

## Supporting Information

*of*

### **Fluorescent probe with aggregation-induced emission characteristic for targeted labelling and imaging cancer cells**

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Na Peng,<sup>†a</sup> Rui Xu,<sup>†a</sup> Michelle Si,<sup>b</sup> Amanda Victorious,<sup>b</sup> Emily Ha,<sup>b</sup> Chun-Yu Chang<sup>\*a</sup> and Xiao-Ding

Xu<sup>\*a,b</sup>

<sup>a</sup> College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, China

10 <sup>b</sup> Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA

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20 <sup>\*</sup>Corresponding author. E-mail address: changcy@whu.edu.cn; xd-xu@whu.edu.cn

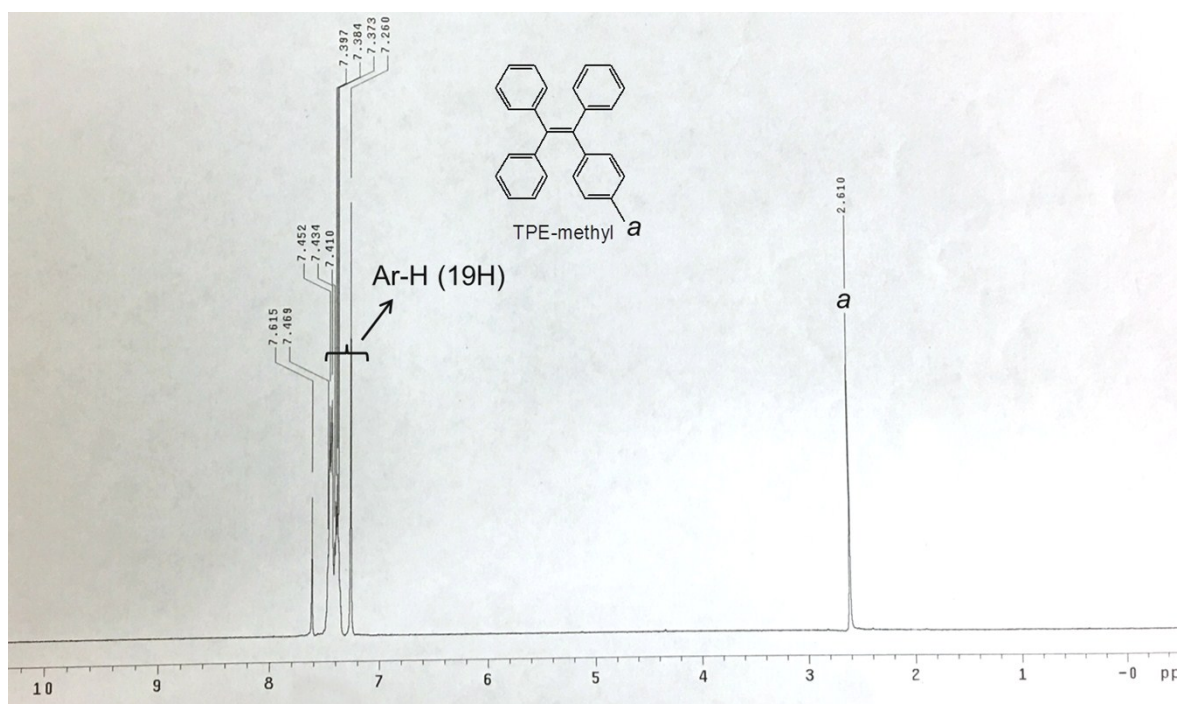
## 1. Materials

*N*-Fluorenyl-9-methoxycarbonyl (Fmoc) protected amino acids (Fmoc-Gly-OH, Fmoc-Asp(OtBu), Fmoc-Lys(Bos)-OH and Fmoc-Ser(tBu)-OH), Rink amide resin (100-200 mesh, loading: 0.56 mmol/g), 9-fluorenylmethoxy carbonyl chloride (Fmoc-Cl), *N*-hydroxybenzotriazole (HOBT), 5-benzotriazole-*N,N,N',N'*-tetramethyluroniumhexafluorophosphate (HBTU) and piperidine were purchased from GL Biochem (Shanghai) Ltd. (China) and used as received. Triisopropylsilane (TIS), 4-carboxyphenylboronic acid, fluorescein 5(6)-isothiocyanate (FITC)-labelled lectin and 5-hexynoic acid were provided by Sigma-Aldrich and used directly. Diphenylmethane, 4-methylbenzophenone, *n*-butyllithium (*n*-BuLi, 2.5 M in hexane), trifluoroacetic acid (TFA), *p*-toluenesulfonic acid (PTSA), *N*-bromosuccinimide (NBS), galactose, glucose, mannose, carbon tetrachloride (CCl<sub>4</sub>), dichloromethane (DCM), sodium azide (NaN<sub>3</sub>), dimethyl sulfoxide (DMSO), diethyl ether, anhydrous magnesium sulfate (MgSO<sub>4</sub>) and benzoyl peroxide were provided by Shanghai Reagent Chemical Co. (China) and used without purification. *N,N'*-Dimethylformamide (DMF), tetrahydrofuran (THF) and diisopropylethylamine (DiEA) were obtained from Shanghai Reagent Chemical Co. (China) and distilled prior to use. Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin and trypsin were purchased from Invitrogen Corp. All other reagents and solvents are of analytical grade and used directly.

