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Supporting Information for

Influence of protein adsorption on cellular uptake of AuNPs conjugated with chiral oligomers

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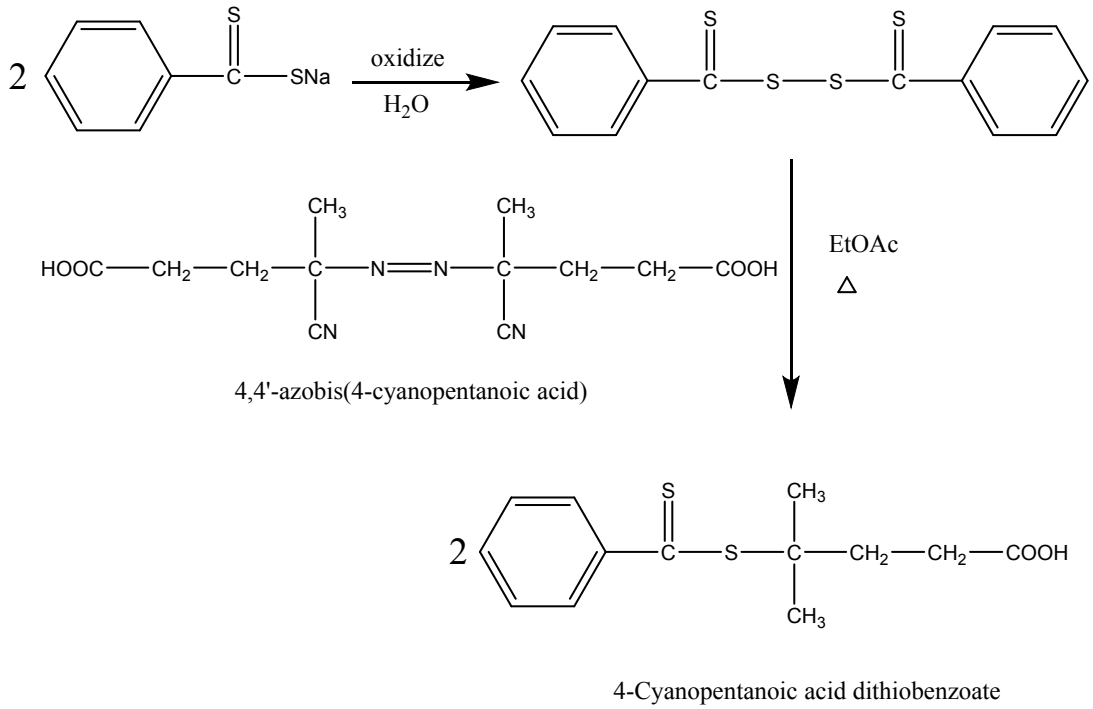
Materials and methods

Materials

Sodium methoxide, elemental sulfur, benzyl chloride, potassium ferricyanide (III), 4,4'-azobis(4-cyanopentanoic acid), L-valine, D-valine and acryloyl chloride were purchased from Aladdin Company (China). Azodiisobutyronitrile (AIBN) and 2-hydroxyethyl methacrylate (HEMA, 97%) were purchased from Sigma-Aldrich (USA). The organic solvents used were purified according the standard methods. The water used in all experiments was purified *via* a Millipore Milli-Q purification system and had a resistivity higher than 18.2 $M\Omega \cdot cm^{-1}$.

Synthesis and characterization of 4-cyanopentanoic acid dithiobenzoate



Scheme S1. Schematic illustration of synthesis of 4-cyanopentanoic acid dithiobenzoate.

