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Supplementary Information for

Bio-inspired Nanotadpoles with Compartmental Functionality

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Experiments

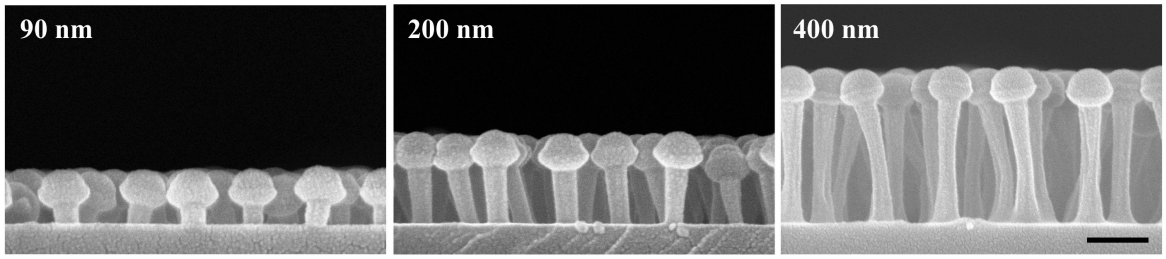
Fabrication of the Tadpole-Like Structures: A colloidal lithographical method was used to fabricate tadpole-like structures. Polyacrylic acid (PAA) and polystyrene (PS) films were sequentially deposited onto a silicon wafer as sacrificial layer and the tail material, respectively. PAA and PS films were subsequently formed by spin-casting a 2 wt.% PAA solution (in ethanol, 1000 rpm) and a 1.5 – 5 wt.% PS solution (in toluene, 2000 rpm). The spin-coating of the hydrophilic silica nanospheres (NSs) onto the hydrophobic PS film was facilitated by modifying the PS surface under mild O_2 plasma for 1 min to render the surface hydrophilic. Monodisperse silica NSs with an average diameter of 165 nm were spin-coated onto the PS film (in ethanol, 2000 rpm), which results in a hexagonally ordered monolayer. The assembled NSs were partially embedded onto the PS film by heating the sample above the glass transition temperature of PS, to 110° C, for 2 min. Reactive ion etching (RIE, Vacuum Science Inc.) with CF_4 gas (60 sccm) was performed at an RF power of 80 W to increase the gap between silica NSs. The PS film under nonclose-packed arrays of silica NSs, which served as a shadow mask for PS film, was further etched using RIE with O_2 gas. The hexagonally arranged tadpole-like nanostructures were formed on the substrate and then released from the substrate in distilled water by dissolving the sacrificial layer under ultra-sonication. Finally, a suspension containing the released NTPs was dialyzed against distilled water to remove any dissolved PAA molecules. The morphology of the tadpole-like structures and NTPs were observed using scanning electron microscopy.

Preparation and Characterization of Functional Nanotadpoles: Dual-fluorescent NTPs were prepared by loading 9-diethylamino-5-benzo[α]phenoxazinone (Nile Red) dyes into the PS tail and FITC dyes into the silica head. The fluorescent tail was prepared by mixing the PS solution with 0.5 wt% Nile Red solution (in toluene, PS : Nile Red = 1 : 0.02, weight ratio). The solution was then spin-coated onto the PAA film at 2000 rpm for 30 sec. The fluorescent head was prepared by embedding FITC (Sigma) into the silica NSs. The FITC dyes were incorporated into the NS matrix through a seeded growth process. Briefly, 0.0711 mL (3-aminopropyl) trimethoxysilane (APS, Sigma) and 4.5 mg FITC were mixed in 6 mL ethanol for 12 h at ambient temperature to form the FITC-APS conjugates. Three milliliters of the silica seed solution containing seeds with a diameter of 55 nm, 3.4 mL deionized water, 15.5 mL ethanol, 0.5 mL as-prepared FITC-APS conjugates, and 1.2 mL ammonia hydroxide were poured into the round bottom flask and gently stirred. Uniform seed growth was achieved by injecting 4 mL tetraethoxysilane (TEOS, Sigma) at 0.8 mL/hour using a syringe pump. The synthesized FITC-incorporated NSs were washed several times by centrifugation and re-suspended in ethanol. The FITC-incorporated silica NSs with an average diameter of 165 nm were spin-coated onto the Nile Red-loaded PS film. The subsequent steps, including annealing and RIE, were the same as those used to prepare the non-fluorescent NTPs. Magnetic NTPs were prepared by loading magnetic iron oxide nanocrystals (MIONs) into the PS tail. The PS solution was mixed with the MIONs having an average diameter of 5 nm (in toluene, PS : MION = 1 : 1.5, weight ratio) and then spin-coated onto the PAA film at 2000 rpm for 30 sec. The subsequent steps were the same as those used to prepare the non-magnetic NTPs. The plasmonic NTPs were prepared

