Supporting information for
A hyaluronidase/ATP tandem stimuli-responsive supramolecular assembly

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1 General methods and materials

1.1 Materials. All the reagents and solvents were commercially available and used as received unless otherwise specified purification. Eosin Y (EY), hyaluronidase (HAase, 400−1000 units/mg) and adenosine triphosphate (ATP) were purchased from Sigma-Aldrich. Hyaluronic acid (HA, $M_n = 10$ kDa) was purchased from Meilunbio. 2-bromoethylamine was purchased from J&K Scientific. $N$-(3-dimethylaminopropyl)-$N'$-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HoBt) were purchased from Aladdin. GC5A-6C was synthesized according to the previous literature.1

1.2 Samples. The HEPES buffer solution of pH 7.4 was prepared by dissolving 2.38 g of 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) in approximate 900 mL double-distilled water. Titrate to pH 7.4 at 25 °C with NaOH and make up volume to 1000 mL with double-distilled water. The pH value of the buffer solution was then verified on a pH-meter calibrated with three standard buffer solutions.

1.3 Instruments. $^1$H data were recorded on a Bruker AV400 spectrometer. The sample solutions for dynamic light scattering measurements were examined on a laser light scattering spectrometer (NanoBrook 173plus) equipped with a digital correlator at 659 nm at a scattering angle of 90°. UV-Vis spectra were recorded in a quartz cell (light path 10 mm) on a Shimadzu UV-3600 spectrophotometer equipped with a PTC-348WI temperature controller. Steady-state fluorescence measurements were recorded in a conventional quartz cell (light path 10 mm) on a Cary Eclipse equipped with a Cary single-cuvette peltier accessory. The singlet oxygen generation of samples were illuminated by a Fenix UC40 LED lamp (960 lumens maximum output).

1.4 The singlet oxygen generation measurements. The singlet oxygen generation was measured by the $p$-nitroso-$N$,$N$-dimethylaniline (RNO, singlet oxygen sensor)/histidine assay based on the oxidation of histidine by singlet oxygen and the subsequent reaction of the oxidized histidine with RNO as previously described.2, 3 In brief, RNO (50 μM) and histidine (10 mM) were mixed with samples. Then the mixed solutions were illuminated by LED lamp and the UV absorption at 440 nm was detected immediately every several minutes to monitor the consumption of RNO.
Control experiments, only having RNO and histidine, were run by repeating the measurements. Meanwhile, all samples were stable in darkness and no degradation of RNO was observed.

2 Synthesis of EY-HA

![Scheme S1. Synthetic route of EY-HA. (i) 2-bromoethylamine, DMF, 30 °C; (ii) HA, EDC, HoBt, DMSO/double-distilled water (1:1, v/v).](image)

**Compound EY-NH$_2$:** EY-NH$_2$ was synthesized and purified according to the procedure reported previously.$^4$ Briefly, to a suspended solution of EY (1.00 mmol, 0.69 g) in DMF (20 mL) was added 2-bromoethylamine (3.00 mmol, 0.62 g) at room temperature. The mixture was stirred for 24 h at 30 °C and then was concentrated under reduced pressure. The residue was diluted with acetone (20 mL) and filtered off. The volatiles were removed under reduced pressure and recrystallized in methanol to give EY-NH$_2$ as a dark red solid (0.32 g, 44.8%).

$^1$H NMR (400 MHz, D$_2$O, $\delta$): 7.77 (H1, d, $J = 7.34$ Hz, 1H), 7.62 (H2-H3, m, 2H), 7.46 (H4-H5, s, 2H), 7.23 (H6, d, $J = 7.25$ Hz, 1H), 3.62 (H7, t, $J = 6.00$ Hz, 2H), 3.40 (H8, t, $J = 6.00$ Hz, 2H) ppm.
Compound EY-HA: EY-HA was synthesized and purified according to the procedure reported previously. HA (100 mg) dissolved in deionized water (30 mL) was added to a 30-fold molar excess of EY-NH₂. The pH value was adjusted to 6.8 with 0.1 M NaOH. To this solution was added DMSO-double-distilled water (1:1, 1 mL) containing EDC (192 mg) and HoBt (135 mg). The pH of the solution was maintained at 6.8 and the reaction was allowed to proceed overnight. The reaction solution was directly transferred to the dialysis tubing (MWCO = 1 kDa) and dialyzed exhaustively against 100 mM NaCl for 2 days and double-distilled water for several days until the dialysate was not detected the fluorescence signal of EY. Purple spongy-like EY-HA was obtained after freeze-drying. The average number of EY within each HA chain was 3, which was estimated from ¹H NMR of EY-HA and UV absorption of EY-NH₂ (Figure S3).

¹H NMR (400 MHz, D₂O, δ): 7.76 (d, J = 7.59 Hz, 1H, H of EY), 7.62 (m, 2H, H of EY), 7.45 (s, 2H, H of EY), 7.23 (d, J = 7.21 Hz, 1H, H of EY), 4.49 (m, 15.59H, H of HA), 3.56 (m, 91.90H), 3.32 (t, J = 8.03 Hz, 9.53H), 2.20 (s, 26.30H, H of methyl group of HA) ppm.
Figure S2 $^1$H NMR spectrum of EY-HA in D$_2$O (400 MHz, 298 K).

3 The calibration curve of EY-NH$_2$

Figure S3 Representative calibration curve with EY-NH$_2$. a) Obtained absorption spectra, and b) the resulting calibration curve ($\lambda_{obs} = 517$ nm).

References


