

Electronic Supplementary Information

Rational design of a ratiometric and lysosome-targetable

AIE dots for imaging endogenous HClO in live cells

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Experimental Section

Synthesis of SA-C2

A solution of stearoyl chloride (903.5 mg, 3.0 mmol) in dry THF (3 mL) was added to the mixture of compound **2** (500 mg, 1.99 mmol) and dry TEA (302 mg, 3.0 mmol) in dry THF (20 mL) at 0 °C. After being stirred at 0 °C for 2 h, the mixture was then stirred at room temperature overnight. After that, the solution was evaporated under reduced pressure. The residue was further purified by column chromatography using DCM/MeOH (40:1) as the eluent to afford a yellow solid (912 mg, 88%). ¹H NMR (500 MHz, CDCl₃) δ 9.05 (d, *J* = 8.5 Hz, 1H), 7.76 (t, 1H), 7.67 (d, *J* = 8.7 Hz, 1H), 7.43 (t, *J* = 7.7 Hz, 1H), 6.79 (s, 1H), 4.55 (t, *J* = 6.3 Hz, 2H), 4.42 (t, *J* = 6.3 Hz, 2H), 2.63 (s, 3H), 2.30 (t, *J* = 7.5 Hz, 2H), 1.61 – 1.54 (m, 2H), 1.26 – 1.25 (m, 28H), 0.88 (t, *J* = 6.8 Hz, 3H).

Synthesis of SA-C2-PCD

The mixture of **SA-C2** (200 mg, 0.387 mmol), 1-pyrenecarboxaldehyde (178 mg, 0.774 mmol), piperidine (5 drops), and dry CH₃CN (15 mL) and dry THF (15 mL) was refluxed under N₂ for 24 h. After the completion of the reaction, the solution was evaporated under reduced pressure. The residue was further purified by column chromatography using DCM/PE (1:1 ~ 10:1) as the eluent to afford a red solid (150 mg, 53%). ¹H NMR (500 MHz, CDCl₃) δ 8.98 (d, *J* = 8.4 Hz, 1H), 8.29 (d, *J* = 3.9 Hz, 1H), 8.27 (d, *J* = 2.6 Hz, 1H), 8.19 (d, *J* = 14.3 Hz, 2H), 8.16 (d, *J* = 2.9 Hz, 1H), 8.12 (d, *J* = 8.7 Hz, 2H), 8.01 – 7.96 (m, 3H), 7.69 (t, *J* = 7.6 Hz, 1H), 7.52 (d, *J* = 8.7 Hz, 1H), 7.38 (t, *J* = 7.7 Hz, 1H), 7.33 (d, *J* = 15.4 Hz, 1H), 7.10 (s, 1H), 4.45 (s, 4H), 2.26 (t, *J* = 7.6 Hz, 2H), 1.53 – 1.45 (m, 2H), 1.32 – 1.04 (m, 28H), 0.87 (t, *J* = 7.0, 6.1 Hz, 3H).

¹³C NMR (126 MHz, CDCl₃) δ 173.48, 153.34, 148.29, 138.18, 137.24, 133.40, 132.61, 131.26, 130.59, 129.33, 128.94, 128.66, 128.58, 127.30, 126.97, 126.40, 126.18, 125.93, 125.28, 124.86, 124.45, 123.94, 122.04, 121.77, 121.32, 119.83, 118.90, 115.88, 107.81, 60.56, 52.76, 46.56, 34.00, 31.94, 29.67, 29.62, 29.55, 29.38, 29.19, 29.07, 24.72, 22.71, 14.15.

MS: TOF-MS *m/z* calculated for C₅₀H₅₅N₃O₂: 729.4, found: 730.4 [M + H]⁺ (Scheme S1 and Fig. S4).

Synthesis of MAA-CO720

A solution of DCC (1030 mg, 5 mmol) in dry DMF (5 mL) was added to the mixture of 4-Morpholineacetic acid (725 mg, 5 mmol), IGEPAL CO-720 (749 mg, 1 mmol), and DMAP (12.2 mg, 0.1 mmol) in dry DMF (20 mL) via the pressure-equalized dropping funnel at 0 °C. After being stirred at room temperature overnight, the solution was evaporated under reduced pressure. The residue was further purified by column chromatography using DCM/MeOH (30:1) as the eluent to afford a light yellow sticky solid (622 mg, 71%). ¹H NMR (500 MHz, CDCl₃) δ 7.06 (d, *J* = 31.0 Hz, 2H), 6.71 (s, 2H), 4.15 (d, *J* = 4.6 Hz, 2H), 3.98 (d, *J* = 4.0 Hz, 2H), 3.80 – 3.42 (m, 48H), 3.18 – 3.00 (m, 3H), 2.71 – 2.32 (m, 7H), 1.70 – 0.27 (m, 21H).

Preparation of AIED-Lyso

AIED-Lyso was prepared via typical procedures as follows: a mixture solution of PEO₁₁₃-*b*-PS₄₆ (8 mg), MAA-CO720 (0.8 mg) and **SA-C2-PCD** (0.8 mg) in THF (1 mL) was firstly prepared. Then, the solution was quickly injected into 10 mL of water under vigorous sonication. After that, the residual THF was removed by rotatory evaporation at 30°C and filtrated by using a 0.22 µm syringe driven filter. Finally, the stable **AIED-Lyso** dispersion (80 µg / mL) was obtained.

