Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Biosensor based on Self-Clickable AIEgen: A Signal Amplification Strategy for Ultrasensitive Immunoassay

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Materials and Methods

Materials and characterization: Copper (II) sulfate ($CuSO_4$), sodium ascorbate (SA), trifluoroacetic acid (TFA), alkaline phosphatase (ALP) and ascorbic acid-phosphate and other chemicals were purchased from Sigma-Aldrich. Primary antibodies and ALP-conjugated antibodies were purchased from Jackson Immuno Research. Anhydrous dimethyl sulfoxide (DMSO) and other chemicals were all purchased from Sigma-Aldrich and used as received. Milli-Q water was supplied by Milli-Q Plus System (Millipore Corporation, United States). Phosphate-buffer saline (PBS, 10×) buffer with pH = 7.4 is a commercial product of 1st BASE (Singapore). Milli-Q water (18.2 MΩ) was used to prepare the buffer solutions from the 10× PBS stock buffer. 1× PBS contains NaCl (137 mM), KCl (2.7 mM), Na_2HPO_4 (10 mM), and KH_2PO_4 (1.8 mM). Tetrahydrofuran (THF) and dichloromethane were dried by distillation using sodium as drying agent and benzophenone as indicator. All non-aqueous reactions were carried out under nitrogen atmosphere in oven-dried glassware. Deuterated solvents with tetramethylsilane (TMS) as internal reference were purchased from Cambridge Isotope Laboratories Inc..

NMR spectra were measured on a Bruker ARX 300/400/500 NMR spectrometer. Chemical shifts are reported in parts per million referenced with respect to residual solvent ($CDCl_3$ = 7.26 ppm and $(CD_3)_2SO$ = 2.50 ppm) for ^1H NMR and ($CDCl_3$ = 77.0 ppm and $(CD_3)_2SO$ = 40.0 ppm) for ^{13}C NMR. The extent of reaction was monitored by thin layer chromatography (TLC) using Merck 60 F254 pre-coated silica gel plates with fluorescent indicator UV254. After the plates were subjected to elution in the TLC chamber, the spots were visualized under UV light or using the appropriate stain (I_2, $KMnO_4$, ninhydrin or ceric ammonium molybdate (CAM)). Flash column chromatography was carried out using Merck silica gel (0.040-0.063). Mass spectra were recorded on Agilent 5975 DIP-MS for electron impact (EI) and the AmaZon X LC-MS for electrospray

ionization (ESI). Particle size and size distribution were determined by laser light scattering (LLS) with a particle size analyzer (90 Plus, Brookhaven Instruments Co., United States) at a fixed angle of 90° at room temperature. UV-vis absorption spectra were taken on a Shimadzu Model UV-1700 spectrometer. Photoluminescence (PL) spectra were measured on a Perkin-Elmer LS 55 spectrofluorometer. All UV and PL spectra were collected at 24 ± 1 °C.

Synthesis of compound 1. 2-Chloro-*N,N*-dimethylethanamine hydrochloride (720 mg, 5.0 mmol) was dissolved in deionized water (20 mL), and then sodium azide (975 mg, 15.0 mmol) was added. The mixture was stirred at 80 ^{o}C for 24 h. After cooling, the mixture was adjusted to pH = 10 by the addition of 1 M sodium hydroxide solution and extracted three times with dichloromethane. The combined extracts were dried over anhydrous sodium sulfate. Finally, compound **1** was obtained after solvent evaporation under reduced pressure as colorless oil (514 mg, 90.1 % yield). ^{1}H NMR (600 MHz, $CDCl_3$, 298 K), δ(TMS, ppm): 2.28 (s, 6H, -NCH_3), 2.51 (t, 2H, J = 6.6 Hz, -NCH_2-), 3.35 (t, 2H, J = 6.0 Hz, -CH_2N_3).

Synthesis of compound 3. 4,4'-Dihydroxybenzophenone (1.07 g, 5.0 mmol) in acetone (30 mL) was added dropwise to a stirred mixture of 1,4-dibromobutane (3.24 g, 15.0 mmol), potassium carbonate (1.73 g, 12.5 mmol), and potassium iodide (166 mg, 1.0 mmol) in acetone (30 mL), and the mixture was stirred at 60 ^{o}C in an oil bath for 12 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in water, and further extracted with dichloromethane. The combined organic extracts were dried over anhydrous sodium sulfate and concentrated by rotary evaporation. The crude product was recrystallized in hexane to afford compound **2** as a white solid (2.12 g, 87.6 % yield). The solid was directly used for the subsequent step without further purification. To a mixture of Zn (1.07 g, 8.0 mmol) and 20 mL of dry THF was added $TiCl_4$ (0.88

