Supporting Information

Targeted Combinatorial Therapy Using Gold Nanostars as Theranostic Platforms

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1. Synthesis of pegylated Au NS

*1.1 Au NPs 15 nm (seeds):*Briefly, 5 mL of 1 wt.% sodium citrate aqueous solution was added under continuous stirring to a boiling aqueous solution of $HAuCl_4$ (100 mL, 0.5 mM) and allowed to react for 15 min.

1.2 PVP modification of seeds: 5 mL of a PVP aqueous solution containing a sufficient amount to provide approximately 60 molecules of PVP per nm^2 of gold was added dropwise to the Au colloid and allowed to react overnight. Finally, the solution was centrifuged at 4000 rpm for 90 min, the supernatant removed, and the particles redispersed in ethanol.

1.3 Synthesis of Au NS: For obtention of Au NS, in a typical synthesis 82 μL of an aqueous solution of 50mM $HAuCl_4$ was mixed with 15 mL of 10 mM PVP solution in DMF. After the complete disappearance of the Au^{3+} CTTS absorption band at 325 nm, a certain amount of preformed-seed dispersion was added under continuous stirring and allowed to react until completion of the reaction (no further changes in the UV-vis-NIR spectra). The ratio of [$HAuCl_4$] to [seed] was set to 90. Au NS solutions were centrifuged three times at 4500 rpm for 45 min, redispersed in water and stored.Au NS were pegylated by mixing 1 mL of Au NS aqueous solution (1.65 mM), 2 mL of HS-PEG-NH_2 (4 mM) and 2 mL of K_2CO_3 (10 mM) under continuous stirring overnight. For removing the unattached HS-PEG-NH_2, the nanoparticle solution was centrifuged three times at 10500 rpm and redispersed at a concentration of 0.25 mM.

2. NIR-laser induced temperature increase

Temperature increment tests as a function of time were used to determine the photothermal activity of the hybrid nanoconjugates. The tests were performed with a continuous wave fiber-coupled diode laser source of 808 nm wavelength (50W, Oclaro Inc, San Jose, CA, USA). The laser was powered by a Newport 5700-80 regulated laser diode driver (Newport Corporation, Irvine, CA, USA). A 200-μm-core optical fiber was used to transfer laser power from the laser unit to the target solution; it was equipped with a lens telescope mounting accessory at the

outputfor tuning of the laser spot size in the range 1-10 mm. The output power was independently calibrated using an optical power meter (Newport 1916-C) and laser spot size was previously measured with a laser beam profiler (Newport LBP-1-USB), which was placed at the plane of the sample. For measuring the temperature change, 2 mL of Au NS-PEG nanoconjugates in cell culture medium (DMEM) supplemented with 10% (v/v) FBS was placed in a quartz cuvette and irradiated for different times (0-10 min) at several laser power densities. The temperature of the samples was measured with a type J thermocouple linked to a digital thermometer inserted into the solution.

3. Cellular uptake by confocal microscopy.

Nanoconjugates (Au NS-PEG-SS-DOXO and Au NS-PEG-SS-DOXO-FA) uptake was followed by confocal microscopy by seeding HeLa cells on poly-L-lysine coated glass coverslips (12 × 12 mm) placed inside 6-well plates (3 mL, $1.5 \cdot 10^4$ cells/well) and grown for 24 h at standard culture conditions (5% CO_2 at 37 ºC in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) FBS, 2 mM L-glutamine, 1% penicillin/streptomycin, 1 mM sodium pyruvate, and 0.1 mM MEM Non-Essential Amino Acids (NEAA)). Then, nanoconjugates at the desired concentration were added to cells (100 μL). After 2 h of incubation the Au NS-containing cells were washed three times with PBS, then, fixed with paraformaldehyde 4% (w/v) for 10 min, washed again with PBS pH 7.4, mounted on glass slides and stained with ProLong® Gold antifade DAPI to stained nuclei, and cured for 24 h at -20 °C. Confocal images of DOXO were made using the autofluorescnece of DOXO with a confocal spectral microscope Leica TCS-SP2 **(***LEICA Microsystems Heidelberg GmbH, Mannheim, Germany*; green channel for DOXO, $\lambda_{exc.}$ 488 nm; blue channel for DAPI, $\lambda_{exc.}$ 355 nm). Both bright-view differential interference contrastimages and confocal fluorescence images were obtained with a 40X (NA 1.2) and 63X (oilimmersion, NA 1.4) objectives.

