Supplementary information

Rational design of HA-AuNPs@AIEDs nanoassembly for activatable fluorescence detection of HAase and imaging in tumor cells

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Experimental Section

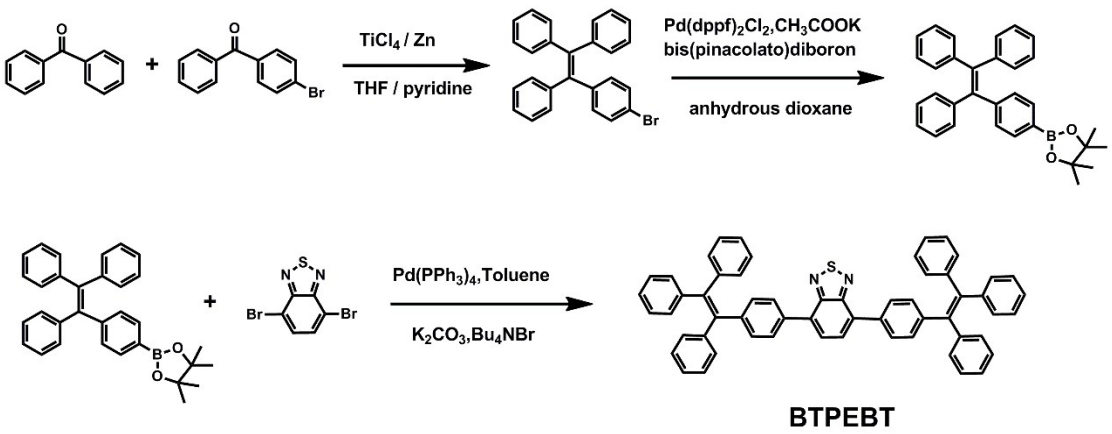
Chemicals and Materials

All reagents and solvents were at least analytical grade and used directly without further purification. Hyaluronic acid and Hyaluronidase were purchased from Sangon Biotech. Bovine serum albumin (BSA), 2-aminoethyl methacrylate hydrochloride (AEMH), Styrene (St, 99%)， Azobisisobutyronitrile (AIBN, 99.99%) and N, N-Dimethylformamide (DMF) were purchased from Sigma Aldrich (St. Louis, MO, U.S.A.). Poly (ethylene glycol) methyl ether (PEO-OH, Mw=5000) were purchased from Alfa. All the other relative raw materials and chemical reagents were got from Aladdin and Energy Chemical. PBS buffer solution and all the metal ions were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All the

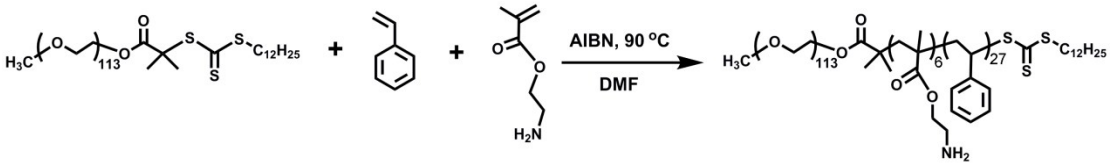
solutions were prepared in double-distilled water which was purified by a Milli-Q system (Millipore, Bedford, MA). The subset of Hela cells with high CD44 expression level was chosen (Shanghai Cell Bank, Shanghai, China).

Instruments and methods

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 500 MHz NMR spectrometer. UV-Vis spectra were recorded on a Shimadzu UV-2501PC spectrophotometer at room temperature (298 K). The number-average molecular weight (M_n) and PDI were determined by using a Waters 2695 gel permeation chromatograph (GPC) at 30 °C and THF was used as the eluent (1.0 $mL \cdot min^{-1}$). The calibration curve was obtained by using polystyrene (PS) as the standard. MS was conducted with a Finnigan LCQ Advantage MAX mass spectrometer. Fluorescence spectra were measured on RF-5301PC fluorescence spectrophotometer at room temperature. The diameter of nanoparticles was determined by a Malvern Nano-ZS90 instrument, and their morphology was recorded on a JEM-2100F transmission electron microscope (TEM, JEOL USA, Inc).



Scheme S1. Synthesis route of compound **BTPEBT**.



Scheme S2. Synthesis route of PEO_{113}-b-($AEMH_6$-co- St_{27}).

