

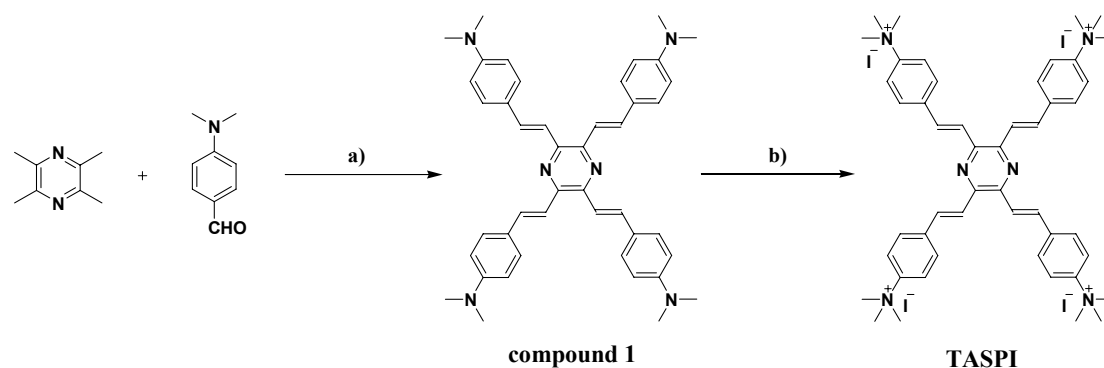
Supporting Information

Materials, methods and instrumentation.

The following compounds and reagents were commercially available: Thrombin was purchased from Sigma (Missouri); bovine serum albumin, trypsin and nuclease S1 were bought from Sangon Biotechnology Co., Ltd. (Shanghai, China); Tyrosinase mushroom was bought from Sigma-Aldrich; thrombin-binding aptamer (TBA, 5'-GGTTGGTGTGGTTGG-3') and oligo-N (5'-ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG-3') were purchased from Invitrogen (China). 2,3,5,6-Tetramethylpyrazine, methyl iodide and 4-(dimethylamino)benzaldehyde were obtained from Alfa Aesar.

^1H and ^{13}C NMR spectra were recorded on Varian Mercury 300 and 600 spectrometers, respectively. HRMS were recorded on a Bruker APEX IV (7.0 T). Fluorescent emission spectra were collected on PerkinElmer LS 55 with an excitation wavelength of 330 nm, the excitation and emission slit widths were both 5nm. CD spectra were recorded on Chirascan CD spectrometer (Applied Photophysics, UK) with scanning range from 220nm to 320nm. Quartz cuvettes with 400 μL volume used for emission measurements. Unless otherwise specified, all spectra were taken at an ambient temperature in 10 mM Tris-HCl at pH 7.5. The aptamer and protein solutions were stored at 4 $^\circ\text{C}$ before use.

General procedure for the synthesis of TASPI



Scheme S1 a) DMF, t-BuOK, 80 $^\circ\text{C}$, 4h, 60%; b) CHCl_3 , CH_3I , reflux, 4h, 85%.

Synthesis of compound 1

(*E,E,E,E*-2,3,5,6-Tetrakis[4-(dimethylamino)styryl]pyrazine)^{1,2}:

Potassium tert-butoxide (448mg, 4 mmol) was added to a solution of 2,3,5,6-tetramethylpyrazine (136mg, 1 mmol) and 4-(dimethylamino)benzaldehyde (1.2g, 8 mmol) in DMF (3 mL) in small portions. The reaction mixture was heated to 80 °C for 4 h under N₂. After cooling to room temperature, the mixture was filtered and washed with ethanol and water. The precipitate was purified by silica gel column chromatography using chloroform as the eluant to afford the compound 1 as a red solid (396mg, 60%).

¹H NMR (CDCl₃, 300 MHz) δ(ppm) 7.87 (d, 4 H, *J* = 15.3 Hz), 7.58 (d, 8 H, *J* = 8.1 Hz), 7.37 (d, 4 H, *J* = 15.3 Hz), 6.75 (d, 8 H, *J* = 8.1 Hz), 3.03 (s, 24 H) ; ¹³C NMR (CDCl₃, 150 MHz) δ: 150.7, 145.1, 135.0, 128.8, 118.8, 112.5, 40.6; HRMS (ESI):661.4008 for [M+H]⁺ (calcd:661.4013 for C₄₄H₄₉N₆).

