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Electronic Supplementary Information for

Targeted Degradation of ABCG2 for Reversing Multidrug Resistance by Hypervalent Bispecific Gold Nanoparticle-Anchored Aptamer Chimeras†

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Supplementary Experimental Section

Supplementary materials and methods

Reagents and materials. Chloroauric acid ($HAuCl_4$), trisodium citrate, and anhydrous ethanol were purchased from Nanjing Reagent Company (Nanjing, China). Doxorubicin (Dox) hydrochloride was purchased from Aladdin Reagents (Shanghai, China). Probenecid was purchased from Shanghai Topscience (Shanghai, China). Ko143 was purchased from Bide Pharmatech (Shanghai, China). Tariquidar was purchased from Macklin (Shanghai, China). All unmodified, thiol-modified, and carboxyfluorescein (FAM)-modified ssDNA sequences were obtained from Sangon Biotech (Shanghai, China) with recommended purification method. Roswell Park Memorial Institute 1640 medium (RPMI-1640), phosphate buffer saline for cell culture (1x PBS), parenzyme cell digestion solution (containing 0.25 % trypase and 0.02 % EDTA) and BCA protein assay kit were purchased from Keygen Biotech (Nanjing, China). Fetal bovine serum (FBS) was purchased from Gibco (Life Technologies, Australia). GAPDH antibody (AF7021) and Goat Anti-Rabbit IgG (H+L) HRP (S0001) were purchased from Affinity Biosciences (Changzhou, China). ABCG2 antibody (100341-T32) for western blotting was purchased from Sino Biological Inc. (Beijing, China). Rabbit monoclonal [EPR21122] to BCRP/ABCG2 (ab229193) and Goat Anti-Rabbit IgG H&L (Alexa Fluor® 647) (ab150079) for confocal imaging were purchased from Abcam (Shanghai, China). Lyso-Tracker Red, CellTiter Lumi™ steady luminescent cell viability assay kit, RIPA lysis buffer, PMSF, BeyoGel™ Plus Precast PAGE Gel for Tris-Gly System (4-20 %), 5x SDS PAGE protein sample loading buffer, BeyoECL Star, BeyoColor™ prestained color protein marker and QuickBlock™ western solution package were from Beyotime (Shanghai, China). Annexin V-Alexa Fluor 647/PI apoptosis detection kit was from Yeasen Biotechnology (Shanghai, China). All other reagents used were analytical grade or higher. Water used in all the experiments was purified by a Milli-Q Advantage A10 water purification system (Millipore, Milford, MA, USA).

Instruments. Transmission electron microscopy (TEM), scanning transmission electron microscopy (STEM) and energy dispersive spectroscopy (EDS) mapping profiles characterization was carried out on a JEOL JEM-2800 field-emission high-resolution transmission electron microscope (JEOL, Tokyo, Japan) operated at 200 kV. Diameter distribution and zeta potential measurement were carried out on a BI-200SM instrument (Brookhaven Instrument Corporation, Holtsville, NY, USA). Ultraviolent (UV) spectral analysis was performed with a NanoDrop 2000/2000C spectrophotometer (Thermo Fisher, MA, USA). Microplate reader analysis was carried out on a BioTek Synergy Mx microplate reader (Winooski, VT, USA). Confocal fluorescence imaging of cells was acquired on an LSM 710 confocal laser scanning microscope (Zeiss, Oberkochen, Germany). Flow cytometric analysis was performed on a Beckman Coulter CytoFlex S system (California, USA). SDS PAGE gel was imaged by Tanon-4600SF Gel Imager (Tanon, Shanghai, China).

Cell lines and cell culture. HepG2 cells were purchased from Keygen Biotech (Nanjing, China). Dox-resistant HepG2 cells (HepG2/ADR) were purchased from Aiyan Biology (Shanghai, China). HepG2 cells and HepG2/ADR cells maintained in RPMI-1640 supplemented with 10 % fetal bovine serum under a 5 % CO_2 atmosphere at 37 °C. In order to maintain the resistant strains, 200 ng/mL Dox was added into the culture medium of HepG2/ADR cells.

Preparation and characterization of AuNPs and AuNP-APTACs.

Preparation of AuNPs and AuNP-APTACs. AuNPs were prepared using the protocol reported in the literature[1]. The diameter of such prepared nanoparticles is about 13 nm. The concentration of the nanoparticles is about 11 nM. The bispecific multivalent aptamer-functionalized AuNPs (AuNP-APTACs) were prepared with freeze-thaw method reported in previous literature[2]. Briefly, thiol-modified aptamers at the 5' terminus were mixed with AuNPs and placed in a freezer (-20 °C) for 2 h, and then thawed at room temperature for standby application.

Characterization of AuNP-APTACs. The morphology features and particle diameters of AuNPs and AuNP-APTACs were characterized by TEM. The EDS mapping of AuNP-APTACs was carried out to verify the existence of Au, P, and S elements on AuNP-APTACs. Before the hydrodynamic sizes and Zeta potential were analyzed, AuNPs and AuNP-APTACs were filtered by 0.22 μm filter. The concentration of AuNPs was quantified by UV-visible spectroscopy and calculated by dividing the UV absorbance by molar extinction coefficient of 13 nm AuNPs (2.7×10^8 $M^{-1}cm^{-1}$)[3]. The UV-Vis spectroscopy of AuNP-APTACs with different molar ratios of AuNPs to aptamers was also measured.

Quantification of the aptamer strands number attached to each AuNP. To evaluated the numbers of IGF2R-binding aptamer (A1) and ABCG2-binding aptamer (A2) attached to each AuNP respectively, FAM labeled thiol-modified aptamers were used to modify AuNPs. Firstly, the fluorescence intensities of different concentrations of free FAM-labeled thiol-modified A1 and A2 were respectively measured with a fluorescence spectrometer to establish standard working curves (Figure S9). The molar ratio of AuNPs to aptamer of the AuNP-APTACs was 1:400, and the molar ratio of A1 to A2 was 1:1. Then, for the sample to quantify the number of conjugated A1, FAM-labeled A1 and equal quantity of no FAM-labeled A2 were functionalized onto AuNPs. After centrifuged at 12,000 rpm for 30 min, the fluorescence intensity of the supernatant was detected. The concentration of A1 in the supernatant was obtained by comparing its fluorescence intensity against the standard working curve of SH-A1-FAM, according to which the molar ratio of AuNP to A1 could be calculated. The same way was applied to quantify the number of A2 attached on each AuNP. The results are shown in Table S2.

Construction bispecific aptamer chimeras in buffer. The construction methods were as reported literature[4]. Aptamers were heated separately to 95 °C in 1×DPBS-Mg buffer (DPBS, 12.5 mM $Mg(Ac)_2$) and cooled down on ice. Then, A1 and A2 with different lengths (A1-12 and A2-12, A1-20 and A2-20, A1-30 and A2-30) of linkers were mixed together in 100 μL buffer at 4 °C for 30 min to form B-12, B-20, and B-30. 12 % native polyacrylamide gel in 1×TBE buffer was run at 120 V for 60 minutes and then was stained with Gel Red staining solution.