Instruments and methods

¹H NMR and ¹³C NMR spectra were measured on a Bruker Avance 500 MHz NMR spectrometer. MS was conducted with a Finnigan LCQ Advantage MAX mass spectrometer. The diameter of nanoparticle was determined by a Malvern Nano-ZS90 instrument and their morphology was observed with a JEM-2100F transmission electron microscope (TEM, JEOL USA, Inc.). UV-Vis spectra were recorded on a Shimadzu UV-2501PC spectrophotometer at room temperature (298 K). Fluorescence spectra were measured on an Edinburgh FLS920 (UK) fluorescence spectrometer at room temperature.

General Procedure for HClO detection

Unless otherwise stated, all the fluorescence measurements were performed in 10 mM PBS buffered solution (pH 4.0) according to the following procedure. In a 5 mL quartz cell, 2.0 mL of PBS and 0.1 mL of 80 µg / mL **AIED-Lyso** (final concentration: 2.67 µg / mL) were mixed together, followed by addition of an appropriate volume of NaClO solution (HClO donor, 0 µM, 1 µM, 2 µM, 3 µM, 4 µM, 5 µM, 6 µM, 7 µM, 8 µM, 9 µM, 10 µM, 11 µM, 12 µM, 13 µM, 14 µM, 15 µM, 16µM, 17 µM, 18 µM, 19 µM, 20 µM), respectively. The final volume of liquid in the cuvette was adjusted to 3.0 mL with PBS. After the incubation at room temperature for 5 min, the solution was transferred to a quartz cell of 1 cm optical length to measure the absorbance or fluorescence.

Cell viability assay and cell imaging

The cytotoxicity of the probe against Hela cells was assessed by MTT assay according to ISO 10993-5.

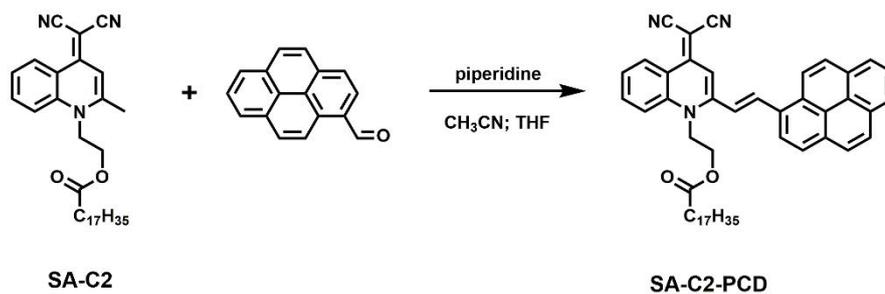
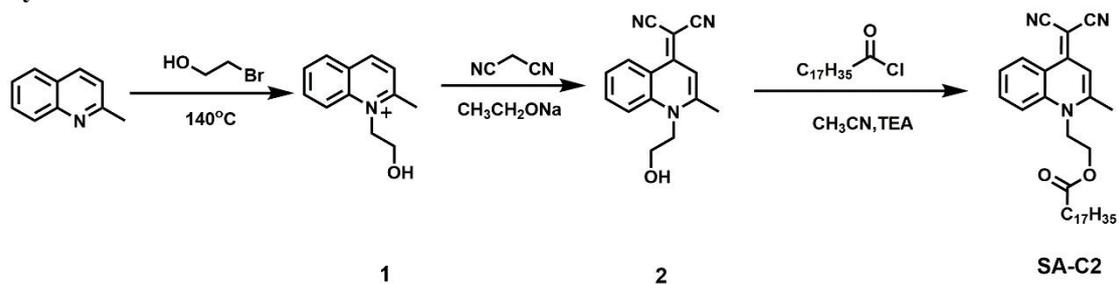
For the co-localization imaging, the cells were stained firstly with the fresh cell growth medium supplemented with **AIED-Lyso** (2.67 µg / mL) for 3 h, then LysoTracker Green (500 nM) in cell culture medium was added to prewashed cells and incubated at 37 °C for 30 min, rinsed with PBS three times, and then the fluorescence images were acquired through a Nikon A1MP confocal microscope equipped with a cooled CCD camera. Channel 1: excitation: 405 nm, red

emission collected: 575-675 nm; Channel 2 (Lyso Tracker): excitation: 468 nm, emission collected: 500-520 nm.