by depositing a gold film onto the tadpole-like structures using a vertical e-beam evaporator. The gold film was deposited onto the top surfaces of the silica NSs to form a gold hemisphere cap on the silica head (2E-6 Torr, 1 Å/sec). The deposition time for the gold film was varied to identify the optimal thickness of the gold hemisphere cap for inducing a strong NIR absorption. The functional tadpole-like structures were then released from the substrate in distilled water by dissolving the sacrificial layer under ultra-sonication. Finally, the suspension containing released NTPs was dialyzed against distilled water to remove the dissolved PAA molecules. The morphologies of the functional tadpole-like structures and NTPs were observed using a scanning electron microscope (Hitachi S-4800). The prepared magnetic and plasmonic NTPs were observed using a transmission electron microscope (Philips Tecnai F20). The hydrodynamic sizes of the plasmonic NTPs were measured in PBS using dynamic light scattering (Zetasizer Nano ZS90, Malvern Instruments). The emission spectra of the dual-fluorescent NTPs were measured using a fluorescence spectrometer (Molecular Devices). Excitation wavelengths of 490 and 550 nm were used to obtain photoluminescence spectra from head and tail, respectively.

Cellular Uptake and Cytotoxicity: Cellular internalization of plasmonic nanoparticles were tested by treating HeLa cells with three types of plasmonic nanoparticles – plasmonic NSs (NSs), and plasmonic NTPs with tail lengths of 90 and 200 nm (NTP-90 and NTP-200) – at a constant particle concentration of 5.04×10^9 particles/mL for 4 h. The cells were then washed with PBS to remove the un-internalized plasmonic particles and then imaged using dark field microscope or confocal laser scanning microscope (Nikon). Cytotoxicity was evaluated by treating HeLa cells with three types of plasmonic particles at a constant particle concentration of 5.04×10^9 particles/mL for 4 h. The cells were then washed and incubated for an additional 24 h. Their cytotoxicity was investigated using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Invitrogen).

Photothermal therapy: The photothermal effects of the plasmonic NTPs under NIR irradiation were evaluated. The plasmonic NTPs were dispersed in distilled water at three different concentrations of 5.04×10^9, 2.52×10^9, and 1.26×10^9 particles/mL. The prepared suspensions were irradiated with an 808 nm laser at 450 mW for 20 min. The temperature change in the suspension of plasmonic NTPs was monitored in real time using a digital thermometer (DAIHAN Scientific). Photothermal ablation of cancer cells was tested by treating HeLa cells with plasmonic NSs and NTP-200s at a constant concentration of 5.6×10^9 particles/mL for 4 h. The cells were then washed with PBS to remove un-internalized plasmonic nanoparticles and exposed to a focused 808 nm laser beam for 1 min at three distinct powers of 100, 200, and 300 mW. The irradiated cells were stained with a LIVE/DEAD assay kit (Invitrogen) for 30 min and then imaged using a fluorescence microscope (Nikon). The assay visualized dead cells as red and viable cells as green.



