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Supporting Information

Aggregation-induced emission based one-step "lighting up" sensor array for rapid protein identification

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EXPERIMENTAL SECTION

Chemicals

Trimethylamine, N,N-Dimethylbenzylamine, N,N-Dimethylcyclohexylamine, 1,4-Dibromobutane, Tetrahydrofuran (THF), K_2CO_3, and anhydrous acetone were obtained from J&K. 4,4',4",4"'-(ethene-1,1,2,2-tetrayl) tetraphenol (TPE-OH）was purchased from Shanghai Tensus Biotech Co., LTD. Guanidine hydrochloride (GndHCl) was purchased from Aladdin. Bovine serum albumin (BSA), human serum albumin (HSA), hemoglobin (Hb), catalse (CAT), cytochrome c (CytC), lysozyme (Lys), myoglobin (Myo), Pepsin (Pep), trypsin (Try) and trandferrin (Tf) were obtained from Sigma-Aldrich (USA). Ultrapure Milli-Q water (18 MΩ·cm) was used in the whole experiments. Serums samples of healthy people and cancer patients were collected and approved by the Affiliated Hospital of Qingdao University under the agreement of the human subjects. Moreover, serums collection was approved by Institutional Review Board of the Affiliated Hospital of Qingdao University.

Instruments

Fluorescence measurements were performed on a FLS-1000 fluorescence spectrometer (Edinburgh, UK). Fluorescence images of 96-well plates were obtained using a VILBER bioimaging system (Quantum, France). Confocal images were collected using a Laser scanning confocal microscope (Olympus FV1000). The docking between probes and proteins was simulated by AutoDock 4.2. All the docking simulations were performed for 500 runs. Dynamic light scattering (DLS) experiment was performed on a Nano-ZS Zetasizer

ZEN3600 (Malvern, UK). ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Bruker Avance 500 spectrometer. Full-scan negative (-) mode was used to obtain the m/z information on a Mass Spectrometer (Thermo-Fisher, Q-Exactive).

Synthesis of TPE-OBr: Synthesis of 1, 1, 2, 2-tetrakis(4-(4-bromobutoxy) phenyl)ethane: Hydroxylated TPE (TPE-OH) (0.5 g, 1.26 mM), 1,4-dibromobutane (3.0 mL, 24.85 mM) and anhydrous K_2CO_3 (1.04 g, 7.56 mM) were dissolved in anhydrous acetone (30 mL) in round-bottomed flask. The solution was refluxed overnight. The product was washed by acetone and rotary evaporated twice. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1: 5) as eluent. After dying under vacuum, product (886 mg, 75%) was obtained as a white powder. ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 6.94 (d, J = 8.0 Hz, 8H), 6.65 (d, J = 4.0 Hz, 8H), 3.95 (t,J = 8.0 Hz, 8H), 3.51 (t, J = 8.0 Hz, 8H), 2.11-2.05 (m, 8H), 1.96-1.91 (m, 8H).

Synthesis of TPE-NRs: The mixture of 1, 1, 2, 2-tetrakis (4-(4-bromobutoxy) phenyl) ethane (TPE-OBr) (500 mg, 0.625 mM) and the corresponding amine (15.75 mM) was refluxed in THF (40 mL) for 48 h. The obtained product was washed several times with THF, and TPE-NRs were obtained by recrystallization. The characterization of three TPE-NRs is followed:

TPE-NR1: ^{1}H-NMR (500 MHz, D_2O):δ (ppm) = 6.91 (d,J=5.0 Hz, 8H), 6.44 (s, 8H), 3.49 (s, 8H), 3.21 (d,J=5.0 Hz, 8H), 3.19 (s,8H), 2.99 (s, 36H), 1.55 (s, 16H). ^{13}C-NMR (500MHz, D_2O):δ (ppm) = 156.49, 139.23, 137.06, 132.13, 114.01, 67.21, 65.94, 59.53, 52.92, 25.42, 19.39. ESI-MS: m/z calculated for $C_{54}H_{84}N_4O_4^{4+}$: 213.2, found: 213.4.

TPE-NR2: ^{1}H-NMR (500 MHz, D_2O):δ (ppm) = 6.75 (s, 8H), 6.46 (s, 8H), 3.63 (s, 8H), 3.55 (t, J=15.0Hz, 4H), 3.21 (s, 8H), 2.88 (s, 24H), 1.93 (s, 8H), 1.77 (s, 8H), 1.60 (s, 8H), 1.38 (d, J=5.0

Hz), 1.14 (d, J=10.0 Hz, 8H), 1.07 (t, J=10.0 Hz, 8H). ^{13}C-NMR (500MHz, D_2O):δ (ppm) = 156.74, 138.74, 136.86, 132.27, 114.04, 71.42, 67.82, 67.12, 62.23, 57.39, 48.28, 25.58, 25.11, 24.32, 18.69, 16.81. ESI-MS: m/z calculated for $C_{74}H_{116}N_4O_4^{4+}$: 277.2, found: 281.2.

TPE-NR3: ^{1}H-NMR (500 MHz, D_2O):δ (ppm) = 7.22 (s, 8H), 7.06 (s, 8H), 6.97 (s, 8H), 6.74 (s, 8H), 6.36 (s, 8H), 4.22 (s, 8H), 3.54 (t, J=15.0 Hz, 8H), 2.85 (s, 32H), 1.76 (s, 8H), 1.51 (s, 8H), 1.07 (t, J=15.0 Hz, 8H).

^{13}C-NMR (500MHz, D_2O):δ (ppm) = 156.69, 138.46, 136.77, 132.63, 129.12, 127.30, 113.87, 66.37, 61.64, 57.38, 50.56, 25.14, 19.12, 16.78. ESI-MS: m/z calculated for $C_{78}H_{100}N_4O_4^{4+}$: 289.2, found: 289.2.