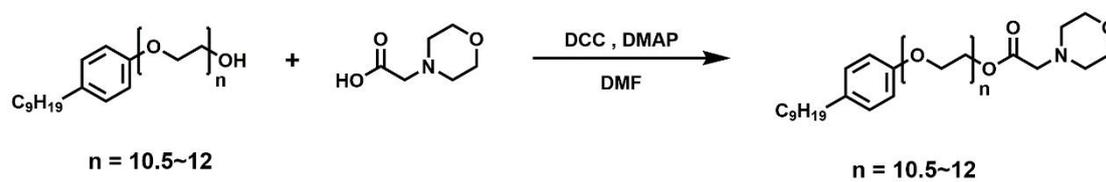
For the ratiometric imaging of exogenous HClO, HeLa cells are incubated in the RPMI1640 medium supplemented with 10% fetal bovine serum (FBS, Invitrogen). One day before imaging, the cells are passed and plated on polylysine-coated cell culture glass slides inside 30 mm glass culture dishes and allowed to grow to 50–70% confluence. Afterwards, the cells (on glass slides) are washed with RPMI1640 and re-incubated in the RPMI1640 medium containing the AIED-Lyso (final concentration: 2.67 $\mu\text{g} / \text{mL}$) for 3.0 h at 37 °C and then washed with PBS three times. After incubating with NaOCl (HClO donor, 10 μM 、 20 μM 、 30 μM) for another 0.5 h at 37 °C, the culture dishes are washed with PBS and then imaged on a Nikon A1MP confocal microscope equipped with a cooled CCD camera. Excitation: 405 nm, blue emission collected: 425-525 nm (Channel A); red emission collected: 575-675 nm (Channel B).

For the ratiometric imaging of endogenous HClO, Raw264.7 murine macrophages were cultured in Dulbeccos Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in an atmosphere of 5% CO₂ and 95% air at 37 °C. The cells were plated in 35 mm glass-bottom culture dishes and allowed to adhere for 24 h. For the detection of endogenously produced HClO, the living RAW 264.7 macrophages were treated with LPS (1 $\mu\text{g} / \text{mL}$) for 12 h, and then further co-incubated with PMA (1 $\mu\text{g} / \text{mL}$) and AIED-Lyso (2.67 $\mu\text{g} / \text{mL}$) for 3.0 h. Prior to imaging, the cells were washed three times with PBS (1 mL), and the fluorescence images were imaged on a Nikon A1MP confocal microscope equipped with a cooled CCD camera. Excitation: 405 nm, blue emission collected: 425-525 nm (Channel A); red emission collected: 575-675 nm (Channel B).

Synthesis routes:



Scheme S1. Synthesis route of **SA-C2-PCD**.



Scheme S2. Synthesis route of **MAA-CO720**.

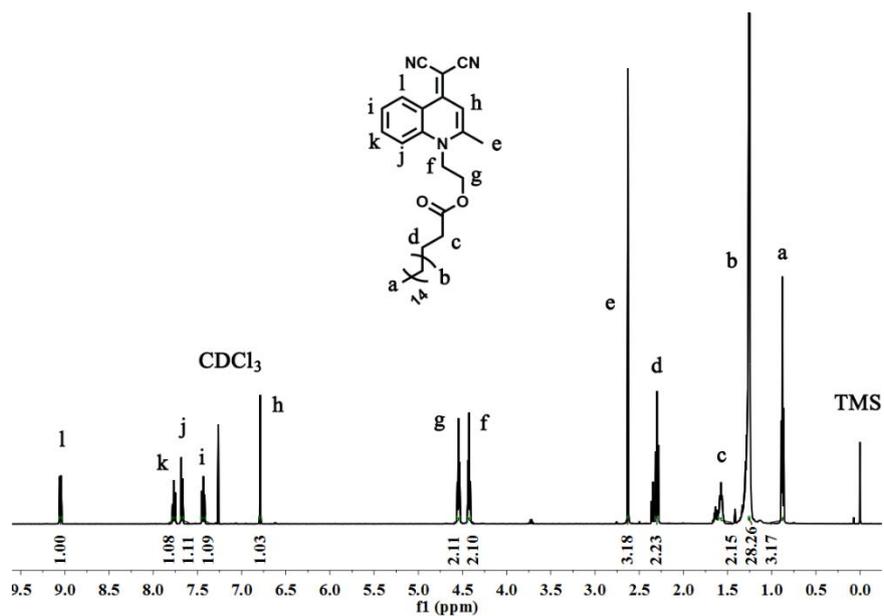


Figure S1. ¹H NMR spectrum (in CDCl₃) of SA-C2.

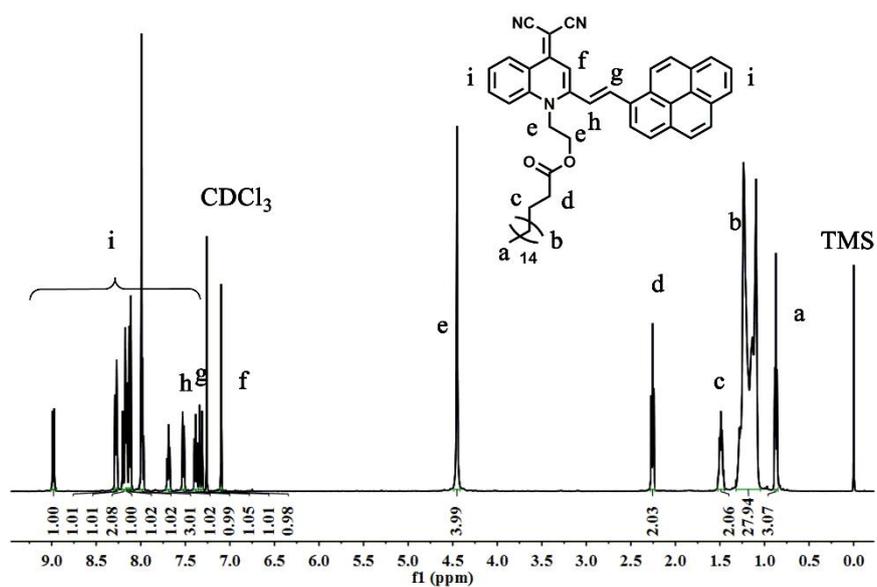


Figure S2. ^1H NMR spectrum (in CDCl_3) of SA-C2-PCD.

