An AIEgen-based “turn-on” probe for sensing cancer cells and tiny tumors with high furin expression

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Experimental

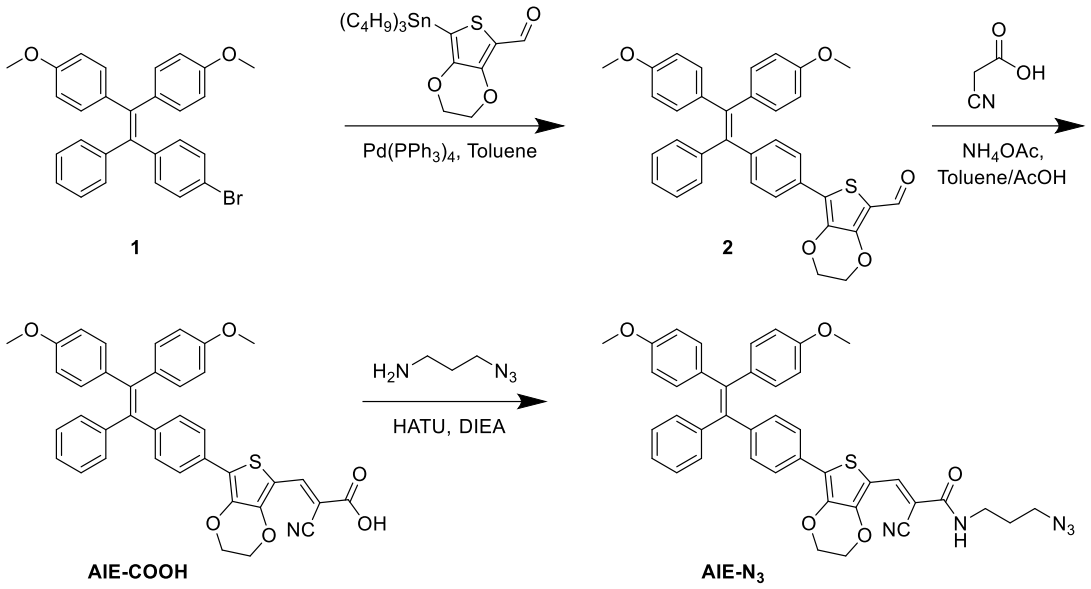
Materials

Fmoc-amino acids, o-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) and 2-chlorotrityl chloride resin were purchased from GL Biochem (Shanghai, China). 3-aminopropyl azide was obtained from Energy Chemical (Beijing). BHQ-3 succinimidyl ester was purchased from Biosearch Technologies. Furin (2000 U/mL) was obtained from New England Biolabs, Inc. Poly-γ-glutamic acid (γ-PGA, molecular weight (Mw) = 5000 Da) was purchased from XABC Biotech Co., Ltd. 9,10-anthracenediyl-bis(methylene)dimalonic acid (ABDA) was purchased from Sigma-Aldrich. Golgi-Tracker green and Lyso-Tracker green were obtained from Beyotime Biotechnology (Shanghai). D-luciferin, potassium salt was purchased from Yeasen (Shanghai). All the other starting materials were purchased from Aladdin Chemistry Co., Ltd. Commercially available reagents were used without further purification. All other chemicals were reagent grade or better. MDA-MB-468 and LoVo cells were purchased from Procell Life Science & Technology (Wuhan, China). 4T1 and HEK 293T cells were maintained in our lab.

Characterization

The synthesized compounds were characterized by ^{1}H NMR and ^{13}C NMR (Bruker Ascend 400). HR-MS was conducted on a Bruker micrOTOF-Q III. HPLC was performed on an LC3000 semi-preparative system (China) using a C18 RP column with MeOH (0.05% of TFA) and water (0.05% of TFA) as the eluents. UV-vis absorption spectra were recorded on a UV759S UV–vis spectrophotometer (YOKE, China) at room temperature. Photoluminescence (PL) spectra were recorded on a Thermo Scientific Varioskan Flash microplate reader. HPLC analyses were conducted at the Shimadzu LCMS-20AD (Japan) system. Dynamic light scattering (DLS) and zeta potential tests were conducted on Malvern Zetasizer Nano ZS90 at room temperature. TEM measurements were carried out on FEI Talos F200X G2. Cell imaging studies were performed by confocal laser scanning microscopy (CLSM, Leica TCS SP8).

Chemical synthesis



Scheme S1. The synthetic route for **AIE-N$_3$**. Compounds **1**, and **2** were synthesized according to our precedent report.[1]

1.1 Synthesis of AIE-COOH

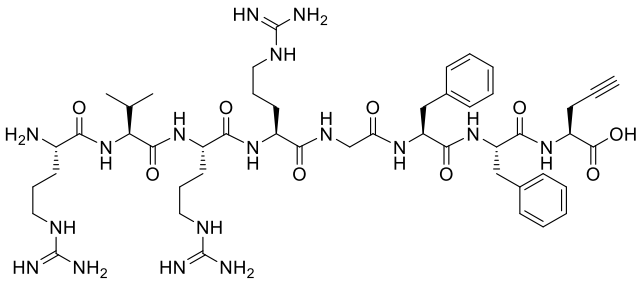
This procedure was modified from the literature.[2] Compound **2** (0.5 mmol, 314 mg), cyanoacetic acid (1.5 mmol, 127 mg), ammonium acetate (1.5 mmol, 115.5 mg), and then 10 mL acetic acid glacial was added into a round-bottom flask. The flask was evacuated under vacuum and flushed with dry nitrogen three times. Then the mixture was heated to 120 ºC overnight. After cooling to room temperature, the mixture was filtered and the residue was washed by ethyl acetate/petroleum ether (v/v 1:3) 3*20 mL and absolute ethyl alcohol 3*20 mL to get a red solid of compound AIE-COOH. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (s, 1H), 7.56 (d, J = 8.1 Hz, 2H), 7.13 (dd, J = 12.0, 7.2 Hz, 3H), 6.98 (m, 4H), 6.88 (dd, J = 16.7, 8.5 Hz, 4H), 6.71 (dd, J = 15.8, 8.6 Hz, 4H), 4.44 (ddd, J = 32.6, 6.7, 3.2 Hz, 4H), 3.68 (d, J = 2.5 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.99, 157.99, 157.88, 144.91, 140.87, 140.18, 138.42, 137.93, 135.44 (d, J = 3.6 Hz), 132.14 (d, J = 6.4 Hz), 131.63, 130.87, 128.79, 128.03, 126.42 (d, J = 17.8 Hz), 126.00, 116.90, 113.31 (d, J = 17.7 Hz), 108.69, 65.66, 64.72, 54.96 (d, J = 2.0 Hz). HR-MS: calcd. [M] = 627.1716; obsvd. [M] = 627.1691.

1.2 Synthesis of AIE-N$_3$

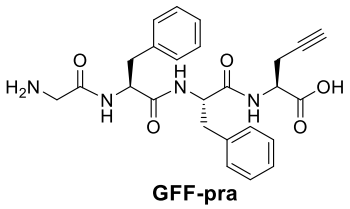
AIE-COOH (125 mg, 0.2 mmol), 3-aminopropyl azide (24 mg, 0.24 mmol) and 2-

(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU, 76 mg, 0.2 mmol) were dissolved in anhydrous N, N'-dimethylformamide (DMF, 5 mL), then N, N-diisopropylethylamine (DIEA, 132 μL, 0.4 mmol) was added. The reaction was stirred overnight. The solvent was removed under vacuum. The residue was purified by silica gel chromatography (petroleum ether/ ethyl acetate = 1/1, v/v) to get a red solid of AIE-N_3 (yield: 76 mg, 53.5%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (d, J = 5.9 Hz, 1H), 8.19 (s, 1H), 7.54 (d, J = 8.1 Hz, 2H), 7.14 (m, 3H), 6.99 (dd, J = 24.8, 7.7 Hz, 4H), 6.87 (dd, J = 17.0, 8.3 Hz, 4H), 6.70 (dd, J = 15.7, 8.2 Hz, 4H), 4.43 (dd, J = 27.0, 4.9 Hz, 4H), 3.67 (m, 6H), 3.25 (d, J = 6.3 Hz, 2H), 1.72 (q, J = 6.8 Hz, 2H), 1.22 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.21, 157.91 (d, J = 9.8 Hz), 148.44, 144.56, 143.45, 140.76, 138.36, 137.97, 137.44, 135.49, 132.13 (d, J = 5.9 Hz), 131.59, 130.87, 128.96, 128.01, 126.49, 125.84, 124.44, 117.20, 113.30 (d, J = 16.6 Hz), 108.87, 96.99, 65.45, 64.73, 54.95, 48.49, 37.23, 28.28. HR-MS: calcd. [M] = 709.2359; obsvd. $[M+Na]^+$ = 732.2247.

1.3 Synthesis of peptides



RVRRGFF-pra



GFF-pra

Scheme S2. Chemical structures of different peptides.

RVRRGFF-pra and **GFF-pra** were synthesized by solid-phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin and the corresponding Fmoc-amino acids. 20% piperidine in DMF was used during the deprotection of the Fmoc group. HBTU was used as a condensation agent. The growth of the peptide chain was according to the

established Fmoc SPPS protocol. At last, the peptide derivative was cleaved using 95% trifluoroacetic acid (TFA) with 2.5% of trimethylsilane (TIS) and 2.5% of H_2O for 3 h. Then the solution was collected, concentrated and dropped into cold methyl tert-butyl ether. After that, the crude product was obtained by centrifugation and purified by HPLC.

RVRRGFF-pra: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (d, J = 7.7 Hz, 1H), 8.40 (d, J = 8.3 Hz, 1H), 8.26 (s, 1H), 8.16 (m, 5H), 8.06 (s, 1H), 7.96 (d, J = 7.6 Hz, 1H), 7.72 (d, J = 5.8 Hz, 1H), 7.61 (dt, J = 21.7, 5.6 Hz, 2H), 7.21 (m, 17H), 4.61 (q, J = 4.4 Hz, 1H), 4.49 (d, J = 4.4 Hz, 1H), 4.36 (m, 1H), 4.24 (m, 3H), 3.90 (s, 1H), 3.64 (m, 2H), 3.07 (m, 9H), 2.93 (t, J = 2.6 Hz, 1H), 2.80 (dd, J = 13.9, 9.5 Hz, 1H), 2.64 (m, 3H), 1.98 (q, J = 6.7 Hz, 1H), 1.66 (m, 4H), 1.48 (td, J = 16.0, 12.7, 6.6 Hz, 8H), 0.86 (dd, J = 13.1, 6.7 Hz, 6H). HR-MS: calcd. [M] = 1031.5777; obsvd. $[M+3H]^{3+}/3$ = 344.8935, $[M+2H]^{2+}/2$ = 516.8306.