mL, 16.4 mmol) under argon atmosphere at 0 °C. The mixture was stirred for 30 min at room temperature and then 2 h at 60 °C. Subsequently, a solution of compound **2** (968 mg, 2.0 mmol) and 4,4'-dibromobenzophenone (680 mg, 2.0 mmol) in THF (30 mL) was added dropwise in 1 h at 0 °C. The mixture was refluxed overnight. After cooling to room temperature, 10 wt% K_2CO_3 (aq) was added and the mixture was filtered. The filtrates were extracted with dichloromethane and the organic layer was washed with brine and dried over anhydrous sodium sulfate. After solvent removal under reduced pressure, the crude product was purified by column chromatography using n-hexane/dichloromethane (2/1, v/v) as eluent to afford compound **3** as a white solid (648 mg, 40.9 % yield). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K), δ(TMS, ppm): 1.92 (m, 4H, -CH_2-), 2.04 (m, 4H, -CH_2-), 3.48 (t, 4H, J = 6.4 Hz, -CH_2Br), 3.93 (t, 4H, J = 6.0 Hz, -NCH_2-), 6.63 (d, 4H, J = 4.4 Hz, ArH), 6.85-6.90 (m, 8H, ArH), 7.22 (d, 4H, J = 4.8 Hz, ArH). ^{13}C NMR (150 MHz, $CDCl_3$, 298 K), δ(ppm): 27.9, 29.5, 33.4, 66.7, 113.8, 120.3, 131.0, 132.5, 133.0, 135.7, 136.6, 141.5, 142.8, 157.8.

Synthesis of compound 4. A mixture of compound **3** (198.1 mg, 0.25 mmol), copper iodide (CuI, 5 mol%), triphenylphosphine (PPh_3, 5 mol%), tetrakis(triphenylphosphine)palladium ($Pd(PPh_3)_4$, 3 mol%), was carefully degassed and charged with nitrogen. THF (5 mL) and Et_3N (1 mL) were then added. After all the compounds were dissolved, (trimethylsilyl)acetylene (196 mg, 2.0 mmol) was injected into the flask, and the mixture was stirred at 65 °C for 24 h. The reaction was quenched by adding saturated NH_4Cl aqueous solution (50 mL). The aqueous phase was extracted with dichloromethane. After solvent removal under reduced pressure, the crude product was purified by column chromatography using n-hexane/dichloromethane (2/1, v/v) as eluent to afford compound **4** as a yellow solid (172 mg, 92.9 %).^{1}H NMR (600 MHz, $CDCl_3$, 298 K), δ(TMS, ppm): 0.25 (s, 18H, -$Si(CH_3)_3$), 1.92 (m, 4H, -CH_2-), 2.04 (m, 4H, -CH_2-), 3.48 (t, 4H, J = 6.6 Hz,

-CH_2Br), 3.93 (t, 4H, J = 6.0 Hz, -NCH_2-), 6.61 (d, 4H, J = 7.2 Hz, ArH), 6.85-6.93 (m, 8H, ArH), 7.20 (d, 4H, J = 8.4 Hz, ArH). ^{13}C NMR (150 MHz, $CDCl_3$, 298 K), δ(ppm): 0.01, 28.0, 29.4, 33.4, 44.8, 66.8, 94.4, 105.3, 113.7, 120.7, 131.4, 132.6, 135.8, 137.7, 141.6, 144.4, 157.7.

Synthesis of compound TPEAl. Compound **4** (82.7 mg, 0.10 mmol) and K_2CO_3 (41.4 mg, 0.30 mmol) were placed into a 100 mL round-bottom flask. The reactants were dissolved in methanol (3 mL), THF (5 mL), and $CHCl_3$ (2 mL). The mixture was stirred for 3 h under nitrogen at room temperature, which was then diluted with dichloromethane, and washed three times with water. The organic layer was collected and dried over $MgSO_4$. After solvent removal under reduced pressure, the crude product was purified by column chromatography using n-hexane/dichloromethane (2/1, v/v) as eluent to afford TPEAl as a yellow solid (54.1 mg, 79.3 % yield).^{1}H NMR (600 MHz, $CDCl_3$, 298 K), δ(TMS, ppm): 1.91 (m, 4H, -CH_2-), 2.05 (m, 4H, -CH_2-), 3.05 (s, 2H, -C≡CH-), 3.48 (t, 4H, J = 6.6 Hz, -CH_2Br), 3.93 (t, 4H, J = 6.0 Hz, -NCH_2-), 6.62 (d, 4H, J = 8.4 Hz, ArH), 6.89 (d, 4H, J = 9.0 Hz, ArH), 6.94 (d, 4H, J = 8.4 Hz, ArH), 7.25 (d, 4H, J = 8.4 Hz, ArH). ^{13}C NMR (150 MHz, $CDCl_3$, 298 K), δ(ppm): 28.0, 29.2, 30.7, 34.7, 46.0, 68.0, 85.1, 115.0, 121.0, 132.6, 132.9, 133.9, 137.0, 138.8, 143.2, 145.9, 159.0.

