Electronic supplementary information

The impact of HPMA polymer structure on the targeting performance of the conjugated hydrophobic ligand

Qingqing Yang, Lian Li, Xi Zhu, Zhou Zhou, Wei Sun, and Yuan Huang*

Key Laboratory of Drug Targeting and Drug Delivery System, Ministry of Education, West China School of Pharmacy, Sichuan University. No. 17, Block 3, Southern Renmin Road, Chengdu 610041, P.R. China.

*Correspondence to: Yuan Huang

Tel.: +86-28-85501617, Fax: +86-28-85501617

E-mail: huangyuan0@163.com

Synthesis of 3,3′-[4,4′-azobis(4-cyano-4-methyl-1-oxo-butane-4,1-diyl)]bis(thiazolidine-2-thione) (ABIK-TT)

ABIK-TT was prepared as previously described.[1] Briefly, thiazolidine-2-thione (TT), 4,4′-azobis -(4-cyanopentanoic acid) and 4-(dimethylamino)pyridine (DMAP) were dissolved in tetrahydrofuran (THF). Dicyclohexylcarbodiimide (DCC) in THF was added dropwise. The reaction mixture was kept under −10 °C for 1 h and then 4°C for 24 h. Dicyclohexylurea (DCU) was removed by filtration. The desired product was obtained by crystallization of the evaporated yellow oily residue from dichloromethane-diethyl ether solution. Yield: 71 %. ^{1}H NMR 400 MHz [$(CD_3)_2SO$]: δ=1.72 (s, 6H, 2 CH_3), 2.45 (m, 4H, 2 (CH_2-C)), 3.15 (m, 4H, 2 ((C=O)-CH_2)), 3.38 (m, 4H, 2 (CH_2S)), 4.52 (m, 4H, 2 (CH_2N)) (Fig. S1).

Synthesis of N-methacryloyl-glycylglycyl-propargyl (MA-GG-C≡CH)

MA-GG-ONp (400 mg, 1.245 mmol) was dissolved in N,N-dimethylformamide (DMF), and propargylamine (60 μL, 1.355 mmol) was added dropwise. The reaction mixture was subsequently stirred for 5 h at room temperature and the progress was monitored by thin layer chromatography (TLC, ethyl acetate: methanol 10:1). DMF was removed under vacuum using a rotavapor. The crude product was purified by column chromatography (silica gel 60 Å, 100–200 mesh, ethyl acetate: methanol 10:1) to give solid in 46.1% yield. FT-IR (ν, cm^{-1}): 3289.40 (-C≡CH) (Fig. S2A). ^{1}H NMR 400 MHz [$(CD_3)_2SO$]: δ=1.88 (s, 3H, CH_3), 3.09 (s, 1H, ≡CH), 3.69 (d, 2H , CO-NH-**CH_2**), 3.75 (d, 2H, CH_2-C≡), 3.86 (d, 2H, **CH_2**-CO-NH-C≡), 5.38 (s, 1H, CH_2=), 5.75 (s, 1H, CH_2=), 8.1-8.2 (d, 3H, 3 NH) (Fig. S1).

Synthesis of 2-(2-pyridyldithio)ethylamin hydrochloride (PDEA)

PDEA was prepared as previously described.[2] Briefly, 2-mercaptoethylamine hydrochloride was dissolved in methanol and added dropwise to a stirred solution of 2,2′- dithiopyridine dissolved in methanol containing glacial acetic acid. The reaction was kept under an argon atmosphere to minimize free thiol oxidation. After 2 h of stirring, the solvent was evaporated in vacuum to give yellow oil. The product was precipitated by the addition of cold ether and purified by redissolving in a small volume of methanol and precipitating with cold ether. This procedure was repeated until the crystals became white. Yield: 56 %. ^{1}H NMR 400 MHz (D_2O): δ=3.14 (t, 2H, CH_2S), 3.45 (t, 2H, CH_2N), 7.38 (t, 1H, Ph-C3H), 7.81 (d, 1H, Ph-C5H), 7.88 (t, 1H, Ph-C4H), 8.51 (d, 1H, Ph-C2H) (Fig. S1).

