Supporting Information

Multifunctional Hyaluronic Acid Ligand-Assisted Construction of CD44-and Mitochondria-Targeted Self-Assembled Upconversion Nanoparticles for Enhanced Photodynamic Therapy

Ze Hao Liu [a], Xin Wang Mo [a], Wei Jiang [a], Changling Liu [a], Yue Yin* [,b], Hong Yu Yang *, [a], Yan Fu *, [a]

[a]College of Materials Science and Engineering, Jilin Institute of Chemical Technology, Jilin City 132022, Jilin Province, PR China

[b]School of Medical Technology, Beijing Institute of Technology, Beijing 100081, PR China.

Corresponding authors

E-mail addresses:

yinyue@bit.edu.cn

yanghy@jlict.edu.cn (H.Y. Yang),

fuyanqd@163.com (Y. Fu)

S1.1. The synthesis of HA-c-mPEG-Deta-g-(Ce6-LA-TPP) polymer ligand.

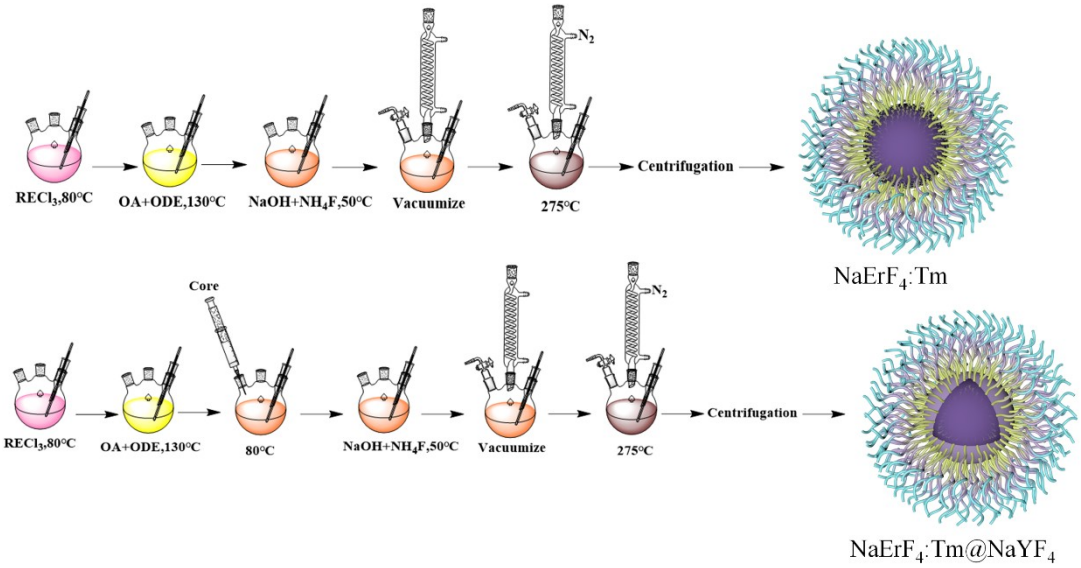
To begin, the intermediate polymer ligand (HA-c-mPEG-Deta) was synthesized by conjugating mPEG-NH_2 and Deta to the side chain of HA via sequential amidation reactions, utilizing EDC and NHS as effective coupling agents. In briefly, 1g of HA was activated in 15 ml of deionized (DI) water using a 1:1 molar ratio of EDC and NHS, which took place under nitrogen (N_2) protection and at RT for 2 hours. After activation, 1.1g of mPEG-NH_2 solution was added to the mixture, and the reaction was allowed to continue for another 24 hours under similar conditions. Subsequently, a solution of 6.21mL (57.69 mmol) of Deta dissolved in 10ml of DI water was dropwise added and allowed to sequentially react for another 24 hours. After the reaction was completed, the mixture was passed through a membrane filter with a molecular weight cut-off (MWCO) of 7000 Da. Dialysis was carried out for three days using ample amounts of DI water to remove impurities and excess reactants. Lastly, the solution was freeze-dried, yielding HA-c-mPEG-Deta with a 90% purity. Afterward, the amino groups persisting in the side chain of HA-c-mPEG-Deta were utilized to conjugate three distinct functional molecules: the photosensitizer Ce6, the mitochondrial targeting ligand TPP, and the LA precursor anchors. Briefly, Ce6 (0.0334 g, 0.16 mmol), TPP (0.0435 g, 0.3785 mmol), and LA (0.0876 g, 0.54 mmol) were individually activated using DCC and NHS as a condensing agent at a molar ratio of 1:1 in 5 mL of DMSO at RT for 2 hours. After activation, three reaction solutions mentioned earlier were individually introduced to 10 mL of DI water containing 100 mg of the HA-c-mPEG-Deta polymer. This reaction was conducted in a dark environment at RT for 24 hours. Subsequently, the final HA-c-mPEG-Deta-g-(Ce6-LA-TPP) polymer ligand was isolated through dialysis against an excess of DI water and further lyophilized. Yield:

83 (^{1}H NMR, 500 MHz, D_2O). It is noteworthy that the synthesis of the polymer ligand without grafted TPP, namely HA-c-mPEG-Deta-g-(Ce6-LA), closely followed the same method as that used for the HA-c-mPEG-Deta-g-(Ce6-LA-TPP) polymer ligand. This prepared non-mitochondria-targeted HA-c-mPEG-Deta-g-(Ce6-LA) polymer ligand was used as a control group.

S1.2. Characterization.

The chemical structures of the polymer ligands were verified at each stage through the utilization of ^{1}H nuclear magnetic resonance (^{1}H NMR) spectroscopy, operating at a frequency of 500 MHz, using a Varian Unity Inova 500NB spectrometer. Transmission electron microscopy (TEM, JEM-ARM 200F) was employed to observe the morphological structures and elemental distribution mappings of UCNPs and CMPNs, operating at an acceleration voltage of 120 kV. Additionally, the crystal structure of UCNPs was confirmed by utilization an X-ray diffractometer (XRD). The hydrodynamic diameters of both UCNPs and CMPNs were accurately measured using a Zetasizer-ZS90 dynamic laser scanning (DLS), maintained at a temperature of 25 °C (Malvern Instrument, U.K.). The in vitro physiological stability of CMPNs incubating in diverse biological media including PBS (pH 7.4), 10% FBS, and RPMI-1640 medium was monitored using DLS for a duration of 24 hours using DLS. The chemical compositions of UCNPs and CMPNs were comprehensively analyzed by Fourier transform infrared spectroscopy (FTIR). UV/visible absorbance measurements were performed using a V-630 Bio UV−vis spectrophotometer, enabling the confirmation of the chemical compositions of UCNPs and CMPNs. Additionally, these measurements were used to estimate the grafted number of Ce6 molecules on the polymer ligand and CMPNs based on calibration curve data obtained from free Ce6. Fluorescence spectroscopy of CMPNs, prepared with varying molar ratios of Ce6 to UCNPs, were

recorded using Fluoromax 4 spectrofluorometer, with excitation at a wavelength of 980 nm.



Scheme S1. Synthesis process of $NaErF_4$:Tm core UCNPs and $NaErF_4$:Tm@$NaYF_4$ core-shell UCNPs using typical hydrothermal methods.

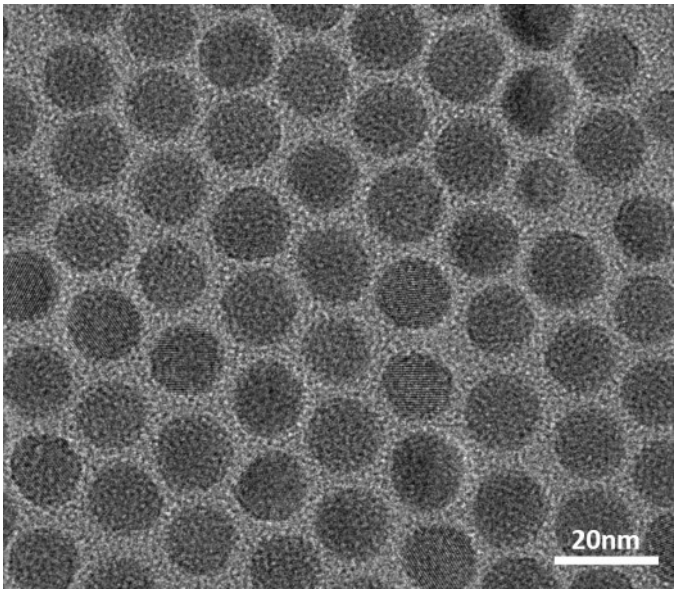


Figure S1. TEM images of $NaErF_4$:Tm core UCNPs.

