Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
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Supporting Information for

A t-PA/nanoparticle conjugate with fully retained enzymatic activity and prolonged circulation time

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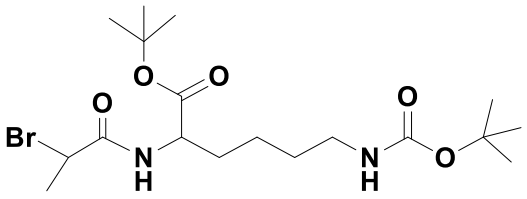
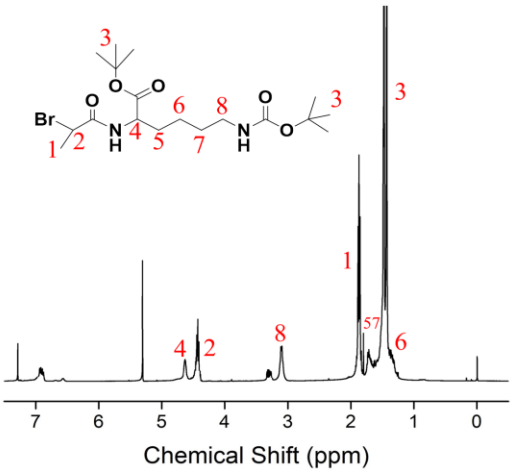
1. Characterization of RAFT agent

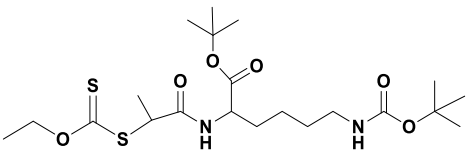


Chemical Formula: $C_{18}H_{33}BrN_2O_5$
Exact Mass: 436.16
Molecular Weight: 437.38

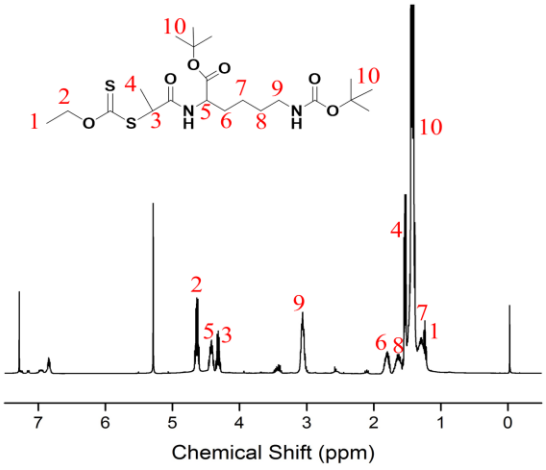


Compound 1

^{1}H NMR (400 MHz,$CDCl_3$) δ: 1.31 (m, 2H, –CH–CH_2–C***H*****$_2$**–); 1.42–1.47 (m, 18H, –C(C***H*****$_3$**$)_3$–); 1.71 (m, 2H, –CH–$(CH_2–)_2$–C***H*****$_2$**–); 1.80 (m, 2H, –CH–C***H*****$_2$**–); 1.91 (m, 3H, C***H*****$_3$**CH(Br)-CH_2–); 3.09 (m, 2H, –C***H*****$_2$**–); 4.44 (m, 1H, CH_3C***H***(Br)-CH_2–); 4.63 (s, 1H, –NHC***H***–).



Chemical Formula: $C_{21}H_{38}N_2O_6S_2$
Exact Mass: 478.22
Molecular Weight: 478.66



Compound 2

^{1}H NMR (400 MHz,CDCl3) δ: 1.24 (m, –C***H*****$_3$**); 1.39 (m, 2H, –CH–CH_2–C***H*****$_2$**–); 1.41–1.44 (m, 18H, –C(C***H*****$_3$**$)_3$–); 1.53 (m, 3H, –S–CH–C***H*****$_3$**–); 1.64 (m, 2H, –CH–$(CH_2–)_2$–C***H*****$_2$**–); 1.81 (m, 2H, –CH–C***H*****$_2$**–); 3.06 (m, 2H, –CH_2–C***H*****$_2$**–NH–);

4.32 (m, 1H, –S–C***H***–CH_3–); 4.42 (m, 1H, –NH–C***H***–CO–); 4.64 (m, 2H, –CH_3–C***H***$_2$–O–)

Fig. S1 ^{1}H NMR spectra of **compound 1**(**A**) and **compound 2**(**B**) in $CDCl_3$.