## 2. Synthesis of 1-(4-methylphenyl)-1,2,2-triphenylethene (TPE-methyl)

The 1-(4-methylphenyl)-1,2,2-triphenylethene was synthesized according to previous report [1]. In brief, under nitrogen atmosphere, diphenylmethane (6.73 g, 40 mmol) was dissolved in 150 mL of THF. After placing the solution in ice-salt bath, *n*-BuLi (20 mL, 37.5 mmol) was added slowly by a syringe. The mixture was stirred at 0 °C for 1 h. Thereafter, 4-methylbenzophenone (6.56 g, 33.5 mmol) was added and the mixture was warmed to room temperature. After stirring overnight, the reaction was quenched by saturated NH<sub>4</sub>Cl solution and then 200 mL of DCM was added. The organic

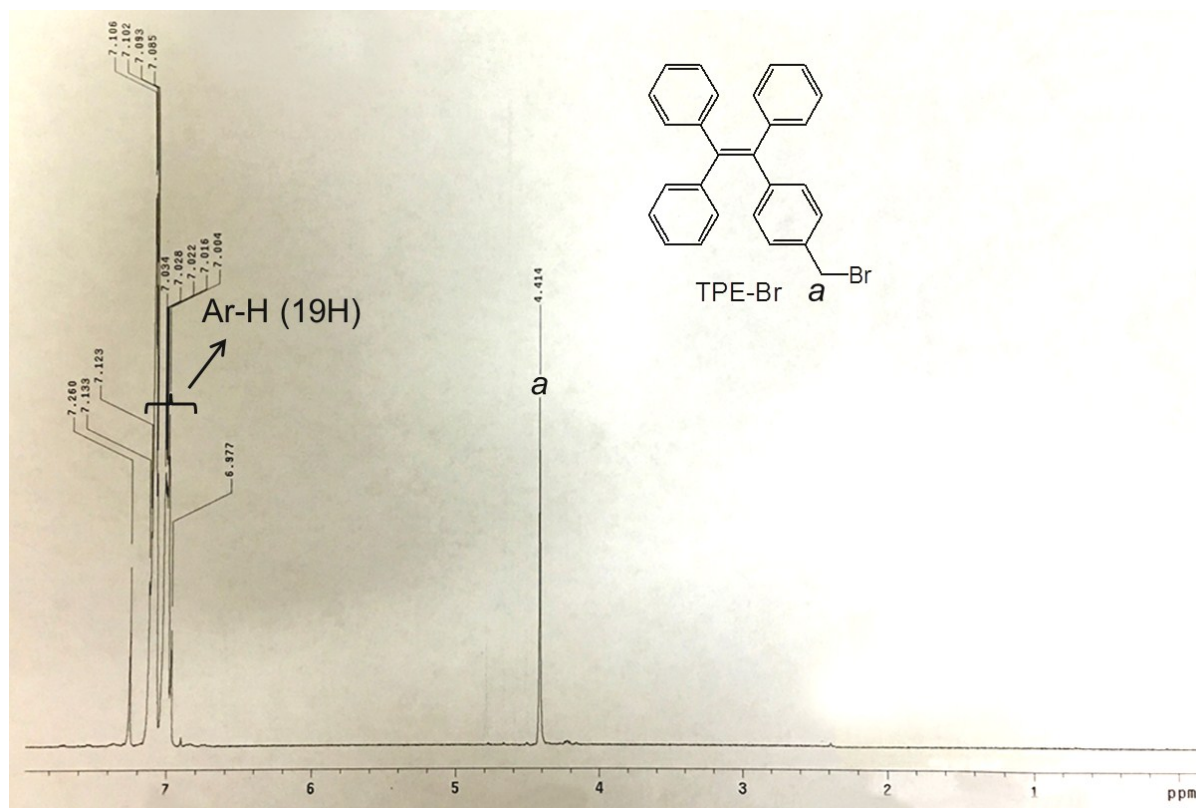
phase was collected and concentrated. The residual was dissolved in 150 mL of toluene and PTSA (0.3 g, 1.7 mmol) was added. After refluxing for 4 h, DCM was added to extract the product. The organic phase was collected and concentrated. The crude product was purified on a silica gel column, eluting with hexane, giving 3.71 g (yield: ~65%) of TPE-methyl as white solid. The reaction scheme is shown 5 in Scheme S1. The  $^1\text{H-NMR}$  spectrum of TPE-methyl is shown in Fig. S1.



