Supporting Information

for

Intraorgan Targeting of Gold Conjugates for Precise Liver Cancer Treatment

Yuan-Yue Gao,[†,#] Huan Chen,[‡,#] Ying-Ying Zhou,[†] Lin-Tao Wang,[□] Yanglong Hou,*[,⊥] Xing-Hua Xia,[‖] Ya Ding*[,†]

[†] State Key Laboratory of Natural Medicines, Department of Pharmaceutical Analysis, China Pharmaceutical University, and [‡] Department of Biochemistry, School of Life Science and Technology, China Pharmaceutical University, Nanjing 210009, China

[□] School of life sciences, and [‖] State Key Laboratory of Analytical Chemistry for Life Science and Collaborative Innovation Center of Chemistry for Life Sciences, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China

[⊥] Department of Materials Science and Engineering, College of Engineering, Peking University, Beijing Key Laboratory for Magnetoelectric Materials and Devices, Beijing 100871, China

[#] The first two authors contributed equally to this work.

* Corresponding author: E-mail: hou@pku.edu.cn (Y. Hou), dingya@cpu.edu.cn (Y. Ding)

Methods

Synthesis of PEG-LA and Gal-PEG-LA

The synthesis procedure of Gal-PEG_{400}-LA was described below as an example of the method for the synthesis of all Gal-PEG-LA ligands (PEG MW=400 and 1,000 Da, Fig. S1). The $^{1}H/^{13}C$ NMR and FT-IR data for all intermediates of Gal-PEG-LA are also presented here.

PEG_{400}-LA. LA (3.7 g, 18 mmol), PEG_{400} (36 g, 90 mmol), and DMAP (0.9 g, 7.2 mmol) were dissolved in dichloromethane (DCM, 120 mL) in a flask with an electromagnetic stir under the protection of N_2. After cooled to 0 °C in an ice-water bath, a solution of DCM (50 mL) containing DCC (5.6 g, 27 mmol) was dropwise added to the reaction mixture and it was stirred at 0 °C for 1 h. The reaction solution was then warmed to room temperature and stirred for 24 h. The precipitate formed in the solution was filtered and the organic filtrate was concentrated under reduced pressure. The residue was dissolved in saturated sodium carbonate solution, and then extracted with ethyl acetate (EA) thrice. The combined organic extracts were dried over anhydrous magnesium ($MgSO_4$), filtered, and evaporated to give a yellow oil. The crude product was purified by column chromatography with DCM: methanol (MeOH) = 30: 1 to obtain 5.4 g (51.0%) yellow oil.

PEG_{400}-LA: ^{1}H NMR ($CDCl_3$): δ 1.48 (m, 2H), 1.69 (m, 4H), 1.80 (m, 1H), 2.35 (m, 2H), 2.48 (m, 1H), 2.85 (s, broad, OH), 3.15 (m, 2H), 3.62~3.75 (m, 36H, broad), 4.25 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 24.52, 28.61, 33.84, 34.50, 38.40, 40.13, 56.22, 61.54, 63.37, 69.05, 70.25, 70.48, 70.52, 72.51, 173.29. IR (KBr): υ_{max} 3429, 288, 1732, 1643, 1351, 1253, 1121, 935, 829 cm^{-1}.

PEG_{1000}-LA: ^{1}H NMR ($CDCl_3$): δ 1.46 (m, 2H), 1.66 (m, 4H), 1.92 (m, 1H), 2.25 (s, broad, OH), 2.35 (m, 2H), 2.37 (m, 1H), 2.46 (m, 1H), 3.18 (m, 2H), 3.54~3.73 (m, 90H), 4.24 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 24.50, 28.58, 33.81, 34.47, 38.37, 40.10, 53.59, 56.19, 61.50, 63.34, 69.03, 70.24, 70.46, 70.56, 72.51, 173.22. IR (KBr): υ_{max} 3456, 2899, 1772, 1652, 1457, 1103, 944, 846 cm^{-1}.

