Supporting Information

Highly Selective Fluorogenic Multianalyte Biosensors Constructed via Enzyme-Catalyzed Coupling and Aggregation-Induced Emission

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Experimental Section

Materials. *N*,*N*'-Dicyclohexylcarbodiimide (DCC), *O*-*tert*-butyl-*L*-tyrosine, *di*-*tert*-butyl dicarbonate, 2-propynylamine 4-dimethylaminopyridine (DMAP) and *N*-hydroxysuccinimide (NHS) were purchased from Sinopharm Chemical Reagent Co., Ltd. Copper (I) bromide (CuBr), *N*,*N*,*N*',*N*'',*N*''-pentamethyldiethylenetriamine (PMDETA), and glucose oxidase (GOx) were purchased from Aldrich. Horseradish peroxidase (HRP) and Human carcinoembryonic antigen (CEA) ELISA Kit (R&D) were purchased from Shanghai Yuanye Biology Technology Co., Ltd. All other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. and used as received without further purification, unless otherwise noted. *Azido*-functionalized tetraphenylethylene, TPE-(N_3)$_4$, was synthesized according to literature procedures.[1,2]

Sample Synthesis. Synthetic schemes employed for the preparation of (*N*-*Boc*-*O*-*tert*-butyl)-*L*-Tyr, *alkynyl*-functionalized (*N*-*Boc*-*O*-*tert*-butyl) *L*-Tyr (**1**), TPE-((*N*-*Boc*-*O*-*tert*-butyl) *L*-Tyr)$_4$ precursor (**2**), and TPE-Tyr (**3**) are shown in Scheme S1.

Synthesis of (N-Boc-O-tert-butyl)-L-Tyr. O-*tert*-butyl-*L*-tyrosine (2.00 g, 8.42 mmol, 1.0 equiv.) and *di*-*tert*-butyl dicarbonate (2.20 g, 10.10 mmol, 1.2 equiv.) were dissolved in a mixture of THF (15 mL) and NaOH (1 N, 30 mL) and stirred at room temperature for 10 h. THF was removed under reduced pressure, and the pH of residual solution was adjusted to 3.0 with dilute hydrochloric acid and the crude product was extracted by ethyl acetate. The combined organic phases was washed with saturated brine (pH 3.0) and dried over anhydrous Na_2SO_4. The organic solvent was removed under reduced pressure and dried in a vacuum oven at room temperature overnight, affording (*N*-*Boc*-*O*-*tert*-butyl)-*L*-Tyr as a yellowish solid (2.64 g, 93%). ^{1}H NMR ($CDCl_3$, δ, ppm, TMS, Figure S1): 7.08 (d, J = 8.4 Hz, 2H), 6.92 (d, J = 8.4 Hz, 2H), 4.95 (s, 1H), 4.58 (s, 1H), 3.07 (dd, J = 16.6, 42.7 Hz, 2H), 1.37 (d, J = 23.5 Hz, 18H).

Synthesis of Alkynyl-Functionalized (N-Boc-O-tert-butyl)-L-Tyr (1). (*N*-*Boc*-*O*-*tert*-butyl)-*L*-Tyr (1.50 g, 4.45 mmol, 1.0 equiv.), DCC (1.10 g, 5.34 mmol,

1.2 equiv.), and DMAP (54 mg, 0.44 mmol, 0.1 equiv.) were dissolved in anhydrous THF (40 mL) and stirred at room temperature. After 20 min, NHS (0.61 g, 5.34 mmol, 1.2 equiv.) was added into the reaction mixture and the reaction was monitored by TLC. After the reaction was completed, 2-propynylamine (0.30 g, 5.34 mmol, 1.2 equiv.) was added and stirred at room temperature overnight. The reaction mixture was filtered and the filtrate was dried under reduced pressure. The crude product was redissolved in ethyl acetate and filtered again. The filtrate was washed with saturated brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and dried in a vacuum oven at room temperature overnight, affording *alkynyl*-functionalized (*N*-*Boc*-*O*-*tert*-butyl)-*L*-Tyr (**1**) as a yellowish viscous liquid (1.45 g, 87%). ^{1}H NMR ($CDCl_3$, *δ*, ppm, TMS, Figure S2a): 7.08 (d, *J* = 11.2 Hz, 2H), 6.91 (d, *J* = 11.1 Hz, 2H), 5.99 (s, 1H), 4.97 (s, 1H), 4.26 (dd, *J* = 9.2, 18.7 Hz, 1H), 3.96 (t, *J* = 7.7 Hz, 2H), 3.09 (d, *J* = 72.8 Hz, 2H), 2.55 (s, 1H), 1.36 (d, *J* = 33.6 Hz, 18H). ^{13}C NMR ($CDCl_3$, *δ*, ppm, TMS, Figure S3): 171.16, 155.43, 154.40, 131.13, 129.91, 124.24, 79.07, 78.24, 78.04, 71.67, 55.75, 37.97, 34.92, 28.97, 28.27.

*Synthesis of TPE-((N-Boc-O-tert-butyl)-L-Tyr)$_4$ (**2**).* Typical CuAAC click reaction procedures employed for the synthesis of TPE-((*N*-*Boc*-*O*-*tert*-butyl)-*L*-Tyr)$_4$ (**2**) are as follows. TPE-(N_3)$_4$ (0.10 g, 0.59 mmol azide moieties, 1.0 equiv.), *alkynyl*-functionalized (*N*-*Boc*-*O*-*tert*-butyl)-*L*-Tyr (1) (0.33 g, 0.89 mmol *alkynyl* moieties, 1.5 equiv.), PMDETA (26 mg, 0.15 mmol, 0.25 equiv.), and DMF (5 mL) were charged into a glass ampoule equipped with a magnetic stirring bar. The glass ampoule was degassed by three freeze-pump-thaw cycles, and CuBr (22 mg, 0.15 mmol, 0.25 equiv.) was then introduced under the protection of nitrogen before freezing and sealing under vacuum. After thermostatting at 35 °C in an oil bath and stirring for 24 h, the reaction was terminated by quenching into liquid nitrogen, exposed to air, and diluted with CH_2Cl_2. The mixture was washed with saturated brine, dried over anhydrous $MgSO_4$. The organic phase was then concentrated under reduced pressure and precipitated into an excess of *n*-hexane. The above dissolution-precipitation cycle was repeated three times, and the product was dried in a vacuum oven at room temperature to afford a white powder (0.24 g, yield: 76%). ^{1}H

NMR ($CDCl_3$, *δ*, ppm, TMS, Figure S2c): 7.50 (s, 4H), 7.02 (d, *J* = 11.1 Hz, 8H), 6.84 (dd, *J* = 3.2, 11.5 Hz, 16H), 6.63 (s, 4H), 6.56 (d, *J* = 11.5 Hz, 8H), 5.08 (d, *J* = 10.9 Hz, 4H), 4.64 (t, *J* = 6.5 Hz, 8H), 4.43 (d, *J* = 7.4 Hz, 8H), 4.26 (s, 4H), 4.24 (t, *J* = 6.4 Hz, 8H), 2.97 (m, 8H), 1.32 (m, 72H).

*Synthesis of TPE-Tyr (**3**).* TPE-((*N*-*Boc*-*O*-*tert*-butyl) *L*-Tyr)$_4$ (**2**) (100 mg) was dissolved in methanol (15 mL) and stirred at room temperature under the protection of nitrogen. Then, 1.5 mL concentrated hydrochloric acid was added. After 24 h, the crude product was concentrated under reduced pressure and then precipitated into an excess of acetone. The above dissolution-precipitation cycle was repeated three times, and the product was dried in a vacuum oven at room temperature to afford a white powder (58 mg, yield: 81%). ^{1}H NMR (D_2O, *δ*, ppm, TMS, Figure S2d): 7.57 (s, 4H), 6.70 (d, *J* = 8.4 Hz, 8H), 6.48 (dd, *J* = 8.5, 12.4 Hz, 16H), 6.27 (d, *J* = 8.3 Hz, 8H), 4.62 (d, *J* = 6.2 Hz, 8H), 4.46 (d, *J* = 15.1 Hz, 4H), 4.00 (dd, *J* = 12.2, 19.9 Hz, 16H), 2.96 (dd, *J* = 5.8, 13.6 Hz, 4H), 2.73 (dd, *J* = 9.6, 13.6 Hz, 4H). RP-HPLC analysis: elution peak at 3.2 min (EtOH/H_2O = 80/20) and ESI-MS (*m/z*): calcd for $[M+H]^+$, 1545.7; found, 1545.8 (Figure S4).