4-Cyanopentanoic acid dithiobenzoate was synthesized by using a modified method reported

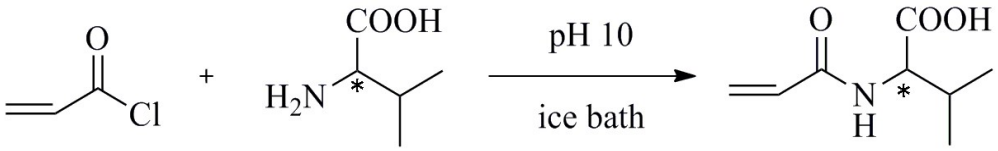
previously [1]. Briefly, 42 g sodium methoxide and 250 mL anhydrous methanol were added to a thoroughly dried 250 mL, three-necked round-bottomed flask equipped with a magnetic stir bar. Then, elemental sulfur (25 g) was added to the flask rapidly. Benzyl chloride (49.44 g) was then added dropwise *via* the addition funnel over a period of 1 h at room temperature under a dry nitrogen atmosphere. The reaction mixture was heated in an oil bath at 90 °C for 10 h. The reaction mixture was then cooled to ~7 °C by using an ice bath. After the precipitated salt was removed by filtration, the solvent was removed by rotary evaporation. To the residue 500 mL deionized water was added. The solution was filtered again. The crude sodium dithiobenzoate solution was then washed with diethyl ether (3×200 mL). After 1 M HCl solution (500 mL) was added, dithiobenzoic acid was extracted into the ether layer. Milli-Q water (300 mL) and 1 M NaOH solution (600 mL) were added, and sodium dithiobenzoate was extracted to the aqueous layer. This washing process was repeated three times to finally yield a solution of sodium dithiobenzoate.

Potassium ferricyanide (III) (32.93 g) was dissolved in deionized water (500 mL). Potassium ferricyanide solution was added dropwise to the above sodium dithiobenzoate (350 mL) *via* an addition funnel over a period of 1 h under vigorous stirring. The red precipitate was filtered and washed with water until the water became colorless. The solid was dried in a vacuum oven at room temperature overnight.

To a 250 mL round-bottomed flask the distilled ethyl acetate (80.0 mL) was added. Dry 4,4'-azobis(4-cyanopentanoic acid) (5.84 g, 21.0 mmol) and di(thiobenzoyl)disulfide (4.25 g, 14.0 mmol) were added to a 250 mL round-bottomed flask containing 80 mL distilled ethyl acetate. The reaction solution was heated at reflux for 18 h. The ethyl acetate was then removed by rotary evaporation. The crude product was isolated by column chromatography (silica gel 60 Å, 70-230 mesh) by using ethyl

acetate: hexane (2:3) as eluent. Fractions with a red color were combined, and dried over anhydrous sodium sulfate overnight. Then the target compound was recrystallized from benzene. The crystal was dried in a vacuum oven and characterized with ^{1}HNMR ($CDCl_3$). CH δ 7.91 (d, 2H), CH δ 7.58 (q, 1H), CH δ 7.41 (q, 2H), CH_2 δ 2.74 (tri, 2H), CH_2 δ 2.47 (tri, 2H), CH_3 δ 1.95 (s, 3H).

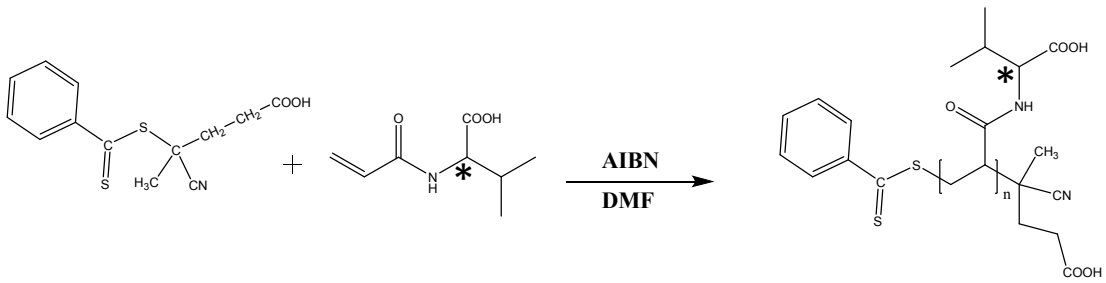
Synthesis of acryloyl-L(D)-valine



Scheme S2. Synthesis of acryloyl-L(D) -valine monomers. * represents chiral center.

The chiral monomers N-acryloyl-L(D)-valine (L(D)-AV) were synthesized according to a previous report [2, 3]. The yield was above 90 %. The ^{1}HNMR (CD3OD, 600MHz DD2, Agilent) spectrum resonance peaks are assigned as follows: CH_3 δ 0.89 (d, 6H), CH δ 2.11 (m,1H), CH δ 4.31 (d, 1H), CH δ 5.58 (tri, 1H), CH_2 δ 6.17, 6.28 (m, 2H).

