

Supplementary Information

Array-based Protein Sensing Using an Aggregation-induced Emission (AIE) Light-up Probe

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S1. Synthesis and characterization

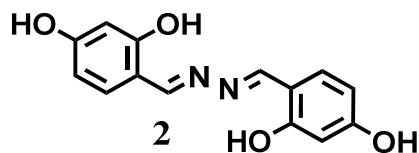
S1.1 Materials and characterization

2,4-dihydroxy benzaldehyde, 1,3-dibromopropane, dimethylamine solution 40% in water and tert-butyl bromoacetate were purchased from Alfa Aesar (USA). Hydrazine monohydrate ($\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}$), trimethylamine 45% aqueous solution in water and all proteins (Bovine serum albumin, Esterase, Fibrinogen, transferrin and β -galactosidase) were purchased from Sigma Aldrich (USA). Trifluoroacetic acid (TFA) was purchased from TCI (Japan). Ethyl acetate (EtOAc) and hexane were obtained from SK Chemical (Korea). Potassium carbonate (K_2CO_3), potassium bicarbonate (KHCO_3), sodium hydroxide (NaOH), sodium chloride (NaCl), and sodium sulfite anhydrous (Na_2SO_3) was obtained from Samchun pure chemical Co.,Ltd (Korea). HPLC grade of tetrahydrofuran (THF) and acetonitrile (ACN) were purchased from Burdick & Jackson Honeywell (USA). Dichloromethane (DCM) HPLC was purchased from Daejung Co.,Ltd (Korea). PBS 1X, pH 7.4 was obtained from Gibco, ThermoFisher SCIENTIFIC (USA)

AIEgens (AIE 1-4) were characterized using 400MHz FT-NMR (Agilent Technologies) and MALDI-TOF/TOF (Bruker Ultraflex III) with MALDI matrix made of 50:50 water/acetonitrile with 0.1% TFA and 2,5-dihydroxybenzoic acid (2,5-DHB). UV-vis spectroscopy (Jasco V-670 spectrophotometer), Fluorometer (Hitachi F-7000), Microplate reader (Molecular devices SpectraMax i3x)

S1.2 Synthesis of AIEgens (AIE1-4)

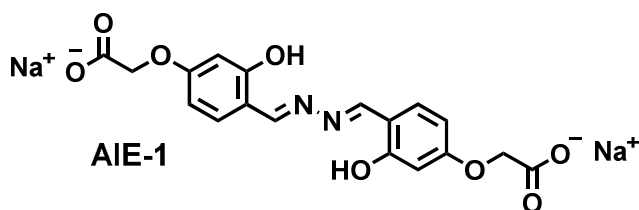
1) Synthesis of compound 2



Compound 2 was synthesized by adding hydrazine monohydrate (90 μL , 1.81 mmol) into compound 1, 2,4-dihydroxy benzaldehyde (0.5 g, 3.65 mmol) in 10 mL of methanol. The mixture was refluxed for 4 h at 75°C. The solution was cooled to room

temperature then yellow solid was formed which was isolated by filtration and dried over under vacuum. Yield (72%, 0.357 g) δ_H (400 MHz, DMSO) 6.29 (2H, s), 6.37 (2H, s), 7.39 (2H, s) 8.73 (2H, s), 10.13 (2H, s) and 11.38 (2H, s). δ_C (400 MHz, DMSO) 102.91, 108.65, 110.71, 133.39, 161.11, 162.20 and 162.51 Calculated [M], $C_{14}H_{12}N_2O_4$, molecular weight: m/z calcd 272.26 ; measured 272.60

2) Synthesis of **AIE-1**



a. Synthesis of *t*-butyl-protected AIE molecule

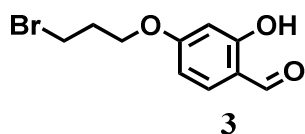
To synthesize **AIE-1**, compound **2** (500 mg, 1.836 mmol) and K_2CO_3 (507.6 mg, 3.673 mmol) were dissolved in 10 mL of acetonitrile. After 30 min, *t*-butyl bromoacetate (542 μ L, 3.673 mmol) was added dropwise into mixture. The reaction was refluxed at 60 °C for overnight. After finishing the reaction, the reaction was then cooled to room temperature. The reaction mixture was treated with water and extracted with ethyl acetate (EA). Organic layer was washed with brine solution. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Purification was done by silica flash column chromatography with hexane: EA = 4:1. Yield (21%, 187 mg) δ_H (400 MHz, DMSO) 1.46 (18H, s), 4.68 (4H, s), 6.43 (2H, s), 6.55 (2H, s), 7.39 (2H, s) 7.52 (2H, s), 8.91 (2H, s). δ_C (400 MHz, DMSO) 27.82, 65.59, 82.79, 102.17, 107.28, 111.44, 133.58, 158.84, 161.76, 163.03, 167.42

