Electronic Supplementary Material (ESI) for Nanoscale.
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Supplementary information for

Photoacoustic Probe for Monitoring and Ratiometric Imaging of Hydrogen Sulfide in Vivo

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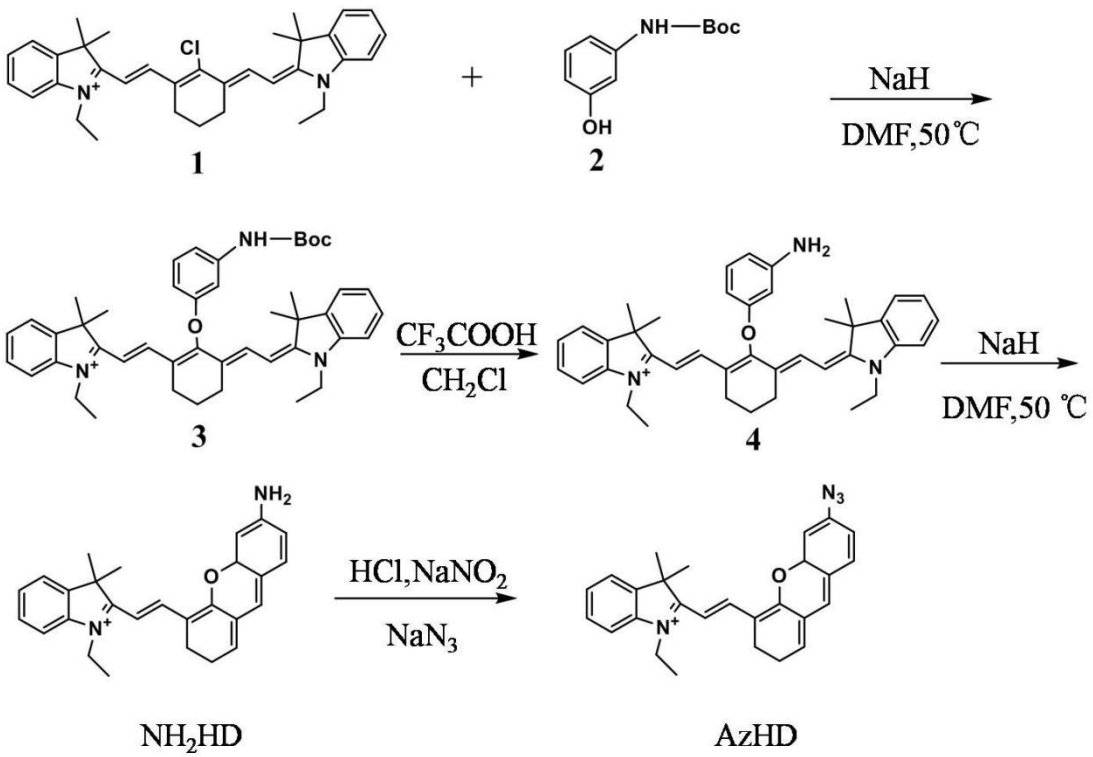

