SUPPORTING INFORMATION

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Silica-encapsulated gold nanoparticle dimers for organelle-targeted cellular delivery

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Abstract: Silica-encapsulated gold nanoparticle dimers were self-assembled through a single-insertion process using capillary force and can be utilized as an advanced drug-delivery and sensing platform for organelle-targeting in cancer cells.

SUPPORTING INFORMATION

Contents

Experimental Procedures

Material. Aluminum foils (Alfa Aesar, 99.99%), oleylamine (ACROS, 80–90%), gold (III) chloride hydrate (Aldrich, 99.999%), ammonium hydroxide (Daejung, 25–28%), tetraethyl orthosilicate (TEOS, Aldrich, 98%), *p*-mercaptobenzoic acid (*p*-MBA, Aldrich, 90%), oxalic acid (OCI company, 99.5%), (3-carboxypropyl)triphenylphosphonium bromide (TPP-COOH, Sig-ma, 98%), MTX (Sigma, 97%), (3-aminopropyl)triethoxysilane (APTES, Sigma, 98%), ELM (Selleckchem, 99.06%), phosphoric acid (Daejung, 85%) were used as supplied without further purification.

Preparation of AuNPs. AuNPs of 36.4 nm diameter were prepared according to the literature.[19] The AuNPs were collected by centrifugation and finally redispersed in toluene.

Preparation of AAO template. To prepare AAO templates containing pores with length of 100 nm and orifice diameter of 100 nm, the template was anodized again for 80 s and then pore-widened in phosphoric acid (25 wt. %) at 30˚C for 30 min. For control experiments, the AAO template with 500-nm long pores were fabricated separately by anodization for 7 min.

Preparation of bare and amine-modified SGDs inside AAO templates. To prepare SGDs without TPP targeting moiety, bare silica nanocages were fabricated by soaking AAO templates in a mixture of mixture of ethanol (15 mL), NH_4OH (3 mL), and TEOS (0.15 mL) for 14 min. In case of SGD with TPP targeting moiety, amine-modified silica nanocages were prepared for the further reaction with TPP-COOH via amide bond formation. The templates were immersed in a mixture of ethanol (15 mL), NH_4OH (2 mL), APTES (1 mL), and TEOS (0.15 mL) for 14 min. In both cases, after finished hydrolysis reaction for the given time to produce silica layer inside the pores of AAO, the templates were removed from the solution and washed with ethanol three times. The resulting tem-plates were then cured in an oven at 120˚C for 5 min.

Preparation of silica-caged gold nanoparticle dimer (SGD). SGDs were fabricated by insertion of AuNPs into the silica nanocages. In brief, a piece of silica-coated AAO (25 × 8 mm) was placed in a vacuum chamber on a rocking platform. Onto the surface of the template, 70 μL of AuNPs in toluene (4 mg/mL) was added dropwise until approximately a half of template's surface was covered with solution. After the solvent was allowed to evaporate under vacuum condition with shaking at 35 rpm for 10 min, the resulting template was sonicated in toluene for 2 min to remove excess surface-bound AuNPs out of silica nanocages. To prepare 500-nm silica nanotube with AuNPs inside, the above AuNP insertion cycles were repeated three times.

Fabrication of SGDs with *p*-MBA inside (*p*-MBA-SGDs). In order to endow SGD with the pH-sensing functionality, *p*-MBA was immobilized onto the surface of AuNP dimer inside silica nanocages via Au-thiol interaction. After immersing the resulting template in a solution of 0.1 mM *p*-MBA in ethanol for 4 h, the unreacted *p*-MBA was removed by washing with ethanol. The free-standing *p*-MBA-SGD were liberated from AAO by dissolving the template in phosphoric acid, washed with distilled (DI) water several times, and stored in DI water for the further use.

Fabrication of SGDs with anticancer agent inside. For anticancer activity of SGDs, MTX and ELM were immobilized onto the AuNPs inside SGDs separately using the same method mentioned above. For MTX-SGDs, an aqueous solution of 0.1 mM MTX was treated for 8 h and DI water was used in the washing step. In case of ELM-SGDs, 0.1 mM ELM in DMSO was treated for 8 h and DMSO and ethanol were used for washing. In both cases, the free-standing SGDs in water were obtained by the same processes as described in *p*-MBA-SGD.

Preparation of SGDs with TPP targeting moiety (SGDs-TPP). In order to modify the surface of SGDs with mitochondria-targeting TPP, amine-modified silica nanocages were used to prepare SGDs. TPP-COOH was then reacted with amine groups of SGDs via an amide coupling reaction as follows: Typically, To a solution of TPP-COOH (50 mg) dissolved in 10 mL PBS buffer (pH 7, 10 mM) was added NHS (0.1 g) and EDC (0.04 g), and the solution was allowed to stand for 15 min. To this solution was then added 0.5 mL of SGDs-NH_2 (3×10^{10} SGD/mL), and the mixture was again stand for an hour. Finally, SGD-TPP were collected by filtration and redispersed in water.