GFF-pra: ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.96 (s, 1H), 8.51 (m, 2H), 7.93 (s, 3H), 7.25 (dp, J = 23.0, 7.5 Hz, 10H), 4.61 (t, J = 8.9 Hz, 2H), 4.38 (d, J = 6.9 Hz, 1H), 3.48 (d, J = 16.3 Hz, 1H), 3.04 (m, 2H), 2.94 (d, J = 2.7 Hz, 1H), 2.80 (d, J = 4.2 Hz, 1H), 2.65 (m, 3H), 2.54 (s, 1H). HR-MS: calcd. [M] = 464.2060; obsvd. $[M-H]^-$ = 463.1985.

1.4 Synthesis of peptide-AIEgens

RVRRGFF-AIE was synthesized by click reaction. Briefly, RVRRGFF-pra (15 mg, 14.5 μmol), AIE-N_3 (5 mg, 7 μmol) and DIEA (1.5 μL) were dissolved in 1 mL DMSO. Then $CuSO_4$ (0.64 mg, 4 μmol) and sodium ascorbate (1.0 mg, 5 μmol) in water were added to the above solution. The reaction was stirred overnight at room temperature in the dark. The product was purified by HPLC (yield: 6.7 mg, 55%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (d, J = 8.3 Hz, 1H), 8.34 (m, 8H), 8.18 (s, 1H), 8.09 (m, 3H), 7.93 (s, 1H), 7.82 (t, J = 5.9 Hz, 2H), 7.69 (m, 1H), 7.55 (d, J = 8.5 Hz, 2H), 7.35 (s, 2H), 7.18 (m, 15H), 6.99 (m, 6H), 6.89 (m, 4H), 6.71 (m, 4H), 4.36 (m, 13H), 4.01 (d, J = 6.5 Hz, 1H), 3.68 (d, J = 3.1 Hz, 6H), 3.09 (m, 14H), 2.81 (dd, J = 14.0, 9.5 Hz, 1H), 2.67 (dd, J = 13.8, 9.8 Hz, 1H), 2.00 (m, 3H), 1.70 (d, J = 24.2 Hz, 4H), 1.51 (d, J = 24.2 Hz, 8H), 0.89 (m, 6H). HR-MS: calcd. [M] = 1740.8136; obsvd.

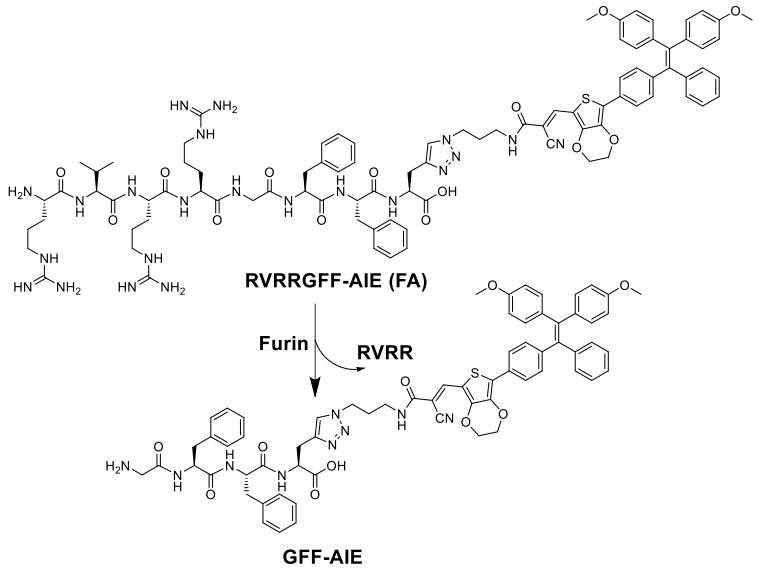
$[M+3H]^{3+}/3$ = 581.2927, $[M+3H]^{3+}/2$ = 871.9236.

GFF-AIE was synthesized in a similar way and obtained in 78.6% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (m, 2H), 8.38 (s, 1H), 8.18 (s, 1H), 7.92 (s, 1H), 7.73 (d, *J* = 4.7 Hz, 2H), 7.68 (s, 1H), 7.55 (d, *J* = 8.1 Hz, 2H), 7.20 (ddd, *J* = 23.1, 17.0, 6.6 Hz, 13H), 7.01 (dd, *J* = 27.0, 7.6 Hz, 4H), 6.88 (dd, *J* = 18.3, 8.3 Hz, 4H), 6.71 (dd, *J* = 16.4, 8.2 Hz, 4H), 4.47 (m, 7H), 4.32 (d, *J* = 7.1 Hz, 2H), 3.68 (d, *J* = 3.1 Hz, 6H), 3.47 (s, 1H), 3.38 (s, 1H), 3.19 (s, 2H), 3.02 (s, 2H), 2.79 (t, *J* = 12.2 Hz, 1H), 2.64 (d, *J* = 14.3 Hz, 1H), 1.97 (dd, *J* = 15.3, 8.1 Hz, 3H), 1.23 (s, 1H). HR-MS: calcd. [M] = 1173.4419; obsvd. $[M+H]^+$ = 1174.4493.

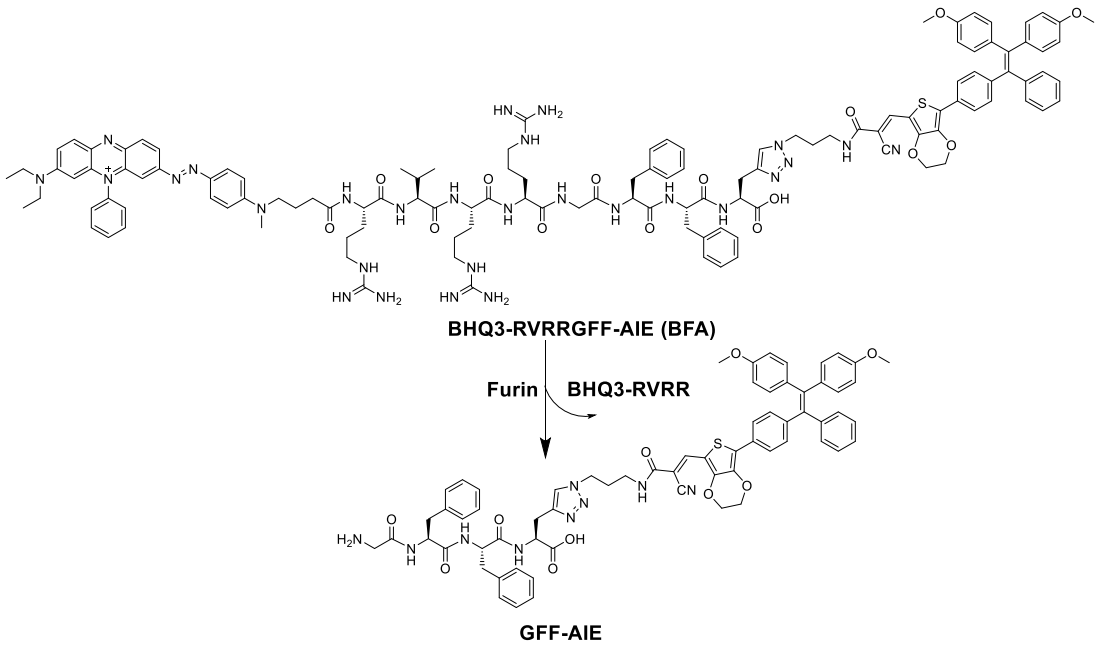
1.5 Synthesis of BHQ3-peptide-AIEgens

BHQ3-RVRRGFF-AIE was synthesized by coupling BHQ-3 succinimidyl ester with RVRRGFF-AIE. Briefly, BHQ-3 succinimidyl ester (5 mg, 6.3 μmol), RVRRGFF-AIE (9 mg, 5.1 μmol), and DIEA (4 μL) were dissolved in 1 mL DMSO and stirred overnight at room temperature in the dark. **BHQ3-RVRRGFF-AIE** was purified by HPLC (yield: 4 mg, 34%). HR-MS: calcd. [M] = 2270.0846; obsvd. $[M+4H]^{4+}/4$ = 568.5421, $[M+3H]^{3+}/3$ = 757.7190.

2.1 Enzymatic cleavage reaction



Scheme S3. Enzymatic cleavage reaction and chemical structures of FA and GFF-AIE.



Scheme S4. Enzymatic cleavage reaction and chemical structure of BHQ3-RVRRGFF-AIE.

RVRRGFF-AIE and BHQ3-RVRRGFF-AIE stock solution (2 mM in DMSO) was diluted with furin working buffer (100 mM HEPES, 1 mM $CaCl_2$, pH 7.4) to make 10 μM working solution. The working solution was incubated with 200 U/mL furin at 37 ^oC for 6 h. Then the absorbance spectra and the change of fluorescence intensity of the probes before and after furin treatment were measured. For HPLC analysis, samples activated by furin and purified GFF-AIE were analyzed with the method (Table S1). The new products of the HPLC trace in "FA + Furin" and "BFA + Furin" groups were collected and characterized by HR-MS.

2.2 Critical micelle concentration (CMC) measurements

CMC was conducted according to the literature.[3] Briefly, a series of FA or BFA solutions with different concentrations (3.125, 4.6875, 6.25, 9.375, 12.5, 18.75, 25, 37.5, 50, 75, 100, 150, 250, 500 and 1000 μM) in furin working buffer were firstly incubated with Rhodamine 6G (5 μM), respectively. Then, the λ_{max} was determined by measuring the absorbance from 520-540 nm using a Thermo Scientific Varioskan Flash microplate reader.

2.3 Preparation of nanoparticles

PGA-FA nanoparticles (NPs) and PGA-BFA NPs were prepared by the same

method. 0.25 mg FA and 1.25 mg γ-PGA were dissolved in 100 μL DMSO, which was slowly added into 0.9 mL Milli-Q water under continuous sonication (200 W output, 2 min) in an ice bath. Then the solution was dialyzed (Mw cutoff 8-14 kDa) for 12 h and concentrated using ultrafiltration tubes (Mw cutoff 10 kDa). Before use, the concentrated NPs need to pass through a 0.2 μm syringe filter and be calibrated with a UV-Vis spectrophotometer.