Figure S1 Synthetic route of AzHD

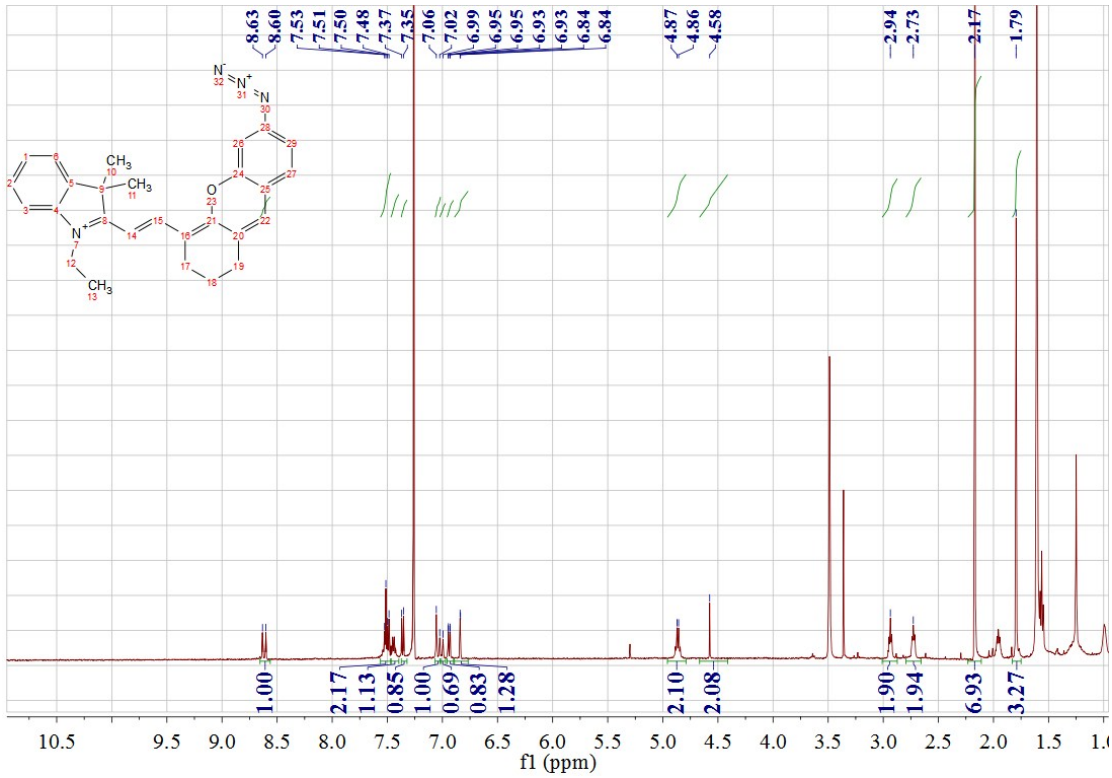

Figure S2 ^{1}H NMR spectrum of the NH_2HD in $CDCl_3$.

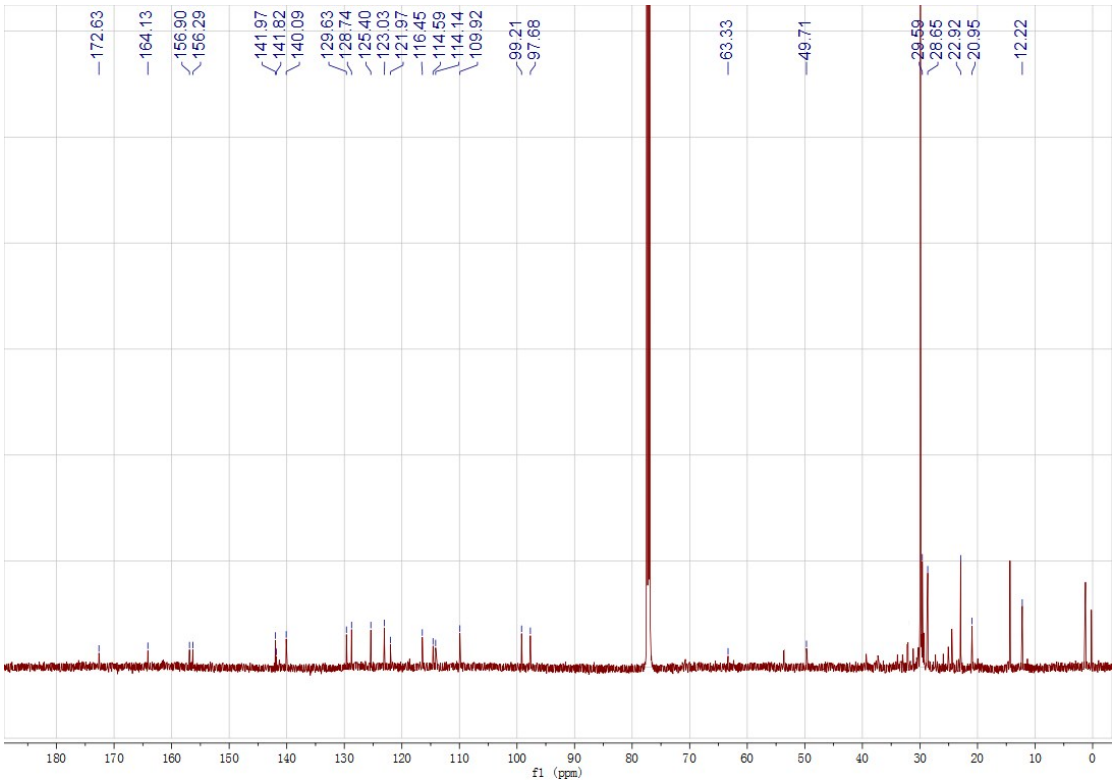

Figure S3 ^{13}C NMR spectrum of the NH_2HD in $CDCl_3$.

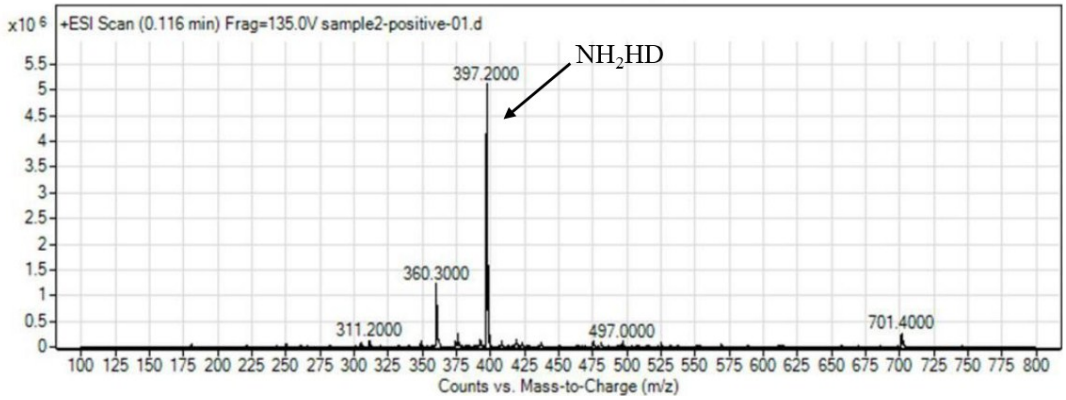

Figure S4 LC-MS of NH_2HD.