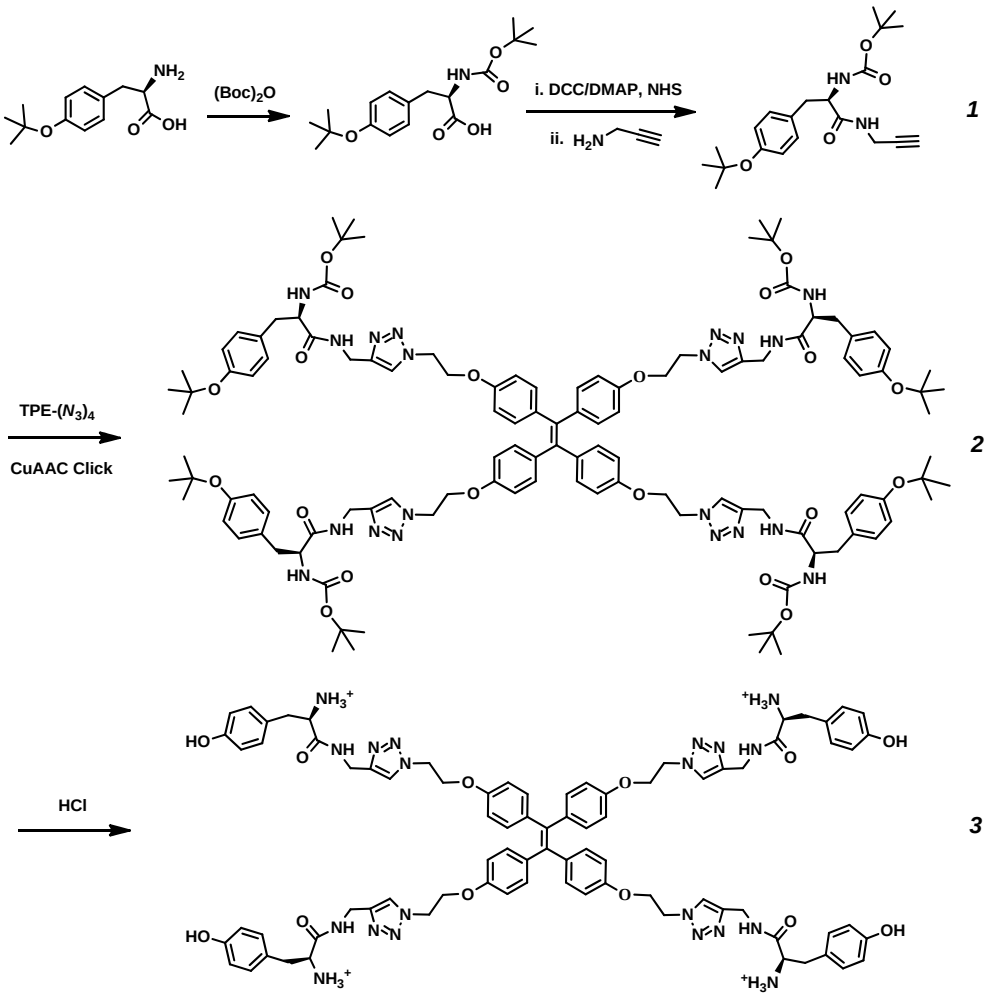
Characterization. ^{1}H and ^{13}C NMR spectra were acquired on a 300MHz Bruker instrument. $CDCl_3$ and D_2O were used as the solvent. ESI-MS experiment was conducted on Thermo Scientific LTQ Orbitrap Mass Spectrometer equipped with an electrospray interface. HPLC analysis was performed with a Shimadzu HPLC system, equipped with a LC-20AP binary pump, a SPD-20A UV-Vis detector, and a Symmetry C18 column. Potentiometric titration was conducted using a Mettler Toledo FE-20 pH meter. Fluorescence spectra were recorded on F-4600 (Hitachi) spectrofluorometer. The slit widths were both set at 5 nm for excitation and emission, and the excitation wavelength was fixed at 360 nm. UV-Vis absorption spectra were acquired on a TU-1910 double-beam UV-Vis spectrophotometer (Puxi. General Instrumental Company, China). Unless otherwise noted, all the samples for fluorescence and absorption measurements were incubated for 5 min at room temperature before the data was acquired. Transmission electron microscopy (TEM) observations were conducted on a JEOL 2010 electron microscope at an acceleration voltage of 200 kV,

and the sample for TEM observations was prepared by placing 20 μL colloidal dispersion on copper grids successively coated with thin films of formvar and carbon. Dynamic light scattering (DLS) experiments were conducted on a Malvern Zeta-sizer Nano ZS Instrument. Confocal laser scanning microscopy (CLSM) images were acquired using a Leica TCS SP5 microscope. The enzyme-linked immunosorbent assay (ELISA) was measured by an enzyme immunoassay microplate reader (Thermo Scientific 3001).

References

[1] Y. N. Hong, M. Haussler, J. W. Y. Lam, Z. Li, K. K. Sin, Y. Q. Dong, H. Tong, J. Z. Liu, A. J. Qin, R. Renneberg, B. Z. Tang, *Chem.-Eur. J.* **2008**, *14*, 6428.

[2] T. Sanji, M. Nakamura, M. Tanaka, *Tetrahedron Lett.* **2011**, *52*, 3283.



Scheme S1. Synthetic routes employed for the preparation of *L*-tyrosine functionalized TPE, TPE-Tyr (**3**).

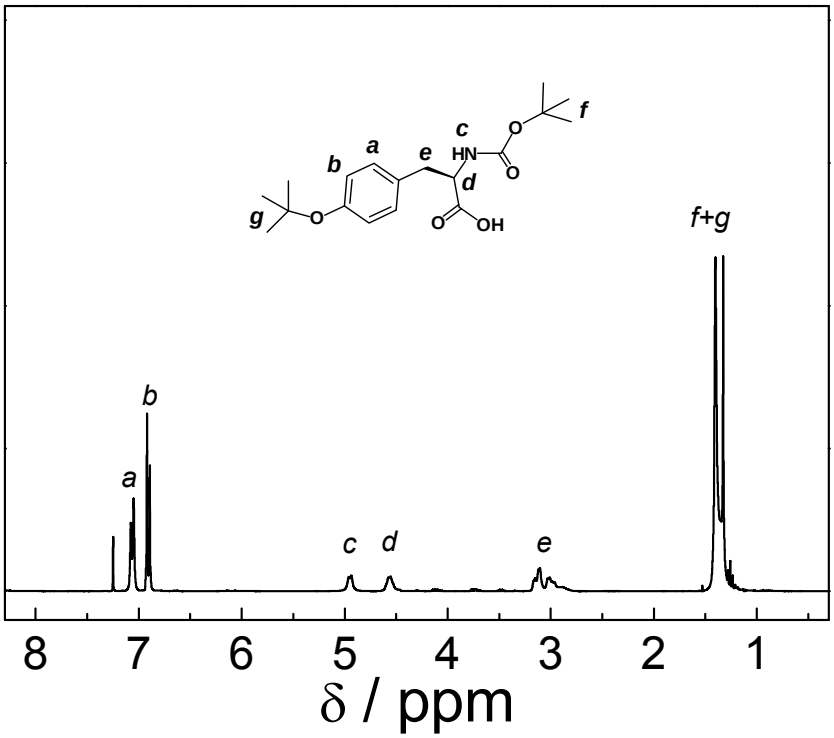


Figure S1. ^{1}H NMR spectrum recorded for *N*-*Boc*-*O*-*tert*-butyl-*L*-tyrosine in $CDCl_3$.

