Electronic Supplementary Information (ESI)

Keto-Salicylaldehyde Azine: A Kind of Novel Building Block for AIE Structure and Their Application in Tracking Lipid Droplets

1. Materials and Measurements

Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), E3 embryo media were purchased from Termo Fisher Scientific (Shanghai, China), 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) was purchased from Tiangen Biotech (Beijing, China)., BODIPY 492/515, Nile Red were purchased from Life Technologies. Oleic acid was purchased from Aladdin. Benzophenone, 9-fluorenone, 1-hydroxy-2-naphthaldehyde and 5-bromosalicylaldehyde were purchased from Energy Chemical. 2-hydroxy-1-naphthaldehyde was purchased from J&K. Milli-Q water was supplied by Milli-Q Plus System (Millipore Corporation, United States). Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under nitrogen immediately prior to use. ^{1}H and ^{13}C NMR spectra were measured on a Bruker 500 spectrometer in $CDCl_3$ at room temperature. UV-vis absorption spectra were measured on a Shimadzu UV-2600 spectrophotometer. Photoluminescence spectra were recorded on a Horiba Fluoromax-4 spectrofluorometer. Solution fluorescence quantum yields were measured using a Hamamatsu absolute PL quantum yield spectrometer C11347 Quantaurus_QY. Particle size measurements were conducted on dynamic light scattering (Malvern ZSE, UK). Single crystal X-ray diffraction was conducted by SuperNova Dual, Mo at zero, Atlas. Confocal laser scanning microscope (CLSM) characterization was conducted with a confocal laser scanning biological microscope (LSM 710, Zeiss, Germany). The absorbance for MTT analysis was recorded on a microplate reader (Thermofisher, USA) at a wavelength of 570 nm. Automated cell counter (Countess II, Invitrogen) was employed for cell counting. The mean fluorescence intensity was recorded by Flow cytometry (BD Accuri C6) and analysed by FlowJo.

2. Methods

2.1 Cell culture

HeLa cervical cancer cell line were purchased from cell culture center of Institute of Basic Medical Sciences, Chinese Academy of Medical Science (Beijing, China). HeLa cells were cultured in the DMEM in a 5% CO_2 humidity incubator at 37°C, and growth media contained 10% FBS, 100 units/mL penicillin and 100 g/mL streptomycin.

2.2 Cytotoxicity studies

HeLa cells were seeded in 96-well plates at a density of 10000 cells/mL. After overnight culture of HeLa cells in the incubator, the cells were exposed to a series of doses of AIE gens varying different concentrations at 37°C. After 24 hours, MTT solution was added into each well and kept 4 hours in the incubator, thereafter MTT solubilization solution was added to dissolve the purple crystals. After 5 h incubation, the absorbance of individual well was then detected at 570 nm by

the microplate reader. Cell viability was expressed by the ratio of the absorbance of the cells incubated with AIEgens to that of the cells incubated with culture medium only.

2.3 Cell imaging

The HeLa cells were grown overnight on a 35 mm petri dish with a cover slip and then incubated with different concentrations of AIEgens for different times. The cells were imaged under a CLSM using different combination of excitation wavelength for each dye.

2.4 Free-wash test

10 μM 1-FAN or CNP-DPAN were mixed with 10 μM NaOH solution in 1 mL DMEM solution, and then incubated with HeLa cells that were grown overnight on a 35 mm petri dish with a cover slip. The cells were imaged under CLSM for different times.

2.5 The model of OA-induced LDs

The Hela cells were grown overnight on a 35 mm petri dish with six well plates and then induced by different concentrations of OA for 0.5 h, 2 h and 4.5 h, respectively. The cells was incubated with different AIEgens when removed medium. After the cells were digested by trypsin and centrifugated and washed for twice times with PBS solution, the 10000 cells from cell solution were recorded by Flow cytometry. After that, the data were analysed by FlowJo with 4000 of effective cells.

2.6 The model of larva zebrafish

Zebrafish were raised, bred, and staged according to standard protocols[1]. Larva zebrafish from liver study were Tg(lfabp:dsRed) type. From 3 d to 7 d postfertilization of the larva zebrafish were seeded into 96 well plates at 1 larva zebrafish per well. The larva zebrafish were only soaked in 3 μM 2-DPAN in E3 embryo media for 24 h at 28 ℃, and were imaged for uptake using CLSM.

2.7 Synthetic routes

2.7.1 Synthesis of 1-FAN

Fluorenone (9.01g, 50 mmol) were dissolve in THF and ethanol, followed with hydrazine hydrate (1.92 g, 60 mmol),). The mixture was reflux for 4 h. After cooling, a large amount of white needle crystals (compounds **1**) were precipitated. The Compounds **1** (7.77g, 40 mmol) and 2-hydroxy-1-naphthaldehyde (7.23g, 42 mmol) were dissolved with THF, ethanol and 2 drops of glacial acetic acid. The reaction was carried out for 4 hours under reflux. The residual solvent was removed by vacuum distillation and then purified by silica gel chromatography with DCM: PE (1:5) as eluent to afford **1-FAN** with nearly 90% yields. ^{1}H NMR ($CDCl_3$, 500M Hz), δ (TMS, ppm): 13.36 (s, 1H), 9.74 (s, 1H), 8.34-8.32 (d, J = 10.0 Hz, 1H), 8.23-8.21 (d, J = 10.0 Hz, 1H), 7.97-7.95 (d, J = 10.0 Hz, 1H), 7.92-7.90 (d, J = 10.0 Hz, 1H), 7.82-7.81 (d, J = 10.0 Hz, 1H), 7.67-7.65 (d, J = 10.0 Hz, 1H), 7.62-7.57 (m, 2H), 7.48-7.40 (m, 3H), 7.36-7.33 (m, 2H), 7.30-7.28 (d, J = 10.0 Hz, 1H).

2.7.2 Synthesis of 1-DPAN

Benzophenone (9.01g, 50 mmol) were dissolve in THF and ethanol, followed with hydrazine

hydrate (1.92 g, 60 mmol),). The mixture was reflux for 4 h. After cooling, a large amount of white needle crystals (compounds **2**) were precipitated. The Compounds **2** (7.85g, 40 mmol) and 2-hydroxy-1-naphthaldehyde (7.23g, 42 mmol) were dissolved with THF, ethanol and 2 drops of glacial acetic acid. The reaction was carried out for 4 hours under reflux. The residual solvent was removed by vacuum distillation and then purified by silica gel chromatography with DCM: PE (1:6) as eluent to afford **1-DPAN** with nearly 90% yields. ^{1}H NMR ($CDCl_3$, 500M Hz), δ(TMS, ppm): 12.83 (s, 1H), 9.74 (s, 1H), 8.19-8.17 (d, J = 10.0 Hz, 1H), 7.81-7.79 (m, 2H), 7.77-7.72 (m, 2H), 7.56-7.53 (m, 4H), 7.48-7.46 (m, 1H), 7.44-7.41 (m, 2H), 7.37-7.35 (m, 3H), 7.08-7.06 (d, J = 10.0 Hz, 1H).

2.7.3 Synthesis of 2-FAN

The Compounds **1** (7.77 g, 40 mmol) and 1-hydroxy-2-naphthaldehyde (7.23g, 42 mmol) were dissolved with THF, ethanol and 2 drops of glacial acetic acid. The reaction was carried out for 4 hours under reflux. The residual solvent was removed by vacuum distillation and then purified by silica gel chromatography with DCM: PE (1:5) as eluent to afford **2-FAN** with nearly 90% yields. ^{1}H NMR ($CDCl_3$, 500M Hz), δ(TMS, ppm): 13.00 (s, 1H), 8.90 (s, 1H), 8.52-8.50 (d, J = 10.0 Hz, 1H), 8.36-8.35 (d, J = 5.0 Hz, 1H), 7.94-7.93 (d, J = 5.0 Hz, 1H), 7.81-7.79 (d, J = 10.0 Hz, 1H), 7.67-7.66 (d, J = 5.0 Hz, 1H), 7.62-7.59 (m, 2H), 7.57-7.54 (m, 1H), 7.48-7.43 (m, 2H), 7.39 (s, 2H), 7.38-7.32 (m, 2H).