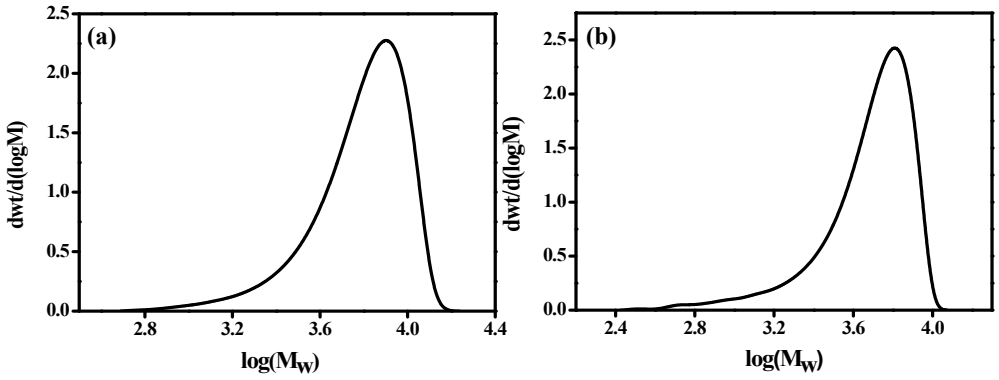
Synthesis and characterization of poly(acryloyl-L(D)-valine)



Scheme S3. Schematic illustration of synthesis of chiral poly(acryloyl-valine). * denotes chiral center.

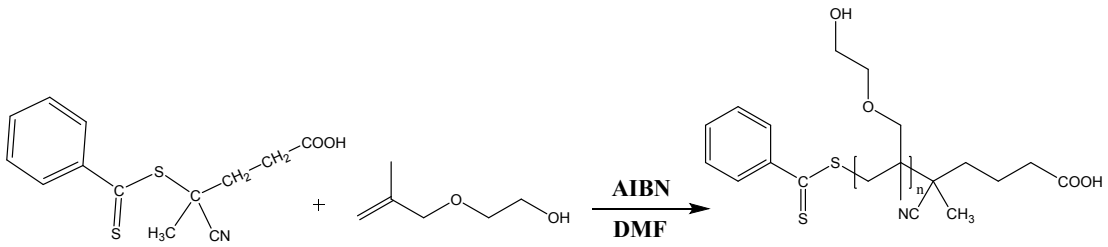
Polymerization was carried out in a 10 mL dry Schlenk flask equipped with a magnetic stirrer. Briefly,

4-cyanopentanoic acid dithiobenzoate (16 mg), azodiisobutyronitrile (AIBN, 4 mg) and acryloyl valine (1.37 g) were dissolved in 5 mL N,N-dimethylformamide (DMF). The mixture was deoxygenated by purging with nitrogen for 20 min, and then heated at 70 ^{o}C for 12 h. The reaction was stopped by exposure to air. The mixture was precipitated in excess diethyl ether, and then separated by centrifugation. The dissolution and precipitation cycle was repeated 3 times. The polymers were dried under high vacuum for 48 h at room temperature to give a pink solid product. The product was characterized by gel permeation chromatography (GPC, eluent tetrahydrofuran (THF), Waters 1515 Isocratic HPLC). The L-PAV and D-PAV had a similar weight average molecular weight (M_w) of 4926 Da and 4997 Da, and narrow polydispersity of 1.24 and 1.15, respectively.