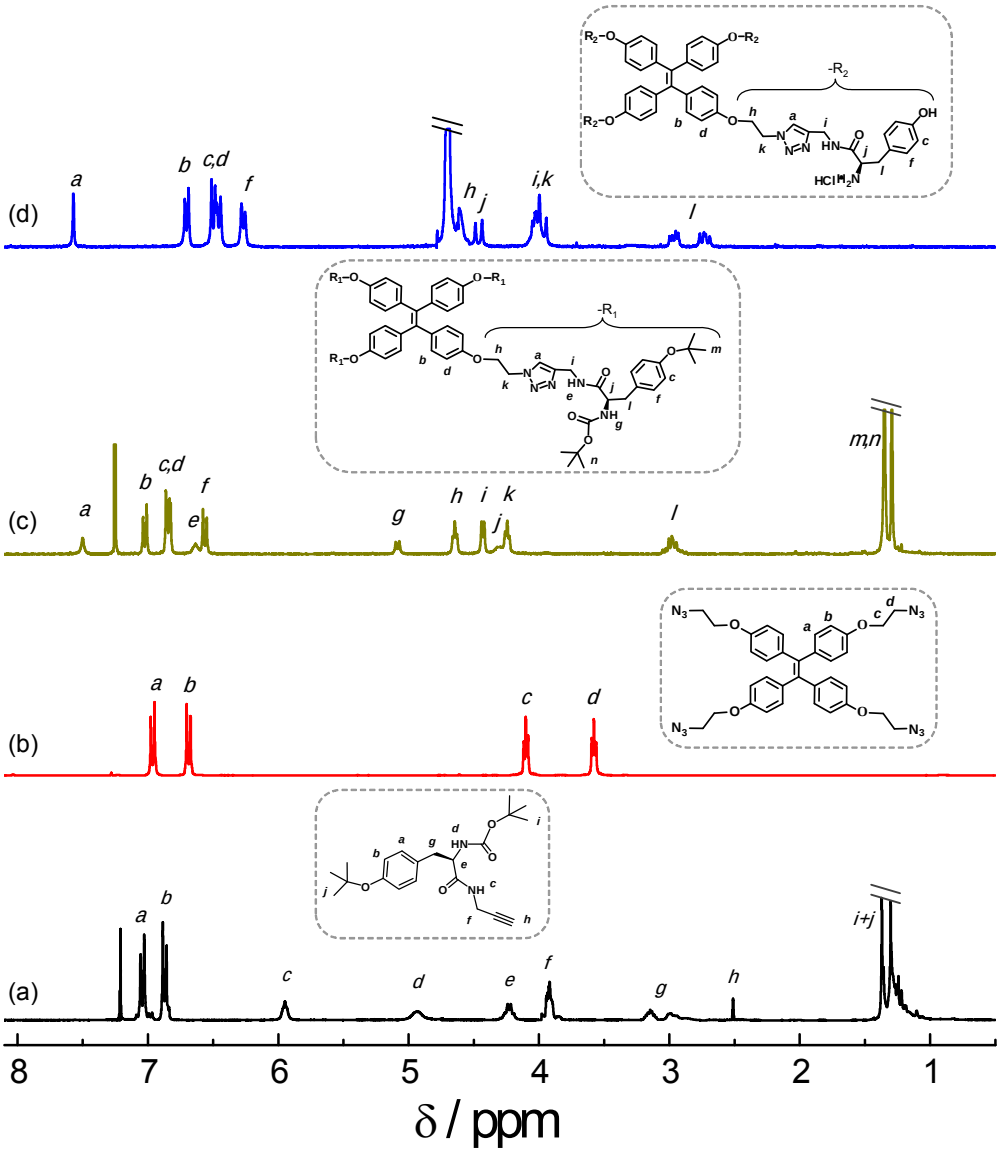


Figure S2. ^{1}H NMR spectra recorded for (a) *alkynyl*-functionalized *N-Boc-O-tert*-butyl-*L*-tyrosine (**1**), (b) TPE-(N_3)$_4$, (c) TPE-(*N-Boc-O-tert*-butyl-*L*-Tyr)$_4$ (**2**) in $CDCl_3$, and (d) TPE-Tyr (**3**) in D_2O, respectively.

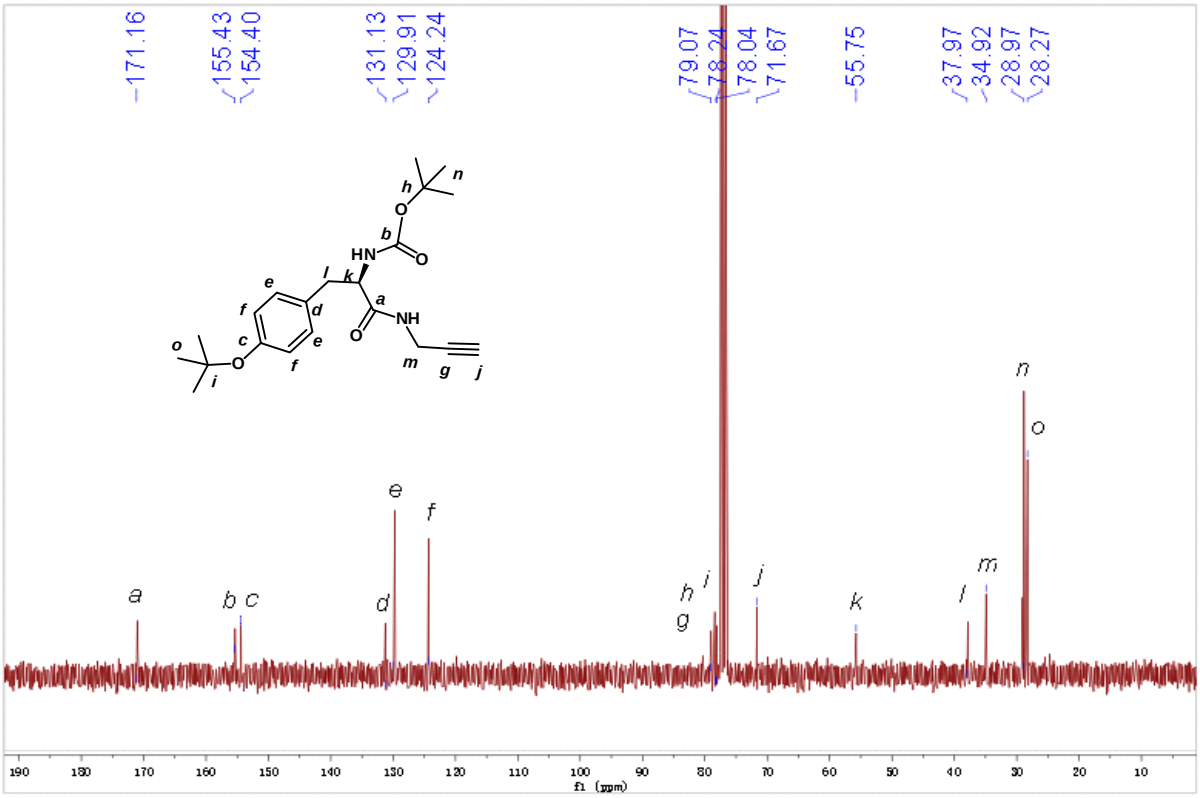


Figure S3. ^{13}C NMR spectrum recorded for *alkynyl*-functionalized *N-Boc-O-tert*-butyl-*L*-tyrosine (**1**) in $CDCl_3$.

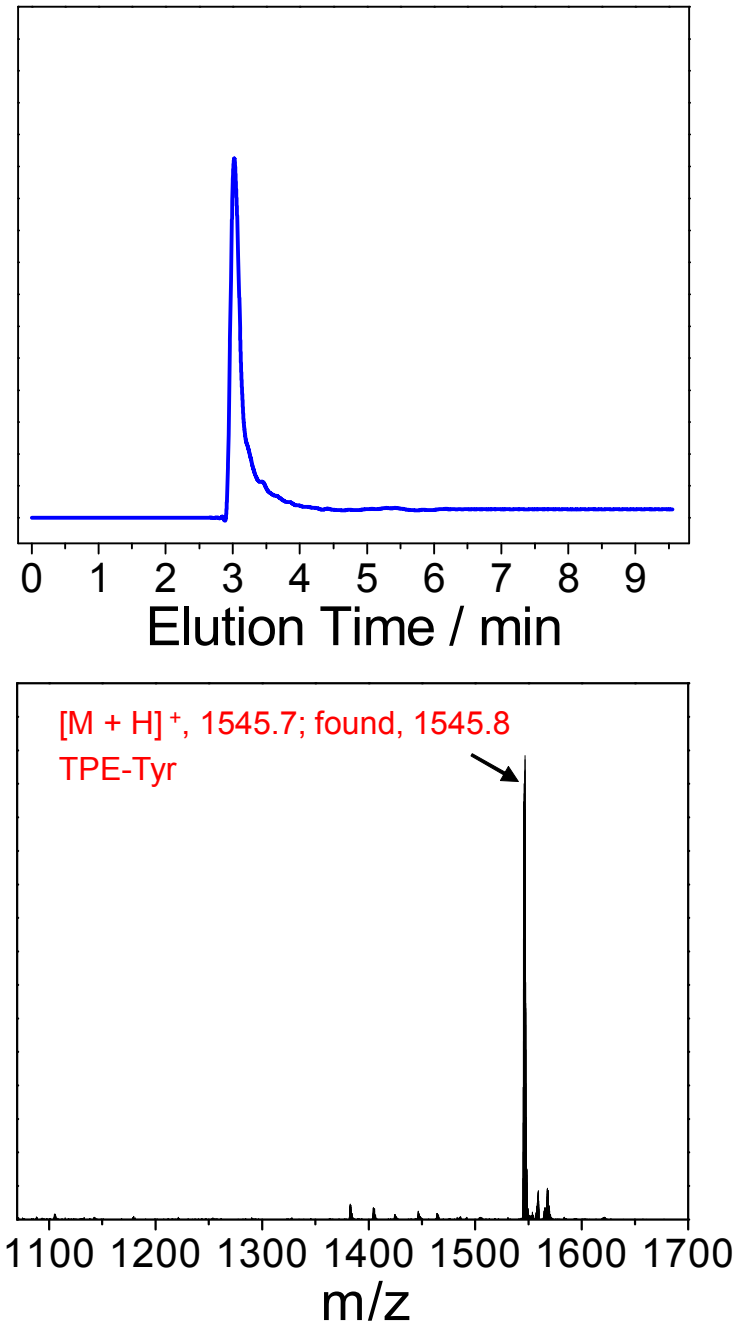


Figure S4. (a) HPLC trace of TPE-Tyr (**3**), the mobile phase was 80/20 ethanol and water at a flow rate of 1.0 mL/min. (b) ESI mass spectrum recorded for TPE-Tyr (**3**).