Protein detection and discrimination: For protein identification: ten kinds of target proteins (2.0 μL, 40 mg/mL) were added to 200 μL of three TPE-NRs probes (2 μM) on a 96-well plate, respectively, and incubated at room temperature for 10 minutes. Subsequently, fluorescent images were obtained directly using a VILBER bioimaging system. The obtained relative fluorescence intensities of TPE-ARs before and after incubation with proteins [$(I - I_0)/I_0$] were processed by principal component analysis (PCA) using statistical analysis software simca (Here, I_0 is the fluorescence intensity of TPE-ARs before adding proteins, I is the fluorescence intensity of TPE-ARs after adding proteins). For protein identification: 2.0 μL of serums from prostate cancer, liver cancer, rectal cancer and healthy people were added to 200 μL of three TPE-NRs probes (2 μM) on a 96-well plate, respectively, and incubated at room temperature for 10 minutes. Then, fluorescent images were obtained directly using a VILBER bioimaging system.

The obtained relative fluorescence intensities of TPE-ARs before and after incubation with serums [$(I - I_0)/I_0$] were processed by principal component analysis (PCA) using statistical analysis software simca (Here, I_0 is the fluorescence intensity of TPE-ARs before adding serums, I is the fluorescence intensity of TPE-ARs after adding serums).

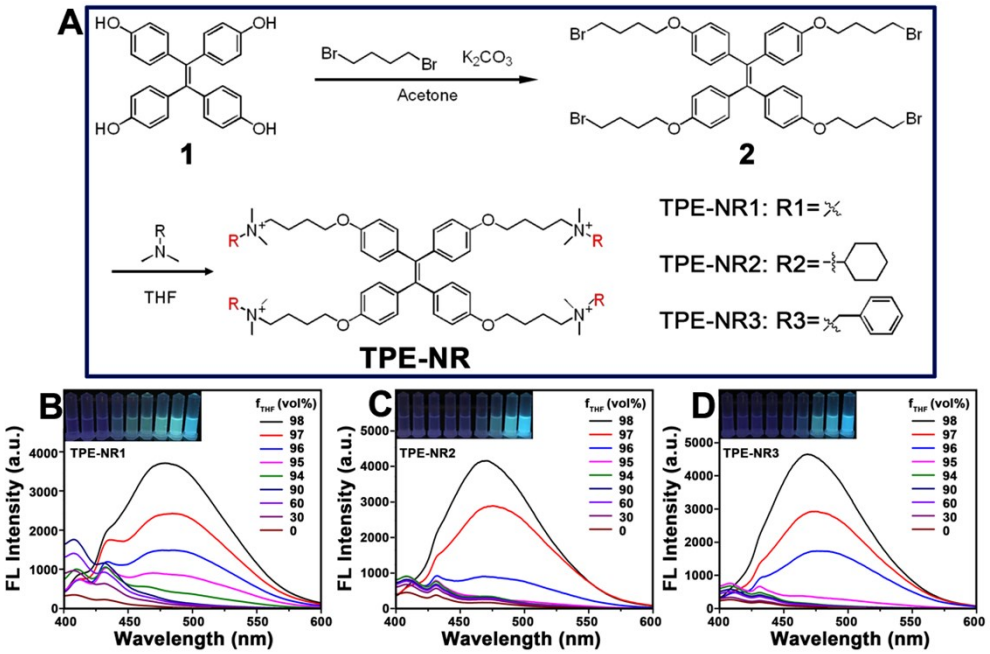

Fig. S1 (A) Synthesis routes of AIE molecules TPE-NR1, TPE-NR2 and TPE-NR3. Fluorescence spectra of (B) TPE-NR1, (C) TPE-NR2 and (D) TPE-NR3 in THF/H_2O mixture with different THF fraction (f_{THF}) (TPE-NRs concentration: 2 μM; λ_{ex}= 380 nm).