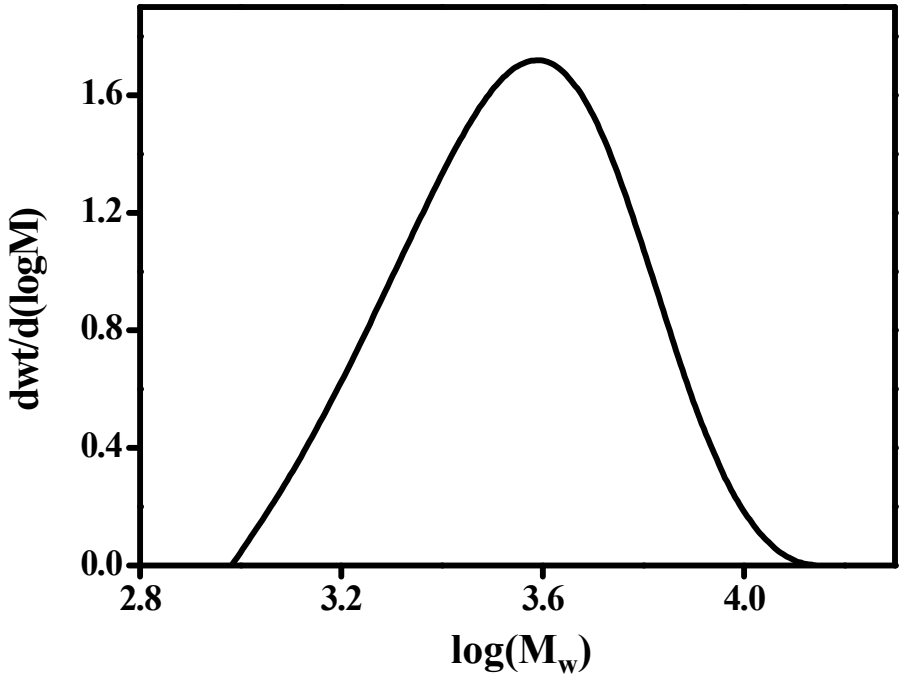
GPC curves of (a) L-PAV and (b) D-PAV.

Synthesis and characterization of poly(2-hydroxyethyl methacrylate) (PHEMA)



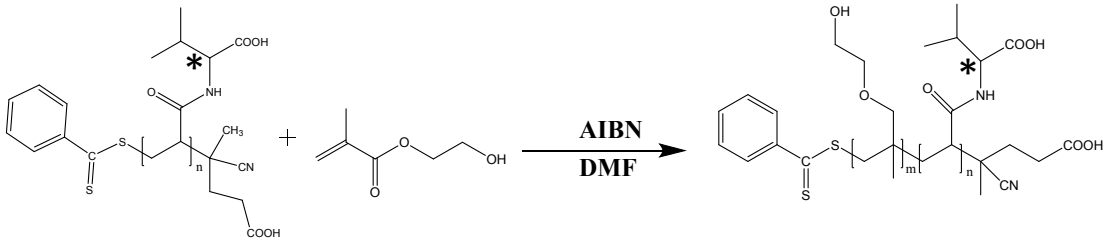
Scheme S4. Schematic illustration of synthesis of PHEMA.

Polymerization was carried out in a 10 mL dry Schlenk flask equipped with a magnetic stirrer. Briefly, 4-cyanopentanoic acid dithiobenzoate (56 mg), azodiisobutyronitrile (AIBN, 4 mg) and HEMA (520 μL) were dissolved in 3 mL N,N-dimethylformamide (DMF). The mixture was deoxygenated by purging with nitrogen for 20 min, and then heated at 70 ^{o}C for 12 h. The reaction was stopped by exposure to air. The mixture was precipitated in excess diethyl ether, and then separated by centrifugation. The dissolution and precipitation cycle was repeated 3 times. The polymers were dried under high vacuum for 48 h at room temperature to obtain a pink solid product. The product was characterized by gel permeation chromatography (GPC, eluent N,N-dimethylformamide (DMF), Waters 1515 Isocratic HPLC). The PHEMA had a weight average molecular weight (M_w) of 3965 Da, and narrow polydispersity of 1.27 .



GPC curve of PHEMA.

Synthesis of L(D)-PAV-*b*-PHEMA



Scheme S5. Schematic illustration of synthesis of L(D)-PAV-*b*-PHEMA. * represents chiral center.