NPC-PEG_{400}-LA. PEG_{400}-LA (1.2 g, 2.0 mmol) and triethylamine (TEA, 0.5 g, 4.8 mmol) were dissolved in anhydrous DCM (40 mL) and stirred at 0 °C. To this solution, DCM solution (20 mL) containing *p*-nitrophenyl chloroformate (NPC, 1.2 g, 6.0 mmol) was added dropwise. The reaction was performed at 0 °C for 1 h and then at room temperature for 24 h under N_2 atmosphere. The resultant solution was diluted by DCM and washed with brine solution thrice. The collected organic phases were dried with anhydrous $MgSO_4$, filtered, and evaporated to give a yellow oil. The crude product was purified by column chromatography with DCM: MeOH = 30: 1 to give 1.2 g (79.8%) yellow oil.

NPC-PEG_{400}-LA: ^{1}H NMR ($CDCl_3$): δ 1.48 (m, 2H), 1.66 (m, 4H), 1.89 (m, 1H), 2.32 (t, 2H), 2.48 (m, 1H), 3.12 (m, 2H), 3.50-3.90 (m, ~32H), 4.20 (t, 2H), 4.44 (t, 2H), 7.40 (d, 2H), 8.28 (d, 2H). ^{13}C NMR δ 24.56, 28.65, 33.87, 34.53, 38.43, 40.16, 56.27, 63.40, 68.27,

68.56, 69.09, 70.50, 70.64, 121.79, 125.25, 145.33, 152.41, 155.50, 173.34. IR (KBr): υ_{max} 3562, 2899, 1767, 1722, 1519, 1360, 1201, 1112, 952, 846 cm^{-1}.

NPC-PEG_{1000}-LA: ^{1}H NMR ($CDCl_3$): δ 1.46 (m, 2H), 1.65 (m, 4H), 1.89 (m, 1H), 2.35 (t, 2H), 2.48 (m, 1H), 3.15 (m, 2H), 3.50-3.90 (m, ~106H), 4.23 (t, 2H), 4.44 (dd, 2H), 7.40 (d, 2H), 8.28 (d, 2H). ^{13}C NMR δ 24.58, 28.68, 33.90, 34.55, 38.44, 40.18, 42.71, 53.51, 56.28, 63.42, 68.29, 68.58, 69.62, 70.62, 71.32, 121.78, 125.27, 145.35, 152.43, 155.30, 173.36. IR (KBr): υ_{max} 3536, 2890, 1757, 1722, 1528, 1351, 1263, 1227, 1112, 944, 846 cm^{-1}.

EDA-PEG_{400}-LA. NPC-PEG_{400}-LA (1.0 g, 1.3 mmol) was dissolved in DCM (20 mL) and a DCM solution (10 mL) containing anhydrous ethylenediamine (EDA, 1.1 g, 18.9 mmol) was added dropwise. After the mixture was stirred at room temperature for 5 h under N_2 atmosphere, it was diluted by DCM and washed with brine solution thrice. The collected organic phases were dried with anhydrous $MgSO_4$, filtered, and evaporated to give a yellow oil. The crude product was purified by column chromatography with DCM: MeOH = 30: 1 to give 0.53 g (51.3%) yellow oil.

EDA-PEG_{400}-LA: ^{1}H NMR (DMSO-d_6): δ 1.38 (m, 2H), 1.54 (m, 3H), 1.66 (m, 1H), 1.87 (m, 1H), 2.32 (t, 2H), 2.41 (m, 1H), 2.55 (m, 2H), 2.95 (m, 4H), 3.15 (m, 2H), 3.50-3.70 (m, ~34H), 4.05 (t, 2H), 4.13 (t, 2H), 7.15 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 24.18, 28.04, 33.22, 34.01, 38.07, 41.47, 43.92, 56.02, 63.04, 68.29, 68.86, 69.77, 156.25, 172.73. IR (KBr): υ_{max} 3439, 2917, 2085, 1961, 1705, 1643, 1528, 1148, 1342, 1263, 1103, 935, 846, 564 cm^{-1}.