2.7.4 Synthesis of 2-DPAN

The Compounds **2** (7.85 g, 40 mmol) and 1-hydroxy-2-naphthaldehyde (7.23g, 42 mmol) were dissolved with THF, ethanol and 2 drops of glacial acetic acid. The reaction was carried out for 4 hours under reflux. The residual solvent was removed by vacuum distillation and then purified by silica gel chromatography with DCM: PE (1:6) as eluent to afford **2-DPAN** with nearly 90% yields. ^{1}H NMR ($CDCl_3$, 500M Hz), δ(TMS, ppm): 12.44 (s, 1H), 8.93 (s, 1H), 8.27-8.25 (d, J = 10.0 Hz, 1H), 7.79-7.77 (d, J = 10.0 Hz, 2H), 7.73-7.71 (d, J = 10.0 Hz, 1H), 7.56-7.50 (m, 4H), 7.47-7.36 (m, 6H), 7.33-7.29 (m, 2H).

2.7.5 Synthesis of CNP-FAS

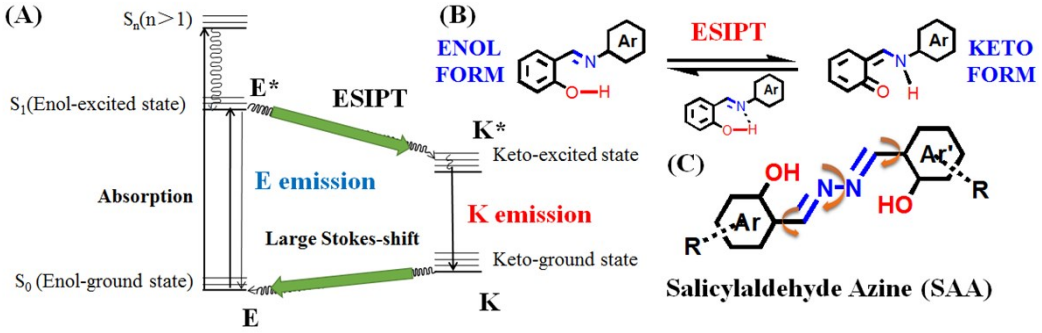
The Compounds **1** (7.77 g, 40 mmol) and 3'-formyl-4'-hydroxy-[1,1'-biphenyl]-4-carbonitrile (9.38 g, 42 mmol) were dissolved with THF, ethanol and 2 drops of glacial acetic acid. The reaction was carried out for 4 hours under reflux. The residual solvent was removed by vacuum distillation and then purified by silica gel chromatography with DCM: PE (1:2) as eluent to afford **CNP-FAS** with nearly 90% yields. ^{1}H NMR ($CDCl_3$, 500M Hz), δ(TMS, ppm): 11.86 (s, 1H), 8.78 (s, 1H), 8.18-8.16 (d, J = 10.0 Hz, 1H), 7.91-7.90 (d, J = 5.0 Hz, 1H), 7.73-7.72 (m, J = 5.0 Hz, 2H), 7.67-7.64 (m, 4H), 7.61-7.60 (m, 2H), 7.47-7.43 (m, 2H), 7.35-7.29 (m, 2H), 7.21-7.19 (d, J = 10.0 Hz, 1H).

2.7.6 Synthesis of CNP-DPAS

The Compounds **1** (7.85 g, 40 mmol) and 3'-formyl-4'-hydroxy-[1,1'-biphenyl]-4-carbonitrile (9.38 g, 42 mmol) were dissolved with THF, ethanol and 2 drops of glacial acetic acid. The reaction was carried out for 4 hours under reflux. The residual solvent was removed by vacuum distillation and then purified by silica gel chromatography with DCM: PE (1:1.5) as eluent to

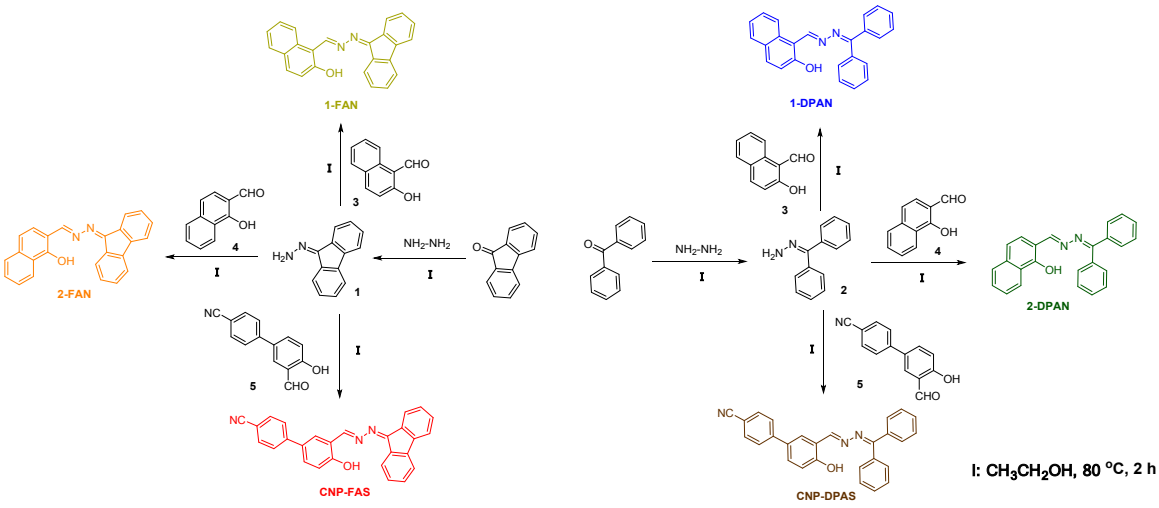
afford **CNP-DPAS** with nearly 90% yields. ^{1}H NMR ($CDCl_3$, 500M Hz), δ(TMS, ppm): 11.40 (s, 1H), 8.86 (s, 1H), 7.79-7.77 (m, 2H), 7.71-7.70 (m, 2H), 7.64-7.62 (m, 2H), 7.54-7.52 (m, 5H), 7.49-7.47 (m, 1H), 7.44-7.40 (m, 2H), 7.33-7.31 (m, 2H), 6.99-6.97 (s, 1H).

3. Diagrammatic description of the ESIPT process



Scheme S1 The process and mechanism of ESIPT (A); the general building block of ESIPT and its E and K forms (B); the SAA framework (C).[2]

4. Synthesis Routes of FAS and DPAS derivatives



Scheme S2 Synthesis Routes of FAS and DPAS derivatives.