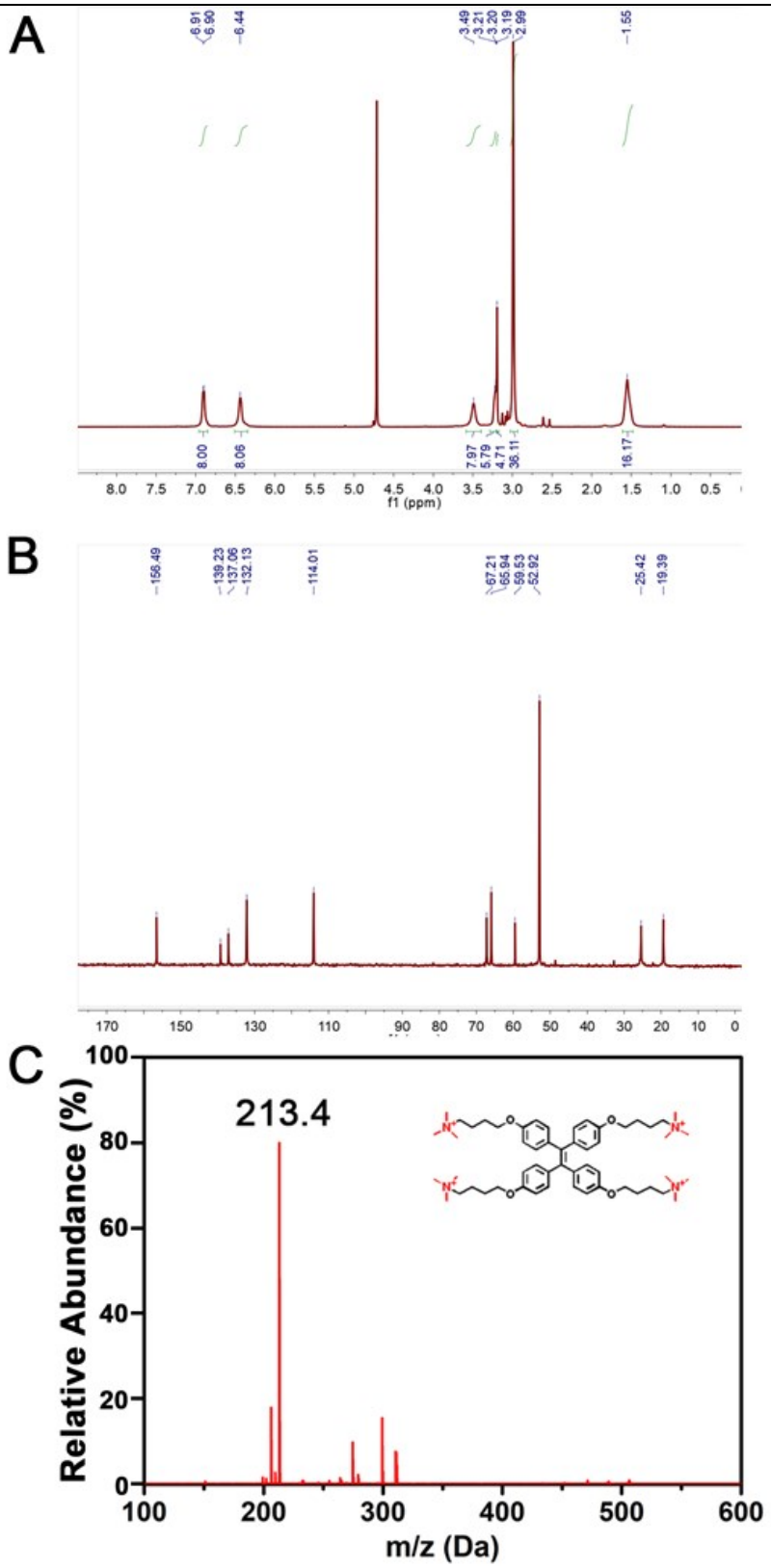

Fig. S2 ^{1}H-NMR (A), ^{13}C-NMR (B) and high-resolution mass spectra (C) of TPE-NR1.

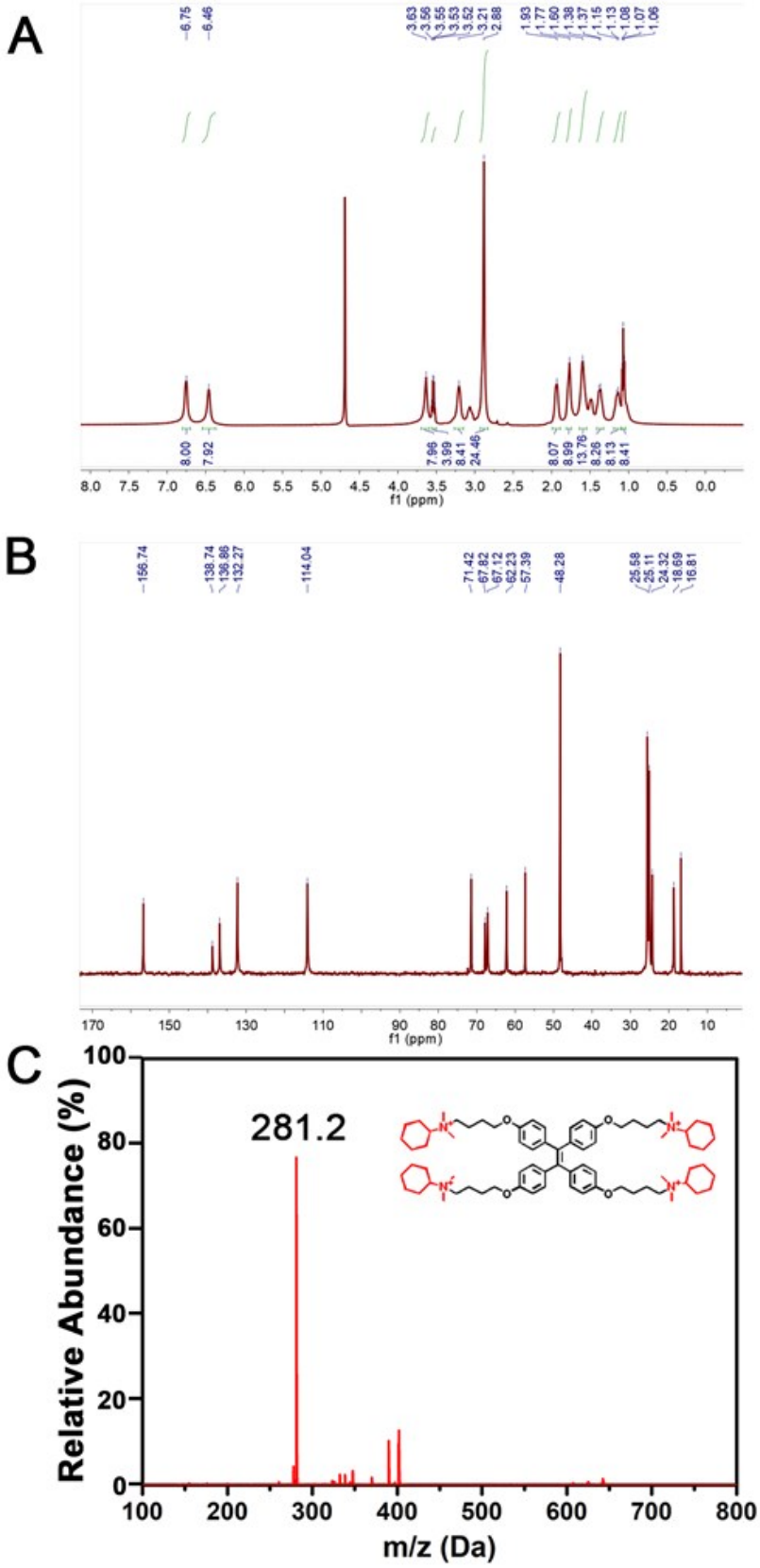

Fig. S3 ^{1}H-NMR (A), ^{13}C-NMR (B) and high-resolution mass spectra (C) of TPE-NR2.