EDA-PEG_{400}-LA: ^{1}H NMR (DMSO-d_6): δ 1.29 (m, 2H), 1.45 (m, 2H), 1.59 (m, 3H), 1.70 (m, 1H), 1.92 (m, 2H), 2.37 (t, 2H), 2.49 (m, 1H), 2.51 (m, 2H), 2.84 (m, 1H), 2.02 (m, 3H), 3.35-3.75 (m, ~84H), 4.09 (m, 2H), 4.17 (m, 2H), 7.23 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 24.18, 28.03, 33.22, 34.00, 38.07, 41.46, 43.86, 56.02, 63.05, 68.29, 68.86, 69.76, 156.24, 172.75. IR (KBr): υ_{max} 3456, 2899, 1732, 1651, 1545, 1457, 1369, 1253, 1103, 952, 846, 564 cm^{-1}.

Gal-PEG_{400}-LA. EDA-PEG_{400}-LA (162 mg, 0.24 mmol), lactobionic acid (144 mg, 0.40 mmol), NHS (192 mg, 1.67 mmol), and EDC·HCl (346 mg, 1.80 mmol) were dissolved in DMSO (8 mL) and then stirred for 3 d. The as-prepared solution was used for the surface conjugation of GNPs without any purification. The product of Gal-PEG_{1000}-LA was also synthesized and used without any purification.

Preparation and characterization of Gal-PEG-GNPs

Gal-PEG-GNP conjugates. The dithiolane group in liver target ligands was conjugated to gold surfaces via a ring-opening reaction by the sodium borohydride ($NaBH_4$) reduction method described previously.[1] Briefly, PEG-LA and Gal-PEG-LA (0.28 mmol, PEG MW of

400 and 1,000 Da, the molar ratio of Gal-PEG-LA: PEG-LA was set to be 0: 100, 50: 50, and 100: 0, respectively) was dissolved in MeOH (2 mL). 1.5 equiv. of $NaBH_4$ (0.42 mmol) in 1.5 mL of ice water was added. The solution was stirred for 1 h at 4 °C and acidified to pH 6 with HCl (1 $mol \cdot L^{-1}$). The linkage of S–Au bond was performed by the addition of citrate-stabilized GNPs (3 mL) into the reaction solution which stirred for 1 h at room temperature. The productions for the Gal-PEG-LA: PEG-LA feeding ratio of 0: 100, 50: 50, and 100: 0 was denoted as Gal-PEG-GNPs (Gal 0, Gal 50, and Gal 100).

All the gold conjugate solutions were then aged overnight and dialyzed in a fresh deionized water solution for 72 h (molecular weight cut off, MWCO= 7,000 Da). After freeze drying, the products were readily dissolved in water and in phosphate-buffered solutions (PBS).

Liver tumor cell target ability of Gal-PEG-GNPs. HepG2 and L02 cells were seeded in 6-well plates at a density of 5×10^5 cells per well and incubated for 24 h to reach 80% confluence. The cells were then incubated for an additional 4 h with Gal-PEG-GNPs with different PEG molecular weights and different galactose densities at a gold dose of 3.62 μg/mL. Subsequently, excess media was removed, and the cells were washed with PBS trice, digested by aqua regia. After the acid was volatilized completely with a water bath, 1 mL H_2O_2 was added and the solution was concentrated under the vacuum to less than 0.5 mL. To confirm the ASGP-R induced cellular uptake mechanism, receptor inhibition by adding free galactose (37.7 mM) in the Gal-PEG_{400}-GNPs (Gal 100) group was also tested. The gold concentration was detected by ICP-MS method.

Preparation of GNPs conjugated with PTX-PEG-TA only.

PTX-PEG-GNP conjugates. The preparation of GNPs conjugated PTX-PEG-TA only was referred to our previous reports.[2,3] PTX-PEG_{1000}-TA (0.28 mmol) was dissolved in 4 mL of methanol. Citrate-protected GNPs (3 mL) were added to the ligand solution, and the mixture was stirred for 1 h to obtain the gold conjugate solution.

All the gold conjugate solutions were then aged overnight and dialyzed in a fresh deionized water solution for 72 h (molecular weight cut off, MWCO=7,000 Da). After freeze drying, the products were readily dissolved in water and in phosphate-buffered solutions (PBS).