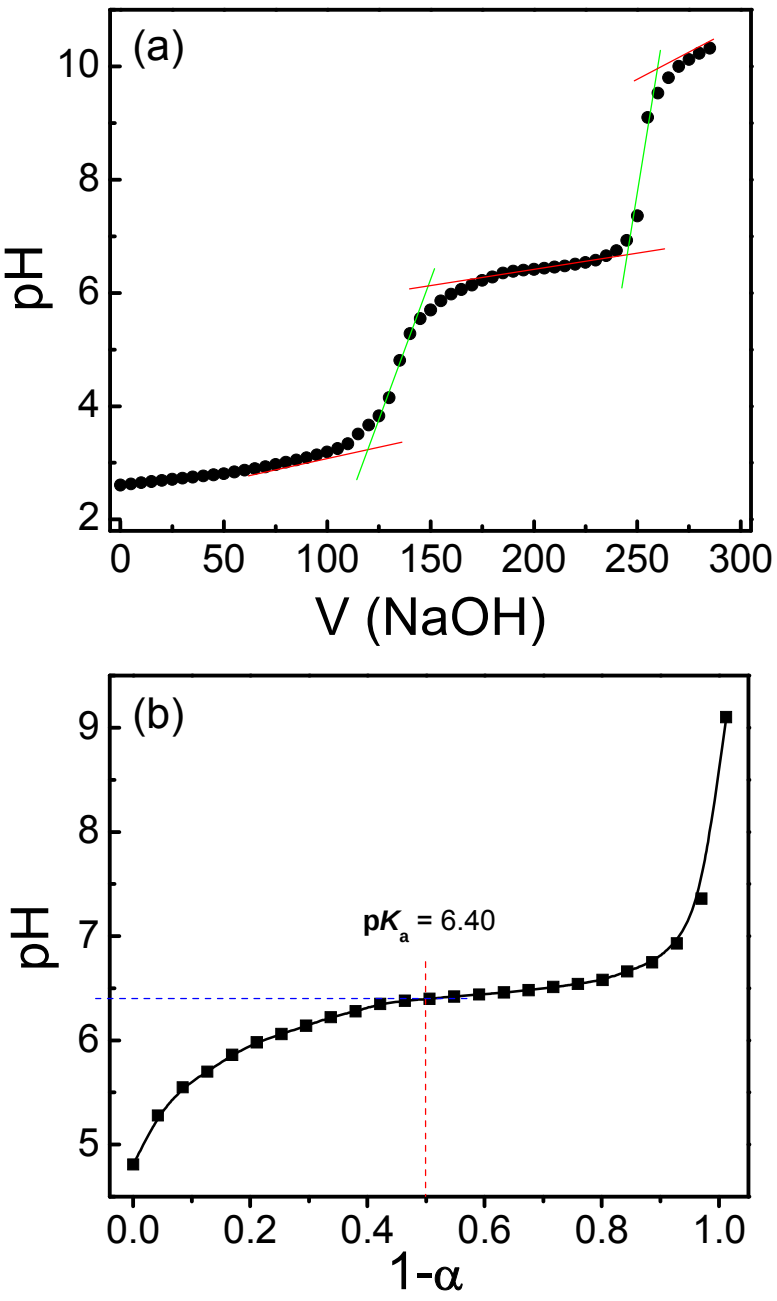


Figure S5. Potentiometric titration curve recorded for TPE-Tyr at 25 °C, where α is the mean degree of protonation of amine moieties of TPE-Tyr.

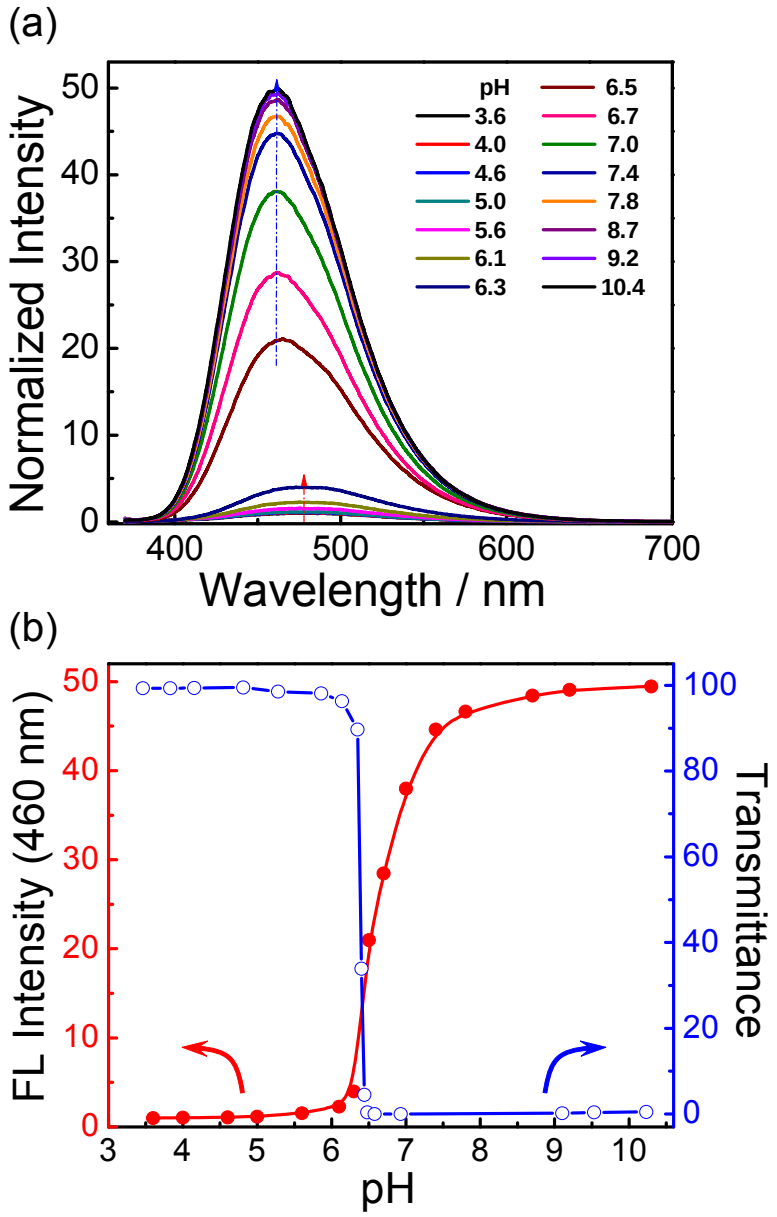


Figure S6. (a) pH-dependent fluorescence emission spectra (λ_{ex} = 360 nm) recorded for the TPE-Tyr aqueous solution. (b) pH-dependent changes in normalized emission intensities at 470 nm (red) and optical transmittance at 700 nm (blue). The TPE-Tyr concentrations for fluorescence experiments and optical transmittance experiments were 0.1 mM and 0.4 mM, respectively.

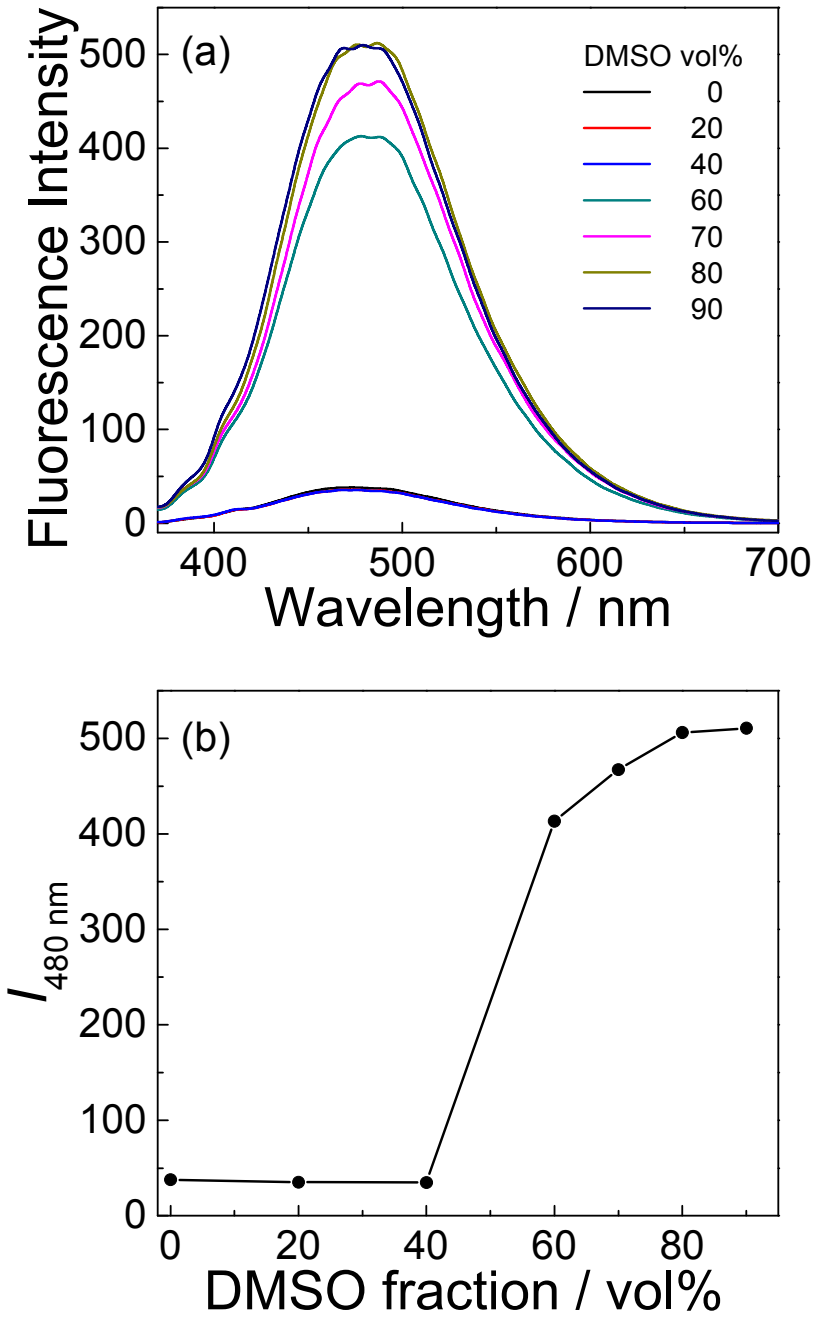


Figure S7. (a) Fluorescence emission spectra (λ_{ex} = 360 nm; slit widths: Ex. 5 nm, Em. 5 nm; 25 °C) and (b) fluorescence intensity changes at λ_{em} = 480 nm recorded for TPE-Tyr (25 μM) in DMSO/PBS buffer (pH 6.0, 10 mM) mixture when the volume fraction of DMSO was varied in the range of 0-90%.

