# Specific and Quantitative Detection of Albumin in Biological Fluids by Tetrazolate-Functionalized Water-Soluble AIEgens

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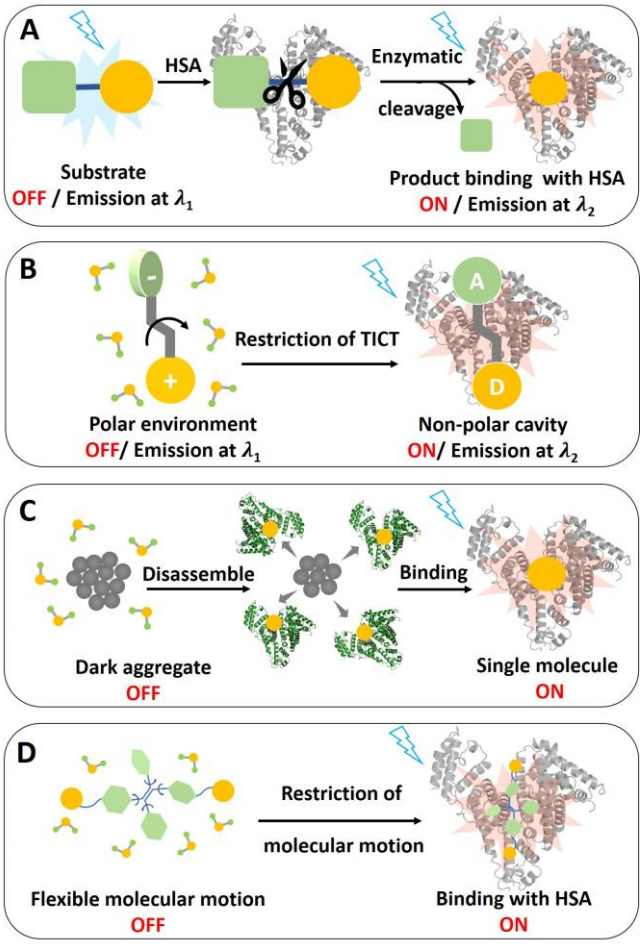



**Figure S1.** Summary of working mechanisms for designing turn-on fluorescent probes in HSA detection. (A) aromatic amides/esters which can undergo enzymatic hydrolysis in the presence of albumins due to albumin's pseudo-esterase activity. (B) push-pull probes by modulating the twisted intramolecular charge transfer (TICT) process between the non-polar albumin binding sites and polar aqueous environment. (C) caged dyes or dark aggregates which can disassemble, disperse into albumin cavities and turn on the emission. (D) water-soluble aggregation-induced emission luminogens which are not emissive when dissolved while can be lighted up after binding with HSA due to the restriction of molecular motion mechanism.

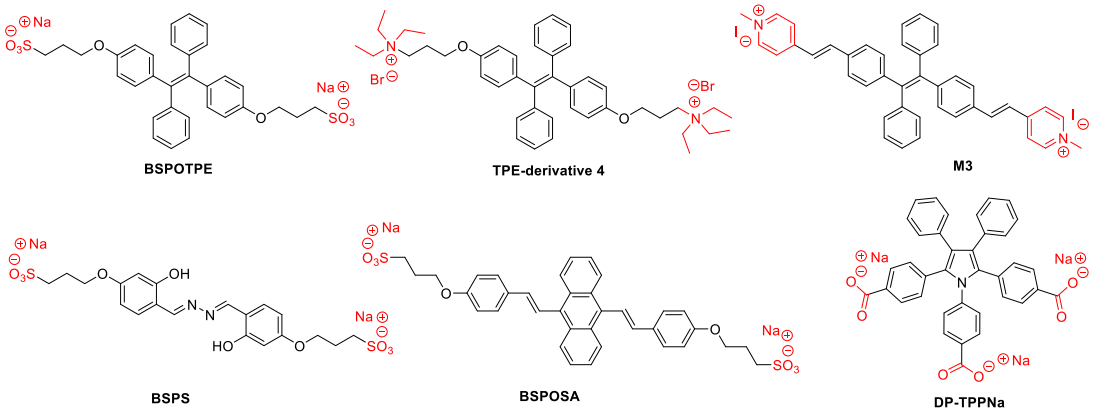
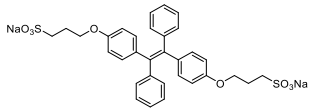
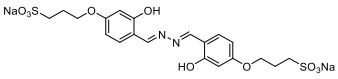
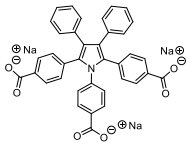
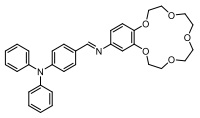
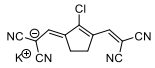
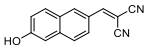
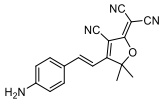
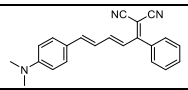
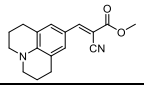
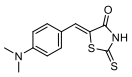
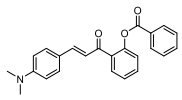
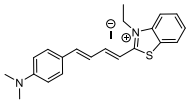
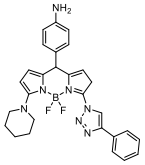
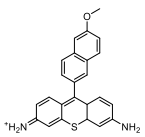



**Figure S2.** AIEgens (BSPOTPE[1], TPE-derivative 4[2], M3[3], BSPS[4], BSPOSA[5], DP-TPPNa[6]) for the albumin detection.