4. In vitro cytotoxicity

HeLa cells with an optical confluence of 80–90% were seeded into 96-well plates (100 µL, $1.5 \cdot 10^4$ cells/well) and grown for 24 h at standard culture conditions. The cells were treated with 100 µL of free DOXO, Au NS-PEG, Au NS-PEG-SS-DOXO, Au NS-PEG-SS-DOXO-FA. FA receptors of HeLa cells in several wells were previously blocked with excess FA prior addition of Au NS-PEG-SS-DOXO-FA. The dose of nanoconjugates was set according to the contained DOXO concentration in Au NS within a range of 0.2–5 µM DOXO.After cells were incubated for 4h, the photothermal treatment was then performedusing a 808 nm laser. The beam diameter was 1.0 cmand the power density of the laser source was fixed at 2.0W/cm^2. The cells were exposed under the 808 nmlaser for 10 min, and then incubated for another 18 h. Afterwards, the culture medium was discarded and the cells were washed with 10 mM PBS, pH 7.4 several times. The cells were shaken at room temperature (300 rpm, 15 min) in the presence of 10 µL of a glutaraldehyde solution (11% (w/v) in water). The solution was discarded and cells were washed 3-4 times with PBS. The cells were then shaken at room temperature (300 rpm, 15 min) in the presence of 100 µL of a crystal violet solution (0.1% w/v in 200 mM orthophosphoric acid, 200 mM formic acid, and 200 mM 2-N-morpholino-ethanesulfonic acid (MES), pH 6). The solution was discarded, and the cells were again washed 3-4 times with milli-Q water. Once washed, the cells were left for incubation at room temperature overnight for drying. Once dried, the cells were shaken at room temperature (300 rpm, 15 min) in the presence of 100 µL of acetic acid (10% w/w in water). Immediately after, the absorbance of the resulting solution was measured with a Microplate Reader (FLUOstar Optima, BMG Labtech GmbH, Germany) operating at 595 nm. All experiments were triplicate carried out. The growth inhibition was quantified as:

$$\%\,\text{Inhibition} = 100 - \frac{100 \cdot \Delta A}{TA} \qquad \text{(Eq. 1)}$$

where OA and TA stand for the absorbance of studied samples and negative controls (cells in the absence of NPs), respectively.

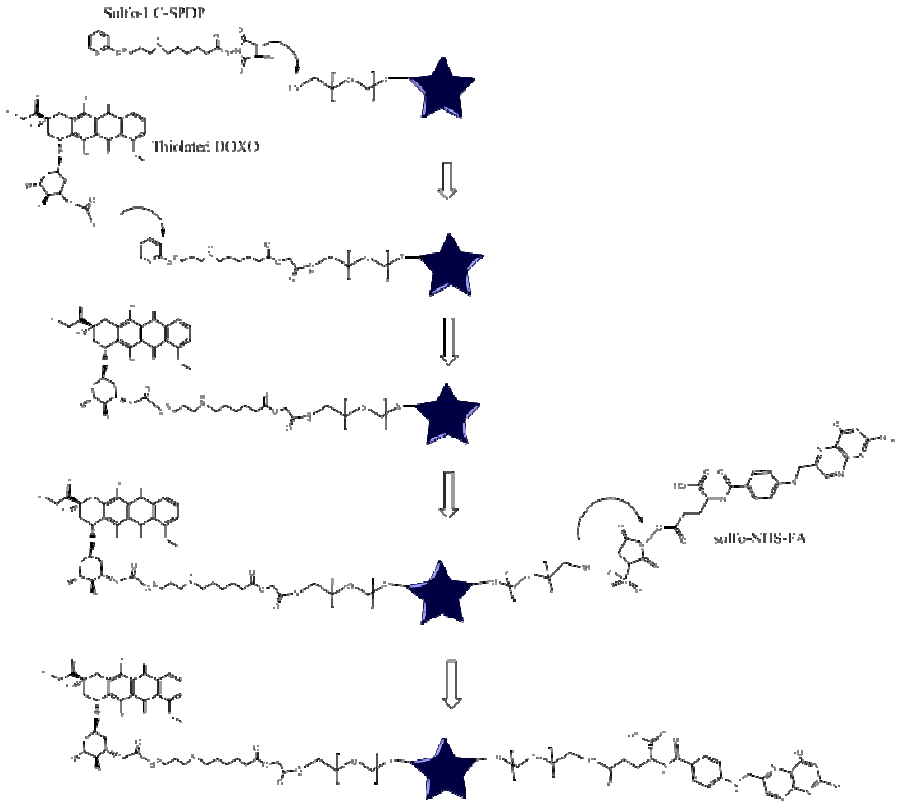

Figure S1: Scheme of the conjugation sequence of DOXO and FA to Au NS.

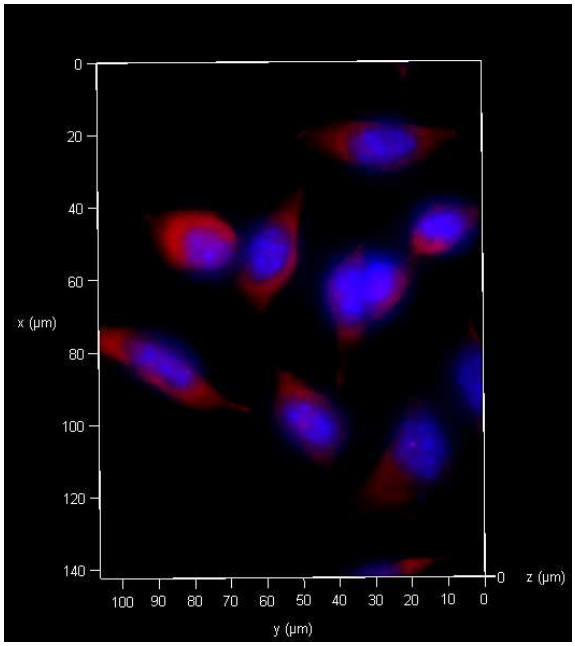

Figure S2: 2D image of HeLa cells with internalized Au NS-PEG-SS-DOXO-FA. Blue channel stands for DAPI (λ_{ex} = 365 nm) and the red one for DOXO (λ_{ex} = 485 nm).