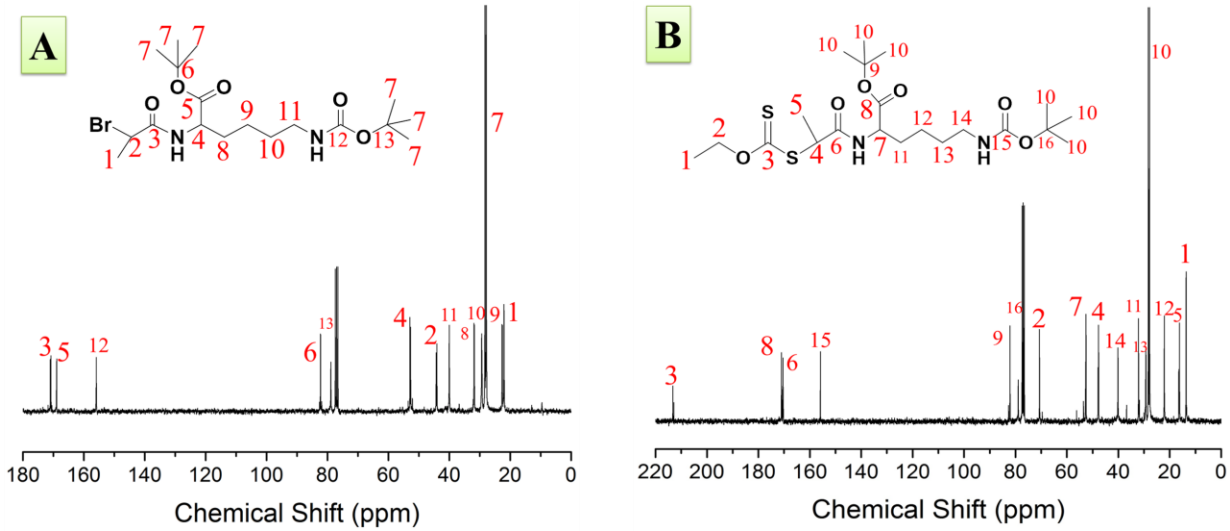


Fig. S2 ^{13}C NMR spectra of **compound 1**(**A**) and **compound 2**(**B**) in $CDCl_3$.

