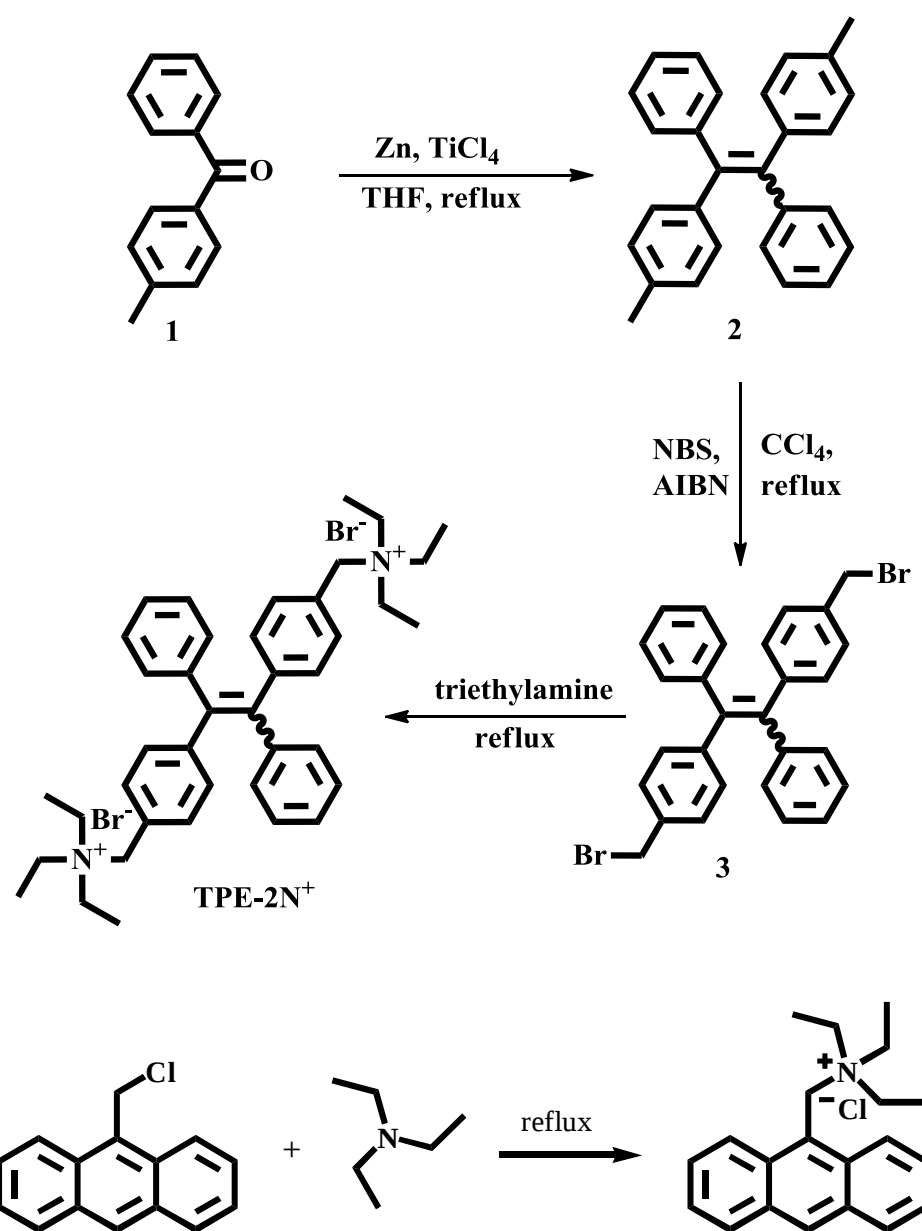


Supporting information

A ratiometric fluorescent biosensor for hyaluronidase with hyaluronan as both nanoparticle scaffold and substrate for enzymatic reaction

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China



Scheme S1. Synthetic route of the two fluorophores (TPE-2N⁺ and AN-N⁺).

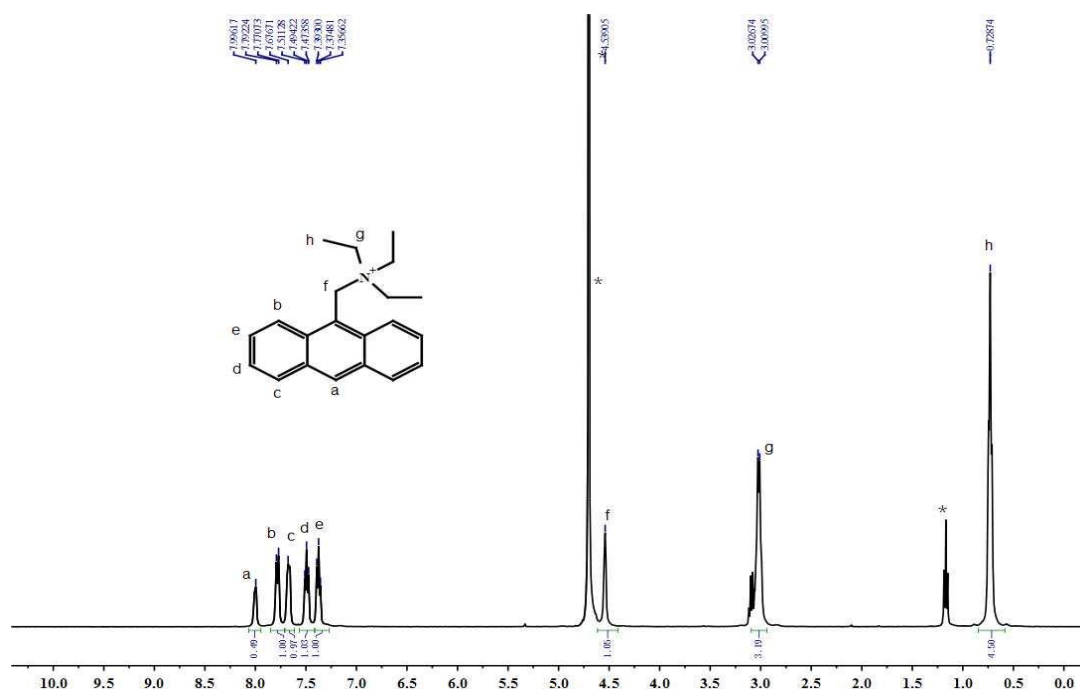


Figure S1. ¹H NMR spectra of the compound AN-N⁺.