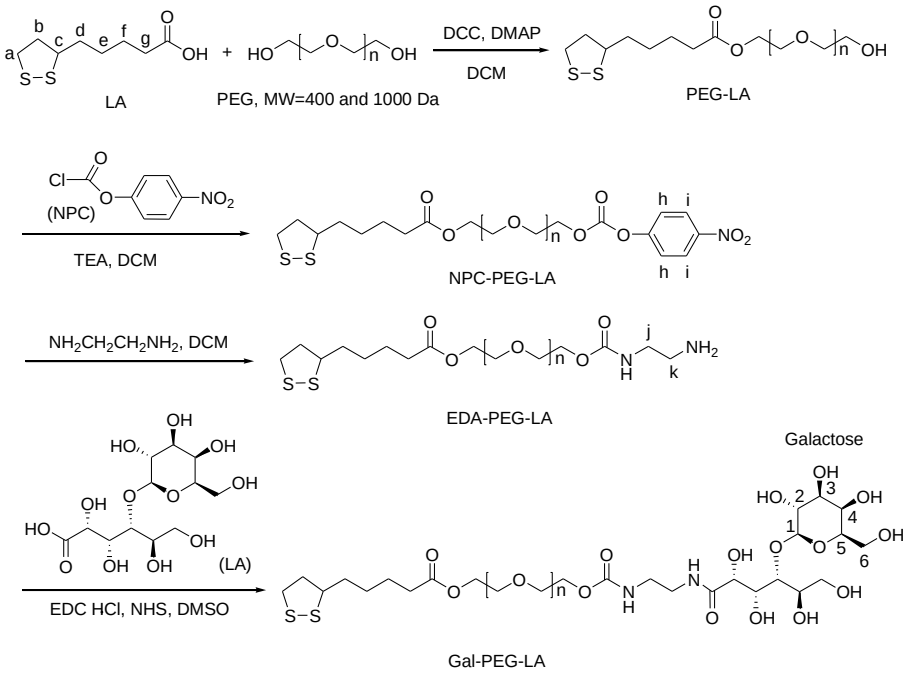


Figure S1. The synthesis route of PEG-LA and Gal-PEG-LA.