**Table S1.** Summary of performances of reported fluorogenic albumin probes.

| Name | Structure | $\lambda_{ex}$/ $\lambda_{em}$ (nm) | LOD (nM) | $K_D$ (μM) | LDR (mg/L) | Ref. |
|---|---|---|---|---|---|---|
| BSPOTPE |  | 350/460 | 1 | - | 0.665~6.65 | [1] |
| BSPS |  | 355/508 | 91.9 | - | 6.11~100 | [4] |
| DP-TPPNa |  | 310/443 | 25.26 | - | 1.68~100 | [6] |
| DB-15C5 |  | 360/500 | 1.7 | 8.87 | 0.112~80 | [7] |
| AB580 |  | 590/616 | 6.02 | 0.077 | 2~200 | [8] |
| HMM |  | 482/562 | 0.75 | 2.7 | ~100 | [9] |
| ACDM |  | 560/612 | 37.6 | 10.87 | 2.5~300 | [10] |
| SA4 |  | 536/685 | 0.00115 | 5.08 | 3.7~853.7 | [11] |
| AL-1 |  | 440/490 | 6.02 | 1.77 | 0.4~67 | [12] |
| DMAR |  | 460/547 | 1.95 | 3.22 | 0.13~33 | [13] |
| HCAB |  | 426/524 | 28.7 | 1.3 | 1.91~100 | [14] |
| NIR-HSA |  | 580/680 | 26.2 | - | 1.73~665 | [15] |
| BDC-9 |  | 460/575 | 4.51 | 12.7 | 0.37~31 | [16] |
| G13 |  | 522/544 | - | 1.25 | ~150 | [17] |

LOD: Limit of detection; $K_D$: dissociation constant; LDR: linear dynamic range.

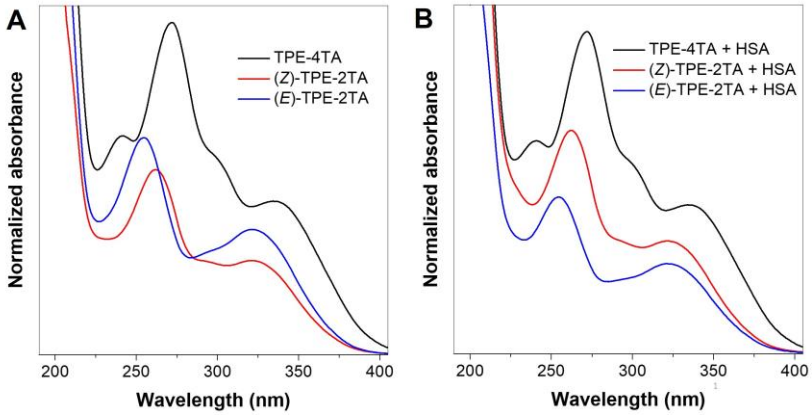



**Figure S3** UV-vis absorption spectra of AIEgens in the absence (A) and presence (B) of HSA

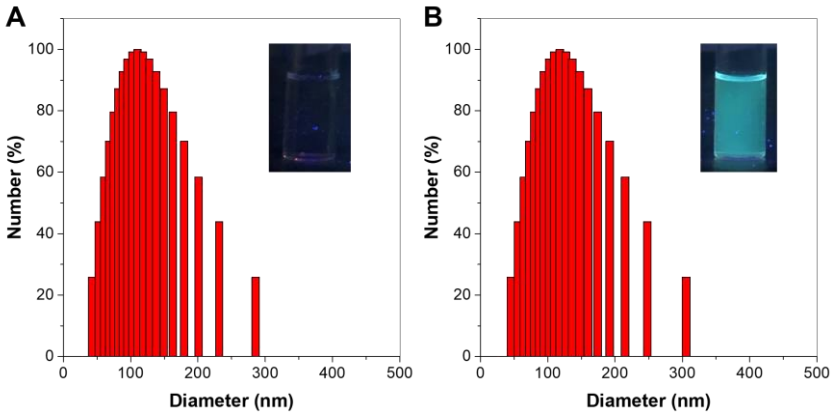



**Figure S4.** Dynamic light scattering analysis of (A) HSA (50mg/L) (B) HSA (50mg/L) in addition of TPE-4TA (10 μM). Inset: Photos of target solution taken under UV light.