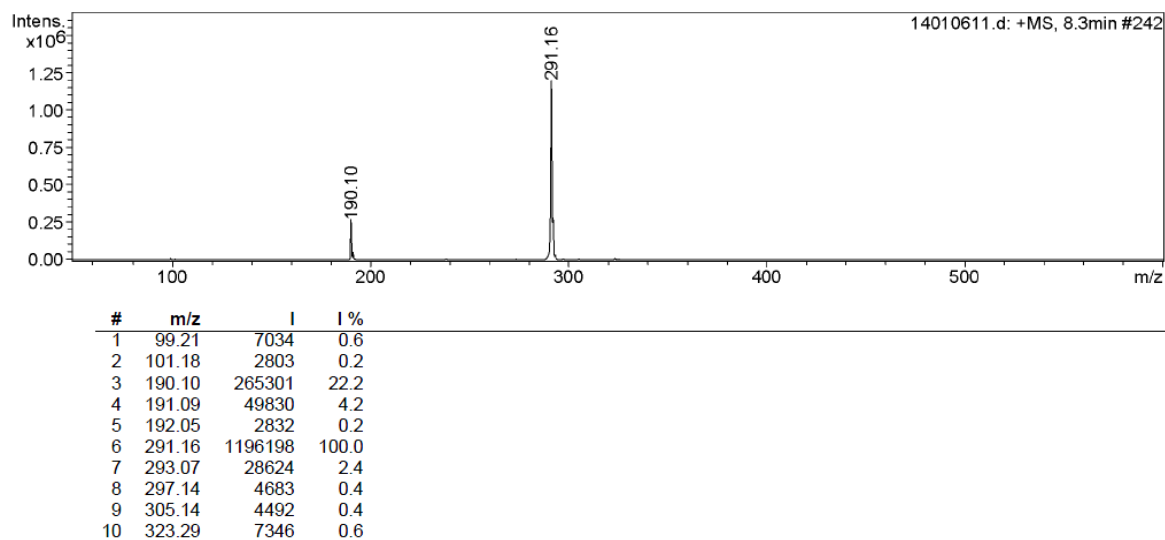


Figure S2. Mass spectrum of AN-N⁺, MS (ESI): m/z 291.16 [M]⁺.

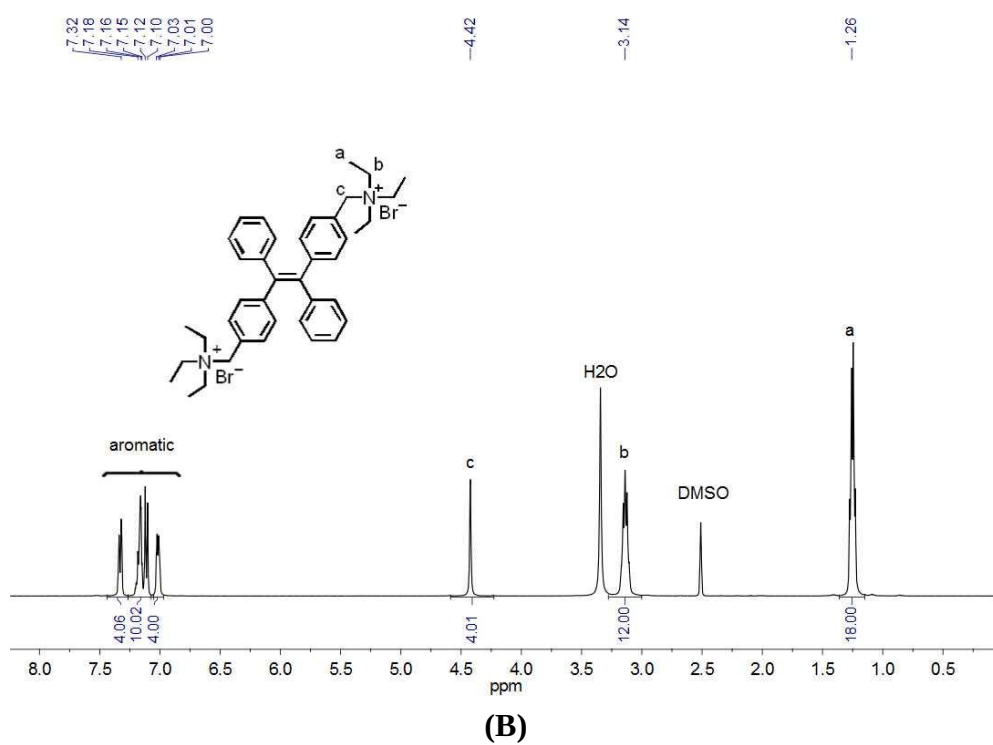
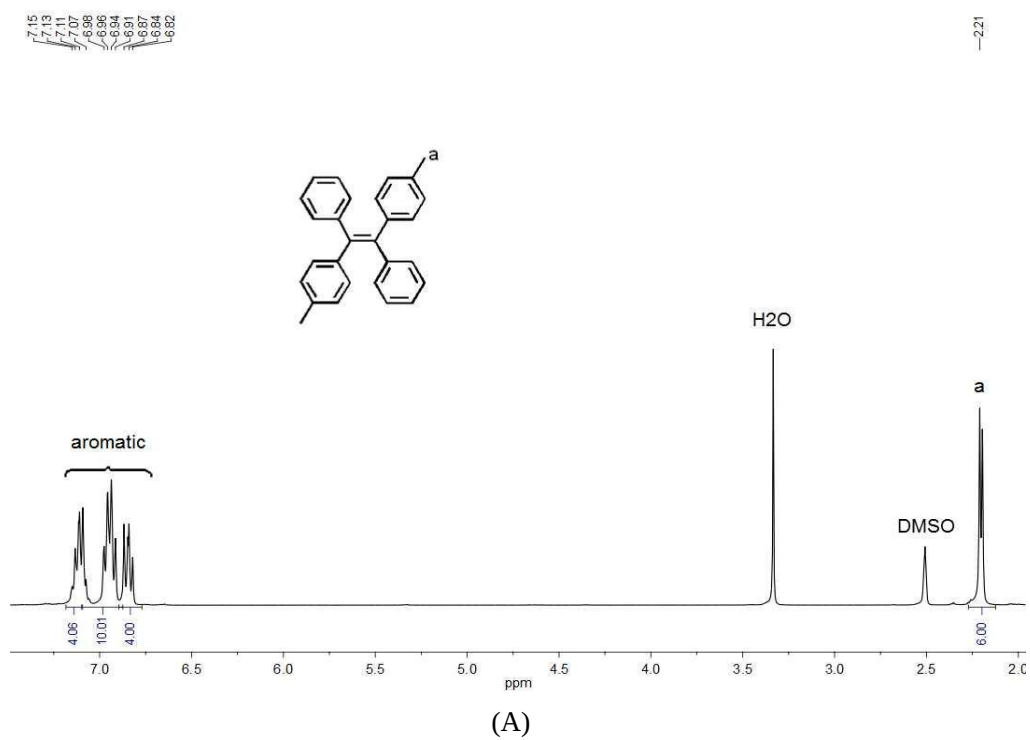
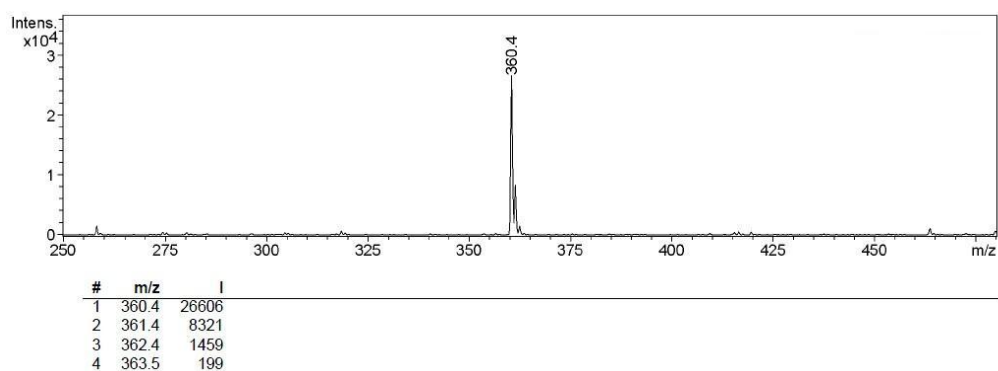
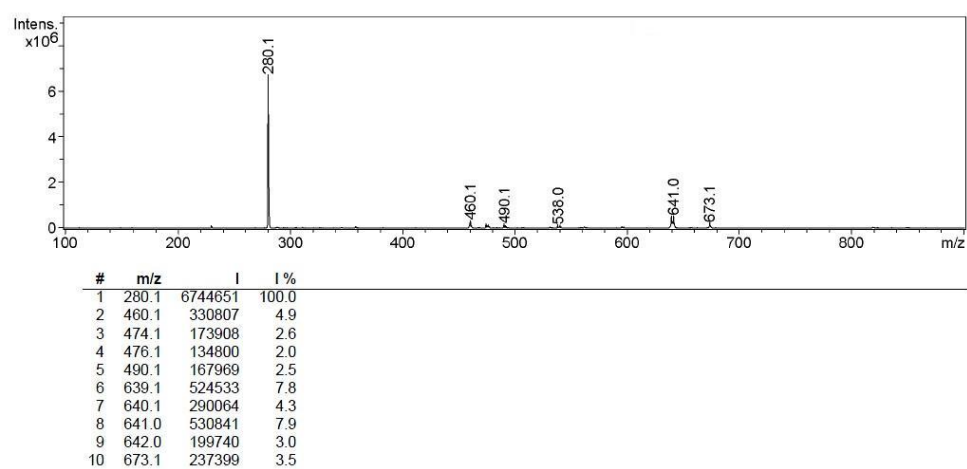