The fabrication and characterization of DNA tetrahedrons. DNA tetrahedrons (TDN) was fabricated by 2 steps. Firstly, TDN was synthesised as previously reported method[5]: single-stranded DNAs (S1, S2, S3, and S4) were mixed together in TM buffer (10 mM Tris-HCl, 50 mM MgCl2, pH 8.0) at the same concentration by gentle vortexing. After that, they were heated at 95 °C for 10 min, rapidly cooled to 4 °C and kept in the thermal cycler for 20 min for tetrahedral structure maintenance. Secondly, for aptamer attaching to form TDN-Aptamer nanostructures (T7 and T17), two kinds of aptamers (A1-T20, and A2-T20) with molar ratio 1:1 was added to the TDN solution and annealed slowly from 35 °C to 20 °C at a ramp of 10 min per degree. The resultant DNA products were stored at 4 °C for further use. 5 % native SDS-PAGE was performed to confirm the successful synthesis. DNA ladder and samples mixed with loading buffer were loaded onto lanes to perform electrophoresis at 80 V for 1 h in 1×TBE running buffer. The resultant polyacrylamide gel was stained with Tanon Nucleic Acid

Gel Stain Red (10,000×) at room temperature for 1h. After that, the gel was imaged by Tanon Gel Imager (Tanon-4600SF).

Serum stability of AuNP-APTACs. A1, A2, or AuNP-APTACs (containing 3 μM aptamer totally) was incubated with RPMI 1640 containing 10 % FBS for different time (0 to 48 h) at 37 ºC. After incubating with 1 mg/mL GSH for 15 min which was used to cleave the thiol-gold bonds[6], the samples were loaded onto 5 % polyacrylamide gel in 1×TBE buffer and run at 120 V for 30 min. After electrophoresis, the gels were stained with nucleic acid staining (Gel Red) and imaged by Tanon Gel Imager. The bands were analyzed by ImageJ.

Degradation experiments. Cells at 70 % confluency were treated with a series of multivalent bispecific AuNP-APTACs or control particles in complete growth medium. At the experiment end point, cells were washed with cold PBS, and tested by either western blotting or confocal laser scanning microscope (CLSM) imaging.

Western blotting. Cells were lysed by RIPA buffer containing 1 mM PMSF on ice for 8 min. The lysates were spun at 20,000 g for 10 min at 4 °C and protein concentrations were normalized using BCA assay. 5× SDS PAGE protein sample loading buffer was added to the lysates and then the lysates were boiled for 10 min. Equal amounts of lysates (20-50 μg protein) were loaded onto a 4-20 % Tris-Gly SDS PAGE gel which ran at 80 V for 30 min firstly and at 120 V for 1 h subsequently. Next, the gel was transferred to a polyvinylidene difluoride (PVDF) membrane (0.45 μm) under 400 mA constant current for 1 h. The membrane was then blocked in QuickBlock™ blocking buffer for 30 min at room temperature and immunoblotted with the ABCG2 antibody diluted 500 times and GAPDH antibody diluted 3,000 times at 4 °C overnight. After washing, secondary antibody conjugated HRP was incubated for 1 h at room temperature, and then the membrane was washed 3 times for imaging. The protein levels were quantified by the gray values of the bands in the resulting images using the control group as the standard.

Lysosomal colocalization experiment using CLSM. The HepG2/ADR cells cultured in confocal dishes were co-incubated with Lyso-Tracker Red at 37 °C for 10 min. Then the cells were washed twice with PBS, followed by addition of 600 nM (the total concentration of aptamers) of FAM-AuNP-APTACs, FAM-A1, or FAM-A2 in 500 μL of complete culture medium at 37 °C for 30 minutes, respectively. Then the cells were washed twice with washing buffer and added 1 ml PBS into confocal dish. Imaging of the cells was performed on an LSM 710 confocal laser scanning microscope. A 488-nm laser was the excitation source for FAM and Dox, 543-nm laser for Lyso-Tracker throughout the experiment.

Immunofluorescence staining. HepG2/ADR cells treated with or without 600 nM Au-A1, Au-A2, or AuNP-APTACs for 24 h were fixed by 4 % paraformaldehyde for 10 min. After blocked for 1 h in PBS with 10 % FBS (v/v) and 5 % BSA (w/v), cells were incubated with ABCG2 antibody for 2 h at room temperature and then with Alexa Flour 647 goat anti-rabbit IgG for 1 h at room temperature, followed by incubation with DAPI for 5 min. The cells were observed under the CLSM. A 405-nm laser was used for DAPI, and 633-nm laser for Alexa Fluor® 647.

Dox loading experiment. To confirm the drug loading capacity and the balanced loading time of AuNP-APTACs, different volumes (0, 6.8, 17, 68, 135 μL) of AuNP-APTACs (ca. 4.4 nM) were mixed with 15 μL of 1 mM Dox to obtain AuNP-APTACs-Dox. The final volume was supplemented to 150 μL with sterile water. After incubation at

room temperature for 2, 6, or 12 h, the fluorescence spectra were monitored by setting the excitation wavelength at 488 nm and used to calculate loading capacity.

CLSM imaging of Dox in cells. The HepG2 cells and HepG2/ADR cells were incubated with 15 μM free Dox alone or together with 600 nM AuNP-APTACs or AuNP-APTACs-Dox (loading 15 μM Dox) immediately at 37 °C for 24 h. Then the cells were washed twice with PBS, followed by fixed with 4 % paraformaldehyde for 10 min and incubated with DAPI for 5 min at room temperature. The confocal dish was above a 40×objective on the confocal microscope. A 488-nm laser was the excitation source for Dox and 405-nm UV laser for DAPI throughout the experiment.

Flow cytometry analysis. To evaluate the binding performance of AuNP-APTACs with HepG2/ADR, about 2×10^5 HepG2/ADR cells were incubated with 600 nM (total aptamer concentration) of FAM-labeled AuNP-APTACs, 600 nM of FAM-labeled monovalent aptamer (A1, A2), or 600 nM of FAM-labeled random sequence in 200 μL RPMI 1640 incomplete culture medium on ice for 30 min. The cells without FAM-aptamer incubation were as a blank group. Cells were washed twice with cold buffer and suspended in 500 μL PBS for flow cytometry analysis by counting 10000 events.

To measure the intracellular accumulation of Dox, cells incubated with 15 μM of Dox and 600 nM of AuNP-APTACs or 1.5 nM of AuNPs (the same concentration of AuNPs in AuNP-APTACs) were cultured at 37 °C for 24 h. Then, the cells were washed twice and resuspended in 200 μL PBS for flow cytometry analysis.

For apoptosis detection experiments, 600 nM of no FAM-labeled AuNP-APTACs were incubated with HepG2/ADR cells at 37 °C for 24 h. And 1 μM of Dox was added into culture medium to incubate cells for another 3 hours. The cells only incubated with free Dox were as a control group, and the cells normally cultured were set as a blank group. Then cells were digested using trypsin without EDTA and collected by centrifugation (300 g, 5 min), followed by washing twice with PBS buffer solution. Consequently, 5 μL of Annexin V-Alexa Fluor 647 and 10 μL of PI with 100 μL buffer were added into collected cells. After incubation avoiding light for 15 min at room temperature and addition of 400 μL buffer, the samples were measured by flow cytometry within 1 hour. The results were analyzed by FlowJo.