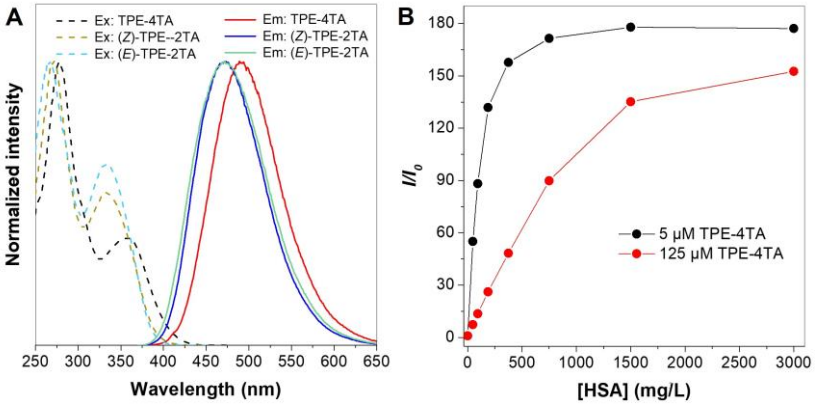



**Figure S5.** (A) Excitation and emission spectra of three AIEgen-HSA complexes. (B) Linear response of TPE-4TA at 5 μM and 125 μM towards human serum albumin. $\lambda_{ex}$ = 370 nm. $I_0$ is the fluorescence intensity at [HSA] = 0 mg/L.

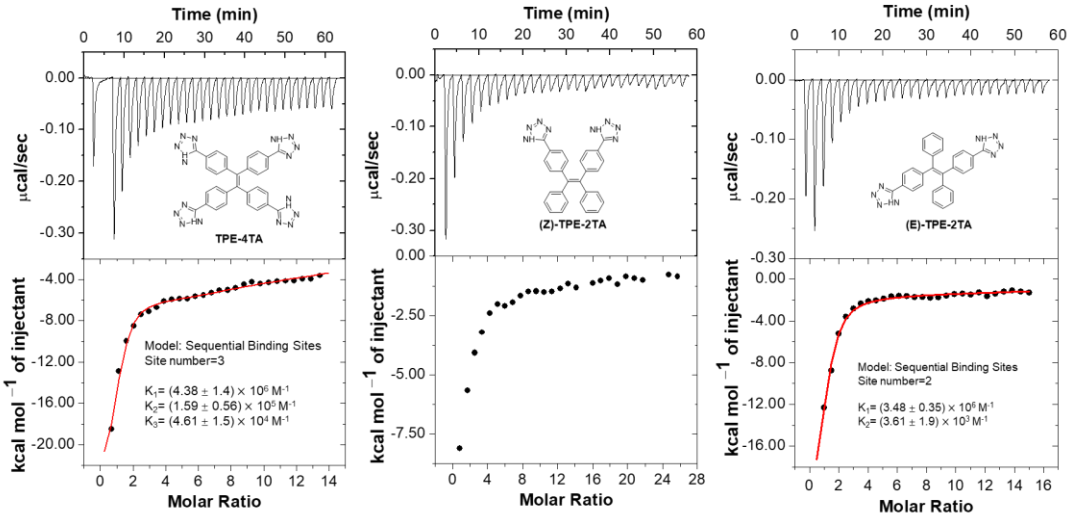



**Figure S6.** Binding isotherms for the interaction between AIEgens ligands and HSA protein. The top panels show the calorimetric traces recorded by titrating HSA solution in a cell with AIEgens solutions in the syringe at 25℃. The bottom panels show the integrated heat of each injections and the fitting lines with sequential binding sites model. [AIEgens] = 0.07 mM.

**Table S2.** Docking energies of TPE-4TA in three binding sites obtained by all-atom MD simulation.

| **Sites** | Electrostatic energy (kJ/mol) | Polar solvation energy (kJ/mol) | van der Waal energy (kJ/mol) | Binding energy (kJ/mol) |
|---|---|---|---|---|
| **Cleft 1** | -50.516 | 64.923 | -201.477 | -207.695 |
| **Cleft 2** | -159.624 | 202.034 | -213.640 | -197.062 |
| **DIII** | -341.260 | 515.419 | -192.627 | -38.615 |