Figure S3. ^1H NMR spectra of Compound **2** (A) and TPE-2N⁺ (B).

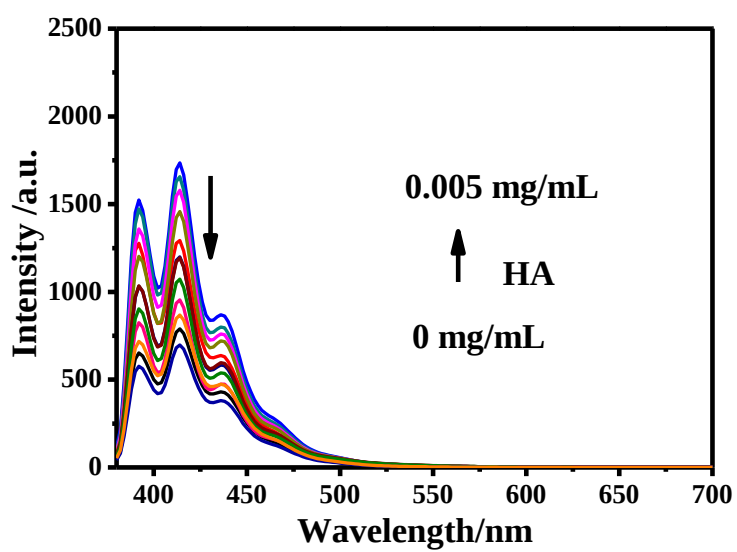


(A)

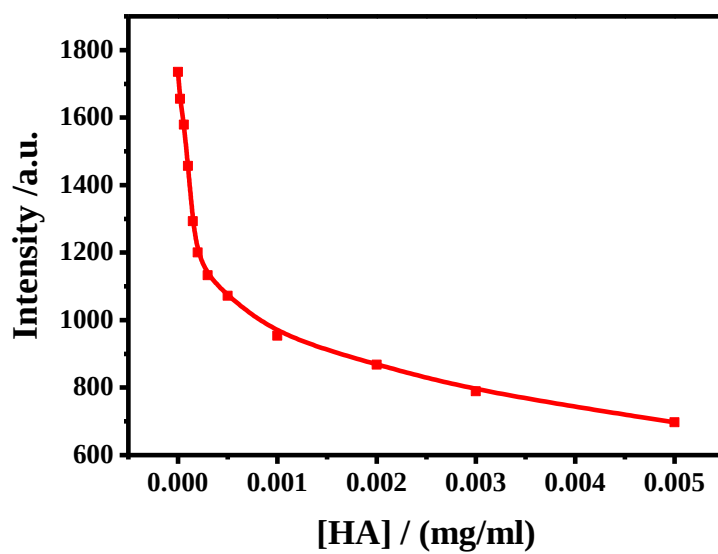


(B)

Figure S4. (A) Mass spectrum of Compound 2, MS (ESI): m/z 360.4 $[M]^+$; (B) Mass spectrum of TPE-2N⁺, MS (ESI): m/z 280.1 $[M-2Br]^{2+}$.

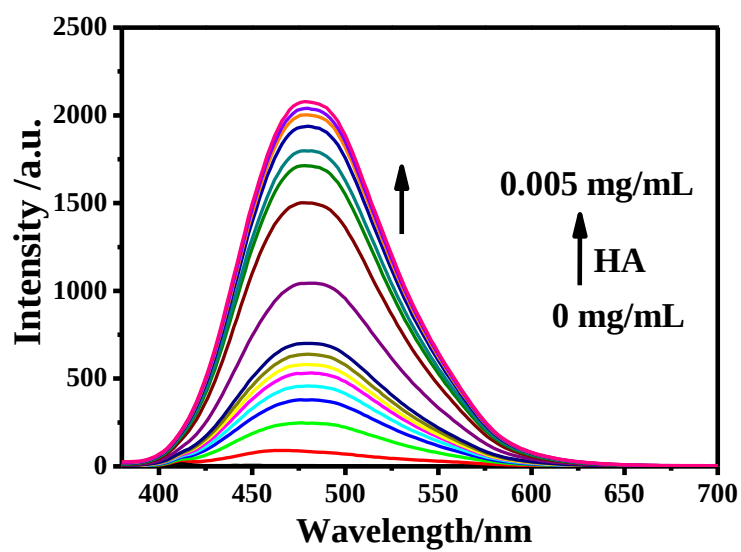


(A)