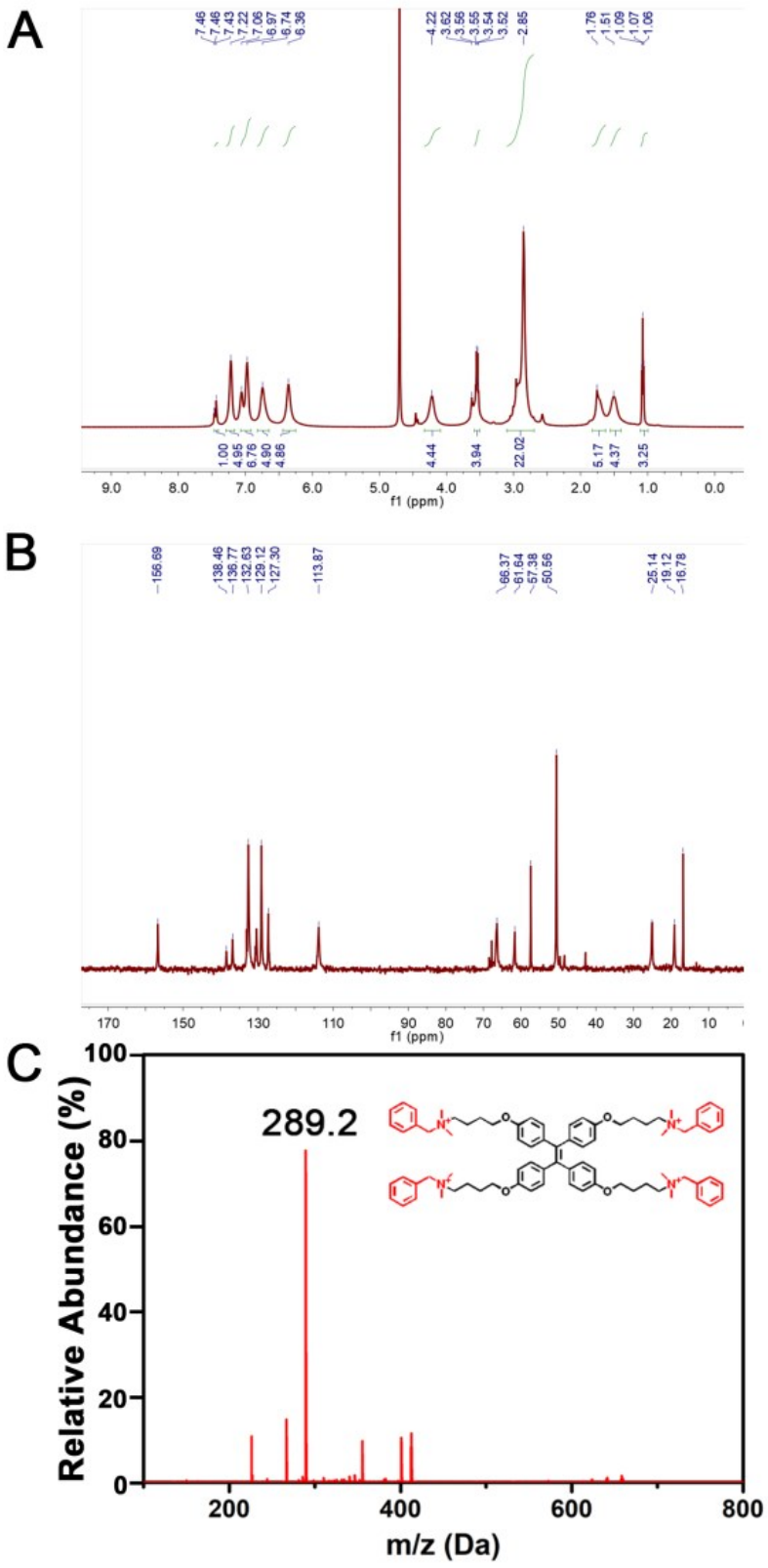

Fig. S4 ^{1}H-NMR (A), ^{13}C-NMR (B) and high-resolution mass spectra (C) of TPE-NR3.

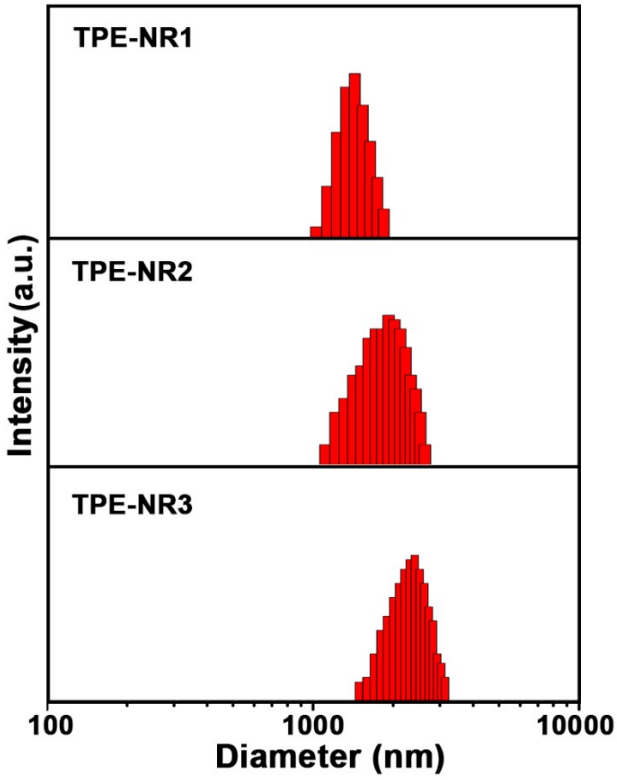

Fig. S5 Size distribution of (A) TPE-NR1, (B) TPE-NR2 and (C) TPE-NR3 aggregates in THF (f_{THF}=96%) (TPE-NRs concentration: 2 μM).