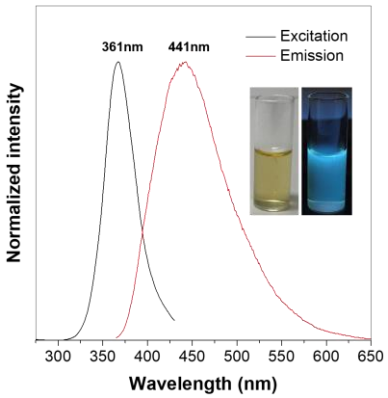



**Figure S7.** Fluorescence spectra of a urine sample showing auto-fluorescence.

**NMR Spectra:**

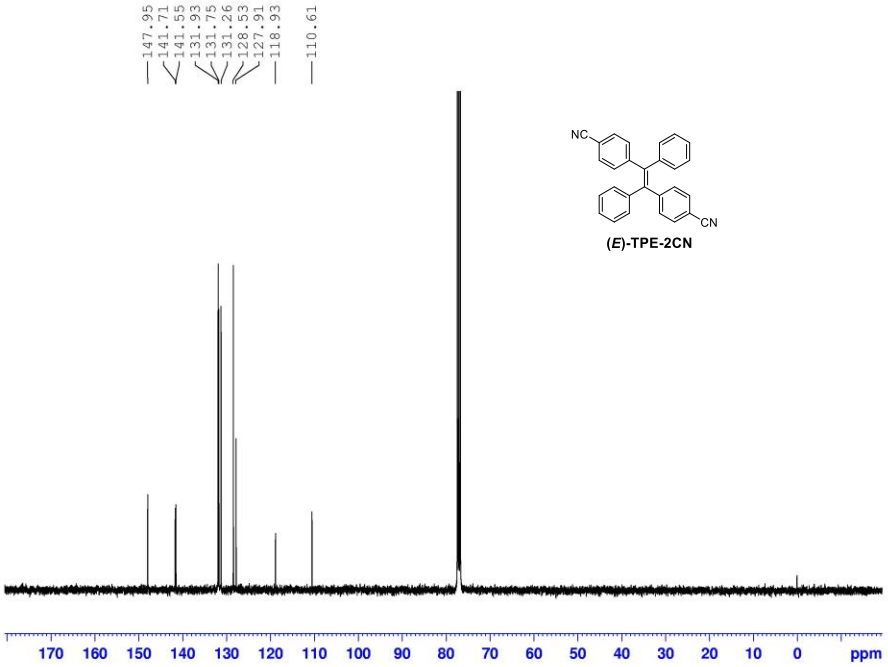



**Figure S9.** $^{13}C$ NMR spectrum of (*E*)-TPE-2CN in $CDCl_3$.

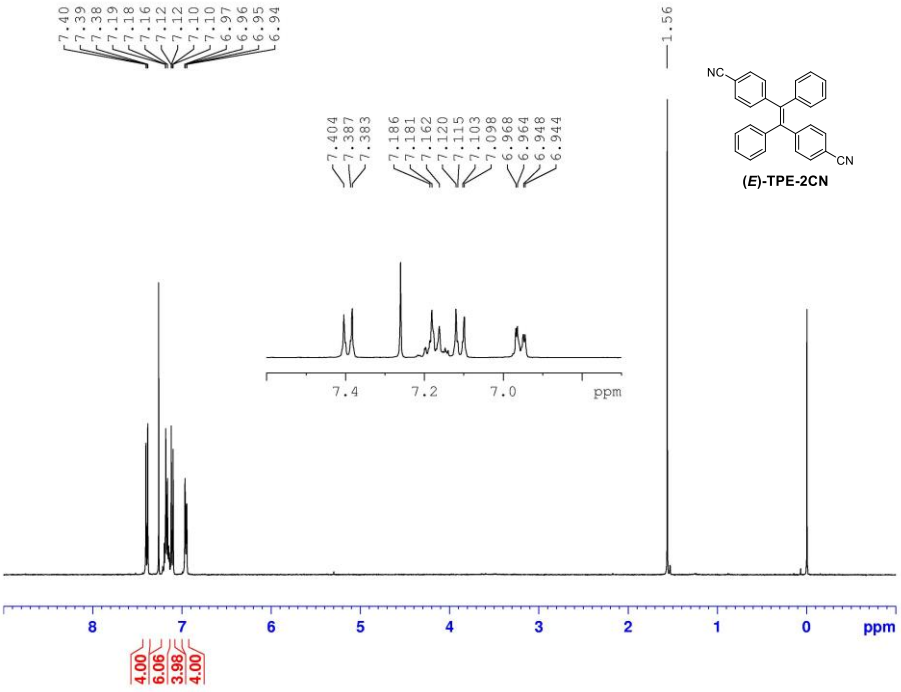