5. NMR spectra of FAS and DPAS derivatives

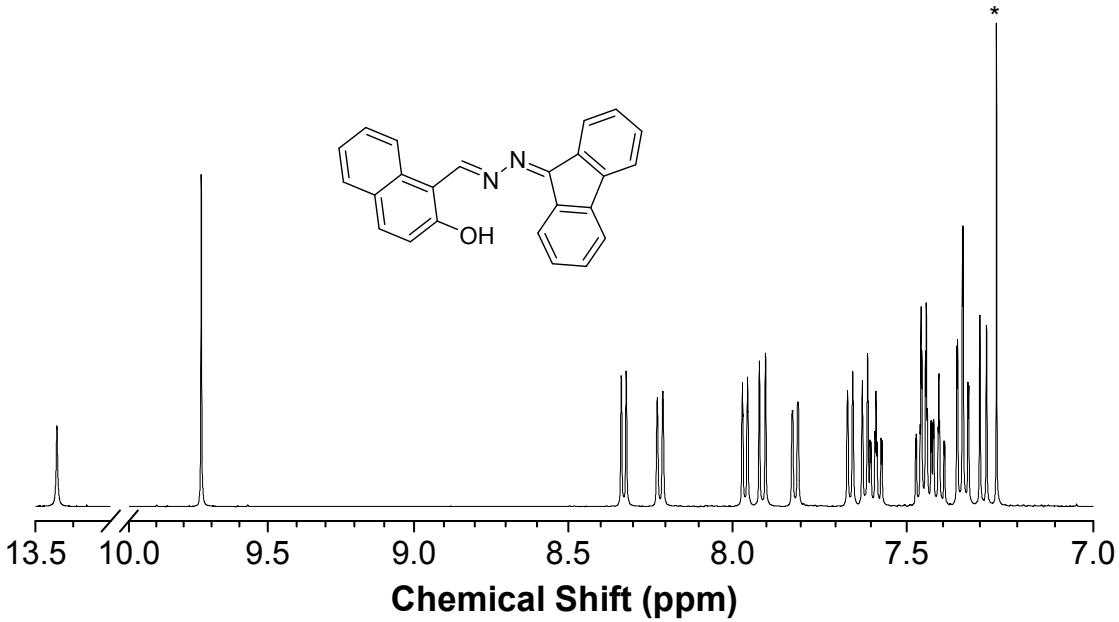


Fig. S1 ^{1}H NMR spectrum of 1-FAN in $CDCl_3$.

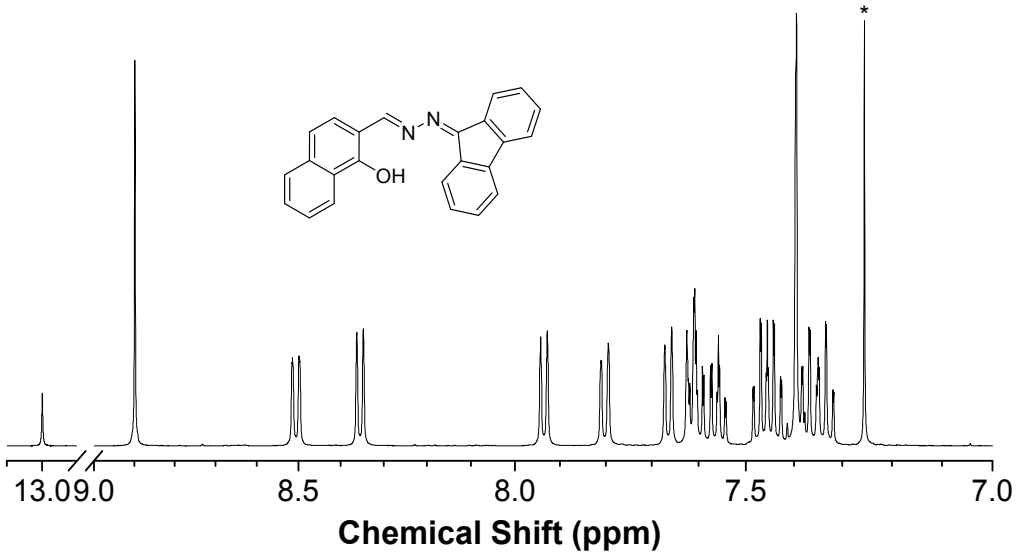


Fig. S2 ^{1}H NMR spectrum of 2-FAN in $CDCl_3$.

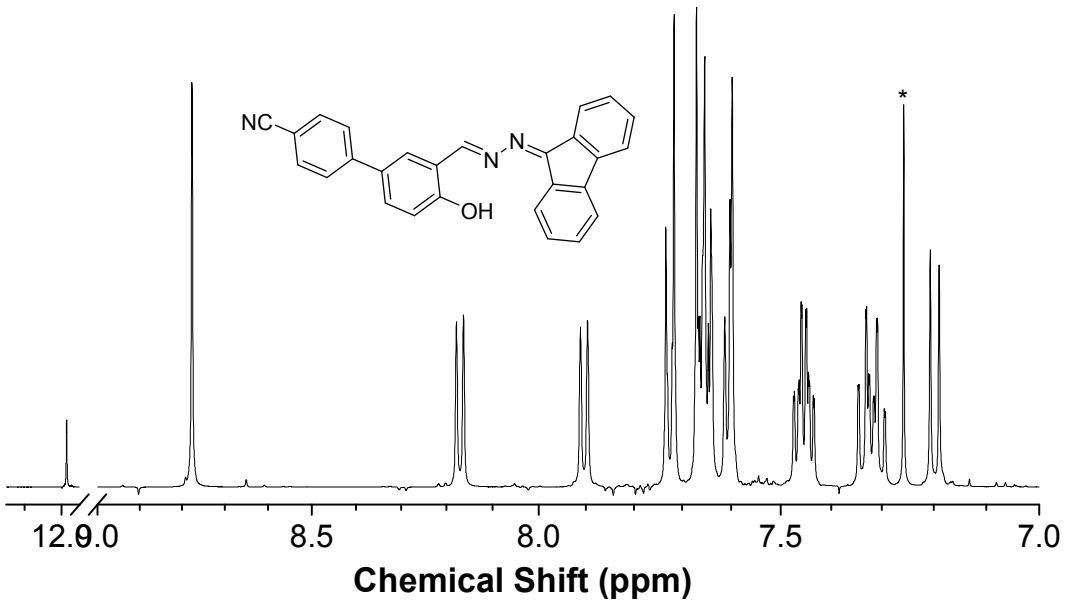


Fig. S3 ^{1}H NMR spectrum of CNP-FAS in $CDCl_3$.

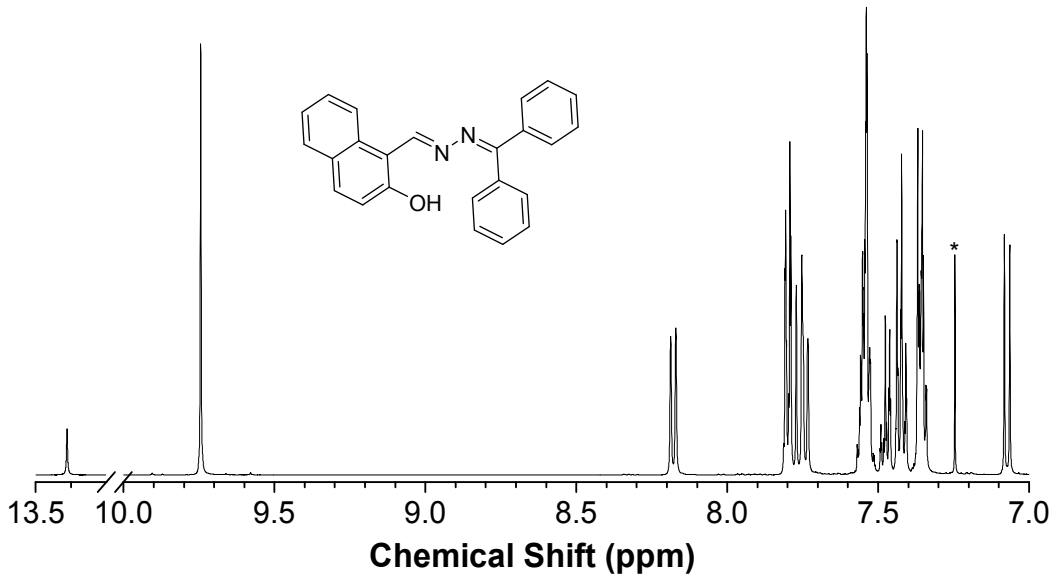


Fig. S4 ^{1}H NMR spectrum of 1-DPAN in $CDCl_3$.