Height of PS	90 nm	200 nm	400 nm
PAA layer	2 wt.% solution (1000 rpm)	2 wt. % solution (1000 rpm)	2 wt. % solution (1000 rpm)
PS layer	1.5 wt.% solution (2000 rpm)	3 wt.% solution (2000 rpm)	5 wt.% solution (2000 rpm)
Silica monolayer	7 wt.% suspension (3000 rpm)	7 wt.% suspension (3000 rpm)	7 wt.% suspension (3000 rpm)
Annealing	110 °C for 2 min	110 °C for 2 min	110 °C for 2 min
CF_4 RIE	80 W, 60 sccm for 90 s	80 W, 60 sccm for 90 s	80 W, 60 sccm for 90 s
O_2 RIE	80 W, 60 sccm for 3 min	80 W, 60 sccm for 5 min	80 W, 60 sccm for 7 min

Table S1 Scanning electron microscopy (SEM) images and detailed fabrication conditions for the nanotadpoles (NTPs) with tail lengths of 90, 200, and 400 nm. The scale bar indicates 200 nm.

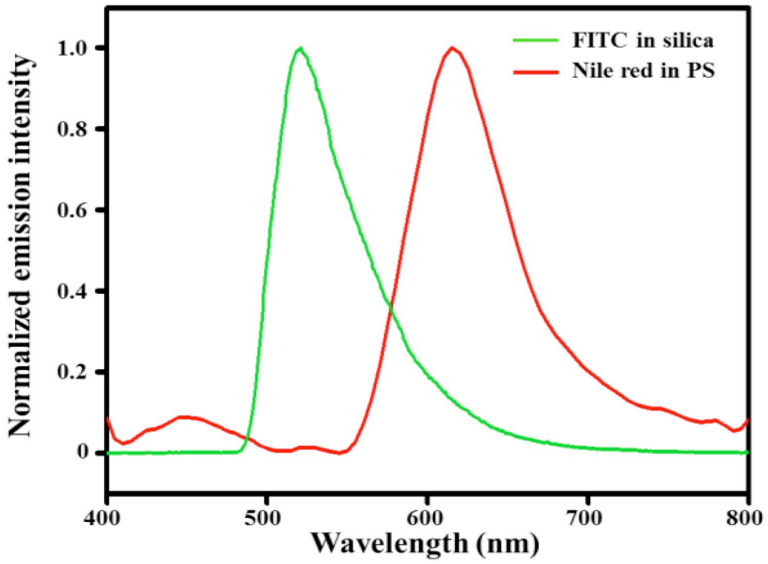


Fig S1. Photoluminescence spectra of NTPs composed of FITC-tagged head and Nile Red-loaded PS tail. Excitation wavelengths of 480 and 550 nm were used to obtain photoluminescence spectra from the head and tail, respectively.

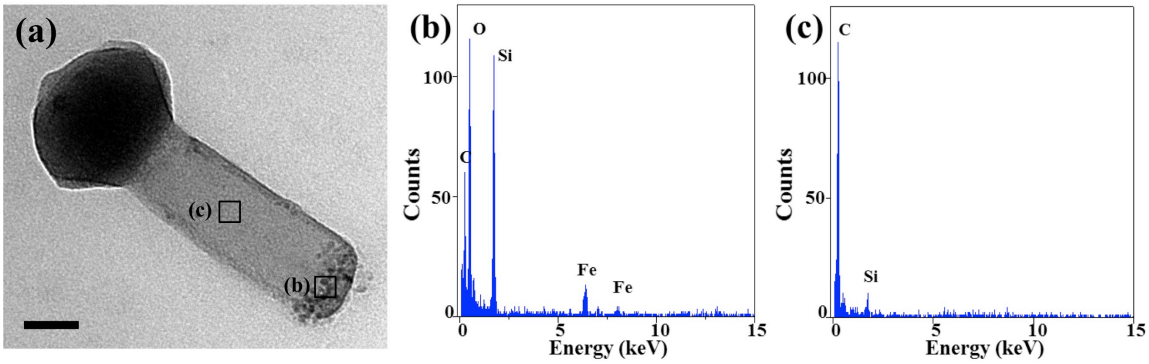


Fig. S2 (a) Transmission electron microscopy (TEM) image and (b, c) energy-dispersive X-ray spectroscopy (EDS) graphs of single magnetic NTP. The EDS analysis was performed at the two different positions denoted with squares in (a). The scale bar indicates 50 nm.