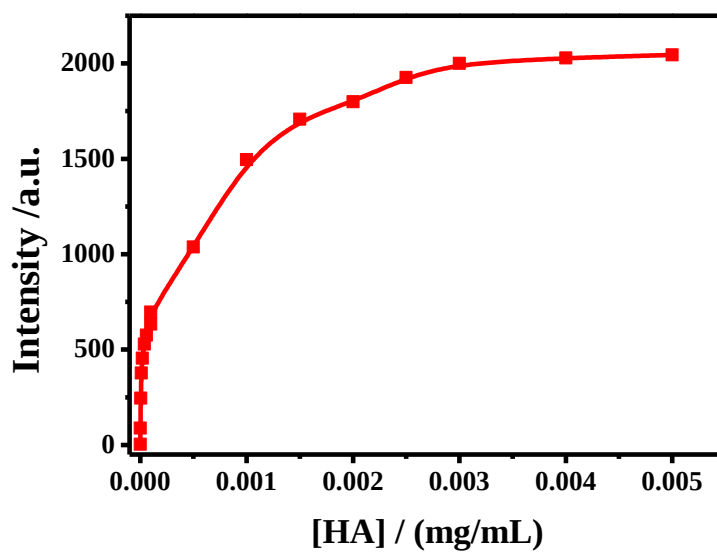


(B)

Figure S5. Fluorescence spectra of 4 μM AN-N⁺ in the presence of different amounts of HA in PBS buffer solution (0.1 mM, pH 4.3).



(A)



(B)

Figure S6. Fluorescence spectra of 20 μM TPE-2N⁺ in the presence of different amounts of HA in PBS buffer solution (0.1 mM, pH 4.3).

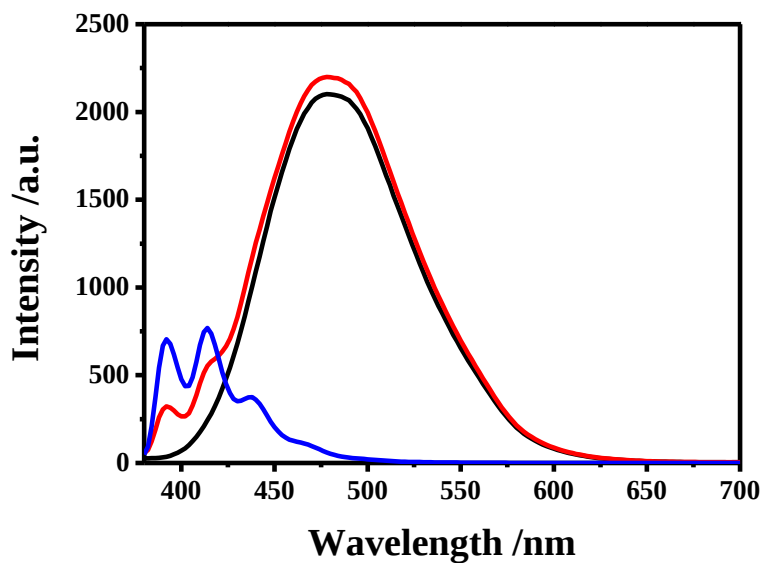


Figure S7. Fluorescence spectra. Black curve: 0.003 mg/mL HA / 26 μM TPE-2N⁺ in PBS buffer; red curve: 4 μM AN-N⁺ / 26 μM TPE-2N⁺ / 0.003 mg/mL HA in PBS buffer; blue curve: 4 μM AN-N⁺ / 0.003 mg/mL HA in PBS buffer.