**Fig. S1.**  $^1\text{H-NMR}$  spectrum of TPE-methyl in  $\text{CDCl}_3$ .

### 3. Synthesis of 1-[(4-bromomethyl)phenyl]-1,2,2-triphenylethene (TPE-Br)

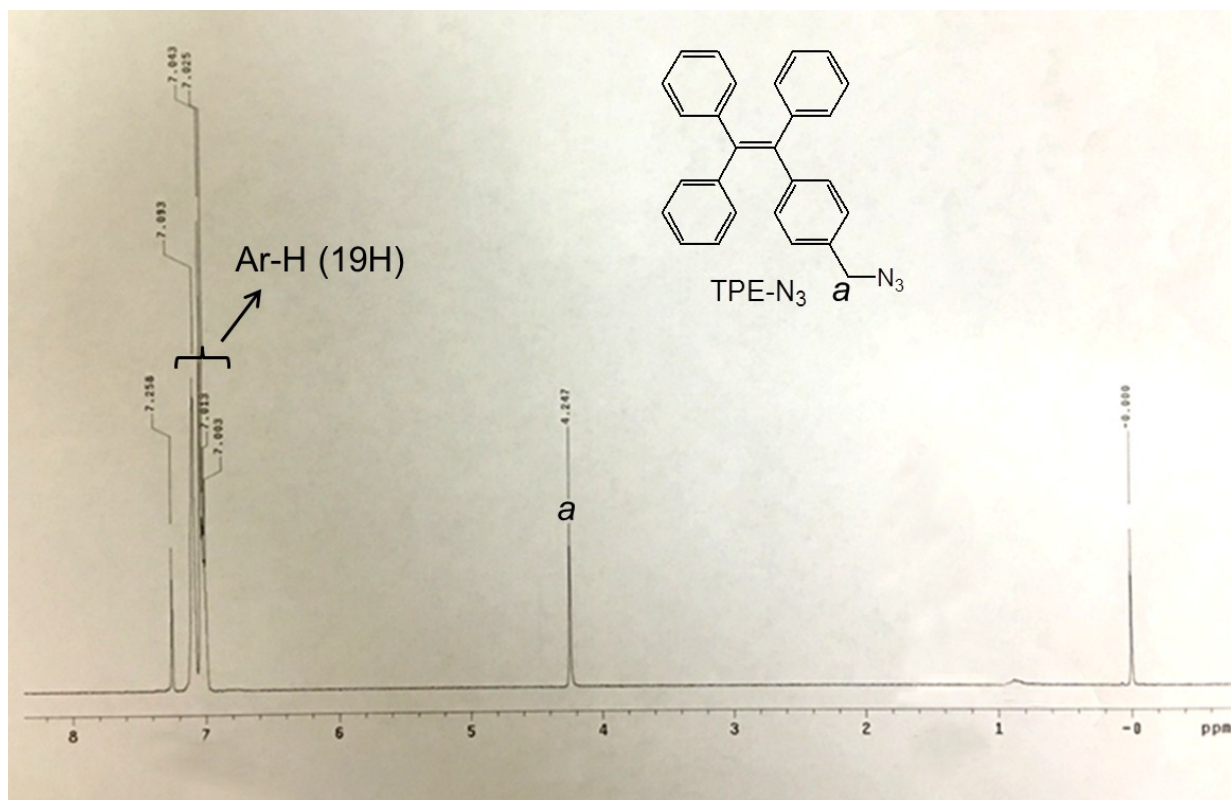
10 TPE-methyl (3.47 g, 10 mmol), NBS (0.89 g, 11 mmol) and benzoyl peroxide (24 mg, 0.1 mmol) were dissolved in 60 mL of  $\text{CCl}_4$ . After refluxing for 12 h, DCM and water were added. The organic phase was collected and dried over anhydrous  $\text{MgSO}_4$ . After removing the solvent, the residual was purified on a silica gel column, eluting with hexane, giving 2.43 g (yield: ~55%) of TPE-Br as white solid. The reaction scheme is shown in Scheme S1. The  $^1\text{H-NMR}$  spectrum of TPE-Br is shown in Fig. S2.



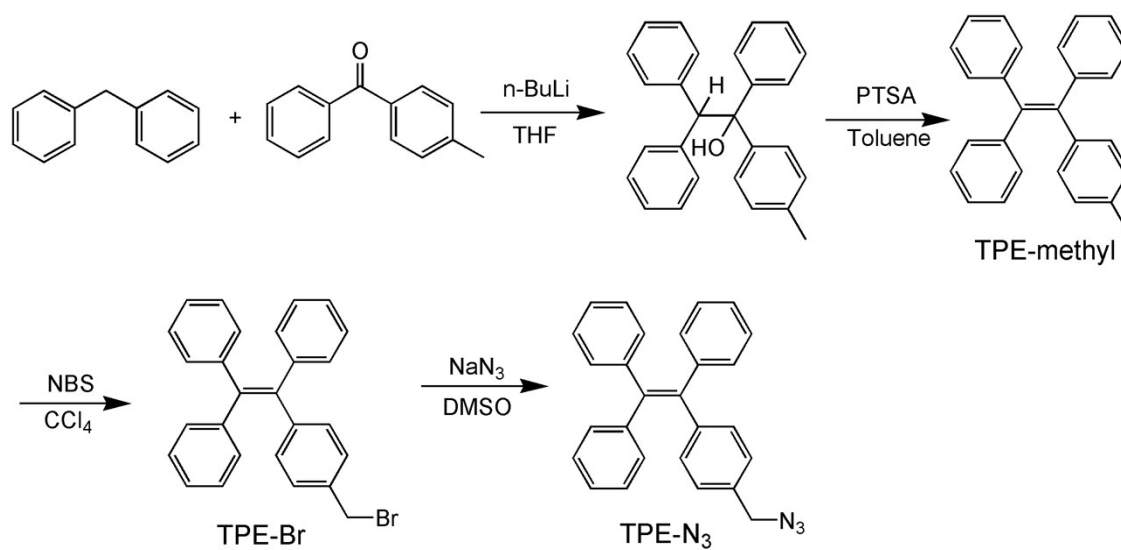
**Fig. S2.** <sup>1</sup>H-NMR spectrum of TPE-Br in CDCl<sub>3</sub>.

