Supporting Information

Tumor-Targeted and Enzyme-Responsive Gold Nanorod-based Nanoplatform with Facilitated Endo-lysosomal Escape for Synergetic Photothermal Therapy and Protein Therapy

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Preparation of CTAB-capped Au nanorods (AuNRs@CTAB)

To synthesize AuNRs capped with a layer of cetyltrimethylammonium bromide (CTAB) (AuNRs@CTAB), a modified method of a previously reported was used. In a first step, a 0.2 M CTAB solution (5 mL) and a 0.0005 M $HAuCl_4$ solution (5 mL) were mixed by gentle vortexing. Then, a 0.01 M NaBH4 solution (0.6 mL) in cold de-ionized water (DIW) was dropped into the mixture and vigorously stirred for 2 mins to obtain a brownish seed solution. Secondly, a growth solution was obtained by mixing a 0.2 M CTAB solution (5 mL), a 0.001 M $HAuCl_4$ solution (5 mL), a 0.01 M AgNO3 solution (100 μL), and a 0.1 M L-ascorbic acid solution (55 μL). Next, the as-prepared seed solution (100 μL) was gradually introduced into the growth solution while gently agitating it. The mixture was subsequently incubated at a temperature of 30 °C for a duration of 3 hours, resulting in the formation of AuNRs@CTAB. Finally, AuNRs@CTAB were collected and subjected to multiple cleaning–centrifugation cycles to remove excess CTAB, followed by lyophilization.

Synthesis of HA-mPEG-Deta-LA polymer ligand

In order to synthesize the transitional polymer HA-mPEG-Deta, an amidation reaction was performed to conjugate mPEG-NH_2 and diethylenetriamine (Deta) onto the backbone of HA. Initially, mPEG-NH_2 (1g, 0.2 mmol) was conjugated with HA (1g, 0.11 mmol) in a solution of 20 mL deionized (DI) water, along with coupling agents (EDC/NHS in a molar ratio of 1:1). This reaction took place at room temperature under a nitrogen environment. After an 8-hour reaction period, the resulting solution was gradually added drop by drop into 10 mL of DI water containing 6.2 mL (57.70 mmol; 21 times the molar ratio to the repeating unit of HA) of Deta. The mixture was continuously reacted for another 8 hours. Subsequently, the mixture was subjected to dialysis against a 0.01 M HCl solution for one day, followed by deionized (DI) water for two days, using a MW cut-off (MWCO) of 3,500 Da. Finally, the product was lyophilized to obtain the HA-mPEG-Deta transitional polymer with a yield of 90%. (^{1}H NMR, 500 MHz, D_2O). The final HA-mPEG-Deta-LA polymer ligand is obtained by modifying the residual amino groups of Deta in the sidechain of HA with LA as an anchor. Briefly, the carboxyl group of LA is activated using CDI. The molar ratio of carboxyl groups to CDI is 1:1, and this reaction is performed in a solution of DMSO. Next, the activated LA solution is stirred for 2 hours at 25 °C under a nitrogen (N_2)

atmosphere. After that, the activated LA solution is added to a 10 mL deionized water (DIW) containing 200 mg HA-mPEG-Deta prepolymers. The mixture is stirred constantly for an additional 8 hours under identical conditions. To purify the HA-mPEG-Deta-LA polymer ligand, it is dialyzed against excess DIW. A dialysis membrane with a molecular weight cutoff of 3000 Da is used for this process. The dialysis lasts for 3 days and is followed by lyophilization.