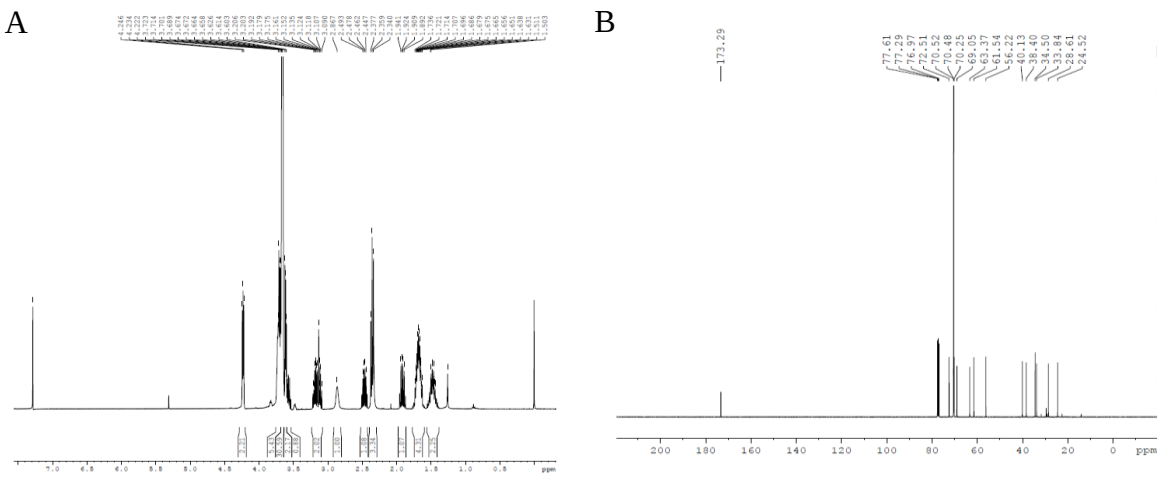


Figure S2. (A) ^{1}H and (B) ^{13}C NMR ($CDCl_3$) spectra of PEG_{400}-LA

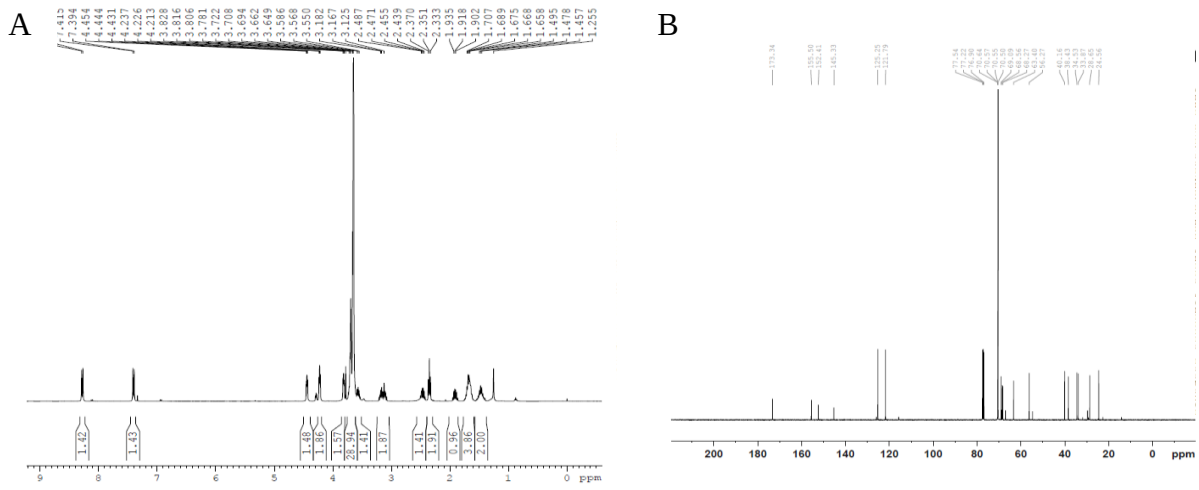


Figure S3. (A) ^{1}H and (B) ^{13}C NMR ($CDCl_3$) spectra of NPC-PEG_{400}-LA.

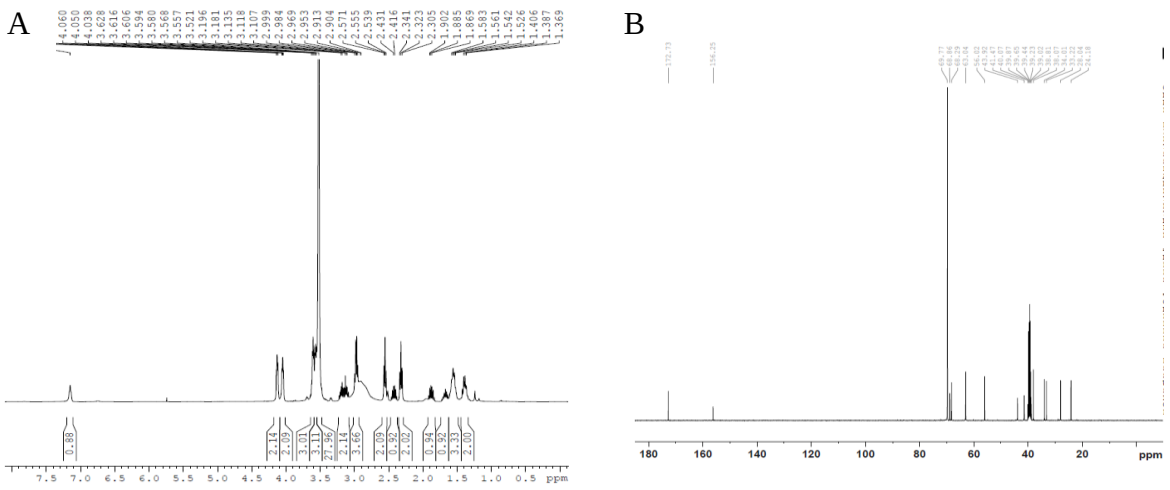


Figure S4. (A) ^{1}H and (B) ^{13}C NMR (DMSO-d_6) spectra of EDA-PEG_{400}-LA.