2.4 DLS and zeta potential

The DLS and zeta potential of the prepared PGA-FA NPs and PGA-BFA NPs in water were conducted on Malvern Zetasizer Nano ZS90 at room temperature. For FA and BFA, the DMSO stock solutions were firstly diluted with furin working buffer to 10 μM working solution and conducted the DLS and zeta potential tests.

2.5 TEM sample preparation

10 μL PGA-BFA or PGA-FA NPs solution was dropped on the carbon-coated grid for 1 min. The prepared TEM samples were stored in a desiccator and dried overnight. Then they were examined as soon as possible.

2.6 ROS detection in solution

The ROS generation of the probes was studied using ABDA as an indicator. 200 μM ABDA and 10 μM probe were mixed in furin working buffer and exposed to white light irradiation (0.25 W/cm^2). The absorbance spectra of the sample were recorded.

2.7 Hemolysis experiment

Hemolysis experiment was performed according to the literature.[4] Briefly, mouse blood was harvested from the eyeball and heparin was added for anticoagulation. Then 3 mL PBS was added and centrifuged at 500 g for 10 min (repeat twice) to isolate red blood cells (RBCs). 800 μL FA or BFA (20, 40, 60, 80, 100, 200 μM) were added to 200 μL diluted RBC suspension. PBS and water were used as the negative and positive control, respectively. Incubation was carried out on a rocking shaker (37 ℃, 2 h) following centrifugation (12000 rpm, 5 min). Then these centrifuge tubes were photographed and the absorbance at 540 nm of the supernatant was determined. Hemolysis ratio (%) = (sample-negative)/(positive-negative) *100.

2.8 Cell culture

MDA-MB-468 human breast cancer cells and HEK 293T human embryonic kidney cells were cultured in high-glucose Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S). 4T1 mouse breast cancer cells were cultured in RPMI 1640 medium containing 10% FBS and 1% P/S. LoVo human colon cancer cells were cultured in Ham's F-12K medium containing 10% FBS and 1% P/S. The cell lines were maintained in a humidified incubator with 5% CO_2 at 37 ºC. Before experiments, the cells were pre-cultured until 80% confluence was reached.

2.9 Confocal imaging

MDA-MB-468, 4T1, HEK 293T and LoVo cells were seeded in confocal imaging chambers at a density of 1.5×10^5 cells, respectively. After 24 h, these cells were incubated with PGA-FA or PGA-BFA NPs (containing 10 μM AIE probe) in culture medium (containing 10% FBS) at 37 ºC for 6 h. Then the live cells were washed three times with 1 × PBS. For the fluorescence of AIE, CLSM images were excited at 488 nm and received at 600-700 nm. For colocalization assay, the PGA-BFA or PGA-FA pre-treated cells were washed with Hanks' Balanced Salt Solution (HBSS with Ca^{2+} & Mg^{2+}) three times. 250 μL Golgi-Tracker green staining solution (33.3 μg/mL) was added. After incubation at 4 ºC for 30 min, cells were washed with 4 ºC pre-cooled cell culture medium three times, then kept in fresh medium and incubated at 37°C for another 30 min. Before imaging, the cells were rinsed three times with PBS. For the fluorescence of Golgi-Tracker green, CLSM images were excited at 488 nm and received at 500-530 nm. For the fluorescence of AIE, CLSM images were excited at 488 nm and received at 600-700 nm. Similarly, for the lysosomal colocalization assay, the PGA-FA pre-treated cells were washed with PBS three times, stained with Lyso-Tracker green DND-26 (200 nM) for 30 min and washed with PBS three times. For the fluorescence of Lyso-Tracker, CLSM images were excited at 488 nm and received at 500-530 nm. Colocalization finder plugin in ImageJ software was used for co-localization analysis.

2.10 Flow cytometry analysis

MDA-MB-468 cells and HEK 293T cells were cultured in 12-well plates at a density of 2×10^5 cells/mL, respectively. After 24 h, these cells were incubated with PGA-FA or PGA-BFA NPs (containing 10 μM AIE probe) in culture medium (containing 10% FBS) at 37 ºC for 6 h. Then the live cells were collected, washed with 1 × PBS, and detected by flow cytometry (guava easyCyte 8).

2.11 Cytotoxicity Test

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were used to assess the cell viability. Each kind of cell in 96-well plates (1.0×10^4 cells/well) was incubated with various concentrations of PGA-FA or PGA-BFA NPs for 6 h. After that, the wells were washed with 1×PBS and 100 μL of freshly prepared MTT solution (0.5 mg/mL) in cell culture medium was added into each well. After 4 h incubation, the supernatant was removed cautiously and 100 μL of DMSO was added to each well. The plate was shaken for 10 min at room temperature and then the absorbance of MTT at 490 nm was determined by a microplate reader (Thermo Scientific Varioskan Flash). To eliminate the UV absorption interference of AIEgen and BHQ3, the cells were also incubated with the same series of concentrations of PGA-FA or PGA-BFA NPs as the control. The control wells were not treated with MTT solution but added with DMSO and read by a microplate reader. The cell viability was expressed by the ratio of the absorbance of pure MTT of the sample-treated cells (subtraction of UV absorption of AIEgen and BHQ3) to that of the cells incubated with culture medium only.

2.12 Animals and tumor-bearing mouse model

The female BALB/c mice were purchased from Skbex Biotechnology (Huaxian, China). All animal procedures were performed following the Guidelines for Care and Use of Laboratory Animals of Xinxiang Medical University and approved by the Animal Ethics Committee of Xinxiang Medical University.

The peritoneal carcinomatosis-bearing mouse model was built according to the literature.[5] Briefly, a total of 1.0×10^6 luciferase transgenic murine 4T1 breast cancer cells in 0.1 mL of PBS were intraperitoneally (i.p.) injected into the healthy female

BALB/c mice. Metastatic tumors were grown for approximately five days.

2.13 In vivo imaging of metastatic tumor nodules

The mice bearing intraperitoneal metastatic tumors were i.p. injected with 100 μL of PGA-BFA (100 μM) and PGA-FA (100 μM), respectively. At 12 h post-injection, the mice were i.p. injected with D-luciferin (100 μL, 15 mg/mL) for 15 min and then were anesthetized. The mouse abdominal cavity was opened, followed by fluorescence imaging during surgery using Xenogen IVIS Lumina II system in fluorescence mode (excitation at 460 nm and emission at 670 nm). The nodules on the intestines were sliced and imaged both in fluorescence mode and bioluminescence mode. At last, intestines and major organs were also obtained and imaged in fluorescence mode and bioluminescence mode.

2.14 In vivo biosafety of PGA-BFA and PGA-FA

BALB/c mice were intraperitoneally injected with 100 μL of PBS, PGA-BFA (100 μM) and PGA-FA (100 μM), respectively. After 12 h, the mice were sacrificed and major organs were excised and fixed in 4% paraformaldehyde, which were then processed routinely into paraffin, sliced and stained with hematoxylin-eosin (H&E) staining.

2.15 Data analysis

Data were expressed as mean ± standard deviation from triplicate experiments performed in a parallel manner. Comparison between more than two groups was conducted with one-way ANOVA by using GraphPad Prism 7.0. A difference of $p < 0.05$ was considered statistically significant.