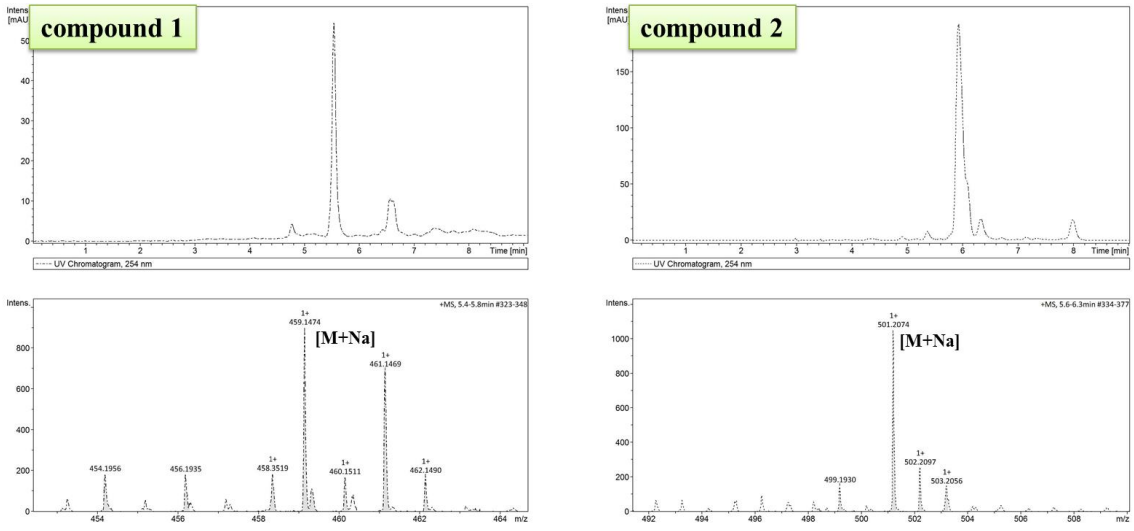


Fig. S3 Mass spectrum of compound 1and compound 2.

2. Characterization of polymers

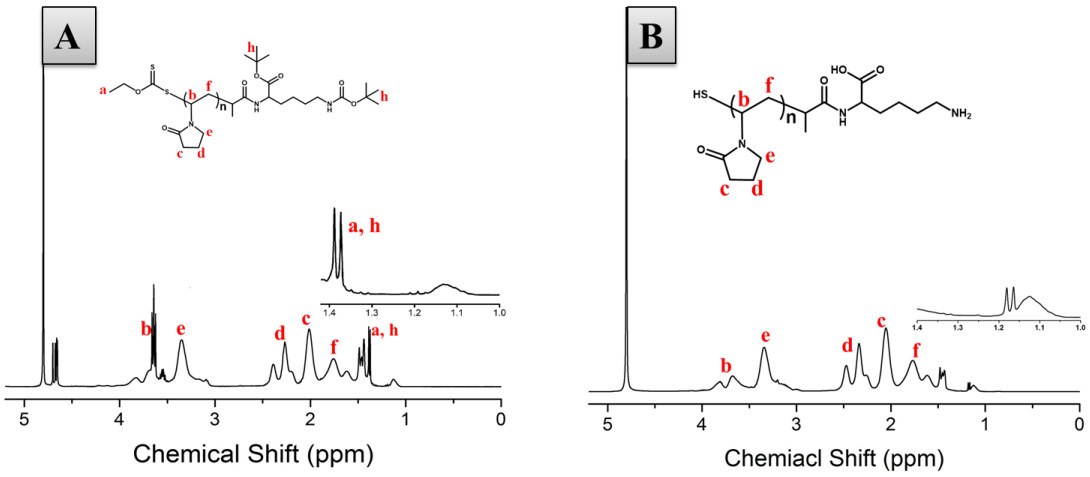


Fig. S4 ^{1}H NMR spectra of PVP-Lys(P)--**A**, and SH-PVP-Lys--**B** in D_2O.

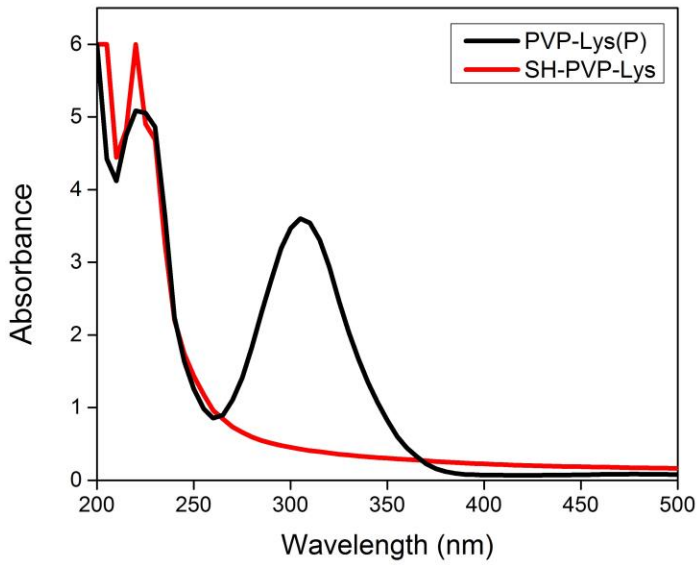


Fig. S5 UV spectra of PVP-Lys(P) and SH-PVP-Lys.