Synthesis of azido- modified folate

Azido-folate (N_3-FA) was synthesized by a method modified from the literature.[3] In the first step, 1-azido-3-aminopropane was prepared as previously described.[4] Then folic acid (0.22 g, 0.5 mmol) was dissolved in dimethylsulfoxide (DMSO) containing triethylamine (600 μL). After addition of DCC (68.7 mg, 1.1 equiv), the mixture was stirred at room temperature in the dark for 24 h. Then, 1-azido-3-aminopropane (65.5 mg, 2 equiv) was added into the mixture under stirring. The reaction was continued for another 24 h. After the precipitated DCU was filtered off, the product was precipitated from the reaction mixture by the addition of an excess amount of cold diethyl ether. The crude product was purified by dissolving in 1 M sodium hydroxide (NaOH) and precipitation by addition of 1 M hydrochloric acid (HCl). The precipitates were collected by centrifugation, washed with ethanol/H_2O (1:1), and dried under vacuum to give an orange solid in 69 % yield. FT-IR (ν, cm^{-1}): 2101.2 (−N_3) (Fig. S2B). ^{1}H NMR 400 MHz [$(CD_3)_2SO$]: δ=1.60 (m, 2H, CH_2N_3), 1.70 (m, 2H, CH_2**C**$\mathbf{H_2}$$CH_2N_3$), 2.05 (m, 2H，$CH_2$**C**$\mathbf{H_2}$CONH), 2.30 (m, 2H, **C**$\mathbf{H_2}$$CH_2$CONH), 3.07 (s, 1H, Pt-NH); 3.38 (m, 2H, **C**$\mathbf{H_2}$$CH_2CH_2N_3$), 4.33 (m, 1H, **CH**COOH), 4.49 (d, 2H, **C**$\mathbf{H_2}$NH), 6.63 (d, 2H, Ph−C3H and Ph−C5H), 6.97 (s, 1H, PtC7H), 7.66 (d, 2H, Ph−C2H and Ph−C6H), 7.89 (s, 1H, CH_2CO**NH**), 8.15 (d, 1H, CO**NH**CHCOOH), 8.20 (d, 1H, CH_2**NH**), 8.65 (s, 2H, NH_2), 10.6 (s, 1H, COOH) (Fig. S1), Pt = pteridine.