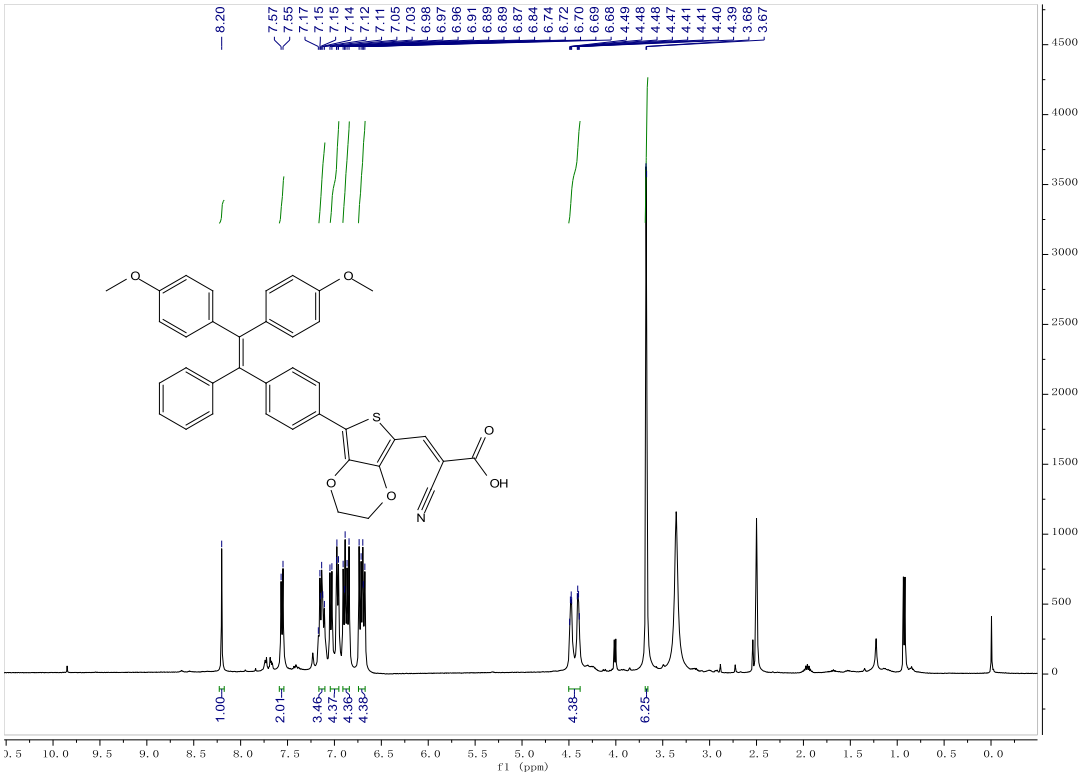


Fig. S1. ^{1}H NMR spectrum of **AIE-COOH**

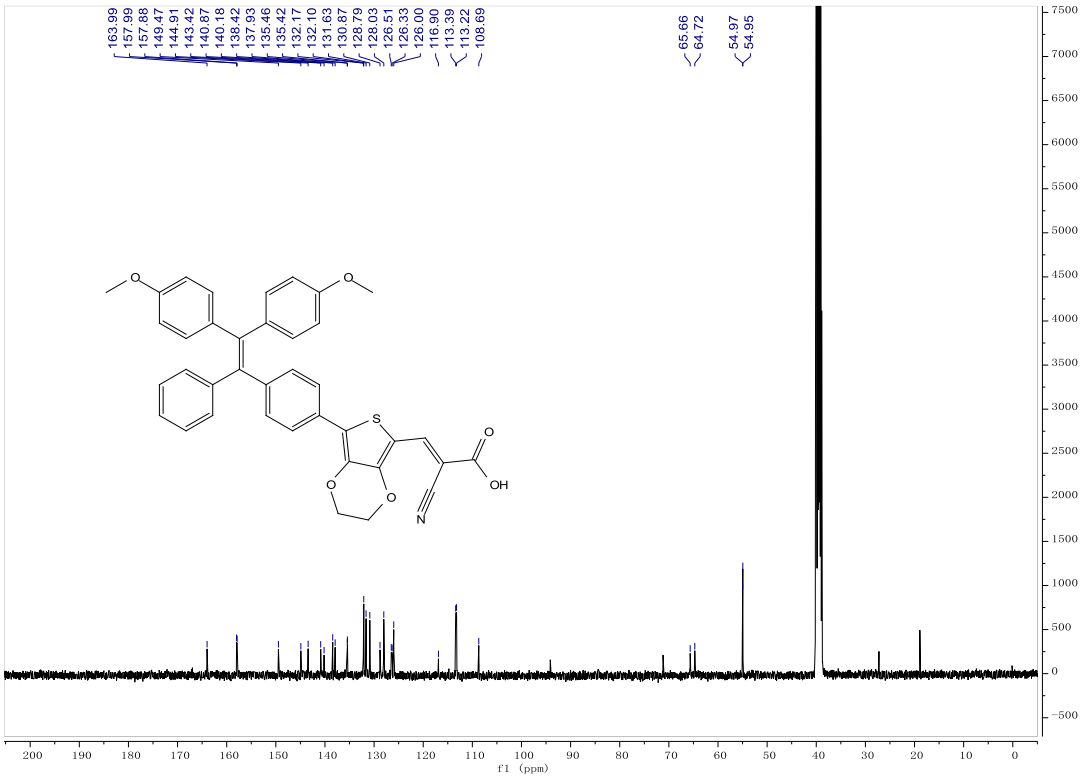


Fig. S2. ^{13}C NMR spectrum of **AIE-COOH**

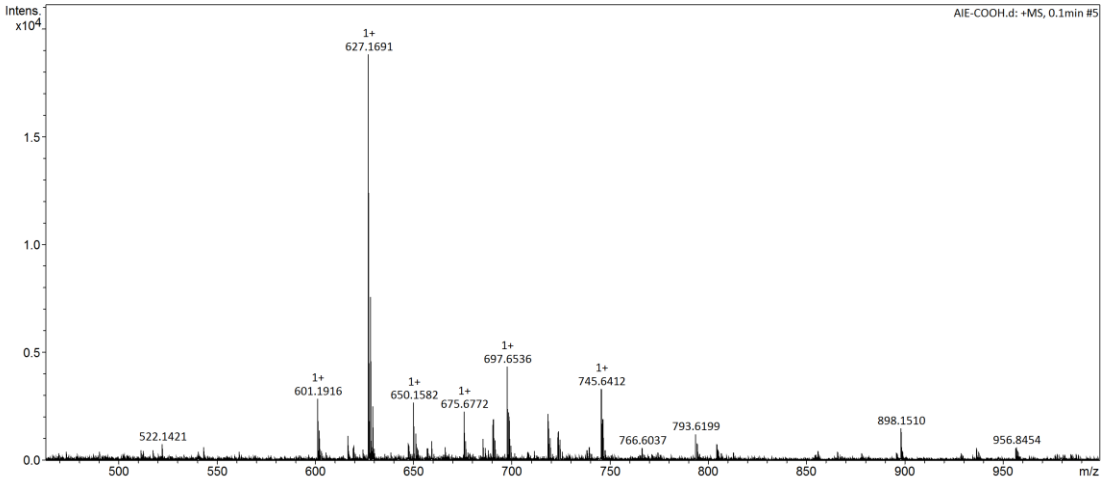


Fig. S3. HR-MS spectrum of **AIE-COOH**

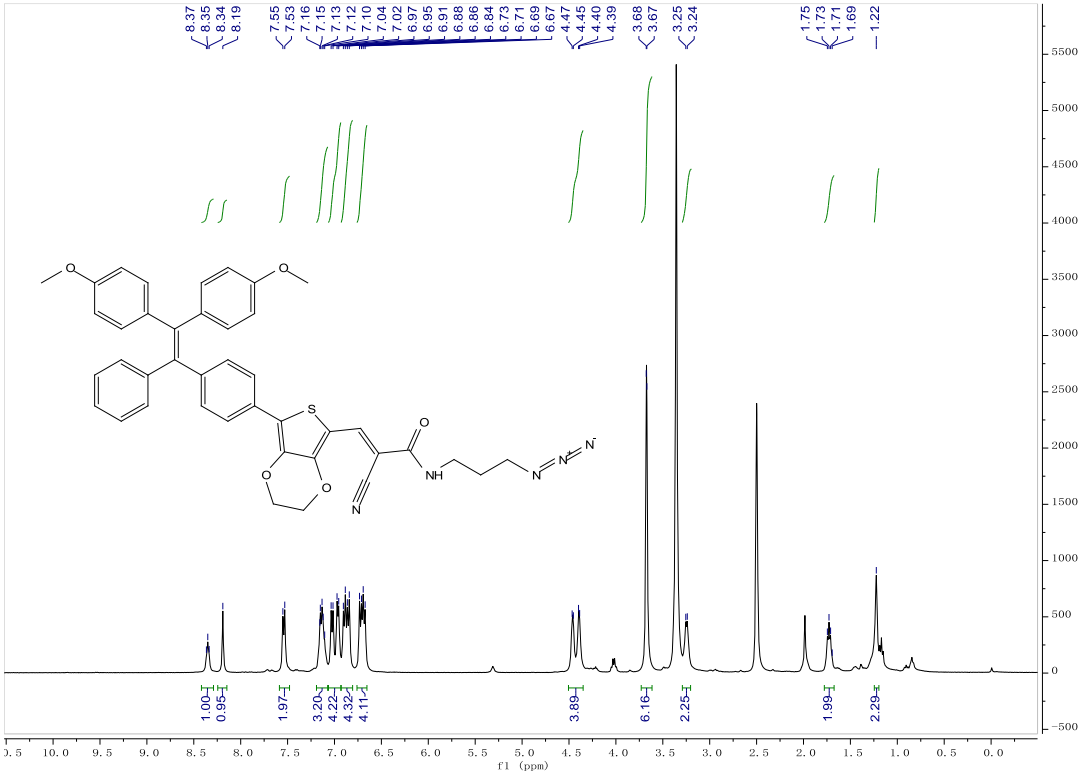


Fig. S4. ^{1}H NMR spectrum of **AIE-N$_3$**

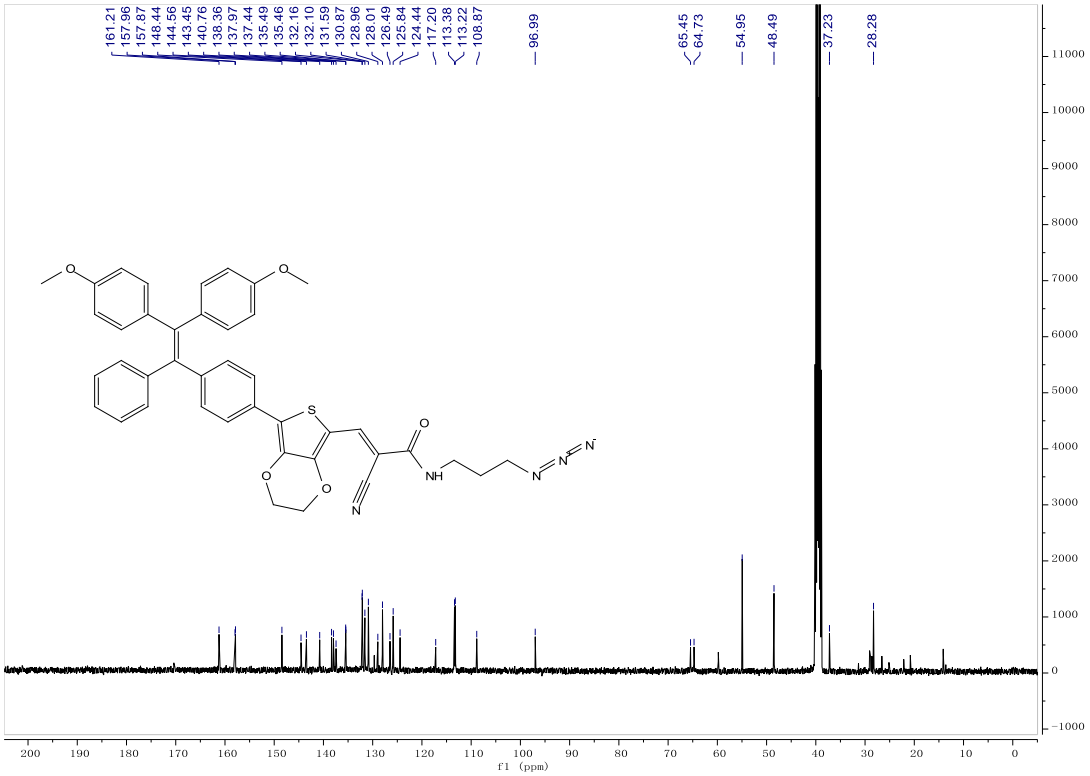


Fig. S5. ^{13}C NMR spectrum of **AIE-N$_3$**

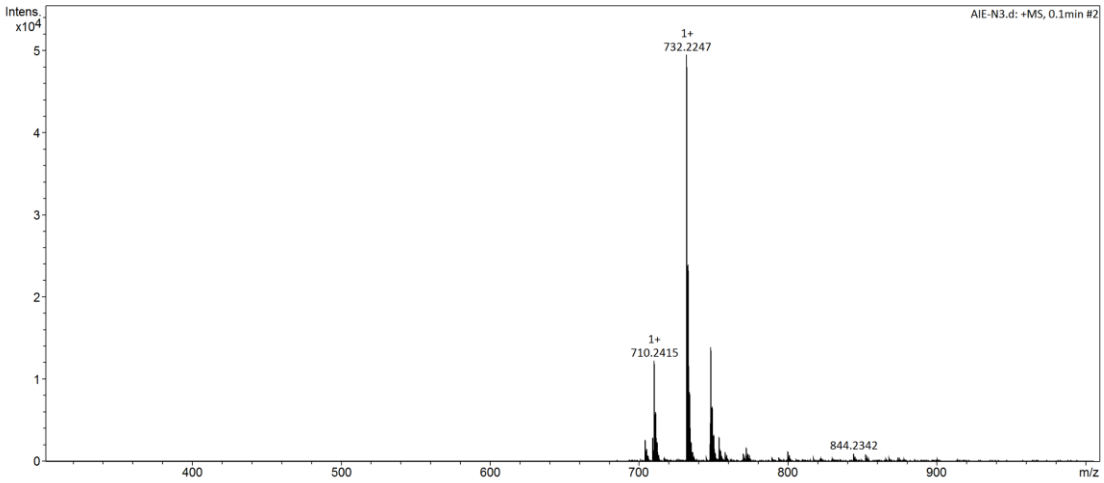


Fig. S6. HR-MS spectrum of **AIE-N$_3$**

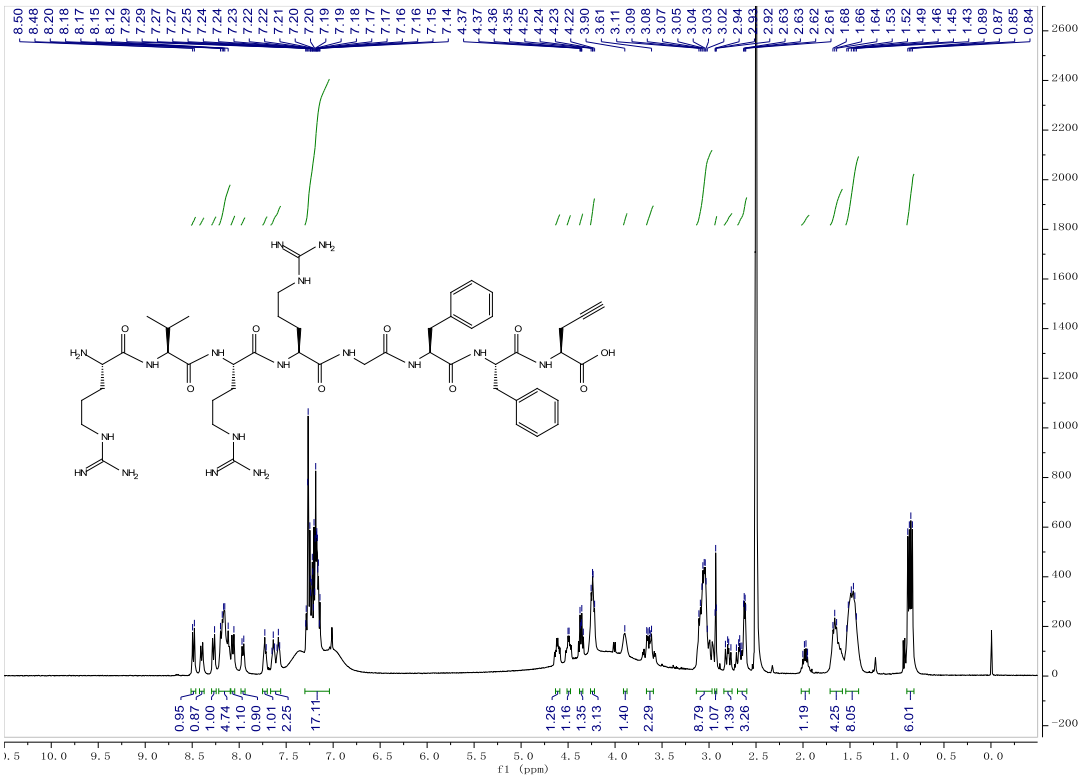


Fig. S7. ^{1}H NMR spectrum of **RVRRGFF-pra**