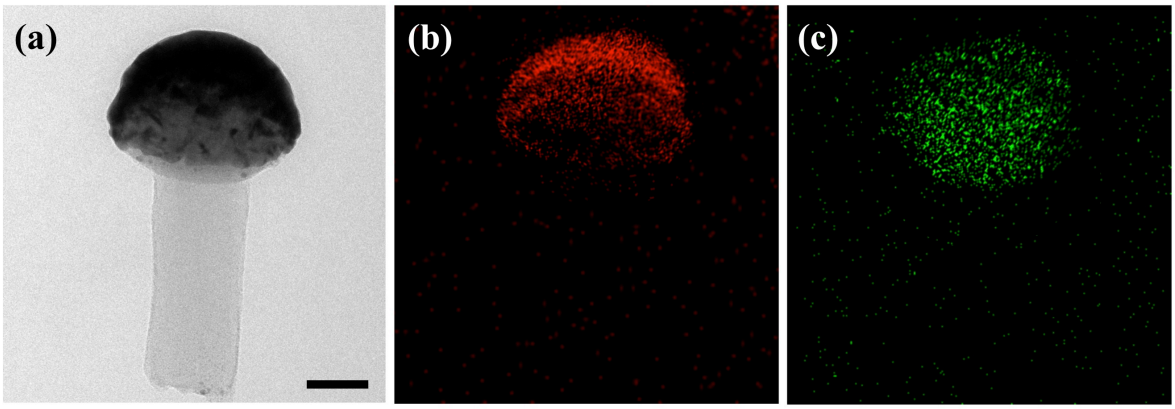


Fig. S3 (a) TEM image and (b, c) EDS maps of the single plasmonic NTP; the EDS maps indicate (b) Au and (c) Si. The scale bar indicates 50 nm.

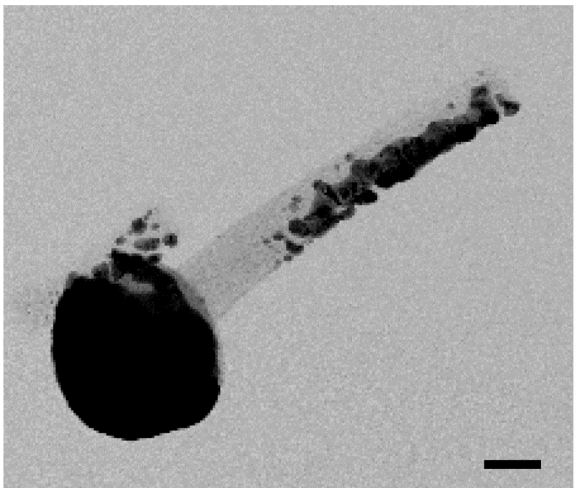


Fig. S4 TEM image of the plasmonic NTP with a tail length of 400 nm. Gold film was partially deposited onto the side wall of the long tail. The scale bar indicates 50 nm.