Synthesis of compound TPEAzAl. TPEAl (34.1 mg, 0.050 mmol) and compound **1** (228.3 mg, 2.0 mmol) were placed in a 100 mL round-bottom flask. The reactants were dissolved in acetonitrile (3 mL). The mixture was stirred under nitrogen at 45 ^{o}C for 3 d. After solvent removal under reduced pressure, the crude product was purified by high performance liquid chromatography (HPLC) using acetonitrile/water with 0.1% CF_3COOH as the eluent to yield the product TPEAzAl as yellow powders after freeze drying (15.9 mg, 35% yield). ^{1}H NMR (600 MHz, $CDCl_3$, 298 K), δ(TMS, ppm): 1.82 (s, br, 4H, -CH_2-), 1.95 (s, br, 4H, -CH_2-), 2.95 (s, br, 6H, -NCH_3), 3.13 (br,

8H, -NCH_3- and -C≡CH-), 3.44 (s, br, 4H, -CH_2N_3-), 3.54 (s, br, 4H, -CH_2N_3-) 3.8-4.0 (br, 8H, -OCH_2- and -NCH_2-), 6.74 (d, 4H, ArH), 6.87 (d, 4H, ArH), 6.95 (d, 4H, ArH), 7.26 (d, 4H, ArH). HRMS (ESI), calcd for ($C_{50}H_{54}F_6N_8O_6$): m/z $[M+H]^+$: 997.5123; found: m/z 997.5135.

Fluorescence change of TPEAzAl upon click reaction. DMSO stock solution of TPEAzAl (1 M) was diluted into a mixture of DMSO and PBS (v/v = 1/99) to a final concentration of 10 μM. Then to the solution was sequentially added $CuSO_4$ (25 μM) and sodium ascorbate (2.5 mM). The fluorescence change of the solution was monitored at different reaction time. The excitation wavelength was 365 nm and the emission spectra were collected from 425 to 675 nm.

Click reaction mediated ALP sensing. DMSO stock solution of TPEAzAl (1 M) was diluted into a mixture of DMSO and PBS (v/v = 1/99) to a final concentration of 10 μM. Then ascorbic acid-phosphate (2.5 mM) was added to the solution. ALP in PBS stock solution (1 mM) was added to the solution to reach a final concentration ranging from 1.0 ng/mL to 50.0 ng/mL. The solution mixture was kept at 37 °C for 1 h. After that, $CuSO_4$ (25 μM) was added to the solution to initiate the click reaction. The fluorescence changes of the solution were monitored at different reaction time. The excitation wavelength was 365 nm and the emission spectra were collected from 425 to 675 nm.

Immunoassay for the model antibody. For the rabbit antihuman IgG immunoassay, we first added 100 μL of human IgG (1.5 μgmL^{-1}) in a 96-well plate and incubated the plate at 4 °C overnight. After discarding the solutions, the wells were washed with 0.05% Tween-20 in PBS buffer and blocked with 2.5% bovine serum albumin (150 μL) at 37 °C for 1 h. The wells were then washed with PBS and rabbit antihuman IgG at different concentrations were added.

After incubation at 37 °C for 30 min, followed by another washing with PBS, goat antirabbit IgG labeled with ALP (100 μL, 1.0 μg mL^{-1}) was subsequently added to the wells. After incubation at 37 °C for 1 h, the wells were washed three times with PBS. Then TPEAzAl (100 μL, 10 μM), $CuSO_4$ (50 μL, 25 μM) and ascorbic acid-phosphate (50 μL, 2.5 mM) were added to the wells and the mixtures were further reacted at 37 °C for 1 h. The fluorescence change of TPEAzAl solution was recorded by a microplate reader. The excitation wavelength was 365 nm and the emission was collected at 530 nm.

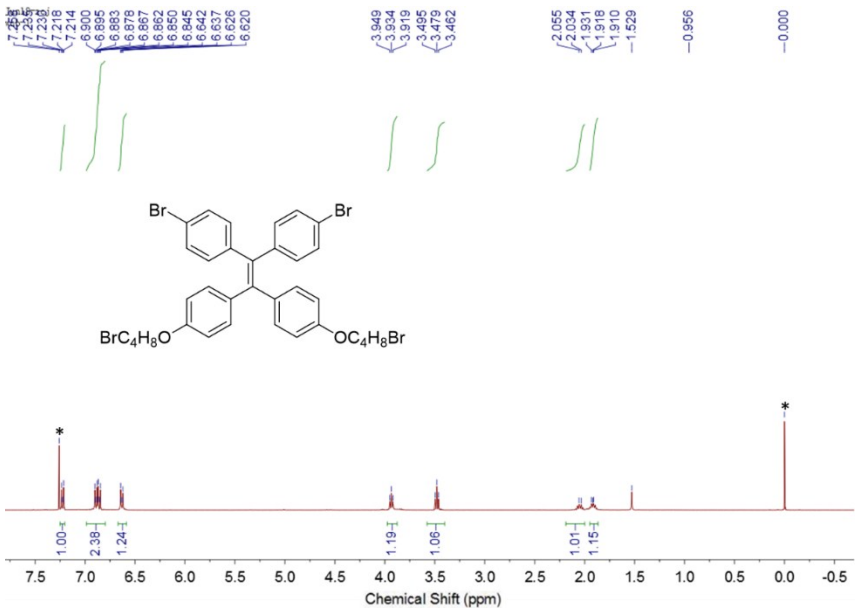

Figure S1. ^{1}H NMR spectrum of compound **3** in chloroform-*d*, and the peaks from residual solvents were marked with *.