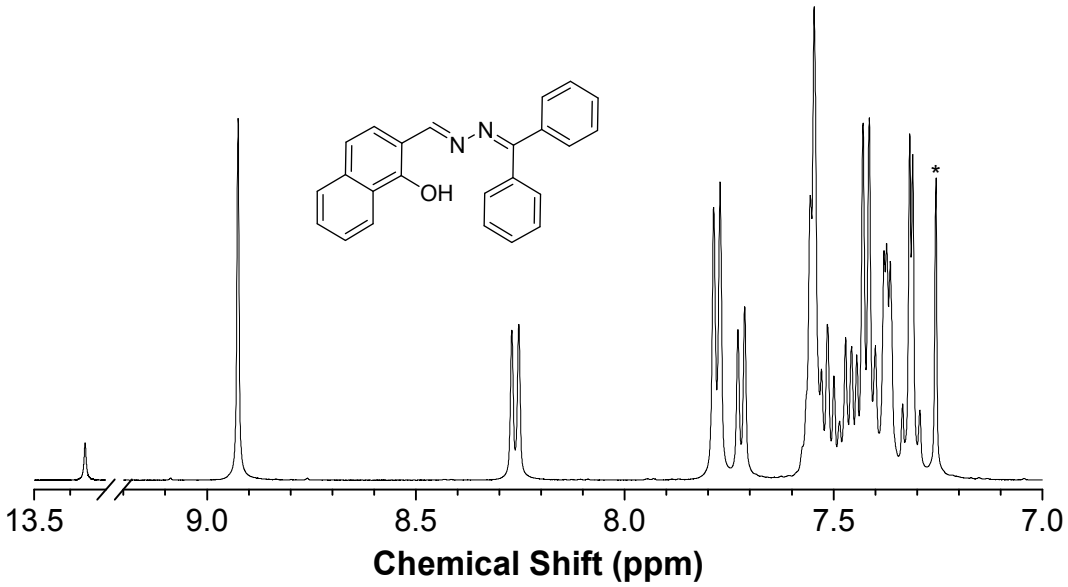


Fig. S5 ^{1}H NMR spectrum of 2-DPAN in $CDCl_3$.

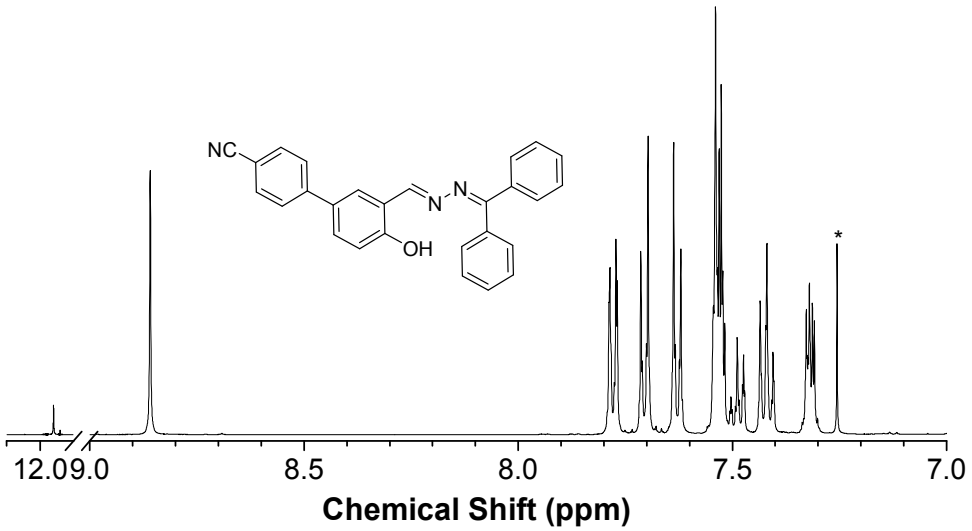


Fig. S6 ^{1}H NMR spectrum of CNP-DPAS in $CDCl_3$.

6. Crystal structures of DPAS

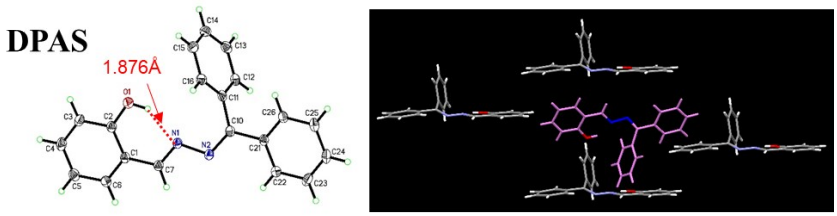


Fig. S7 ORTEP drawings of single-crystal structures and molecular packing patterns of DPAS.

7. Photophysical properties

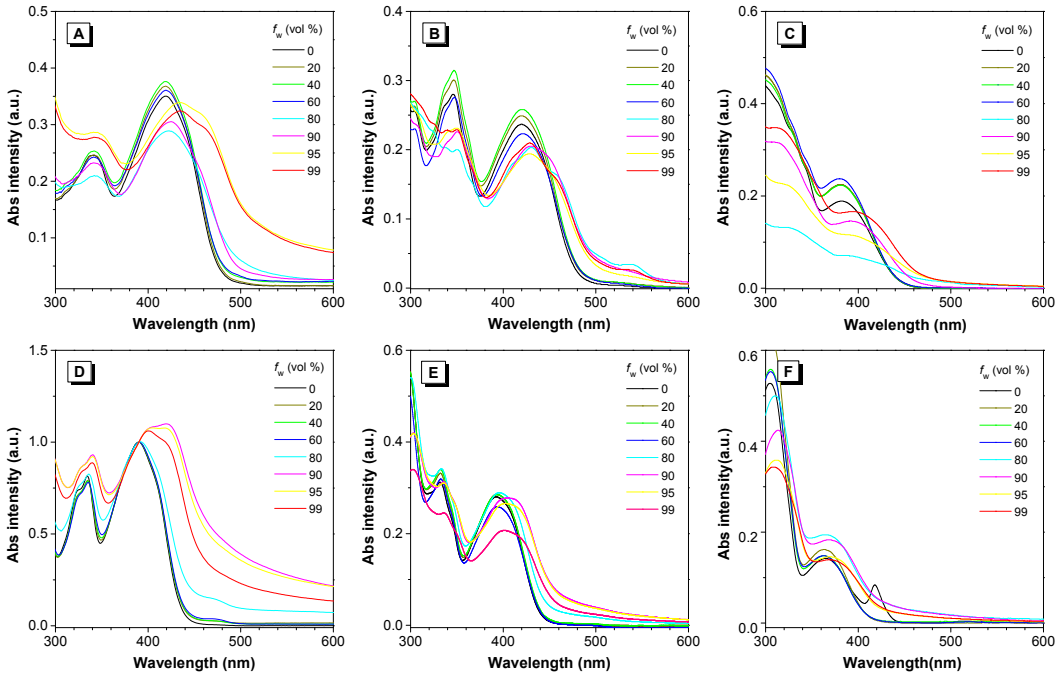


Fig. S8 The absorption spectra of (A) 1-FAN, (B) 2-FAN, (C) CNP-FAS, (D) 1-DPAN, (E) 2-DPAN, and (F) CNP-DPAS (10 μM) in THF-water mixtures with different water fractions (f_w).