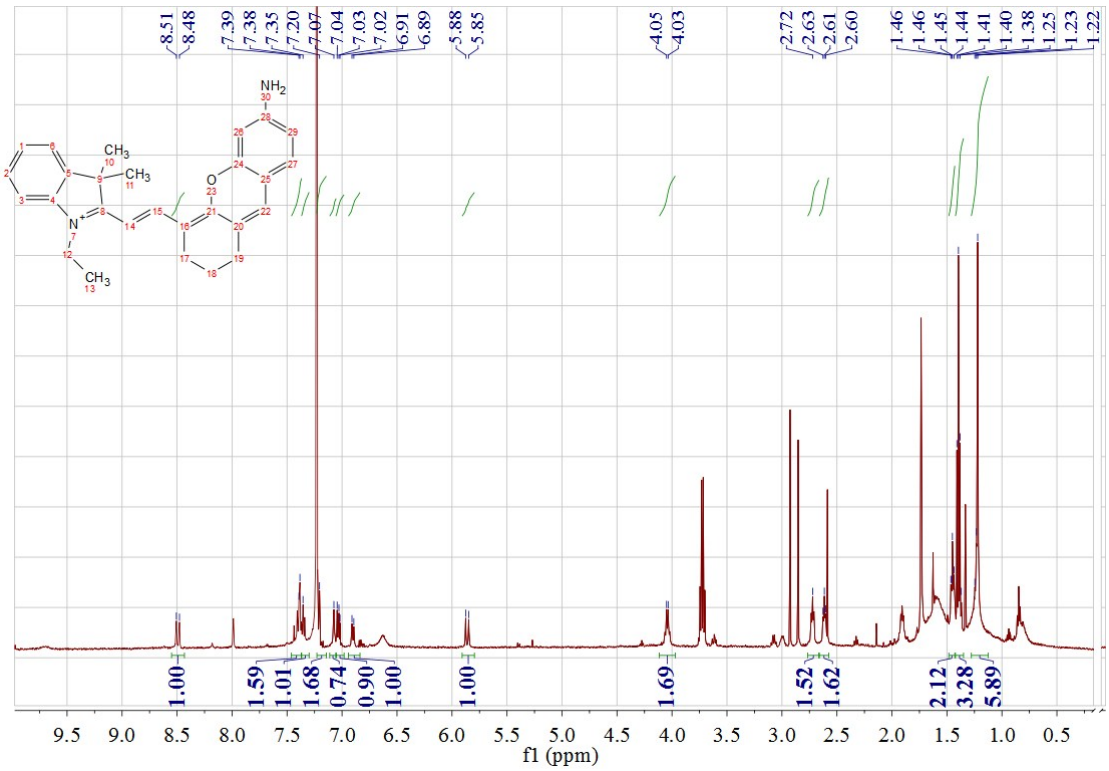

Figure S5 ^{1}H NMR spectrum of AzHD in $CDCl_3$.