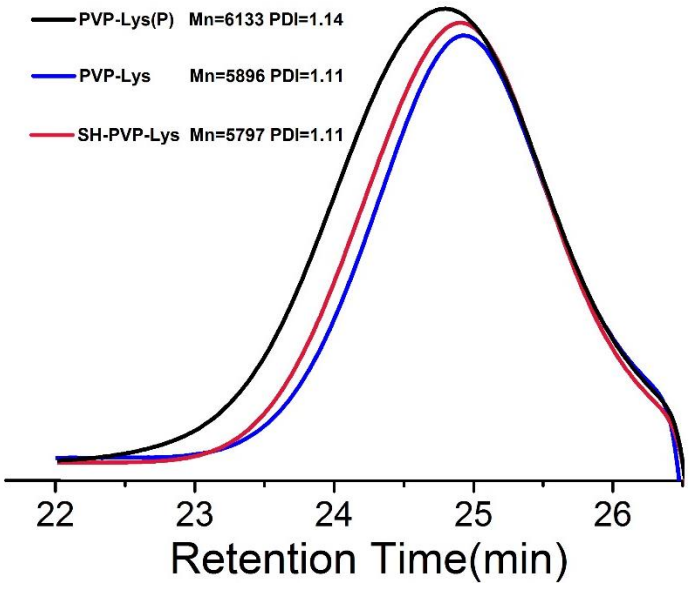


Fig. S6 Molecular weight of PVP-Lys(P), PVP-Lys and SH-PVP-Lys determined by GPC.

3. Mass of the polymer grafted on AuNPs

Thermal Gravimetric Analysis (TGA) was performed on a PerkinElmer Pyris1 TGA with a thermal analysis gas station and under N_2 purge with a heating rate of 10 ^{o}C/min.

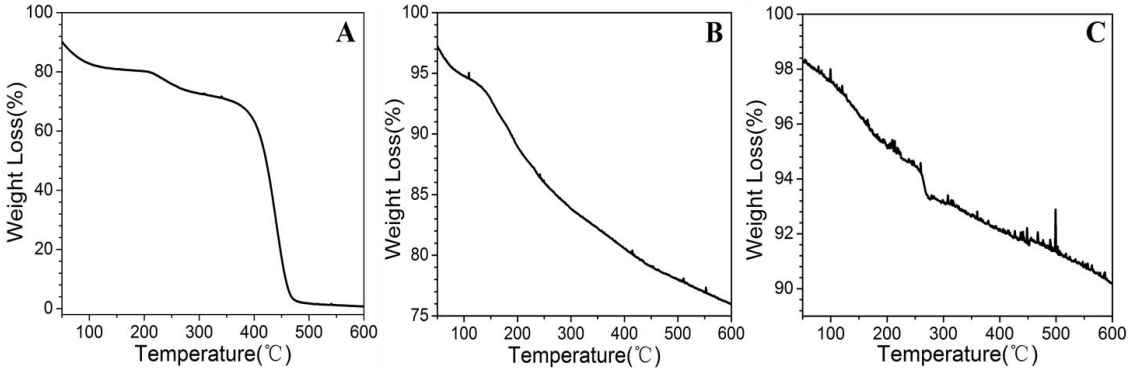


Fig. S7 Representative TGA results for SH-PVP-Lys (A), AuNPs-PVP-Lys (B) (weight loss ≈24 %) and Au (C) (weight loss ≈10 %). Based on the weight of each polymer chain (Mn ≈5800 g/mol), the Au particle diameter (D = 14.6 nm), the average number of PVP-Lys per Au particle was calculated to be 370 ± 2.