Synthesis of TASPI

(*E,E,E,E*-2,3,5,6-Tetrakis[4-(trimethylamino)styryl]pyrazinyl iodide) :

E,E,E,E-2,3,5,6-Tetrakis[4-(dimethylamino)styryl]pyrazine (100mg, 0.15mmol) was dissolved in 2mL of chloroform, followed by addition 2mL methyl iodide, and refluxed for 4 h under N₂. After cooling to room temperature, the mixture was filtered and washed with chloroform and diethyl ether to give a red solid (158mg, 85%).

¹H NMR (DMSO-*d*₆, 300 MHz) δ(ppm) 8.21 (d, 8 H, *J* = 8.7 Hz), 8.15 (s, 8 H), 8.07 (d, 8 H, *J* = 8.4 Hz), 3.68 (s, 36 H) ; ¹³C NMR (DMSO-*d*₆, 150 MHz) δ: 147.6, 145.7, 138.7, 134.5, 129.9, 125.1, 121.5, 57.2; HRMS (ESI):180.1218 for [M⁴⁺/4] (calcd:180.1215 for C₄₈H₆₀N₆) ; Elemental analysis: found (%) C 44.28, H 5.10, N 6.53(calcd (%):C 44.32, H 5.27, N 6.46 for C₄₈H₆₀I₄N₆·4H₂O).