Cell viability analysis. HepG2/ADR cells or HepG2 cells were seeded in 96-well microplates with a density around 10^4 cells per well and allowed to adhere for 12 h prior before adding certain reagents. For the cell safety analysis of AuNP-APTACs, the HepG2/ADR cells were incubated with different concentrations of AuNP-APTACs for 24 h. For the determination of IC_{50}, the cells were incubated with different concentrations of Dox and 600 nM (the total aptamer concentration) of AuNP-APTACs, AuNPs, or the inhibitors of ABCG2, P-gp, and MRP1 at 37 °C for 24 h. For the cytotoxicity comparation, 15 μM of free Dox with different particles (AuNPs, Au-A1, Au-A2, AuNP-APTACs) or AuNP-APTACs-Dox (loading 15 μM of Dox) were incubated with cells at 37 °C for 48 h. The wells without the variable reagents were set as control group, and the wells without cells were used as background group. The other wells were set as test groups. Then cell viability was evaluated by MTT assay or CellTiter Lumi™ steady luminescent cell viability assay kit (to avoid the effect of Dox absorbance) according to the instructions.

The cell viability was expressed as a percentage of the absorbance of test cells over that of control experiment (both were deducted by the background absorbance), which was calculated by the following equation:

$$\text{Cell viability (\%)} = \frac{\text{Abs(test)} - \text{Abs(background)}}{\text{Abs(control)} - \text{Abs(background)}} \times 100\%$$

Cell viability analysis was conducted for 3 independent times.

References

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6 Y. Song, Y. Shi, M. Huang, W. Wang, Y. Wang, J. Cheng, Z. Lei, Z. Zhu and C. Yang, *Angew. Chem. Int. Ed*., 2019, **58**, 2236-2240.

Supplementary Figures

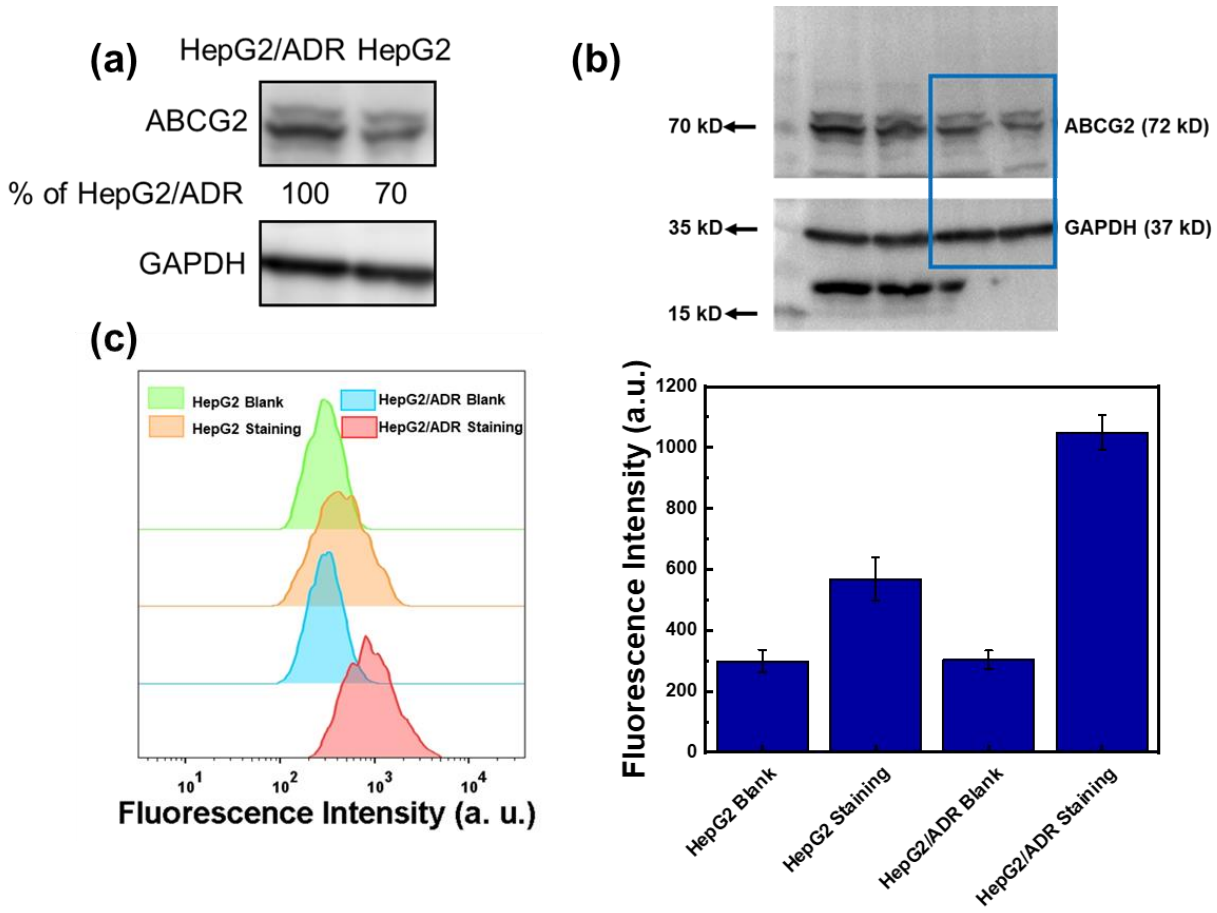

Fig S1. (a) The expression of ABCG2 in HepG2 and HepG2/ADR cells analyzed by western blotting. (b) Full raw image of the western blotting for the expression analysis of ABCG2 in HepG2 and HepG2/ADR cells in (a) (Lane 4 and Lane 5). The final western blotting images were obtained by overlaying the chemiluminescence image and the molecular weight markers image. Lane details: (1) Molecular weight markers; (2), (3) and (4) HepG2/ADR cells; (4) HepG2 cells. (c) The expression of ABCG2 in HepG2 and HepG2/ADR cells analyzed by flow cytometry. (d) Quantitative analysis of flow cytometry results in (c).