**Figure S10.** $^{1}H$ NMR spectrum of (*E*)-TPE-2CN in $CDCl_3$.

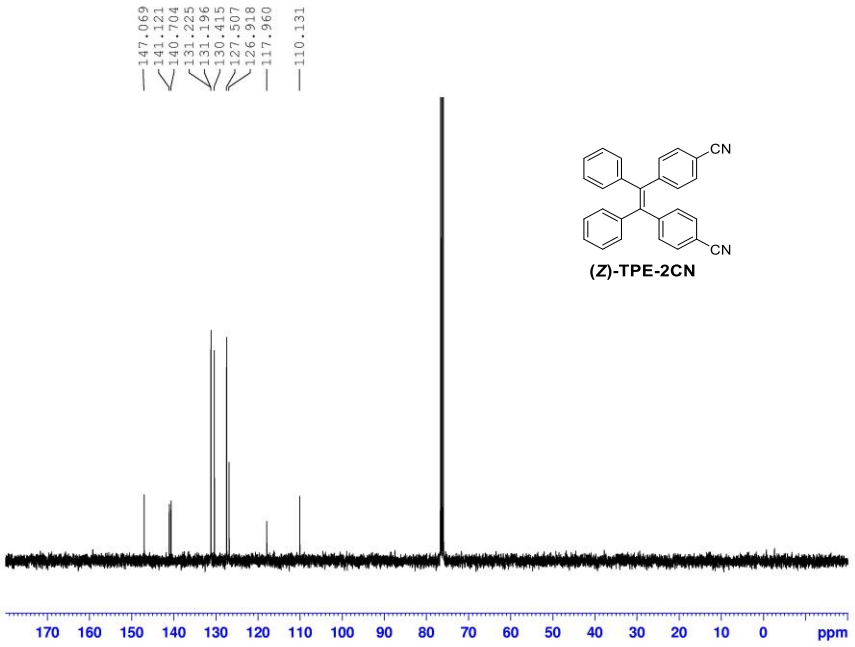



**Figure S11.** $^{13}C$ NMR spectrum of (*Z*)-TPE-2CN in $CDCl_3$.

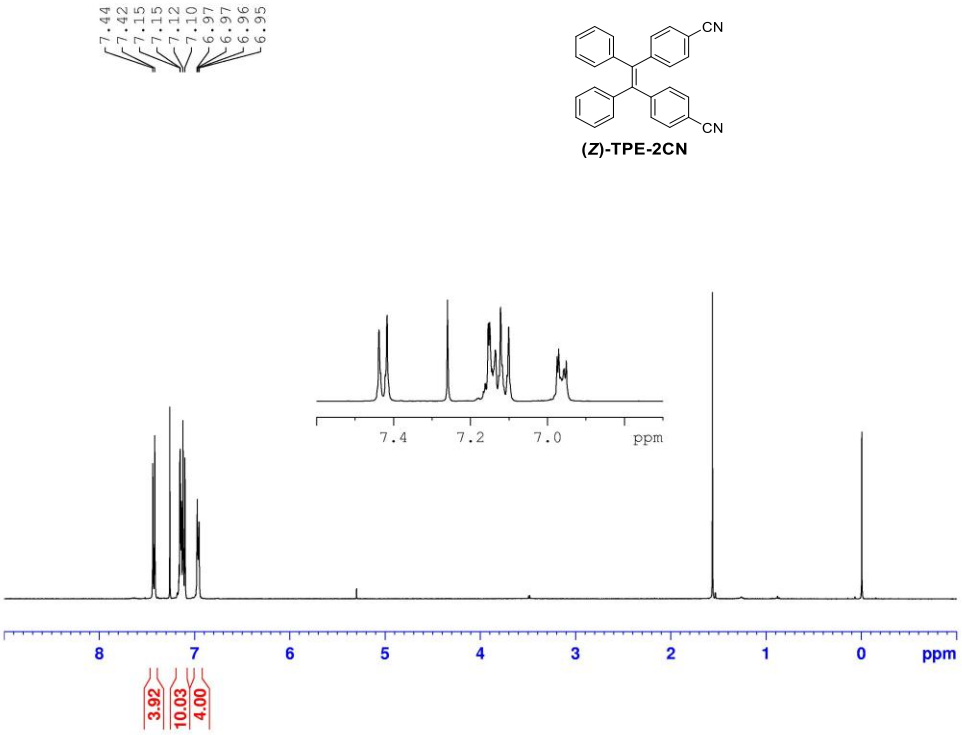



**Figure S12.** $^{1}H$ NMR spectrum of (*Z*)-TPE-2CN in $CDCl_3$.

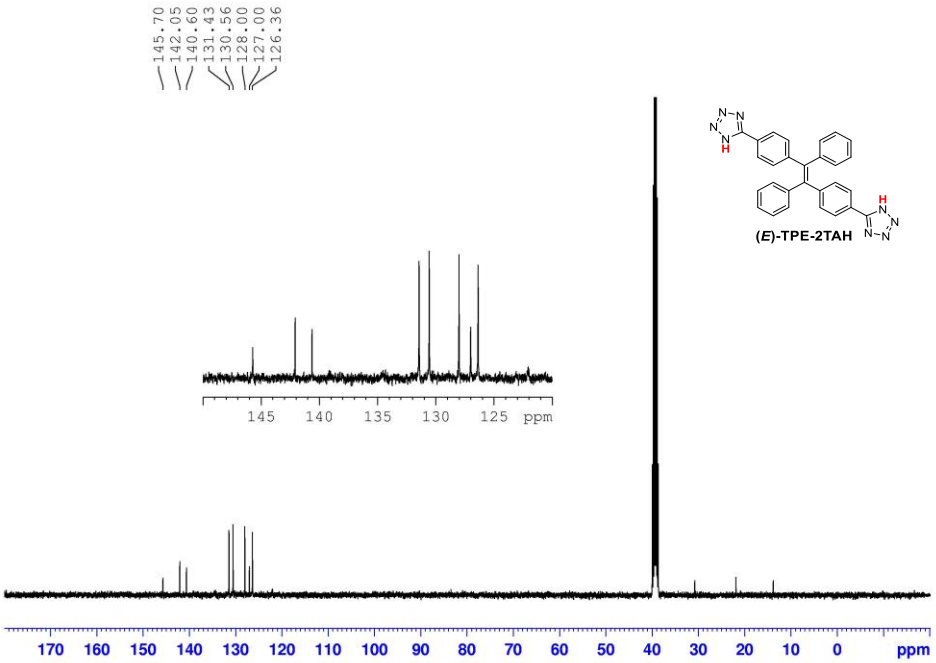



