Supporting Information

Plasmon Resonance Energy Transfer (PRET)-based Molecular Imaging of Cytochrome c in Living Cells

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Methods

Preparation of 3-Mercaptopropylcarboxylic Acid (MPA)-conjugated Plasmonic Probes on a Glass Slide Glass slides were cleaned in a piranha solution (30 min, *warning: strong acidic oxidant, very harmful to personal contact*). A cleaned glass slide was modified with 3-mercaptotrimethoxysilane (MPTMS, Fluka) by incubation in 1 mM MPTMS isopropyl alcohol (IPA) for 24 h. A solution of MPTMS reacts with the surface to form a surface presenting thiol groups. The glass slide was then rinsed with acetone, IPA. 50 nm spherical gold nanoparticles (TedPella inc) were immobilized on the MPTMS-modified glass slide (24 h, under mild sonication). Then freshly prepared particles on the glass slide were immersed into 1 mM 3-mercaptopropionic acid (MPA) for 24 h.

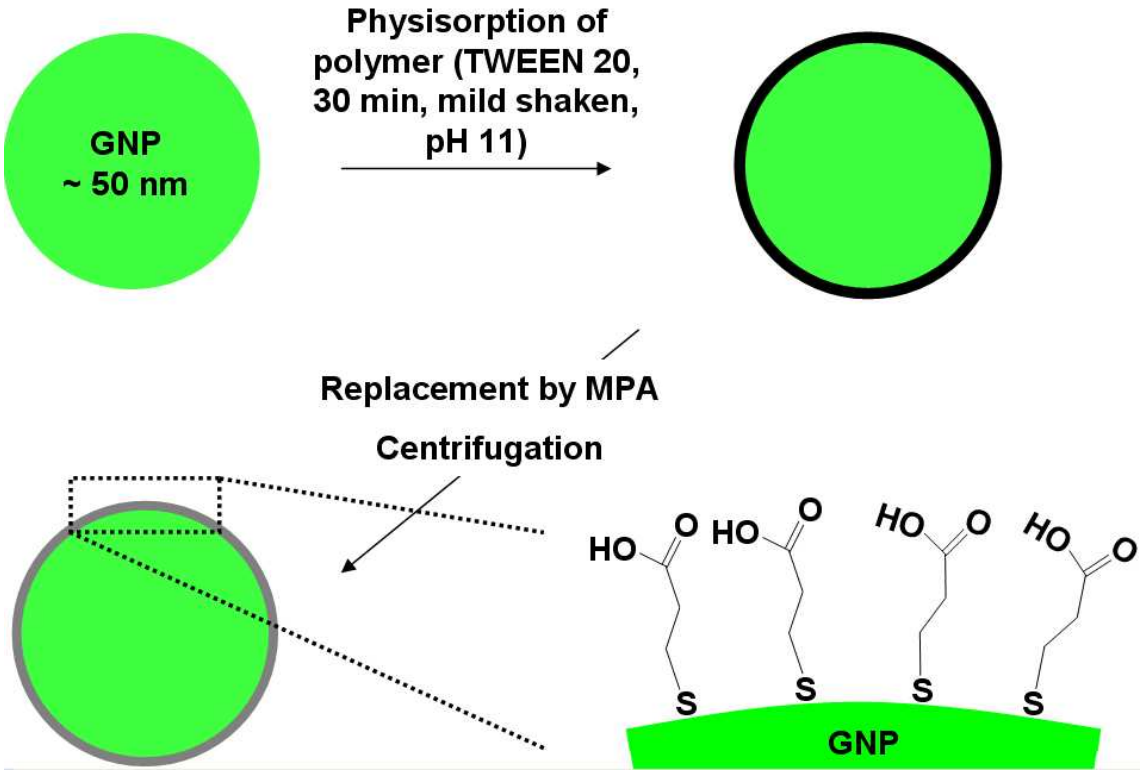
Plasmon Resonance Energy Transfer (PRET) Imaging without HepG2 Cells The microscopy system depicted as Fig. 4a consisted of a Carl Zeiss Axiovert 200 inverted microscope (Carl Zeiss) equipped with a dark-field condenser (NA 1.2 ~ 1.4), a true-colour digital camera (CoolSNAP cf, Roper Scientific) and a 300 mm focal length and 300 grooves per mm monochromator (Acton Research) with a 1024 X 256 pixel cooled spectrograph CCD camera (Roper Scientific). A 2-μm-wide aperture was placed in front of the monochromator to

keep only a single particle in the region of interest.