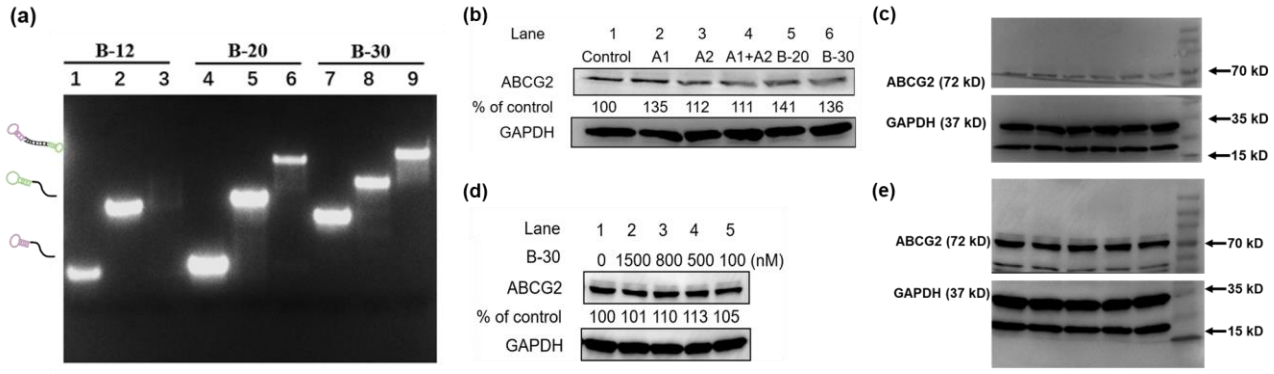

Fig. S2. (a) The construction verification of bispecific aptamer linked by dsDNA with 12, 20, and 30 bp. Lane 1, 4, 7, corresponding to A1 with 12, 20, and 30 bp linkers. Lane 2, 5, 8, corresponding to A2 with 12, 20, and 30 bp linkers. Lane 3, 6, 9, corresponding to B-12, B-20, and B-30. (b) The degradation degree of ABCG2 in HepG2/ADR cells with 1 μM of A1, A2, mixture of A1 and A2, 500 nM of B-20, and B-30 treatments for 24 h. (c) Full raw image of the western blotting the degradation degree evaluation of ABCG2 in HepG2/ADR cells with different treatments for 24 h in (b). The final western blotting images were obtained by overlaying the chemiluminescence image and the molecular weight markers image. Lane details: (1) No treatment control; (2) 1 μM A1; (3) 1μM A2; (4) Mixture of 500 nM A1 and 500 nM A2; (5) 500 nM B-20; (6) 500 nM B-30; (7) Molecular weight markers. (d) Degradation degree for ABCG2 in HepG2/ADR cells incubated with 100 nM, 500 nM, 800 nM, or 1500 nM B-30 for 24 h. (e) Full raw image of the western blotting in (d) for the degradation degree evaluation of ABCG2 in HepG2/ADR cells incubated with different concentrations of B-30 for 24 h. The final western blotting images were obtained by overlaying the chemiluminescence image and the molecular weight markers image. Lane details: (1) No treatment control; (2) 1500 nM B-30; (3) 800 nM B-30; (4) 500 nM B-30; (5) 100 nM B-30; (6) Molecular weight markers.

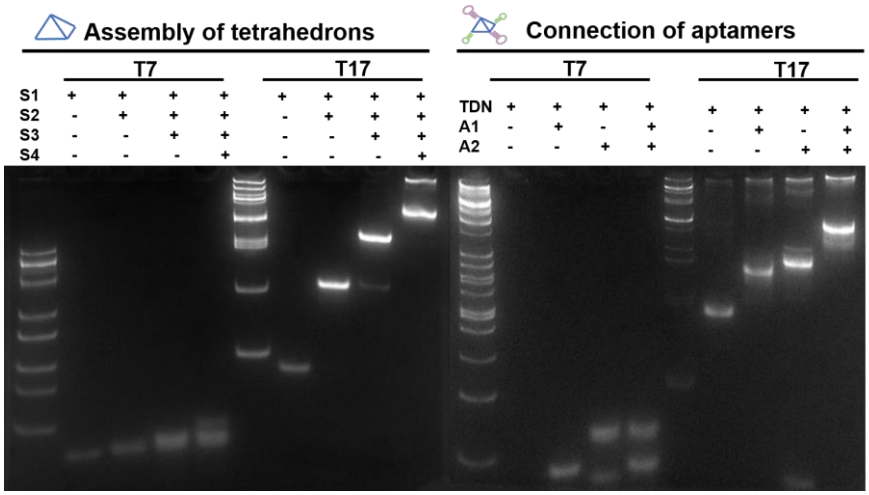

Fig. S3. The construction verification of bispecific aptamer anchored by TDN with 7 and 17 bp per side.

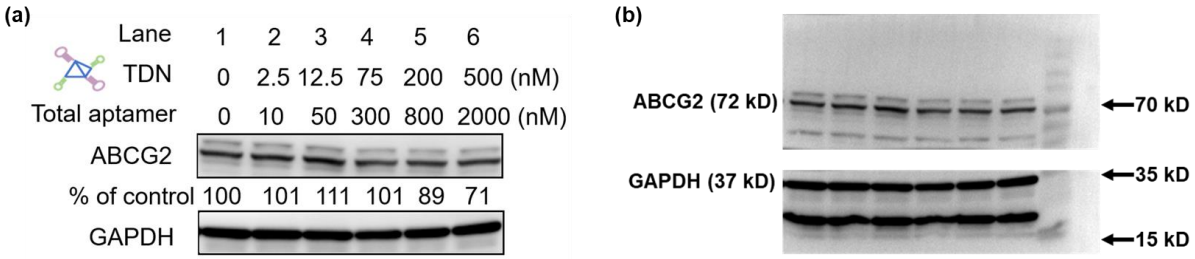

Fig. S4. (a) Degradation degree for ABCG2 in HepG2/ADR cells incubated with 0-2000 nM T17 for 24 h. (b) Full raw image of the western blotting in (a) for the degradation degree evaluation of ABCG2 in HepG2/ADR cells incubated with 0-200 nM T17 for 24 h. The final western blotting images were obtained by overlaying the chemiluminescence image and the molecular weight markers image. Lane details: (1) No treatment control; (2) 10 nM T17; (3) 50 nM T17; (4) 300 nM T17; (5) 800 nM T17; (6) 2000 nM T17; (7) Molecular weight markers.

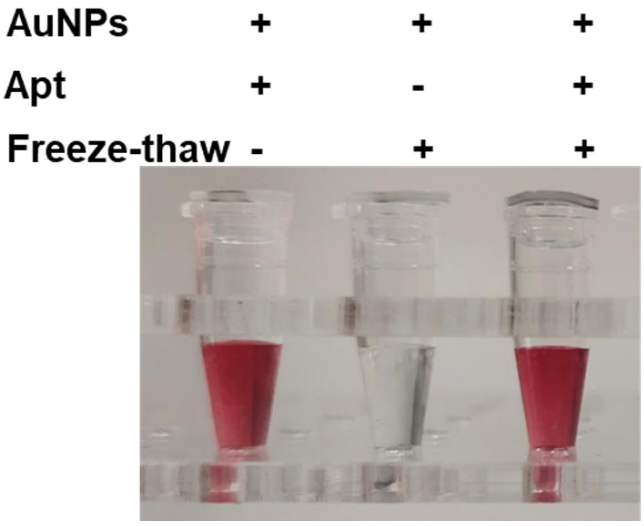

Fig. S5. The photo of AuNPs with aptamer addition before and after freeze-thaw method (keeping red), and AuNPs without addition of aptamer treated with that method (occurring precipitate).

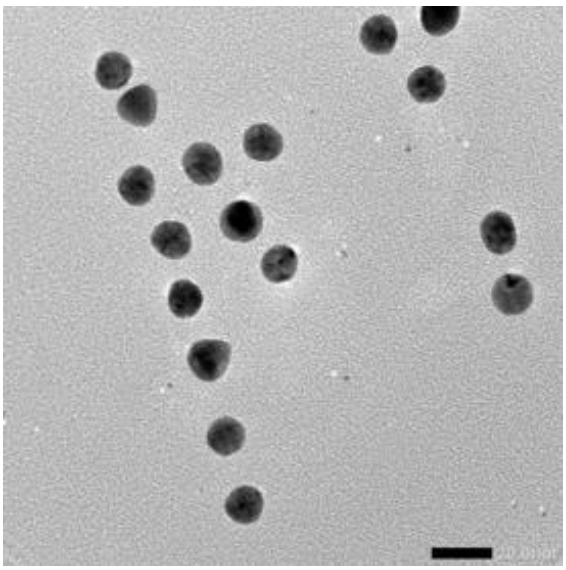

Fig. S6. The TEM image of AuNPs. Scale bar=20 nm.