b. Deprotection of *t*-butyl group

To synthesize **AIE-1**, boc-protected AIE molecules (244mg, 0.487 mmol) was dissolved in 5 mL of DCM. TFA (0.2 mL , excess amount) was added into boc-protected AIE in DCM. The reaction was stirred at room temperature for 12 hours. After reaction, DCM and TFA were removed using rotary evaporator and dried using high vacuum pump. To make negatively charged **AIE-1**, 2 equivalent of NaOH was

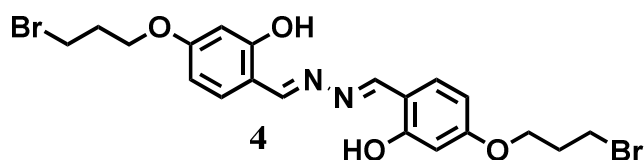
added into water. Yield (180 mg, 95%) δ_H (400 MHz, DMSO) 4.72 (4H, s), 6.48 (2H, s), 6.65 (2H, s), 7.54 (2H, s), 8.93 (2H, s). δ_C (400 MHz, DMSO) 64.88, 102.18, 107.52, 112.22, 132.80, 159.09, 160.52, 162, and 170.08 Calculated [M], $C_{18}H_{14}N_2O_8^{2-}$, molecular weight: m/z calcd 386.32 ; measured 386.03

3) Synthesis of compound 3



Compound 3 was synthesized through reference [1]. Compound 1, 2,4-dihydroxy benzaldehyde (2 g, 14.48 mmol) and potassium bicarbonate ($KHCO_3$, 1.448 g, 14.48 mmol) were dissolved in dry acetone and then 1,3-dibromopropane (1.47 mL, 14.48 mmol) was added dropwise into mixture. The reaction was refluxed at 60 °C for 60 h. After the reaction, solvent was evaporated under pressure. 20 mL of water was added and extracted with 30 mL of chloroform for three times. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Purification was done by silica flash column chromatography with hexane: EA = 1:1. Yield (17%, 1.2 g) δ_H (400 MHz, $CDCl_3$) 2.36 (4H, m), 3.57 (4H, t), 4.12 (4H, t), 6.48 (2H, s), 6.59 (2H, s), 7.48 (2H, s), 9.85 (2H, s), and 11.50 (2H, s). δ_C (400 MHz, $CDCl_3$) 29.47, 31.90, 65.79, 101.27, 108.50, 115.30, 135.30, 164.41, 165.81, 194.37. Calculated [M], $C_{10}H_{11}BrO_3$, molecular weight: m/z calcd 259.10 ; measured 258.36

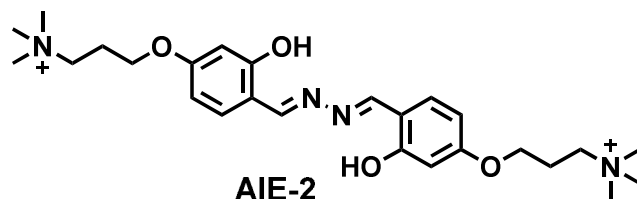
4) Synthesis of compound 4



Compound 4 was synthesized by adding hydrazine monohydrate (7.5 μ L, 0.09 mmol) into compound 3 (92.6 mg, 0.18 mmol) in 3 mL of acetonitrile. The mixture was

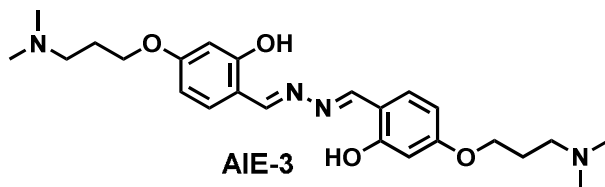
refluxed for 12 h at 60 °C. After the reaction, solvent was dried over under vacuum. Yield (91%, 168 mg) δ_H (400 MHz, $CDCl_3$) 2.34 (4H, m), 3.60 (4H, t), 4.15 (4H, t), 6.53 (4H, s), 8.61 (2H, s), and 11.73 (2H, s). δ_C (400 MHz, $CDCl_3$) 29.65, 32.11, 50.86, 65.52, 101.82, 107.72, 111.28, 133.52, 161.71, 162.80, 16.89 Calculated [M], $C_{20}H_{22}Br_2 N_2O_4$, molecular weight: m/z calcd 514.21; measured 515.12