Polymerization was carried out in a 10 mL dry Schlenk flask equipped with a magnetic stirrer. Briefly, L-PAV (987 mg) or D-PAV (1002 mg), AIBN (4 mg) and HEMA (520 μL) were dissolved in 3 mL N,N-dimethylformamide (DMF). The mixture was deoxygenated by purging with nitrogen for 20 min, and then heated at 70 ^{o}C for 12 h. The reaction was stopped by exposure to air. The mixture was

precipitated in excess diethyl ether, and then separated by centrifugation. The dissolution and precipitation cycle was repeated 3 times. The polymers were dried under high vacuum for 48 h at room temperature to obtain a pink solid product. The weight average molecular weight (M_w) of L-PAV-*b*-PHEMA (8891 Da) or D-PAV-*b*-PHEMA (8962 Da) was calculated as a combination of the M_w of L-PAV (4926 Da) or D-PAV (4997 Da) and M_w of PHEMA (3965 Da).

Size distribution

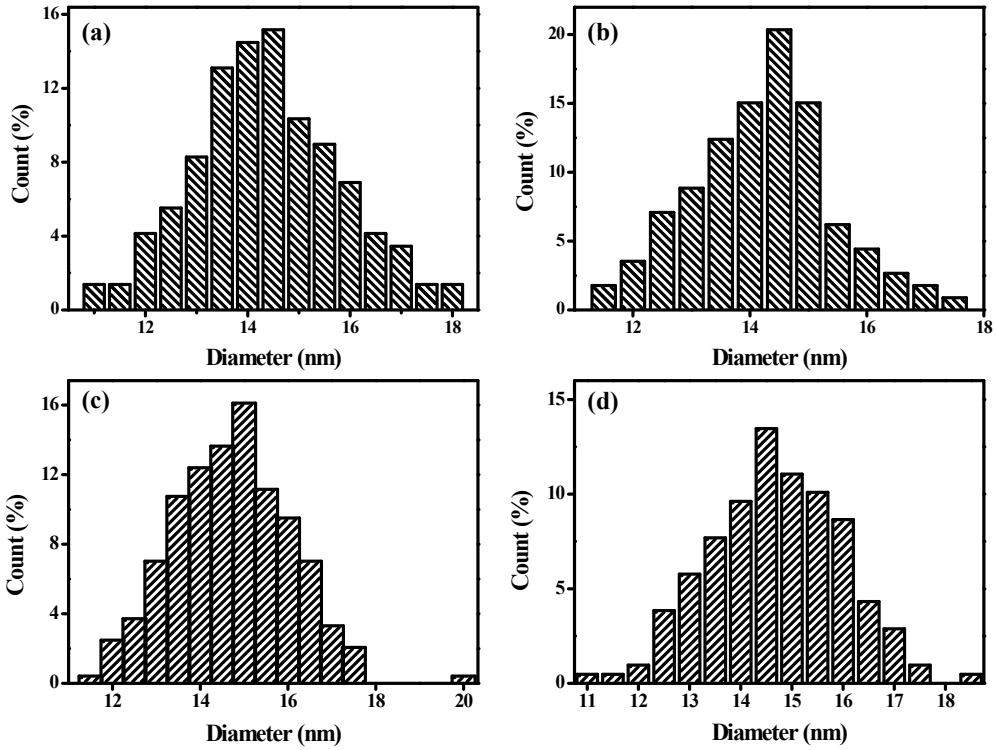


Figure S1. Size distribution calculated for (a) L-PAV-AuNPs, (b) D-PAV-AuNPs, (c) L-PAV-*b*-PHEMA-AuNPs and (d) D-PAV-*b*-PHEMA-AuNPs from TEM images (based on random counting of more than 600 particles). These diameters were used to calculate the total particle concentration by using ICP-MS measurements.

Thermogravimetric analysis (TGA)

The density of L(D)-PAV and L(D)-PAV-*b*-PHEMA grafted on AuNPs was measured by TGA (Q50 V20.13 Build 39) (Figure S4)**.** The analysis was performed from 60 °C up to 800 °C at a fixed heating rate of 10 °C·min^{-1} under a continuous flux of nitrogen.