**Figure S13.** $^{13}$C NMR spectrum of (*E*)-TPE-2TAH in DMSO-d6.

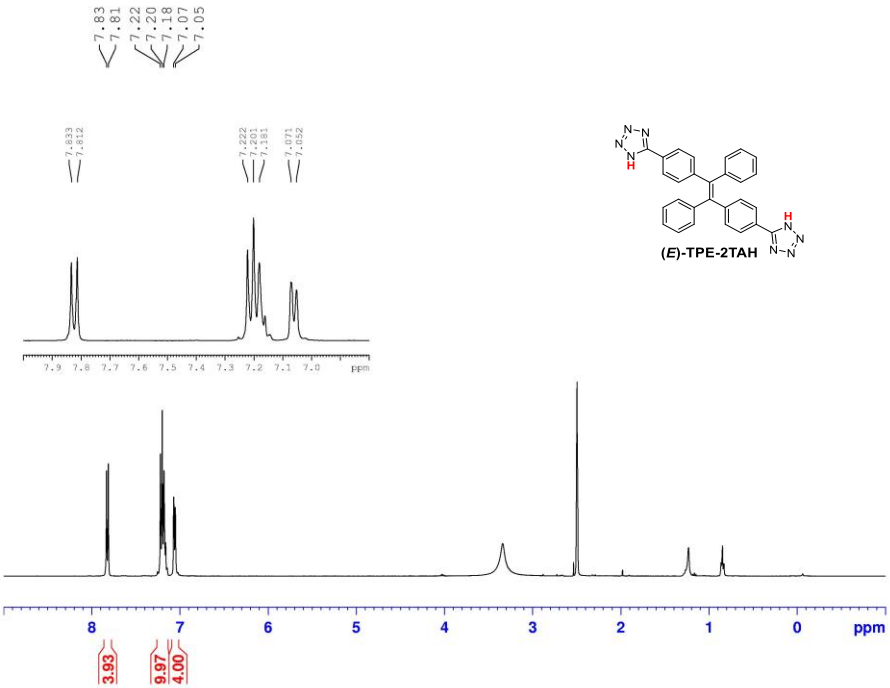



**Figure S14.** $^{1}$H NMR spectrum of (*E*)-TPE-2TAH in DMSO-d6.

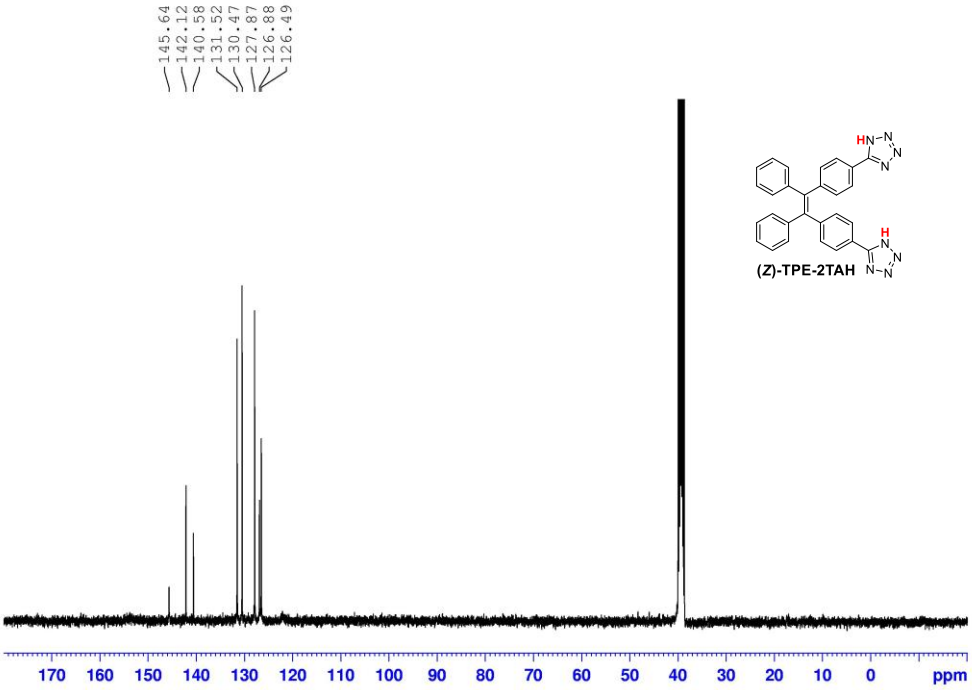