Physicochemical characterization. The morphologies of SNTs were checked using a FEI TECNAI G2 transmission electron microscope (TEM). We checked the serum protein adsorption on *p*-MBA-coated AuNPs using a thermoelectron 6700 Fourier-transform infrared spectrometer with a nominal resolution of 4 cm^{-1} and 256 scanning times. The Inductively coupled plasma mass spectrometry data were obtained using a Varian 820-MS spectrometer.

Cell culture. HeLa cells (ATCC CCL 243) were cultured in DMEM medium supplemented with 10% (v/v) fetal bovine serum (FBS) from WelGene (Seoul, Korea) and 1% (v/v) penicillin–streptomycin at 37^{o}C in a 5% CO_2 atmosphere incubator.

DFM and confocal Raman spectroscopy. Confocal microRaman spectroscopy was performed using a Renishaw RM 1000 microscope equipped with a Cytoviva high-resolution adaptor for DFM at the excitation wavelength of 632.8 nm.

Finite-dimension time domain (FDTD) calculations. The FDTD calculation was performed using Lumerical FDTD solutions. We had used the FDTD solutions of Lumerical software for

our 2D-FDTD simulations. The ambient conditions of the simulation domain was perfectly well matched layer absorbance boundaries. An electromagnetic pulse fixed at 633 nm was launched into a box containing the target SGDs to simulate a propagating plane wave interacting with the nanostructure. In our simulation, the dielectric function of AuNPs and silica nanotube were described by the Palik model. The Au nanostructure and its surrounding space were divided into 1.0 nm meshes and the gap region between AuNPs dimer was divided into 0.5 nm meshes. The refractive index of the surrounding medium was taken to be 1.0. The AuNPs were modeled as 30 nm-diameter spheres, the thickness of SiO_2 was 30 nm, and the separation between AuNPs and Silica wall distance was 0.5 nm.

Fluorescent images of living cells. HeLa cells were seeded onto glass bottom confocal dishes at a density of 5×10^4 cells in 2 mL of DMEM medium and incubated 24 hours at 37°C. The cells were washed with PBS (pH 7.4) solution, added 1.8 mL of fresh DMEM and 0.2 mL of substances to be tested to the confocal dish, incubated overnight. HeLa cells were washed with PBS, suspended in 2 mL of fresh DMEM and 0.5 μL of Mito Tracker Green (1 mM), incubated for 30 min. Fluorescence imaging was performed after washing with PBS, the cells were kept in 2 mL fresh DMEM medium for living imaging. The cells were imaged under a confocal fluorescence microscope, using different excitation for each dye: for MTX, excitation wavelength: 649 nm; for Mito Tracker Green, excitation wave-length: 482 nm. Mitochondria were stained using a MitoTracker Green FM packing (Lifescience technology, USA). The anticancer drug MTX also exhibited fluorescence emission spectra at 680 nm. The white irradiation experiment was performed using a Xe lamp with ~60 W/cm^2 for 0.5-5 min by means of an Eurosep spot.il.5180 fiber optic illuminator (Christophe, France).

Cell cytotoxicity assay. Cell viability test was per-formed using a CCK-8 kit (Dojindo, Japan). To measure cell viability, dispensed 100 μL of HeLa (either A549 or MG-63) cell suspension (1.5×10^3 cells/well) in a 96-well white-plate. Pre-incubate the plate for 24 hours in a humidified incubator (at 37°C, 5% CO_2). Added 10 μL of various substances to be tested to the plate, incubated overnight. Each substance was tested in 6 wells. Added 10 μL of Cell Counting Kit-8 solution to each well of the plate. After further incubation for 2 hours at 37°C, the absorbance of each well was measured at 450 nm using a microplate reader. The cell viability rate was estimated by the following equation: Viable cells (%) = $(O.D._{treated}/O.D._{control}) \times 100\%$. In the case of cell photothermal cytotoxicity assay, the white light had been used before added 10 μL of Cell Counting Kit-8 solution to each well of the plate.

***In vivo* fluorescence imaging.** To estimate the biodistribution of subcutaneoul and tail-vein injected SGDs, the fluorescence images were obtained using a Neoscience FOBI image spectrometer under the **Seoul National University Research Ethics** Guidelines.