#### 4. Synthesis of 1-((4-azidomethyl)phenyl)-1,2,2-triphenylethene (TPE-N<sub>3</sub>)

5 TPE-Br (2.2 g, 5.18 mmol) and NaN<sub>3</sub> (0.51 g, 7.8 mmol) were dissolved in DMSO. Under nitrogen atmosphere, the mixture was stirred at room temperature for 24 h. Subsequently, 150 mL of water was then added and the solution was extracted with diethyl ether (3 × 200 mL). The organic phase was combined and then dried over anhydrous MgSO<sub>4</sub>. The crude product was purified on a silica gel column, eluting with hexane/chloroform (v/v = 3:1), giving 1.9 g (yield: ~95%) of TPE-N<sub>3</sub> as white solid. The reaction scheme is shown in Scheme S1. The <sup>1</sup>H-NMR spectrum of TPE-N<sub>3</sub> is shown in Fig. S2.



**Fig. S3.** <sup>1</sup>H-NMR spectrum of TPE-N<sub>3</sub> in CDCl<sub>3</sub>.



**Scheme S1.** Synthesis route of TPE-N<sub>3</sub>.

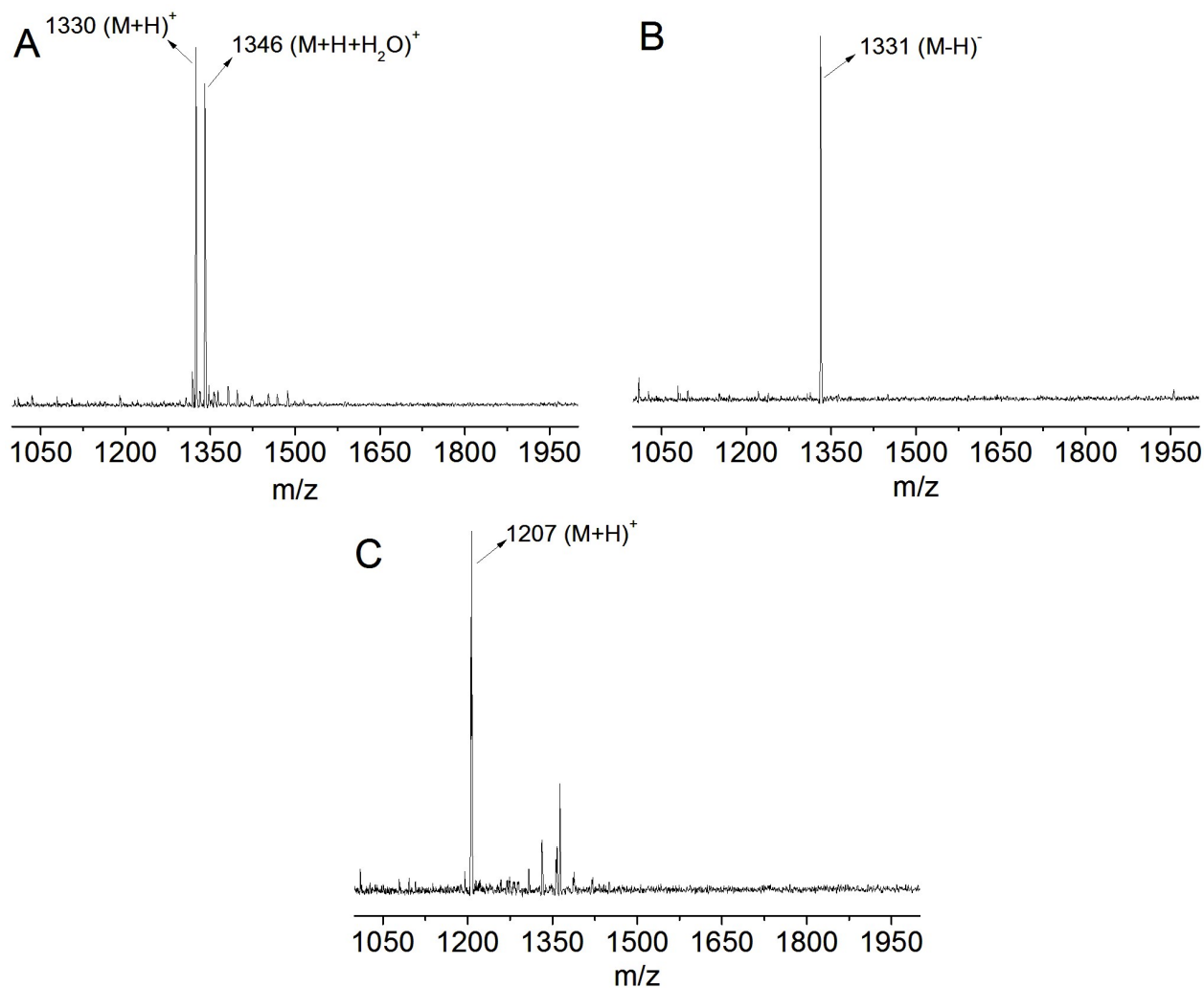
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## 5. Synthesis of TPE-tagged peptidyl boronic acids (TPBAs)