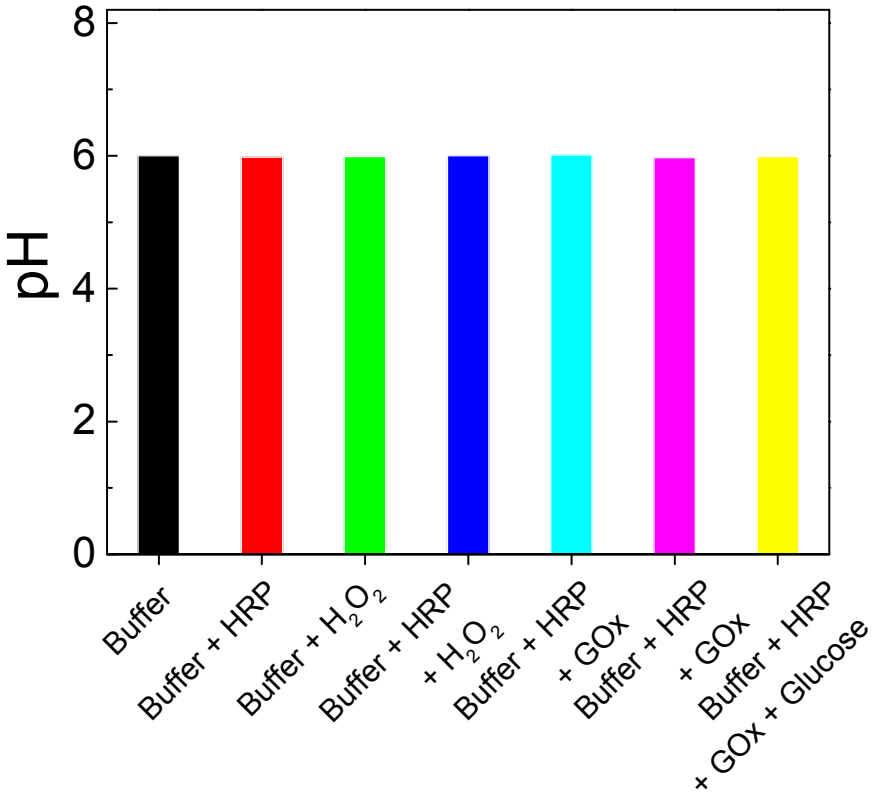


Figure S8. Measured pH values of TPE-Tyr solution in PBS buffer (pH 6.0, 10 mM, 25 ^{o}C) in the absence and presence of HRP, H_2O_2, GOx, glucose or the combination of them (HRP 0.01 g/L, H_2O_2 50 μM, GOx 0.01 g/L, glucose 1.0 mM).

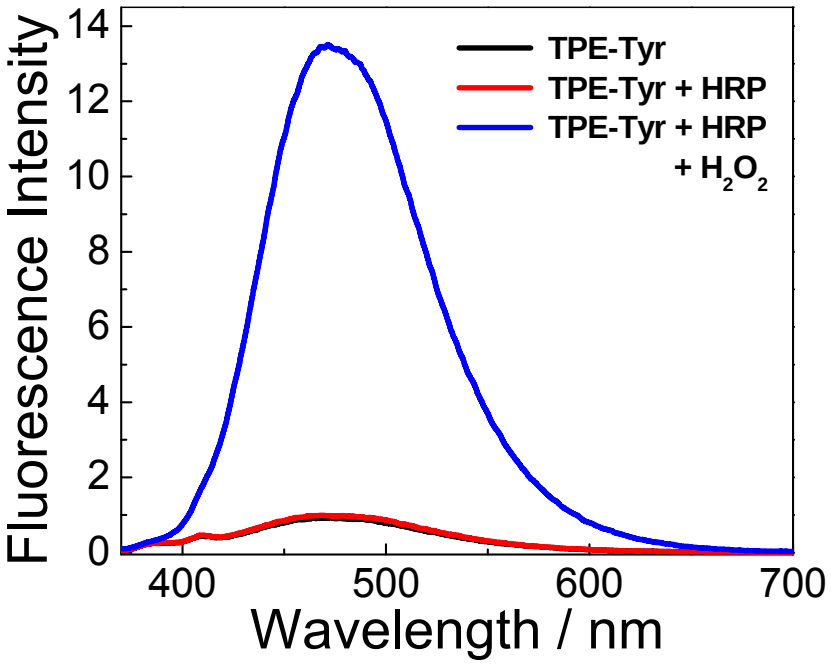


Figure S9. Normalized fluorescence emission spectra of TPE-Tyr only, TPE-Tyr added HRP, and TPE-Tyr added HRP + H_2O_2, respectively. TPE-Tyr 25 μM, HRP 0.01 g/L; PBS buffer (pH 6.0, 10 mM, 25 ^oC; λ_{ex} = 360 nm; [H_2O_2] 50 μM; the reaction time was fixed to be 5 minutes in all cases.

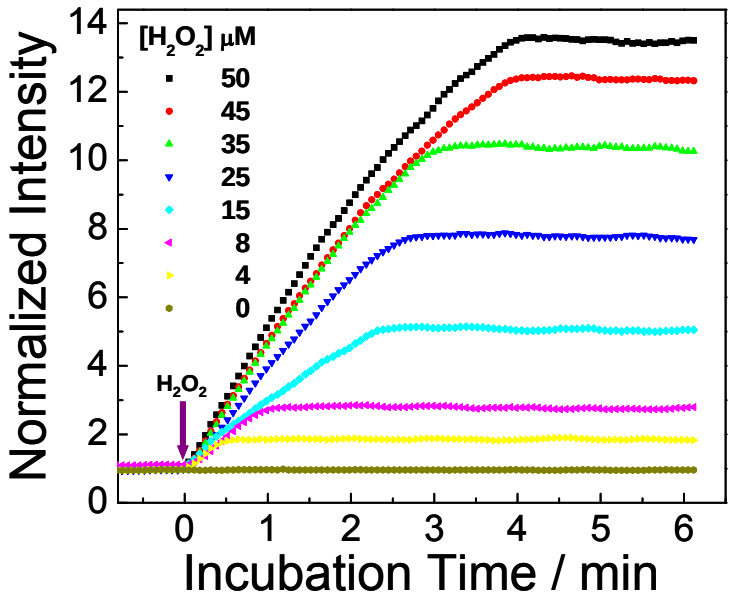


Figure S10. Normalized time-dependent fluorescence intensity changes (λ_{ex} = 360 nm, λ_{em} = 470 nm) recorded for TPE-Tyr/HRP aqueous mixture (10 mM PBS buffer, pH 6.0) upon addition of varying concentrations of H_2O_2 (TPE-Tyr = 25 μM, HRP = 0.01 g/L, 25 ^{o}C).

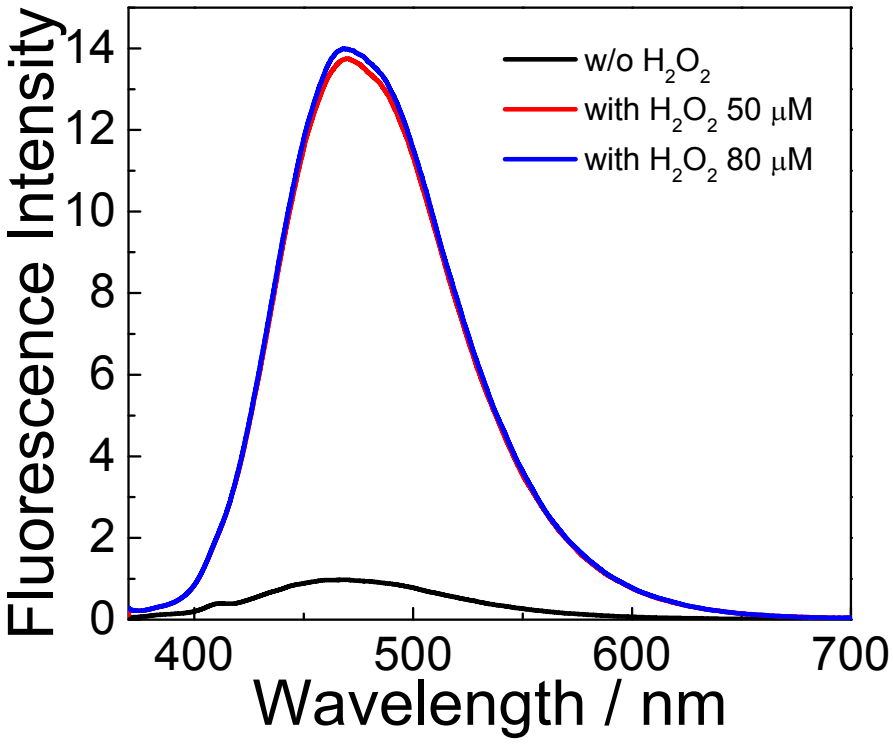


Figure S11. Normalized fluorescence emission spectra of TPE-Tyr only, TPE-Tyr added HRP + H_2O_2 with [H_2O_2] 50 μM and 80 μM, respectively. TPE-Tyr 25 μM, HRP 0.01 g/L; PBS buffer (pH 6.0, 10 mM, 25 ^{o}C; λ_{ex} = 360 nm; the reaction time was fixed to be 5 minutes in all cases.