5) Synthesis of **AIE-2**



Compound **4** (20 mg, 0.039 mmol) and dimethylamine 40% solution in water (17 μ L, 0.155 mmol) in THF and water co-solvent was stirred and heated up to 60 °C for overnight. After the reaction, the solvent (THF and water) was unreacted trimethylamine removed by evaporation. The 16 mg (98%) of product **AIE-3** was obtained. δ_H (400 MHz, D_2O) 1.98 (4H, m), 2.68 (4H, t), 4.13 (4H, t), 6.38 (4H, s), 6.62 (4H, s), 7.16 (2H, s), and 7.85 (2H, s), 11.70 (2H, s). δ_C (400 MHz, D_2O) 22.44, 52.84, 63.74, 64.60, 101.62, 107.58, 111.80, 133.88, 159.85, 161.87, 162.89 Calculated [M]⁺, $C_{26}H_{40}N_4O_4^{2+}$, molecular weight: m/z calcd 472.63; measured 471.39

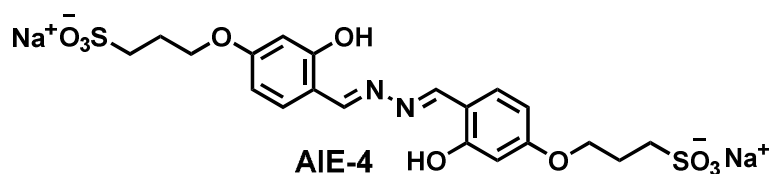
6) Synthesis of **AIE-3**



Compound **4** (20 mg, 0.039 mmol) and dimethylamine 40% solution in water (17 μ L,

0.155 mmol) in 1 mL of THF and water co-solvent was stirred and heated up to 60 °C for overnight. After the reaction, the solvent (THF and water) and unreacted dimethylamine was removed by evaporation. The 16 mg (98%) of product **AIE-3** was obtained. δ_H (400 MHz, DMSO) 1.98 (4H, m), 2.68 (4H, t), 4.13 (4H, t), 6.53 (4H, s), 7.51 (2H, s), and 8.80 (2H, s). δ_C (400 MHz, DMSO) 34.41, 42.99, 54.68, 65.55, 101.82, 107.84, 112.01, 133.06, 160.84, 162.32, 162.73 Calculated [M], C₂₄H₃₄N₄O₄, molecular weight: m/z calcd 442.56; measured 443.32

7) Synthesis of **AIE-4**



Compound **4** (10 mg, 0.019 mmol) and sodium sulfite (NaSO₃, 6 mg, 0.042 mmol) in 2 mL of THF and water co-solvent was stirred and heated up to 100 °C under nitrogen atmosphere for overnight. After the reaction, the mixture kept at room temperature. Acetone (diethyl ether) was added to get white crystals. The 4 mg (80%) of product **AIE-4** was obtained. δ_H (400 MHz, D₂O) 1.98 (4H, m), 2.68 (4H, t), 4.13 (4H, t), 6.53 (4H, s), 7.51 (2H, s), and 8.80 (2H, s). δ_C (400 MHz, D₂O) 24.10, 47.67, 66.48, 101.39, 107.51, 111.99, 133.25, 159.80, 162.05, 162.50 Calculated [M], C₂₆H₄₀N₄O₄²⁻, molecular weight: m/z calcd 514.52; measured; 514.42

S2. Spectrometer experiment

S2.1 Characterization of AIEgens (AIE 1-4)

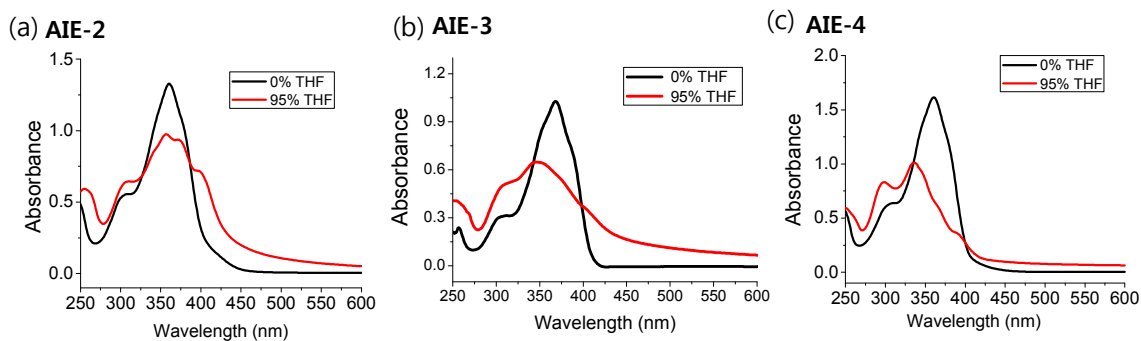


Figure S1. UV-Vis absorption spectra of (a) **AIE-2**, (b) **AIE-3** and (c) **AIE-4** in water (black line) and in a mixture of **H₂O** /THF (v/v, 5:95) (red line). [AIEgens] = 40 μ M

