

Supplementary Information

A sensitive polymeric dark quencher-based sensing platform for fluorescence “turn on” detection of proteins

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Experimental

Materials. Tetrakis(triphenylphosphine) palladium ($\text{Pd}(\text{PPh}_3)_4$), cuprous iodide (CuI) and tetrabutylammonium fluoride were purchased from Energy Chemical Co. (Shanghai, China). The carboxyfluorescein (FAM)-labeled DNA probe (5'-FAM-GGTTGGTGTGGTTGG, TBA-FAM) was purchased from Thermo-Fisher Scientific (Shanghai, China). The carboxytetramethylrhodamine (TAMRA)-labeled peptide probe (5'-TAMRA-RRRRRRRN LWAAQRYGRELRRMSDKFVD, PEP-TAMRA) was purchased from GL Biochem (Shanghai, China) Ltd. Thrombin, bovine serum albumin (BSA) and lysozyme were purchased from Sigma-Aldrich (Shanghai, China). The expression and purification of Bcl-xL protein were referred to the method previously reported.¹⁻³ The water used in all experiments was prepared on a Milli-Q water purification system and displayed a resistivity of $\geq 18.2 \text{ M}\Omega \text{ cm}^{-1}$. The concentration of stock solution concentrations of TBA-FAM and PEP-TAMRA were both $100 \text{ }\mu\text{M}$ in water and diluted as needed for spectroscopic experiments. The concentrations of stock solution of azo-PPE(-) and azo-PPE(+) were 0.62 and 7.11 mM in water, respectively, (all concentrations are provided as polymer repeat unit concentration, [PRU]), and diluted as needed for spectroscopic experiments. Final concentrations of the diluted azo-PPE(+) and azo-PPE(-) were determined based on their extinction coefficients $\epsilon_{451} = 3.7 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ and $\epsilon_{479} = 1.0 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$, respectively. The buffer solution used in this study was 20 mM tris (pH 7.0) with 140 mM Na^+ , 10 mM K^+ , 10 mM Mg^{2+} and 1 mM Ca^{2+} .

Instrumentation. ^1H NMR spectra was acquired on a Bruker 400MHz spectrometer. All mass spectra were acquired by a 4700 Proteomics Analyzer MALDI-TOF/TOF-MS (Applied Biosystems, Framingham, MA). The absorption spectra were recorded on a Beckman DU 800

spectrometer. All the fluorescence spectra were recorded on a SPEX Fluorolog 3-TCSPC instrument with 1 cm path length cuvettes. Fluorescence decays were analyzed using Data Station developed by Horiba Jobin Yvon.

Synthesis. In this study, two azo-PPE polymers with different charged side chains were obtained as in our previously reported procedure.^{4, 5} Detailed procedures are listed below for clarity purpose.

Azo-PPE(+). A solution of 5, 5'-((2, 5-diethynyl-1, 4-phenylene) bis (oxy)) bis (N, N, N-triethyl-pentan-1-aminium) (249 mg, 0.5mmol) and 1, 2-bis (4-iodophenyl) diazene (217 mg, 0.5mmol) in 20 mL of dry THF/Et₃N (v/v = 2/1) fitted with a condenser were degassed with argon for 5 minutes. Then 17.4 mg of Pd(PPh₃)₄ (15 μmol) and 8 mg of CuI (15 μmol) were added under argon. The reaction mixture was stirred at 60 °C for 20 hr. The obtained reaction solution was poured into 300 mL of methanol, and the precipitation were further purified by two repeated cycles of dissolution in THF and precipitation into methanol and dark red solid was collected and dried (359 mg, 85%). ¹H NMR (400 MHz, DMSO-d₆) 7.98 (br, d, 4H), 7.76 (br, s, 4H), 7.29 (br, s, 2H), 4.12 (br, d, 4H), 3.21 (br, d, 12H), 3.11 (br, s, 4H), 1.83 (br, s, 4H), 1.59 (br, s, 8H), 1.43 (br, s, 4H), 1.14 (br, t, 18H).