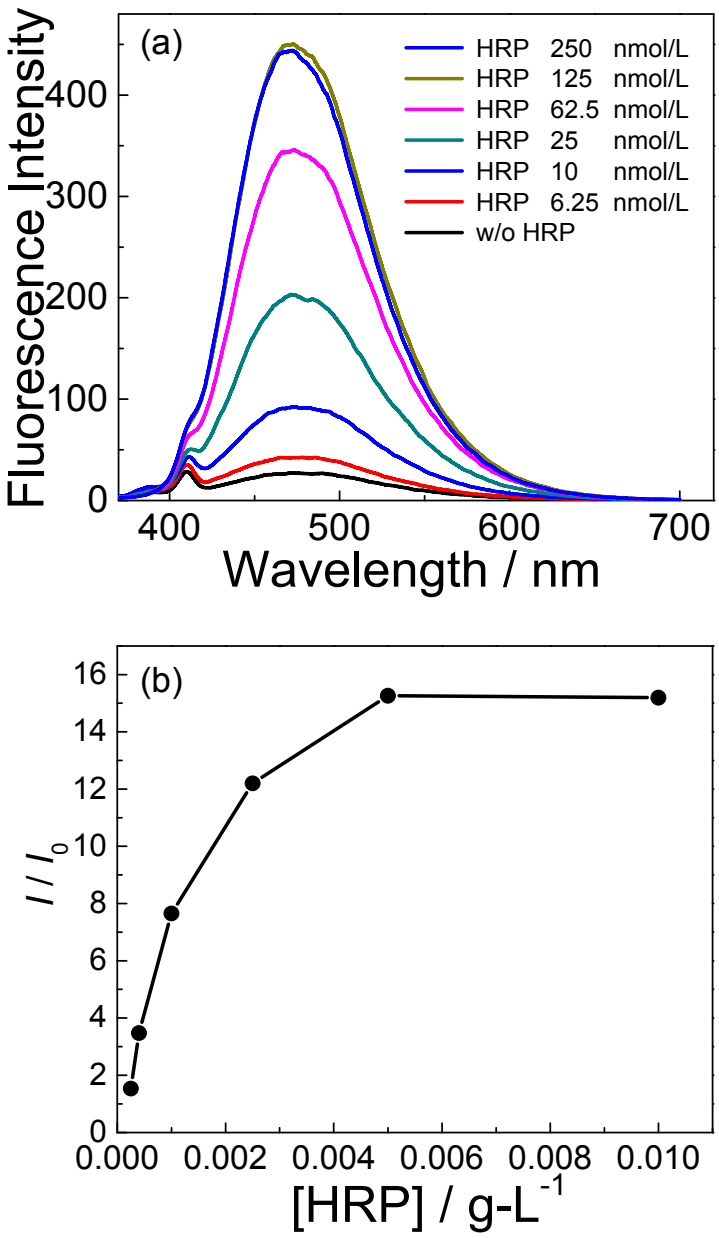


Figure S12. (a) Fluorescence emission spectra recorded for TPE-Tyr aqueous solution in the presence of H_2O_2 with different concentrations of HRP; (b) The HRP concentration dependence fluorescence emission change. TPE-Tyr 25 μM, H_2O_2 50 μM, in PBS buffer (pH 6.0, 10 mM), 25 ^oC; λ_{ex} = 360 nm, λ_{em} = 470 nm; the reaction time was fixed to be 5 minutes in all cases.

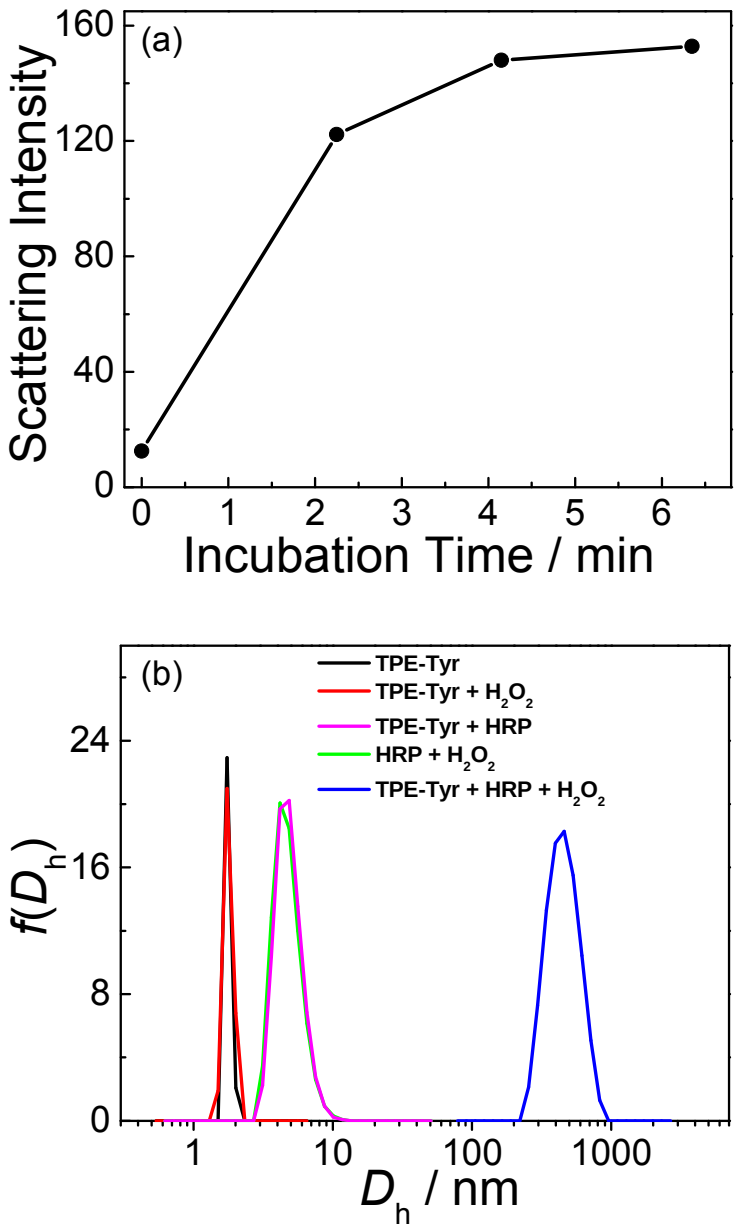


Figure S13. (a) Incubation duration-dependent changes in scattering light intensities of TPE-Tyr aqueous solution (10 mM PBS buffer, pH 6.0) in the presence of 50 μM H_2O_2 (TPE-Tyr = 25 μM, HRP = 0.01 g/L, 25 ^{o}C). (b) Intensity-average hydrodynamic diameter distributions recorded for aqueous solutions (10 mM PBS buffer, pH 6.0) of (black) TPE-Tyr, (red) TPE-Tyr/H_2O_2, (pink) TPE-Tyr/HRP, (green) HRP/H_2O_2, and (blue) TPE-Tyr/HRP/H_2O_2 mixtures upon incubating for 5 min (TPE-Tyr = 25 μM, H_2O_2 = 50 μM, HRP = 0.01 g/L, 25 ^{o}C).