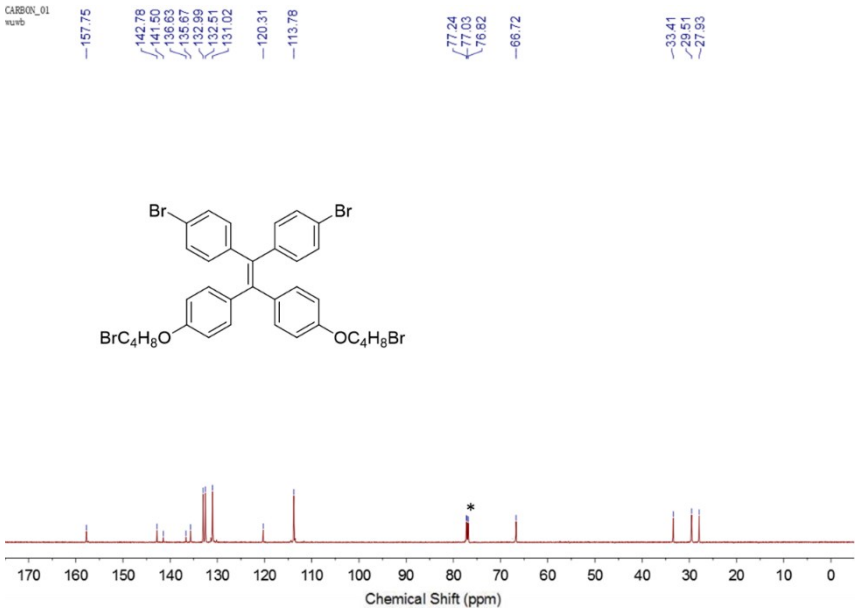

Figure S2. ^{13}C NMR spectrum of compound **3** in chloroform-*d*, and the peaks from residual solvents were marked with *.

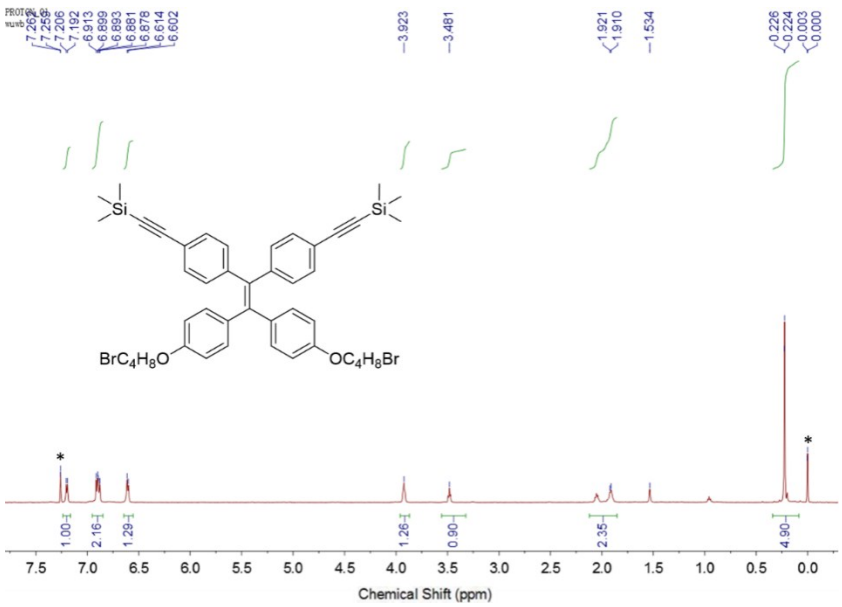

Figure S3. ^{1}H NMR spectrum of compound **4** in chloroform-*d*, and the peaks from residual solvents were marked with *.

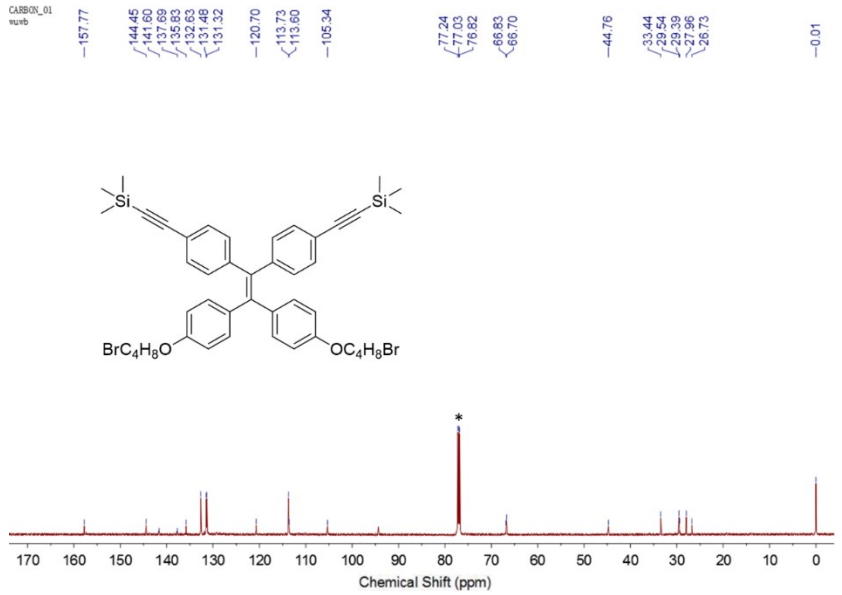

Figure S4. ^{13}C NMR spectrum of compound **4** in chloroform-*d*, and the peaks from residual solvents were marked with *.

