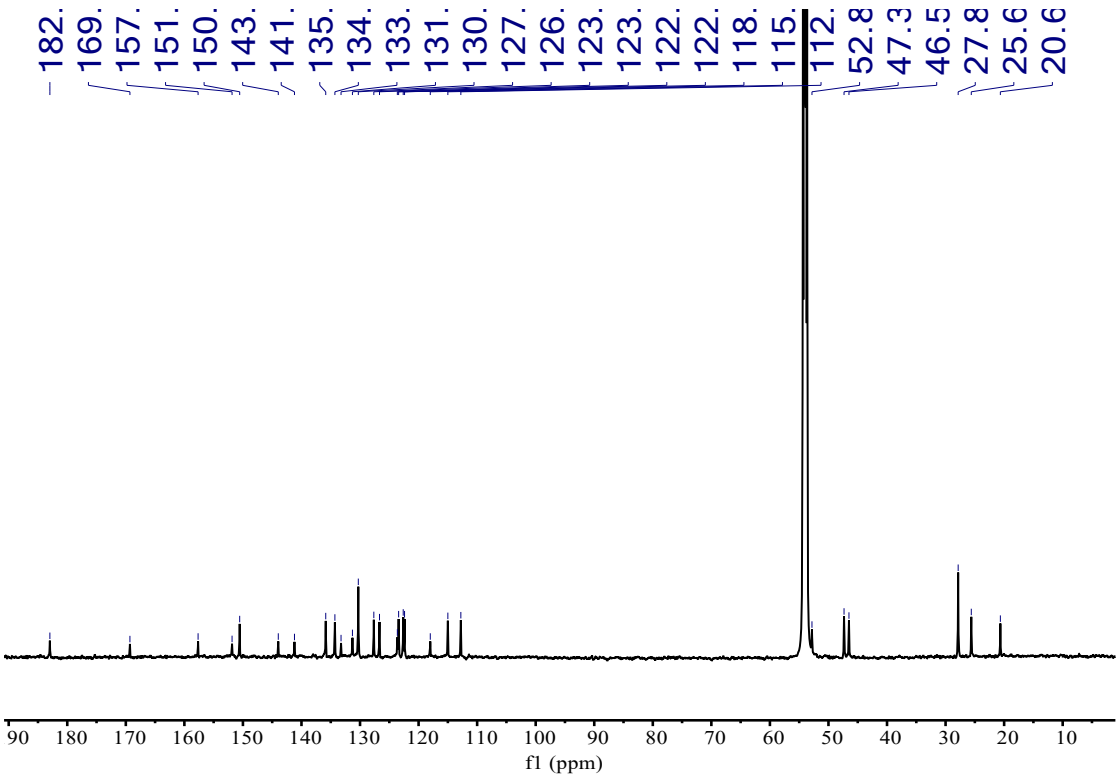


Fig. S21 Confocal microscopy images of the HEK293 cells incubated with **CynE-P123** nano micelles for 6 h: (a) red channel (713 nm), excitation at 488 nm; (b) green channel (540 nm), excitation at 384 nm; and (c) merged images of red and green channels. (scale bar: 50 μm).

