Supporting Information For

SV119-Gold Nanocage Conjugates: A New Platform for Targeting Cancer Cells via Sigma-2 Receptors

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Experimental Section

Synthesis of Gold Nanocages (AuNCs). AuNCs were prepared through galvanic replacement reaction between Ag nanocubes and chloroauric acid ($HAuCl_4$) in an aqueous solution according to our previously reported protocol.[1] The samples were examined using a Tecnai G^2 Spirit transmission electron microscope (TEM) operated at 120 kV (FEI, Hillsboro, OR). The UV-Vis extinction spectra were recorded using a Cary 50 spectrometer (Varian, Palo Alto, CA).

Conjugation of SV119 with AuNCs. SV119 was synthesized and purified according to a previously published protocol.[2] SV119 and succinimidyl propionyl PEG disulfide (SVA-PEG-OPSS, Mw≈5000, Laysan Bio, Arab, AL) were dissolved in aqueous solution and stirred at 4 °C for 24 h to generate conjugate SV119-PEG-OPSS, which was then mixed with SVA-PEG-OPSS at different ratios at a total concentration of 0.12 mM. After adding PVP-stabilized AuNCs (0.3 nM), the mixture was stirred at 4 °C for 12 h. Thereafter, the solution was centrifuged at a speed of 1.2×10^4 rpm for 8 min to remove unconjugated polymers and washed three times with ultrapurified water (Millipore, Billerica, MA), and resuspended in phosphate buffered saline (PBS, 0.02 M) to obtain SV119-PEG-AuNCs. The concentration of SV119-PEG-OPSS in the

supernatant before and after conjugation was determined by UV-Vis (together with a calibration curve), and thus the conjugation efficiency of PEG calculated as 9.3%, with the number of PEG chains on the surface of AuNCs being about 3.7×10^4 per AuNC (see Fig. S1).

Cell Culture. MDA-MB-231 and MDA-MB-435 cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA). The cells were cultured in MEM medium, supplemented with 5% fetal bovine serum, penicillin (10^4 IU)/streptomycin (10 mg mL^{-1}), 2 mM L-glutamine, 1 mM sodium pyruvate, 1 mM non-essential amino acids and 20 mM vitamins for MEM at 37 ºC using a humidified 5% CO_2 incubator. PC-3 and Hela cell lines were obtained from ATCC, and cultured in F-12K Medium, supplemented with 10% fetal bovine serum.

***In Vitro* Cell Studies with AuNCs using Inductively Coupled Plasma Mass Spectromety (ICP-MS).** Cells were seeded in 24-well plates at a density of 1×10^6 cells/well and incubated overnight. The cells were washed and treated with the culture medium containing PEG-AuNCs or SV119-PEG-AuNCs at different concentrations. At different time intervals, the cells were washed three times with cold PBS, treated with 0.2 mL of trypsin solution (containing 0.25% EDTA) and counted by hematocytometer. The cell pellets were freeze-dried, and 400 μL aqua regia was added to completely dissolve the cells and AuNCs. The amount of Au was measured by Elan DRC II ICP-MS (Perkin Elmer, Waltham, MA), converted to the total number of AuNCs and then normalized to the cell number.

Flow Cytometry Analyses of Cells. MDA-MB-435 cells were seeded in 24-well plates at a density of 1×10^6 cells/well and incubated overnight. The medium was replaced with fresh medium containing SV119 (10 μM) or (+)-pentazocine (10 μM) or SV119-PEG-AuNCs (1.2 nM). After 1 h pre-incubation, the medium was replaced with fresh medium containing 50 nM of SW120 together with 10 μM SV119 or (+)-pentazocine, or 1.2 nM SV119-PEG-AuNCs for another 0.5 h. The cells were washed with PBS twice, harvested, and then suspended in 200 μL of PBS for analyses using a FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ). Cells with PBS treatment were used as a control. The data were analyzed using WinMDI 2.9 software.

Two-Photon Confocal. MDA-MB-435 cells were seeded in 6-well plates at 5×10^7 cells/well in 2 mL of complete MEM medium and incubated overnight. The medium was replaced with fresh medium containing PEG-AuNCs or SV119-PEG-AuNCs at a concentration of 0.02 nM. After incubation for 3 h, FM4-64 was added at a final concentration of 5 g/mL. After incubation, the

cells were washed with PBS three times. The cells were then covered with a coverslip and sealed with mounting medium. They were kept in the dark and imaged within the next few hours. The imaging was performed using an upright Zeiss LSM 510 system (Carl Zeiss, Thornwood, NY) as described in our previous publication.[3]

1. S. E. Skrabalak, L.Au, X. D. Li, and Y. Xia, *Nat. Protoc.* 2007, **2**, 2182-2190.
2. S. Vangveravong, J. Xu, C. Zeng, R. H. Mach, *Bioorg. Med. Chem.* 2006, **14**, 6988-6997.
3. L. Au, D. Zheng, F. Zhou, Z. Li, X. Li and Y. Xia, *ACS Nano*, 2008, **2**, 1645-1652.

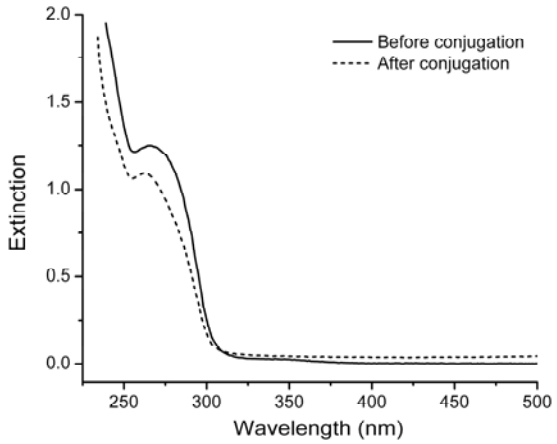

Fig. S1 UV-Vis spectra of SV119-PEG-OPSS solution before and after conjugation with AuNCs.

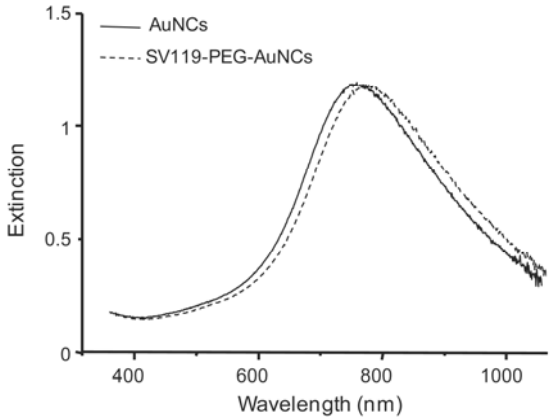

Fig. S2 UV-Vis spectra of AuNCs and SV119-PEG-AuNCs in aqueous suspensions.