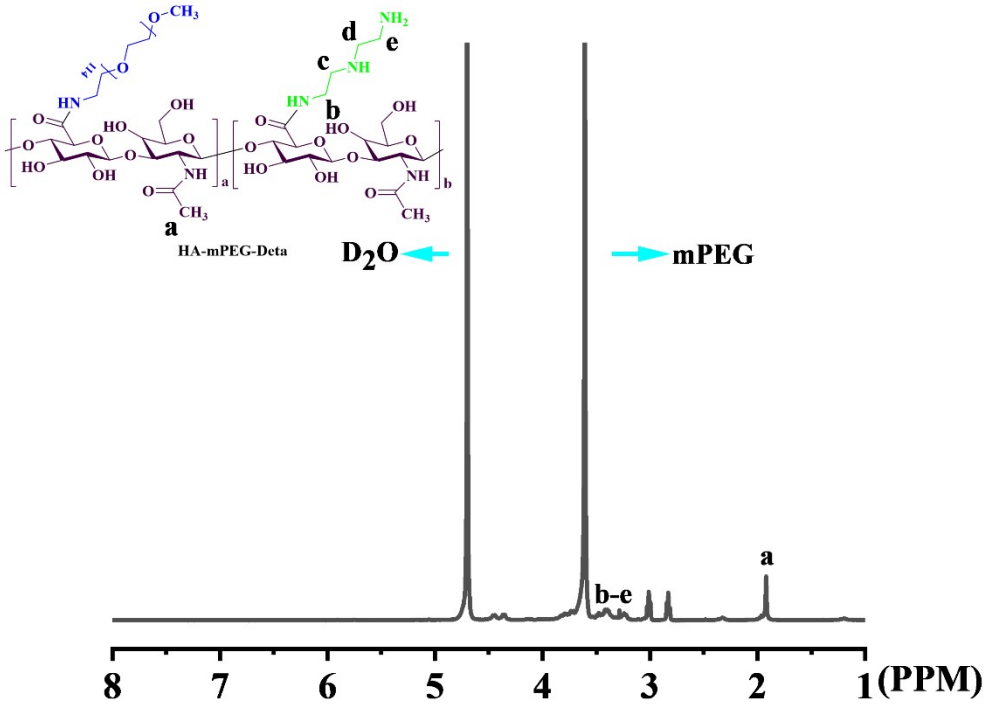


Figure S2. ^{1}H NMR spectrum (500 MHz) of HA-mPEG-Deta ligand polymer in D_2O.

Table S1. Structural characteristics of the prepared HA-c-mPEG-Deta-g-(Ce6-LA-TPP) polymer ligand.

Polymer Ligand	DP (HA)	CR (mPEG)[a]	CR (Deta)[b]	CR (LA)[c]	CR (Ce6)[d]	CR (TPP)[e]	Mn[f]
HA-c-mPEG-Deta-g-(Ce6-LA-TPP)	23	~2	~18	~5	~1	~3	~23300

[a]Number of conjugations of mPEG based on the ^{1}H-NMR results.