Azo-PPE(-). To a solution of azo-PPE-CO₂R (the ester precursor of azo-PPE(-)) (198 mg, 0.25 mmol) in 30 mL of dioxane/THF (v/v = 5/1) was added 1.5 mL of 1 M (n-Bu)₄NOH in methanol, and stirred at room temperature for 24 hr. During the course of the hydrolysis reaction, 2 mL of water was systematically added in order to keep the solution clear. Then a solution of 0.20 g of NaIO₄ in 3 mL of water was added to the hydrolyzed polymer solution, and the resulting mixture was poured into 400 mL of cold acetone, resulting in the precipitation

of azo-PPE-CO₂R as orange powders. The polymer was then dissolved in 50 mL of deionized water (several drops of 1 M NaOH solution were added) and was purified by dialysis against deionized water using a regenerated cellulose membrane (7 kD molecular weight cut-off). After dialysis, the solution was stored as the stock solution in the refrigerator.

Steady state fluorescence quenching. For a typical quenching experiment, 2 mL of the probe tris-HCl solution was put in a 1 cm quartz fluorescence cell. The fluorescence spectra was recorded at room temperature. Then fluorescence spectra was repeatedly obtained after the addition of microliter aliquots of a concentrated polymer quencher solution by use of a calibrated Eppendorf microliter. Excitation and emission slits were set as 5 nm, and the integration time was 0.1 s. 450 and 535 nm was used as the excitation wavelength for TBA-FAM and PEP-TAMRA, respectively.

Time-resolved fluorescence quenching. The time-resolved fluorescence spectra measurements were performed at room temperature. 461 nm NanoLed was used as the excitation light source. Fluorescence decays at 520 and 581 nm were recorded for TBA-FAM and PEP-TAMRA, respectively. Fluorescence decays were analyzed using DAS6 developed by Horiba Jobin Yvon.

Protein sensing experiments. Probes were mixed with azo-PPE polymers for 10-30 minutes at room temperature. Then the proteins of different concentrations were added to the complex of probe and corresponding azo-PPE. The resulting mixture was further incubated for 1 hr at room temperature and then the emission spectra were recorded. The final thrombin concentration in TBA-FAM/azo-PPE(+) ranged from 0 to 196 nM and the final bcl-xL protein concentration in PEP-TAMRA/azo-PPE(-) ranged from 0 to 318 nM.

Results

1. FRET parameters and Stern-Volmer quenching constant (K_{SV}) of the probe/quencher pairs.

Table S1. FRET parameters and K_{SV} of the probe/quencher pairs.

Donor	Acceptor	Q_D	$J(\lambda)$	R_0 (Å)	K_{SV} (M ⁻¹)
TBA-FAM	Azo-PPE(+)	0.92	1.11×10^{15}	51.74	5.60×10^6
PEP-TAMRA	Azo-PPE(-)	0.31	3.11×10^{14}	34.92	9.80×10^6

The Förster radius R_0 is calculated by the equation:

$$R_0 = 0.211 \times [\kappa^2 n^4 Q_D J(\lambda)]^{1/6},$$

where κ^2 is the dipole orientation factor, ($\kappa^2=2/3$ for randomly oriented donors and acceptors), n is the refractive index of the solvent, Q_D is the fluorescence quantum yield of the donor in the absence of the acceptor, $J(\lambda)$ is the spectral overlap integral.

K_{SV} is obtained according to the Stern-Volmer (SV) equation:

$$F_0/F = 1 + K_{SV} [Q],$$

where Q is the quencher, F_0 and F are the fluorescence intensities without Q and with Q in low concentration, respectively.