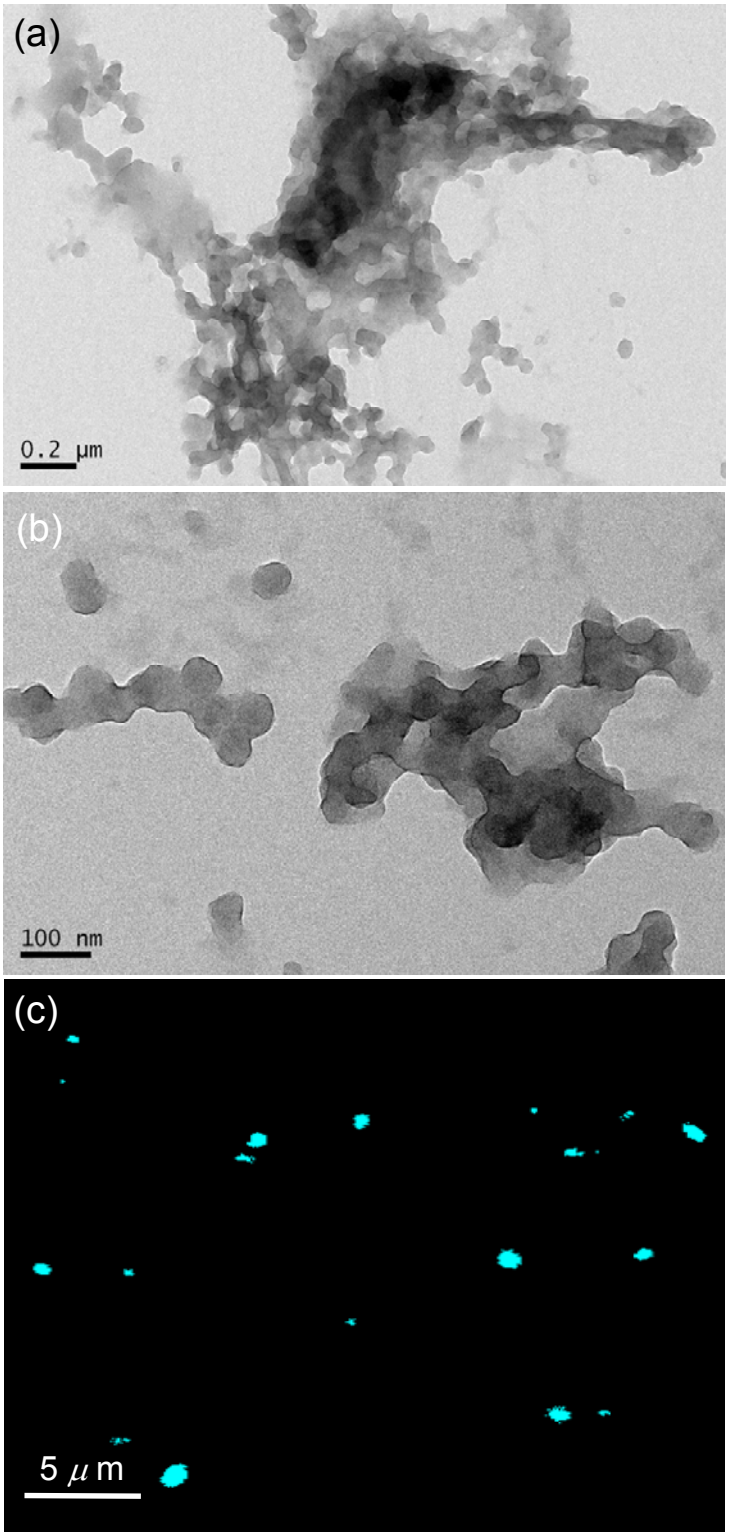


Figure S14. (a and b) Typical TEM images and (c) confocal fluorescence image recorded for the aqueous dispersion (10 mM PBS buffer, pH 6.0) of TPE-Tyr/HRP mixture upon co-incubating with 50 μM H_2O_2 for 5 min (TPE-Tyr = 25 μM, HRP = 0.01 g/L, 25 ^oC).

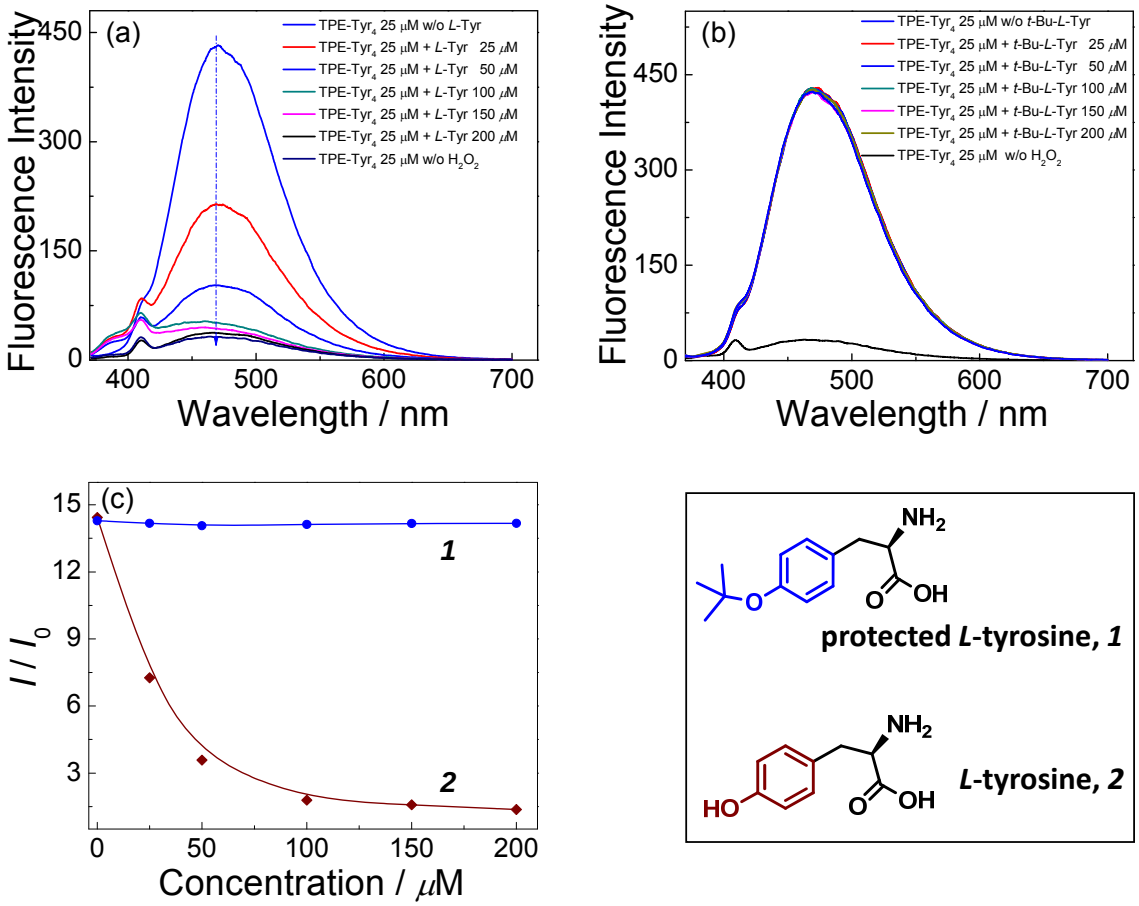


Figure S15. (a and b) Fluorescence emission spectra (λ_{ex} = 360 nm) and (c) corresponding fluorescence intensity changes at 470 nm recorded for TPE-Tyr/H_2O_2 aqueous solution (10 mM PBS buffer, pH 6.0) in the presence of varying concentrations of (a) *L*-tyrosine as a competitive substance and (b) *O*-*tert*-butyl ether protected *L*-tyrosine as a control substance, respectively (TPE-Tyr = 25 μM, H_2O_2 = 50 μM, HRP = 0.01 g/L, 25 ^oC).

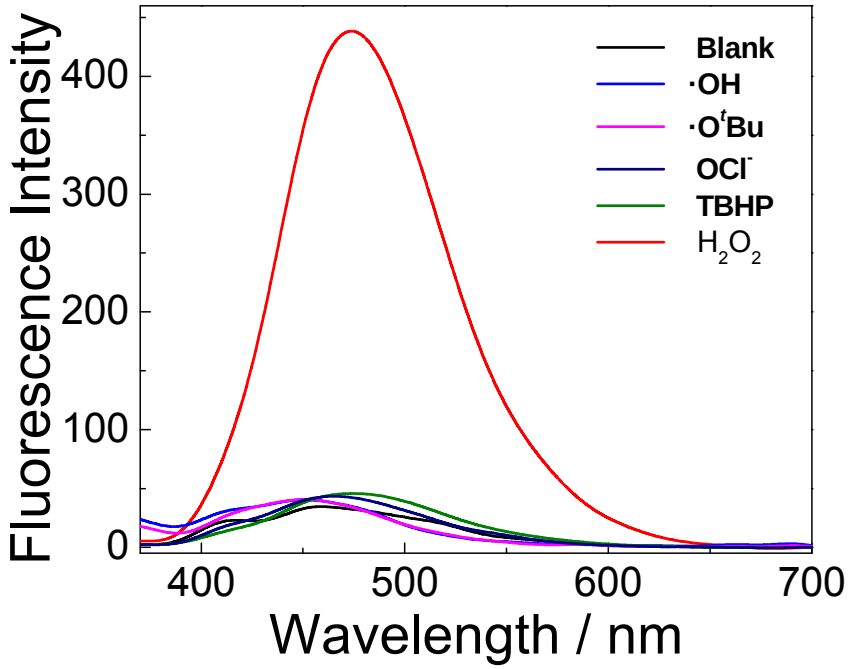


Figure S16. Fluorescence emission spectra (λ_{ex} = 360 nm) of aqueous solution (10 mM PBS buffer, pH 6.0) of TPE-Tyr/HRP in the presence of various ROS species (50 μM): H_2O_2, hydroxyl radical (·OH), *tert*-butoxy radical (·O^{t}Bu), hypochlorite (OCl^-), and *tert*-butyl hydroperoxide (TBHP), respectively (TPE-Tyr = 25 μM, HRP = 0.01 g/L; 25 ^{o}C). Note: ·OH was generated by the reaction of Fe^{2+} with H_2O_2; ·O^{t}Bu was produced by the reaction of Fe^{2+} with TBHP.