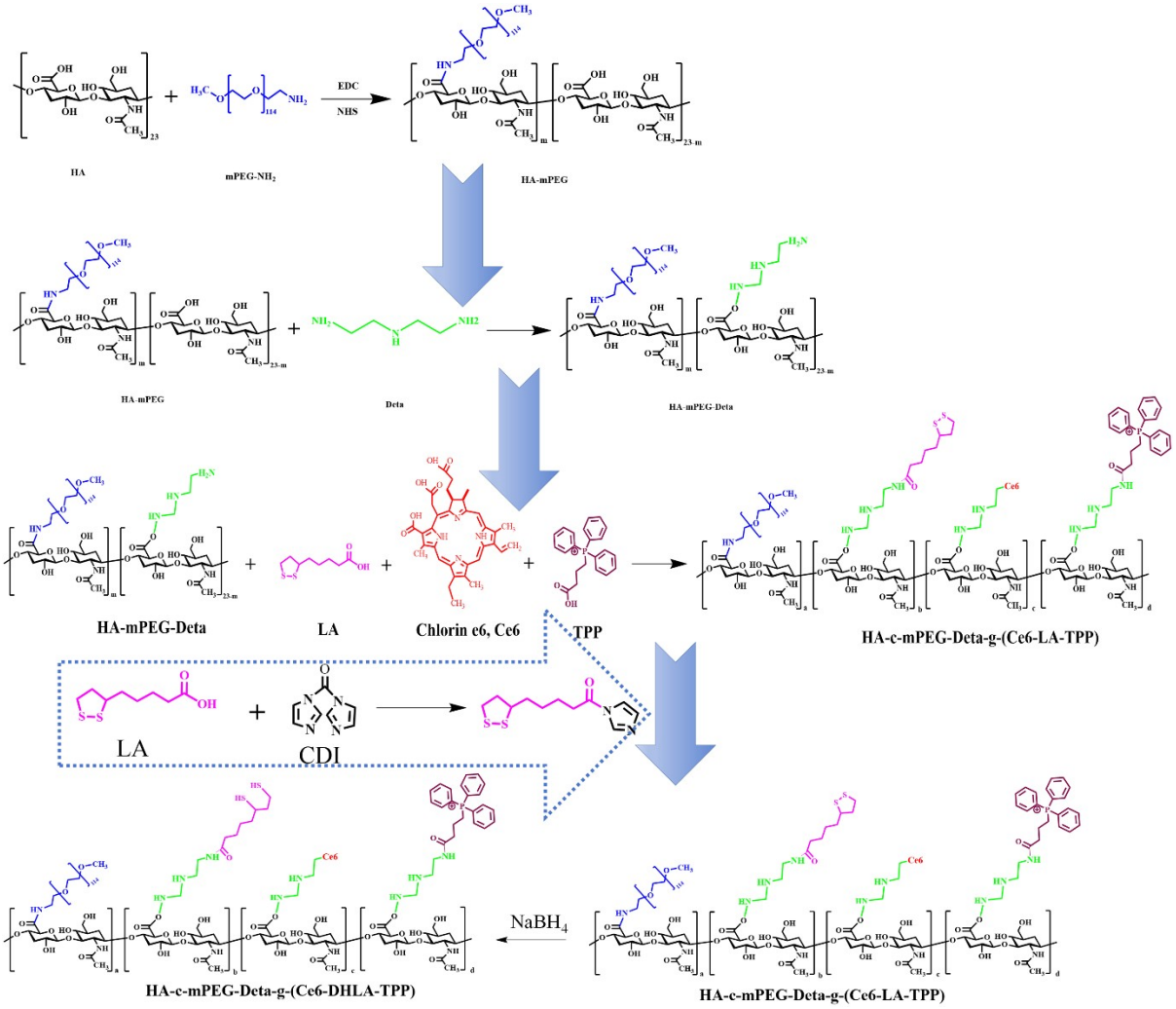
[b]Number of conjugations of Deta based on the ^{1}H-NMR results.

[c]Number of grafts of LA based on the ^{1}H-NMR results.

[e]Number of grafts of TPP based on the ^{1}H-NMR results

[d]Number of grafts of Ce6 according to the UV-vis spectroscopy.

[f]Calculated of Mn from ^{1}H-NMR.



Scheme S2. The synthesis route of HA-c-mPEG-Deta-g-(Ce6-DHLA-TPP) polymer ligand.