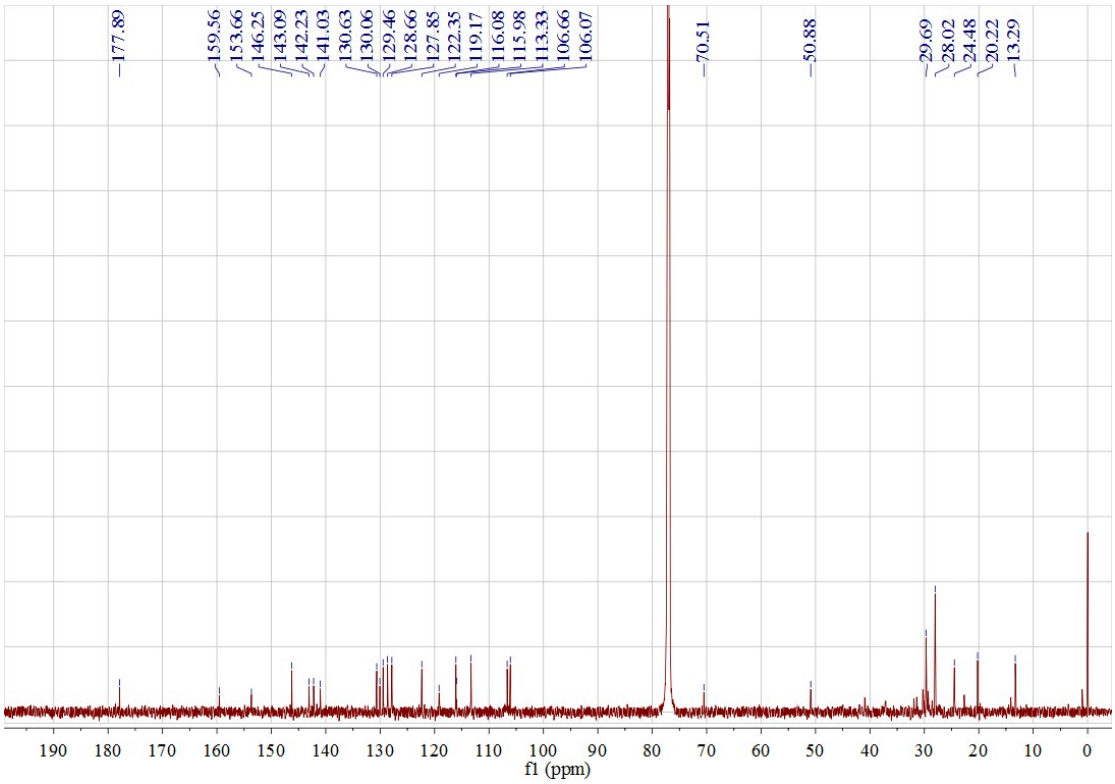

Figure S6 ^{13}C NMR spectrum of the AzHD in $CDCl_3$.

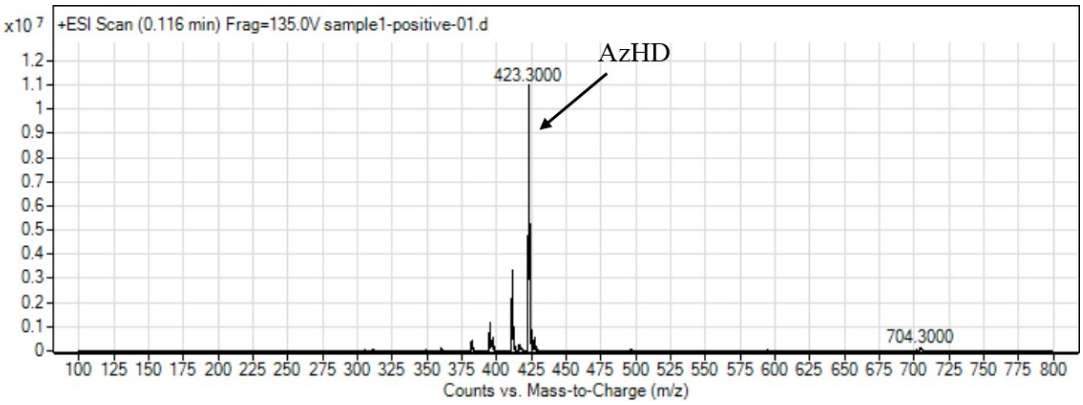

Figure S7 LC-MS of AzHD.

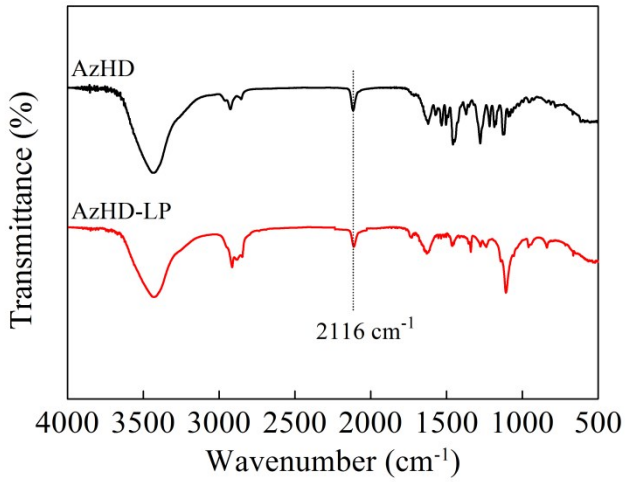

Figure S8 FT-IR transmission spectra of AzHD and AzHD-LP. Both of them have a common characteristic peak for the azido group at 2116 cm^{-1}.[ref1]

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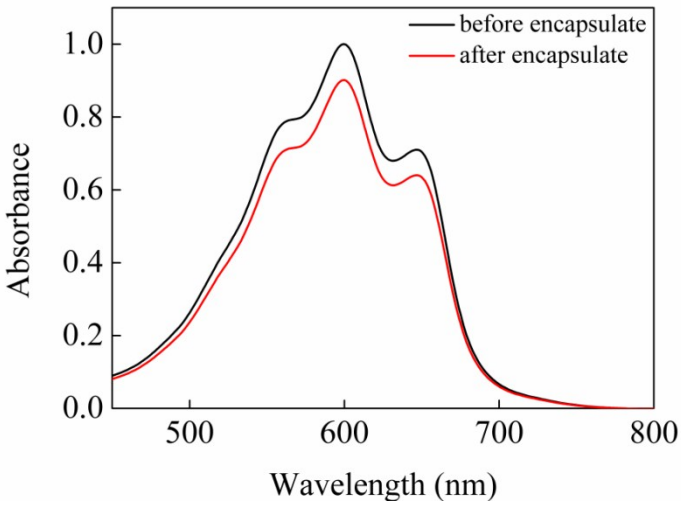

Figure S9 Encapsulation efficiency of AzHD within the nanoparticles AzHD-LP.