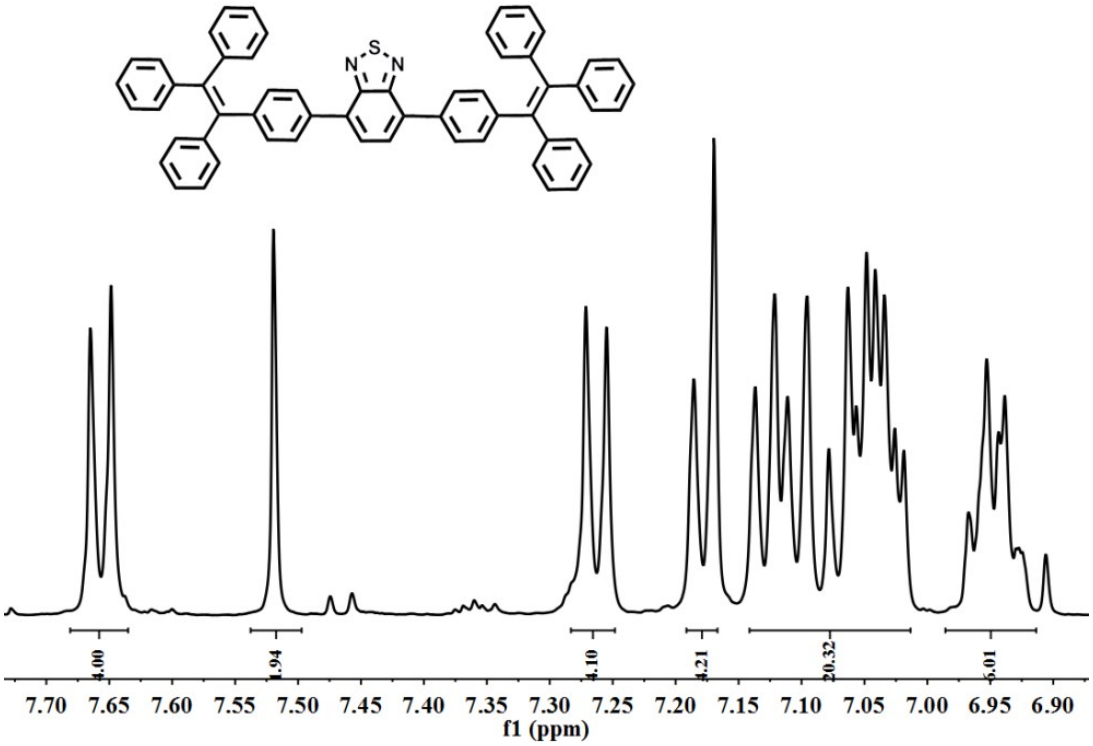


Figure S1. ^1H NMR spectrum (in $CDCl_3$) of the **BTPEBT**.

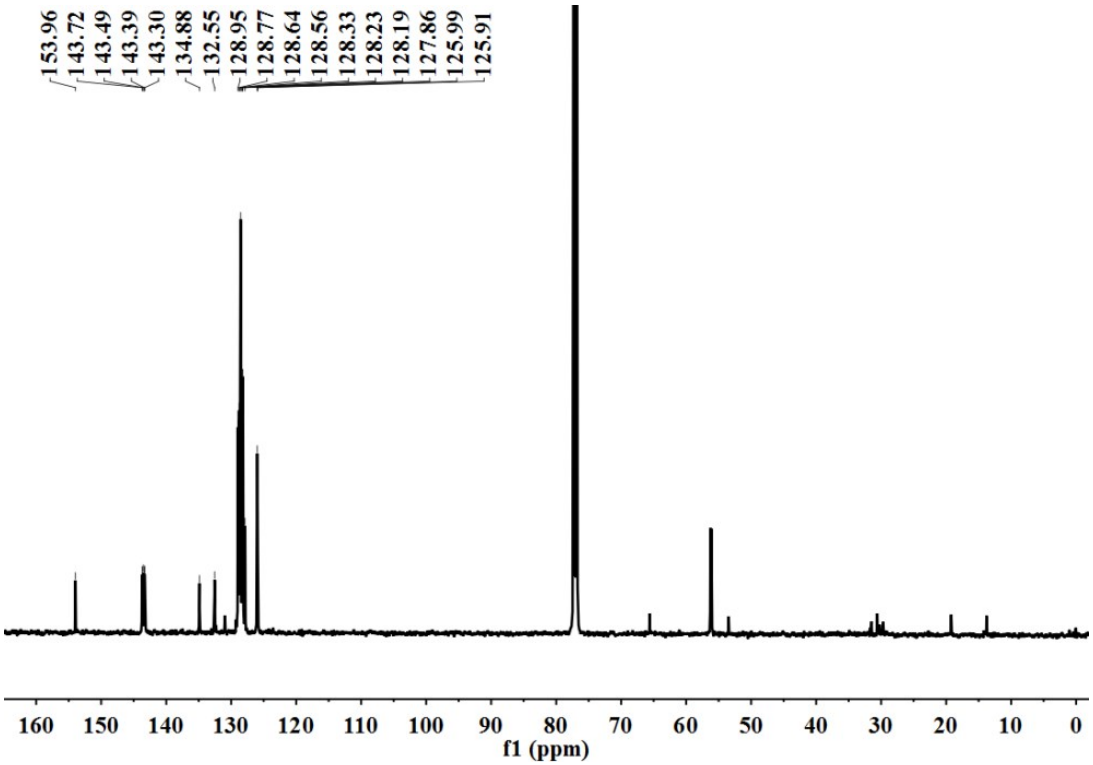


Figure S2. ^{13}C NMR spectrum (in $CDCl_3$) of the **BTPEBT**.