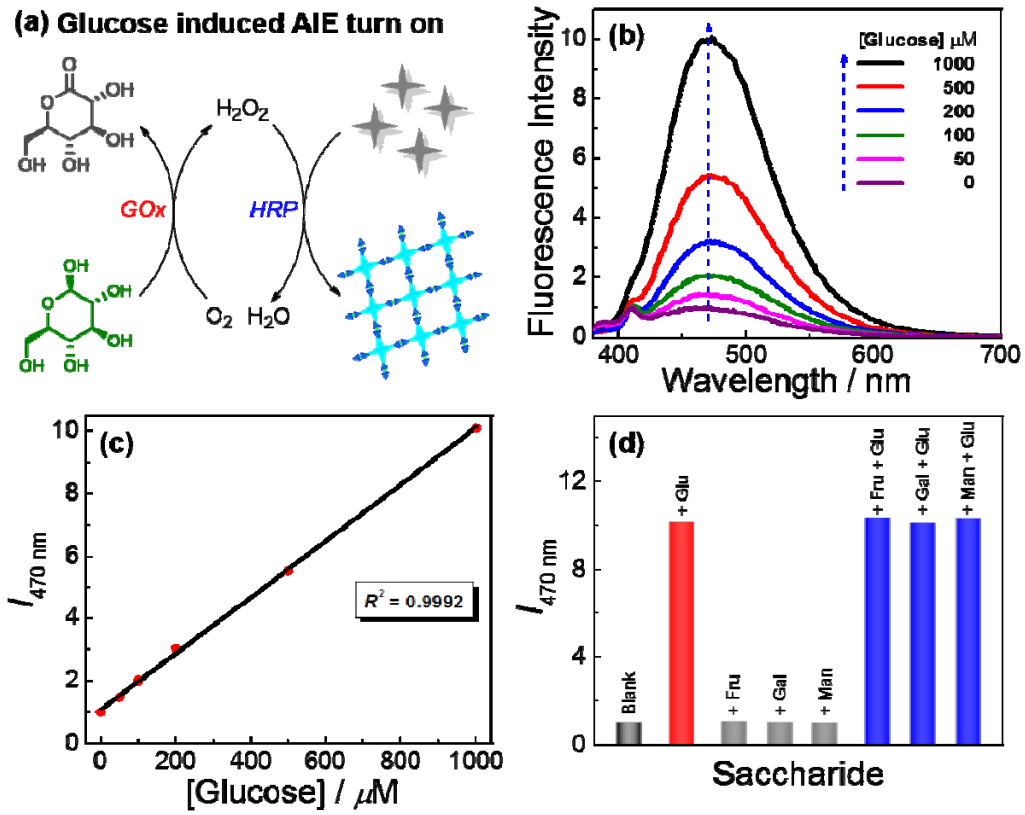


Figure S17. (a) Schematics of the fluorometric *D*-glucose detection strategy via cascade enzymatic oxidative coupling of TPE-Tyr and activated AIE process (GOx: glucose oxidase; HRP: horseradish peroxidase). (b) Normalized emission spectra of TPE-Tyr aqueous solution in the presence of glucose at different concentrations; (c) Glucose concentration dependence of normalized emission intensities at 470 nm. TPE-Tyr 25 μM, HRP 0.01 g/L, GOx 0.01 g/L, Glucose 0-1.0 mM; the reaction time was fixed at 5 min in all cases. (d) Response of TPE-Tyr to various saccharides (1.0 mM). Normalized emission intensities at 470 nm are shown. Glucose (Glu), fructose (Fru), galactose (Gal), and mannose (Man).

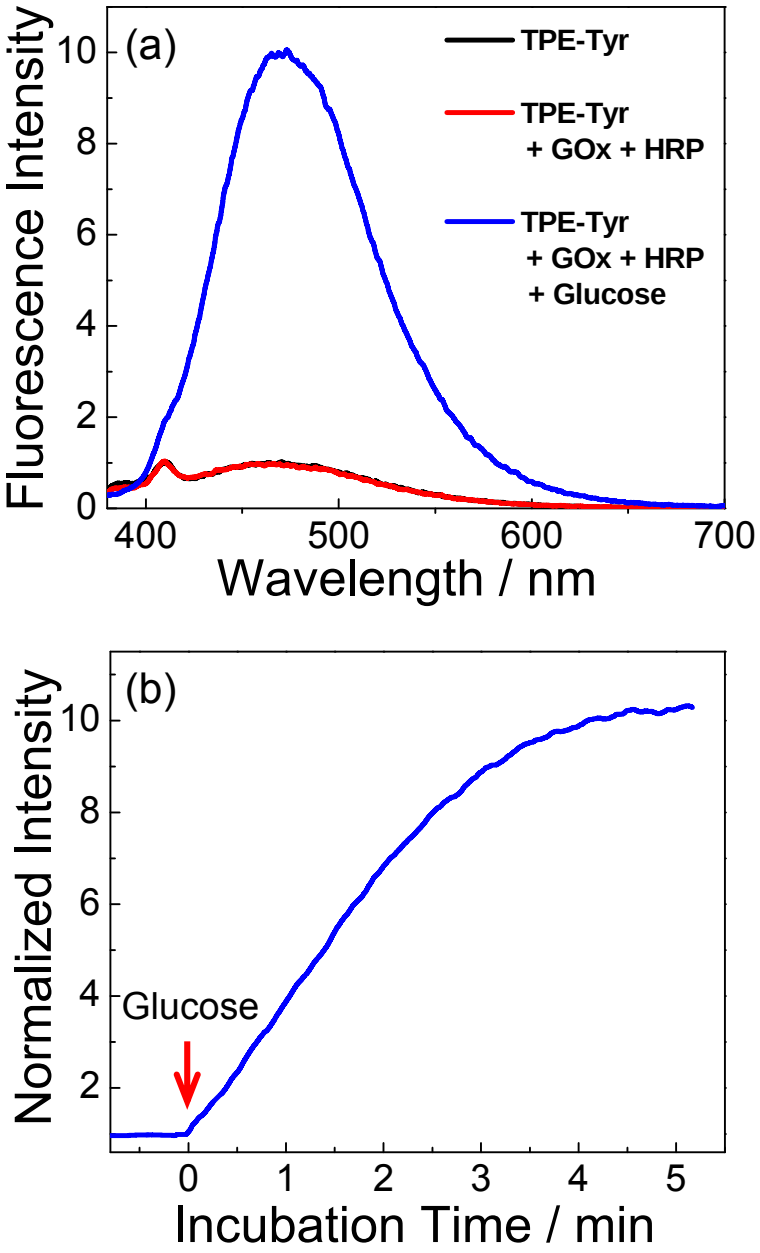


Figure S18. (a) Normalized fluorescence emission spectra of TPE-Tyr only, TPE-Tyr added HRP + GOx, and TPE-Tyr added HRP + GOx + Glucose, respectively; (b) incubation time dependence of fluorescence intensity of TPE-Tyr in the presence glucose. TPE-Tyr 25 μM, HRP 0.01 g/L, GOx 0.01 g/L, Glucose 1.0 mM; in PBS buffer (pH 6.0, 10 mM, 25 ^{o}C, λ_{ex} = 360 nm, λ_{em} = 470 nm; the reaction time was fixed to be 5 minutes in all cases.

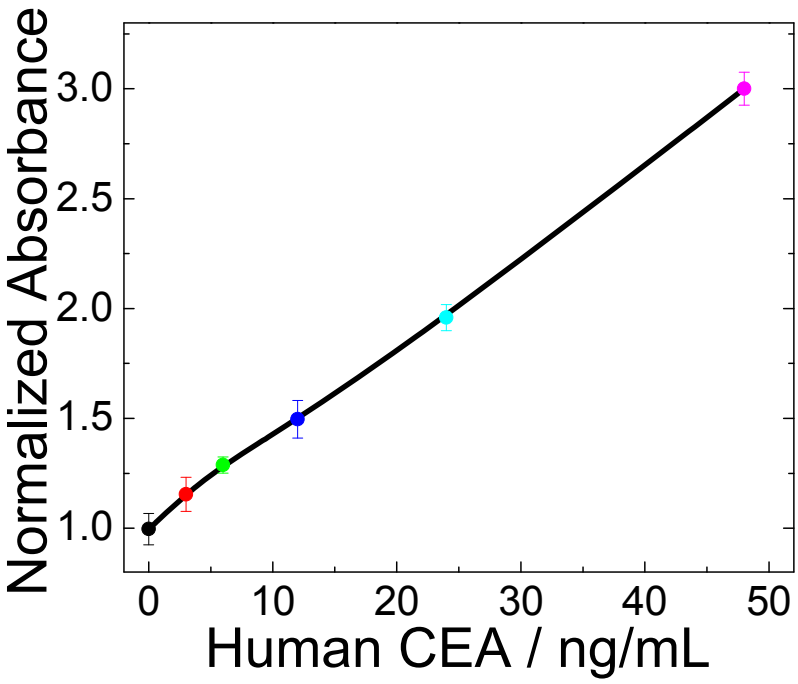


Figure S19. Human CEA concentration-dependent changes in optical absorption at 450 nm obtained from standard Elisa analysis using TMB (3,3',5,5'-Tetramethylbenzidine) as substrate molecules.