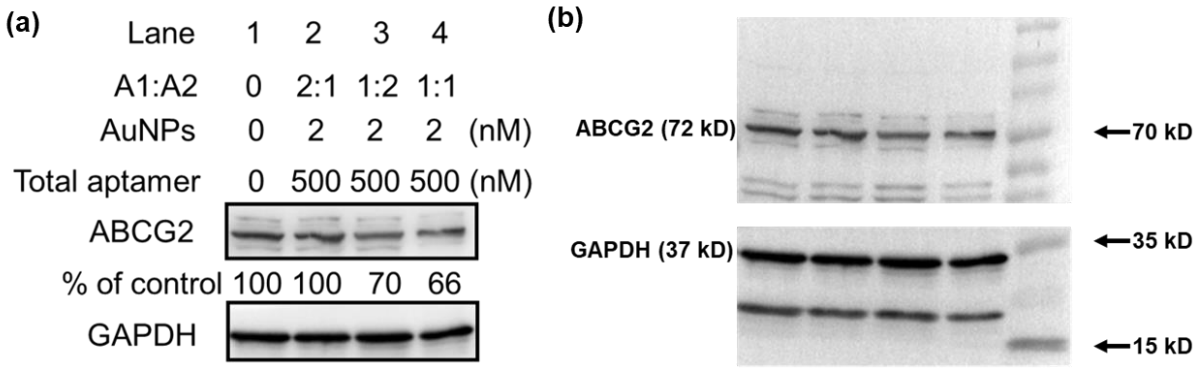

Fig. S7. (a) Optimization of the molar ratio of A1 to A2 modified on AuNPs according to the ABCG2 degradation degree in HepG2/ADR cells evaluated by western blotting. (b) Full raw image of the western blotting in (a) for the optimization of the molar ratio of A1 to A2 modified on AuNPs. The final western blotting images were obtained by overlaying the chemiluminescence image and the molecular weight markers image. Lane details: (1) No treatment control; (2) A1:A2=2:1; (3) A1:A2=1:2; (4) A1:A2=1:1; (5) Molecular weight markers.

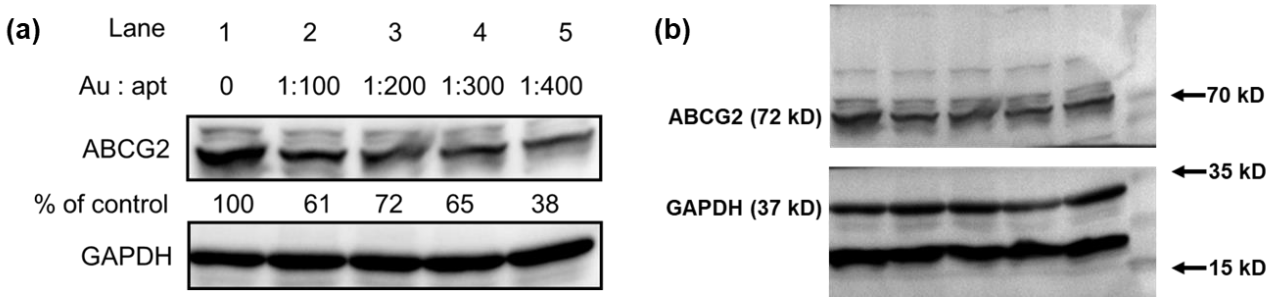

Fig. S8. (a) Optimization of the molar ratio of AuNP to aptamers modified on AuNP-APTACs according to the ABCG2 degradation degree in HepG2/ADR cells evaluated by western blotting. (b) Full raw image of the western blotting in (a) for the optimization of the molar ratio of AuNP to aptamers modified on AuNP-APTACs. The final western blotting images were obtained by overlaying the chemiluminescence image and the molecular weight markers image. Lane details: (1) No treatment control; (2) 1.6 μM AuNPs-APTACs with AuNPs:aptamers=1:100; (3) 1.6 μM AuNPs-APTACs with AuNPs:aptamers=1:200; (4) 1.6 μM AuNPs-APTACs with AuNPs:aptamers=1:300; (5) 1.6 μM AuNPs-APTACs with AuNPs:aptamers=1:400; (6) Molecular weight markers.

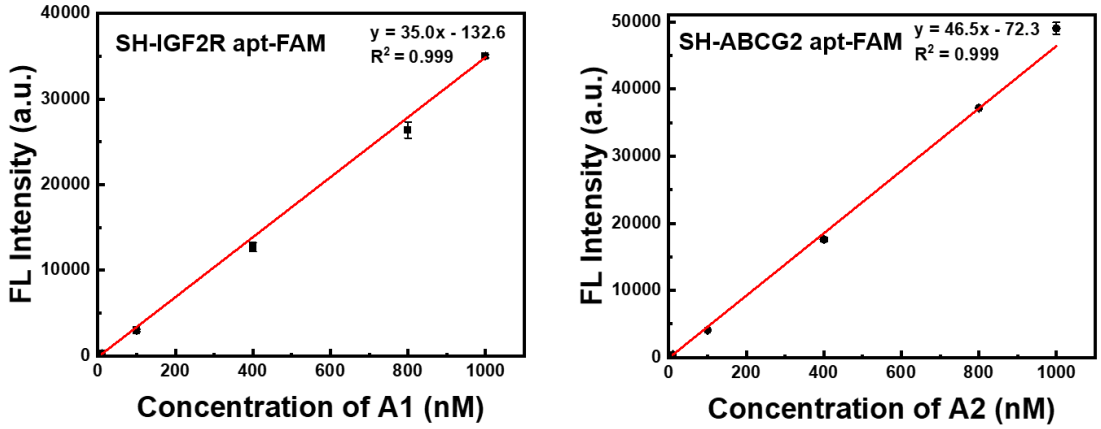

Fig. S9. Fluorescence intensity standard curves of A1 and A2 for quantification of the number of aptamers modified on each AuNP.

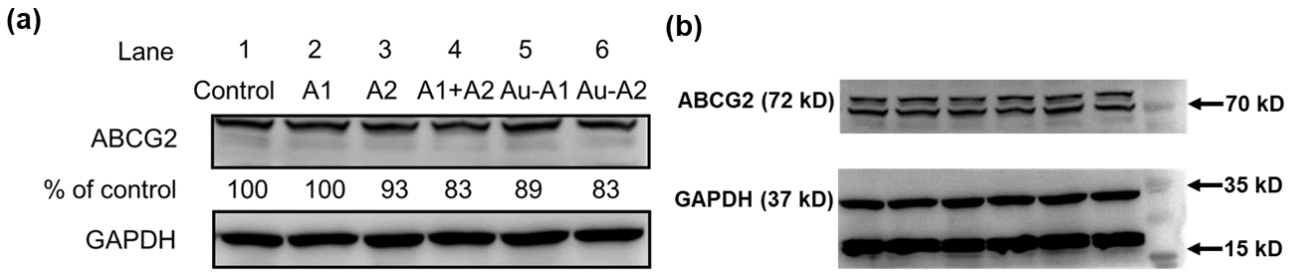

Fig. S10. (a) The capacity of different treatments to induce ABCG2 degradation. (b) Full raw image of the western blotting in (a) for the comparation of ABCG2 degradation degrees induced by control treatments. The final western blotting images were obtained by overlaying the chemiluminescence image and the molecular weight markers image. Lane details: (1) No treatment control; (2) 600 nM A1; (3) 600 nM A2; (4) The mixture of 300 nM A1 and 300 nM A2; (5) 600 nM Au-A1; (6) 600 nM Au-A2; (7) Molecular weight markers