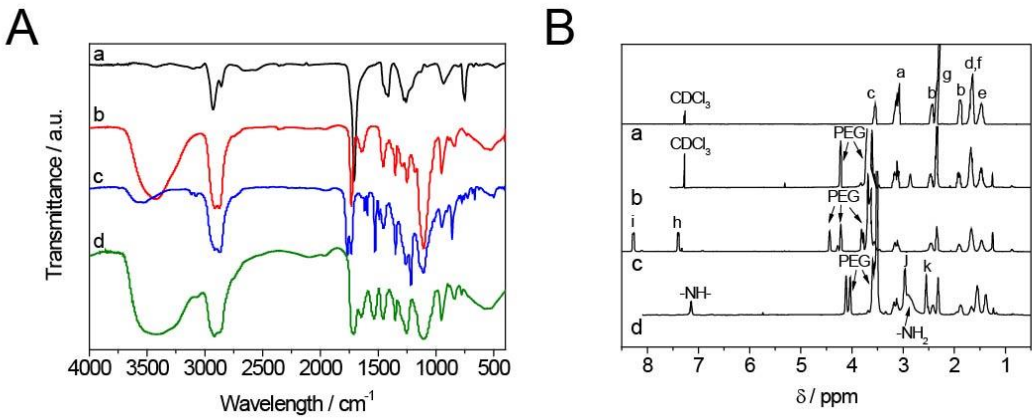


Figure S5. (A) FT-IR spectra and (B) ^{1}H NMR spectra of the intermediates of liver target ligands: (a) LA, (b) PEG_{400}-LA, (c) NPC-PEG_{400}-LA, and (d) EDA-PEG_{400}-LA.

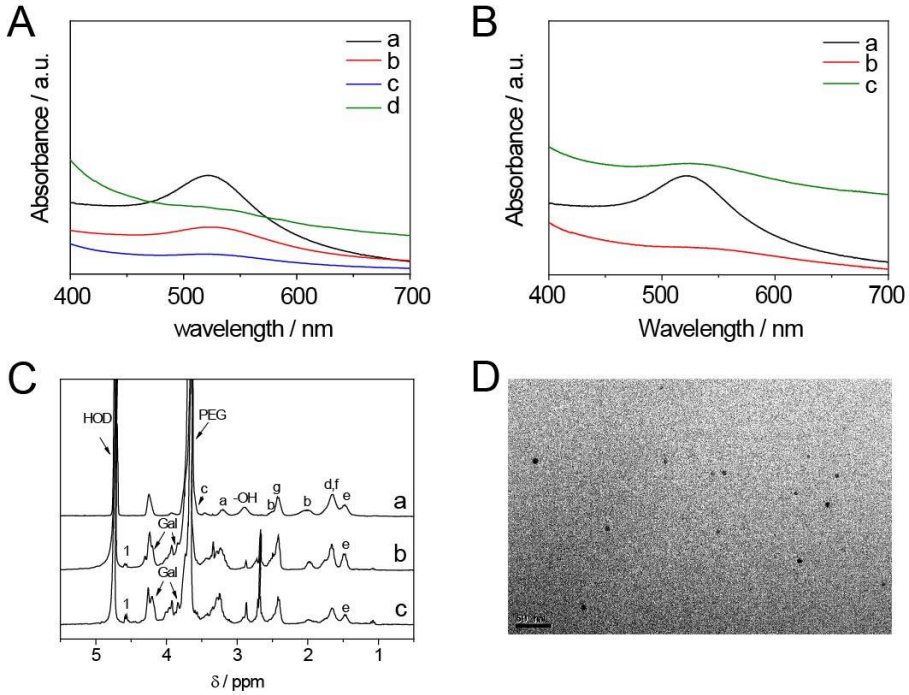


Figure S6. Characterizations of Gal-PEG-GNPs. UV-Vis spectra of (A) (a) GNPs and Gal-PEG$_{400}$-GNPs with same PEG MW of 400 Da but different Gal density. The molar ratio of Gal-PEG-LA: PEG-LA is (b) 0:100 (Gal 0), (c) 50:50 (Gal 50), and (d) 100: 0 (Gal 100) in the preparation. (B) (a) GNPs and Gal-PEG-GNPs with same Gal density of Gal 100 but different PEG MW of (b) 400 Da and (c) 1,000 Da. (C) ^{1}H NMR spectra of Gal-PEG$_{400}$-GNPs with Gal density of (a) Gal 0, (b) Gal 50, and (c) Gal 100. (D) TEM image of Gal-PEG$_{400}$-GNPs (Gal 50).