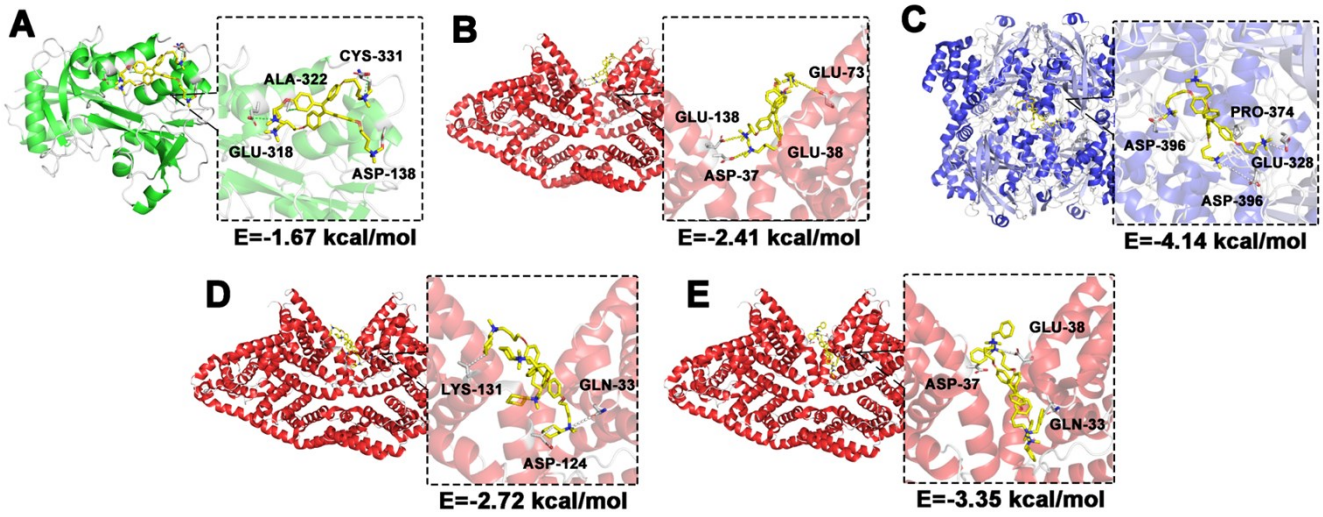

Fig. S6 The best docked complex between (A) TPE-NR1 and Tf, (B) TPE-NR1 and BSA, (C) TPE-NR1 and CAT, (D) TPE-NR2 and BSA, (E) TPE-NR3 and BSA. The TPE-NRs are represented as in ball and stick mode, with carbon, oxygen, and nitrogen atoms colored in yellow, red, and blue, respectively. The crystal structure of proteins are represented as cartoon, with α-helices, β-sheets, and fibril. The α-helices of Tf, BSA and CAT are colored in green, red, and blue, respectively. The

key amino acid residues in the crystal structures of proteins are pointed out in enlarged images. PDB number： Tf: 1D3K; BSA: 3V03; HSA:1DGB.

Table S1 Basic properties of the target proteins

Proteins	M_w(kDa)	pI
Pep	35	1.0-2.5
HSA	69.4	5.2
BSA	68	4.6-5.8
Tf	77	5.6-6.6
Hb	64.5	6.8
Myo	17.8	7.6
CAT	247.0	8.3
Try	24.0	10.5
Lys	14.4	9.6-11.0
CytC	12.3	10.7

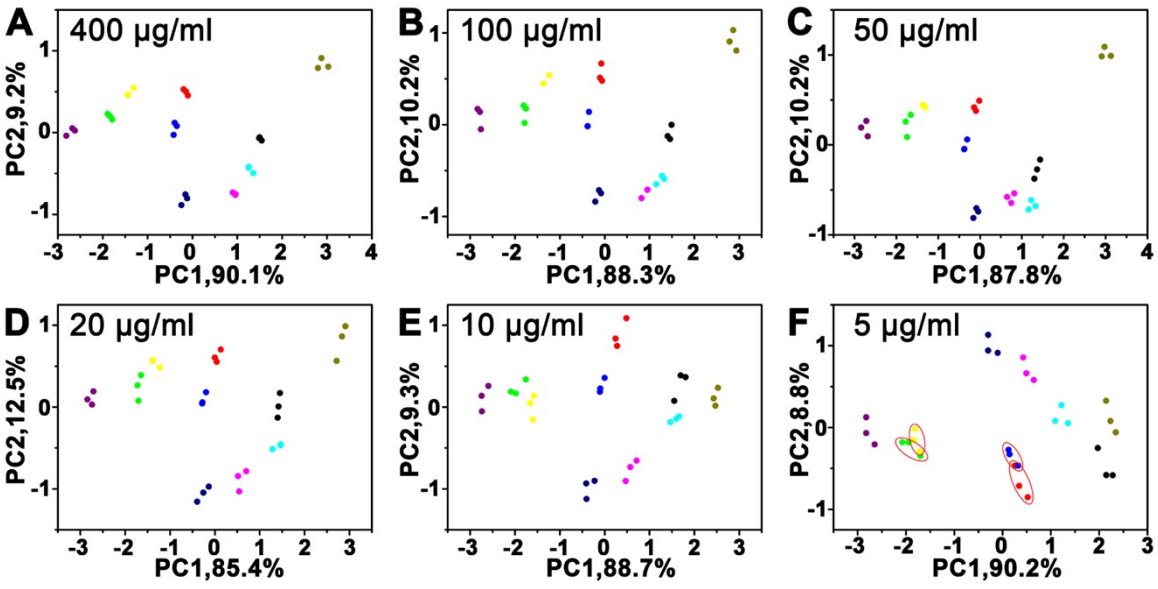

Fig. S7 Canonical score plot for the discrimination of ten proteins with different concentrations (n = 3). (TPE-NRs concentration: 2 μM)