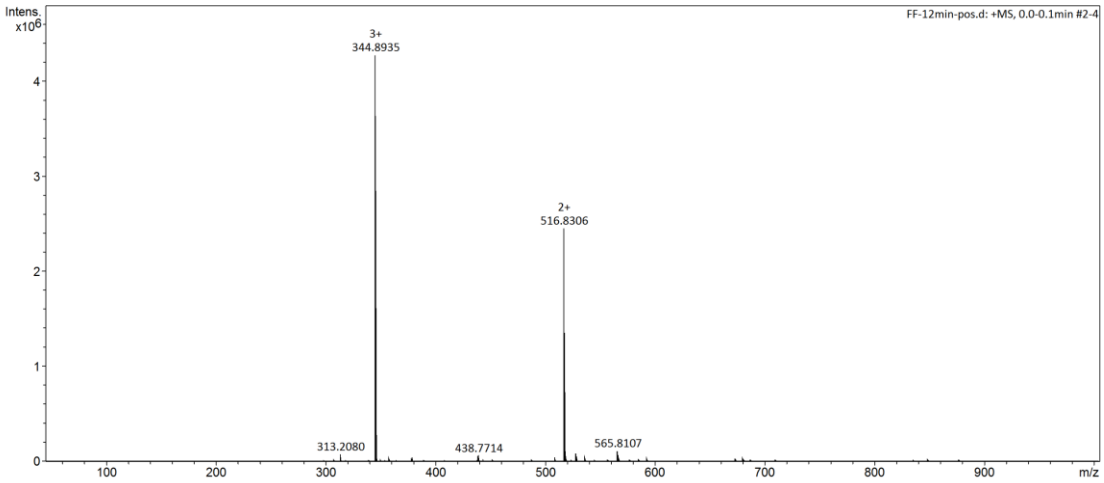


Fig. S8. HR-MS spectrum of **RVRRGFF-pra**

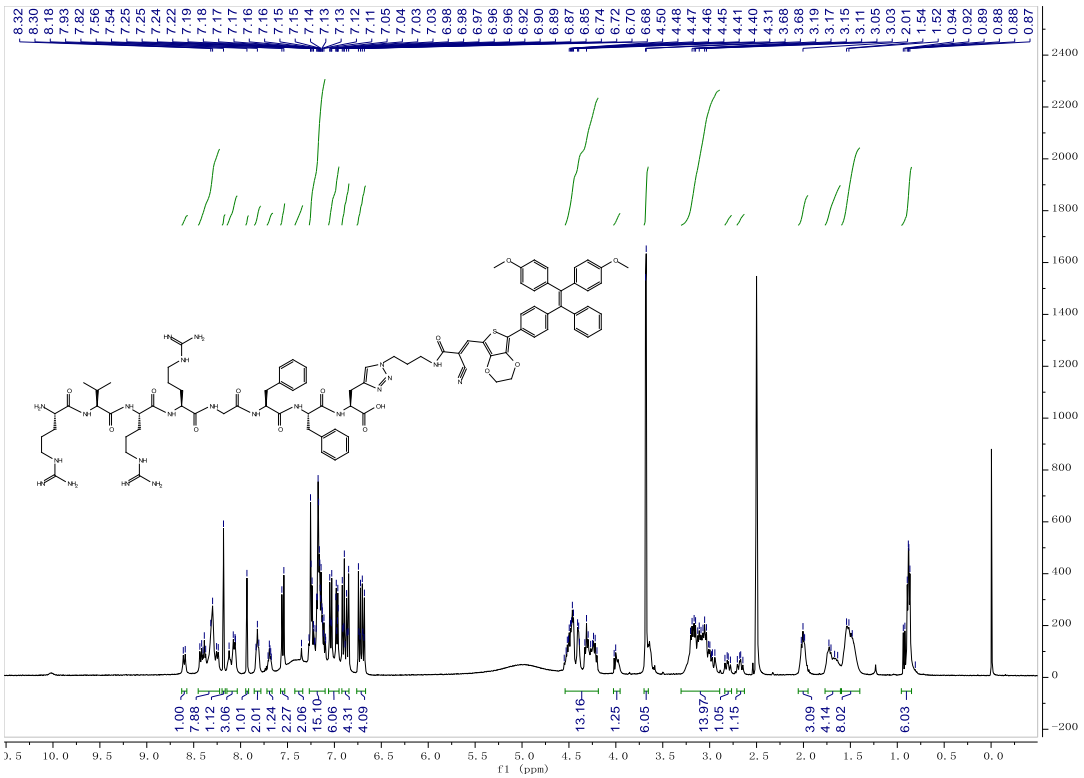


Fig. S9. ^{1}H NMR spectrum of **RVRRGFF-AIE**

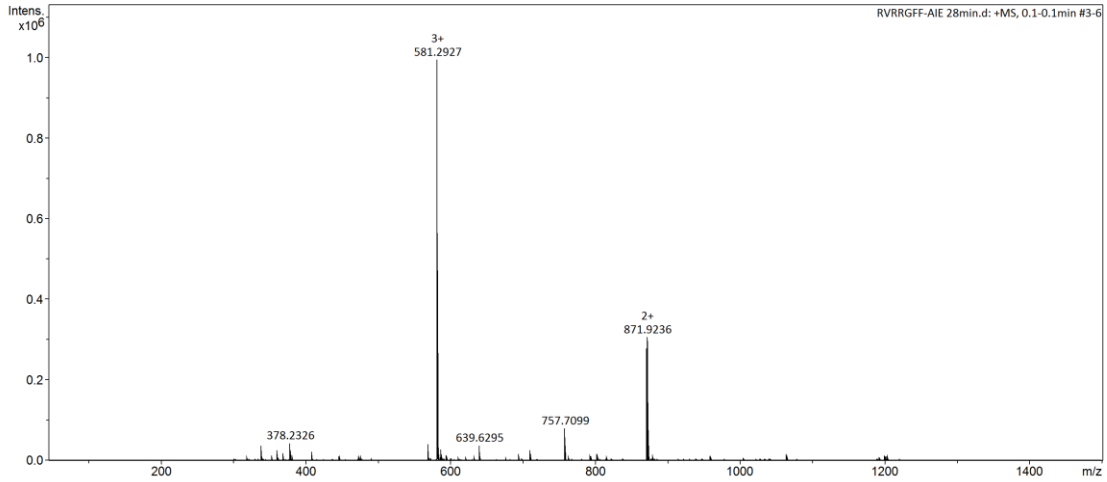


Fig. S10. HR-MS spectrum of **RVRRGFF-AIE**

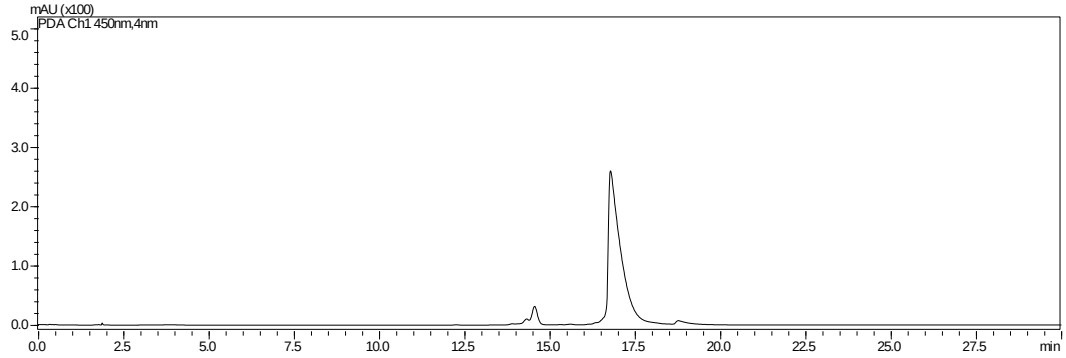


Fig. S11. HPLC spectrum of **BHQ3-RVRRGFF-AIE**

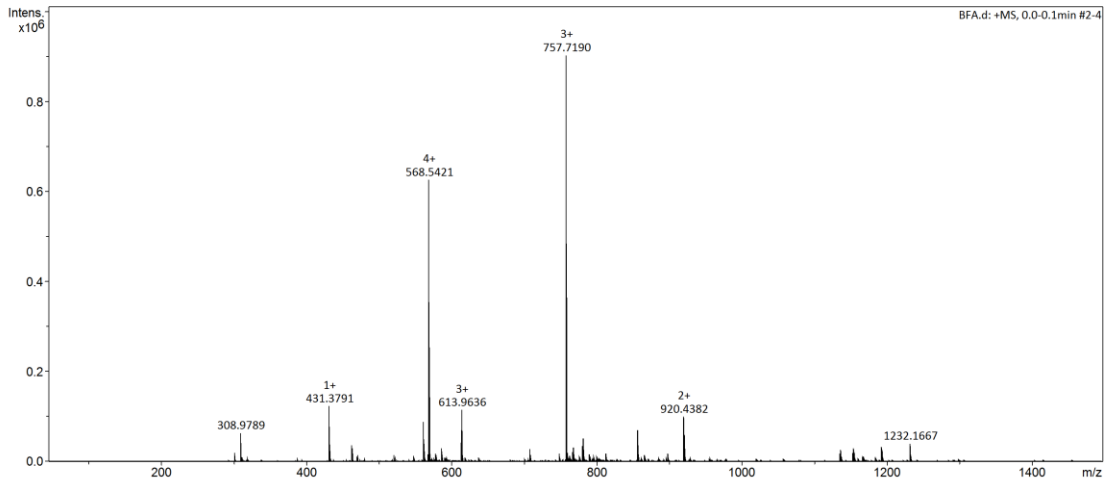


Fig. S12. HR-MS spectrum of **BHQ3-RVRRGFF-AIE**

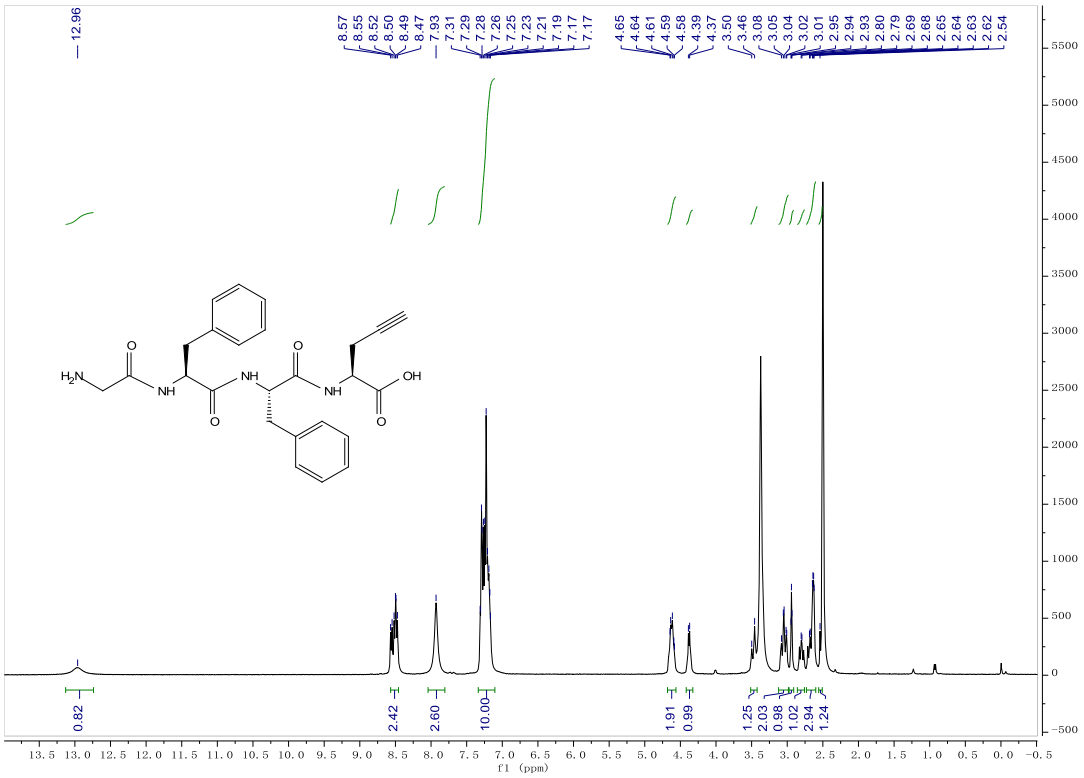


Fig. S13. ^{1}H NMR spectrum of **GFF-pra**

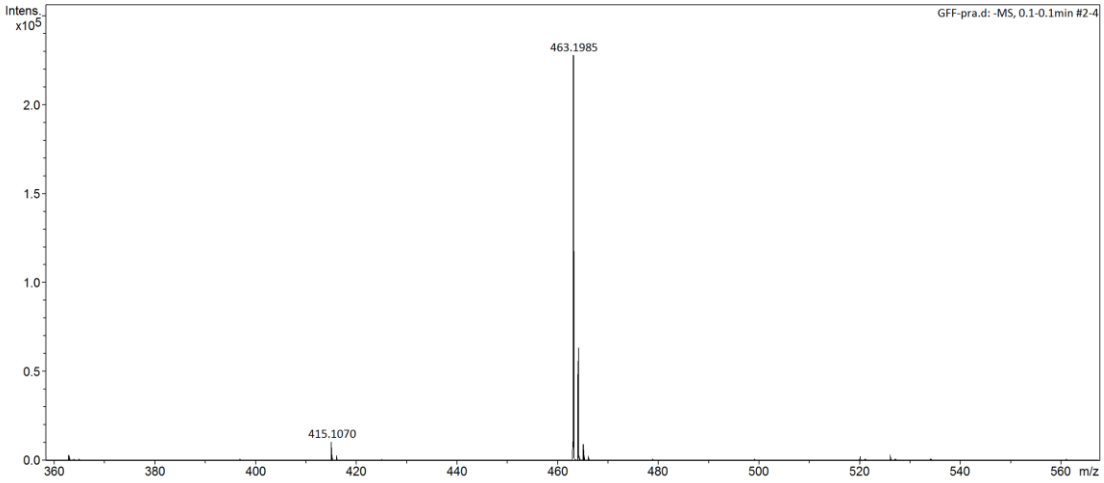