To synthesize TPBAs, peptidyl boronic acids containing alkyne functional groups were first prepared manually on the Rink amide resin employing a standard Fmoc solid phase peptide synthesis (SPPS) technique. Before the reaction, the resin was washed with DCM (three times) and DMF (three times) and then immersed in DMF for 30 min. After draining off DMF solution, 20% piperidine/DMF (V/V) solution was added to the resin to remove the Fmoc protected groups. The presence of free amino groups was indicated by a blue color in the Kaiser test. Thereafter, a DMF solution of the mixture of Fmoc-Lys(Boc)-OH (4 equiv), HBTU (4 equiv), HOBT (4 equiv) and DiEA (6 equiv) was added. After shaking at room temperature for 1.5 h, the reaction solution was drained off and the resin was washed with DMF (three times). The absence of free amino groups was indicated by a yellow color in the Kaiser test. Subsequently, 20% TFA/DCM (V/V) solution was added to the resin to remove Boc protected group. After shaking for 30 min and washing the resin with DMF (three times), a DMF solution of the mixture of 5-hexynoic acid (4 equiv), HBTU (4 equiv), HOBT (4 equiv) and DiEA (6 equiv) was added to the resin. After shaking at room temperature for 1.5 h, the reaction solution was drained off and the resin was washed with DMF (three times). Subsequently, 20% piperidine/DMF (V/V) solution was introduced to the resin to remove the Fmoc protected groups. Thereafter, a DMF solution of the mixture of Fmoc protected amino acid (4 equiv), HBTU (4 equiv), HOBT (4 equiv) and DiEA (6 equiv) was added. After shaking at room temperature for 1.5 h, the reaction solution was drained off and the resin was washed with DMF (three times). After the repetition of the deprotection and acylation reaction to obtain the expected peptide sequence, a DMF solution of the mixture of 4-carboxyphenylboronic acid (4 equiv), HBTU (4 equiv), HOBT (4 equiv) and DiEA (6 equiv) was added. After shaking at room temperature for 1.5 h, the resin was washed with DMF (three times) and DCM (three times). Cleavage of the peptide from the resin was performed using a mixture of TFA, TIS and H<sub>2</sub>O in the ratio of 95:2.5:2.5. After 2 h shaking at room temperature, the cleavage mixture and TFA washing were collected. The combined solution was concentrated to a viscous solution by rotary evaporation. Cold ether was then added to precipitate the product. After washing with cold ether

(three times), the crude peptidyl boronic acids were obtained after drying under vacuum for 24 h.

The synthesis of TPBAs was conducted by using the "click" reaction between TPE-N<sub>3</sub> and peptidyl boronic acids containing alkyne functional groups. In brief, peptidyl boronic acid, TPE-N<sub>3</sub> (1.2 equiv) and CuBr (0.2 equiv) were dissolved in DMSO. Under nitrogen atmosphere, the mixture was stirred for 24 h. After removing CuBr on a neutral Al<sub>2</sub>O<sub>3</sub> column, the crude product was obtained via precipitating in cold ether. The final TPBAs were purified by high-performance liquid chromatography (HPLC) with a C18 column and using a linear gradient of acetonitrile and DI water containing 0.1% TFA. The synthesis scheme is shown in Scheme S2. The MALDI-TOF-MS spectra of the TPBAs are shown in Fig. S4.



**Fig. S4.** MALDI-TOF-MS spectra of TPBA1 (A), TPBA2 (B) and TPBA3 (C).





spectrometry (Perkin-Elmer) with excitation at 312 nm and emission data range between 320 and 700 nm. The TPE-N<sub>3</sub> aqueous solution was prepared by dispersing the DMSO solution of TPE-N<sub>3</sub> in water.