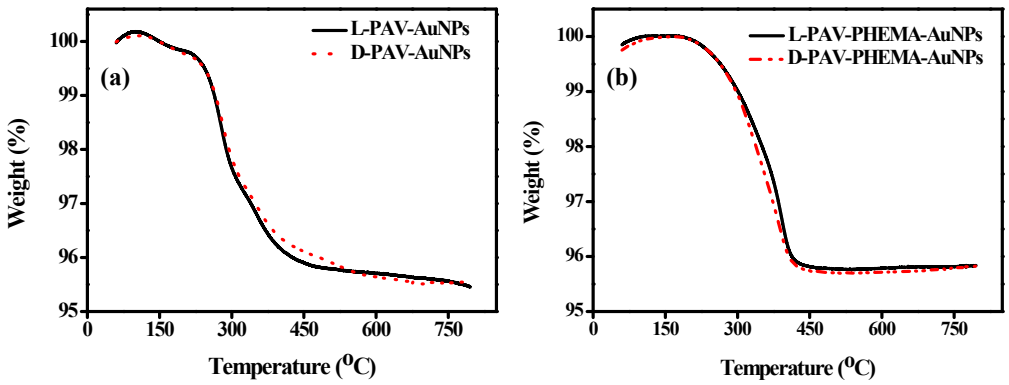


Figure S2. TGA curves of (a) L-PAV-AuNPs and D-PAV-AuNPs, and (b) L-PAV-*b*-PHEMA-AuNPs and D-PAV-*b*-PHEMA-AuNPs.

Conjugate characterization

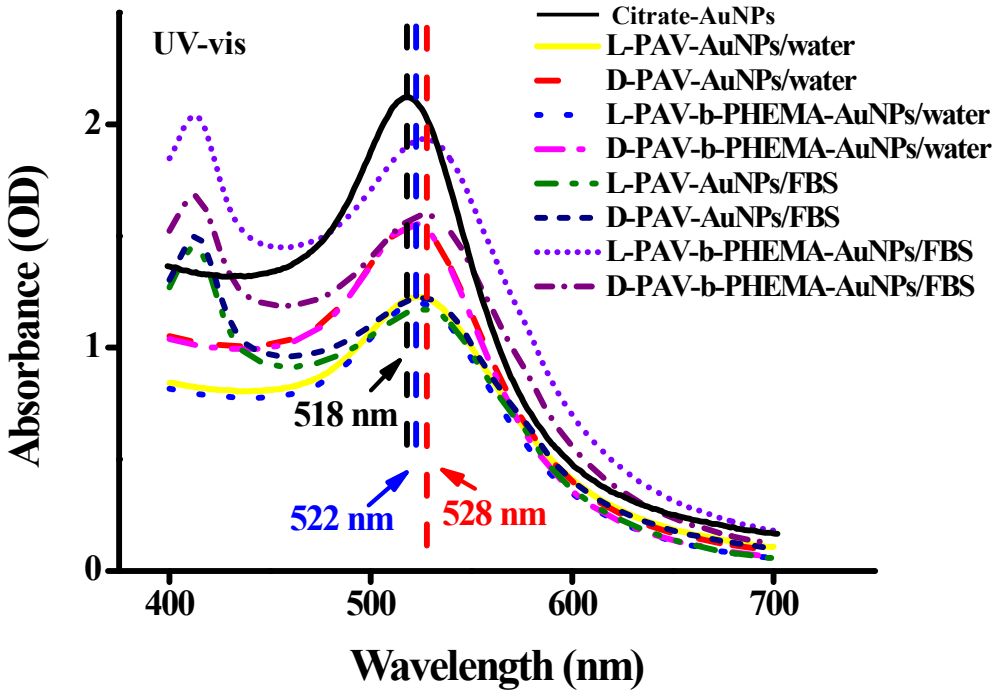


Figure S3. SPR spectra of PAV-AuNPs and PAV-*b*-PHEMA-AuNPs in water and medium containing 50 % FBS, respectively. Here phenol red-free culture medium was used.