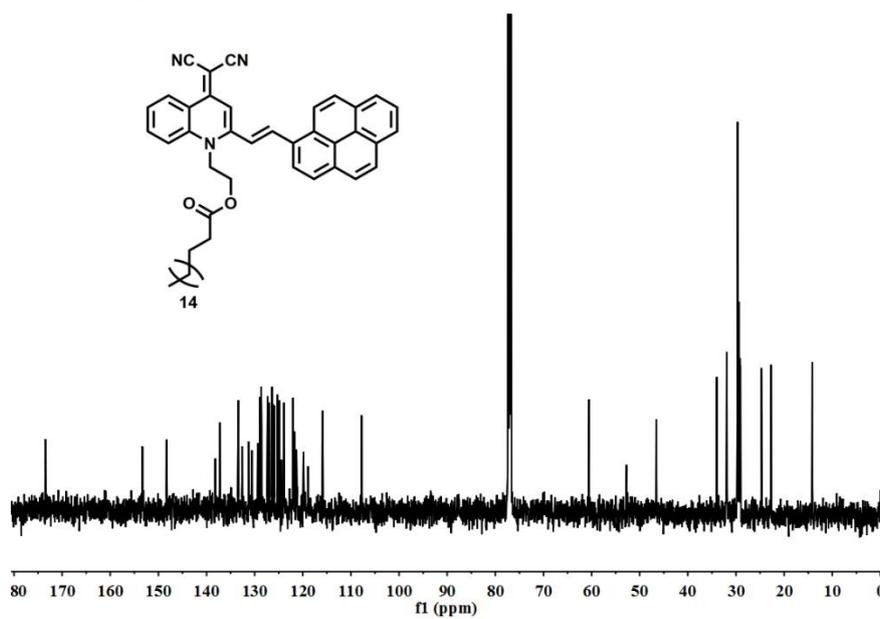


Figure S3. ^{13}C NMR spectrum (in CDCl_3) of SA-C2-PCD.

730 #120 RT: 1.34 AV: 1 NL: 4.33E4
T: + c ESI Full ms [500.00-900.00]

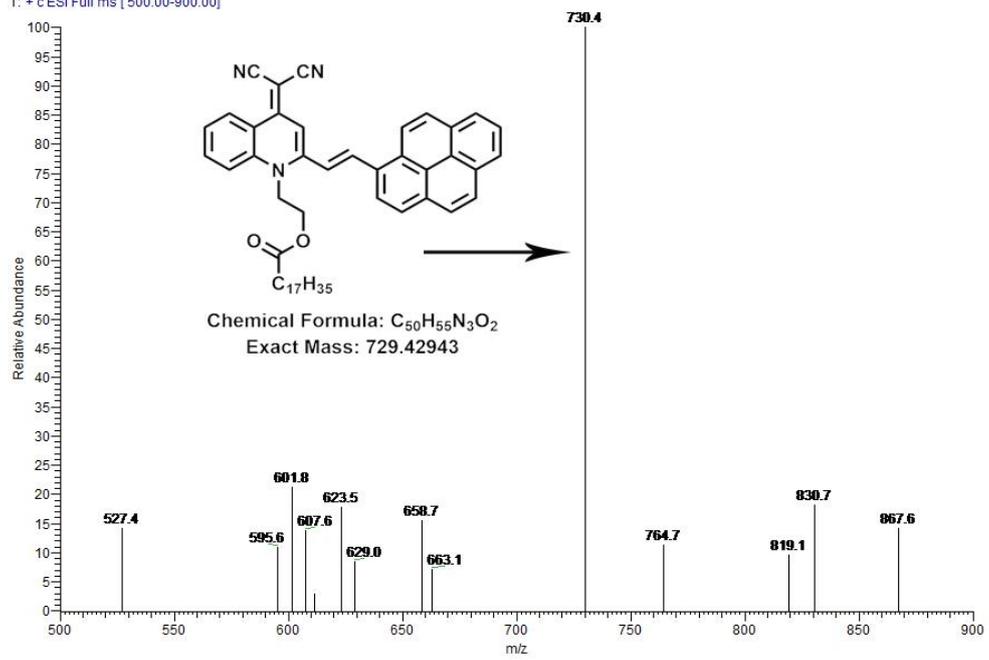


Figure S4. Mass spectrum of SA-C2-PCD.