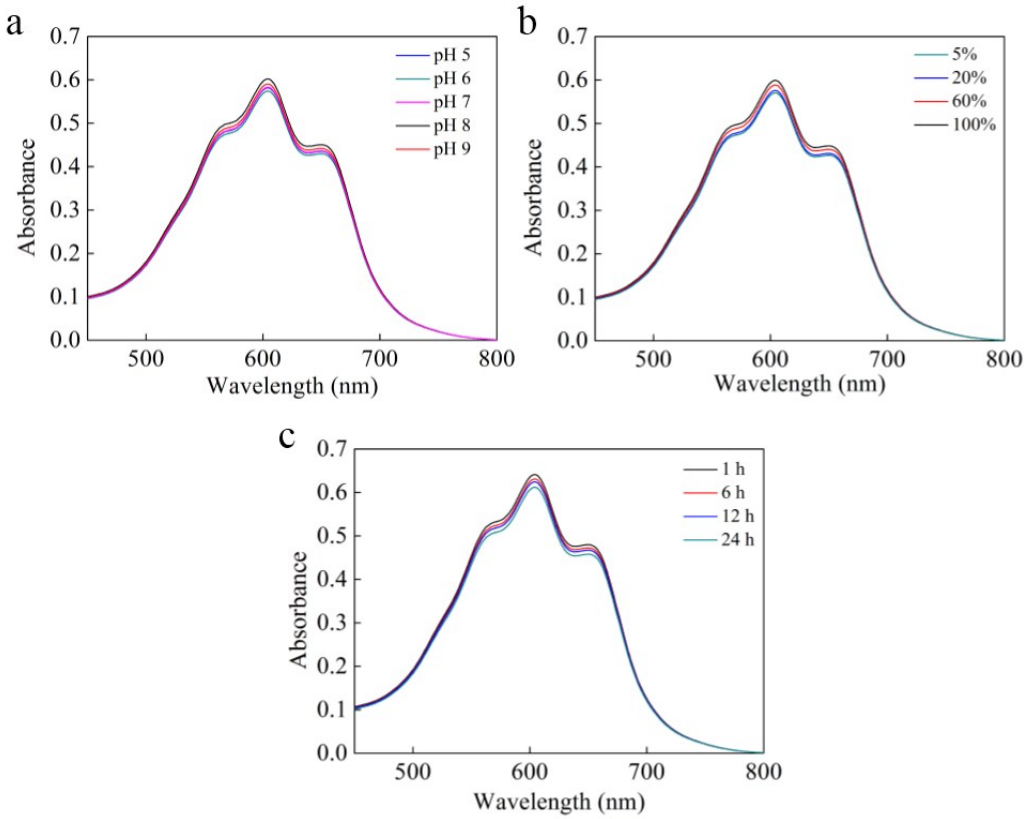

Figure S10 Absorption changes of AzHD-LP in (a) different pH (from 5 to 9), (b) different serum concentration (from 5 to 100%) and (c) 100% serum keeping for various time-points (from 1 to 24 h).

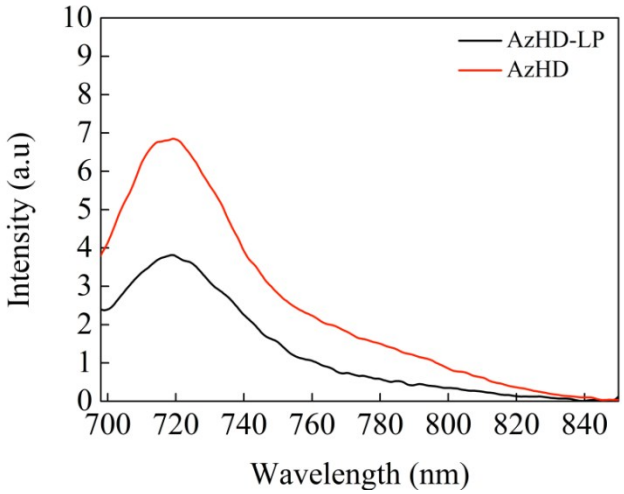

Figure S11 Fluorescence spectra of AzHD (20 μM) and AzHD-LP (30 μg/mL).

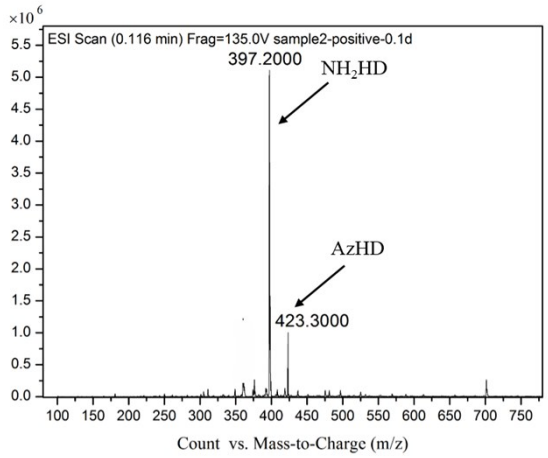

Figure S12 LC-MS of AzHD in the presence of NaSH.