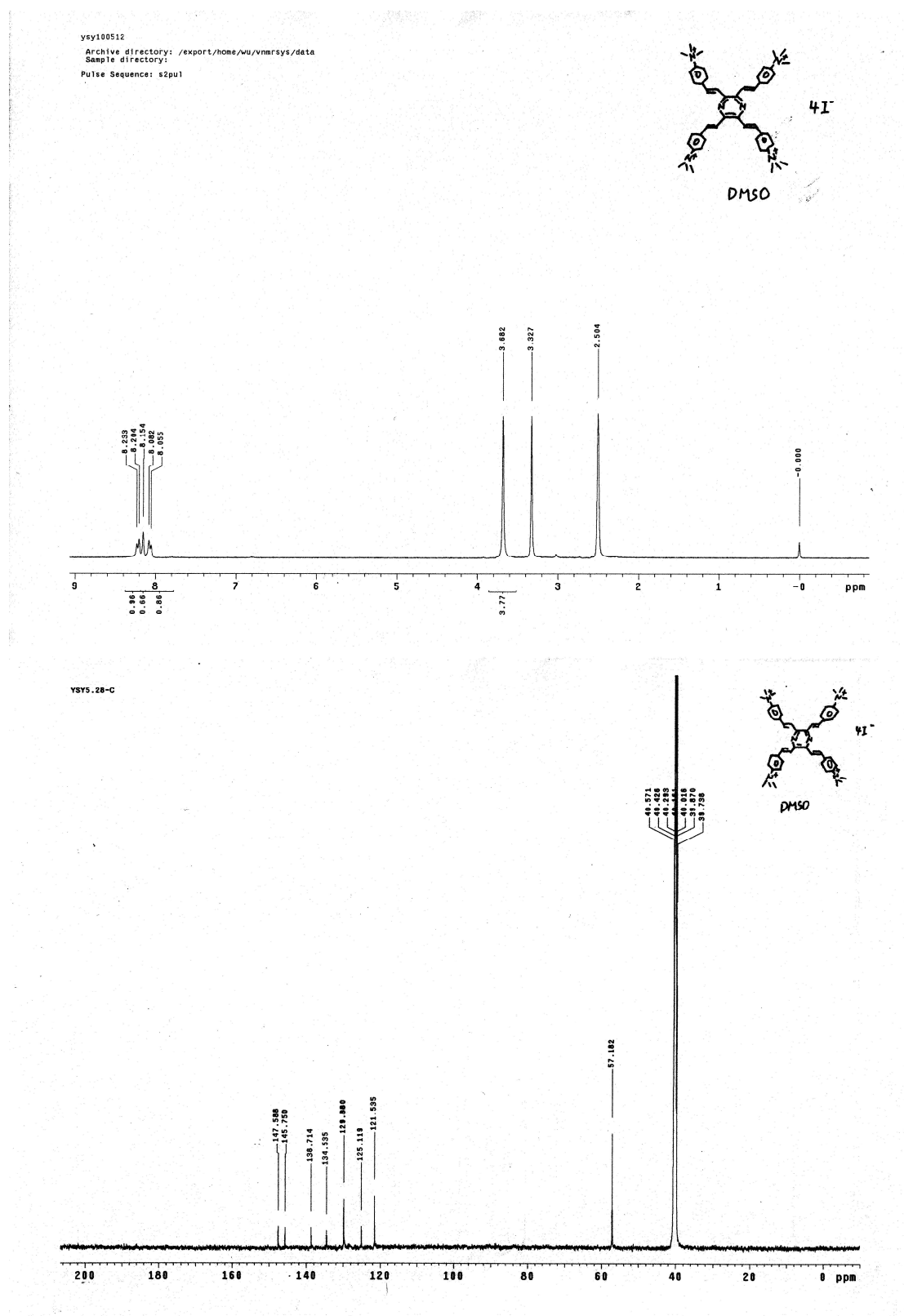


Figure S1. The ¹H NMR and ¹³C NMR spectra of TASPI.

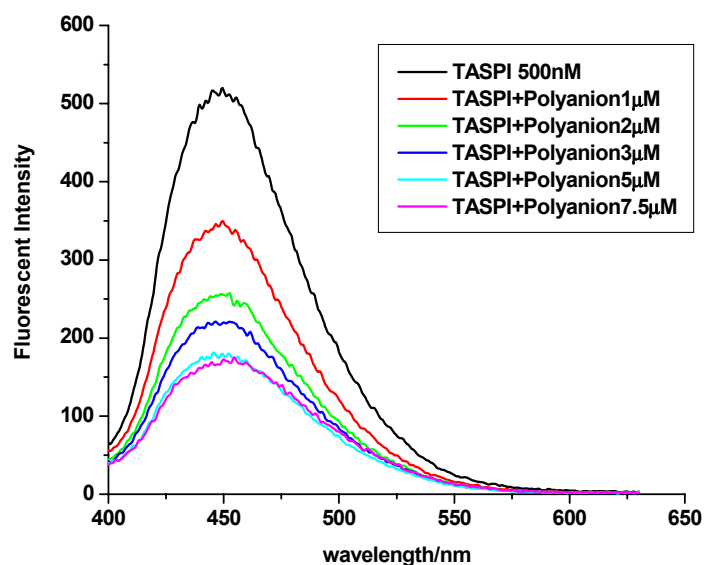


Figure S2. Poly-(vinylsulfonic acid) induced TASPI aggregation and fluorescent intensity decreased gradually. Concentration of the polyanion was increased from 0 to 7.5 μM (concentration of the negatively charged sulfonate functional group). For easy comparison, 1 nM of the thrombin-binding aptamer DNA (TBA) concentration equals 15 nM of the concentration of the negatively charged phosphate functional group.

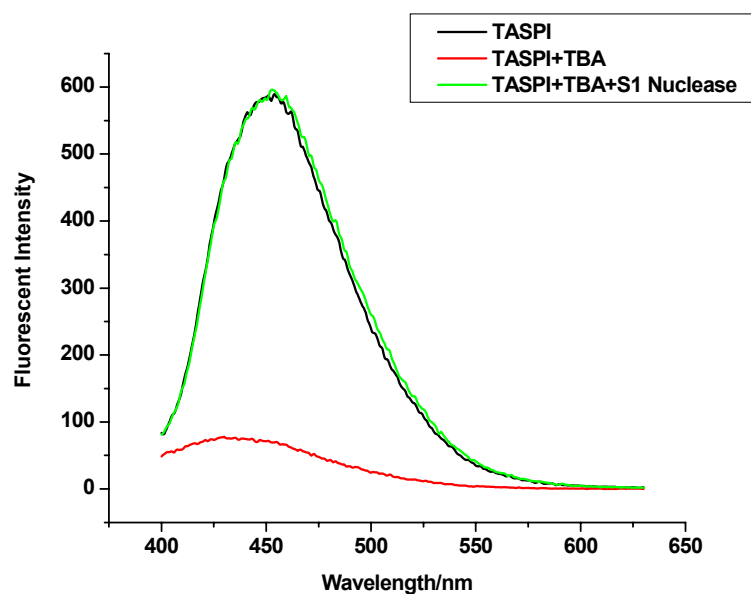


Figure S3. Investigation of the quenching effect of aptamer (TBA) on the fluorescent