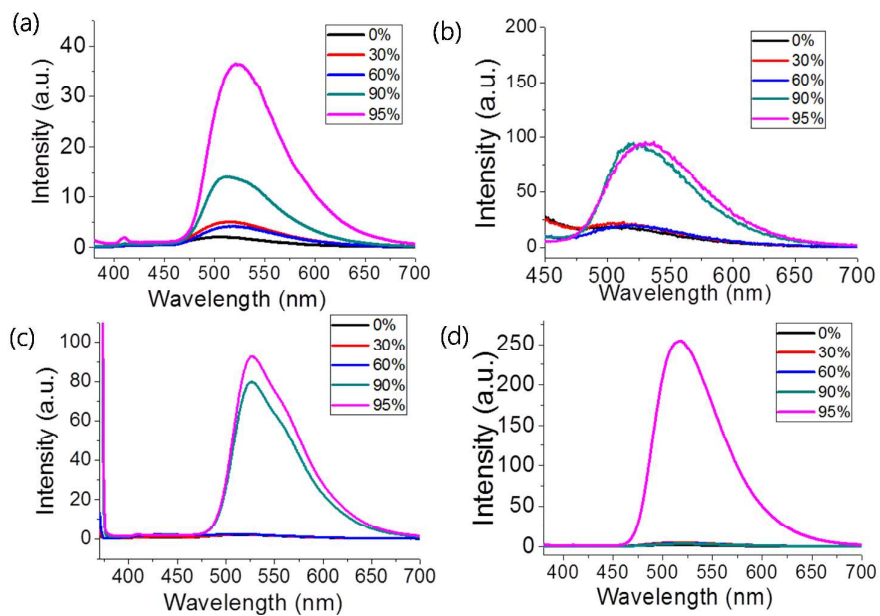


Figure S2 Fluorescence spectra of (a) **AIE-1**, (b) **AIE-2**, (c) **AIE-3**, and (d) **AIE-4** in gradual addition (0%, 30%, 60%, 90% and 95%) of THF to water. λ_{ex} = 365 nm [AIEgens] = 40 μ M

S2.2 Method for AIE fluorescence in presence of five proteins

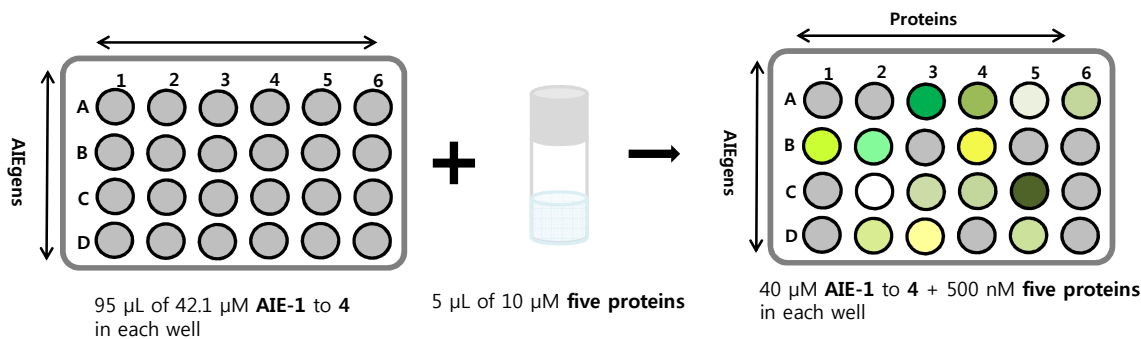


Figure S3. Schematic illustration of fluorescence experiments with four AIEgens and five proteins

All four AIEgens solution in water were prepared 42.1 uM stock solution using 3rd distilled water. Also, five proteins were prepared 10 uM in 1X PBS pH 7.4. In individual 96 wells, 95 uL of 42.1 uM AIE stock solution was added followed by addition 5 uL of 10 uM protein solutions. We measured fluorescence using microplate reader right after adding proteins.