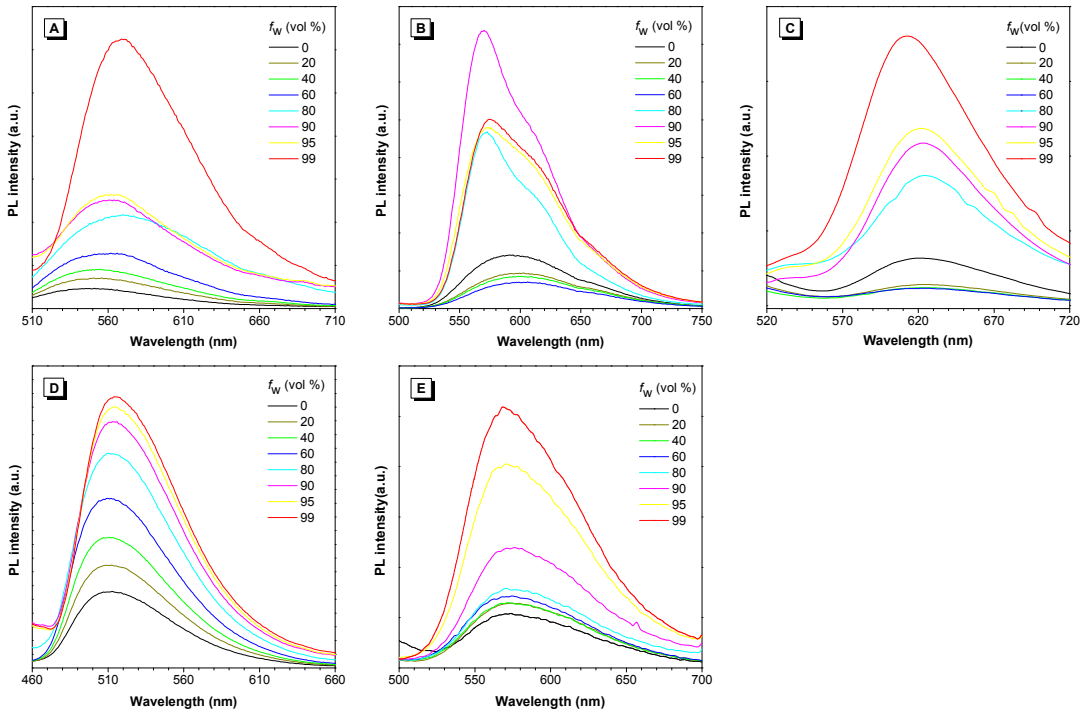


Fig. S9 The PL spectra of (A) 1-FAN, (B) 2-FAN, (C) CNP-FAS, (D) 1-DPAN, and (E) CNP-DPAS (10 μM) in THF-water mixtures with different water fractions (f_w).

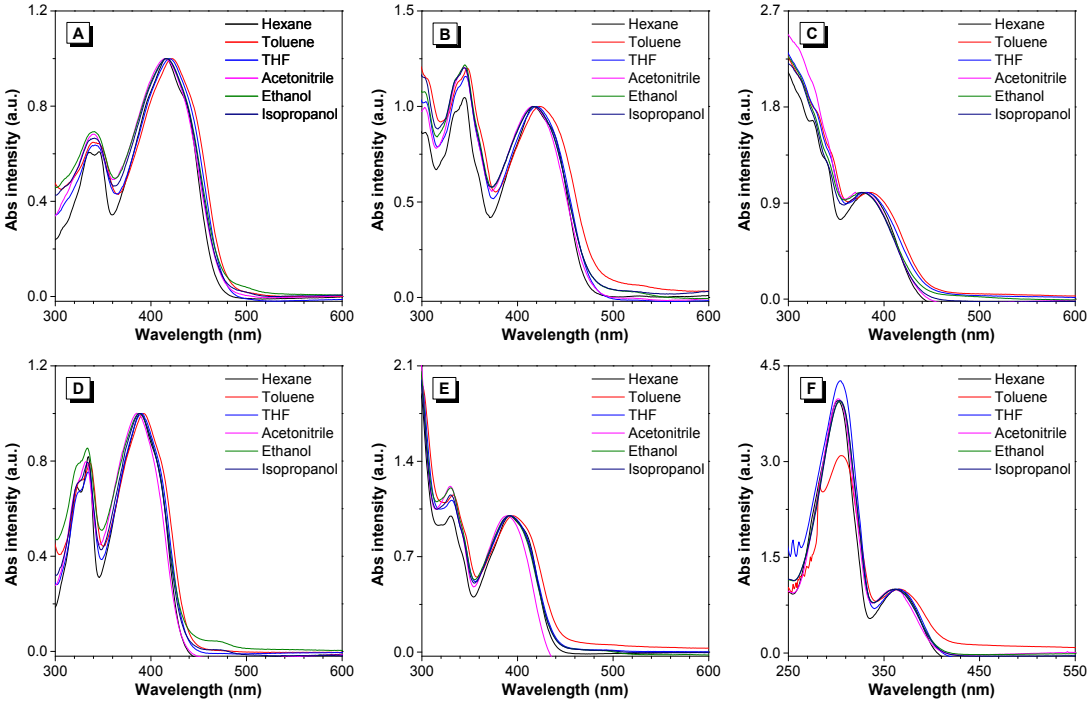


Fig. S10 The absorption spectra of (A) 1-FAN, (B) 2-FAN, (C) CNP-FAS, (D) 1-DPAN, (E) 2-DPAN, and (F) CNP-DPAS (10 μM) in different solvents.

8. pH and solvation effects

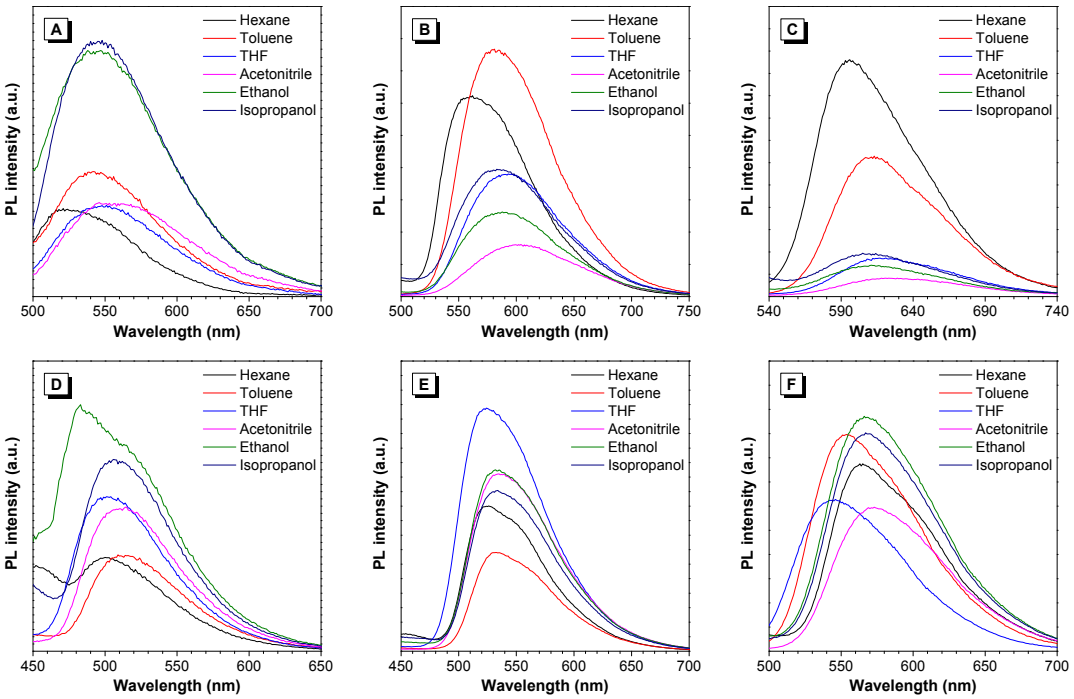


Fig. S11 The photoluminescence (PL) spectra of (A) 1-FAN, (B) 2-FAN, (C) CNP-FAS, (D) 1-DPAN, (E) 2-DPAN, and (F) CNP-DPAS (10 μM) in different solvents. Excitation wavelength: 360 nm (CNP-DPAS), 380 nm (CNP-FAS), 390 nm (1-DPAN, 2-DPAN), 420 nm (1-FAN, 2-FAN).