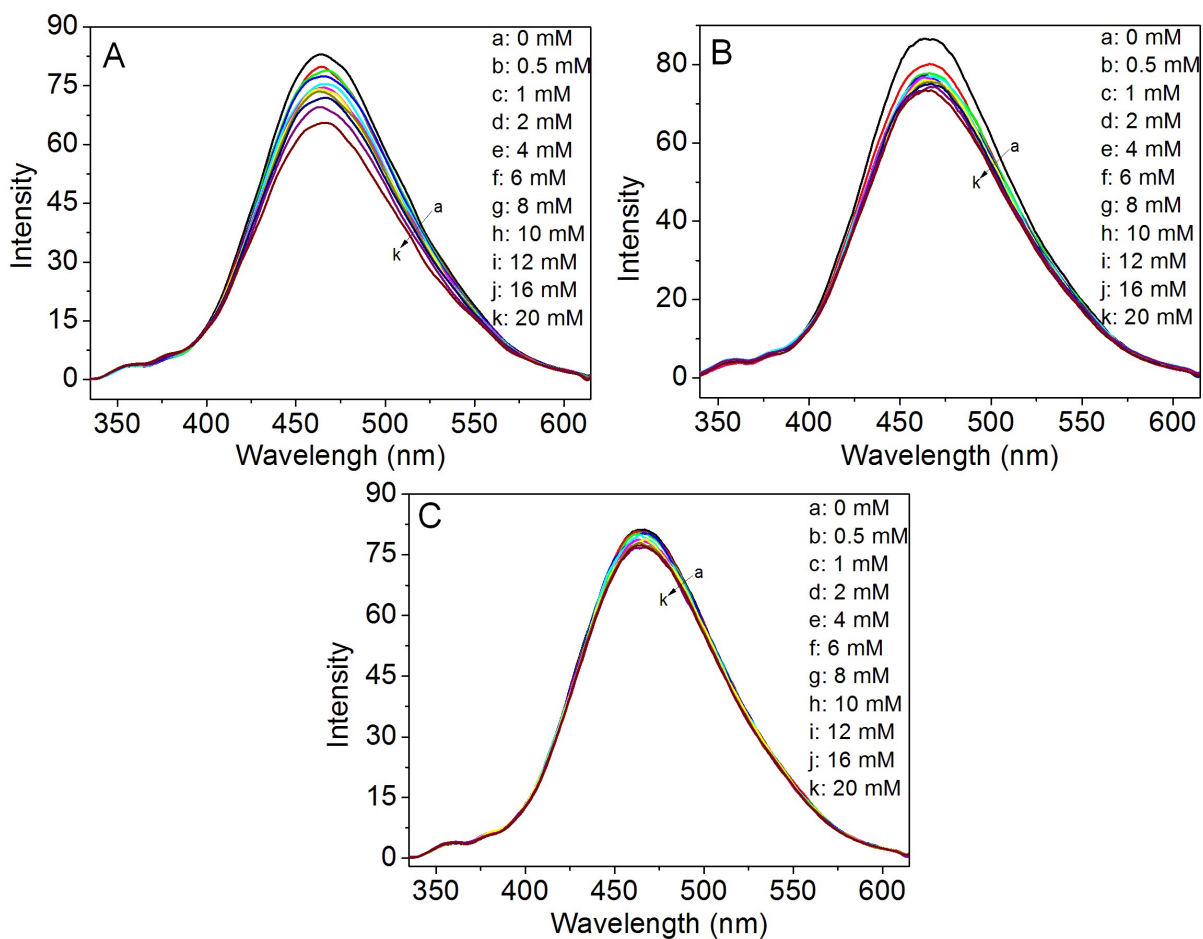
## 5 7. Transmission electron microscopy (TEM)

The morphology of the self-assembled TPBAs and TPE-N<sub>3</sub> was observed on a Tecnai G20 S-TWIN transmission electron microscope (TEM). Before the observation, the TPBAs or the TPE-N<sub>3</sub> aqueous solution was first applied to a copper grid with Formvar film and stained by a 0.2% (w/v) phosphotungstic acid solution.

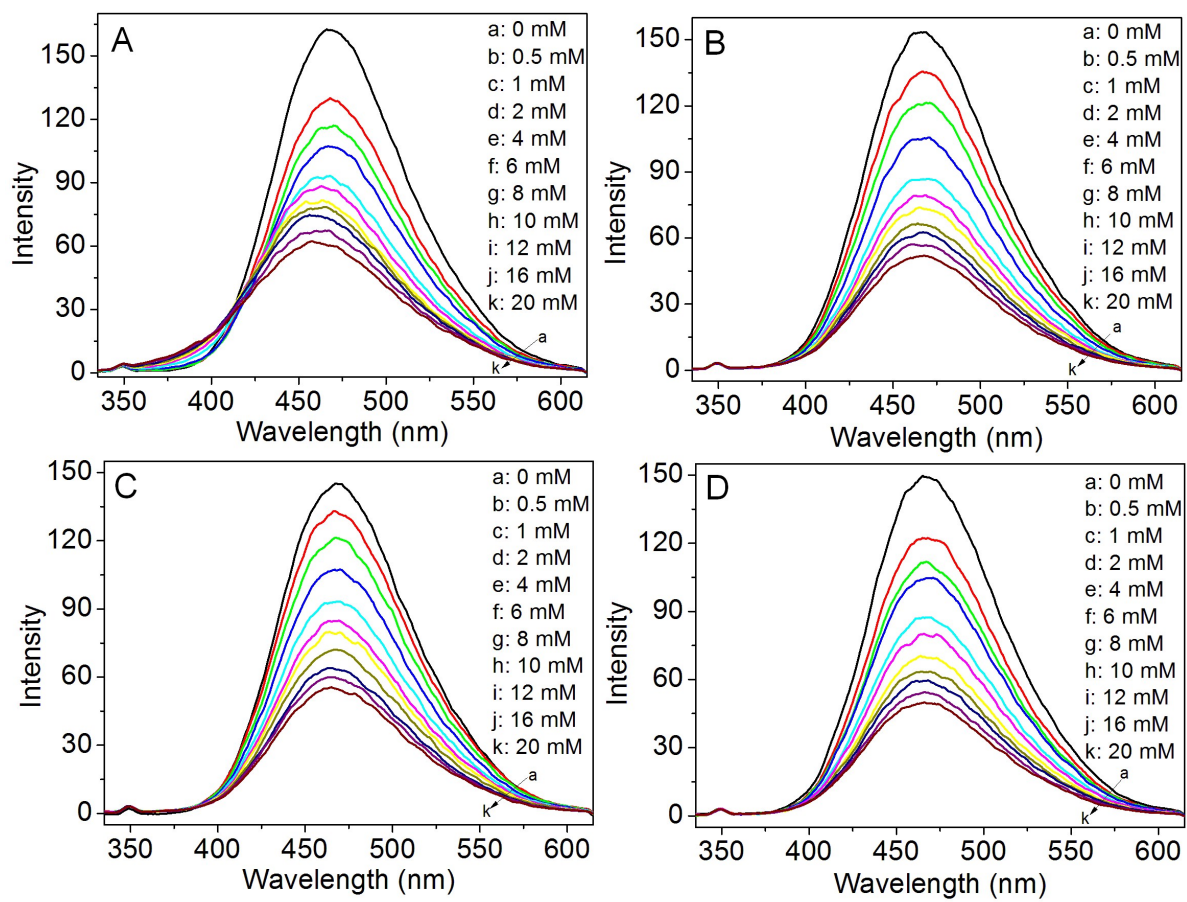
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## 8. Screening of TPBAs