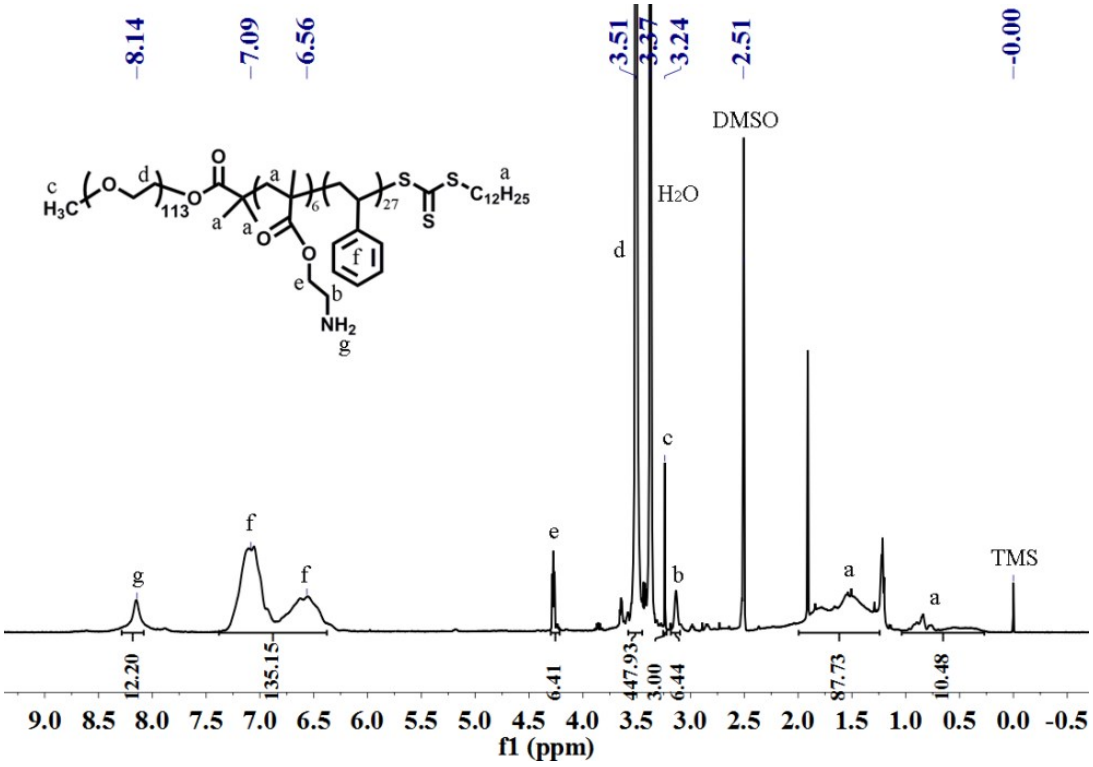


Figure S3. ^{1}H NMR of the PEO_{113}-*b*-($AEMH_6$-*co*- St_{27}).