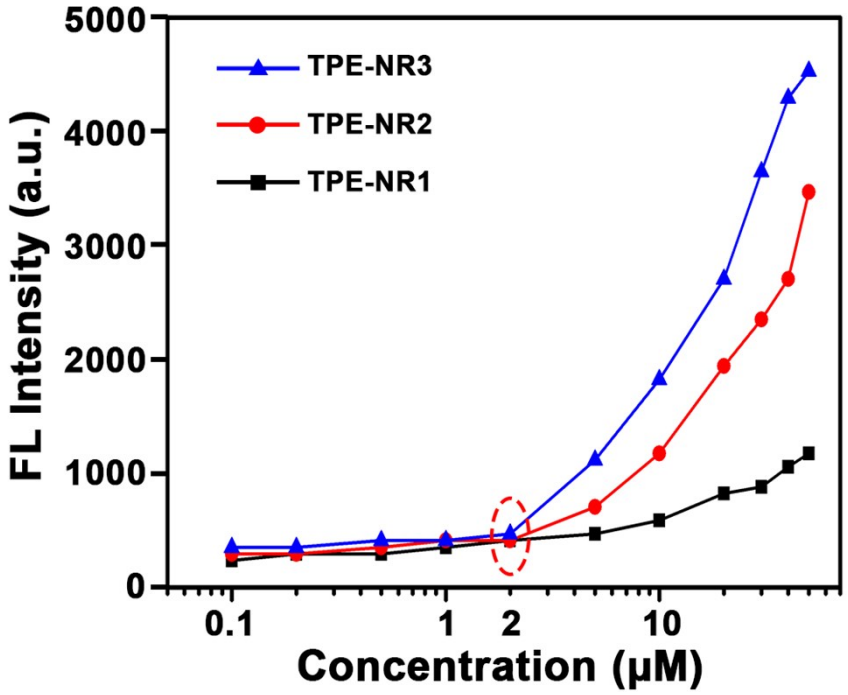

Fig. S8 Plots of fluorescence intensity of TPE-NRs with different concentrations.

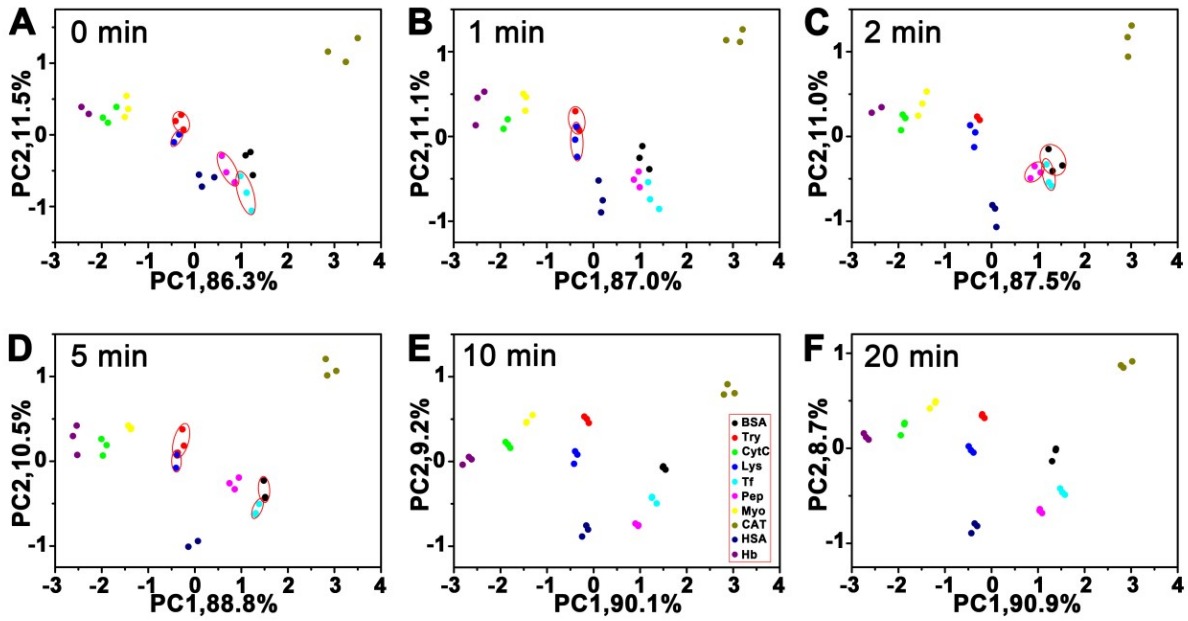

Fig. S9 The influence of incubation time on the protein identification (n = 3). (TPE-NRs concentration: 2 μM)