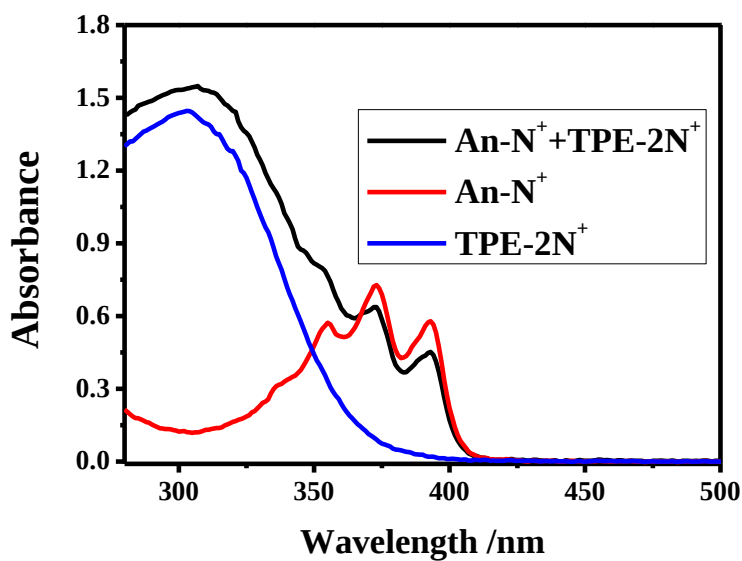
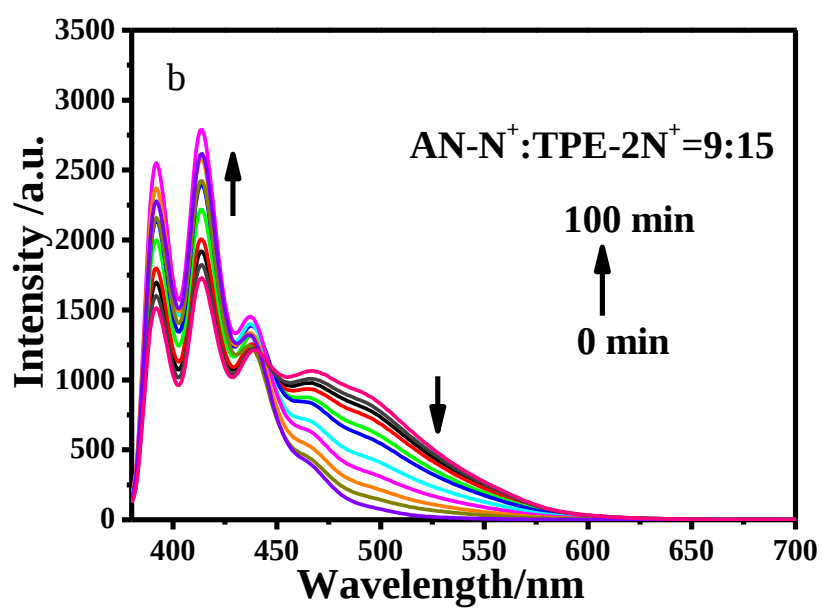
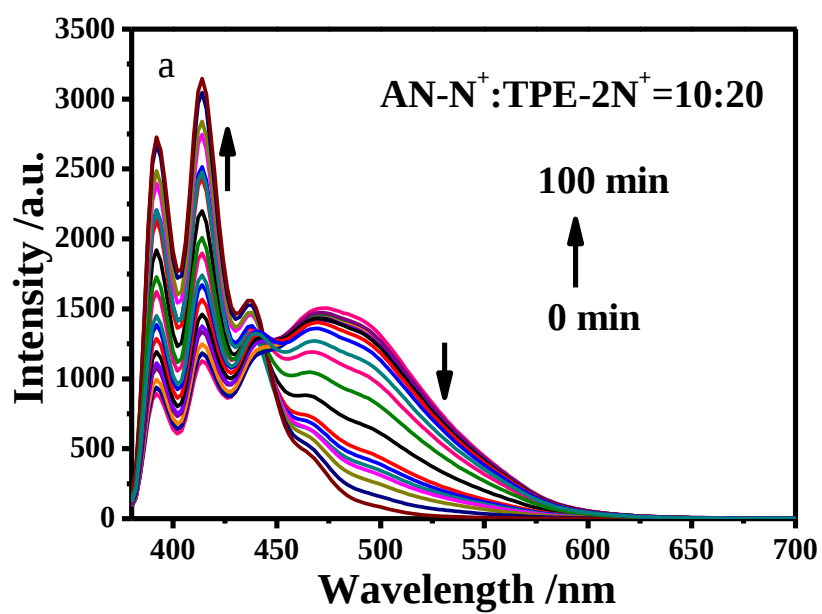
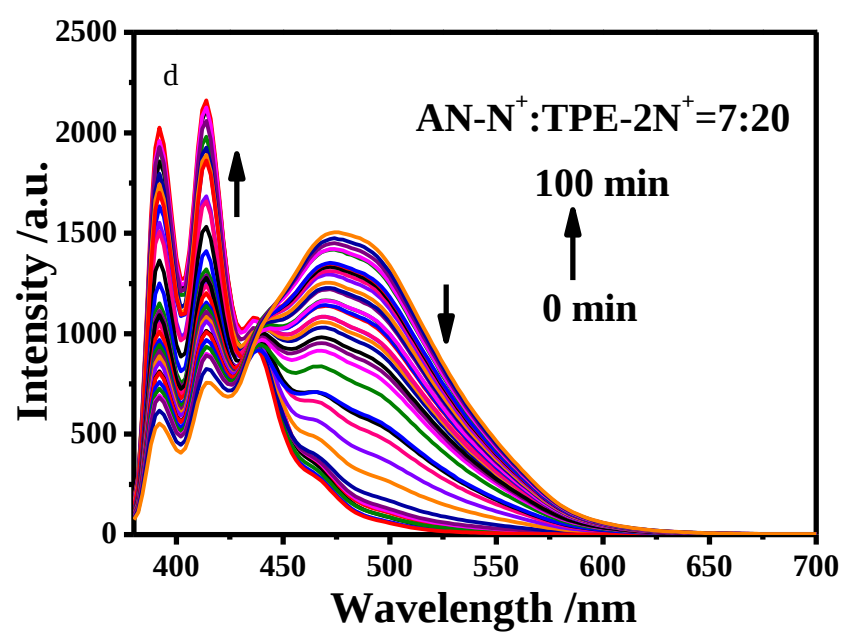
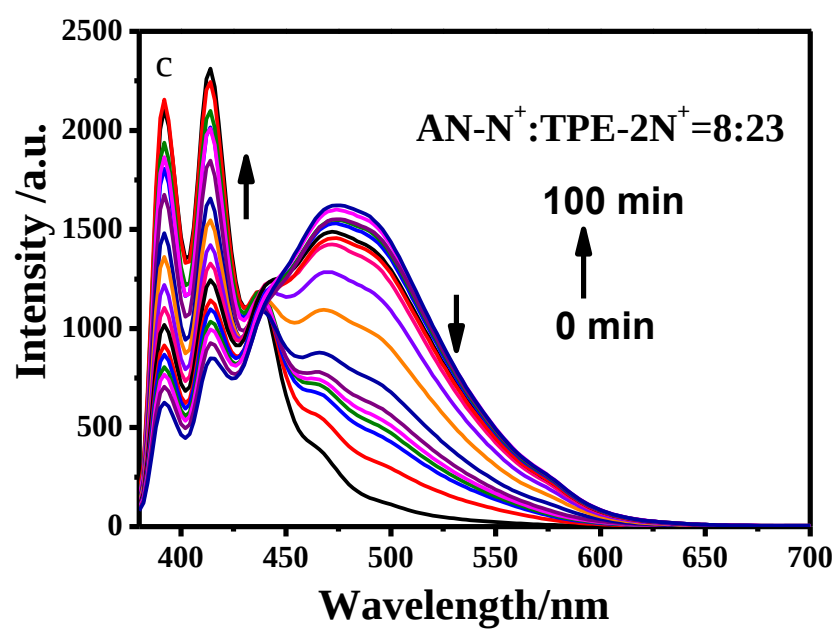
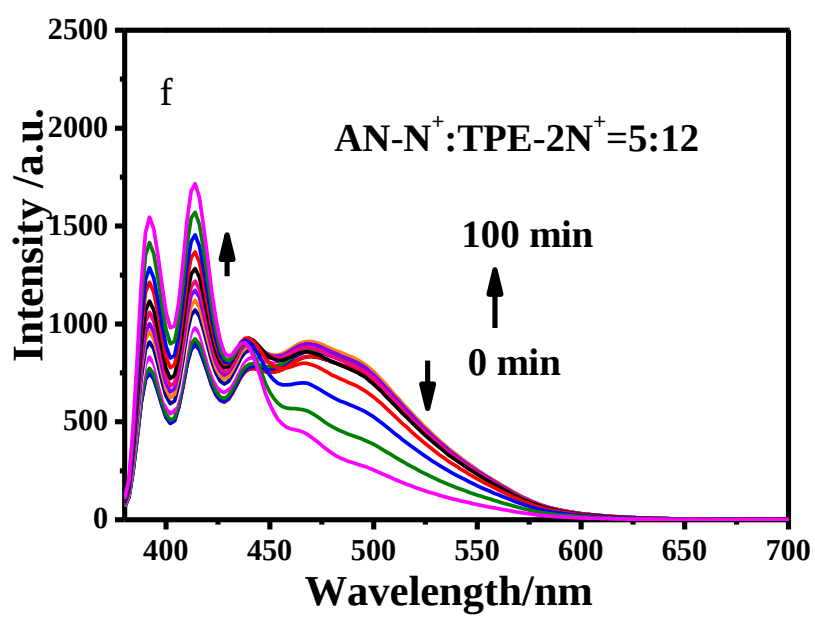
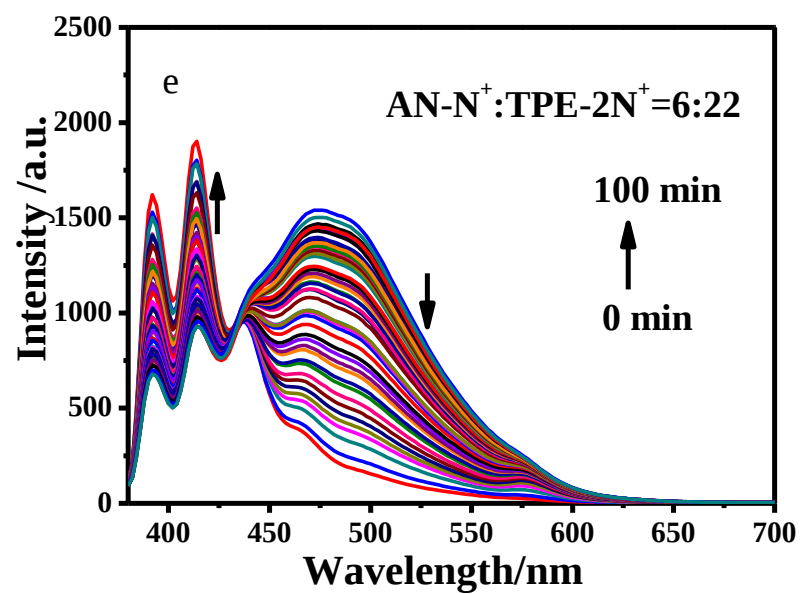