Figures and Table

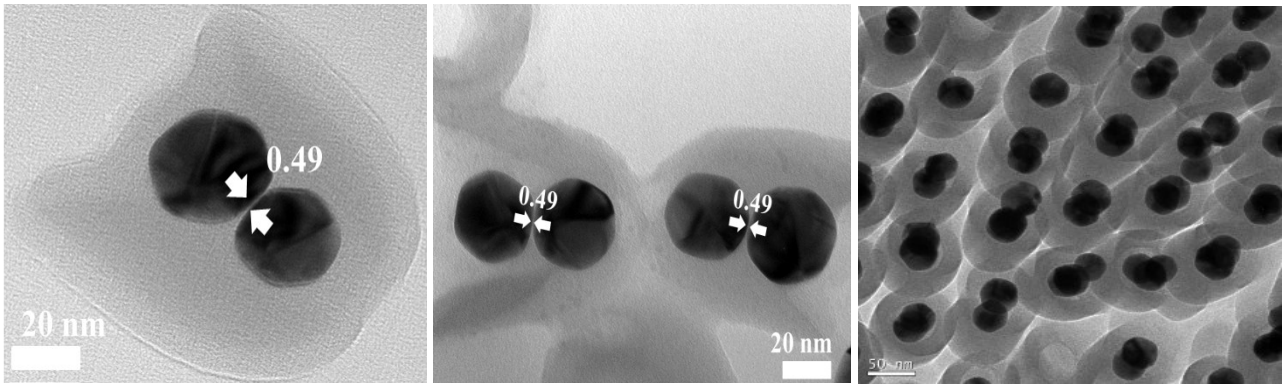


Figure S1 TEM images of SGDs (~100 nm) inside SNCs.

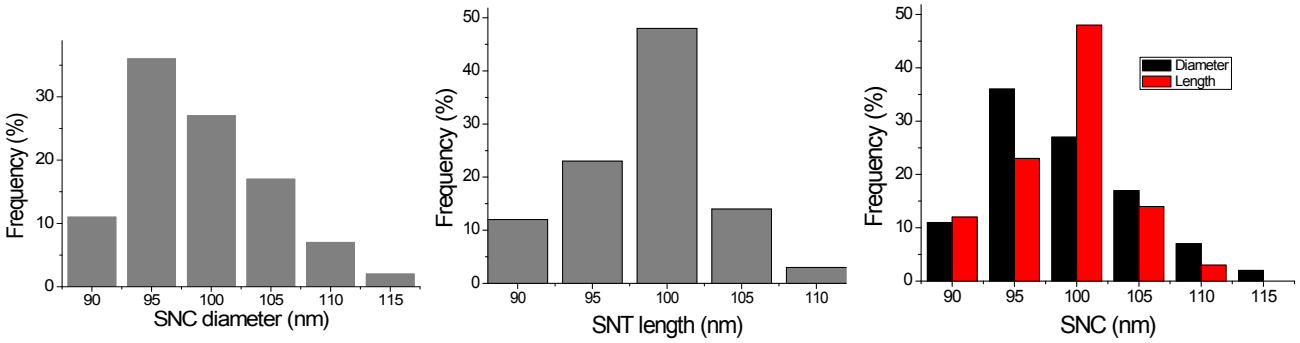


Figure S2 The statistics of length and diameter of SGDs.

Table S1 Size distribution of SNTP and AuNPs. Statistics summary of dimer AuNP@SNT. The units are nm.

	N total	Mean	SD	Minimum	Maximum
SNC length (nm)	100	102.13	4.22	93.6	110.9
SNC diameter (nm)	100	98.43	5.71	95.2	115.4