	BSA	Esterase	transferrin	fibrinogen	β -galactosidase
AIE-1	1.239416	1.056613	1.059014	0.818529	1.096484
	1.216347	1.04249	1.092795	0.864261	1.073643
	1.262054	1.037588	1.098455	0.873736	1.105933
	1.233958	1.047265	1.082385	0.910473	1.218116
	1.179534	1.018032	1.097318	0.929701	1.108485
	1.33237	1.10467	1.112301	0.913101	1.11177
AIE-2	0.95884	1.934575	1.039262	1.031855	1.290872
	0.962982	1.896937	1.060973	1.024958	1.28009
	0.936983	1.981135	1.10684	1.022423	1.271752

	0.951488	1.933937	1.076116	1.006586	1.263268
	0.962325	1.887742	1.051304	0.99564	1.297167
	0.926256	1.823174	1.069183	0.956779	1.239423
AIE-3	1.100433	2.893602	1.246832	1.407829	1.900978
	1.013874	2.760429	1.244907	1.300993	1.946048
	1.192766	2.843424	1.340327	1.327254	1.987403
	1.147104	2.941761	1.353363	1.287405	1.953862
	1.116288	2.985459	1.274084	1.254207	2.022221
	1.211556	2.812856	1.125703	1.235092	2.00545
AIE-4	1.911631	1.699986	1.336614	1.206542	1.382391
	1.952883	1.641435	1.299267	1.167729	1.344156
	1.89577	1.62924	1.268687	1.190794	1.348033
	1.96701	1.595404	1.276893	1.127111	1.343663
	1.802932	1.585535	1.279092	1.082842	1.339222
	1.945707	1.595178	1.243127	1.059213	1.278711

Table S1 Raw data of normalized fluorescence intensity (F/F_0) of **AIE-1** to **AIE-4** (40 μM) with 5 μM of five proteins.

S3. Determination of the binding stoichiometry and binding constant

Stoichiometry of the AIEgen-protein was determined by saturation point of fluorescence intensity with increasing AIEgens' concentrations at fixed concentration of proteins. The fluorescence intensity was measured when protein concentration was fixed at 5 μM by increasing AIE-1 and AIE-2 from 0 to 64 μM . Normalized fluorescence intensity was calculated by deducting fluorescence intensity of only AIE-1 and AIE-2 solution from 0 to 64

μM from proteins with AIE-1 and AIE-2.

Furthermore, binding constant was calculated from the emission intensity-titration plot of $1/(F-F_0)$ as a function of $1/[\text{protein}]$ in micromoles. F and F_0 are the fluorescence intensities of AIEgens with or without proteins. Four AIEgens solutions with $100 \mu\text{M}$ solution in water and proteins solution (50 and $200 \mu\text{M}$) in PBS were prepared for fluorescence titration studies. The concentration of five proteins was varied from 0 to $20 \mu\text{M}$. The binding constant for the AIEgens and proteins was evaluated using a Benesi-Hildebrand plot.

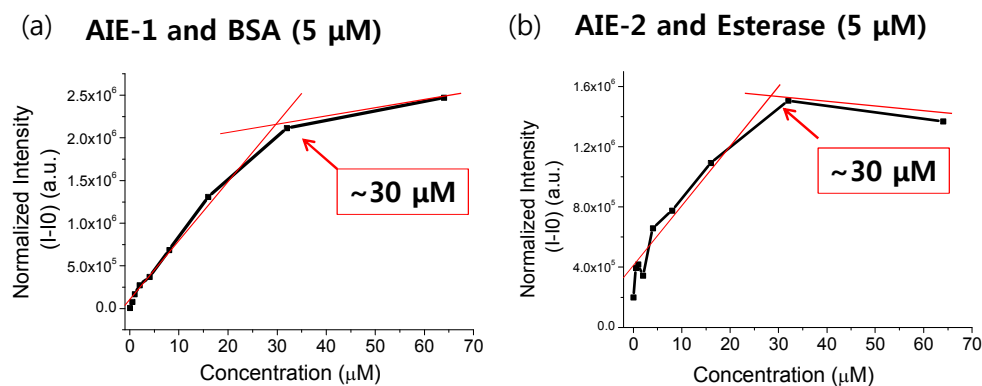


Figure S4. Normalized fluorescence intensity of (a) **AIE-1** and BSA (b) **AIE-2** and esterase [proteins] = $5 \mu\text{M}$ [AIE-1] and [AIE-4] = 0 to $64 \mu\text{M}$.