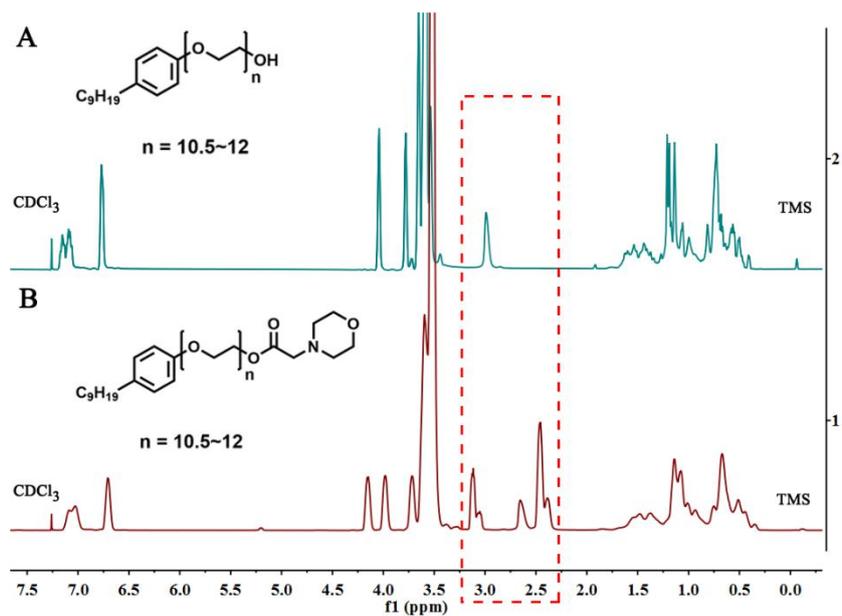


Figure S5. ¹H NMR spectrum (in CDCl₃) of (A) **IGEPAL CO-720**; (B) **MAA-CO720**.

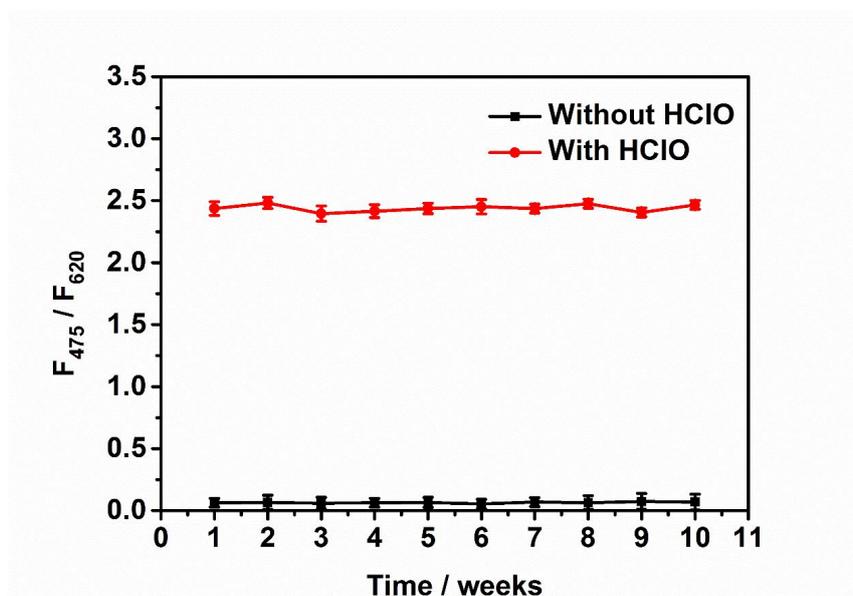


Figure S6. Fluorescence long-term stability of AIED-Lyso (2.6 $\mu\text{g} / \text{mL}$) without and with HClO (20 μM). $\lambda_{\text{ex}} = 399 \text{ nm}$.

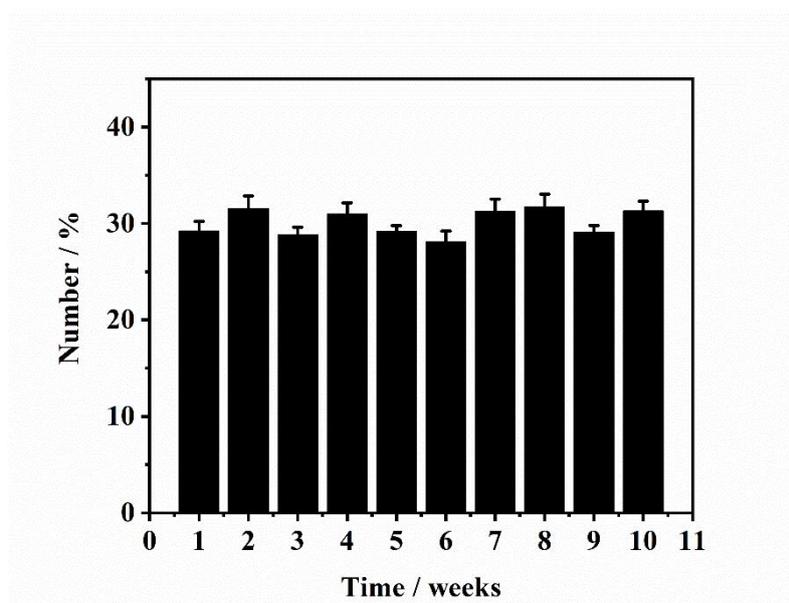


Figure S7. Average diameter of AIED-Lyso (2.6 µg/mL) at different time slots.