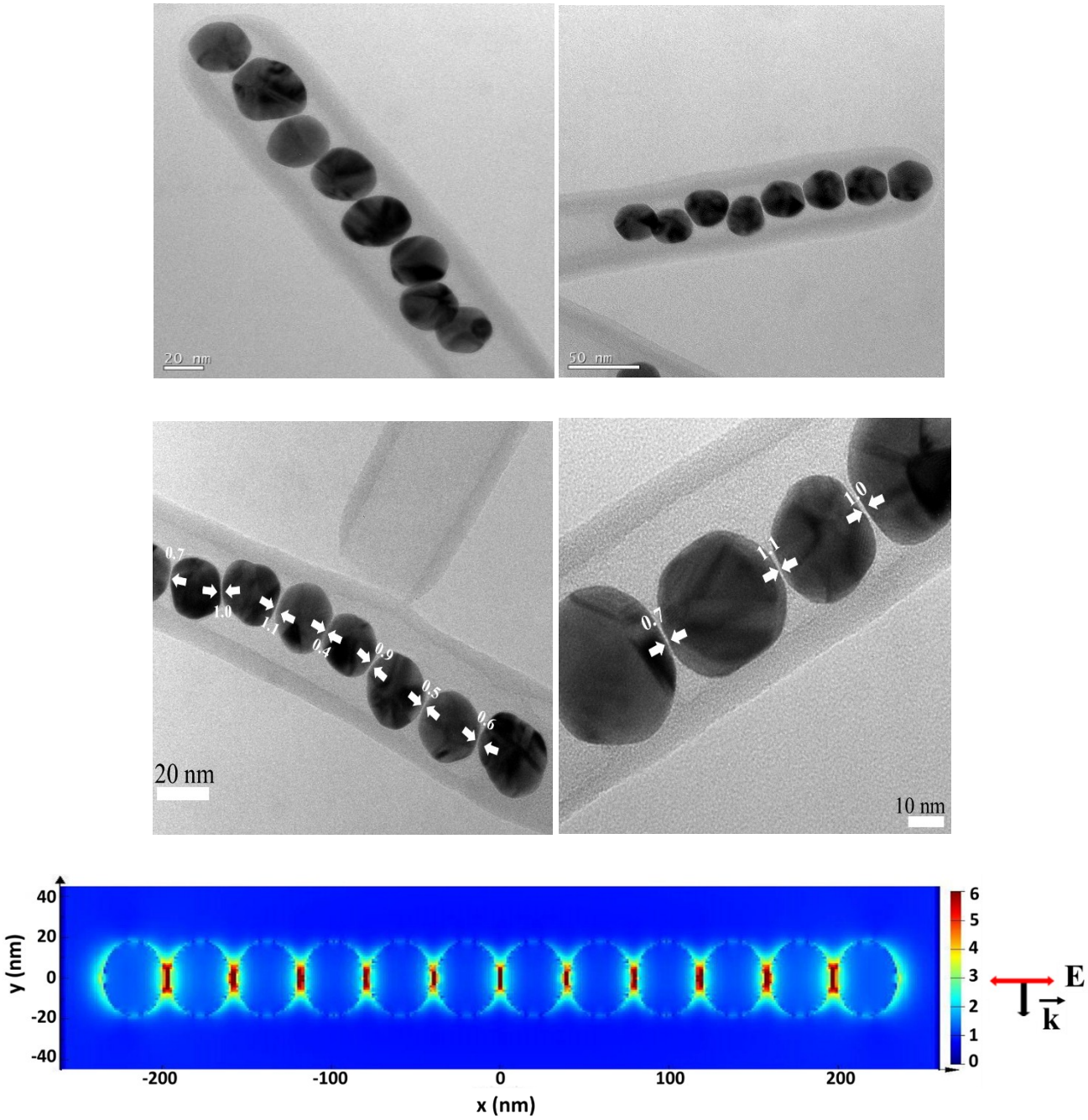
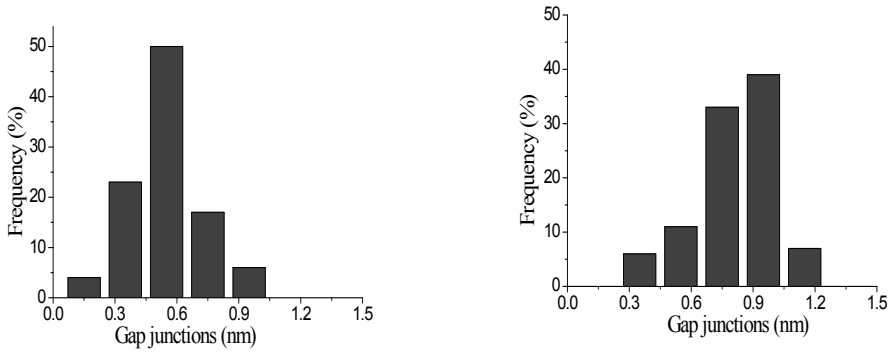


Figure S3 TEM images of longer ~500 nm nanopeapods. FDTD calculations were also depicted.

Table S2 The number distribution of AuNPs inside SGDs, and ~500 nm nanopeapods.

AuNP@SNT	N total	Mean	SD
100 nm	100	2	0.46
500 nm	100	11.5	2.62



Gap junction of 100 nm in SGDs 500 nm AuNP@SNT

Figure S4 The statistics of gap junction of SGDs 100 nm, and 500 nm nanopeapods.

Table S3 The statistics of gap junction of SGDs 100 nm, and 500 nm nanopeapods.

AuNP@SNT	N total	Mean	SD
100 nm	502	0.492	0.063
500 nm	502	0.91	0.25

Table S4. Zeta potential of bare SNC and SNC-NH2. The data supports that there are amine groups at the outer surface of SNCs.