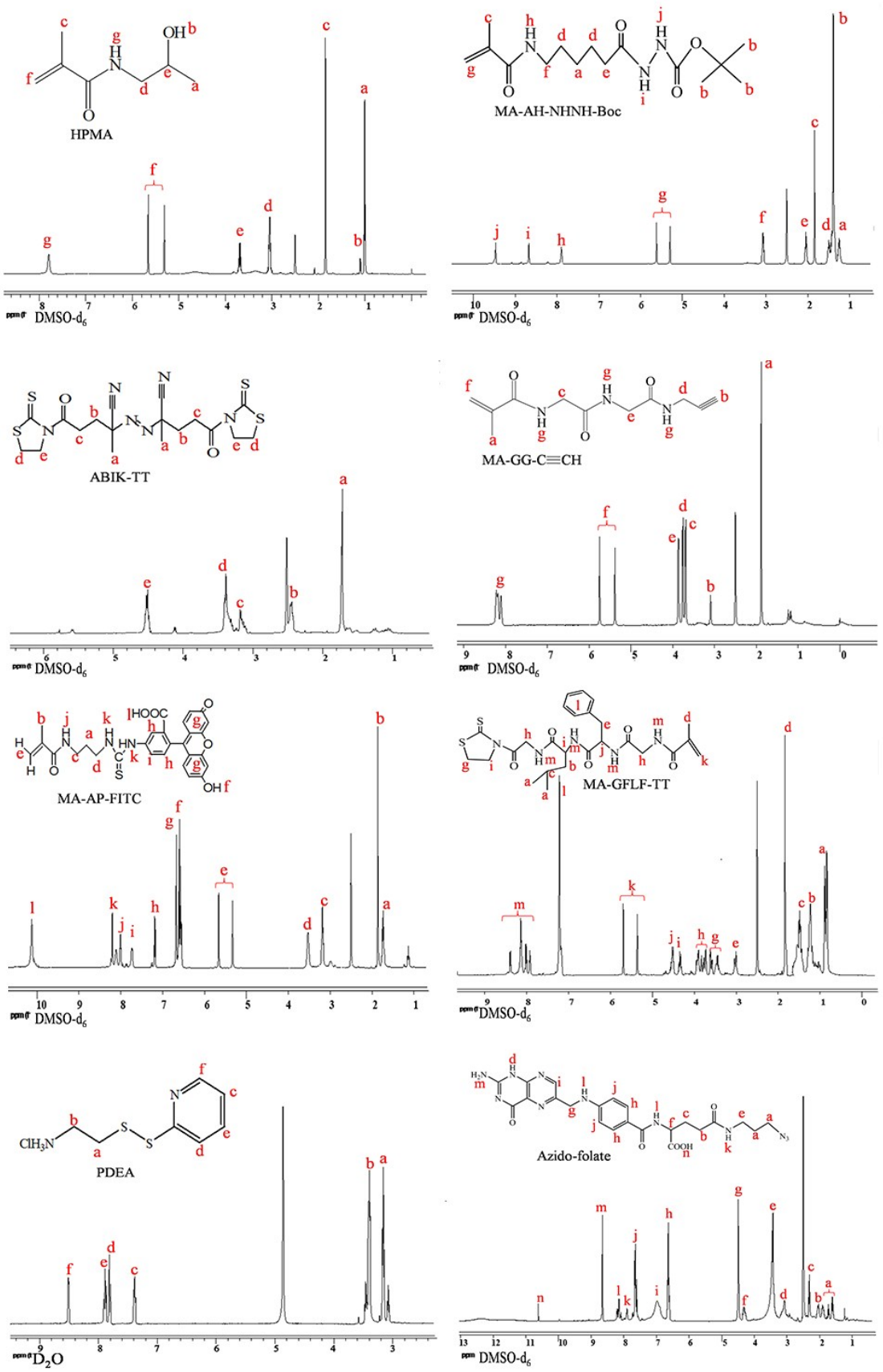

Fig. S1 ^{1}H NMR spectra of each compound, including HPMA, MA-AH-NHNH-Boc, ABIK-TT, MA-GG-C≡CH, MA-AP-FITC, MA-GFLG-TT, PDEA and azido-folate.

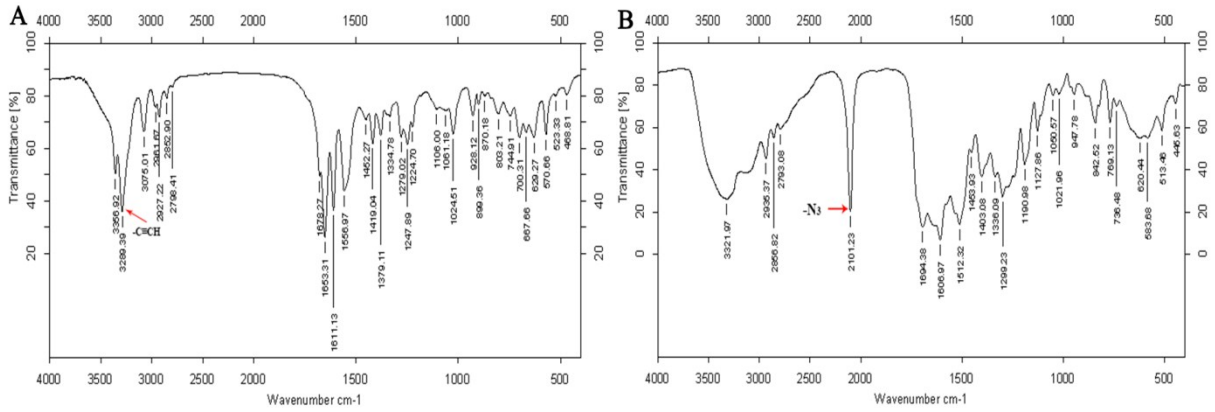

Fig. S2 IR spectra of (A) MA-GG-C≡CH and (B) azido-folate.