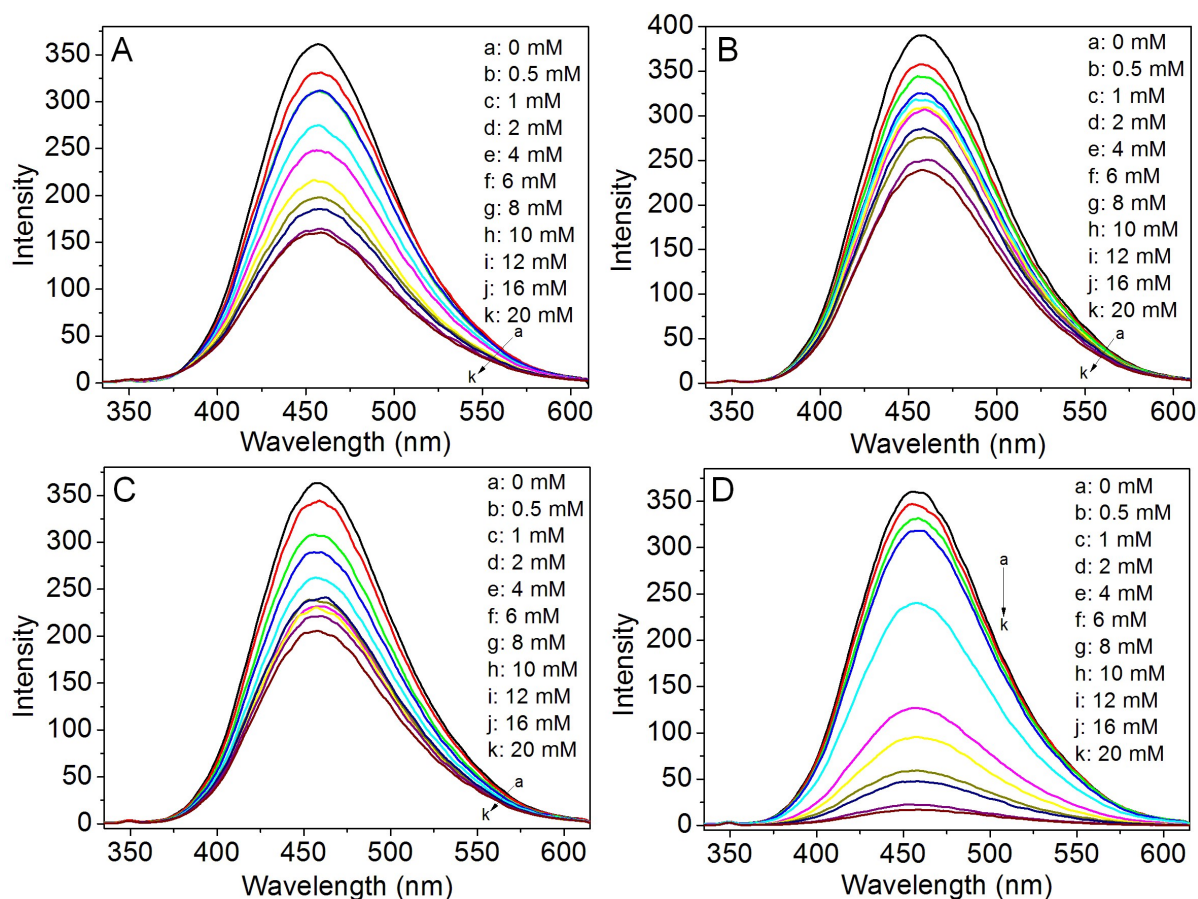
Fluorescence spectroscopy was used to determine the affinity and selectivity of FSs over SA. To evaluate the affinity of FSs for SA, the aqueous solution of TPBAs at a concentration of 20  $\mu$ M was prepared and the fluorescence emission spectra with the addition of different amount of SA were recorded on a LS55 luminescence spectrometry. The solution of all TPBAs shows different degree of decrease in the fluorescence intensity when the concentration of SA increasing from 0.5 to 20 mM. If further increasing the concentration of SA, the fluorescence intensity changes slightly. Therefore, the fluorescence intensity change at which the concentration of SA is 20 mM was used to evaluate the affinity of TPBAs for SA. To evaluate the selectivity of TPBAs over SA, the fluorescence emission spectra of the solution of TPBAs solution with the presence of 20 mM of SA, mannose, galactose and glucose were examined and the fluorescence intensity changes at 460 nm were used to evaluate the selectivity of TPBAs over SA.