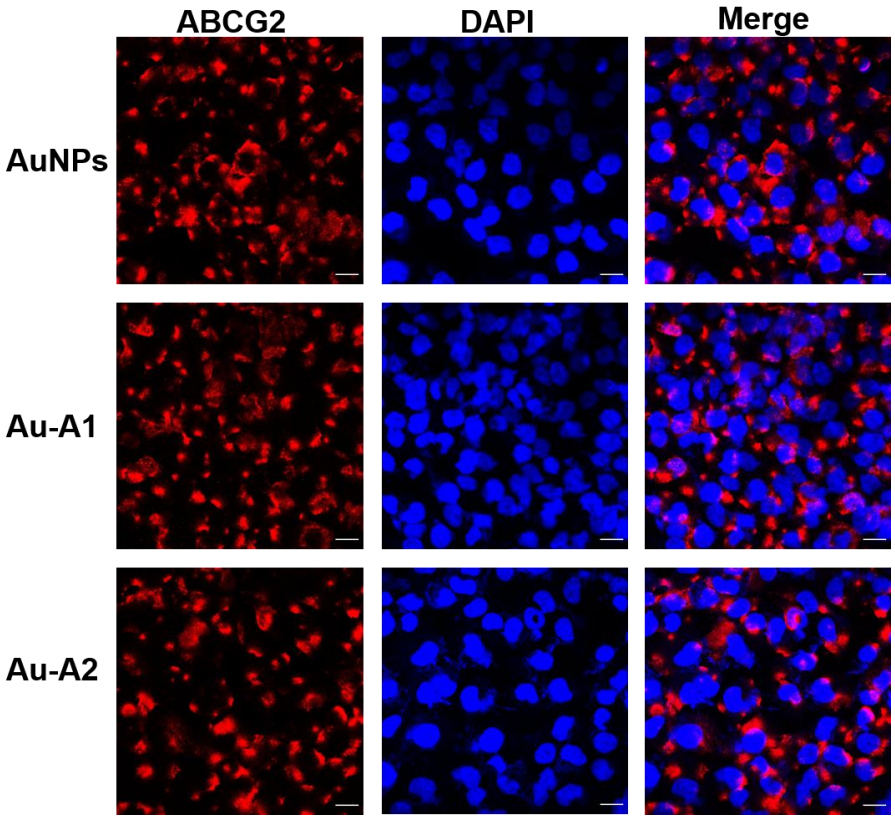

Fig. S11. Visualization of ABCG2 degradation on HepG2/ADR cells by confocal microscopy after treatment with control particles (AuNPs, Au-A1, or Au-A2) for 12 h. Scale bars = 20 μm.

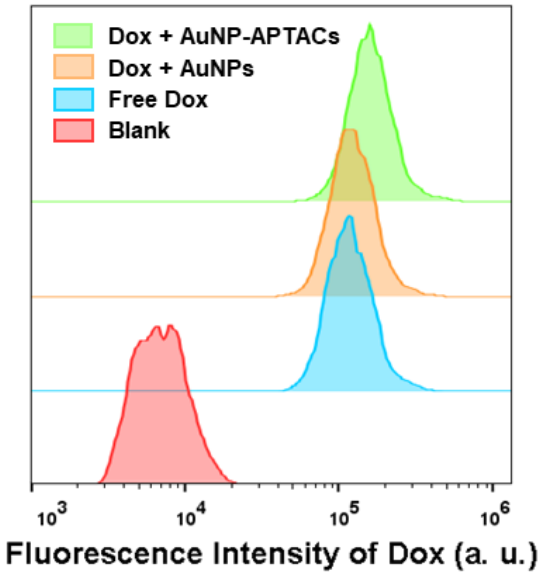

Fig. S12. Flow cytometry analysis of the fluorescence intensity of Dox in HepG2/ADR cells after incubation with 15 μM free DOX and 600 nM AuNP-APTACs (1.5 nM AuNPs modified with 600 nM aptamers) or 1.5 nM AuNPs for 24 h at 37 °C.

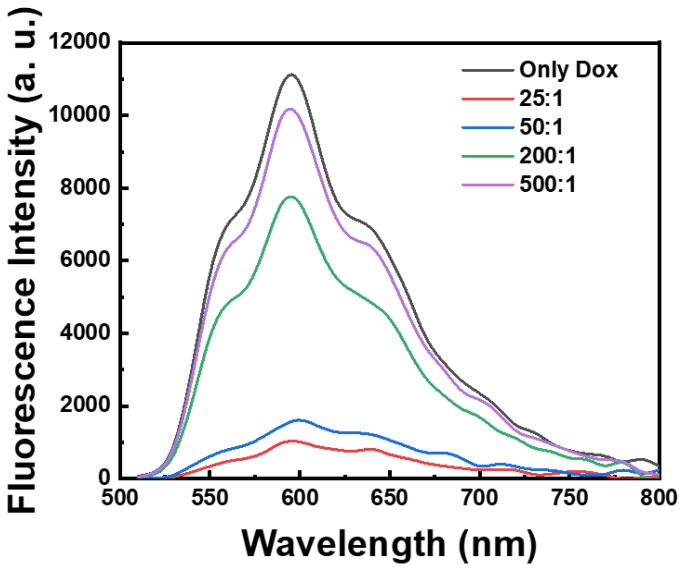

Fig. S13. Fluorescence spectrometry at different molar ratios of AuNP-APTACs to Dox.

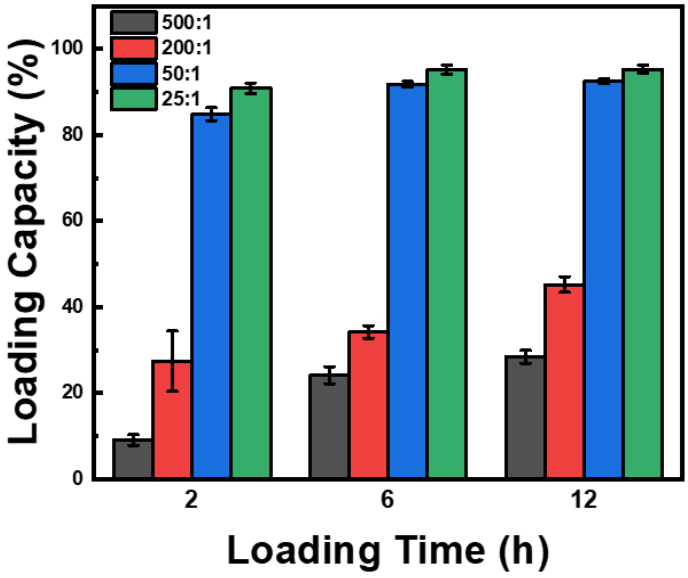

Fig. S14. Effect of loading time on the loading capacity at different molar ratios of AuNP-APTACs to Dox.

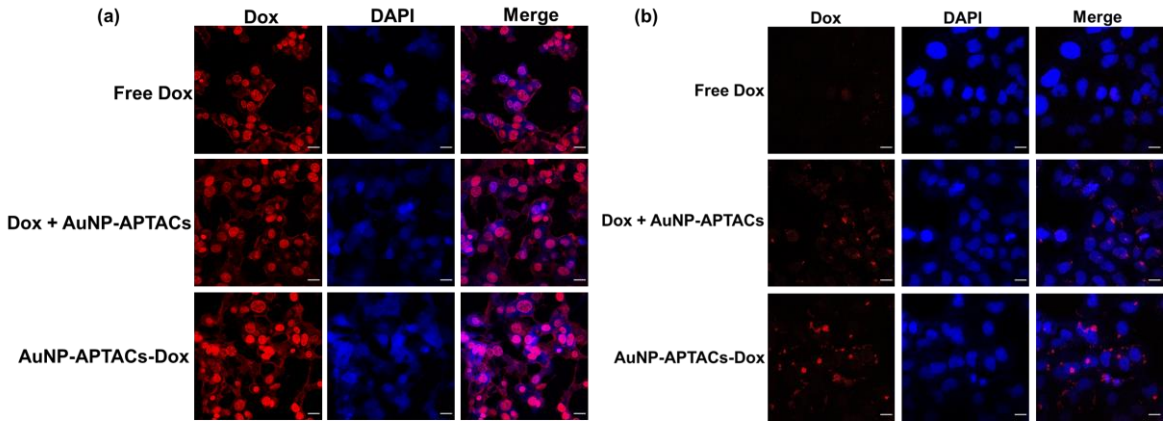

Fig. S15. CLSM images of Dox in HepG2 cells (a) and HepG2/ADR cells (b) after treatments of 15 μM free dox, the mixture of 15 μM Dox and 600 nM AuNP-APTACs, or AuNP-APTACs-Dox (AuNP-APTACs loading 15 μM Dox) for 24 h. Scale bars = 20 μm.

