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

Bovine serum albumin nanoparticles modified with multilayers and aptamer for pH-responsive and targeted anti-cancer drug delivery

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Characterization of ^{1}HNMR spectroscopy

Characterization was performed by ^{1}HNMR spectroscopy, using D_2O as a solvent on a 500 MHz Bruker instrument. The characteristic peaks were found at 3.4-3.7 ppm (-CH_2CH_2O- of PEG) and 2.6-3.1 ppm (-CH_2-N- of PAH). The area of the PEG peak (87.1) was compared with the area of the allylamine side-chain peak (2.2) to determine the graft ratio of the comb copolymer. The PAH-g-PEG-$COOH_{41.7}$ with a grafting ratio of 41.7% was thus obtained according to:

$$\frac{\text{area } of \ PEG \ chains}{\text{area } of \ \text{allylamine side-chain peak}} * \frac{number \ of \ H \ in \ (-CH_2-N-)}{number \ of \ H \ in \ (-CH_2CH_2O-) * units \ of \ PEG} * 100\% = \frac{87.1}{2.2} * \frac{2}{4*47.5} * 100\% = 41.7\%$$

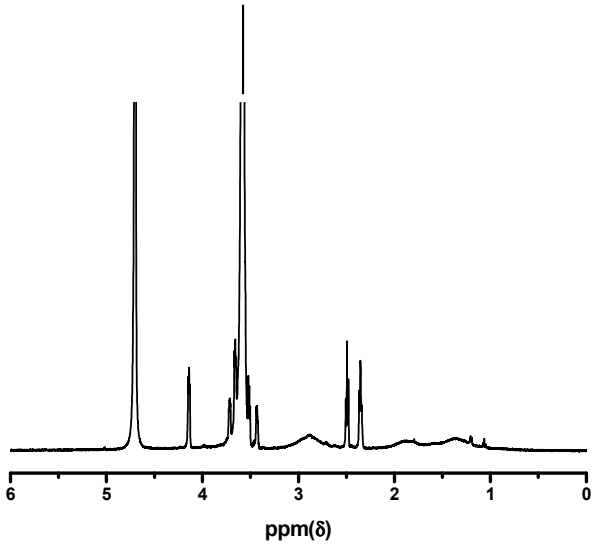

Fig. S1 ^{1}HNMR spectrum of PAH-g-PEG-$COOH_{41.7}$ grafting copolymer.

Targeting ability of AS1411 to QGY-7703 cells

The Hepli and QGY-7703 cells were incubated with 2 μg/mL Cy3-AS1411-NH_2 at 4°C for 30min. After washed with PBS for 3 times, the cells were observed by a fluorescence microscope. All the parameters of the microscope were set identical.

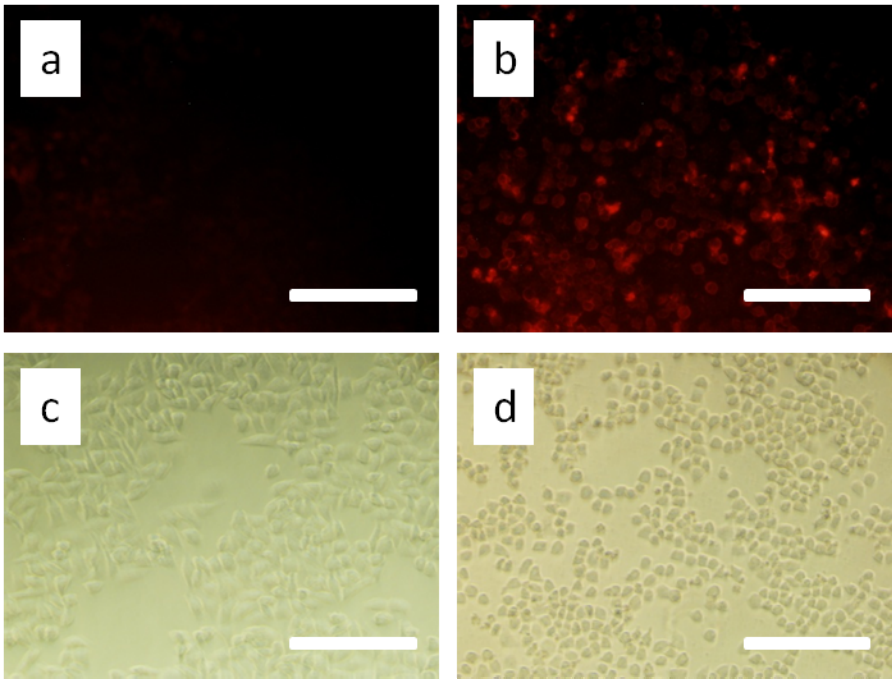

Fig. S2 Fluorescence microscopy images (a,b) and bright field images (c,d) of Hepli (a,c) and QGY-7703 cells (b,d) incubated with Cy3-AS1411-NH_2 at 4 °C for 30 min. Scale bar =100 μm.