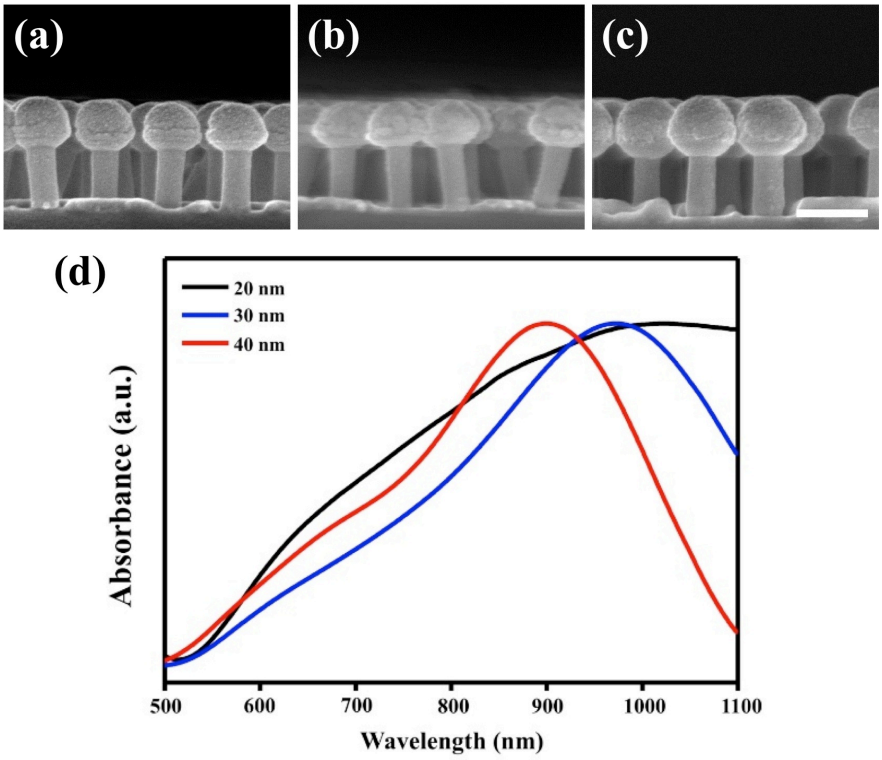


Fig. S5 (a–c) SEM images of plasmonic NTPs with a tail length of 200 nm, of which heads possess gold hemisphere caps with three different thicknesses: (a) 20, (b) 30, and (c) 40 nm. (d) Absorption spectra of the aqueous suspensions of plasmonic NTPs with three different thicknesses of gold hemisphere caps shown in (a-c). The scale bar indicates 200 nm.

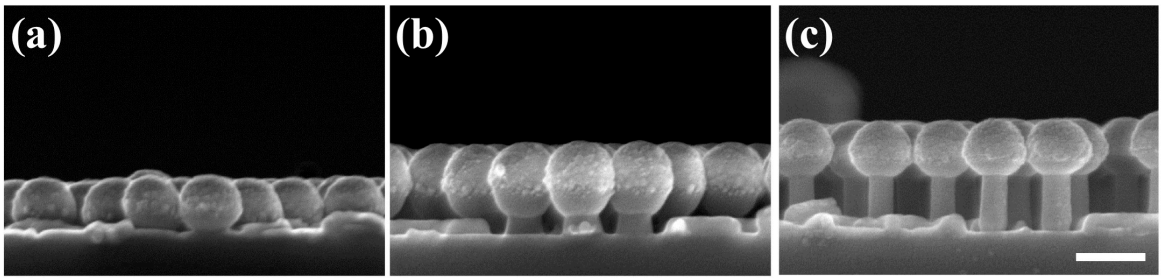


Fig. S6 SEM images of (a) plasmonic NSs, and plasmonic NTPs with tail lengths of (b) 90 and (c) 200 nm. Thickness of gold hemisphere cap was controlled to be 40 nm for all three particles. The scale bar indicates 200 nm.