**Figure S15.** $^{13}$C NMR spectrum of (*Z*)-TPE-2TAH in DMSO-d6.

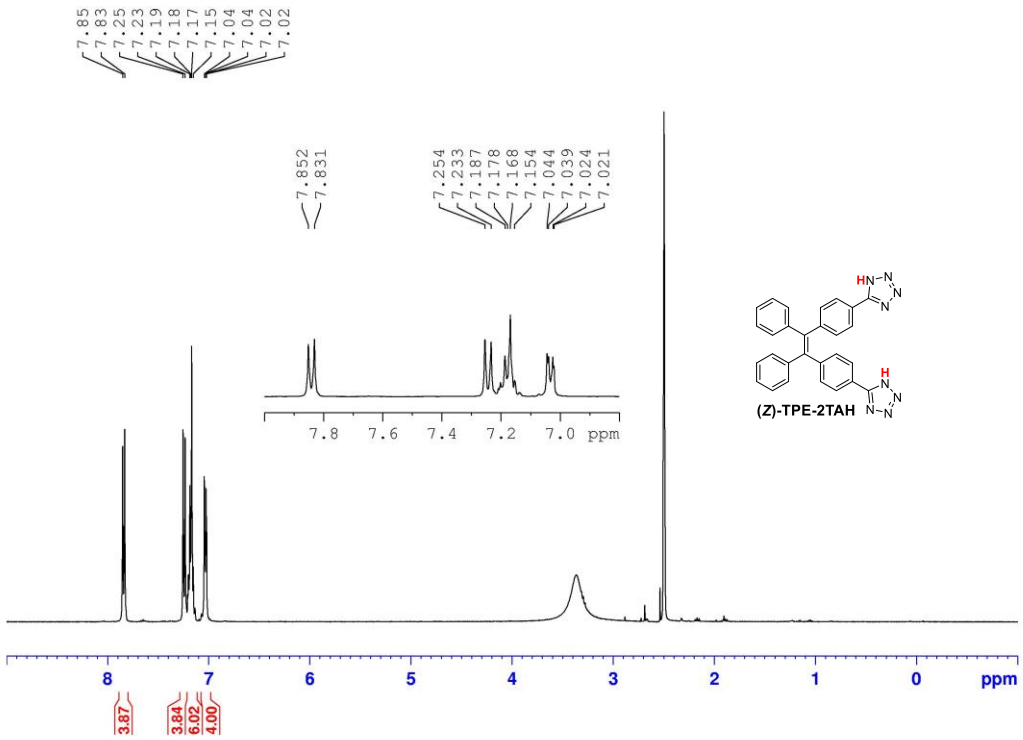



**Figure S16.** $^{1}$H NMR spectrum of (*Z*)-TPE-2TAH in DMSO-d6.

**Mass Spectra:**

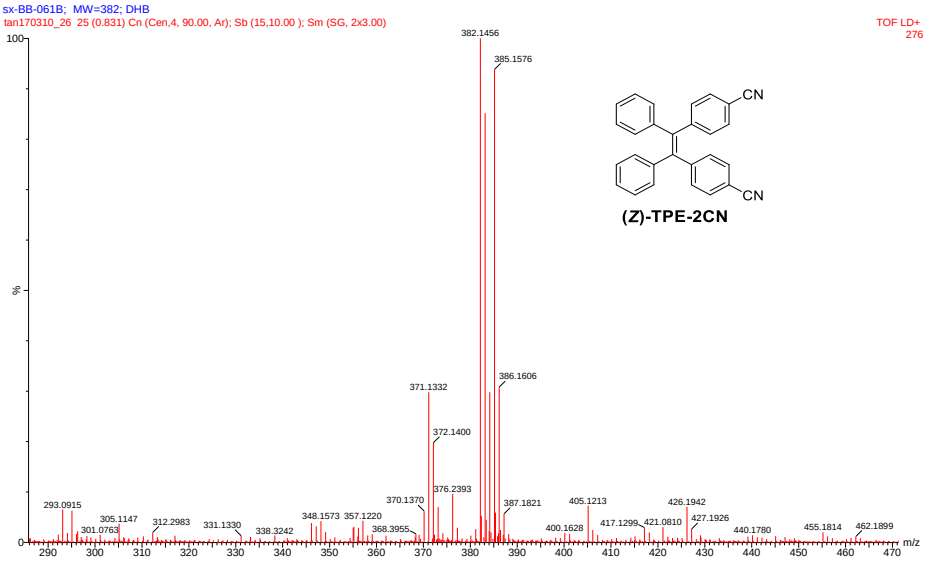



**Figure S17.** Mass spectrum of (*Z*)-TPE-2CN.

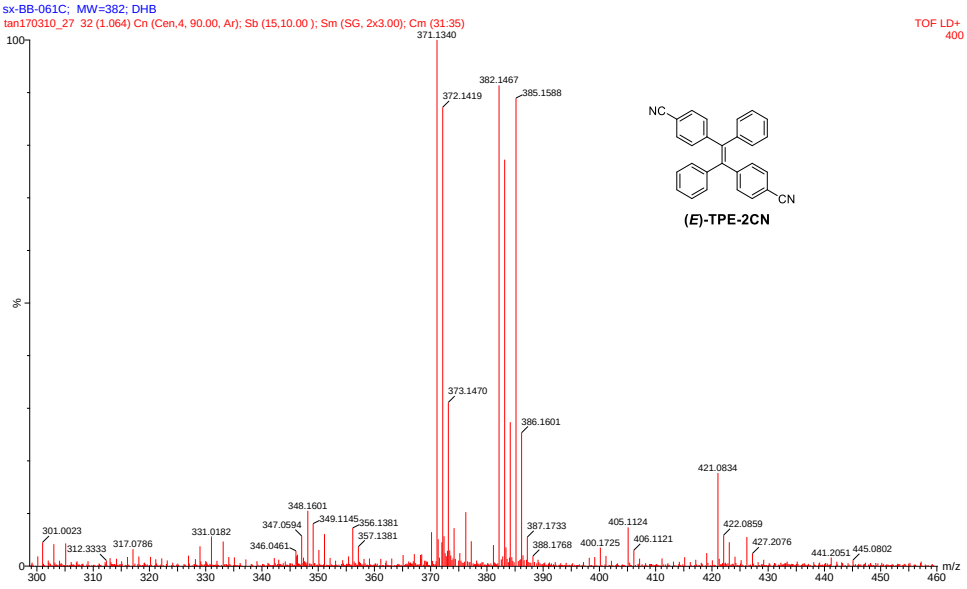