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Cellular uptake of BSA NPs

Cellular uptake of the FITC labeled BNPs and BNPs-Apt was determined by flow cytometry (FACS Calibur, Becton Dickinson BD). The cells were seeded on a 24-well plate at a density of $8x10^4$ cells per well and allowed to attach for 24 h. To determine the particles uptake rate and amount, the cells were incubated with 50 μg/ml NPs for different time (up to 48 h). At desired time intervals, the cells were washed 3 times with PBS and harvested by trypsinization. The FITC incorporated in the BNPs and BNPs-Apt served as a marker to quantitatively determine their cellular uptake by flow cytometry and Cell Quest Pro software.

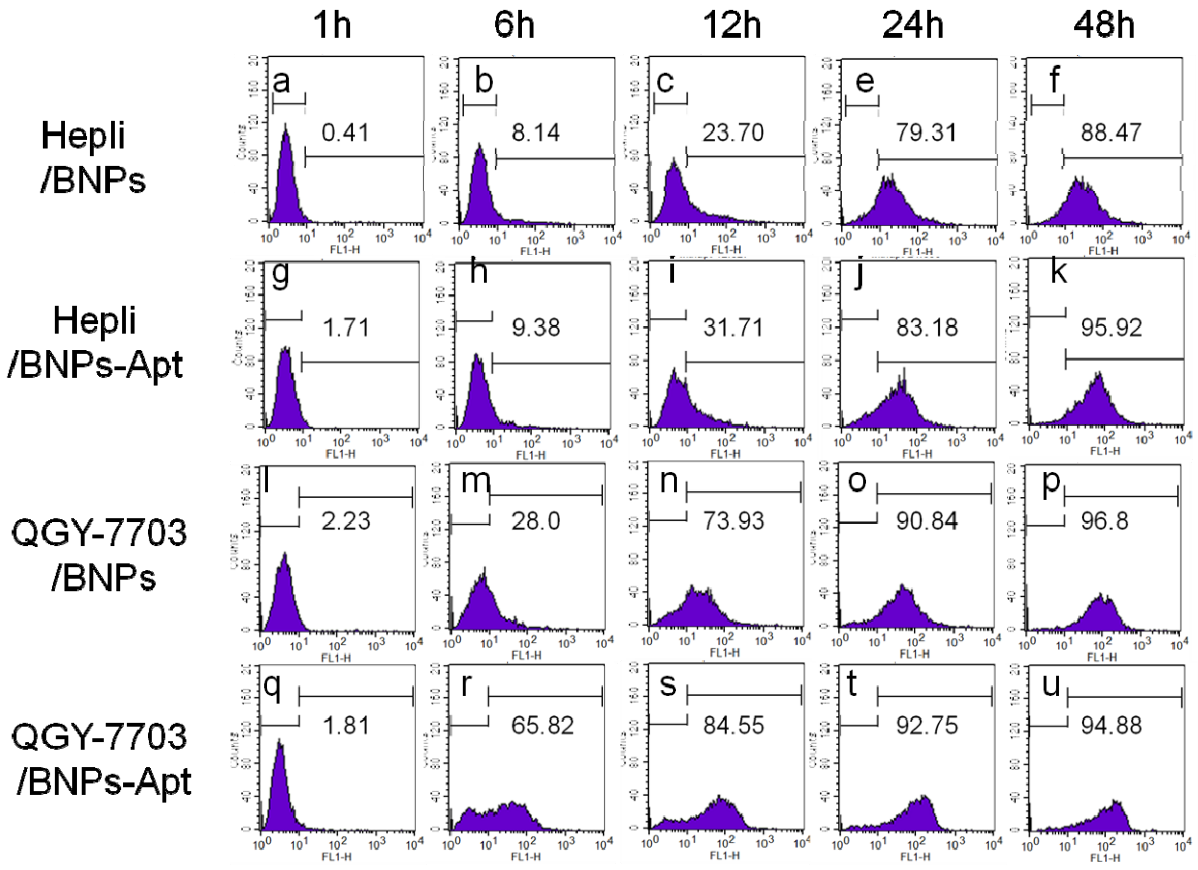

Fig. S3 Typical flow cytometry histograms of cells internalized with BNPs (BSA@(PAH/PSS)2/PAH-g-PEG-COOH NPs) and BNPs-Apt (BSA@(PAH/PSS)2/PAH-g-PEG-CONH-Apt NPs) as a function of time. The Hepli and QGY-7703 cells were incubated with 50 μg/ml NPs.