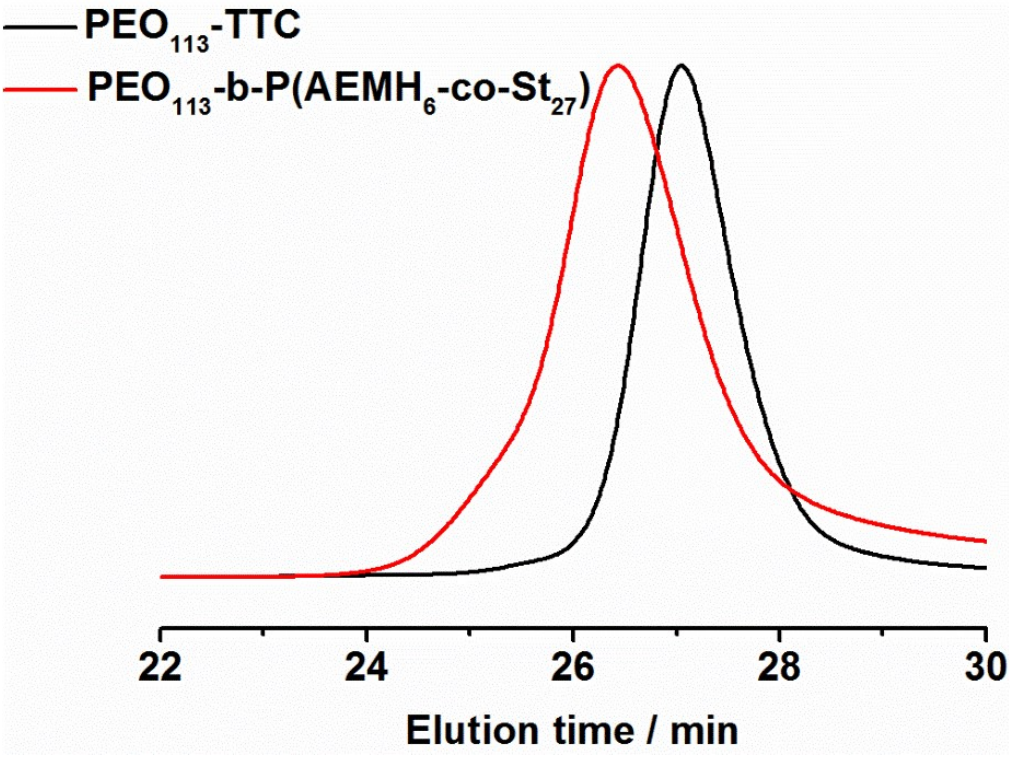


Figure S4. GPC trace of PEO_{113}-TTC and PEO_{113}-*b*-($AEMH_6$-*co*- St_{27}).

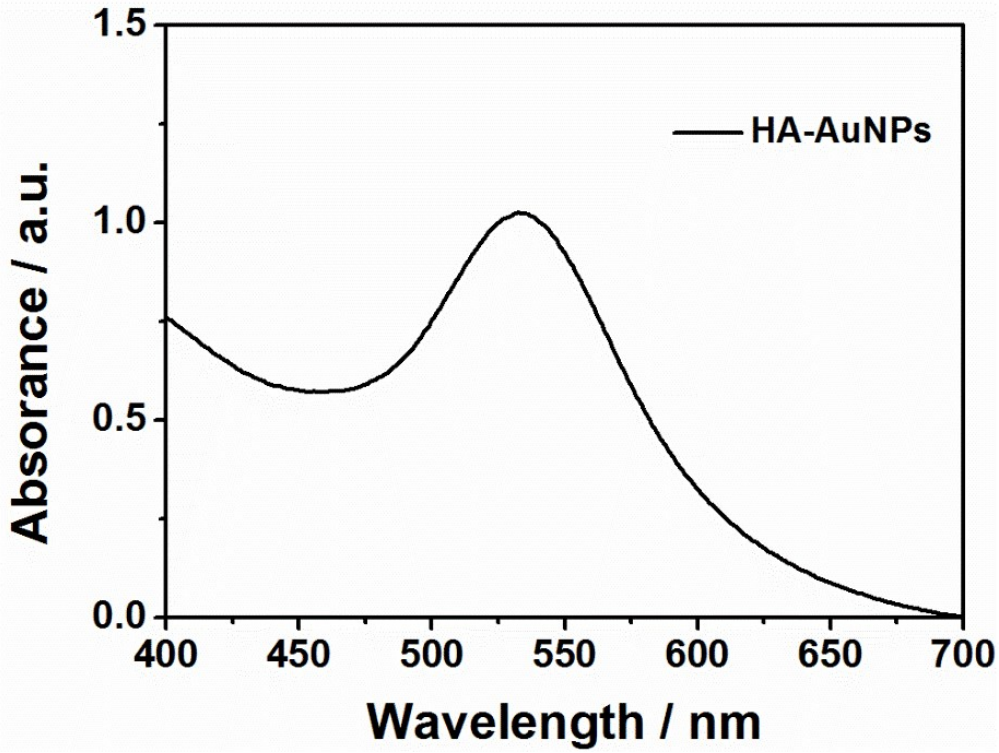


Figure S5. Absorption spectrum of the HA-AuNPs in in pH 7.4 PBS buffered water.