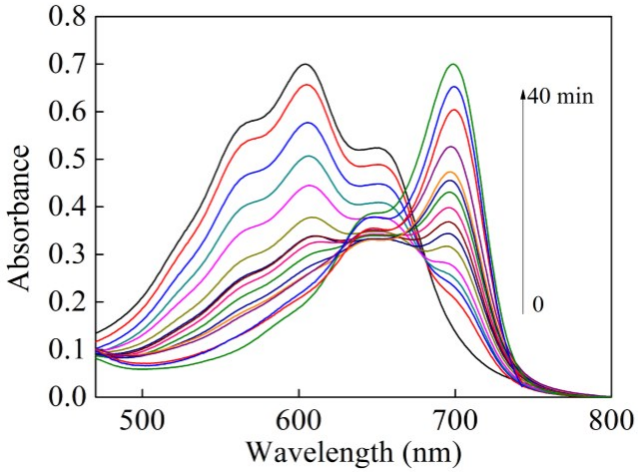

Figure S13 Time-dependent absorption spectra changes of AzHD-LP (20 μM) in the presence of NaHS (100μM).

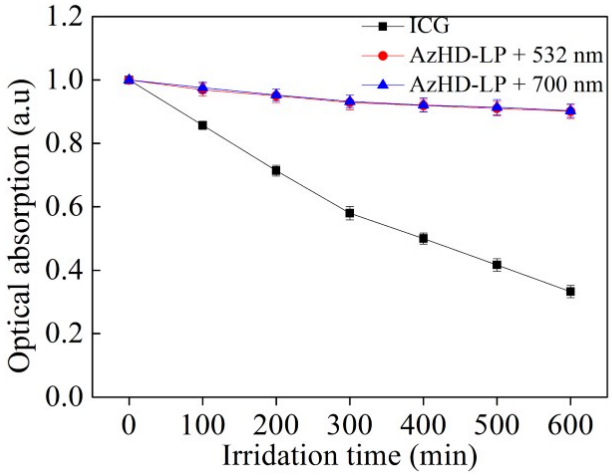

Figure S14 Photostability of AzHD-LP under the irradiation of 532-nm and 700-nm pulsed laser (0.1 W/cm^2) and ICG under the irradiation of 780 nm pulsed laser (0.1 W/cm^2).

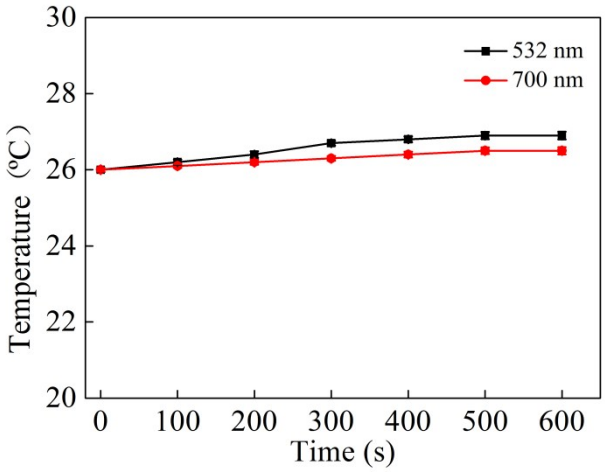

Figure S15 Temperature changes of AzHD-LP solution (200 μg/ml) under irradiation of 532-nm and 700-nm pulsed laser (0.1 W/cm^2).

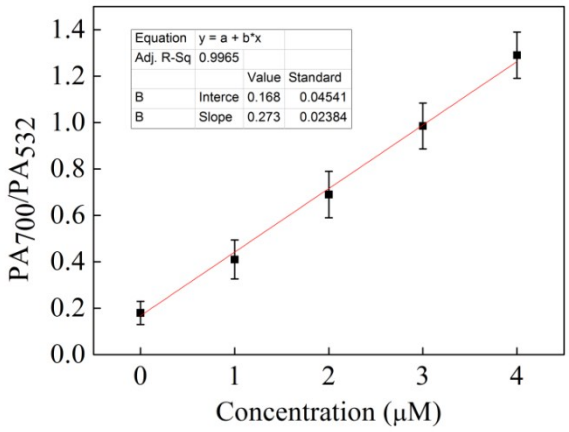

Figure S16 The ratio of PA700/PA532 linearly related to the concentrations of NaSH (0 ~ 4 μM). Linear regression equation: PA700/PA532 = 0.168 + 0.27368 × [NaSH] (μM), R^2 = 0.9965.

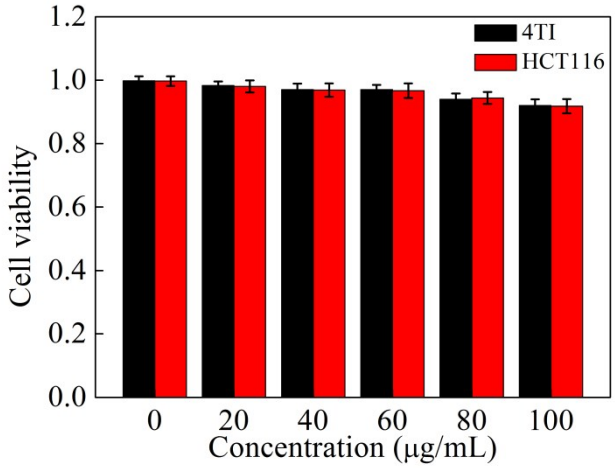

Figure S17 The cell viability of 4T1 cells and HCT116 cells treated with AzHD-LP.