2. Quenching mechanisms of probes by corresponding azo-PPE polymer.

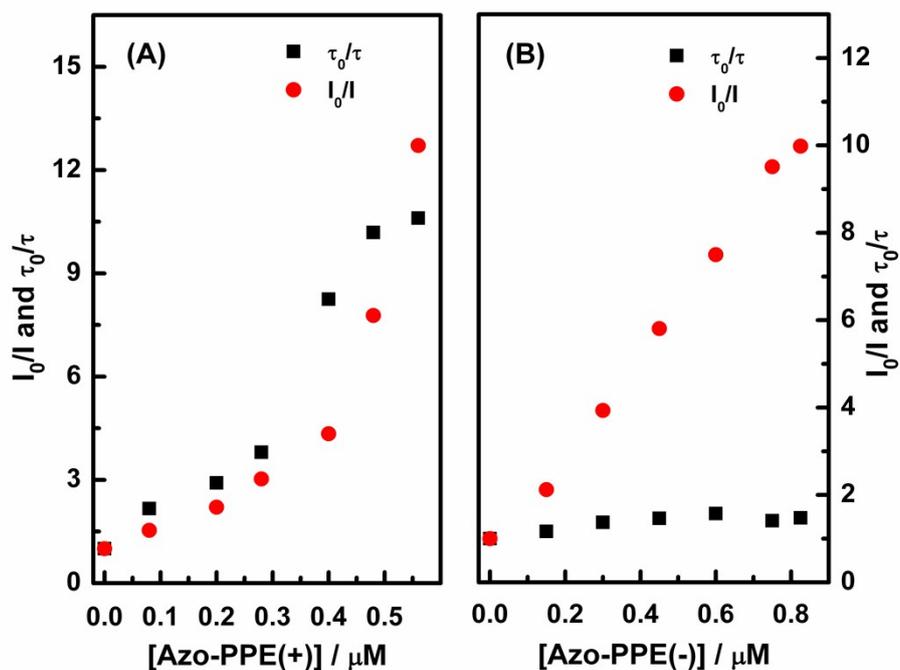


Figure S1. The ratio of fluorescence intensities and fluorescence lifetimes of (A) TBA-FAM and (B) PEP-TAMRA changed with the concentrations of azo-PPE(+) (0, 0.08, 0.20, 0.28, 0.40, 0.48 and 0.56 μM) and azo-PPE(-) (0, 0.15, 0.30, 0.45, 0.60, 0.75 and 0.83 μM), respectively.