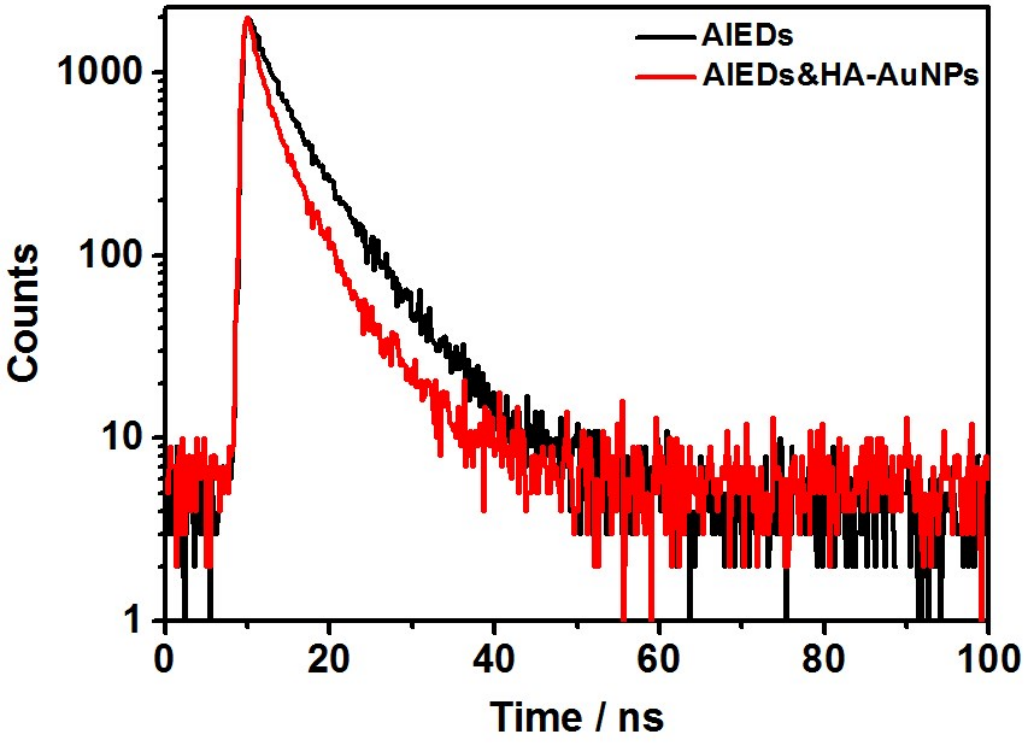


Figure S6. Fluorescence decay curves of AIEDs and HA-AuNPs@AIEDs(λ_{ex}=415 nm).

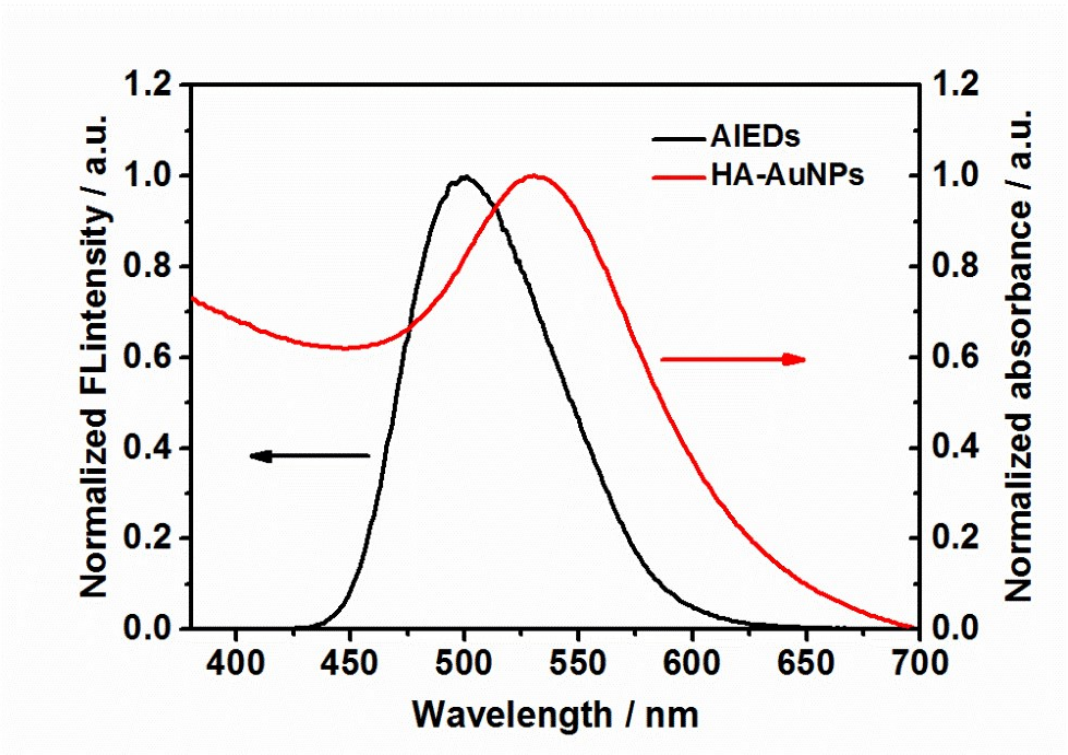


Figure S7. The normalized absorption spectra of the HA-AuNPs (red dashed curve) and the Normalized fluorescence emission spectra of the AIEDs (black solid curve) in pH 7.4 PBS buffered water.