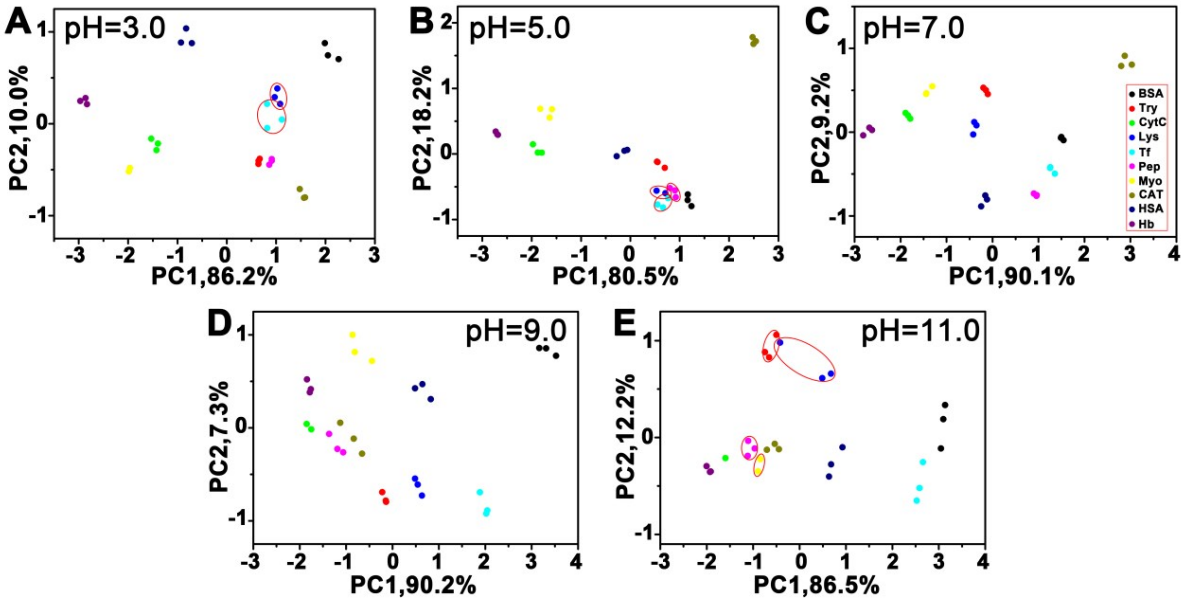

Fig. S10 The influence of pH on the protein identification (n = 3). (TPE-NRs concentration: 2 μM; protein concentration: 400 μg/mL)

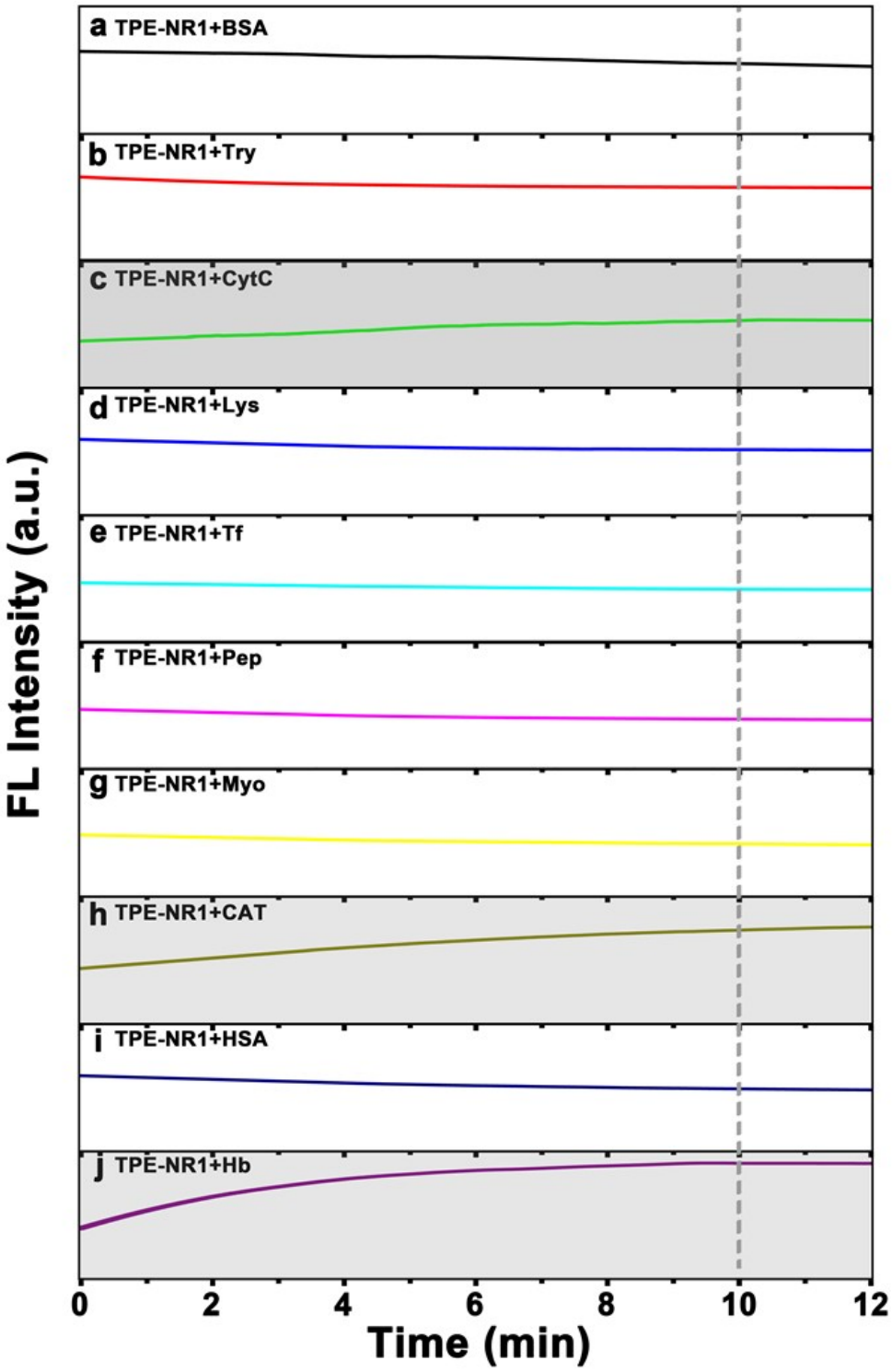

Fig. S11 The kinetics curves of fluorescence intensity of TPE-NR1 at 480 nm after the addition of different proteins over time. (λ_{ex}=380 nm)