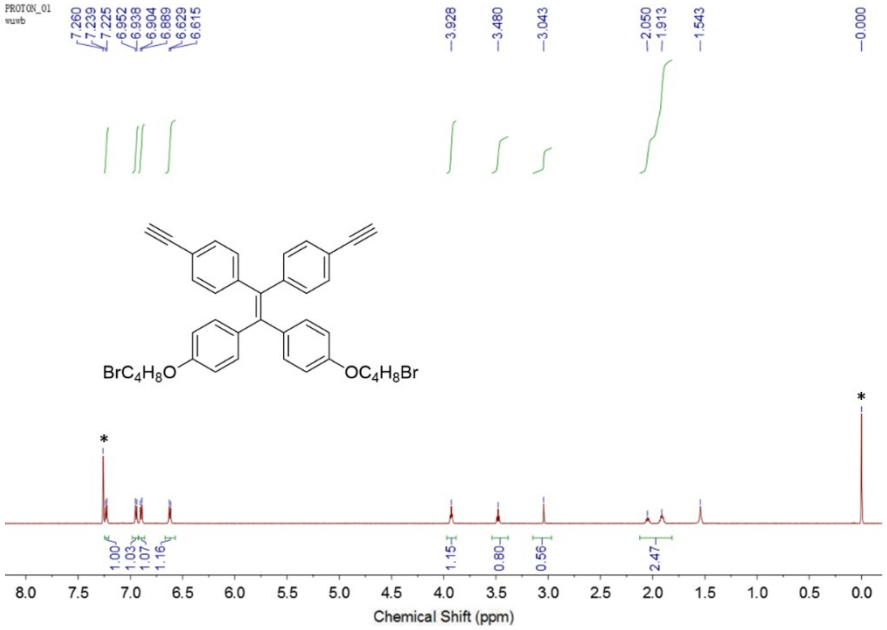

Figure S5. ^{1}H NMR spectrum of TPEAl in chloroform-*d*, and the peaks from residual solvents were marked with *.

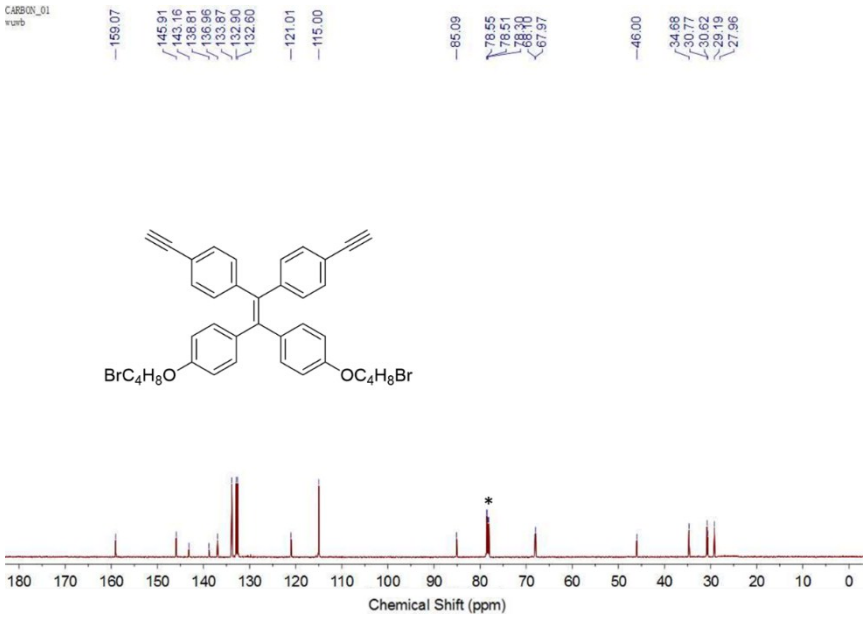

Figure S6. ^{13}C NMR spectrum of TPEAl in chloroform-*d*, and the peaks from residual solvents were marked with *.