Samples	**Zeta potential**
Bare SNC	-48.06 ± 3.41
SNC-NH_2	-28.22 ± 2.29

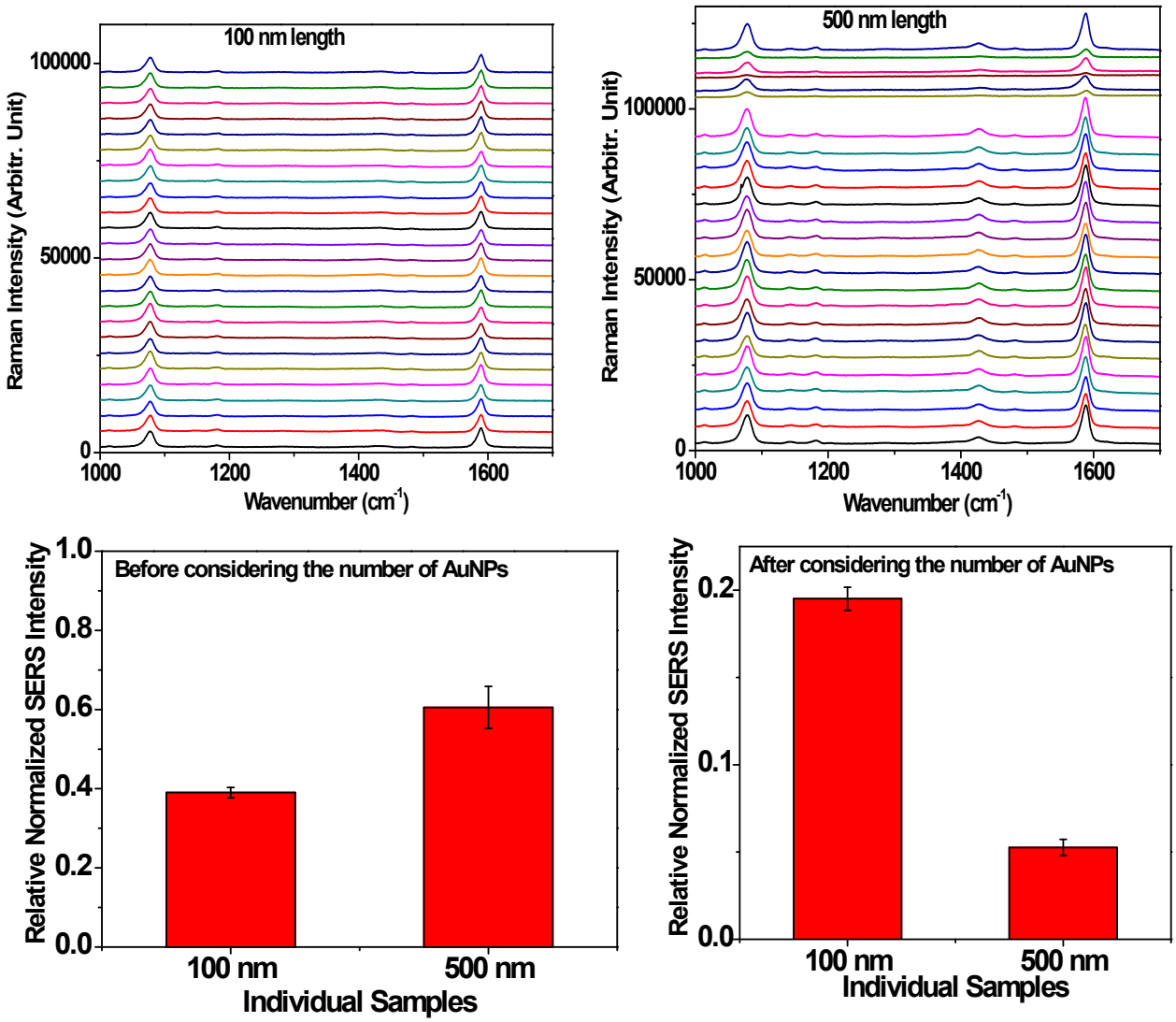


Figure S5 SERS spectra showing the intensity variations depending on the lengths. The stick diagram shows the relative intensities before and after considering the number of AuNPs.

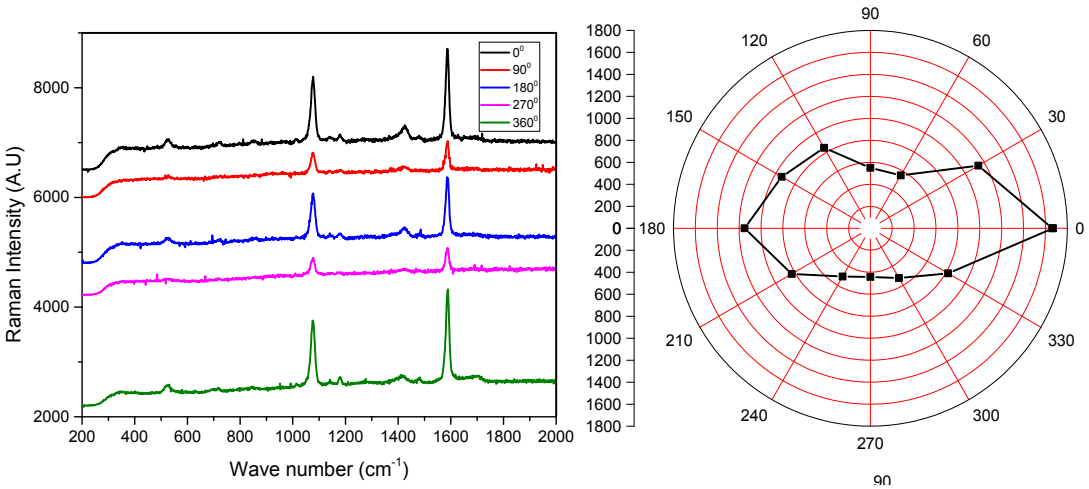


Figure S6 Polarization plots of 500 nm nanopeapods.