Characterizations

The Varian Unity Inova 500NB NMR spectrometer (500 MHz) was utilized, with D_2O or DMSO-d6 as the solvent, to calculate the molecular weight (MW) of HA-mPEG-Deta-LA and the degree of grafting of each component. For the examination of morphological structures, a high-resolution transmission electron microscopy (HR-TEM) technique was employed. The naked AuNRs@CTAB and AuNRs@HA-mPEG-Deta-LA nanoparticles were imaged using a JEM-ARM 200F microscope operating at 120 kV. The element assay of AuNRs@HA-mPEG-Deta-LA nanoparticles were performed by the TEM-EDS. The crystal structure of AuNRs@HA-mPEG-Deta-LA nanoparticles had been confirmed by powder X-ray diffractometer (XRD) using Ultima IV X-ray powder diffractometer (Rigaku Co., Japan). The UV-vis absorbance measurements of the samples were conducted using a V-630 Bio UV-vis spectrophotometer. Infrared (IR) spectrums of polymer ligand, AuNRs@CTAB and AuNRs@HA-mPEG-Deta-LA nanoparticles were recorded FT-IR spectrophotometer (JASCO, 5300). Furthermore, the hydrodynamic size and zeta potential of both AuNRs@CTAB and AuNRs@HA-mPEG-Deta-LA nanoparticles were monitored at 25 °C using a Zetasizer-ZS90 dynamic light scattering (DLS) instrument manufactured by Malvern Instruments based in the U.K.

Analysis of physiological stability of AuNRs@HA-mPEG-Deta-LA in an in vitro setting

The physiological stability of AuNRs@HA-mPEG-Deta-LA was assessed by monitoring the precipitation or aggregation changes using visual white-light images technology in various media, such as PBS, Foetal Bovine Serum (FBS) and RPMI-1640 solution, at predetermined time intervals.

Loading and triggered in vitro release of protein

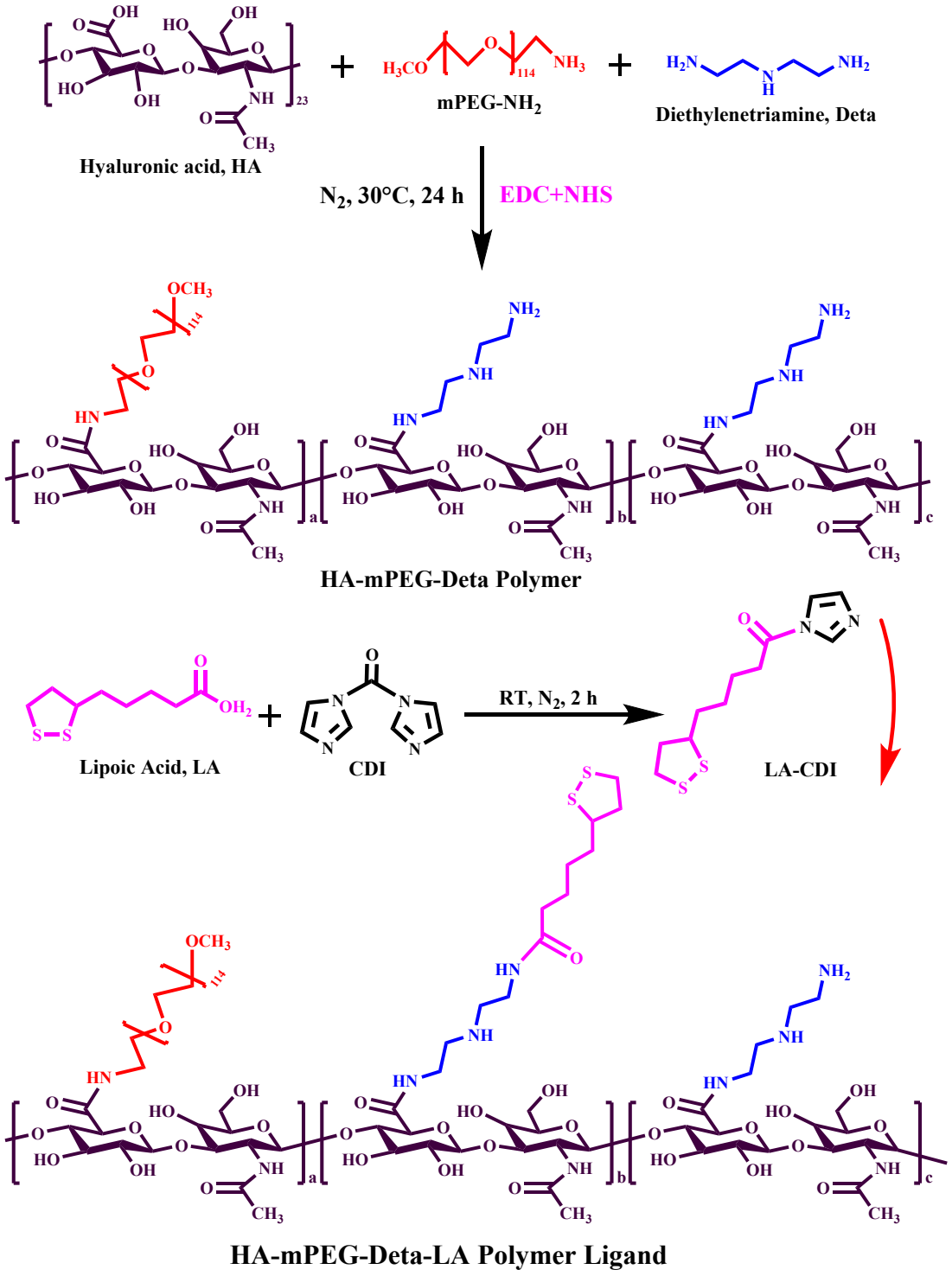
In this experiment, CC was used as a protein drug model. Cy5-CC or CC were placed in 10 mL of PBS solution that contained a specific quantity of AuNRs@HA-