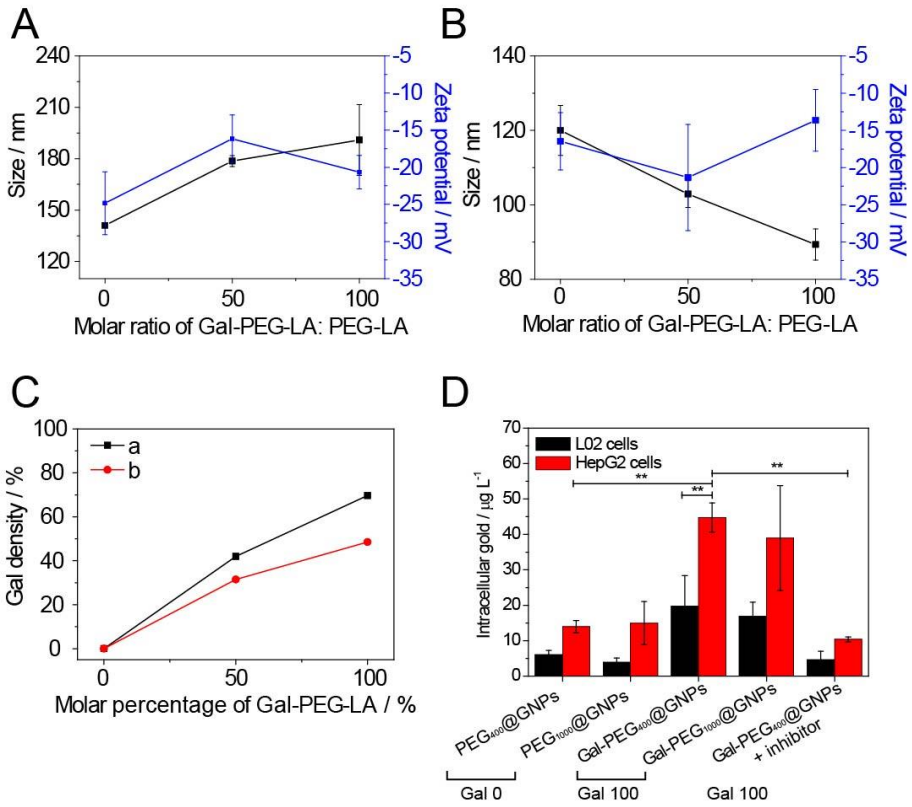


Figure S7. Hydrodynamic diameters and zeta potentials of Gal-PEG-GNPs with different PEG molecular weight (A) 400 Da and (B) 1,000 Da under the condition of different molar ratio of Gal-PEG-LA: PEG-LA in the preparation. (C) Gal density in Gal-PEG-GNPs with PEG MW of (a) 400 Da and (b) 1000 Da adjusted by different molar percentage of Gal-PEG-LA: PEG-LA in the preparation solution and detected by the integration in ^{1}H NMR spectrum. (D) Intracellular gold content in L02 and HepG2 cells after 4 h incubation with Gal-PEG-GNPs with different PEG MW of 400 and 1,000 Da and Gal density of Gal 0 and Gal 100, detected by ICP-MS method, comparing with the group pre-treated with the ASGP-R inhibitor (galactose, 37.7 mM) for 30 min before the incubation of gold conjugates.