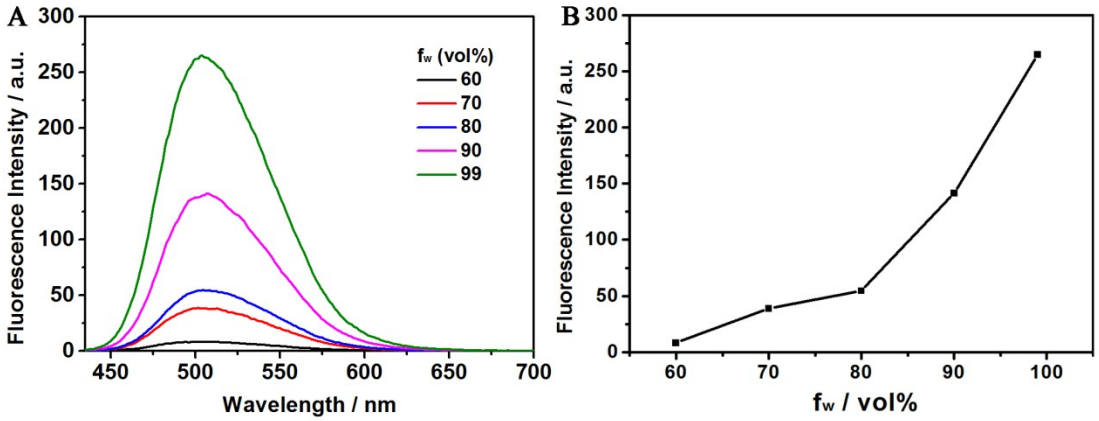


Figure S8. (A) Fluorescence emission spectra of BTPEBT in water / DMSO mixtures with varied water fractions, λex = 415 nm. (B) Variations in fluorescence intensity of BTPEBT with fw.

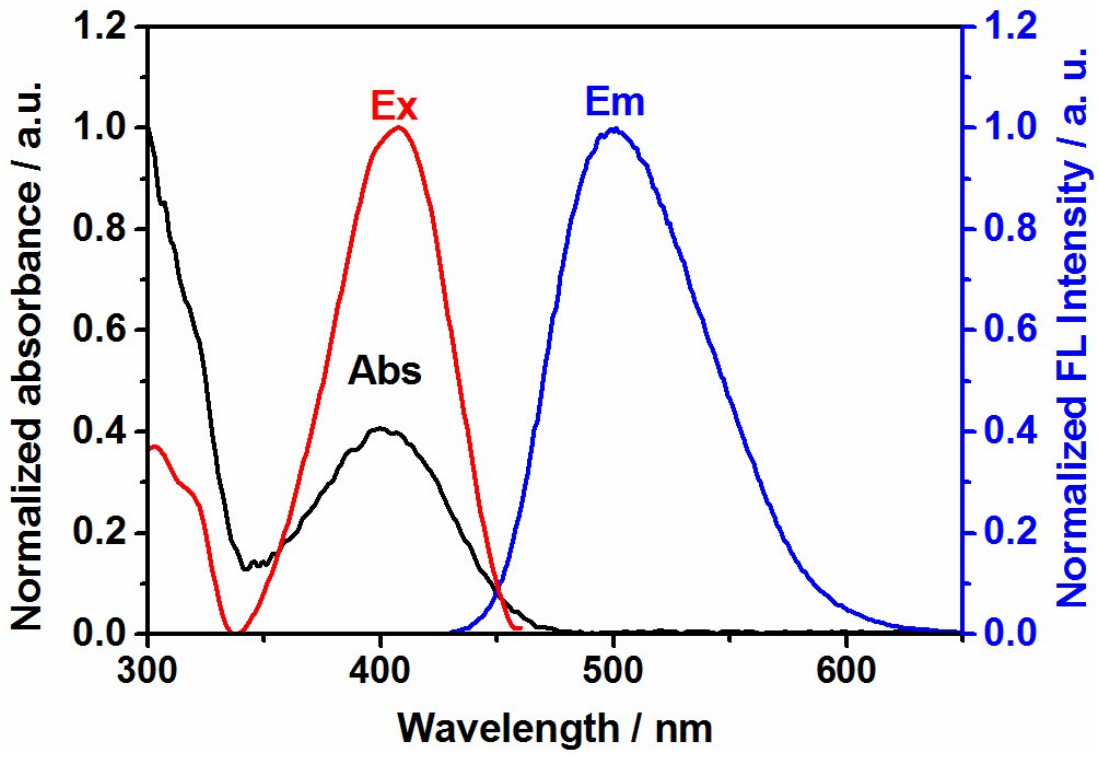


Figure S9. Absorption spectrum (black solid curve) and fluorescence spectrum (red and blue solid curve) of the AIEDs.