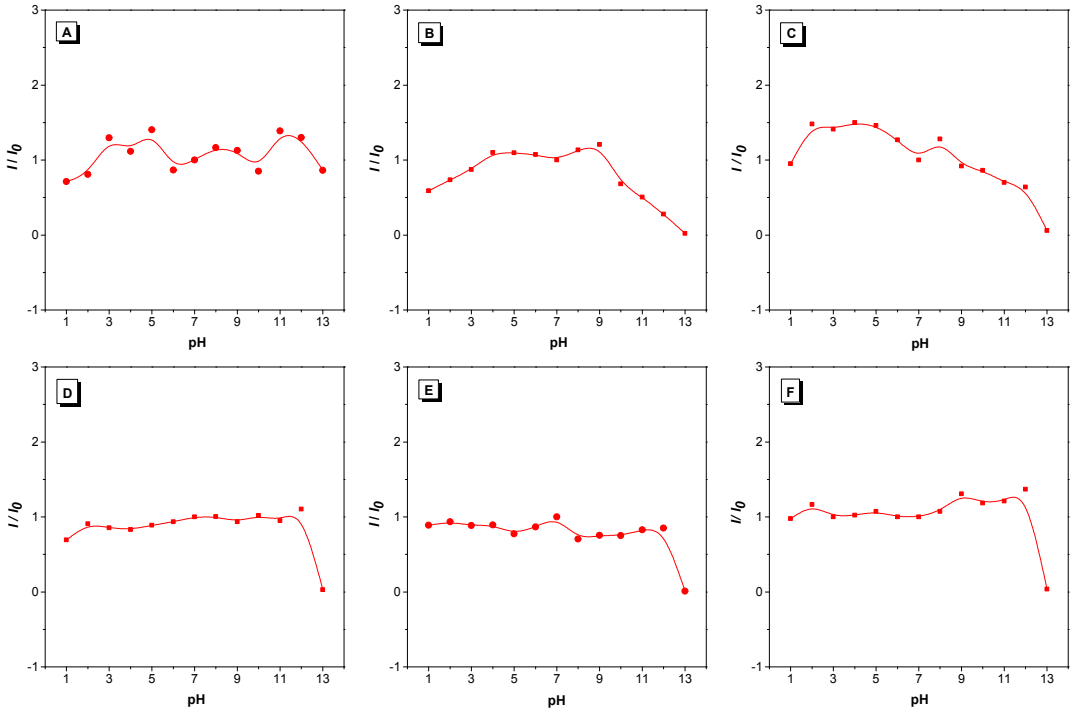


Fig. S12 The I/I_0 plot of (A) 1-FAN, (B) 2-FAN, (C) CNP-FAS, (D) 1-DPAN, (E) 2-DPAN, and (F) CNP-DPAS under different pH. I_0 means the intensity of 1-FAN, 2-FAN, CNP-FAS, 1-DPAN, 2-DPAN, and CNP-DPAS under pH=7.

9. Application in fluorescence imaging

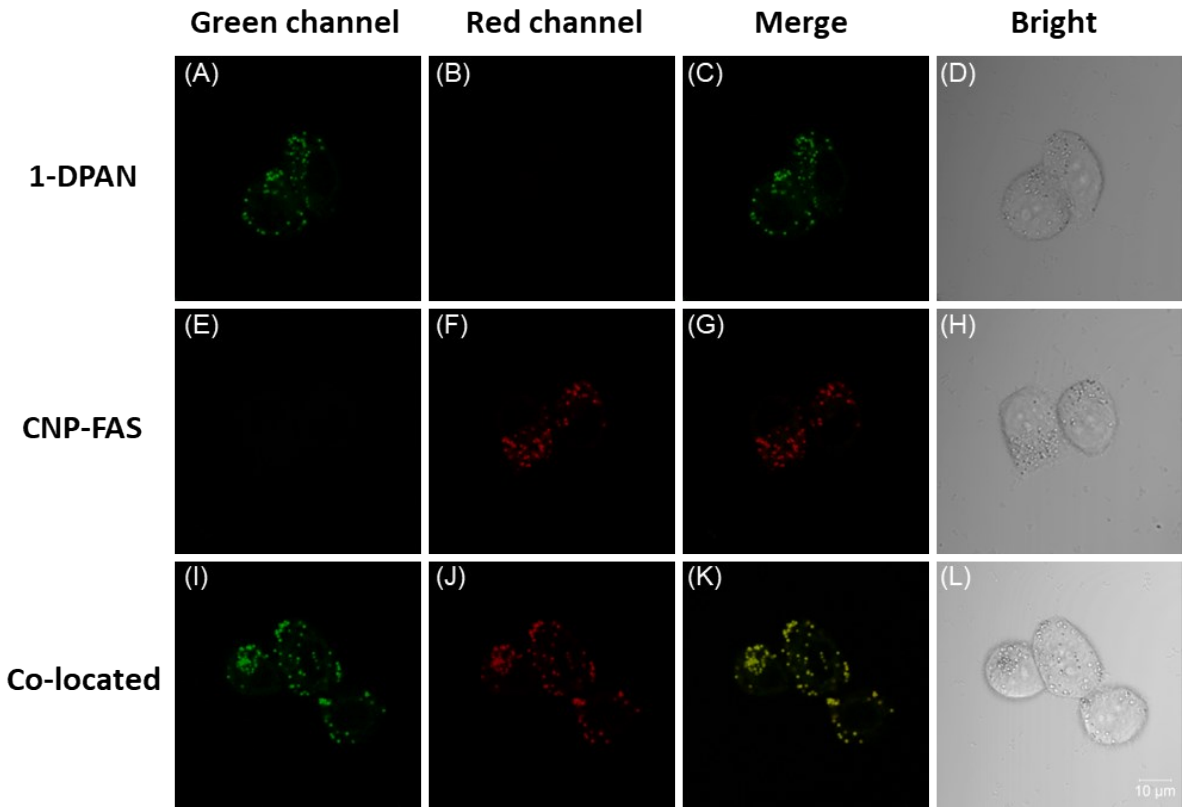


Fig. S13 Confocal images of Hela cells after incubation with 1-DPAN (10 μM) and CNP-FAS

(10 μM) for 30 min observed in the green channel (410-540 nm), the red channel (620-740 nm), the merged field of green and red channels, and the bright field. Excitation wavelength: 405 nm. All images share the same scale bar, 10 μm.

Table S1 Pearson's colocalization coefficients (PCC) of AIEgens with commercial dyes.