**Fig. S5.** Emission spectra of the TPBA1 (20  $\mu\text{M}$ ) mixed with galactose (A), glucose (B) and mannose (C) at different concentrations.



**Fig. S6.** Emission spectra of the TPBA2 (20  $\mu\text{M}$ ) mixed with SA (A), galactose (B), glucose (C) and mannose (D) at different concentrations.



**Fig. S7.** Emission spectra of the TPBA3 (20  $\mu$ M) mixed with SA (A), galactose (B), glucose (C) and mannose (D) at different concentrations.

## 5 9. Cell culture

The cells were first incubated in DMEM or DEME:F12 (V/V; 1/1) containing 10% FBS and 1% antibiotics (penicillin-streptomycin, 10,000 U/mL) at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>.

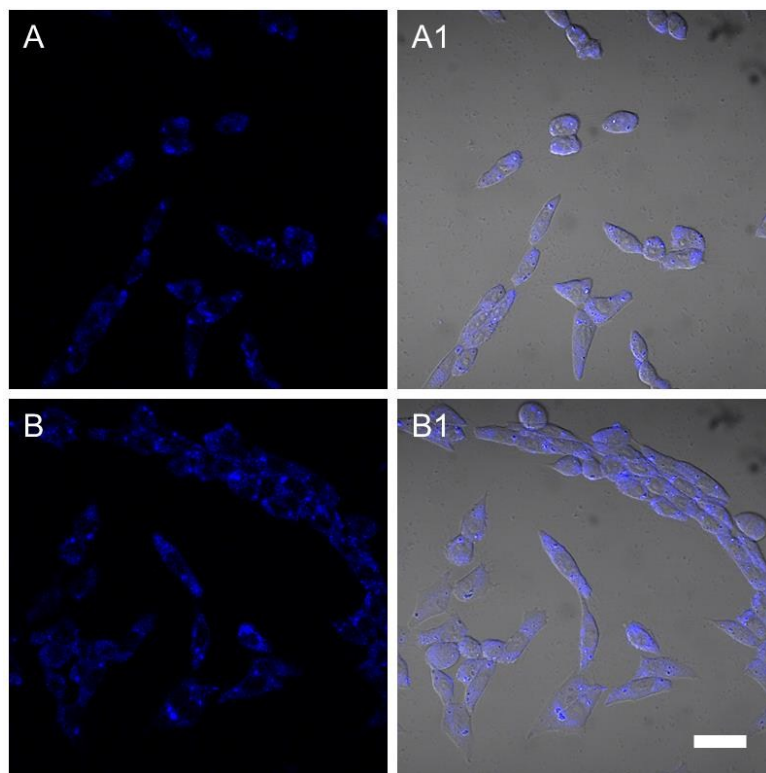
## 10 10. Determination the SA expression

The cells were seeded in 12-well plates and incubated in DMEM (HepG2 cells) or DMEM:F12 (AML-12 cells). After 24 h incubation, SA-specific fluorescein 5(6)-isothiocyanate (FITC)-labelled lectin (0.01 mg/mL) was added and cells were further incubated for another 15 min. Subsequently, the cells

were washed with PBS for three times and then viewed under a lasers canning confocal microscope (Nikon C1-si TE2000, Japan) with excitation at 405 nm and collected for flow cytometry quantitative analysis (BD FACSAria™ III, USA) with excitation at 375 nm.

### 5 11. Fluorescent imaging of cancer cells

The cells were seeded in 12-well plates and incubated in DMEM (HepG2 cells) or DMEM:F12 (AML-12 cells). After 24 h incubation, TPBA1 (5  $\mu\text{m}$ ) was added and incubated with the cells for different periods. After the incubation, the cells were washed with PBS for three times and then viewed under a lasers canning confocal microscope (Nikon C1-si TE2000, Japan) with excitation at 405 nm and 10 collected for flow cytometry quantitative analysis (BD FACSAria™ III, USA) with excitation at 375 nm.



**Fig. S8.** CLSM images of TPBA1 incubated with HepG2 cells for 15 min (A, A1) and 30 min (B, B1).

(A, B) Fluorescent field; (A1, B1) Overlapped field. Scale bar 30  $\mu\text{m}$ .

## Reference

- [1] H. Shi, R. T. K. Kwok, J. Liu, B. Xing, B. Z. Tang, B. Liu. *J. Am. Chem. Soc.* 2012, **134**, 17972-17981.