3. Protein sensing based on azo-PPE polymers.

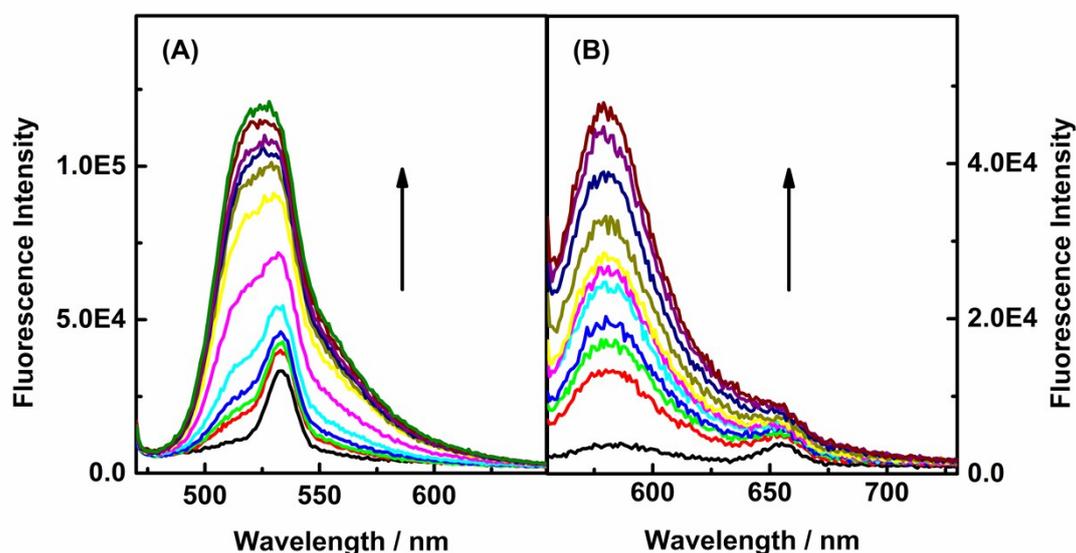


Figure S2. (A) Fluorescence emission spectra of TBA-FAM/azo-PPE(+) in the presence of different concentrations of thrombin (0, 0.7, 1.4, 2.8, 4.2, 9.8, 19.6, 39.2, 78.4, 156.8 and 196 nM). (B) Fluorescence emission spectra of PEP-TAMRA/azo-PPE(-) in the presence of different concentrations of bcl-xL protein (0, 5.3, 10.6, 15.9, 21.2, 26.5, 31.8, 42.2, 106, 212 and 318 nM).

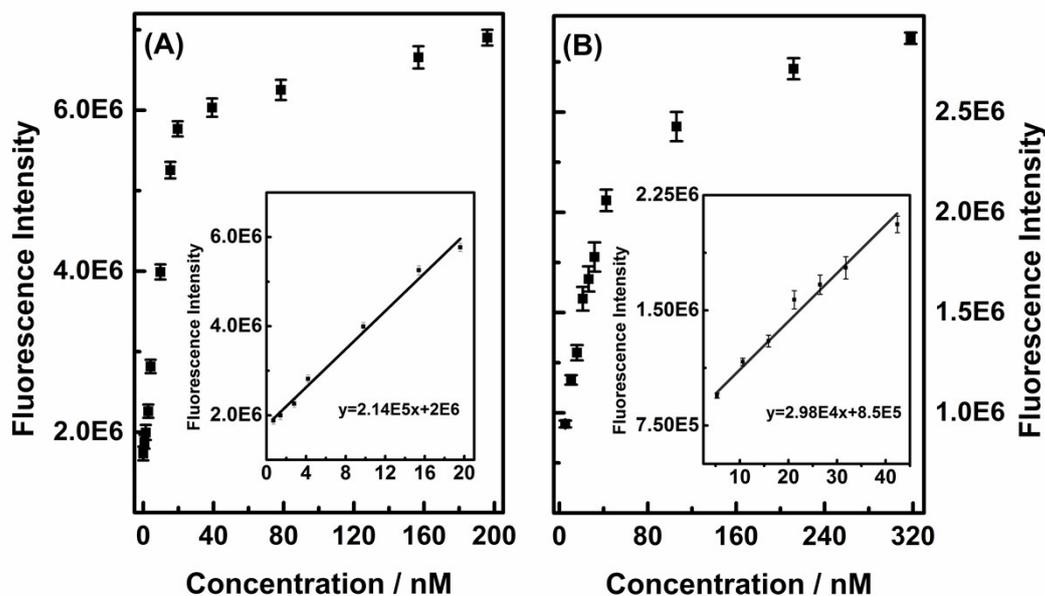


Figure S3. Calibration curves for (A) thrombin and (B) bcl-xL detection. Inset: calibration curve of low protein concentration region.

4. Protein sensing based on graphene oxide.

In recent years, graphene oxide (GO) has been attracting wide attention in the application of biosensing as dark quencher for the fluorophore. In previous researches, GO was introduced to bind with TBA-FAM for detection of thrombin as a sensing platform, which is similar with our TBA-FAM/azo-PPE(+) sensing method. Herein, the fluorescence quenching of TBA-FAM by GO, as well as the fluorescence recovery (Figure S4) due to the introduction of thrombin experiences were carried out to compare these two sensing methods. As a result, the detection limit of TBA-FAM/GO was calculated to be about 0.115 nM in the same experimental conditions, which compares favorably with that of TBA-FAM/azo-PPE(+) (0.108 nM).