	BSA	Esterase	Transferrin	Fibrinogen	β -galactosidase
AIE-1	$2.524 \times 10^5 \text{ M}^{-1}$	$5.003 \times 10^4 \text{ M}^{-1}$	$3.009 \times 10^3 \text{ M}^{-1}$	$6.627 \times 10^4 \text{ M}^{-1}$	$9.454 \times 10^4 \text{ M}^{-1}$
AIE-2	$3.615 \times 10^6 \text{ M}^{-1}$	$7.292 \times 10^5 \text{ M}^{-1}$	-	-	$9.690 \times 10^5 \text{ M}^{-1}$
AIE-3	$1.129 \times 10^6 \text{ M}^{-1}$	$9.738 \times 10^5 \text{ M}^{-1}$	-	-	-
AIE-4	$3.125 \times 10^5 \text{ M}^{-1}$	$2.001 \times 10^6 \text{ M}^{-1}$	$2.539 \times 10^6 \text{ M}^{-1}$	$2.771 \times 10^6 \text{ M}^{-1}$	$2.079 \times 10^6 \text{ M}^{-1}$

Table S2. Binding constant between four AIEgens and five proteins at $25 \text{ }^\circ\text{C}$

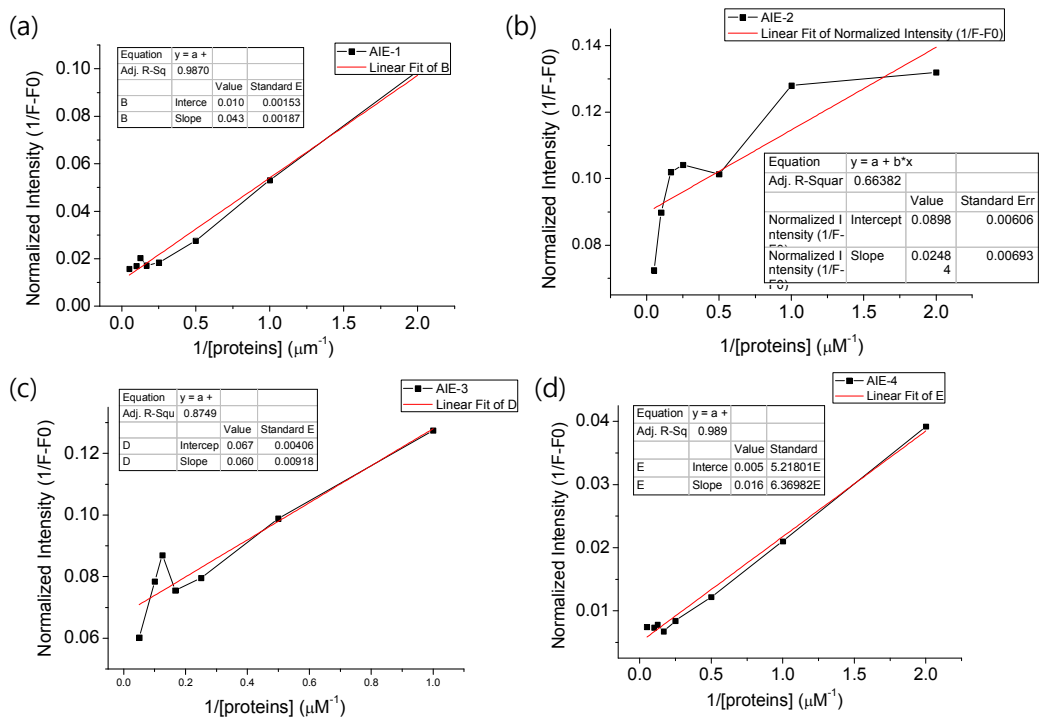


Figure S5. Benesi-Hildebrand plot (emission at 520 nm) of (a) **AIE-1**, (b) **AIE-2**, (c) **AIE-3**, and (d) **AIE-4** by plotting $1/(F-F_0)$ as a function of $1/[BSA]$ [**AIEgens**] = 40 μM .