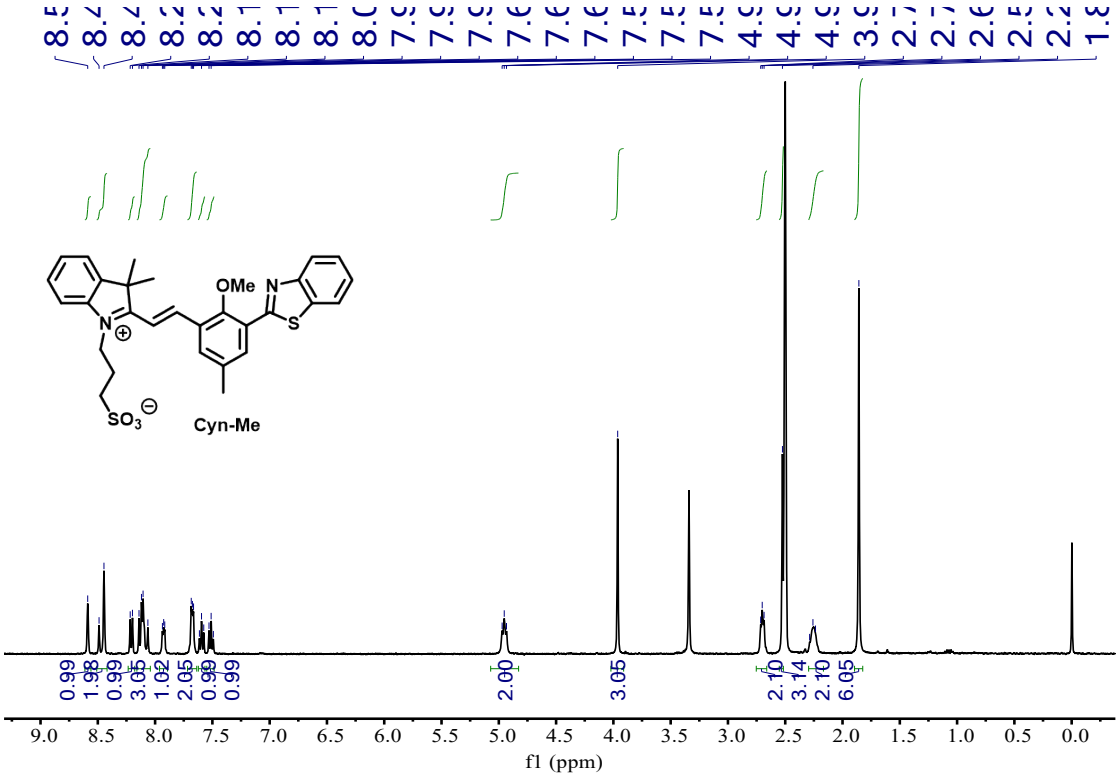
3. ^{1}H NMR and ^{13}C NMR spectra

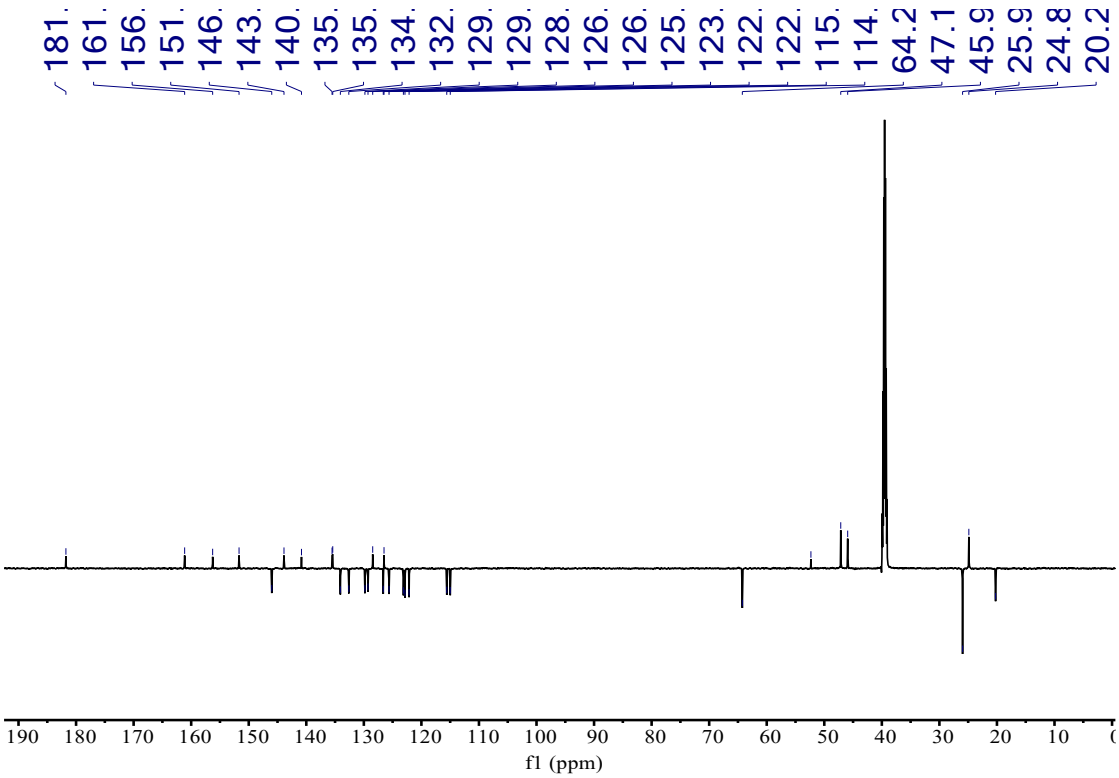
^{1}H NMR and ^{13}C NMR of CynE



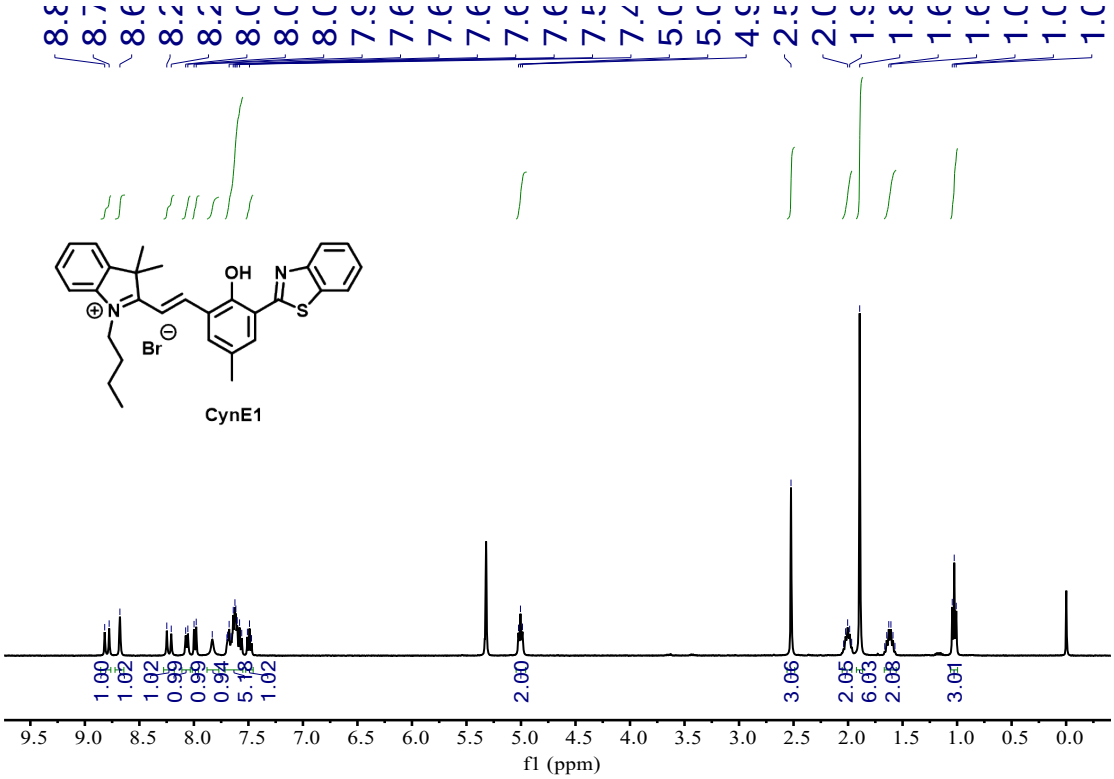


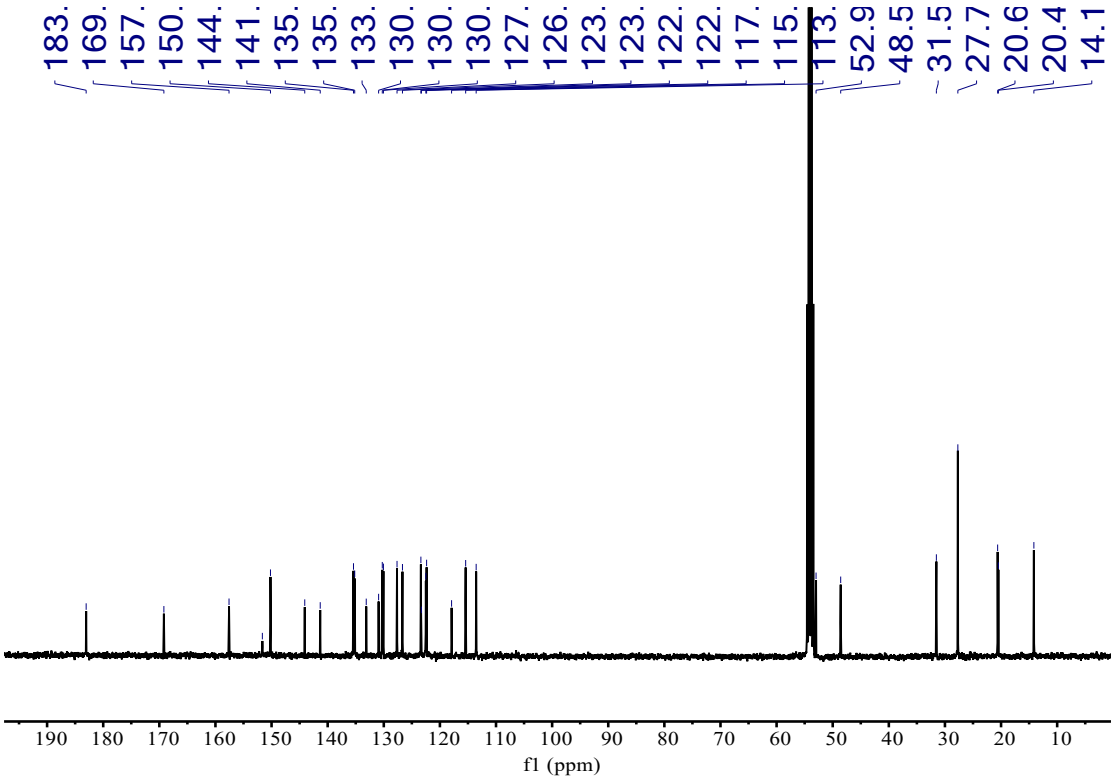
^{1}H NMR and ^{13}C NMR of Cyn-Me





^{1}H NMR and ^{13}C NMR of CynE1





4. Reference

[1] Z. Shi, P. Peng, D. Strohecker, Y. Liao, Long-lived photoacid based upon a photochromic reaction. *J. Am. Chem. Soc.* **2011***, 133*, 14699-14703.

[2] A. Paul, R. Mengji, O. A. Chandy, S. Nandi, M. Bera, A. Jana, A. Anoop, N. D. P. Singh, ESIPT-induced fluorescent o-hydroxycinnamate: a self-monitoring phototrigger for prompt image-guided uncaging of alcohols. *Org Biomol Chem* **2017**, *15*, 8544-8552.

[3] H. Zhang, Z. Huang, G. Feng, Colorimetric and ratiometric fluorescent detection of bisulfite by a new HBT-hemicyanine hybrid. *Anal. Chim. Acta* **2016**, *920*, 72-79.