AIEgens	1-FAN & Nile Red	2-FAN & BODIPY	CNP-FAS & BODIPY	1-DPAN & Nile Red	2-DPAN & Nile Red	CNP-DPAS & BODIPY	1-DPAN & CNP-FAS
PCC / %	98	93	90	96	96	90	96

10. Cytotoxicity and photostability

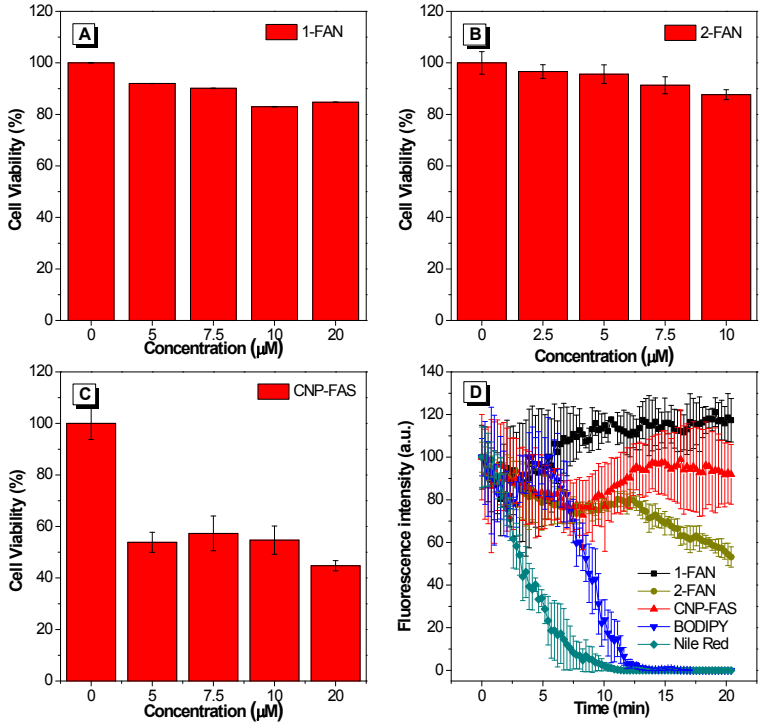


Fig. S14 Cytotoxicity of (A) 1-FAN, (B) 2-FAN, (E) CNP-FAS on Hela cells determined by MTT assay. The signal loss of fluorescence intensity of (G) 1-FAN, 2-FAN, CNP-FAS, Nile Red and BODIPY under different light irradiation time. Laser power: 2%.

11. Washing-free characteristic

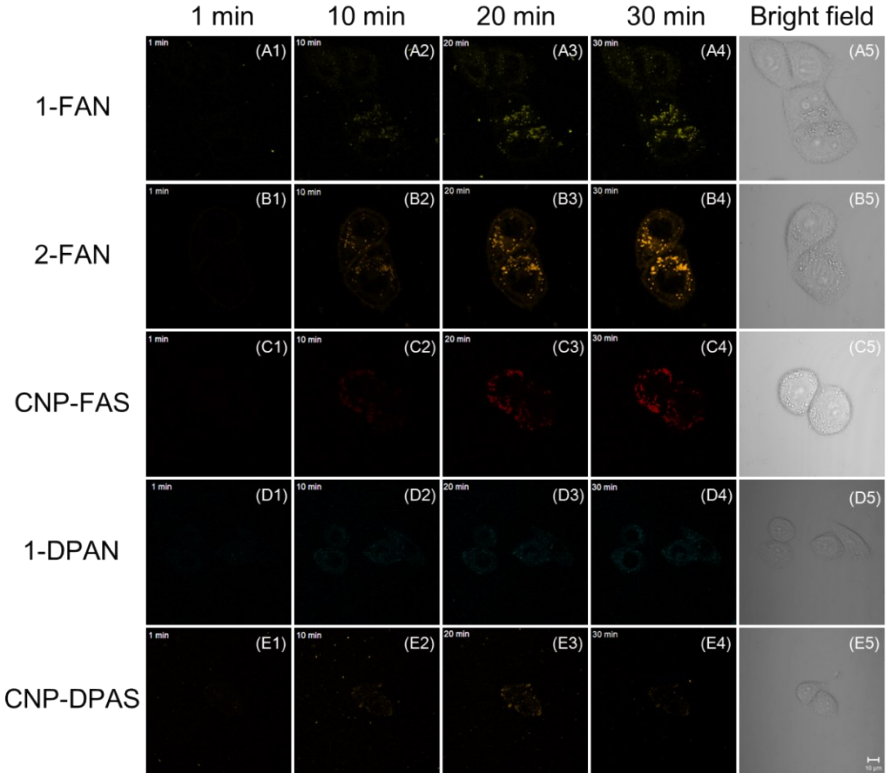
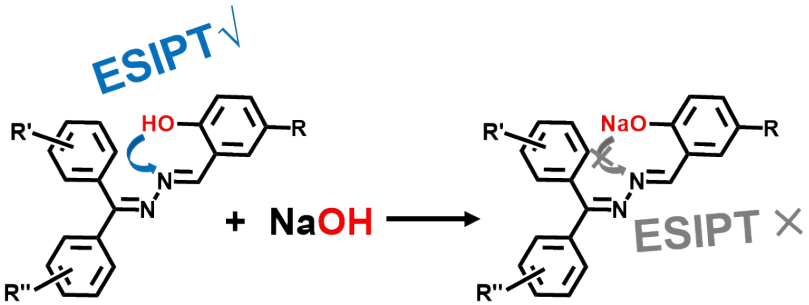


Fig. S15 Confocal images of living Hela cells incubated with 10 μM 1-FAN, and 10 μM 2-FAN, 10 μM CNP-FAS, 10 μM 1-DPAN, 10 μM CNP-DPAS with different times. Excitation wavelength: 405 nm. All images share the same scale bar, 10 μm.



Scheme S3 The diagrammatic sketch of KSA-based probes react with NaOH solution.

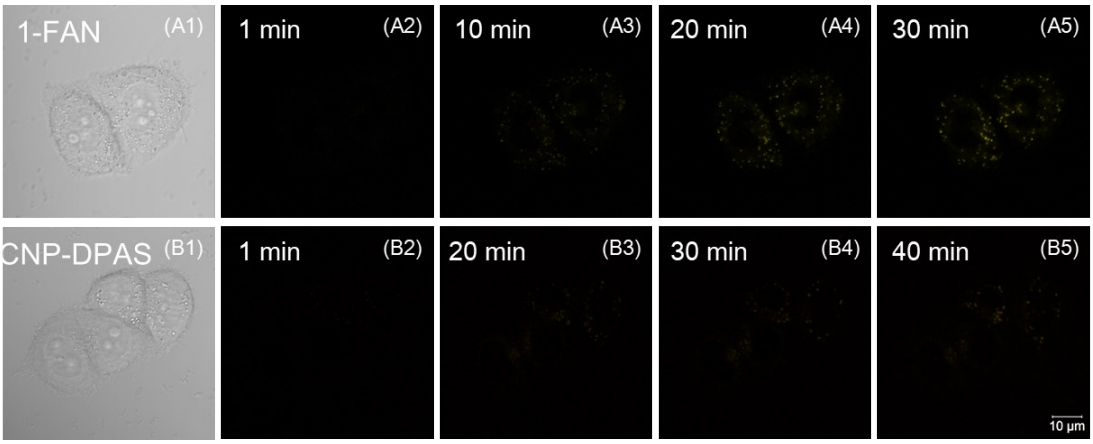


Fig. S16 Confocal images of living Hela cells incubated with 10 μM 1-FAN, and 10 μM CNP-DPAS with different times, which both treated with 12 μM NaOH in advance. Excitation wavelength: 405 nm. All images share the same scale bar, 10 μm.