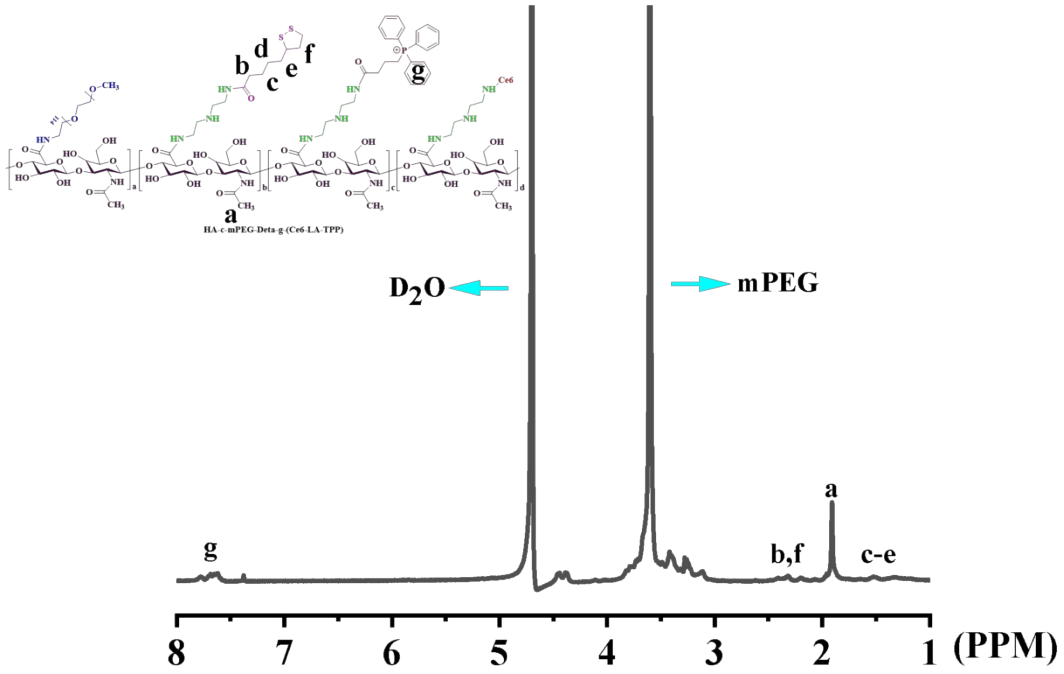


Figure S3.[1]H NMR spectrum (500 MHz) of HA-c-mPEG-Deta-g-(Ce6-LA-TPP) ligand polymer in D_2O.

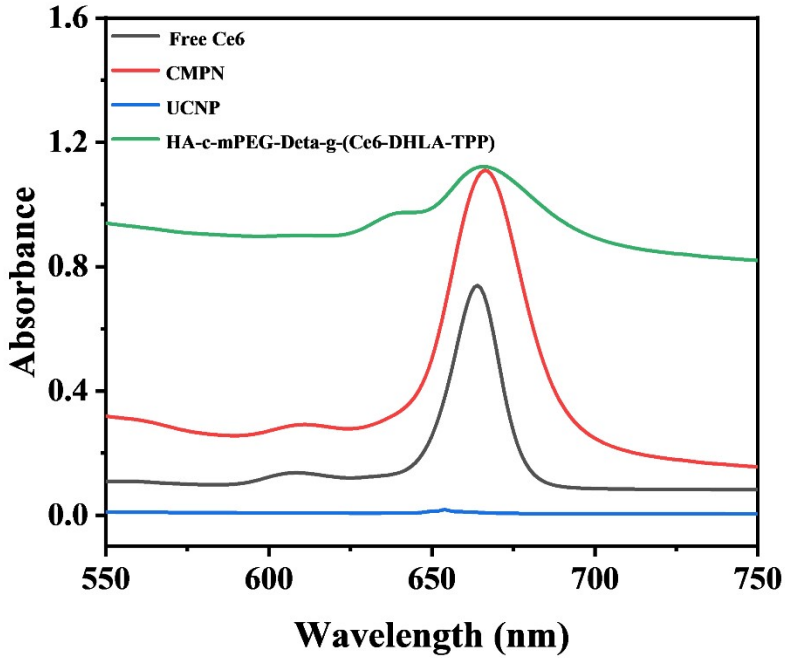


Figure S4. UV-vis absorbance spectra of free Ce6, HA-c-mPEG-Deta-g-(Ce6-DHLA-TPP), UCNP and CMPN.

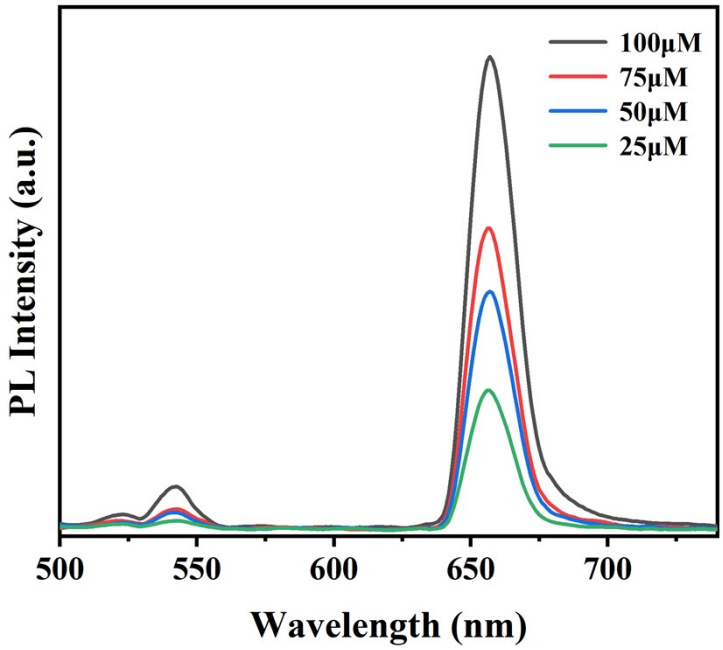


Figure S5. PL emission spectrum of CMPN complexes under different Ce6 loading concentrations.