Fig. S14. HR-MS spectrum of **GFF-pra**

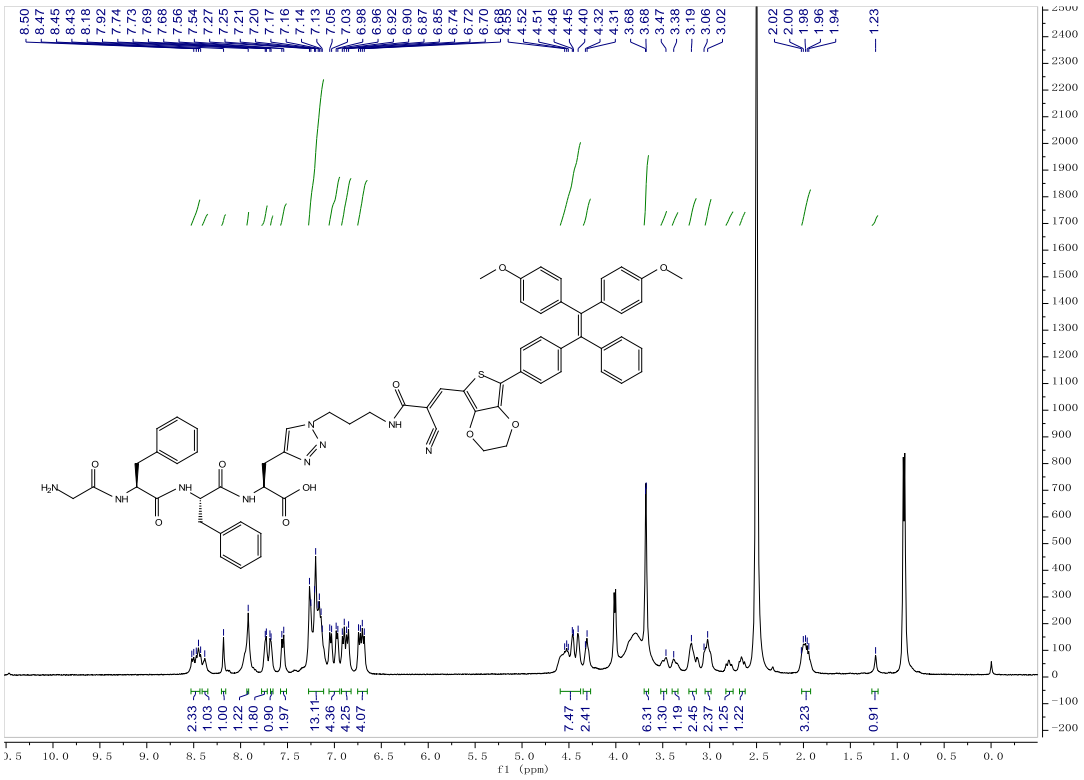


Fig. S15. ^{1}H NMR spectrum of **GFF-AIE**

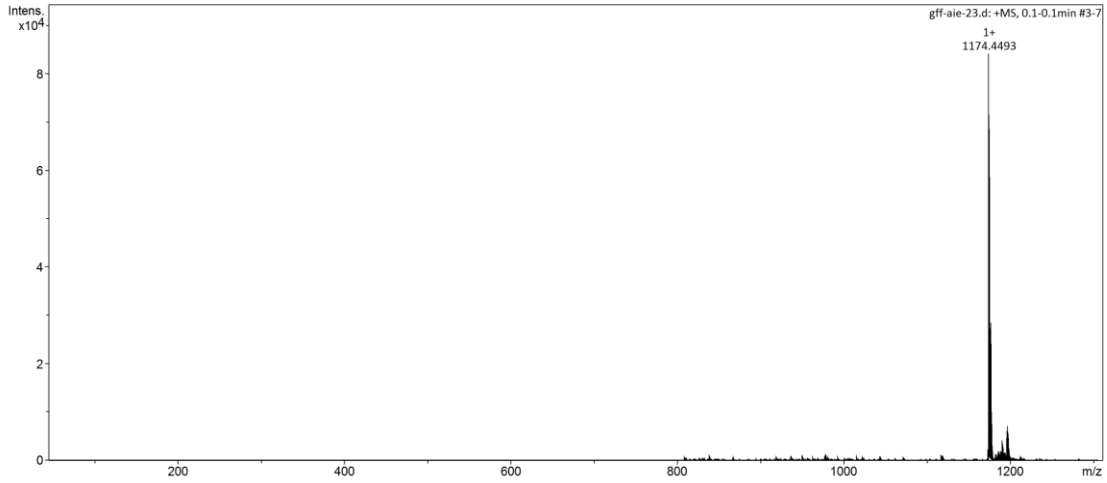


Fig. S16. HR-MS spectrum of **GFF-AIE**

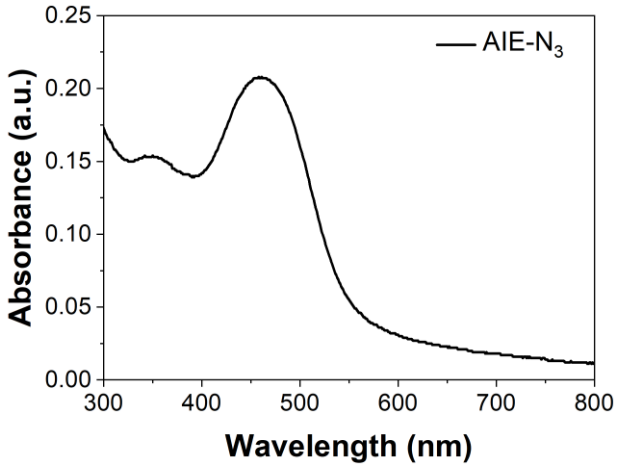


Fig. S17. The absorbance of AIE-N_3.

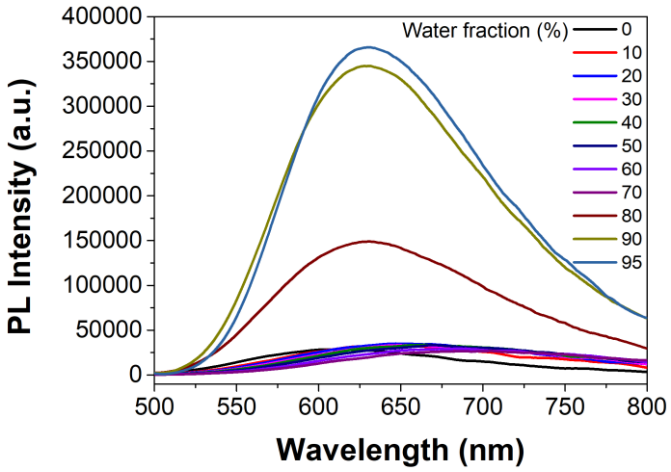


Fig. S18. Plot of PL intensity of 10 μM AIE-N_3 in THF-water mixtures with different water fractions. λex = 450 nm.

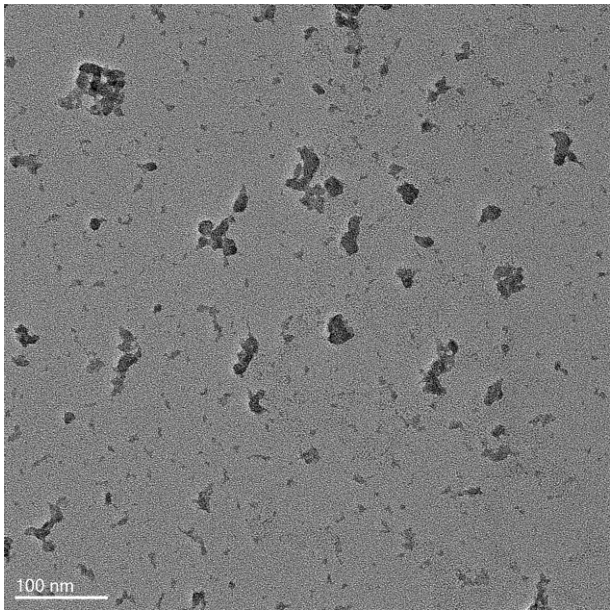


Fig. S19. TEM image of AIE-N_3 in water.

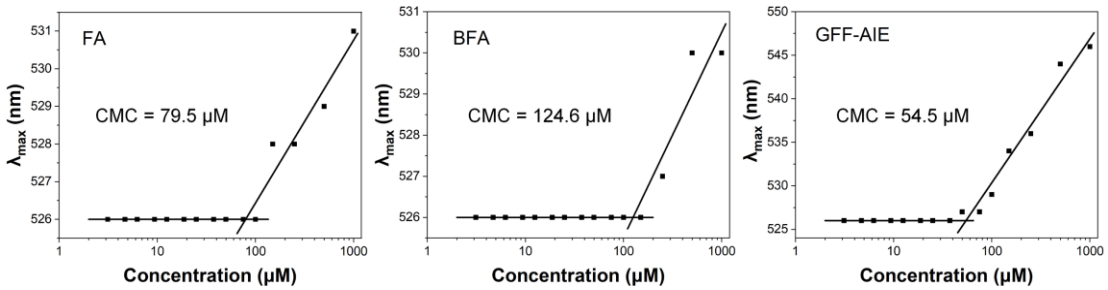