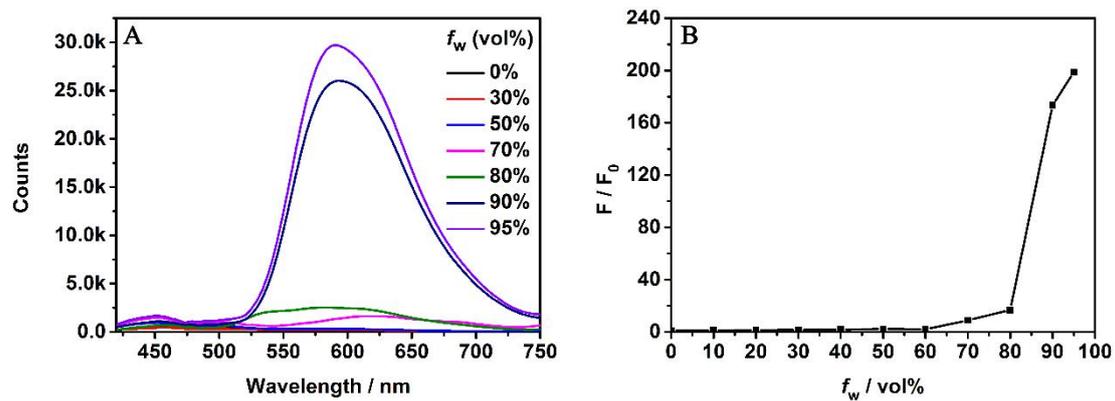


Figure S8. (A) Fluorescence emission spectra of SA-C2-PCD in water / THF mixtures with varied water fractions, $\lambda_{\text{ex}} = 399$ nm. (B) Variations in F / F_0 of SA-C2-PCD with f_w . F_0 and F are the PL intensities in THF ($f_w = 0$) and a water / THF mixture with a specific f_w , respectively.

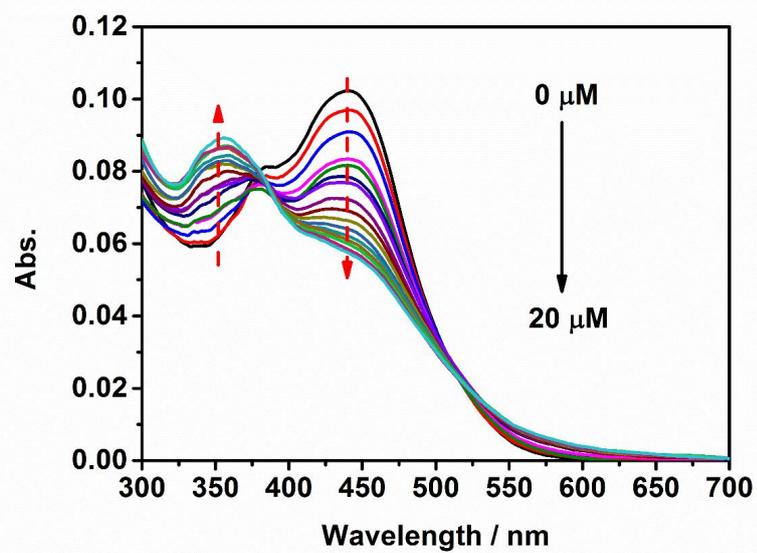


Figure S9. Absorbance spectra of AIED-Lyso (2.67 μg / mL) in pH 4.0 PBS buffer under different concentration of HClO (0 ~ 20 μM).

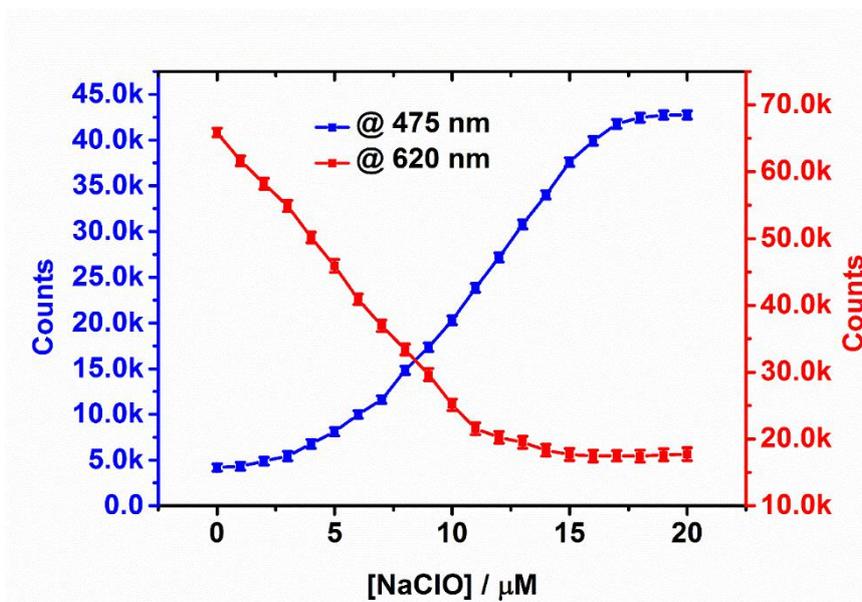


Figure S10. Plot of the fluorescence intensity at 475 nm (blue) and 620 nm (red) versus HClO concentration (0 ~ 20 μM) of AIED-Lyso (2.67 $\mu\text{g} / \text{mL}$) in pH 4.0 PBS buffer.