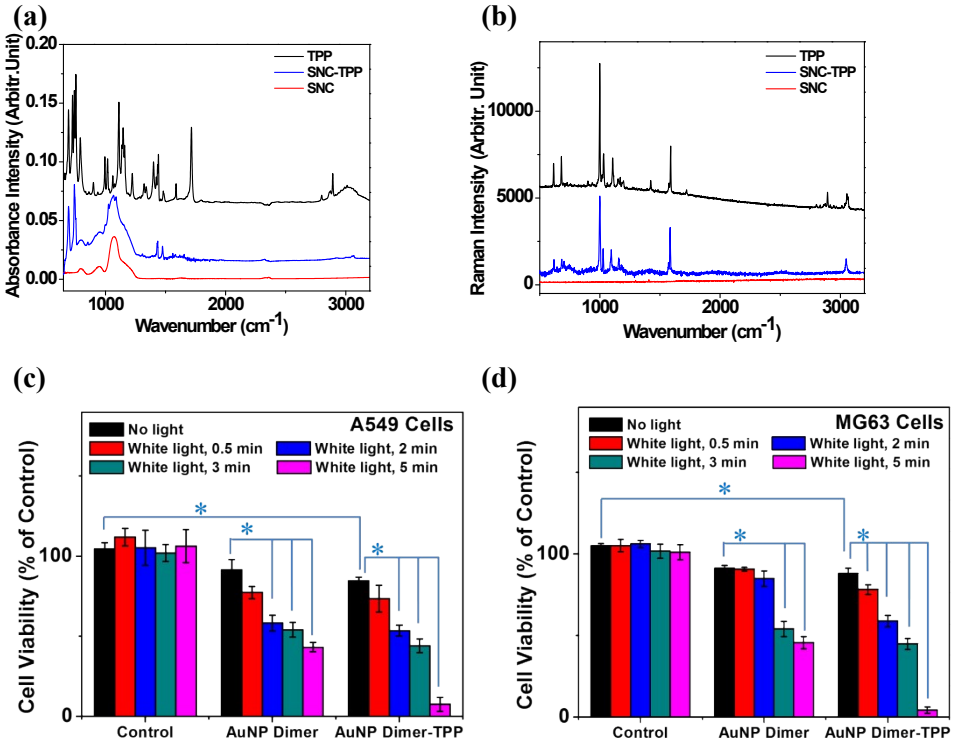


Figure S7 (a) IR and (b) Raman spectra of SGDs showing TPP peaks. Cell viability tests of (c) A549, and (d) MG63 cells (* $p < 0.05$ based on a t-test).

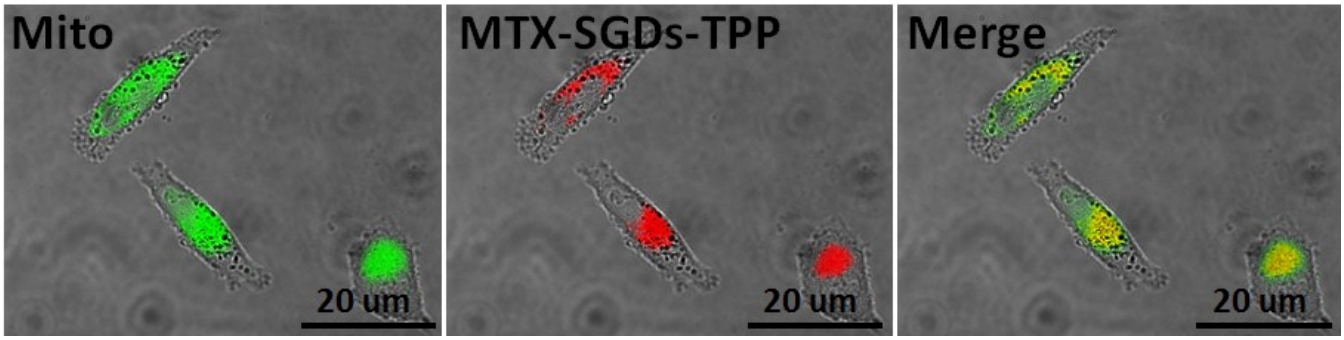


Figure S8. The confocal colocalization images also supproted the targetting efficiencies in A549 cells.

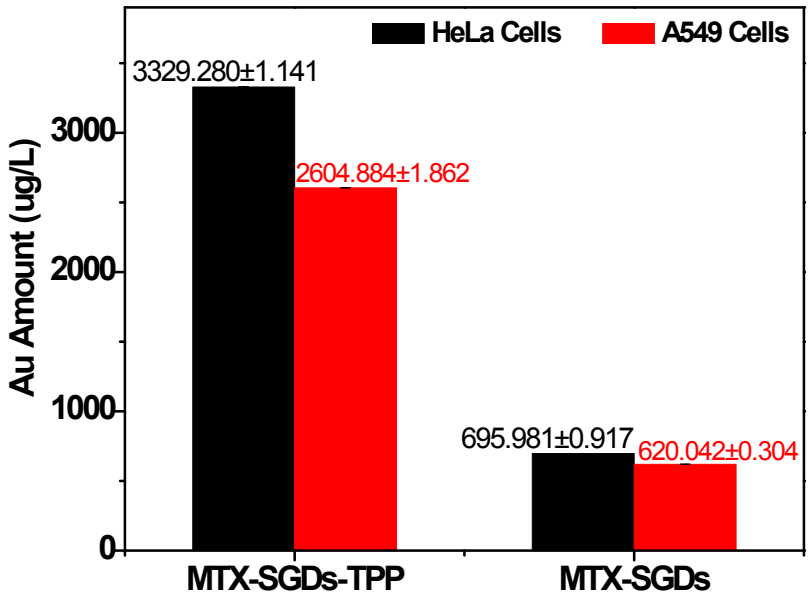


Figure S9. The ICP-MS data for cellular uptake of the TPP-modified and non-TPP SGDs in HeLa and A549 cells.