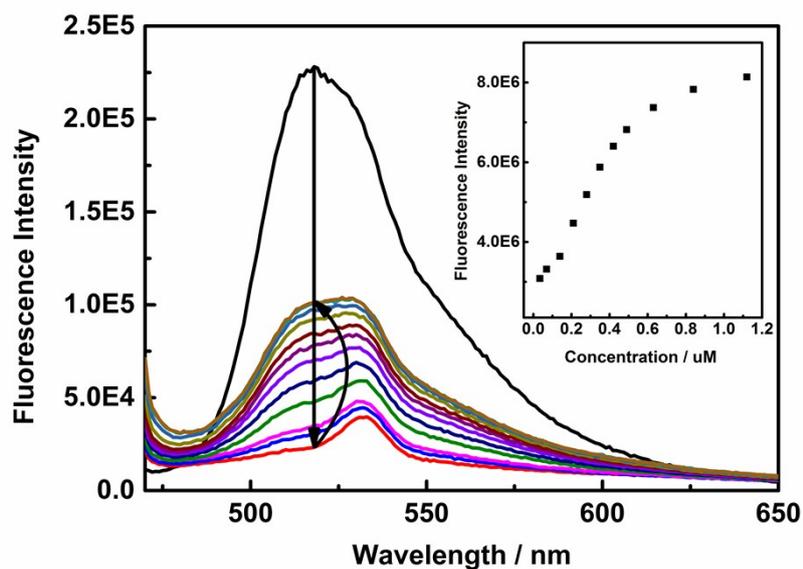


Figure S4. Fluorescence emission spectra of TBA-FAM (20 nM, black) and TBA-FAM/GO in the presence of different concentrations of thrombin (color, 35, 70, 140, 210, 280, 350, 420, 490, 630, 840 and 1120 nM). Inset: Plot fluorescence intensity on the concentration of thrombin.

5. Specificity of the sensing methods.

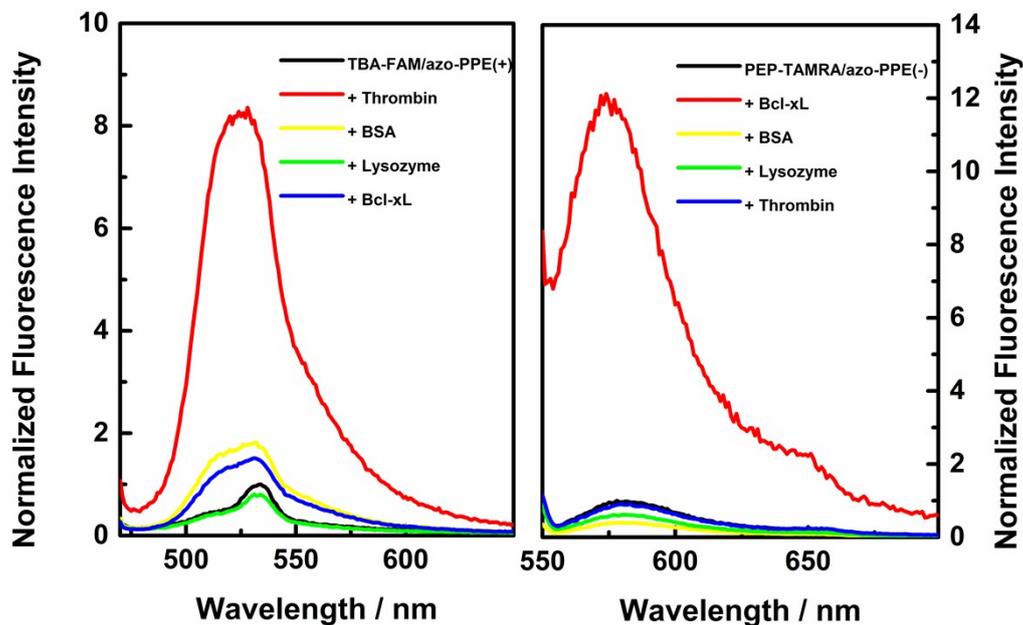


Figure S5. Fluorescence recovery of (A) TBA-FAM/azo-PPE(+) towards thrombin (200 nM), BSA (500 nM), lysozyme (500 nM) and bcl-xL (500 nM), and (B) PEP-TAMRA/azo-PPE(-) towards bcl-xL (320 nM), BSA (500 nM), lysozyme (500 nM) and thrombin (500 nM).

Additional References:

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