The investigation of the conjugates revealed the extent of the ligand and FBS concentration specific differences. Conjugate stability was checked through absorption measurement of the particle surface plasmon resonance (SPR), a parameter determined by coherent oscillation of conduction electrons in the AuNP surface that are exquisitely sensitive to their local dielectric environment. The SPR of the AuNPs conjugated with PAV or PAV-*b*-PHEMA was determined by recording the absorbance of AuNPs (400-700 nm) in water and DMEM containing 50 % FBS on a UV-vis spectrophotometer (Shimadzu UV2550). Each spectrum was an average of those of 3 individual samples recorded twice. Compared to that of the citrate-AuNPs, the SPR peak of the L(D)-PAV-AuNPs and L(D)-PAV-*b*-PHEMA-AuNPs was red-shifted for 4 nm (Figure S3). When being incubated in 50 % FBS/DMEM, the SPR peak of the PAV-AuNPs and PAV-*b*-PHEMA-AuNPs had a red-shift of 6 nm, and the width

of the spectra showed no significant difference (Figure S3). Moreover, the SPR peak of the L-PAV-AuNPs and D-PAV-AuNPs (or L-PAV-*b*-PHEMA-AuNPs and D-PAV-*b*-PHEMA-AuNPs) was kept at 522 nm and 528 nm without change in water and FBS/DMEM, respectively (Figure S3). The intensity difference was caused by the slight difference in concentrations used here (Figure S3). These results reveal that the PAV-AuNPs and PAV-*b*-PHEMA-AuNPs are very stable in all the above mediums.

Cytoviability

To determine the viability of HepG2 or A549 cells, the cells were plated at a density of 5×10^4 cells/cm^2 in a 24-well plate and cultured for 24 h. The medium was replaced with fresh one containing the L(D)-PAV-AuNPs or the L(D)-PAV-*b*-PHEMA-AuNPs (0.9 mL for each well) with a given Au concentration (50 μg/mL). After treatment for another 24 h, 100 μL 5 mg/mL 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) solution was added to each well, and the cells were further cultured at 37 °C for 3 h. The dark blue formazan crystals generated by the mitochondria dehydrogenase in live cells were dissolved with dimethyl sulfoxide. After the sample was centrifuged at 12000 g/min for 5 min, the absorbance of supernatant was measured by a microplate reader (MODEL 680, Bio Rad) at 570 nm. The results are shown in Figure S4.

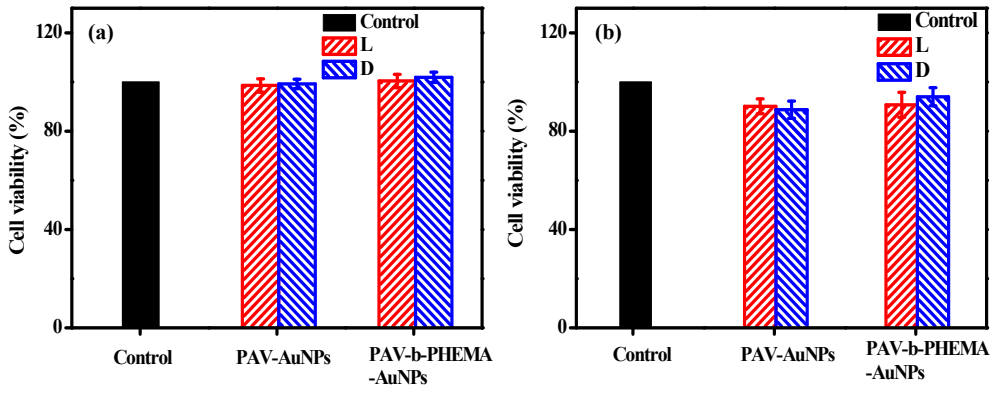


Figure S4. Influence of PAV-AuNPs and PAV-*b*-PHEMA-AuNPs on viability of (a) HepG2 and (b) A549 cells, respectively. The cells were cultured with 50 μg·mL^{-1} PAV-AuNPs and PAV-*b*-PHEMA-AuNPs for 24 h, respectively.

References

1 Y. Mitsukami, M. S. Donovan, A. B. Lowe and C. L. McCormick, *Macromolecules*, 2001, **34**, 2248-2256.

2 J. Deng, L. Zheng, M. Yao and C. Gao, *Langmuir*, 2016, **32**, 5608–5616.

3 B. Hu, J. Deng, H. Zheng, S. Yu and C. Gao, *Macromolecular Rapid Communications*, 2016, doi 10.1002/marc.201600250.