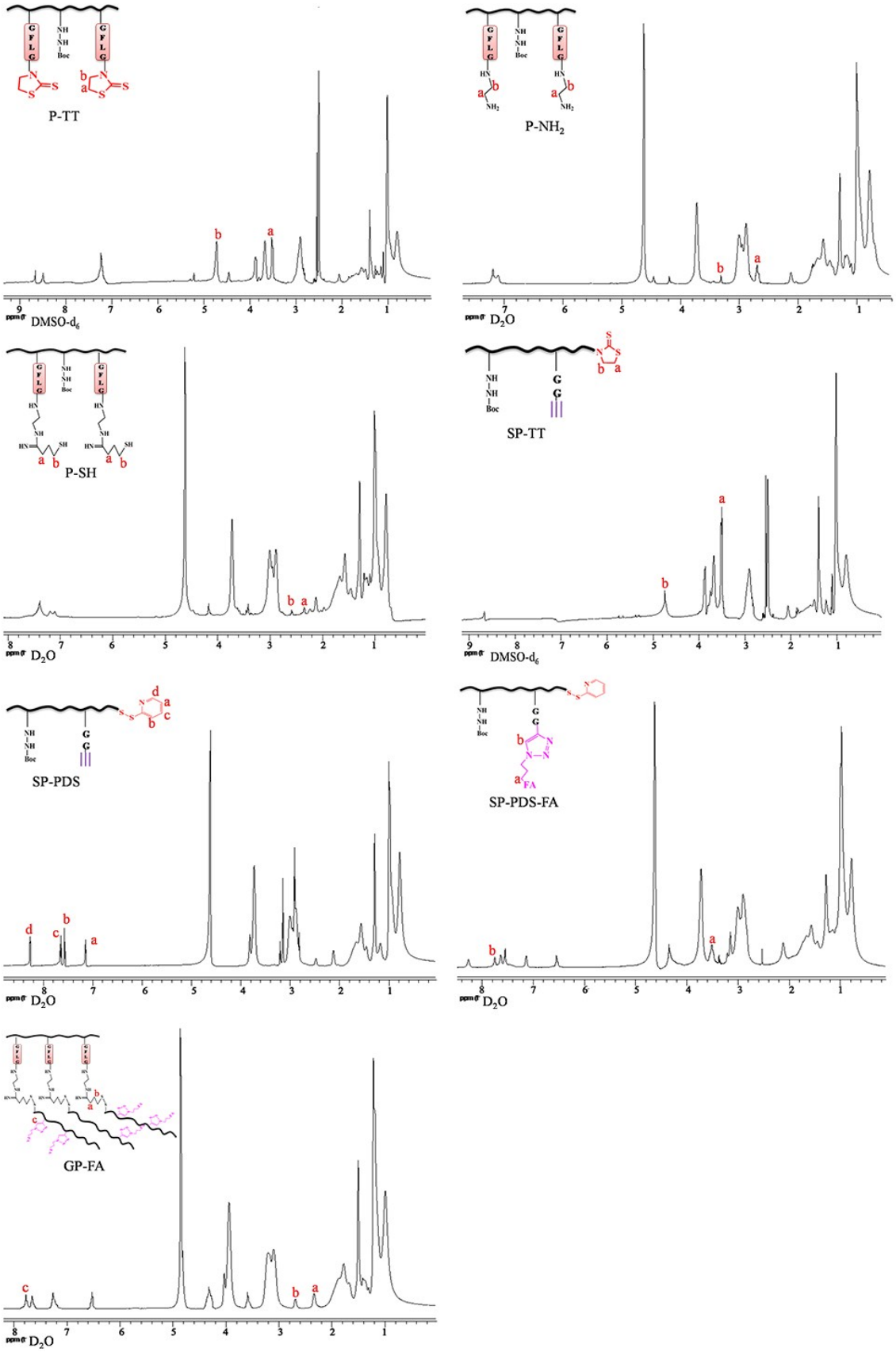

Fig. S3 ^{1}H NMR spectra of polymer precursors and graft polymers, including P-TT, P-NH_2, P-SH, SP-TT, SP-PDS, SP-PDS-FA and GP-FA.

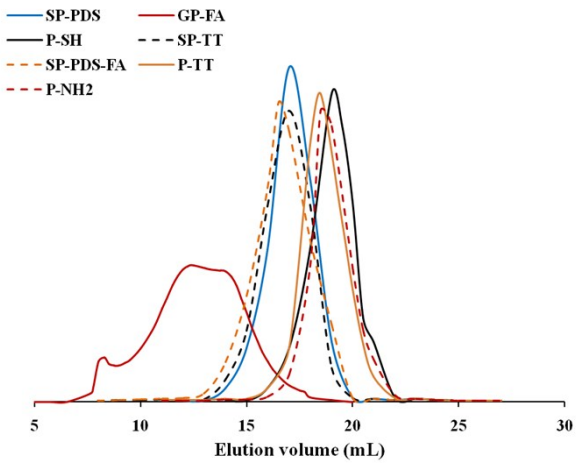

Fig. S4 Gel permeation chromatography (GPC) profiles of polymer precursors and graft polymers.