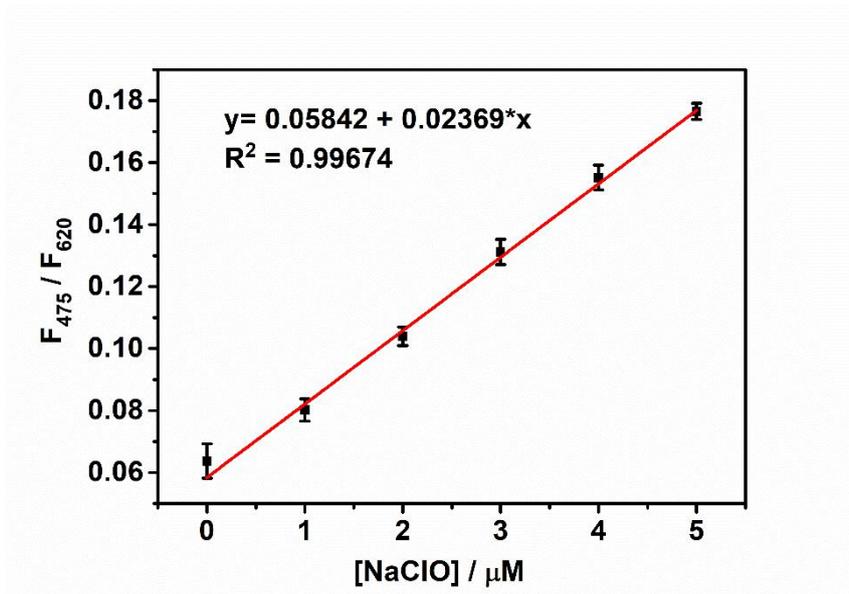


Figure S11. Linear relationship curve of F_{475} / F_{620} versus concentration of HClO ($0 \sim 5 \mu\text{M}$), $\lambda_{\text{ex}} = 399 \text{ nm}$.

Determination of the detection limit:

First the calibration curve was obtained from the plot of F_{475} / F_{620} versus HClO concentration.

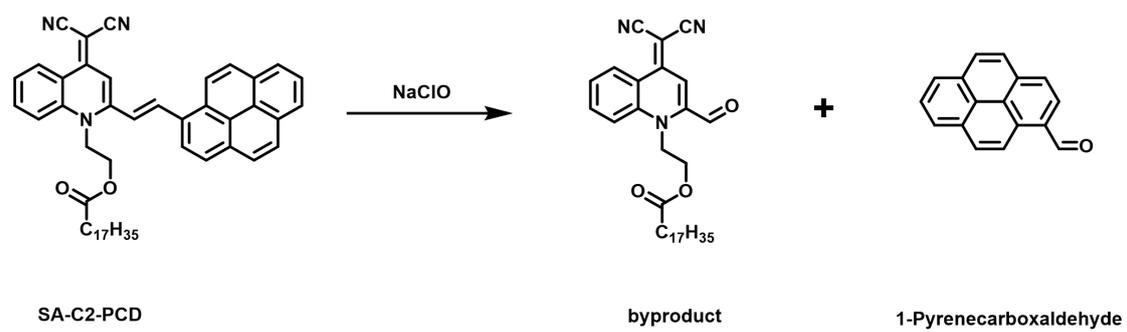
The regression curve equation was then obtained for the lower concentration part.

The detection limit = $3 \times \text{S.D.} / k$

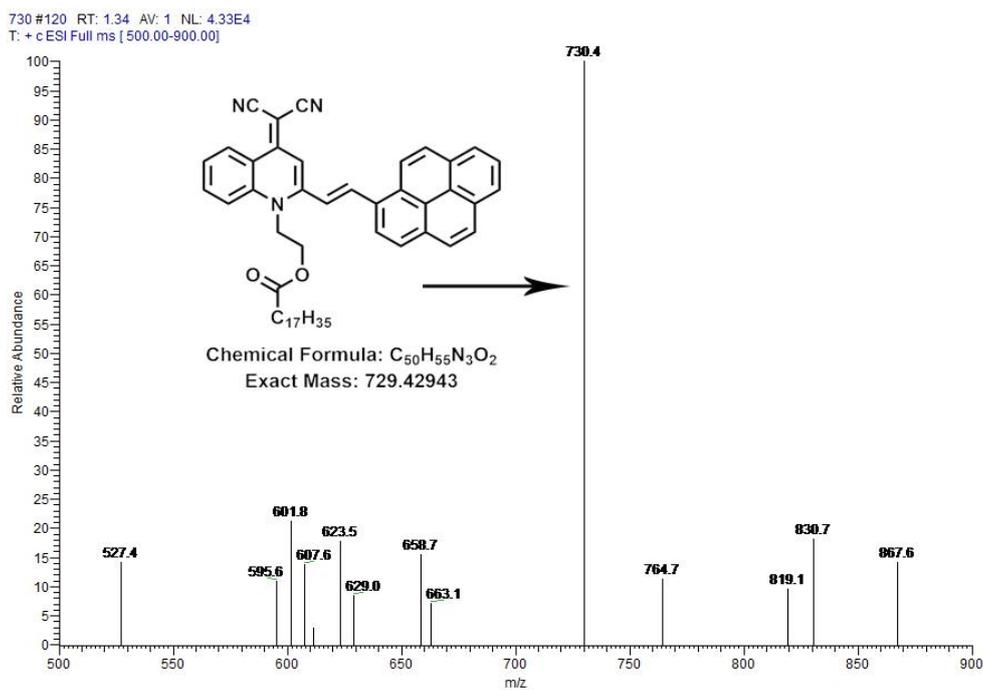
Where k is the slope of the curve equation, and S.D. represents the standard deviation for the F_{475} / F_{620} of **AIED-Lyso** in the absence of HClO.