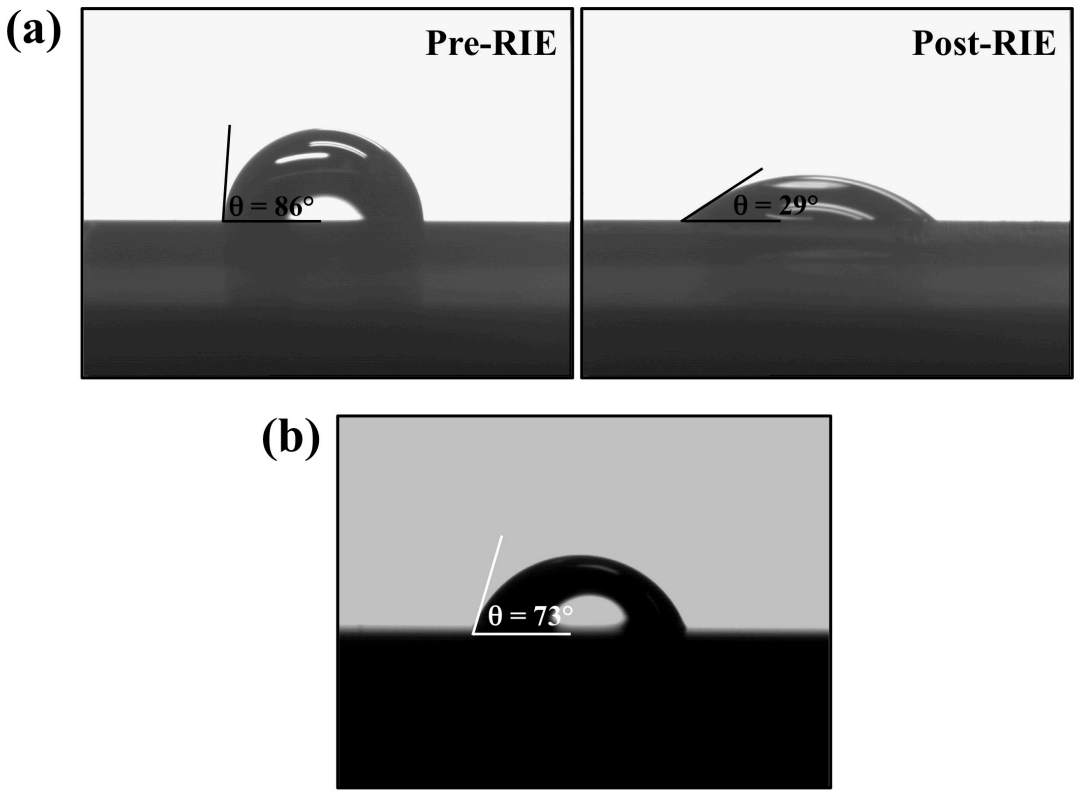


Fig. S7 (a, b) Contact angles of water drops on the surface of (a) bare PS film and reactive ion etching (RIE)-treated PS film with oxygen gas and (b) gold film.

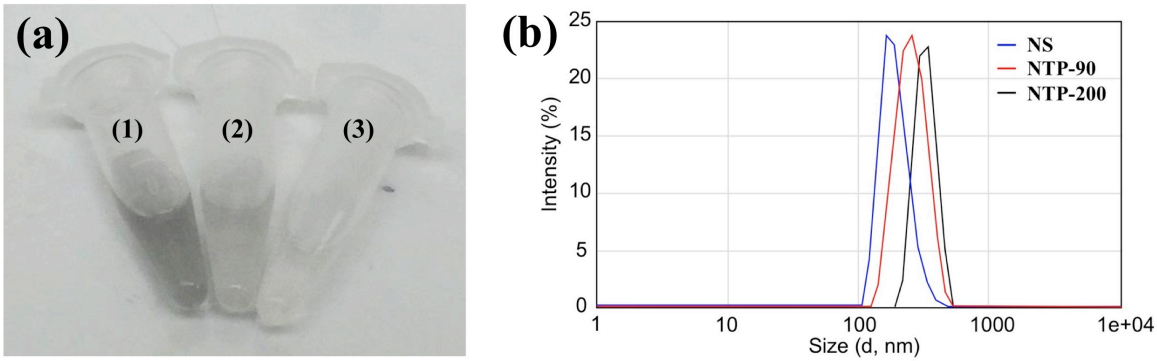


Fig. S8 (a) Photographic image of the aqueous suspension of plasmonic NTPs with a tail length of 200 nm at three different concentrations of 5.04×10^9 (1), 2.52×10^9 (2), and 1.26×10^9 (3) particles/mL, where PBS is used as a suspension medium. (b) Hydrodynamic diameters of plasmonic particles determined from dynamic light scattering (DLS) measurements; plasmonic nanosphere (NS), and two kinds of plasmonic NTPs with a tail length of 90 (NTP-90), and 200 nm (NTP-200) showed average hydrodynamic diameters of 194.1 ± 1.3, 257.6 ± 1.2, and 329.4 ± 1.8 nm, respectively.