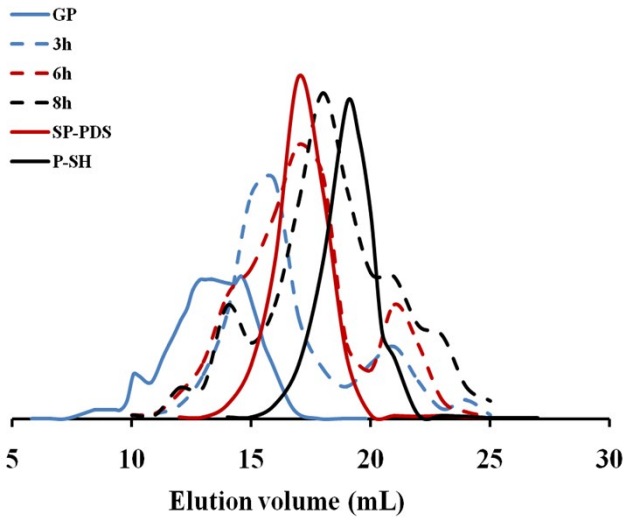

Fig. S5 GPC profiles of the degradation product (Mw < 42 kDa) after incubation of GP solution (10 mg/mL) in citrate-phosphate buffer (pH 6.0, 37 °C) containing 3 mM GSH and 2μM papain at different time points and comparison with initial PDS-terminated semitelechelic HPMA polymer (SP-PDS, Mw 30.7 kDa).