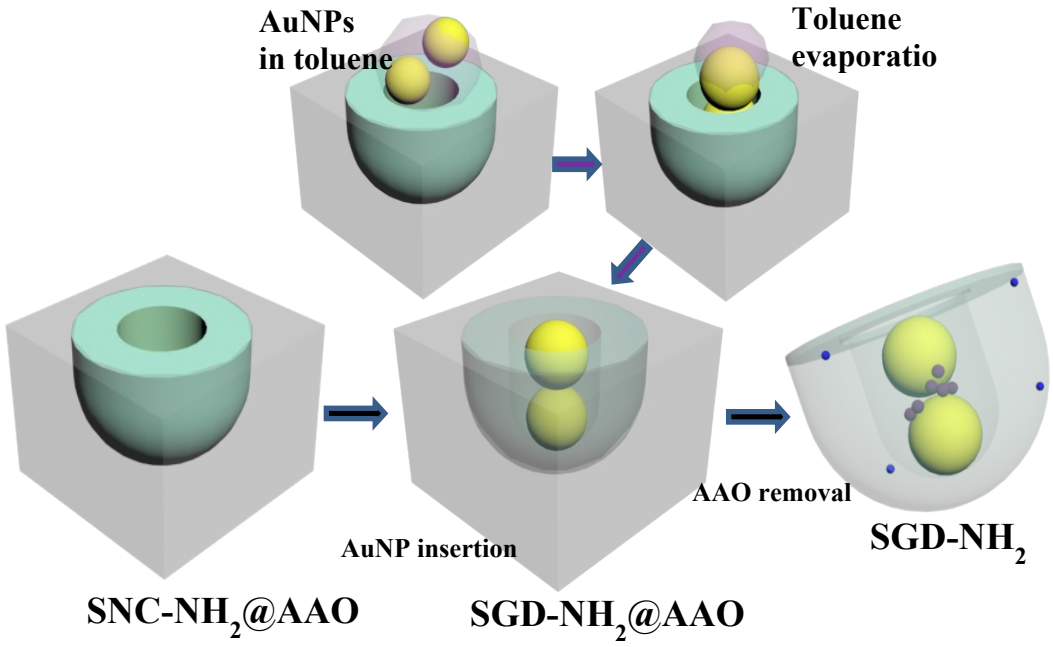


Figure S10. Proposed mechanisms of SGDs.