Fig. S20. Critical micelle concentration (CMC) values of FA, BFA and GFF-AIE.

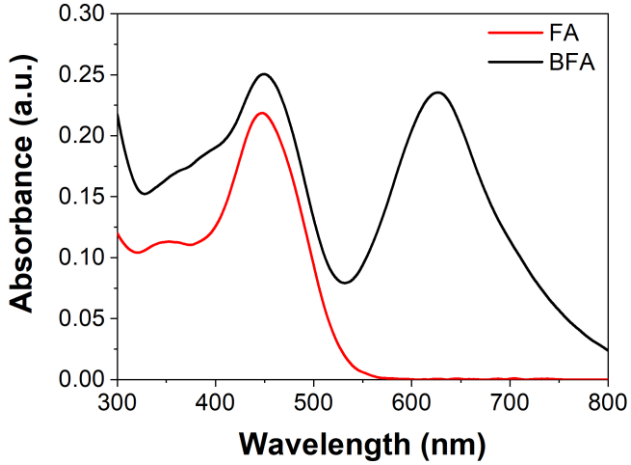


Fig. S21. The absorbance of 10 μM FA and 10 μM BFA in furin buffer.

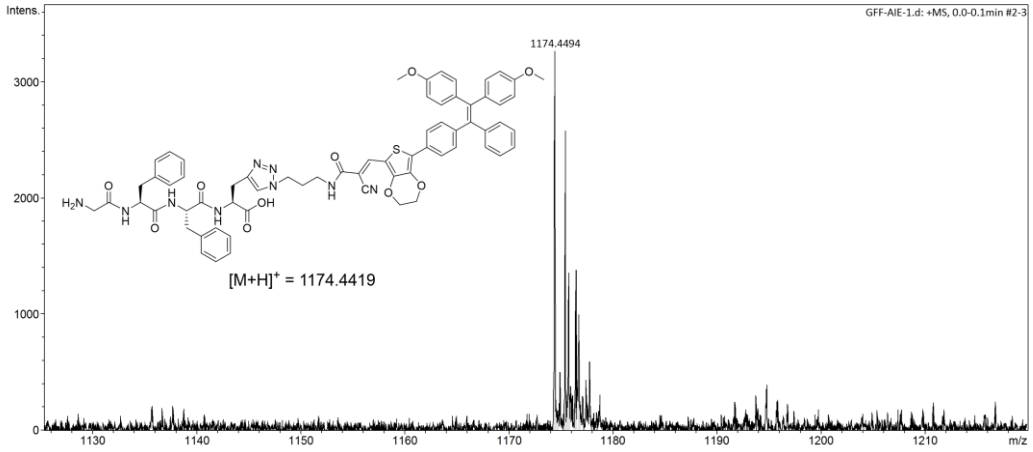


Fig. S22. HR-MS spectrum of the product peak at the retention time of 23.5 min in the red HPLC trace in Figure 2f, confirming the product of FA by furin was GFF-AIE.

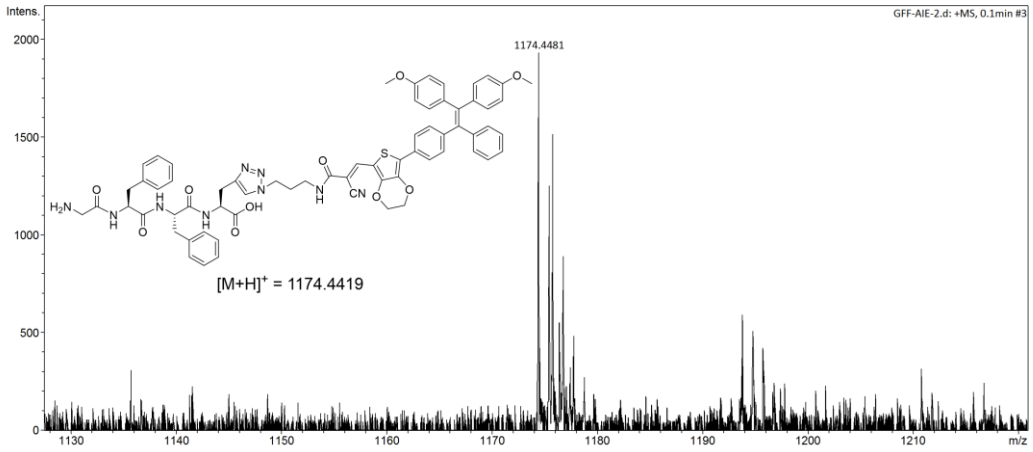


Fig. S23. HR-MS spectrum of the product peak at the retention time of 23.5 min in the green HPLC trace in Figure 2f, confirming the product of BFA by furin was GFF-AIE.