12. Dynamic tracking the movements of LDs

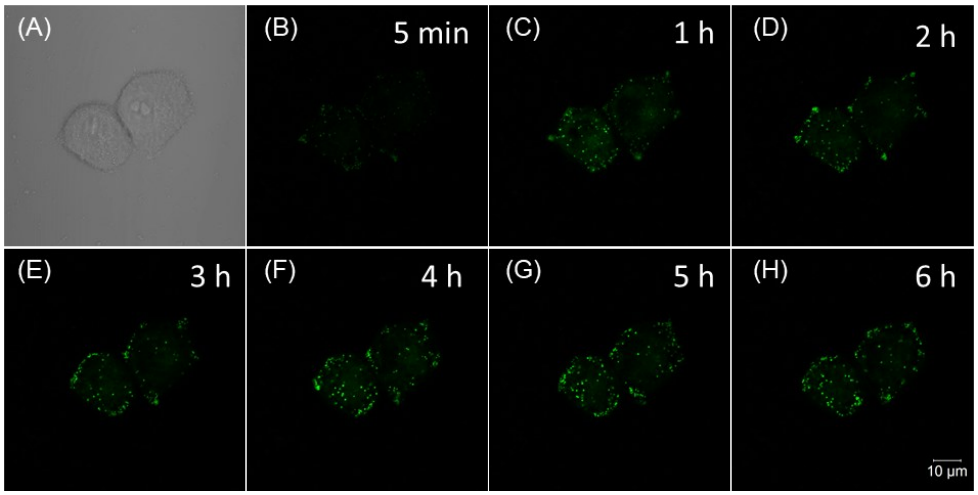


Fig. S17 Confocal images of living Hela cells incubated with 3 μM 2-DPAN with different times. Excitation wavelength: 405 nm. All images share the same scale bar, 10 μm.

13. The model of larva zebrafish

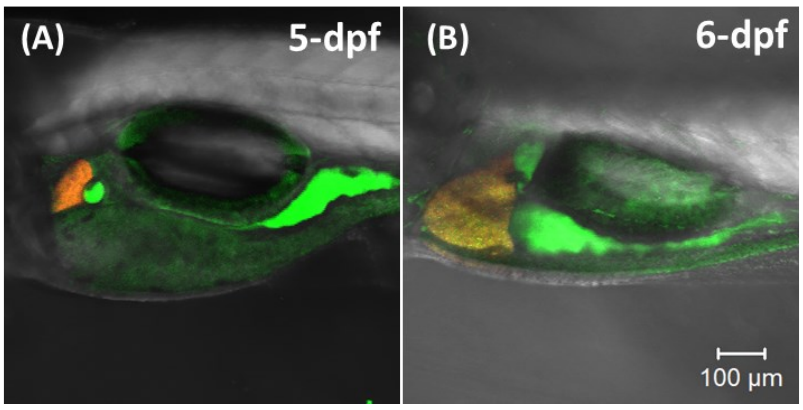
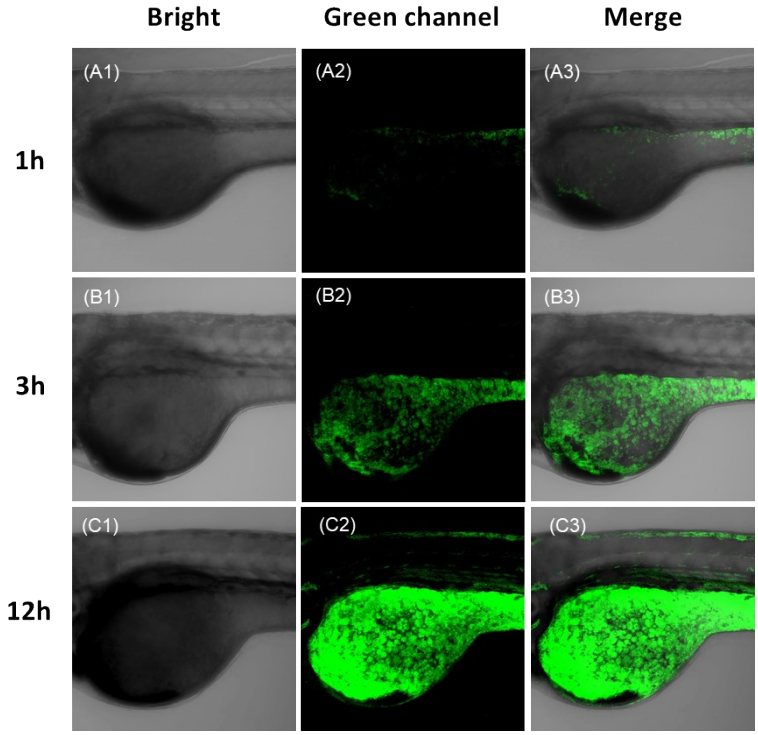


Fig. S18 Confocal images of Tg(lfabp:dsRed) zebrafish by 2-DPAN (3 μM) in E3 embryo media for 24 h at 28 ℃ on (A) 5-dpf and (B) 6-dpf. All CLSM images share the same scale bar, 100 μM.

Fig. S19 Bright field (A1, B1, C1), Confocal images (A2, B2, C2) and Merged images (A3, B3, C3) of zebrafish by 2-DPAN (3 μM) for different times.



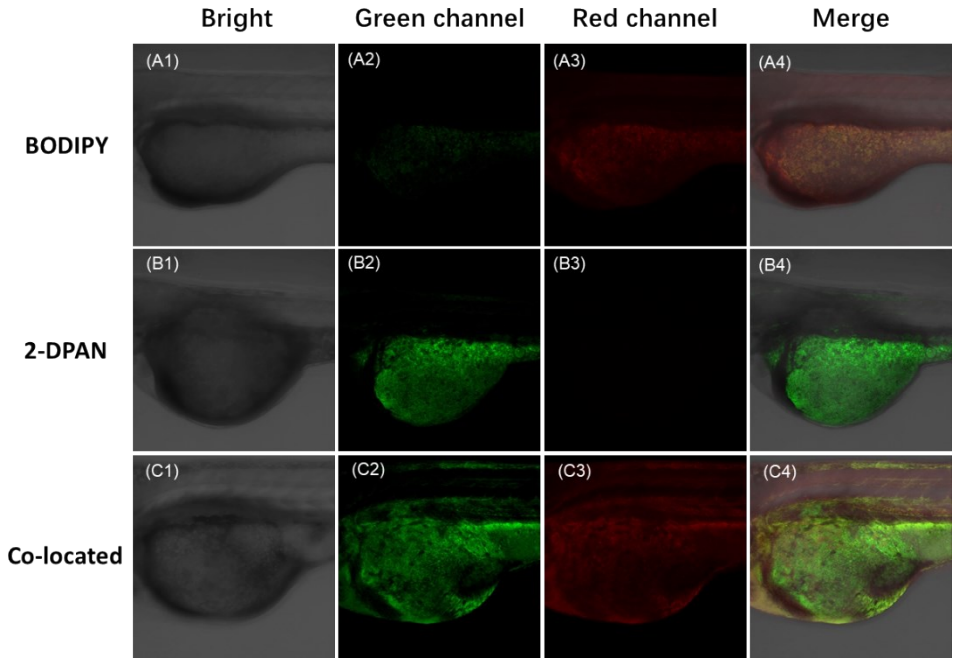


Fig. S20 Bright field (A1, B1, C1), Confocal images (A2-C2, A3-C3) and Merged images (A4, B4, C4) of zebrafish incubated with BODIPY (A1-A4), and 3 μM 2-DPAN (B1-B4) for 24h. Excitation wavelength: 405 nm.

References

1. S. Korzh, X. Pan, M. Garcia-Lecea, C. L. Winata, X. Pan, T. Wohland, V. Korzh and Z. Gong, Requirement of vasculogenesis and blood circulation in late stages of liver growth in zebrafish, *BMC Dev. Biol*., 2008, **8**, 84.
2. Z. Wang, F. Zhou, J. Wang, Z. Zhao, A. Qin, Z. Yu and B. Z. Tang, Electronic effect on the optical properties and sensing ability of AIEgens with ESIPT process based on salicylaldehyde azine, *Science China Chemistry*, 2018, **61**, 76-87.