Figure S8. Absorbance spectra. Blue curve: 26 μM TPE-2N⁺ in PBS buffer; black curve: 4 μM AN-N⁺ / 26 μM TPE-2N⁺ in PBS buffer; red curve: 4 μM AN-N⁺ in PBS buffer.







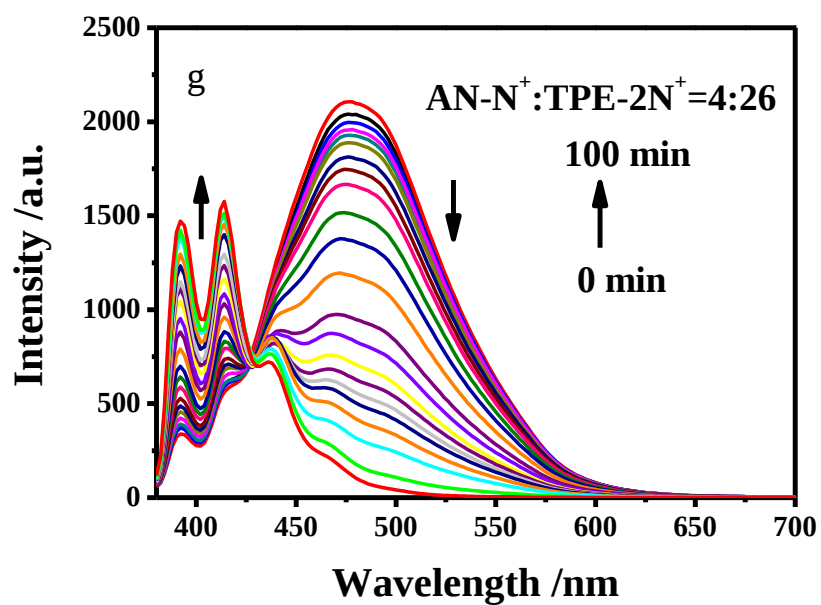


Figure S9. Fluorescence spectra of the ensemble containing 0.003 mg/mL HA and AN-N⁺ / TPE-2N⁺ with different molar ratios with the addition of 15 U/ml HAase after being incubated for 100 min, the AN-N⁺ / TPE-2N⁺ molar ratios are: (a) 10:20, (b) 9:15, (c) 8:23, (d) 7:20, (e) 6:22, (f) 5:12, (g) 4:26.

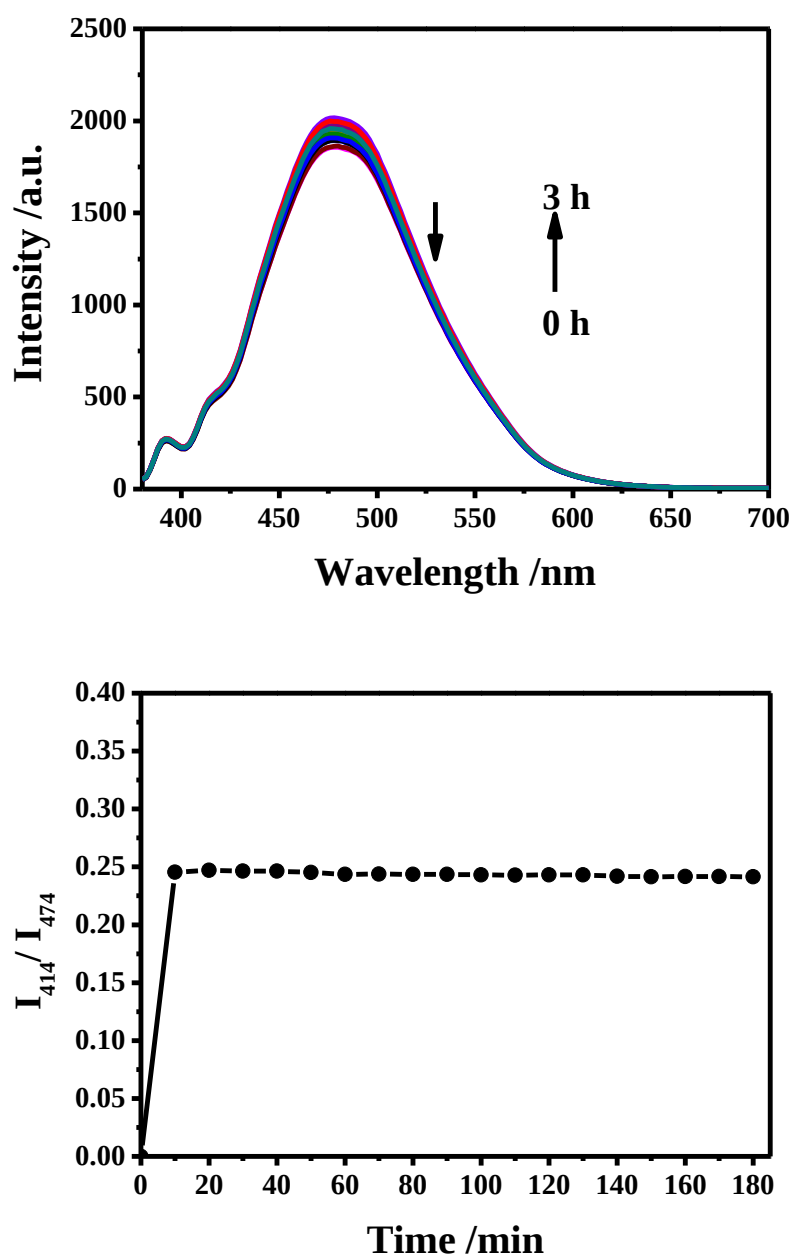


Figure S10. The photobleaching of the sensing system under the UV light irradiation (254 and 365 nm at the same time) for different time periods in PBS buffer solution (0.1 mM, pH 4.3). $\lambda_{\text{ex}} = 365 \text{ nm}$.