mPEG-Deta-LA nanoparticles. The mass ratio of CC or Cy5-CC to AuNRs@HA-mPEG-Deta-LA nanoparticles were maintained at 20 wt %. The mixture solution was then stirred for 2 hours in a dark environment. After that, the superfluous CC or Cy5-CC was eliminated through dialysis (molecular weight cut-off = 300 kDa) against an abundant amount of PBS (10 mM, pH 7.4). The purified Cy5-CC-loaded AuNRs@HA-mPEG-Deta-LA nanoparticles were then obtained after freeze-drying. The loading content (LC) and efficiency (LE) of CC were determined by subtracting the amount of unloaded CC from the total amount of CC. This calculation was performed using a calibration UV-Vis absorbance curve that had known concentrations of Cy5-CC. The LC and LE were determined using the utilizing equations:

LC% = (amount of loaded protein/amount of protein-loaded AuNRs@HA-mPEG-Deta-LA×100%

LE% = (amount of loaded protein/total amount of feeding protein)×100%

The in vitro release of Cy5-CC from Cy5-CC-loaded AuNRs@HA-mPEG-Deta-LA was achieved using dialysis method. In this process, a specific quantity of Cy5-CC-loaded AuNRs@HA-mPEG-Deta-LA were distributed in 5 mL of PBS (pH 7.4), either with or without 100 U/mL of hyaluronidase. Subsequently, the mixture was then transferred into a dialysis membrane with a MWCO of 300 kDa. 3 mL of the medium was periodically withdrawn at specific time intervals and substituted with an equivalent volume of fresh medium. The amount of released CC was determined using a UV/vis spectrometer by measuring the absorption peak at 650 nm.



Scheme S1. Synthetic route for the preparation of HA-mPEG-Deta transitional polymer ligand and targeted HA-mPEG-Deta-LA polymer Ligand.

Table S1. Structural characteristics of the synthetic HA-mPEG-Deta-LA polymer ligand

polymer	DP (HA)	CR (mPEG)[a]	CR (Deta)[b]	CR (LA)[c]	Mn[d]
HA-mPEG-Deta-LA	~23	~2	~21	~6	~21900

[a]Cojugating ratio of mPEG according to the ^{1}H-NMR results.

[b]Cojugating ratio of Deta according to the ^{1}H-NMR results.

[c]Cojugating ratio of LA according to the ^{1}H-NMR results.

[d]Calculated from ^{1}H-NMR.

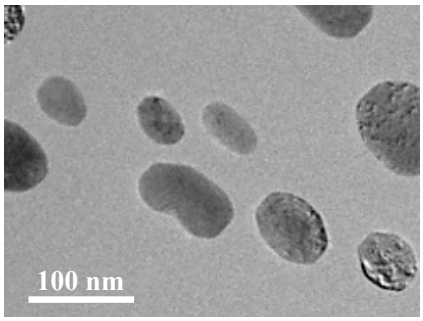


Figure S1. TEM image of AuNRs@HA-mPEG-Deta-LA at pH7.4.