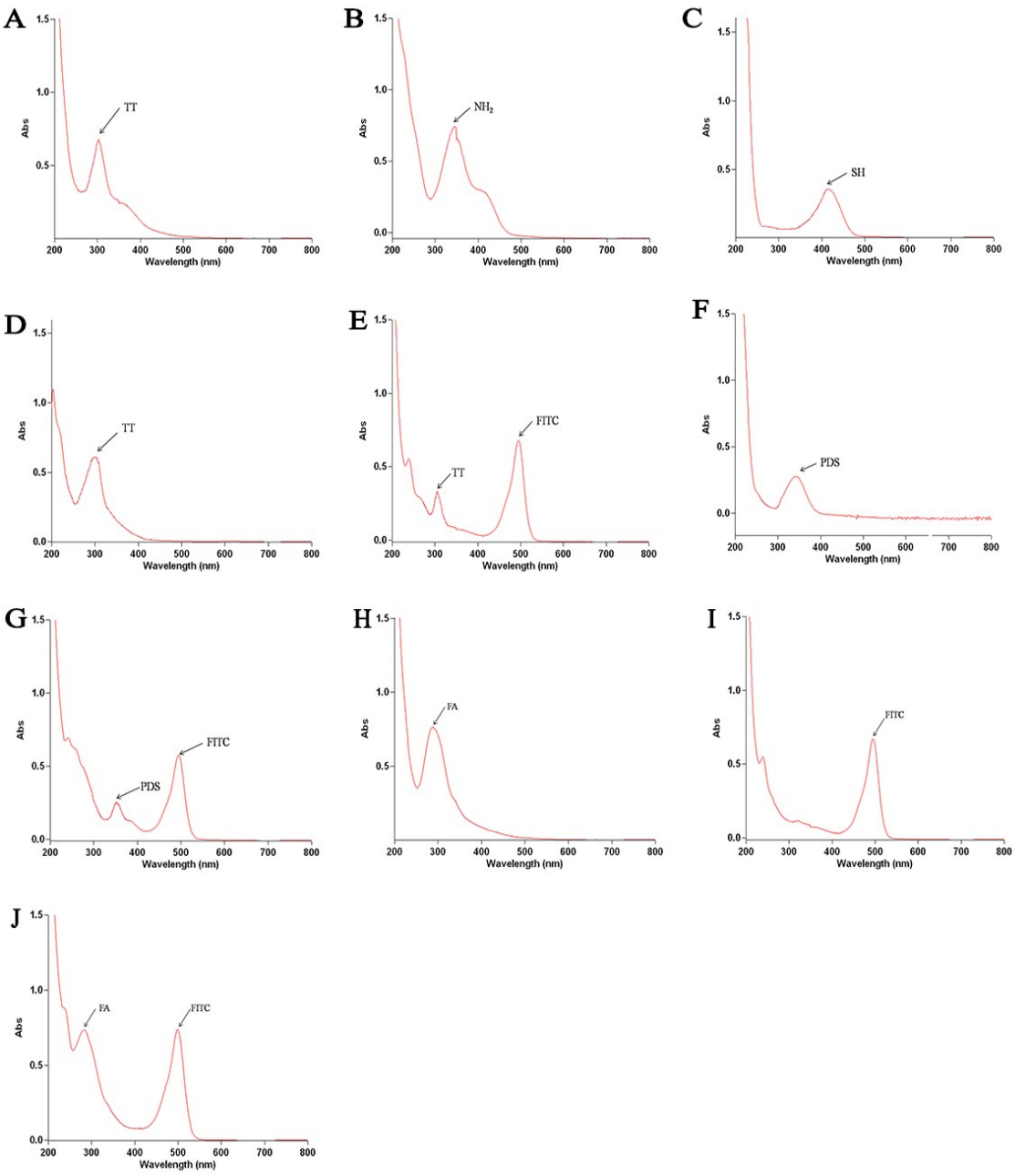

Fig. S6 UV-vis spectra of (A) P-TT (λ_{max}=305 nm), (B) P-NH_2 after reaction with 2,4,6-trinitrobenzene sulfonic acid (λ_{max}=335 nm), (C) P-SH after reaction with 2,2'-dinitro-5,5'-dithiobenzoic acid (λ_{max}=412 nm), (D) SP-TT (λ_{max}=305 nm), (E) SP-TT-FITC, (F) SP-PDS after reaction with dithiothreitol (λ_{max}=343 nm), (G) SP-PDS-FITC after reaction with DTT (λ_{max}=343 nm), (H) GP-FA (λ_{max}=281 nm), (I) GP-FITC (λ_{max}=494 nm) and (J) GP-FA.

References

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