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Cytotoxity of AS1411,BNPs and BNPs-Apt particles

The cells were plated at a density of $1x10^4$ cells per well in a 96-well plate and cultured for 24 h. The medium was exchanged with fresh medium containing free AS1411-NH_2, BNPs or BNPs-Apt of varying concentrations for 6 h and 48 h. After treatment for different time, 20 μL MTT (5 mg/mL) was added to each well and the cells were continually cultured at 37 ^oC for 4 h. The dark blue formazan crystals generated by the mitochondria dehydrogenase in live cells were dissolved with dimethyl sulfoxide to measure the absorbance at 570 nm by a microplate reader (MODEL 550, Bio Rad).

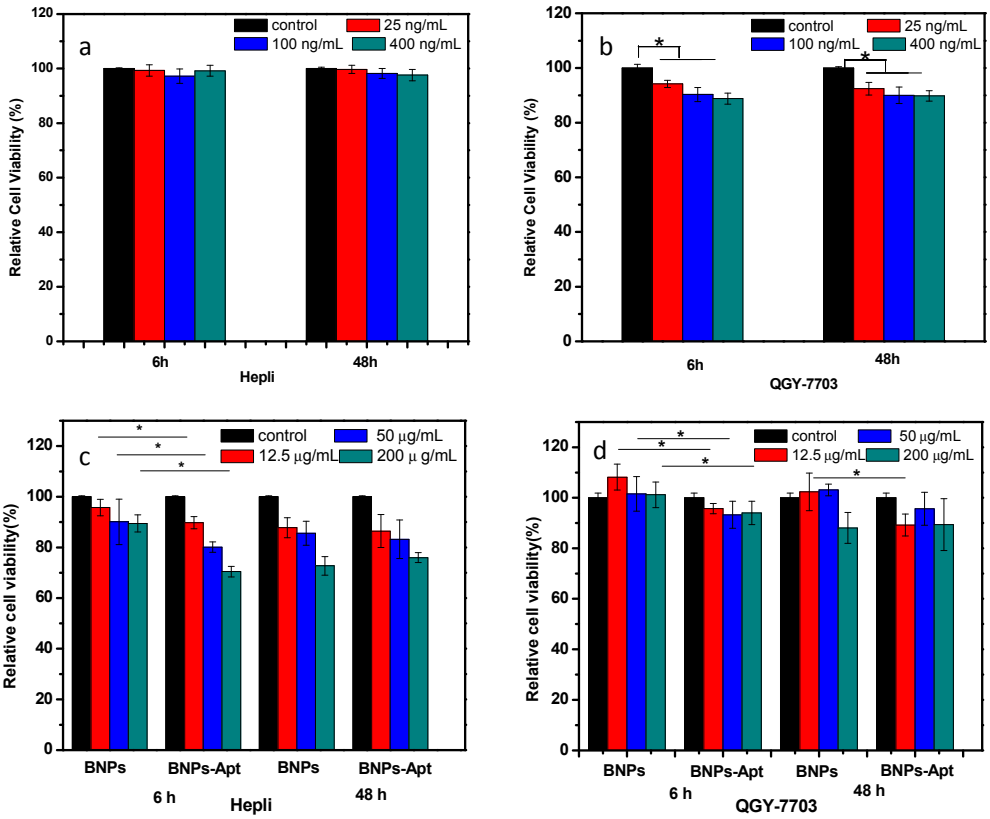

Fig. S4 In vitro cell viability of Hepli (a,c) and QGY-7703 (b,d) cells incubated with free AS1411-NH_2 (a,b), BNPs (BSA@$(PAH/PSS)_2$/PAH-g-PEG-COOH NPs) and BNPs-Apt (BSA@$(PAH/PSS)_2$/PAH-g-PEG-CONH-Apt NPs) (c,d) at different concentrations for 6 h and 48 h, respectively.