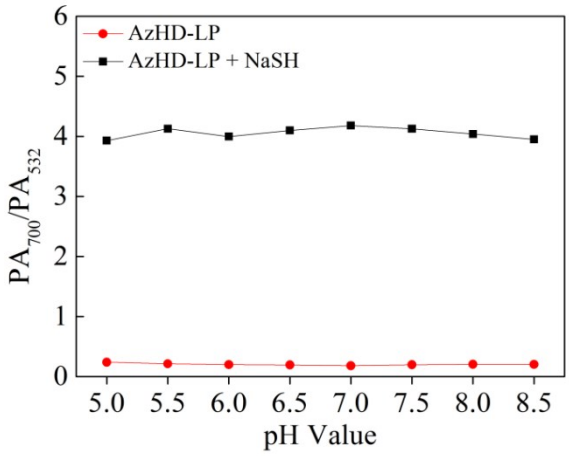

Figure S18 The ratio of PA700/PA532 of AzHD-LP (20 μM AzHD) in the presence and absence of NaHS (100 μM) in physiological environment pH.

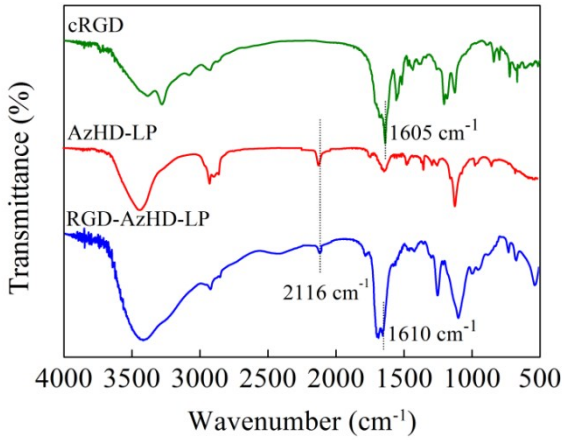

Figure S19 FT-IR transmission spectra of cRGD, AzHD-LP and RGD-AzHD-LP. AzHD-LP and RGD-AzHD-LP exhibited the same characteristic peak for the azido group at 2116 cm^{-1}. The 1605 cm^{-1} band observed in the cRGD-AzHD-LP spectrum was assigned to the arginine guanidinium group ($CN_3H_5^+$ stretch) of RGD, which was also observed in the RGD spectrum (1610 cm^{-1}).[ref2]

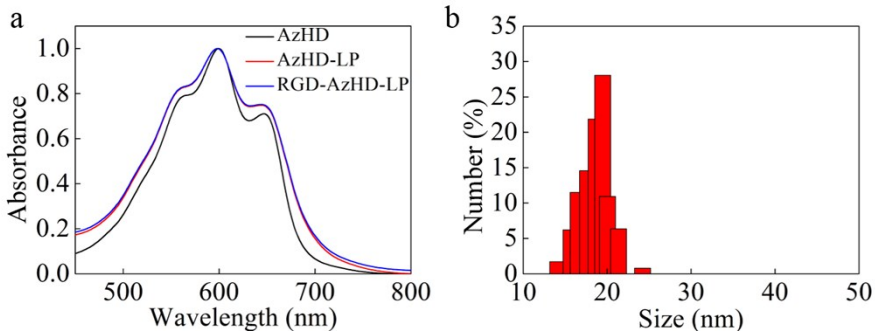

Figure S20 (a) Absorption spectra of AzHD, AzHD-LP and RGD-AzHD-LP; (b) DLS result of RGD-AzHD-LP.

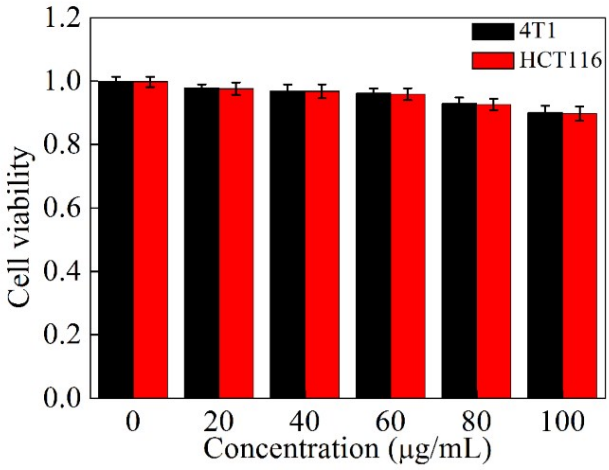

Figure S21 The cell viability of 4T1 cells and HCT116 cells treated with RGD-AzHD-LP.