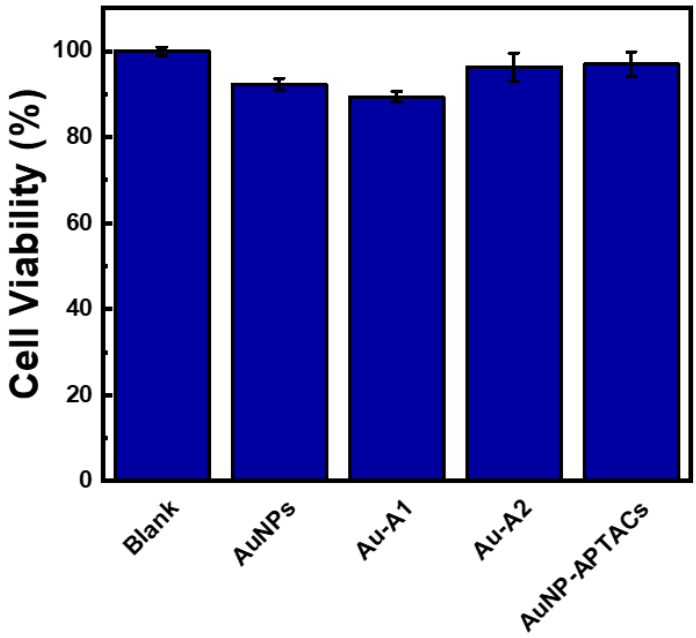

Fig. S16. Cell viability of HepG2/ADR after treatment with AuNP-APTACs or other control particles (Au-A1, Au-A2, and AuNPs, containing 1.5 nM AuNPs) for 48 h.

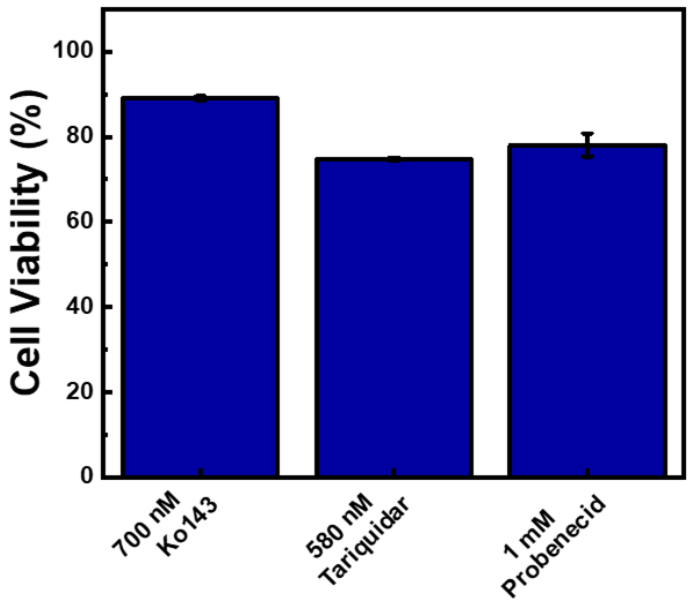

Fig. S17. The cytotoxicity of 700 nM Ko143, 580 nM Tariquidar, and 1 mM Probenecid.

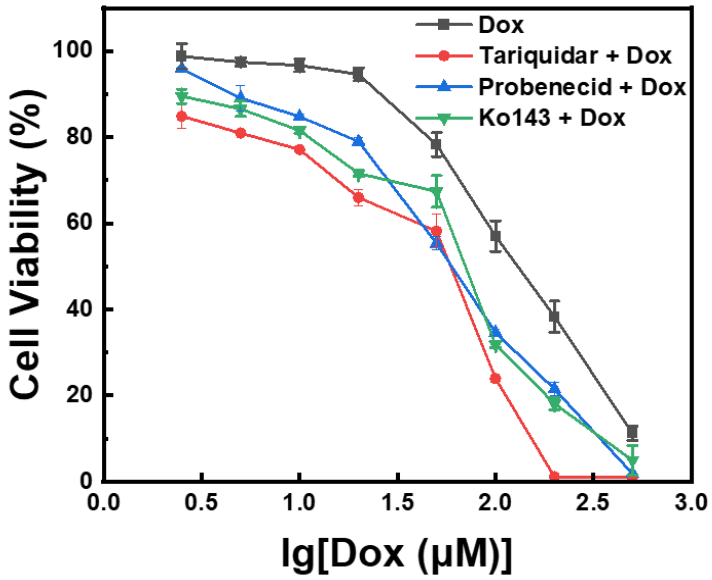

Fig. S18. Cell viability of HepG2/ADR after treatment with Dox alone or together with tariquidar, Ko143, or probenecid (the inhibitor of P-gp, ABCG2, and MRP1 respectively) for 24 h.

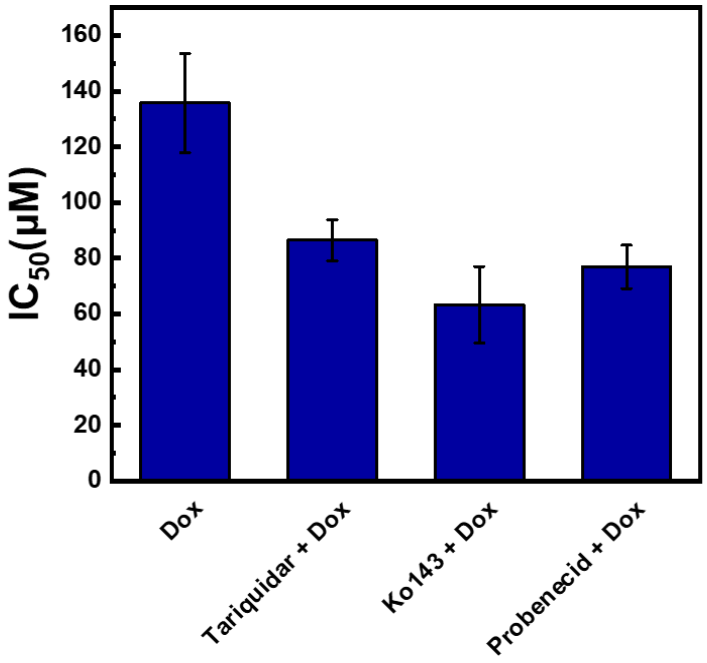

Fig. S19. The IC_{50} values of Dox for HepG2/ADR calculated from Figure S17 after nonlinear curve fitting.