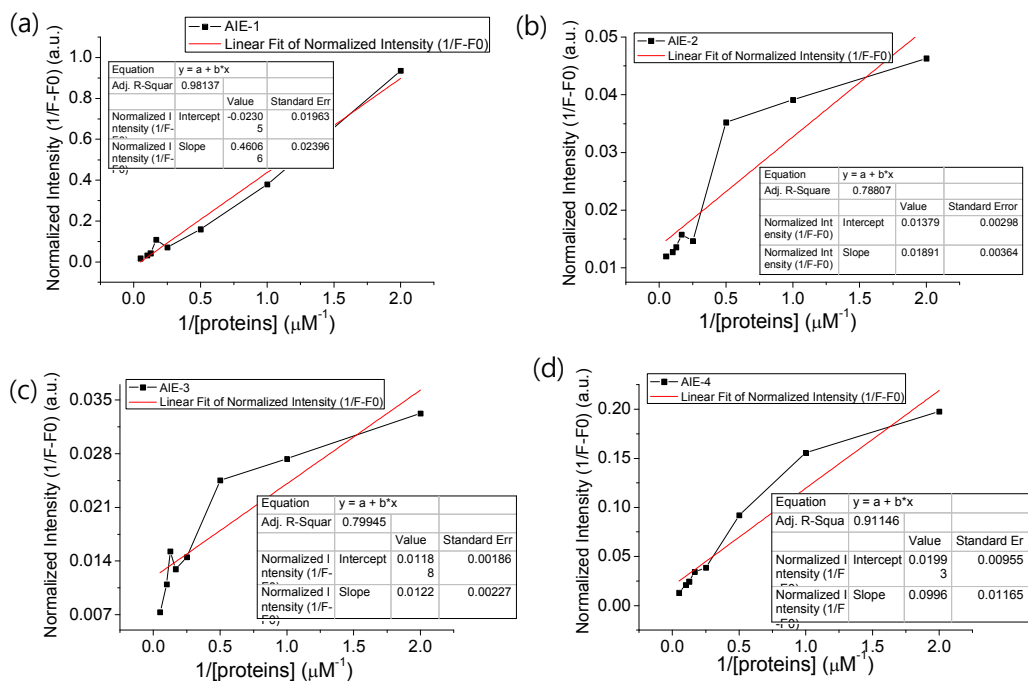


Figure S6. Benesi-Hildebrand plot (emission at 520 nm) of (a) **AIE-1**, (b) **AIE-2**, (c) **AIE-3**, and (d) **AIE-4** by plotting $1/(F-F_0)$ as a function of $1/[Esterase]$ [**AIEgens**] = 40 μM

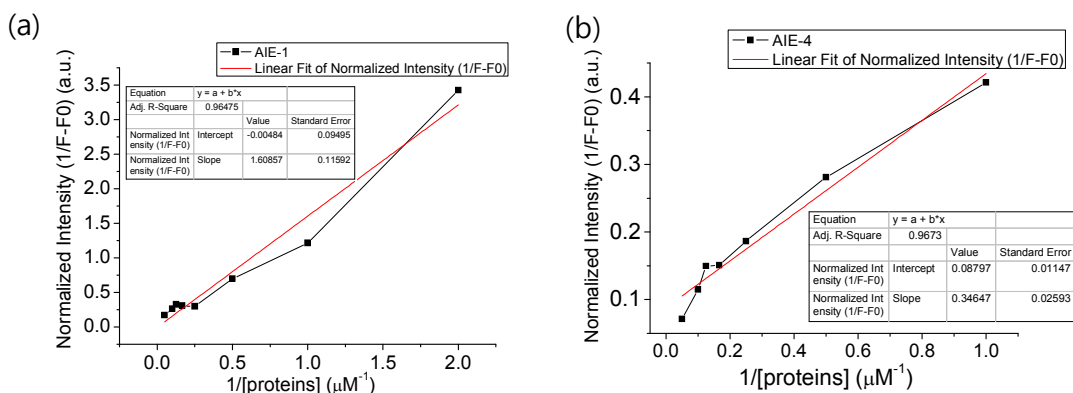


Figure S7. Benesi-Hildebrand plot (emission at 520 nm) of (a) AIE-1 and (b) AIE-4 by plotting $1/(F-F_0)$ as a function of $1/[\text{transferrin}]$ [AIE-1 and AIE-4] = 40 μM .

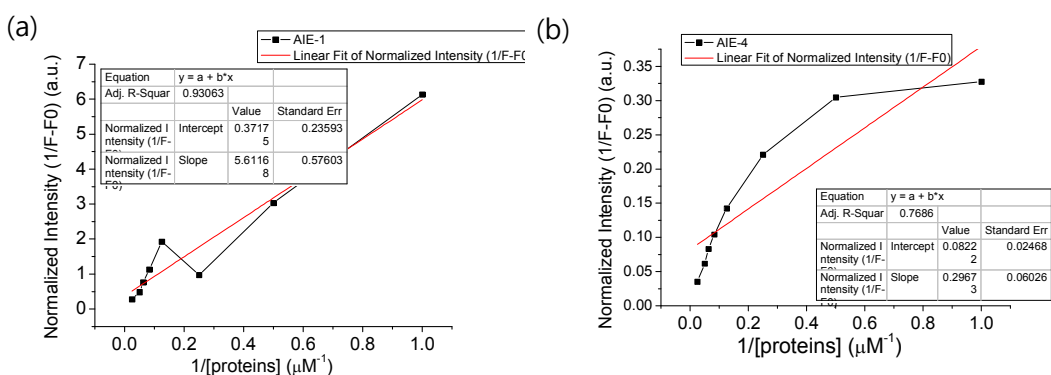


Figure S8. Benesi-Hildebrand plot (emission at 520 nm) of (a) AIE-1 and (b) AIE-4 by plotting $1/(F-F_0)$ as a function of $1/[\text{Fibrinogen}]$ [AIE-1 and AIE-4] = 40 μM .