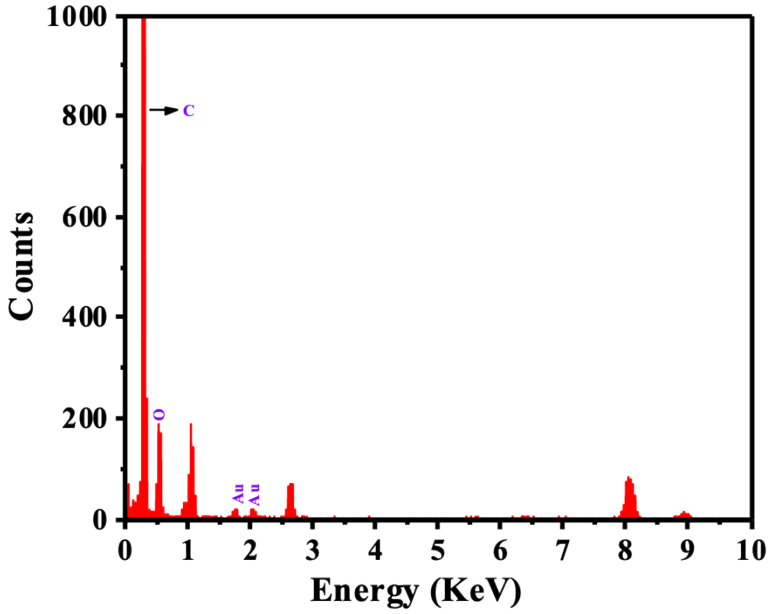


Figure S2. EDS analysis of the AuNRs@HA-mPEG-Deta-LA.

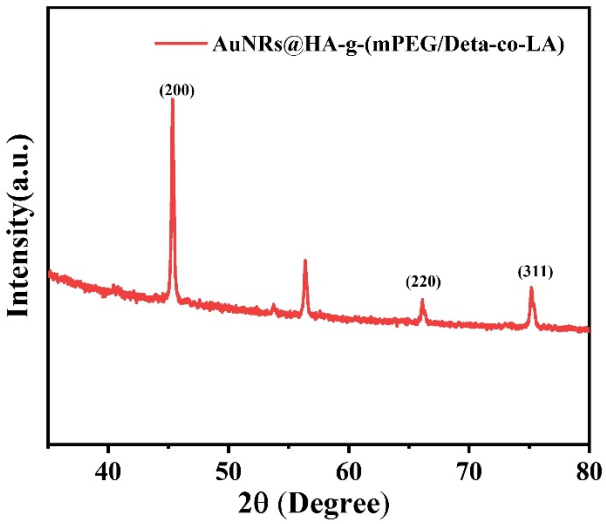


Figure S3. XRD pattern of the AuNRs@HA-mPEG-Deta-LA.

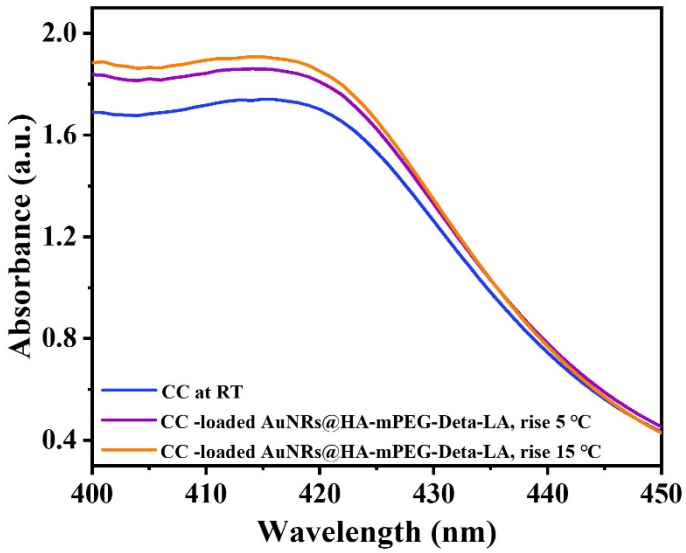


Figure S4. Protein active assay of CC-loaded AuNRs@HA-mPEG-Deta-LA-CC by raising the temperature by 5 or 15 °C under 808 nm laser irradiation (a power density of 2 W/cm^2).