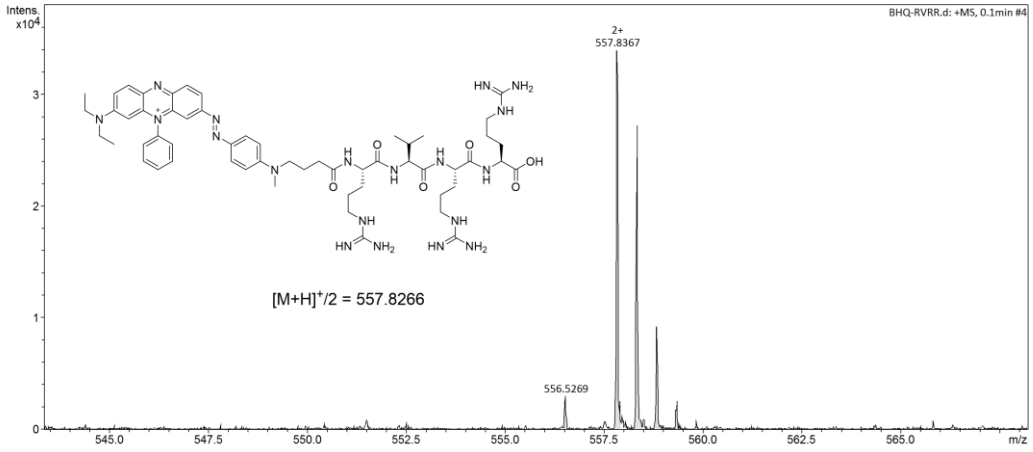


Fig. S24. HR-MS spectrum of the product peak at the retention time of 12.5 min in the green HPLC trace in Figure 2f, confirming the product of BFA by furin was BHQ3-RVRR.

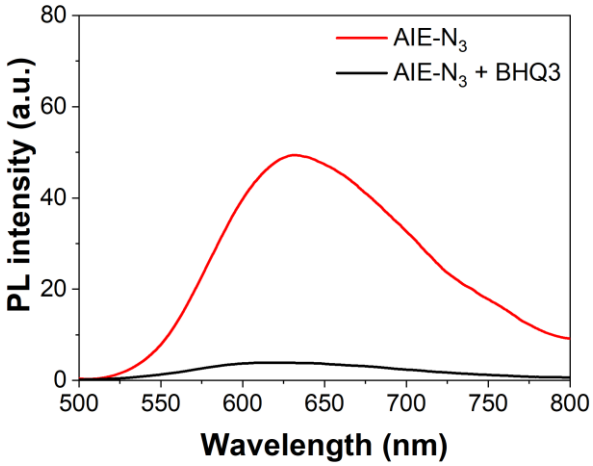


Fig. S25. PL intensity of 10 μM AIE-N_3 with or without 10 μM BHQ3 in furin buffer. The result shows that BHQ3 can quench the fluorescence of AIE-N_3 by simple mixing.

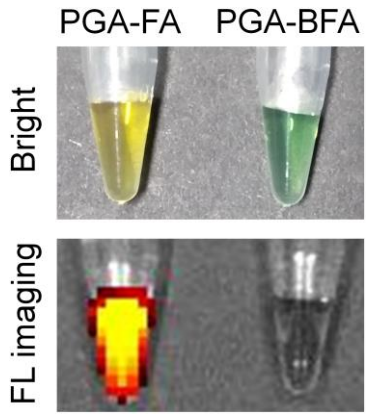


Fig. S26. Photographs of PGA-FA and PGA-BFA solution obtained from digital camera and IVIS system.

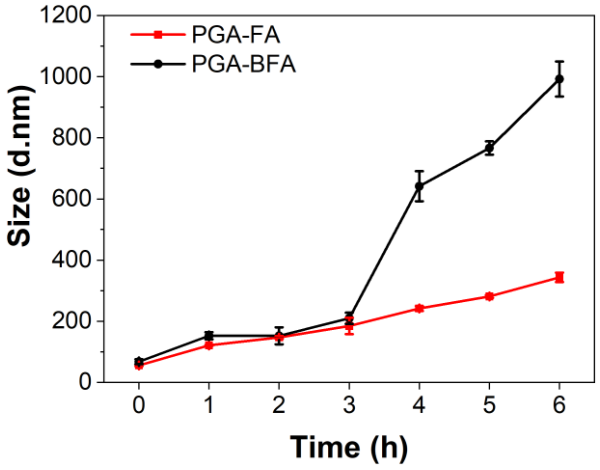


Fig. S27. The DLS result showed that the diameter of PGA-FA and PGA-BFA was increased in PBS (pH 4.5) with increasing time.

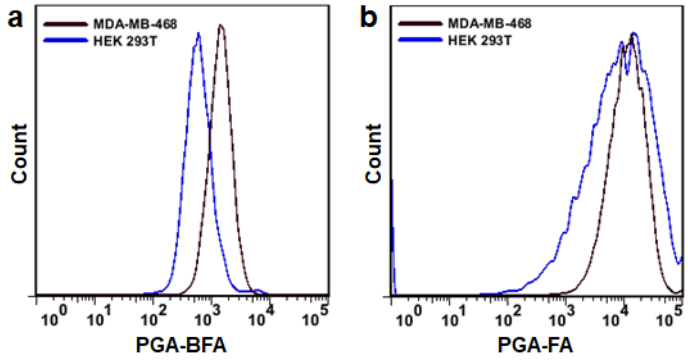


Fig. S28. Flow cytometry analyses show the average fluorescence intensity of MDA-MB-468 cancer cells and HEK 293T normal cells after being incubated with 10 μM PGA-BFA or PGA-FA for 6 h.

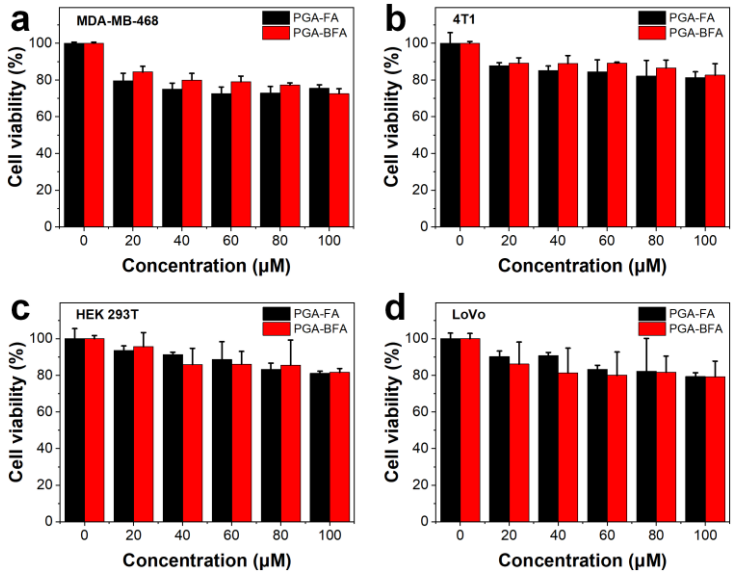


Fig. S29. Cell viability of (a) MDA-MB-468, (b) 4T1, (c) HEK 293T and (d) LoVo cells after incubation with the PGA-FA or PGA-BFA for 6 h.

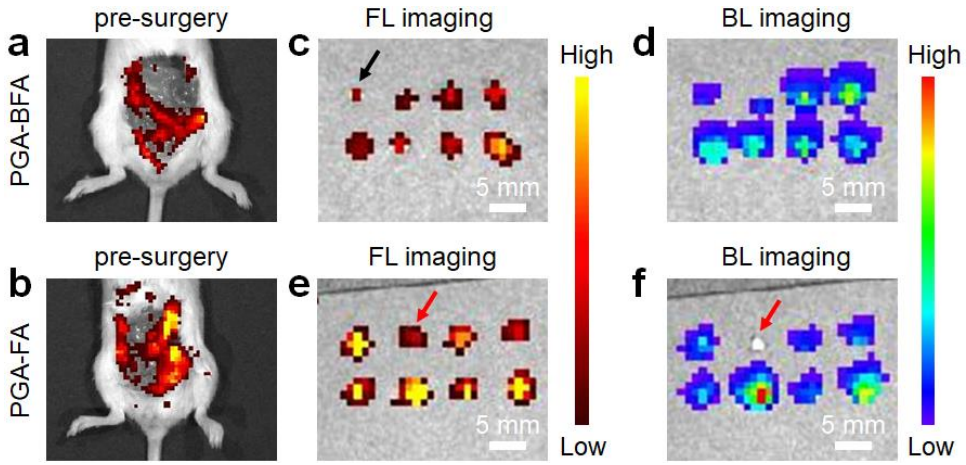


Fig. S30. Fluorescence (FL) imaging and bioluminescence (BL) imaging of the metastatic tumors in the peritoneal cavity. The tumor-bearing mice were intraperitoneally injected with (a) PGA-BFA (100 μM) and (b) PGA-FA (100 μM) for 12 h, respectively. (c) FL imaging and (d) BL imaging of tumor nodules excised from (a) with the guidance of fluorescence. (e) FL imaging and (f) BL imaging of tumor nodules excised from (b) with the guidance of fluorescence. The results showed that PGA-BFA could be used for fluorescence image-guided cancer surgery for that all the excised tumor nodules had BL signal and one of the excised tumor nodules was around 1 mm (black arrow). However, PGA-FA had relatively bad results due to its "always on" property. As shown in (e, f), the red arrow indicated that the excised tissue was not a tumor due to the negative BL signal.

Table S1. HPLC condition for the traces in **Fig. 2f**

Time (min)	Flow (mL/min)	H_2O%	CH_3CN%
0	1.0	70	30
3	1.0	70	30
25	1.0	10	90
27	1.0	0	100
37	1.0	0	100
38	1.0	70	30
40	1.0	70	30

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