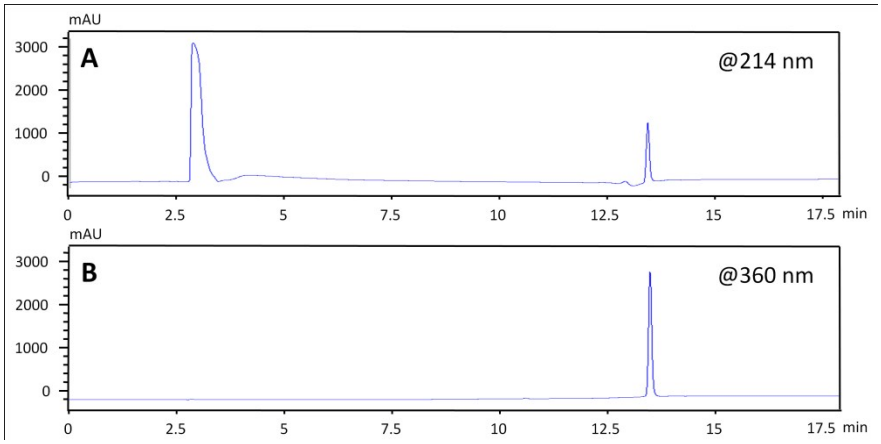

Figure S7. HPLC spectra of TPEAzAl recorded at absorbance of 214nm (A) and 360 nm (B). The peak with elution time at 3.2 min in A is solvent peak.

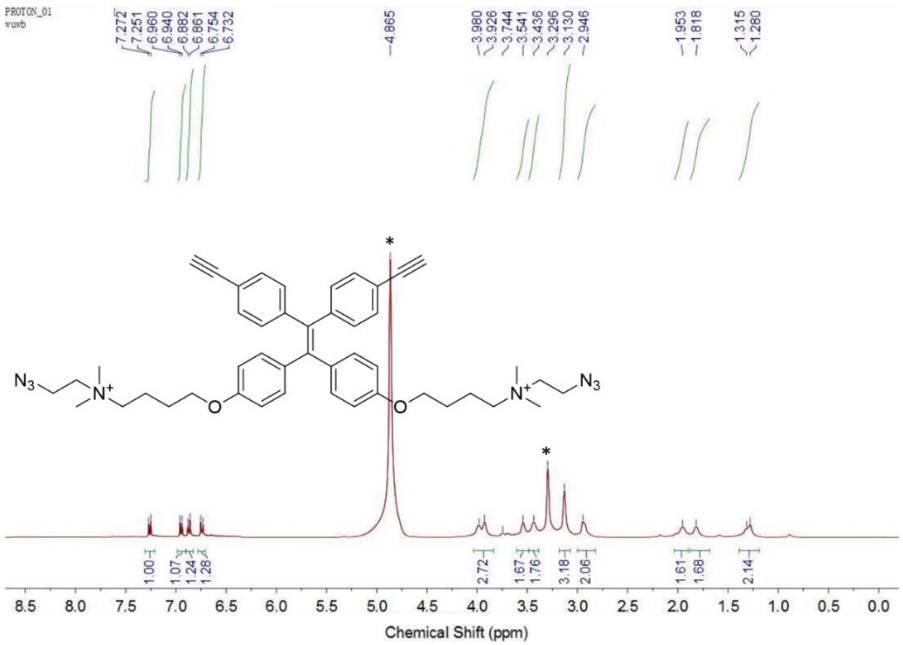

Figure S8. ^{1}H NMR spectrum of TPEAzAl in methonal-d_4, and the peaks from residual solvents were marked with *.