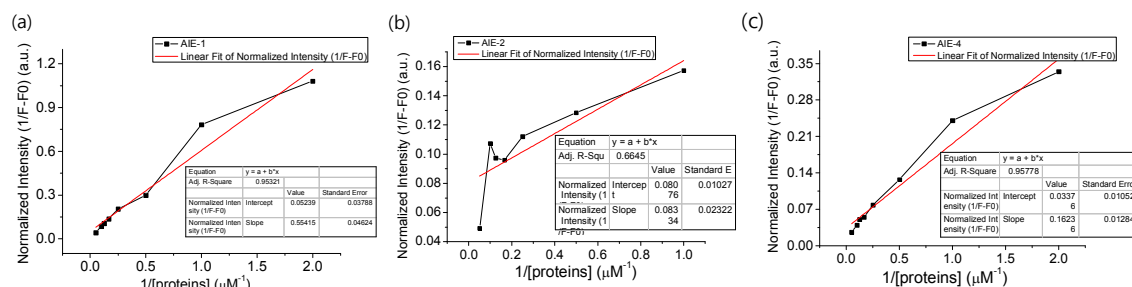


Figure S9. Benesi-Hildebrand plot (emission at 520 nm) of (a) AIE-1, (b) AIE-2, (c) AIE-4 by plotting $1/(F-F_0)$ as a function of $1/[\beta\text{-galactosidase}]$ [AIE-1, AIE-2, and AIE-4] = 40 μM .

S4. Classification proteins in the protein mixtures

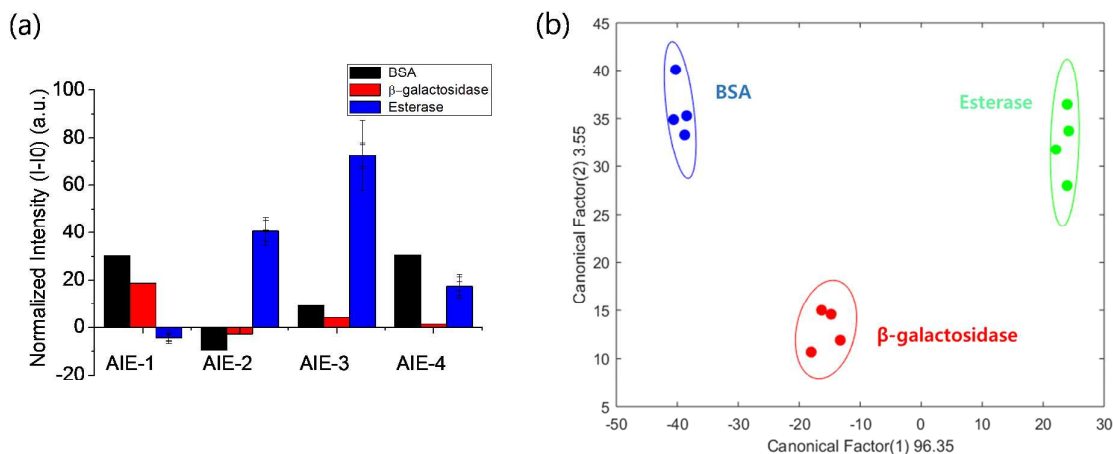


Figure S10 Array-based sensing of proteins (1 μ M) in 4% FBS solution using three AIEgens (40 μ M) at room temperature. (a) Fluorescence pattern of the synthesized AIEgens (AIE-1–4) in the presence of the proteins (BSA, esterase, and β -galactosidase) for subsequent fluorescence experiments. Each value is an average of four parallel measurements. (b) LDA analysis using 2D with 95% ellipse confidence

S5. Reference

- (1) Ali, A., Kamra, M., Roy, S., Muniyappa, K., Bhattacharya, S. Novel Oligopyrrole Carboxamide based Nickel(II) and Palladium(II) Salens, Their Targeting of Human G-quadruplex DNA, and Selective Cancer Cell Toxicity. *Chem. Asian. J.* **2016**, *11*, 2542-2254.