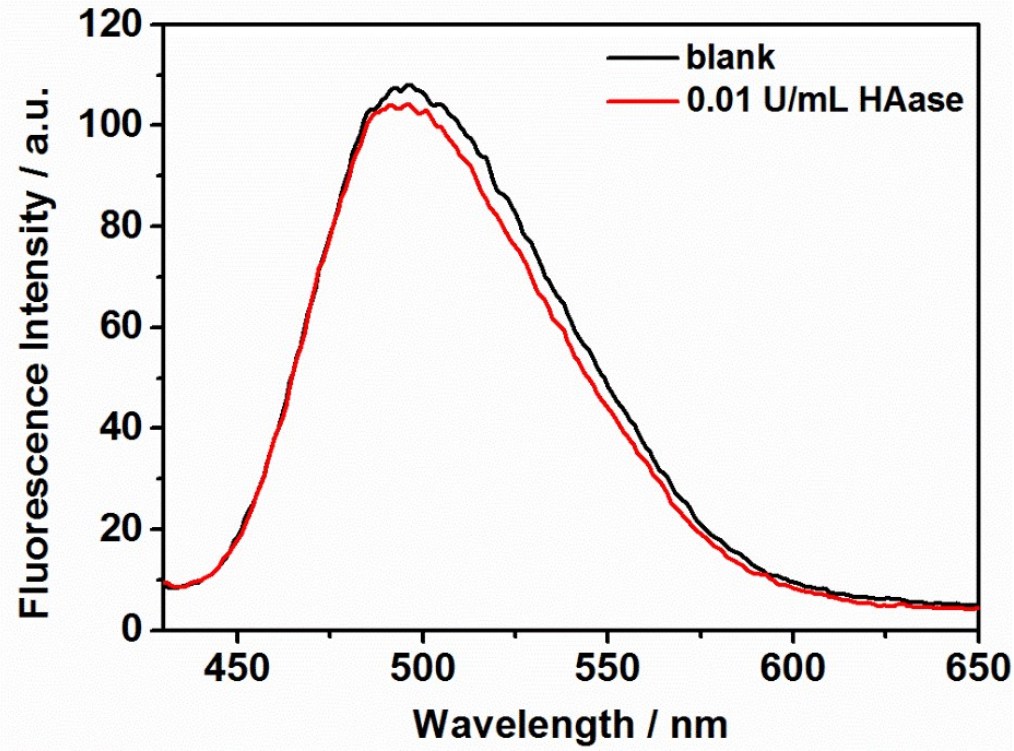


Figure S10. The fluorescence intensity of the nanoprobe at 500 nm under 0 and 0.01 U/mL HAase concentration.

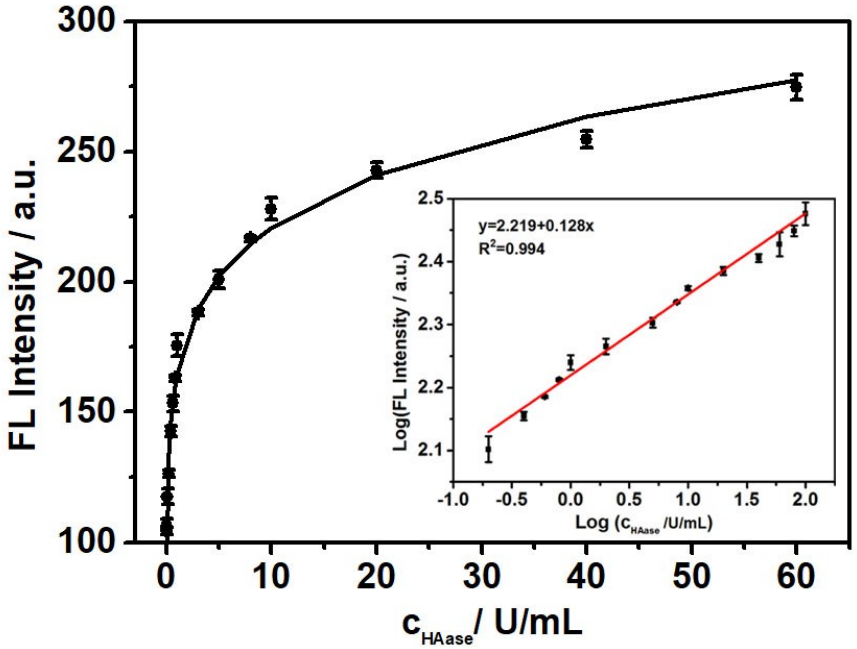


Figure S11. The fluorescence intensity at 500 nm as a function of HAase concentration (0.01-60 U/mL). Error bars are standard deviations of three repetitive experiments.