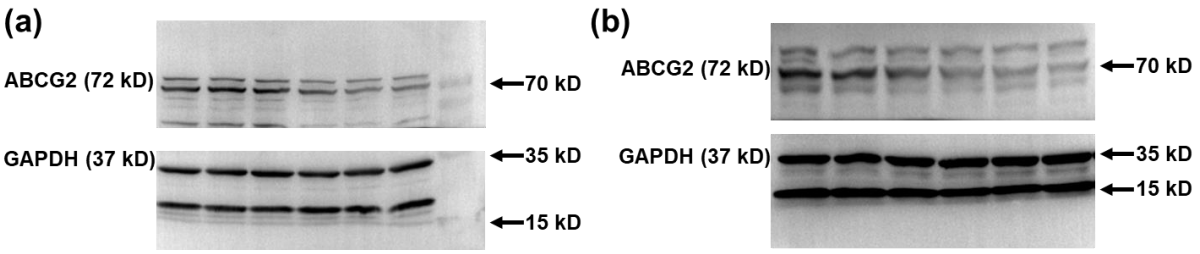

Fig. S20. (a) Full raw image of the western blotting in Fig. 3a for time course evaluation of ABCG2 degradation induced by 800 nM AuNPs-APTACs in HepG2/ADR cells. The final western blotting images were obtained by overlaying the chemiluminescence image and the molecular weight markers image. Lane details: (1) 0 h control; (2) 2 h; (3) 6 h; (4) 12 h; (5) 24 h; (6) 36 h and (7) Molecular weight markers. (b) Full raw image of the western blotting in Fig. 3b for dose-response degradation degree evaluation of ABCG2 induced by a serial concentration of AuNPs-APTACs in HepG2/ADR cells for 24 h. The final western blotting images were obtained by chemiluminescence imaging. Lane details: (1) control without AuNP-APTACs treatment; (2) 50 nM AuNP-APTACs treatment; (3) 300 nM AuNP-APTACs treatment; (4) 600 nM AuNP-APTACs treatment; (5) 800 nM AuNP-APTACs treatment; (6) 1600 nM AuNP-APTACs treatment.

Supplementary Tables

Table S1. Oligonucleotides sequences used in this study.

Name	Sequences
A1	5’-GGGCGCGTAGATGACGAGCAGTCCTAACATCGTTTAGGAC-3’
A2	5’-GCCTCCTTCCCCCACGGCTCGGGCGAGGAAGCGCCGCCGTGCTTTGCTCGGAGACGAGATAGGC-3’
SH-A1	5’-SH-TTTTTGGGCGCGTAGATGACGAGCAGTCCTAACATCGTTTAGGAC-3’
SH-A2	5’-SH-GCCTCCTTCCCCCACGGCTCGGGCGAGGAAGCGCCGCCGTGCTTTGCTCGGAGACGAGATAGGC-3’
SH-A1-FAM	5’-SH-TTTTTGGGCGCGTAGATGACGAGCAGTCCTAACATCGTTTAGGAC-FAM-3’
SH-A2-FAM	5’-SH-GCCTCCTTCCCCCACGGCTCGGGCGAGGAAGCGCCGCCGTGCTTTGCTCGGAGACGAGATAGGC-FAM-3’
A1-12	5’-GGGCGCGTAGATGACGAGCAGTCCTAACATCGTTTAGGACTAGTTATAACGTA-3’
A2-12	5’-GCCTCCTTCCCCCACGGCTCGGGCGAGGAAGCGCCGCCGTGCTTTGCTCGGAGACGAGATAGGC AATACGTTATAACT-3’
A1-20	5’-GGGCGCGTAGATGACGAGCAGTCCTAACATCGTTTAGGACTCATTAGTCATAACGTTCTAC-3’
A2-20	5’-GCCTCCTTCCCCCACGGCTCGGGCGAGGAAGCGCCGCCGTGCTTTGCTCGGAGACGAGATAGGCAAGTAGAACGTTATGACTAATG-3’
A1-30	5’-GGGCGCGTAGATGACGAGCAGTCCTAACATCGTTTAGGACTATTTCAATTTCATTAGTCATAACGTTCTAC-3’
A2-30	5’-GCCTCCTTCCCCCACGGCTCGGGCGAGGAAGCGCCGCCGTGCTTTGCTCGGAGACGAGATAGGCAAGTAGAACGTTATGACTAATGAAATTGAAAT-3’
T7-S1	5’-GTAGAACGTTATGACTAATGTTTTTTTGAGCGTTAGCCACACACACAGTC-3’
T7-S2	5’-GTAGAACGTTATGACTAATGTTTTTTTTTAGGCGAGTGTGGCAGAGGTGT-3’

T7-S3	5’-ATTTCAATTTCATTAGTCATTTTTTTTCGCCTAAACAAGTGGAGACTGTG-3’
T7-S4	5’-ATTTCAATTTCATTAGTCATTTTTTTTAACGCTCACCACTTGAACACCTC-3’
T17-S1	5’-GTAGAACGTTATGACTAATGTTTTTTTACATTCCTAAGTCTGAAACATTACA GCTTGCTACACGAGAAGAGCCGCCATAGTA-3’
T17-S2	5’-GTAGAACGTTATGACTAATGTTTTTTTTATCACCAGGCAGTTGACAGTGTA GCAAGCTGTAATAGATGCGAGGGTCCAATAC-3’
T17-S3	5’-ATTTCAATTTCATTAGTCATTTTTTTTTCAACTGCCTGGTGATAAAACGACA CTACGTGGGAATCTACTATGGCGGCTCTTC-3’
T17-S4	5’-ATTTCAATTTCATTAGTCATTTTTTTTTTCAGACTTAGGAATGTGCTTCCCA CGTAGTGTCGTTTGTATTGGACCCTCGCAT-3’
A1-T20	5’-CATTAGTCATAACGTTCTACTTGGGCGCGTAGATGACGAGCAGTCCTAACA TCGTTTAGGAC-3’
A2-T20	5’-ATGACTAATGAAATTGAAATTTGCCTCCTTCCCCCACGGCTCGGGCGAGGAA GCGCCGCCGTGCTTTGCTCGGAGACGAGATAGGC-3’

Table S2. Comparison of degradation percentages of ABCG2.

Linker type	dsDNA	TDN	AuNPs
Maximum degradation	No effect	29 %	62 %
Total aptamer concentration	3 μM	2 μM	800 nM

Table S3. Quantification of the number of aptamers modified on AuNPs.

AuNP-APTACs (1:400)	Group 1	Group 2	AuNP:apt (molar ratio)
AuNPs	Adding	Adding	/
A1	No FAM modified	FAM modified	1:192
A2	FAM modified	No FAM modified	1:184

Table S4. The IC_{50} values of Dox after different treatments. [a]

Treatment		IC_{50} mean ± SD (μM, resistance fold[b])
HepG2/ADR	Dox alone	135.799 ± 17.828 (6.70)
	+ AuNPs (1.5 nM, containing equal AuNPs with AuNP-APTACs)	113.978 ± 14.836 (5.62)
	+ AuNP-APTACs 600 nM	65.148 ± 12.911 (2.47)
	+ Probenecid 1 mM	76.925 ± 7.853 (3.21)
	+ Tariquidar 580 nM	86.470 ± 7.436 (4.27)
	+ Ko143 700 nM	63.320 ± 13.771 (3.13)
HepG2	Dox alone	20.264 ± 5.377 (1.00)
	+ AuNP-APTACs 600 nM	17.649 ± 5.921 (0.87)

[a] Data are shown as mean ± SD from three independent experiments (n = 3).
[b] Rf: Resistance fold was calculated by dividing the IC_{50} values of Dox in the presence or absence of AuNP-APTACs and inhibitor by the IC_{50} of no treatment HepG2 cells.