$$F_{475} / F_{620} = 0.05842 + 0.02369 \times [\text{H}_2\text{S}] \quad (R^2 = 0.99674)$$

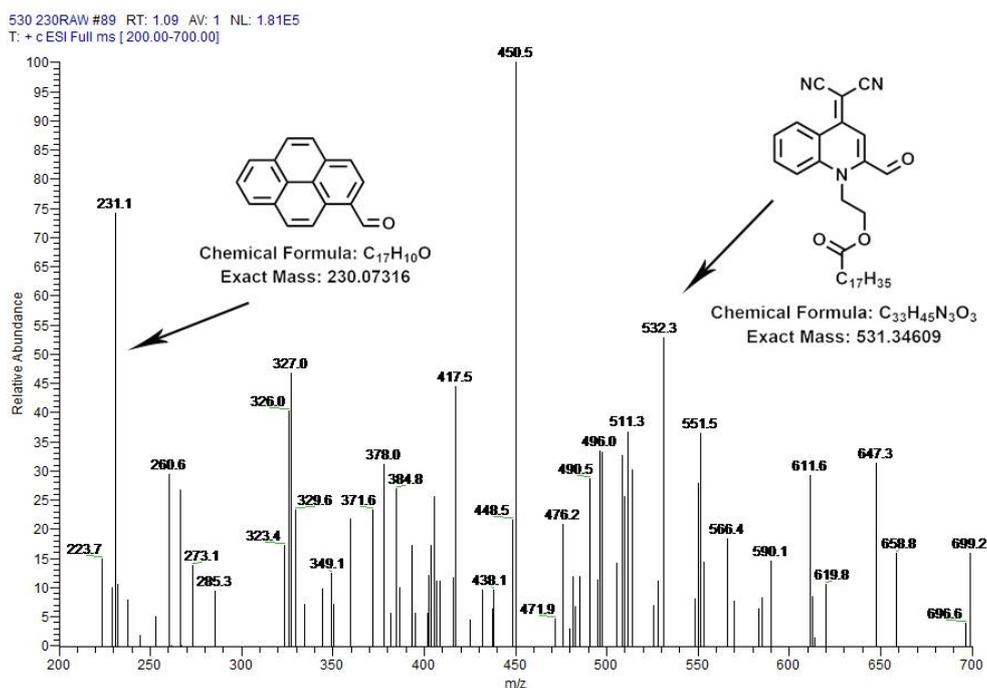
$$\text{LOD} = 3 \times 0.000556 / 0.02369 = 0.0704 \mu\text{M} = 70.4 \text{ nM}.$$



Scheme S3. Possible reaction mechanism of **SA-C2-PCD** with HClO.



(A)



(B)

Figure S12. Mass spectra of SA-C2-PCD before (A) and after (B) addition HClO. For A: the signals at m/z 730.4 are [(SA-C2-PCD) + H]⁺; For B: the signals at m/z 231.1 and 532.3 are [(1-Pyrenecarboxaldehyde) + H]⁺, [(Byproduct) + H]⁺ respectively.

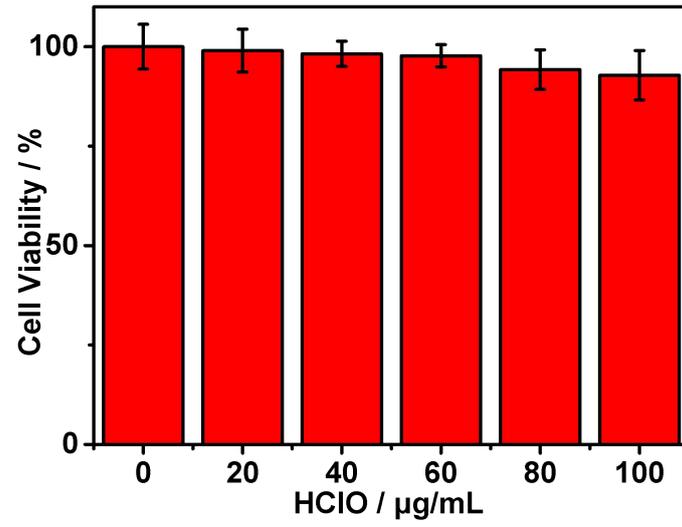


Figure S13. Viability for HeLa cells treated with the varied concentrations of AIED-Lyso for 24 h.