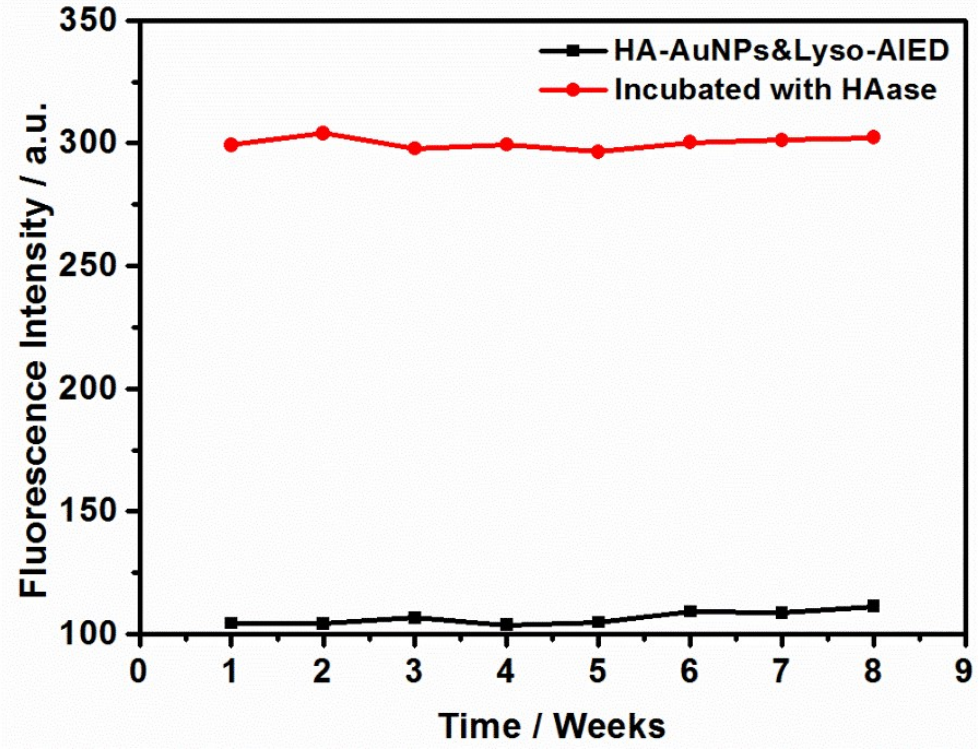


Figure S12. Fluorescence long-term photostability of HA-AuNPs@AIEDs (22 μg/mL) and nanoprobe incubated with HAase (100 U/mL) under ambient temperature and kept in the dark.

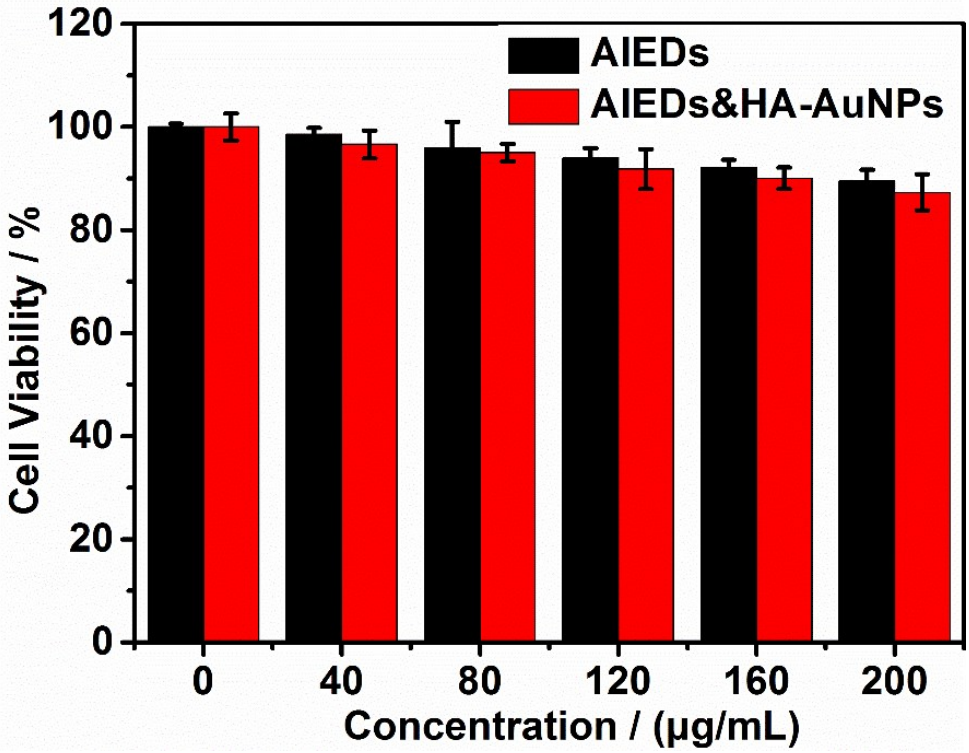


Figure S13. Viability for HeLa cells treated with the varied concentrations of AIEDs and HA-AuNPs@AIEDs for 24 h. The results are the mean standard deviation of three separate measurements. Error bars are standard deviations of three repetitive

experiments.

Determination of the detection limit:
First the calibration curve was obtained from the fluorescence intensity at 500 nm (F_{500}) versus HAase concentration. The regression curve equation was then obtained for the lower concentration part.
The detection limit = 3 ×S.D. / k
Where k is the slope of the curve equation, and S.D. represents the standard deviation for the fluorescence intensity at 500 nm of HA-AuNPs@AIEDs in the absence of HAase.
lgIF = 2.28 + 0.128lgC (R^2=0.994)
LOD =3×0.00031/0.128=0.0072 U/mL=7.2 mU/mL.

Table S1. Molecular weight distribution data of starting linear polymers.

Sample	$M_{n,\ GPC}$ [a]	$M_{w,\ GPC}$ [a]	PDI
PEO_{113}-TTC	7486	8038	1.07
PEO_{113}-*b*-P($AEMH_6$-*co*-St_{27})	9798	11581	1.18

[a]The data were acquired using SEC based on a polystyrene calibration curve and obtained from GPC analysis was using THF as eluent at a flow rate of 1.0 mL/min.