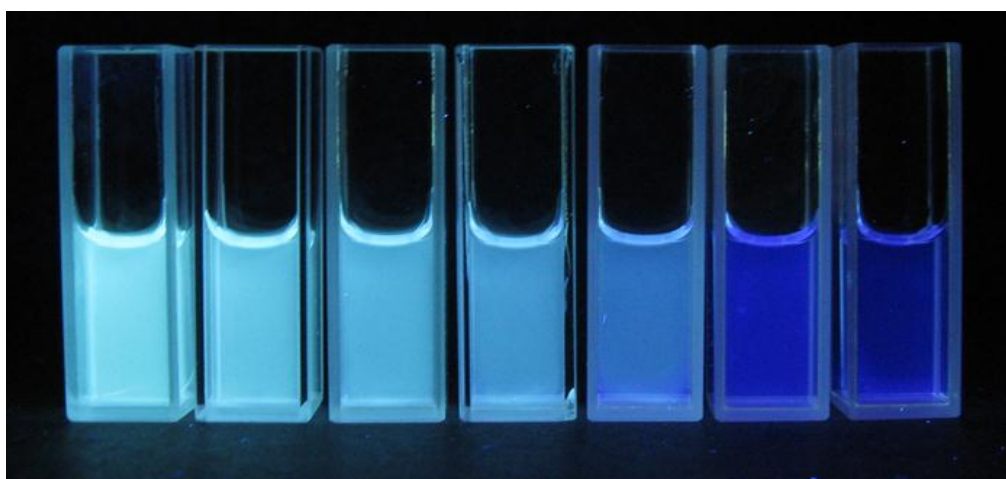


Figure S11. Fluorescence change of the sensing system ($4\ \mu\text{M AN-N}^+$ / $26\ \mu\text{M TPE-2N}^+$ / $0.003\ \text{mg/mL HA}$) in the absence and presence of different amounts of HAase in PBS buffer solution (pH 4.3, $0.1\ \text{mM}$) after 100 min of reaction. (From left to right: $0\ \text{U/mL}$; $0.2\ \text{U/mL}$; $0.5\ \text{U/mL}$; $1\ \text{U/mL}$; $2\ \text{U/mL}$; $5\ \text{U/mL}$; $8\ \text{U/mL}$). Photographs were taken under a hand-held 365 nm UV lamp.

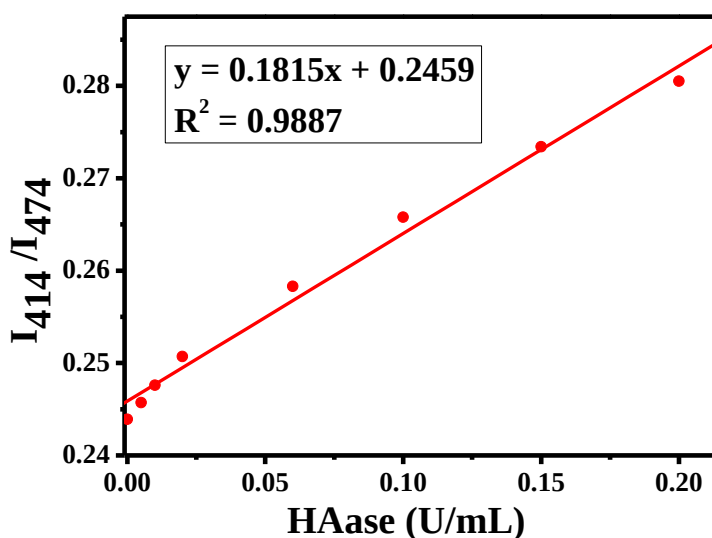


Figure S12. Plot of the fluorescence intensity ratio of I_{414}/I_{474} of the sensing system ($4\ \mu\text{M AN-N}^+ / 26\ \mu\text{M TPE-2N}^+ / 0.003\ \text{mg/mL HA}$) in PBS buffer solution ($0.1\ \text{mM}$, $\text{pH } 4.3$) against the corresponding HAase level showing a linear relationship ($y = 0.1815x + 0.2459$, $R^2 = 0.9887$).

Determination of the detection limit:

First the calibration curve was obtained from the plot of fluorescence intensity ratio of I_{414}/I_{474} as a function of the analyte concentration (HAase). The regression curve equation was then obtained for the lower concentration part.

The detection limit = $3 \times \text{S.D.} / k$

where k is the slope of the curve equation, and S.D. represents the standard deviation for the fluorescence intensity ratio of the assay system in the absence of HAase.

$$I_{414}/I_{474} = 0.2459 + 0.1815 \times [\text{HAase}] \quad (R^2 = 0.9887)$$

$$\text{LOD} = 3 \times 1.0285 \times 10^{-4} / 0.1815 = 0.0017\ \text{U/mL}$$

References:

V. Thomsen, D. Schatzlein and D. Mercurio, *Spectroscopy*, **2003**, *18*, 112-114.

A. D. McNaught and A. Wilkinson, *IUPAC Compendium of Chemical Terminology*, **1997**.

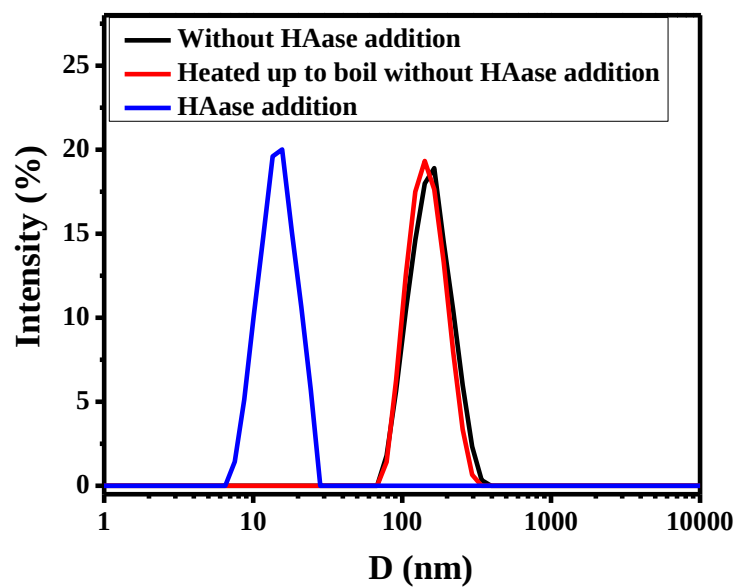


Figure S13. Size distribution of the nanoaggregates determined by dynamic light scattering (DLS) under three different conditions.