emission properties of TASPI. The thrombin-binding aptamer(TBA) was predigested with nuclease S1 (a single-stranded nucleic acid specific nuclease) in specific reaction buffer. Black curve: TASPI only; red curve: TASPI + aptamer digested with nuclease; green curve: TASPI + aptamer without nuclease digestion. TASPI: 500 nM; TBA: 400 nM. DNA aptamer, mixed with TASPI, was digested with 100 U of nuclease S1 for two hours at 37 °C and the emission spectrum was measured.

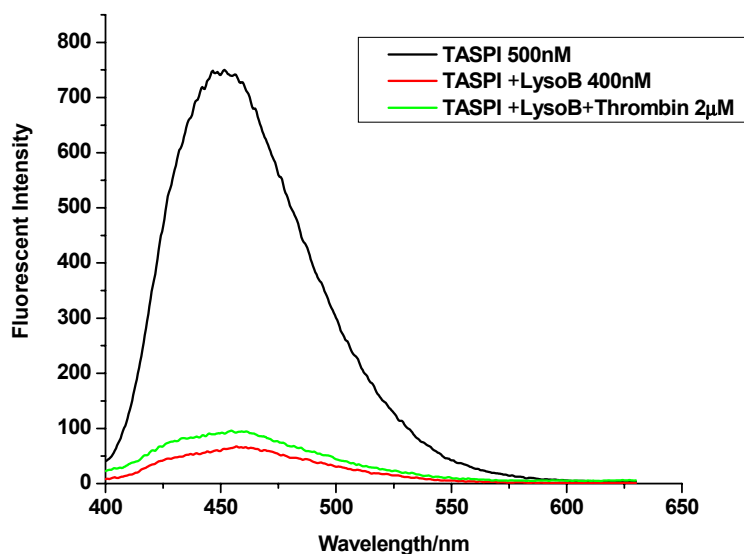


Figure S4. Investigation of the interference effect of oligo-N (5'-ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG-3') on the emission properties of TASPI. Black curve: TASPI only; red curve: TASPI + oligo-N; green curve: TASPI + oligo-N+thrombin. TASPI concentration: 500 nM; oligo-N: 400 nM; thrombin: 2μM. The results show that no fluorescent recovery was observed if oligo-N took place of TBA.

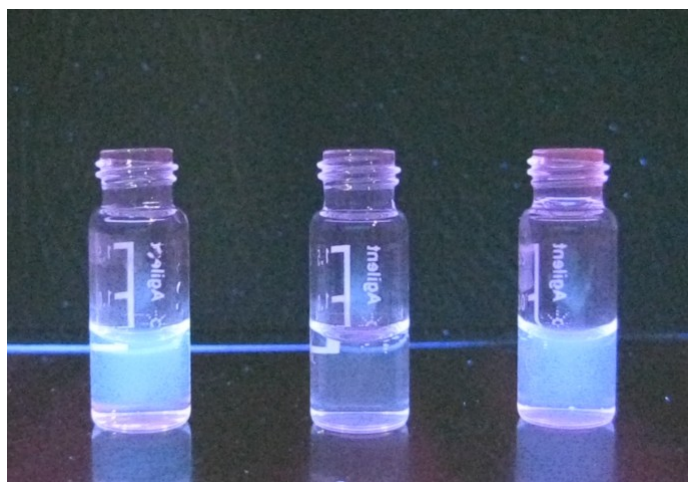


Figure S5. Photograph of TASPI (500nM) itself (left) and in presence of TBA (400nM) (middle) and after addition of thrombin (5 μ M) to the solution of TASPI and TBA (right) under UV excitation.

References:

- 1 M. Rumi, S. J. K. Pond, T. M. Friedrichsen, Q. Zhang, M. Bishop, Y. Zhang, S. Barlow, S. R. Marder, J. W. Perry, *J. Phys. Chem.*, 2008, **112**, 8061.
- 2 J. C. Collette, A. W. Harper, *Proceedings of SPIE-The International Society for Optical Engineering*, 2002, 122.