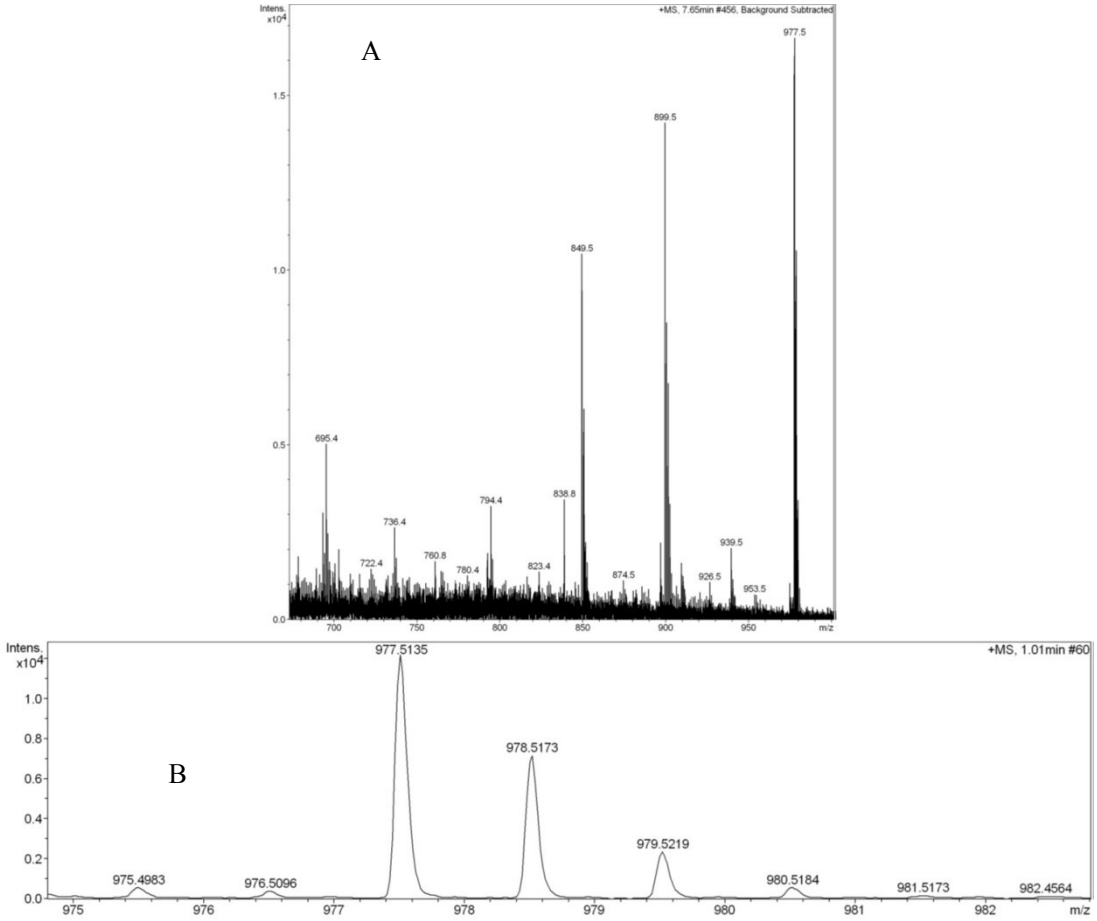

Figure S9. (A)The mass spectrum (ESI) of TPEAzAl; (B) enlarged mass spectrum.

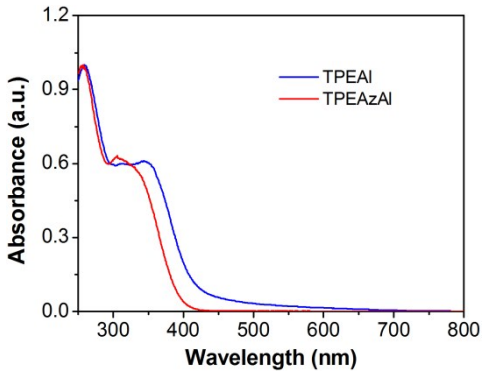

Figure S10. UV-vis absorption spectrum of TPEAl and TPEAzAl (10 μM) in 1×PBS.

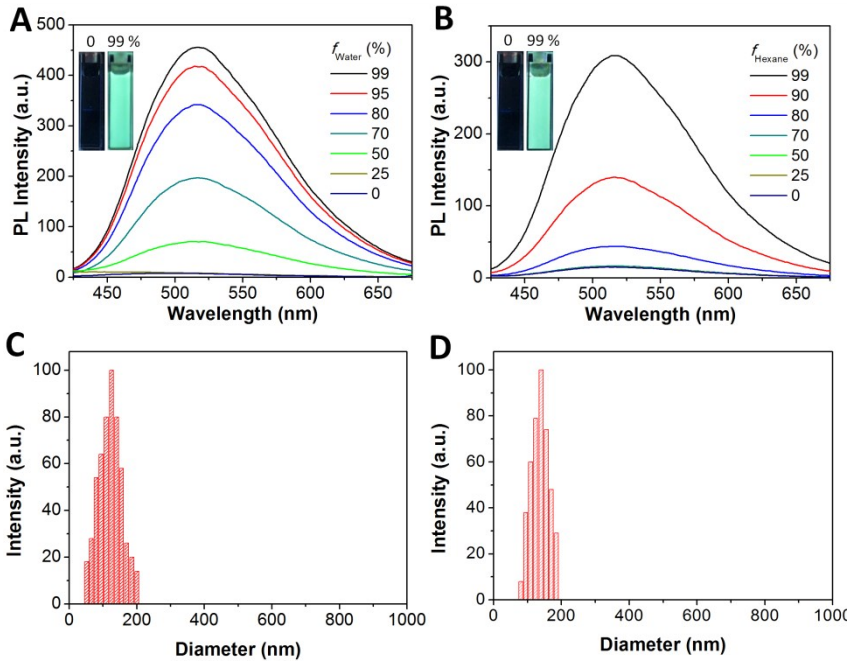

Figure S11. (A) Photoluminescence (PL) spectra of TPEAl (10 μM) in DMSO/water mixtures with different fractions of water (f_{water}). (B) PL spectra of TPEAzAl (10 μM) in isopropyl alcohol/hexane mixtures with different fractions of hexane (f_{hexane}). (C) Laser light scattering data of TPEAl (10 μM) in THF/water mixtures (v/v = 1/99). (D) Laser light scattering data of TPEAzAl (10 μM) in water/THF mixtures (v/v = 1/99).

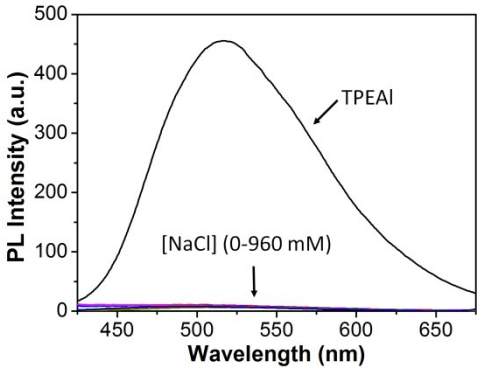

Figure S12. (A) PL spectra of TPEAzAl (10 μM) in the presence of various [NaCl] ranging from 0, 250, 500 to 960 mM. The spectrum of TPEAl (10 μM) in PBS is shown for reference.