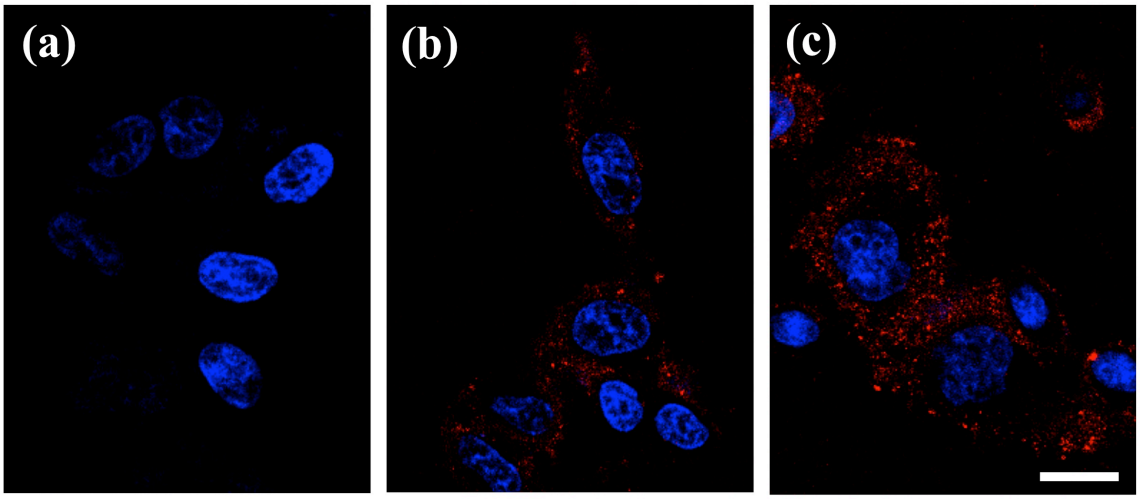


Fig. S9 (a–c) Confocal laser scanning microscopy images of HeLa cells treated with three types of plasmonic nanoparticles where the cylinder of NTPs was loaded with fluorescent nile red molecules (red). The images were obtained after 4 h incubation with particles and subsequent washing of un-internalized particles: (a) plasmonic NSs (no fluorescence), and (b, c) two kinds of plasmonic NTPs with a tail length of (b) 90, and (c) 200 nm. Nuclei were stained with Hoechst (blue). Scale bar indicates 50 μm.

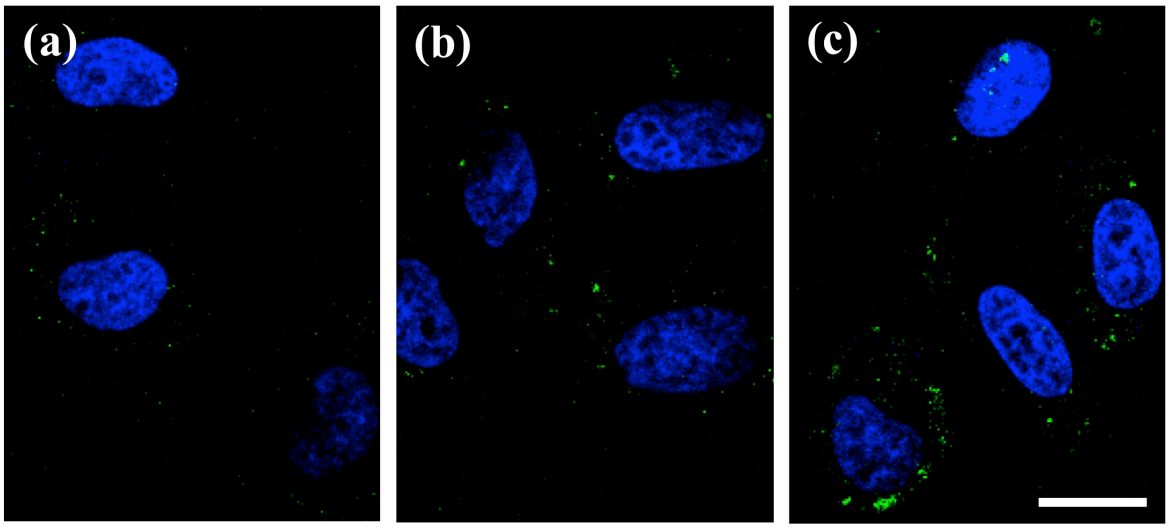


Fig. S10 (a–c) Confocal laser scanning microscopy images of HeLa cells treated with three types of particles where fluorescent FITC molecules (green) were conjugated to the surface of silica head. The images were obtained after 4 h incubation with particles and subsequent washing of un-internalized particles: (a) NSs, and (b, c) two kinds of NTPs with a tail length of (b) 90, and (c) 200 nm. Nuclei were stained with Hoechst (blue). Scale bar indicates 50 μm.