4. Stability of AuNPs-PVP-Lys

The stability of both AuNPs and AuNPs-PVP-Lys in saline solution was tested under 2 M NaCl concentration. The tests were carried out in a 96-well microtiter plate, with a

200 µL final volume per well. The nanoparticle dispersion was added into the saline solution and thoroughly mixed for 30 s and periodically checked by UV−vis spectroscopy every 2 min for 60 min. The stability of gold nanoparticles was also tested under various pH conditions by UV-vis measurements.

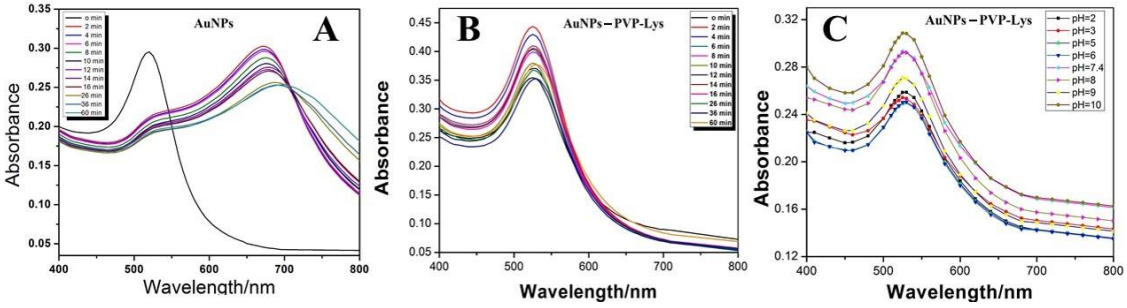


Fig. S8 Time-dependent absorption spectra (0−60 min) of (A) unmodified gold nanoparticles and (B) Au-PVP-Lys nanoparticles under extreme conditions. Au-PVP-Lys nanoparticles under various pH conditions (C). The concentration of gold nanoparticles was 2 nM.

5. Cytotoxicity of AuNPs-PVP-Lys

The cytotoxicity of the PVP-Lys modified gold nanoparticles was assessed by an MTT viability assay against mouse L929 cells. Cells were seeded at a density of 1×105 cells/well of a 96-well tissue culture plate and incubated overnight. The cells were treated with different concentrations (0.5, 1.0, 1.5, and 2.5 nM) of PVP-Lys modified gold nanoparticles (AuNPs- PVP-Lys) for different times (24, 48, and 72 h). Control cells were used without gold nanoparticle treatment. At the end of each exposure, the toxicity level of gold nanoparticles was assessed by 3-(4,5-dimethylazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. All experiments were performed 3 times, and the average of all of the experiments has been shown as cell-viability percentage in comparison with the control experiment.

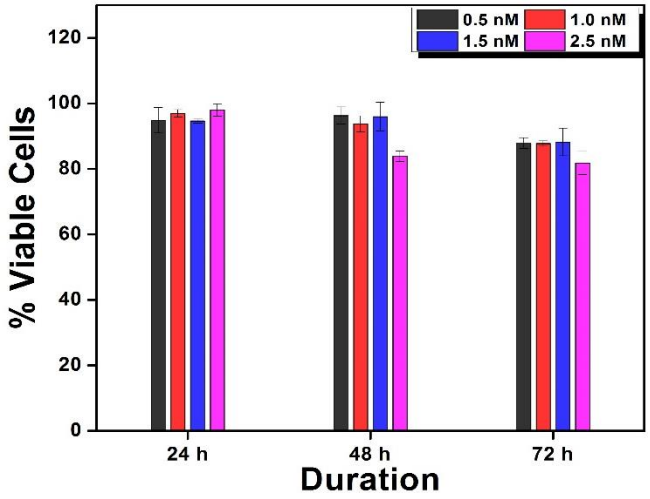


Fig. S9 Cytotoxicity studies of gold nanoparticles on L929 cells. MTT assay showing cell viability during exposure of cells to 0.5, 1.0, 1.5, and 2.5 nM colloidal gold for 24, 48, and 72 h, respectively.