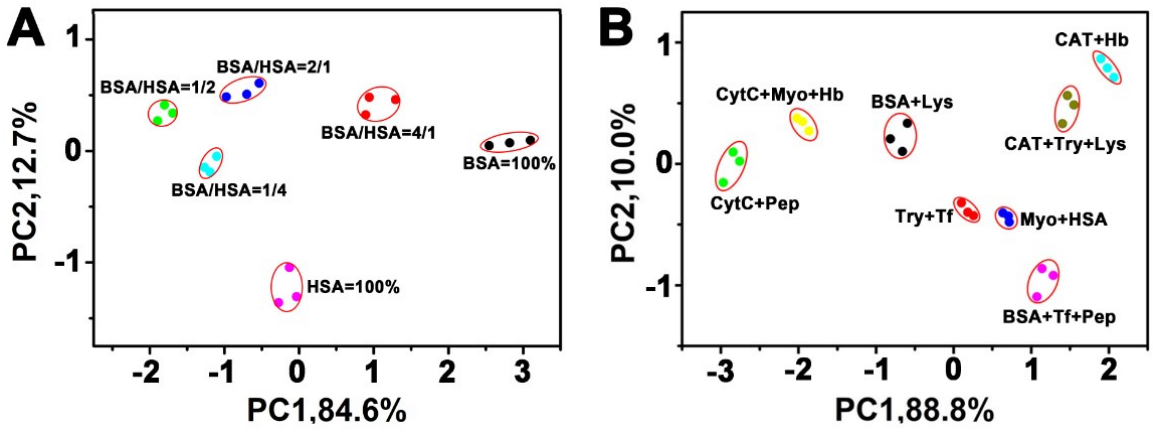

Fig. S12 (A) PCA plot for identification the mixtures of BSA and HSA at different molar ratios (total protein concentration: 400 μg/mL). (B) PCA plot for binary or ternary mixtures of proteins (total protein concentration: 400 μg/mL, n = 3, TPE-NRs concentration: 2 μM).

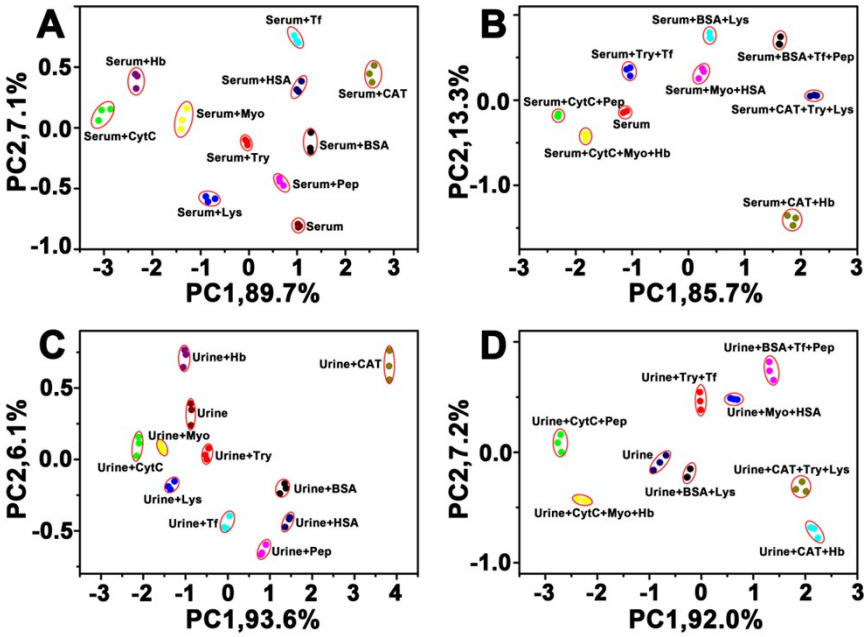

Fig. S13 PCA plots for the identification of (A) ten proteins (400 μg/mL) and (B) random binary or ternary mixtures of proteins in the presence of human serum (total protein concentration: 400 μg/mL). PCA plots for the identification of (C) ten proteins (400 μg/mL) and (D) random binary or ternary mixtures of proteins in the presence of human urine (total protein concentration: 400 μg/mL, n = 3). (TPE-NRs concentration: 2 μM)

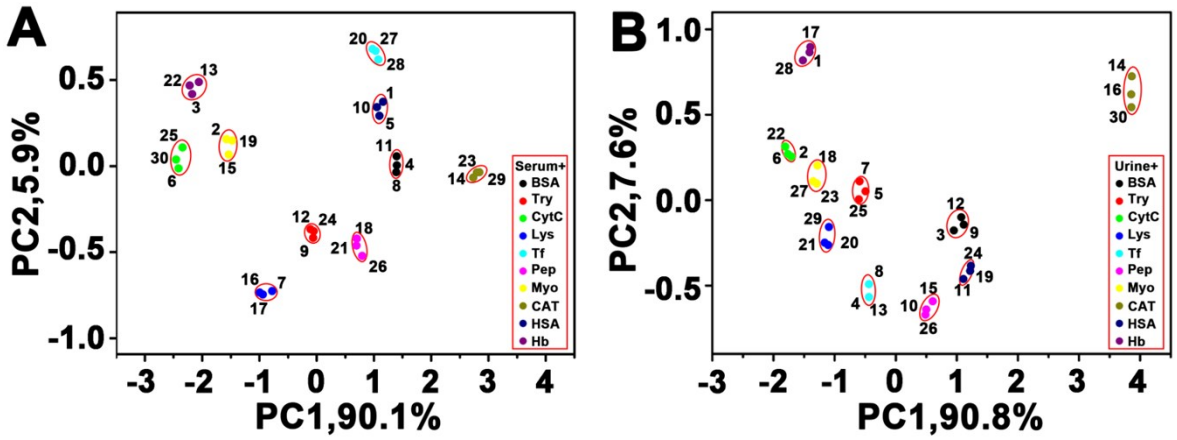

Fig. S14 PCA plot for identification 30 blind samples in the presence of (A) human serum and (B) urine. (n = 3)

For blind testing, we randomly selected 30 samples from these 10 proteins and added them to human serum and urine, respectively, to obtain 30 blind test samples (The final added protein concentration was 400 g/mL for each blind sample). Each blind sample is covered with a numbered label paper. Then, these 30 blind sample were identified by the AIE based sensor array. By comparing the result (**Fig. S14A** and **Fig. S14B**) with the standard data (Data in Figure 13A and C), 30 blind test samples were confirmed to be identified with a discriminative accuracy of 100%, respectively.