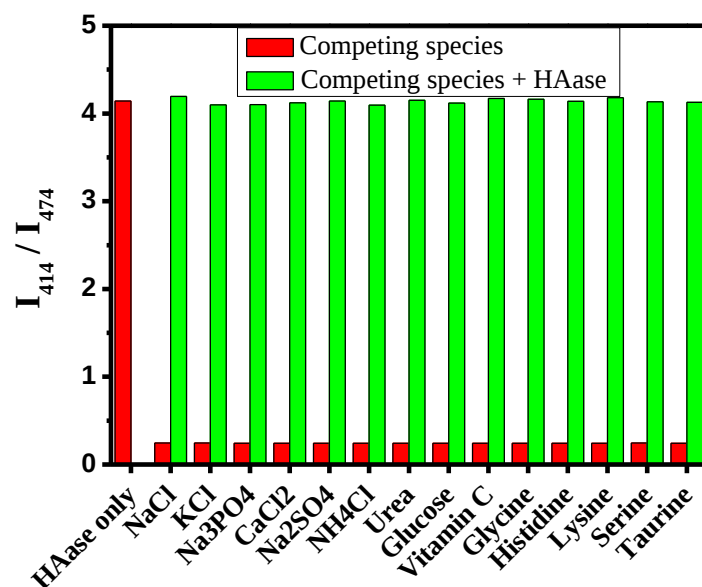


Figure S14. Fluorescence intensity ratio for the sensing system (4 μM AN- N^+ / 26 μM TPE-2 N^+ / 0.003 mg/mL HA) in the presence of competing species only or simultaneous in the presence of 10 U/mL HAase in PBS buffer solution (pH 4.3, 0.1 mM).

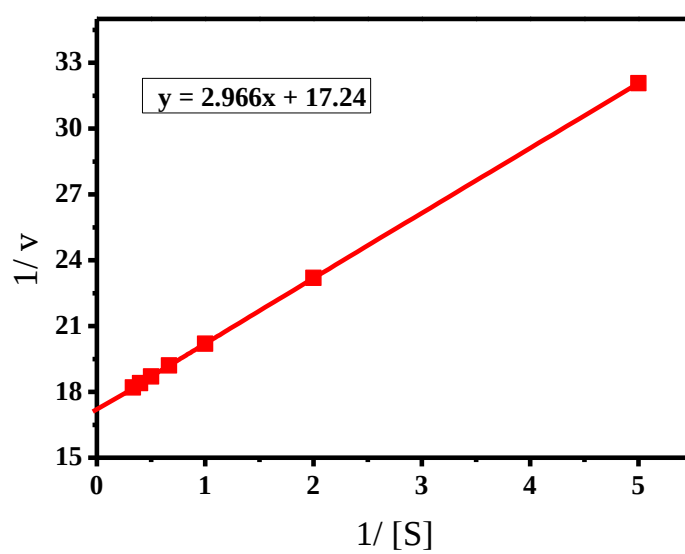
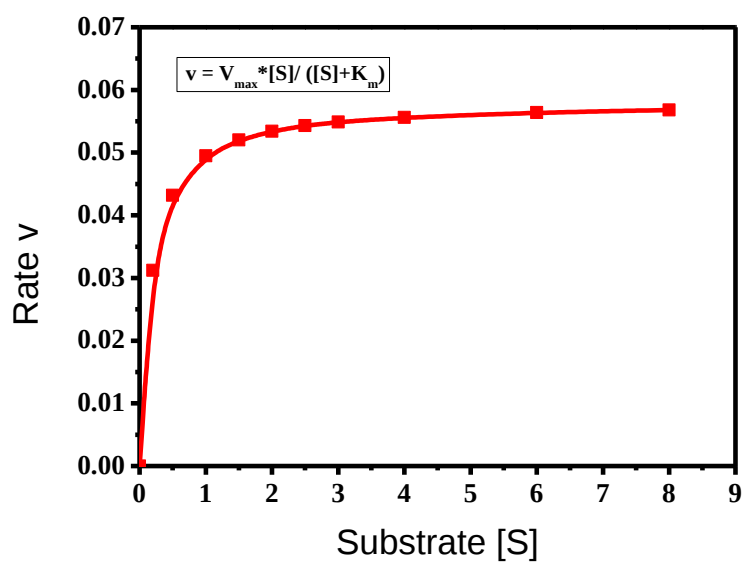


Figure S15. Substrate dependence of the initial degradation velocities for HAase. Initial velocities were plotted against the substrate concentrations and fit to the Michaelis-Menten model (The kinetic parameters: $V_{\max} = 0.058$ mM/min, $K_m = 0.172$ mM). Data show the means $\pm$ SD from triplicate samples.

Table S1. Detection of HAase level in serum

Determined HAase (mU/mL)	Added HAase (mU/mL)	Combined HAase (mU/mL)	Measured (mU/mL)	Recovery (%)
Median: 6.34 ^(a) [6.51 ^(b)]	-	-	-	-
	1	7.34	7.16	97.55
	5	11.34	11.13	98.15
	10	16.34	16.52	101.10
	20	26.34	26.87	102.15
	40	46.34	46.11	99.51

Note: (a) The final concentration of serum in the analytical solutions is 50-fold diluted. The determined HAase level in the diluted serum sample without spiking sulfite is 0.00634 U/mL, which means the HAase concentration in undiluted serum sample is 0.317 U/mL. Each sample measured three times and the middle value was taken.

(b) Determined according to colorimetric Morgan-Elson assay method with the deviation of less than 10%.

Table S2. Detection of HAase in Urine

Determined HAase (mU/mL)	Added HAase (mU/mL)	Combined HAase (mU/mL)	Measured (mU/mL)	Recovery (%)
Median: 5.12 ^(a) [5.29 ^(b)]	-	-	-	-
	1	6.12	6.29	102.77
	5	10.12	11.03	108.92
	10	15.12	15.06	99.69
	20	25.12	25.31	100.75
	40	45.12	44.65	98.95

Note: (a) Each sample measured three times and the middle value was taken.

(b) Determined according to colorimetric Morgan-Elson assay method with the deviation of less than 10%.