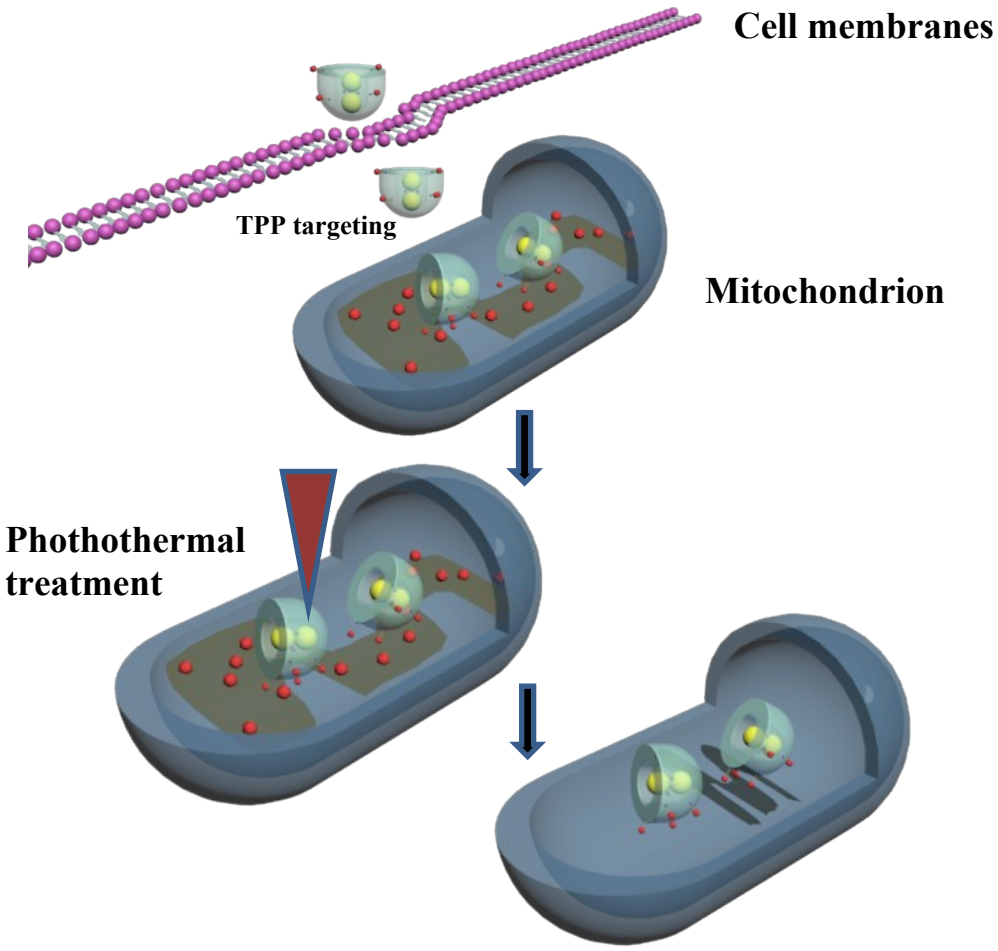


Figure S11. Mitochondria target mechanisms.

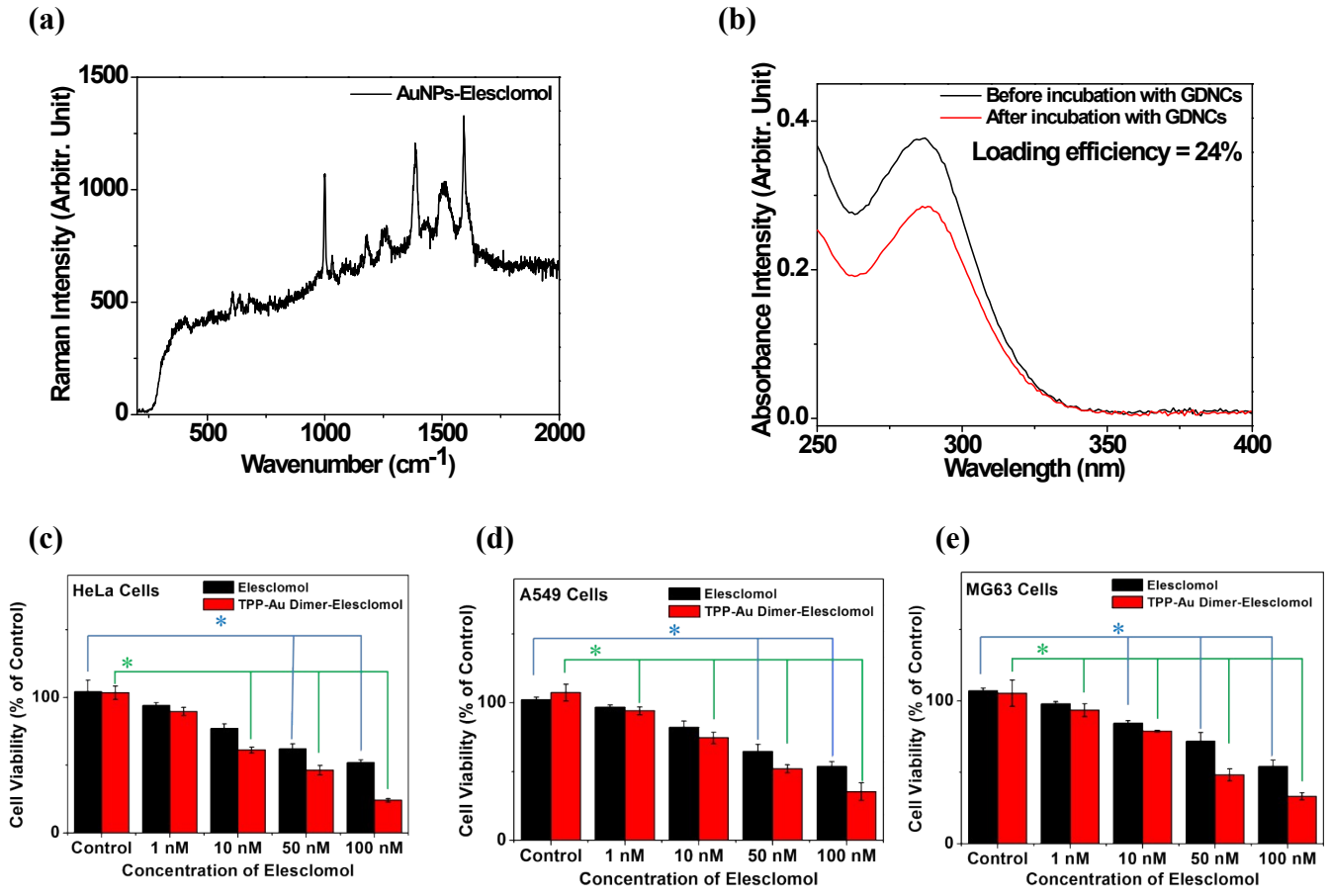


Figure S12. Enhanced cancer cell killing via mitochondria target ELM. (a) Raman and (b) Adsorption spectrum of EL on SGDs. CCK-8 data of (c) HeLa, (d) A549, and (e) MG63 cells (*indicates $p < 0.05$ based on a t-test).