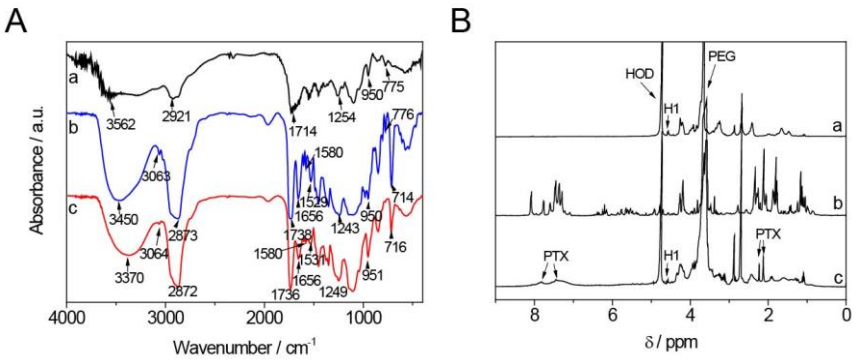


Figure S8. (A) FT-IR and (B) ^{1}H NMR spectra of (a) Gal-PEG$_{400}$-GNPs (Gal 100), (b) GNPs conjugating PTX-PEG-TA only, and (c) Gal/PTX-GNPs.

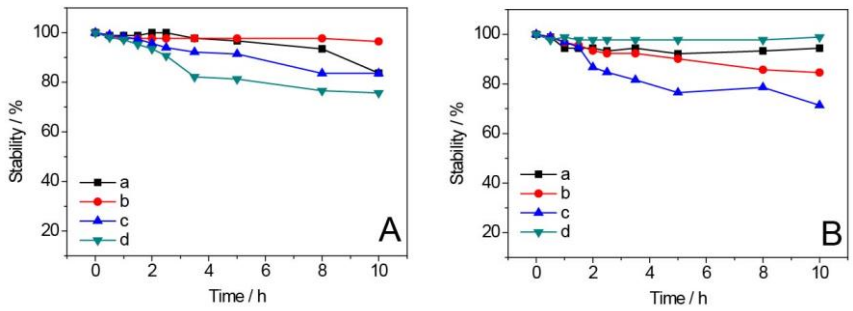
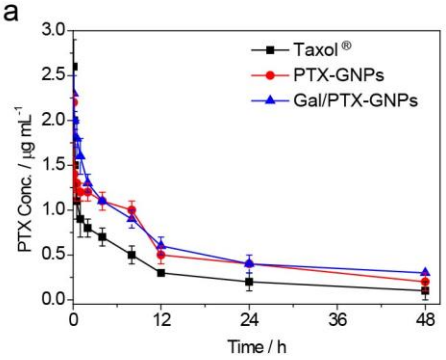


Figure S9. Dispersion stability curves of (A) PTX-GNPs and (B) Gal/PTX-GNPs as a function of time in the following environments: 0.03 M PBS at pH 7.4 (curve a), 0.03 M PBS at pH 5.5 (curve b), 0.2 M PBS at pH 7.4 (curve c), and 0.03 M PBS with 2% serum (curve d).

a



b

Parameter	Taxol®	PTX-GNPs	Gal/PTX-GNPs
C_{max} (μg/mL)[a]	3.49 ± 0.28	2.65 ± 0.16*	2.63 ± 0.24*
AUC_{0-t} (μg/mL/kg)[b]	13.73 ± 1.41	23.26 ± 1.81**	26.04 ± 0.94**
$t_{1/2}$ (h)[c]	28.30±16.06	40.73±25.37**	43.83 ± 7.26**
MRT (h)[d]	36.65 ± 22.27	54.05 ± 32.04**	59.26 ± 8.42**
V_d (L)[e]	13.87 ± 3.77	9.99 ± 2.44**	9.67 ± 1.15**
CL (L/h)[f]	0.39 ± 0.13	0.20 ± 0.07*	0.15 ± 0.01*

[a] C_{max}, the maximum (or peak) plasma concentration
[b] AUC_{0-t}, area under the plasma concentration-time curve
[c] $t_{1/2}$, plasma half life
[d] MRT, mean residence time
[e] V_d, apparent volume of distribution
[f] CL, clearance rate

Figure S10. Pharmacokinetic studies. (a) Plasma concentration time profiles and (b) pharmacokinetic parameters of PTX in rats following intravenous administration of Taxol®, PTX-GNPs, and Gal/PTX-GNPs, respectively, with the dose of 7 mg/kg (mean ± SD, n = 4). *P < 0.05, **P < 0.01 vs. Taxol® group (one-sample t-test).

References

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