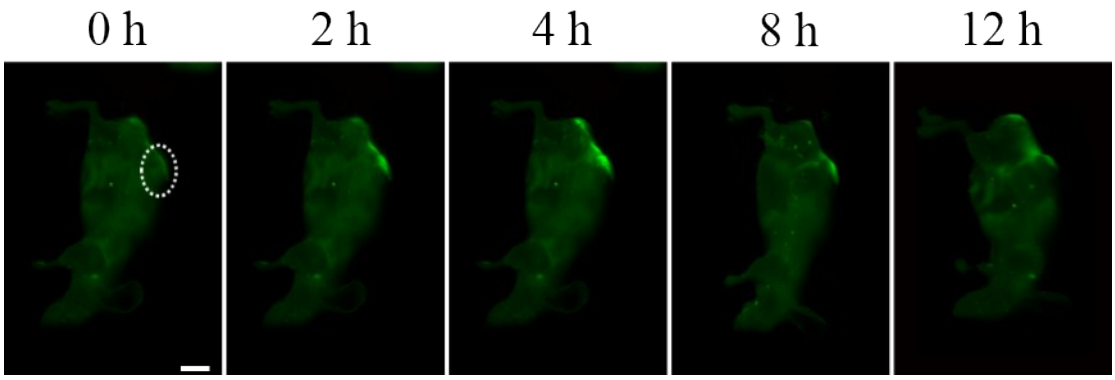

Figure S22 In vivo NIR fluorescent imaging of HCT116 tumor-bearing mice were taken at different times after intravenous injection of RGD-AzHD-LP. The fluorescence signals were collected at λ_{em} = 720 nm under excitation of 680-nm continuous laser. Scale bar: 1 cm.

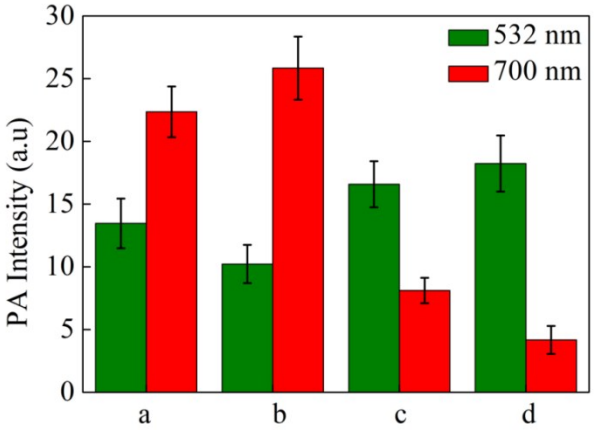

Figure S23 PA intensity of each group at 4 h after injected RGD-AzHD-LP through tail vein: a, RGD-AzHD-LP only; b, pre-injected 300 nmol SAM for 12 h in the tumor region; c, pre-injected 100 nmol AOAA for 12 h in the tumor region; d, $ZnCl_2$-treated mice in tumor region (n = 4 per group).

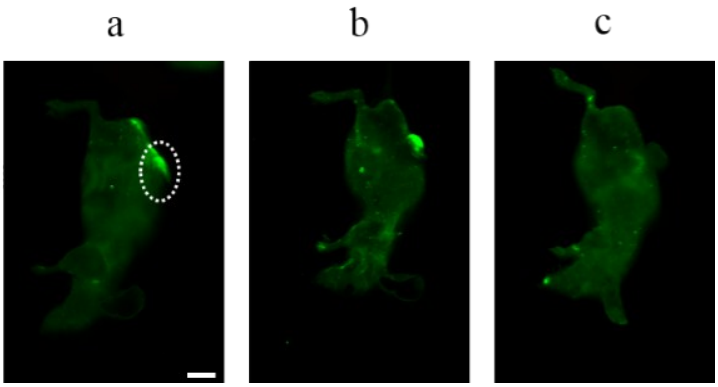

Figure S24 Fluorescence images of each group at 4 h after injected RGD-AzHD-LP through tail vein: a, RGD-AzHD-LP only; b, pre-injected 300 nmol SAM for 12 h in the tumor region; c, pre-injected 100 nmol AOAA for 12 h in the tumor region. The fluorescence signals were collected at λ_{em} = 720 nm under excitation of 680-nm continuous laser. Scale bar: 1 cm.

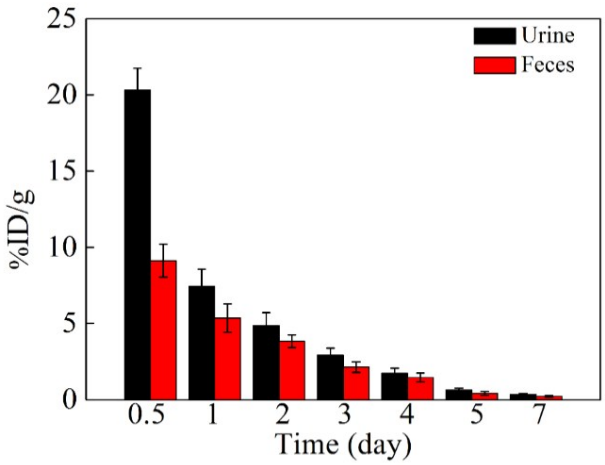

Figure S25 Excretion profile of RGD-AzHD-LP.

References

1. S. Goldmann Anja, C. Schödel, A. Walther, J. Yuan, K. Loos and H. E. Müller Axel, *Macromol. Rap. Commun*., 2010, **31**, 1608-1615.
2. E. Morales-Avila, G. Ferro-Flores, B. E. Ocampo-García, and L. M. Gómez-Oliván, *J. Biomed. Nanotech*., 2012, **8**, 991-999.