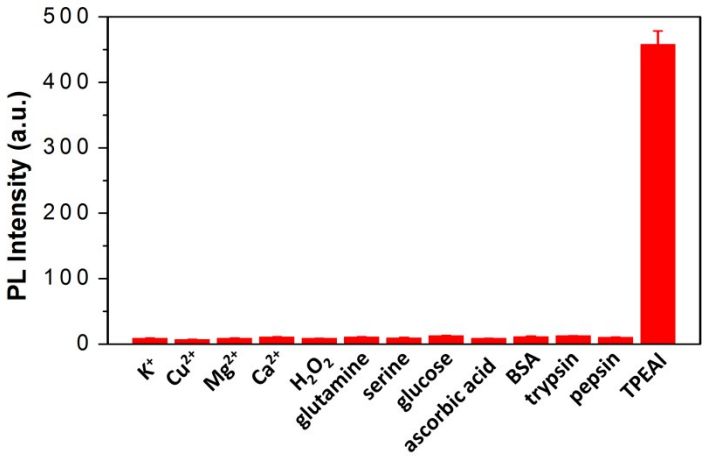

Figure S13. PL intensity of TPEAzAl (10 μM) at 530 nm in PBS in the presence of various species (2.5 mM): KCl, $CuSO_4$, $MgCl_2$, $CaCl_2$, H_2O_2, glutamine, serine, glucose, ascorbic acid, bovine serum albumin (BSA), trypsin, pepsin.

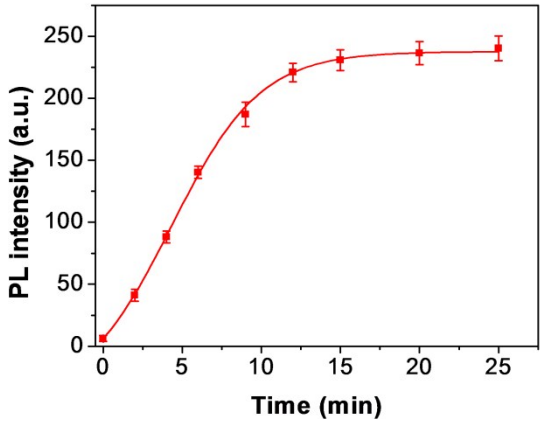

Figure S14. Time-dependent PL intensity at 530 nm of TPEAzAl (10 μM) upon addition of $CuSO_4$ and sodium ascorbate (SA). Data represent mean values ± standard deviation, n = 3.

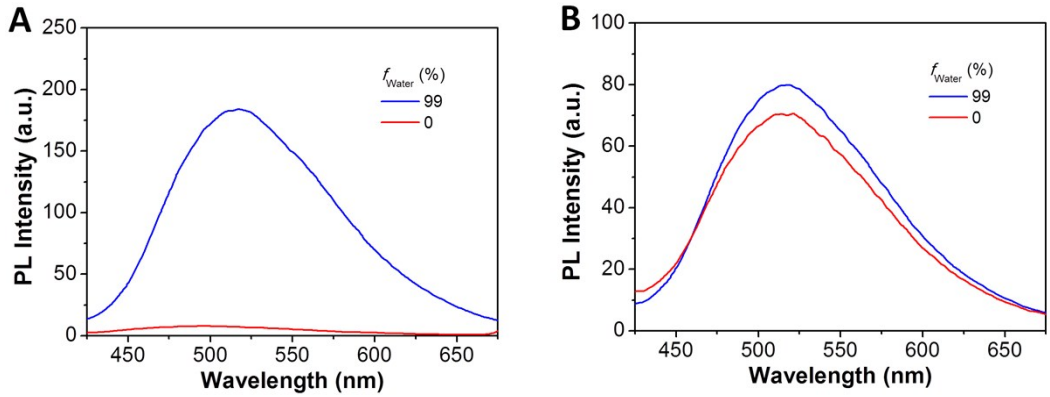

Figure S15. PL spectra of TPEAl(A) and TPEAzAl (B) after the click reaction in DMSO/water mixture at different water fractions.

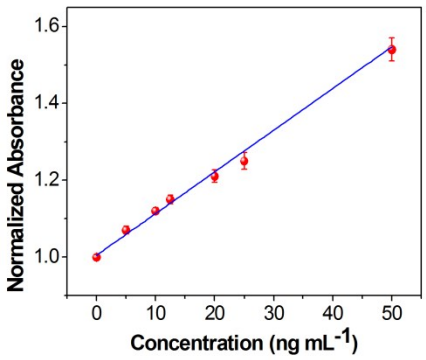

Figure S16. Rabbit antihuman IgG concentration-dependent changes in optical absorption at405 nm obtained from conventional ELISA using pNPP as substrate.