**Figure S18.** Mass spectrum of (*E*)-TPE-2CN.

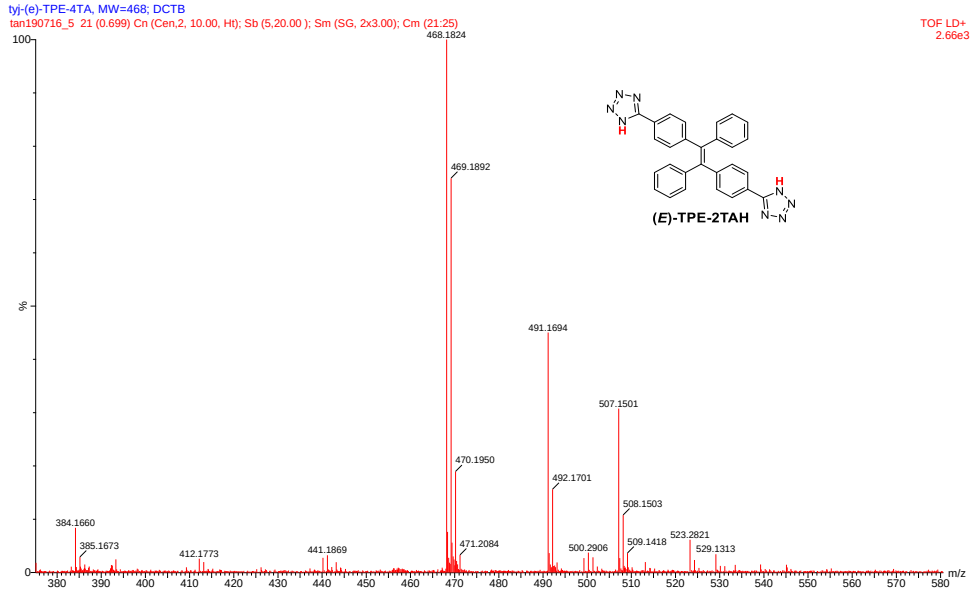



**Figure S19.** Mass spectrum of (*E*)-TPE-2TAH.

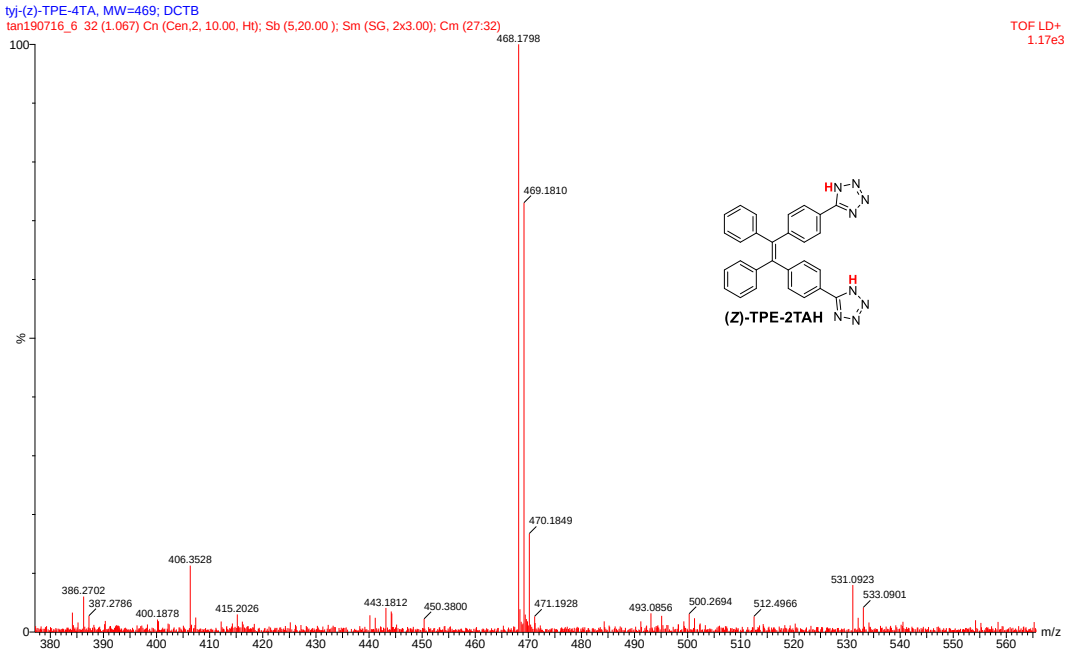



**Figure S20.** Mass spectrum of (*Z*)-TPE-2TAH.

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