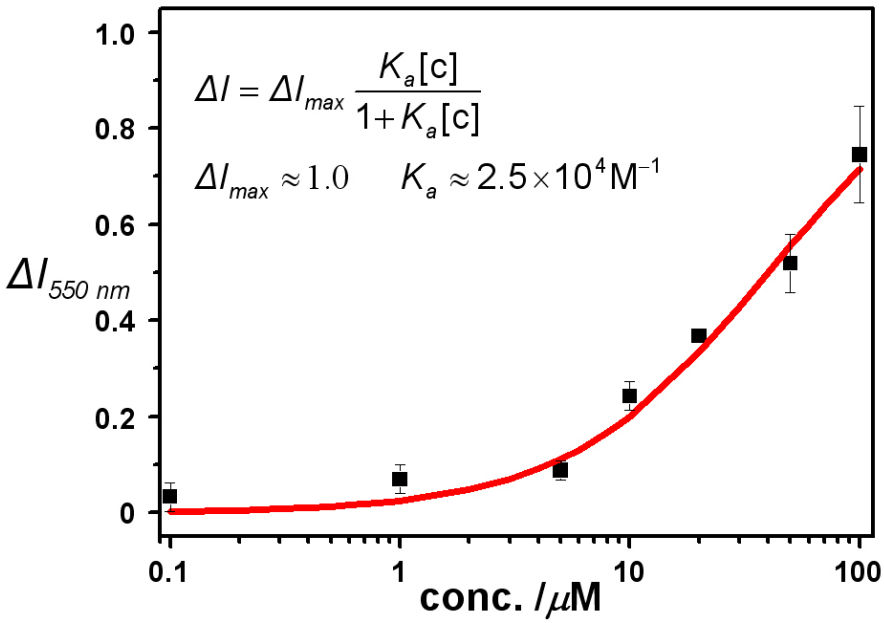
Preparation of 3-Mercaptopropylcarboxylic Acid (MPA)-conjugated GNP for Internalization into Live HepG2 Cells We prepared MPA-conjugated gold nanoparticles according to the procedures in the literature.[1] In typical experiments, 12 mg of TWEEN 20 was mixed with 4 ml of 50 nm gold colloidal solution (1 h) in order to stabilize colloidal particles due to its strong Van der Waals repulsion while MPA are immobilized onto a surface of the particles. 3-MPA was added (final concentration, 10 mM) and the final mixtures were allowed to stand up for 6 h. Excess MPA, anions, TWEEN, and reducing agents were from MPA-conjugated particles by centrifugation for 30 min at 4000 rpm.

Cell Culture CO_2 independent media (Invitrogen inc.) supplemented with 10 % [v/v] fatal bovine serum and 1 % [v/v] penicillin was used. HepG2 cells (~10^6 cells/dish) were counted, moved to a 2'' cell culturing dish with 5 ml of media including approximately 10^9 probes, and followed by the incubation at 37 °C in 5 % CO_2 incubator for 24 h at least in order to internalize the probes into cells. To monitor the fluctuations of intracellular Cyt *c* concentrations, media was replaced with 100 mM ethanol to remove uninternalized particles and induce the apoptosis.

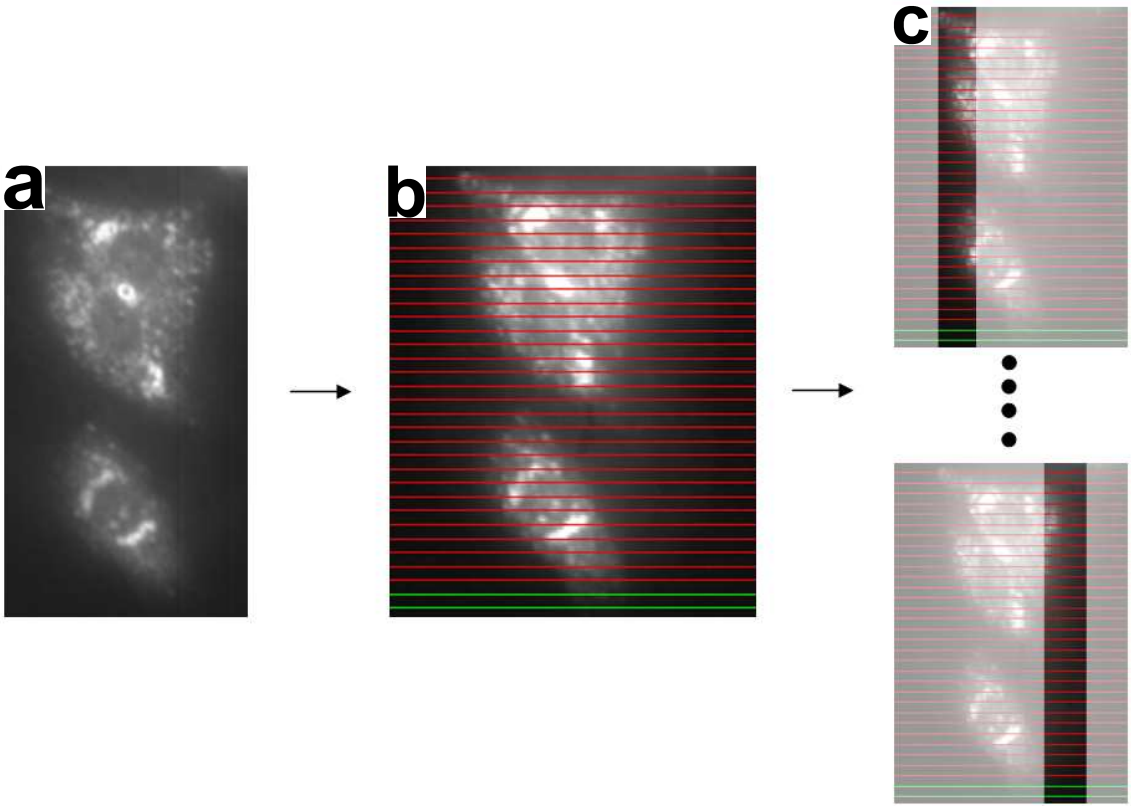
PRET Imaging with HepG2 Cells The sample (HepG2 cells with the probes) was mounted on a Carl Zeiss Axiovert 200 inverted microscope (Fig. 4A). White light (Xenon Arc Lamp, Storzt 300W) was illuminated to the sample only when the measurements were performed (integration time taken for spectra and dark field image were 1 s and 0.1 s, respectively) in order to avoid any thermal effect due to the long exposure time from the light source. Scattering spectra from the probes at different positions inside a single cell (for details, see Fig. S2) were collected with monochromator (300 mm focal length and 300 grooves per mm, Acton Research) with a 1024 X 256 pixel cooled spectrograph CCD camera (Roper Scientific).



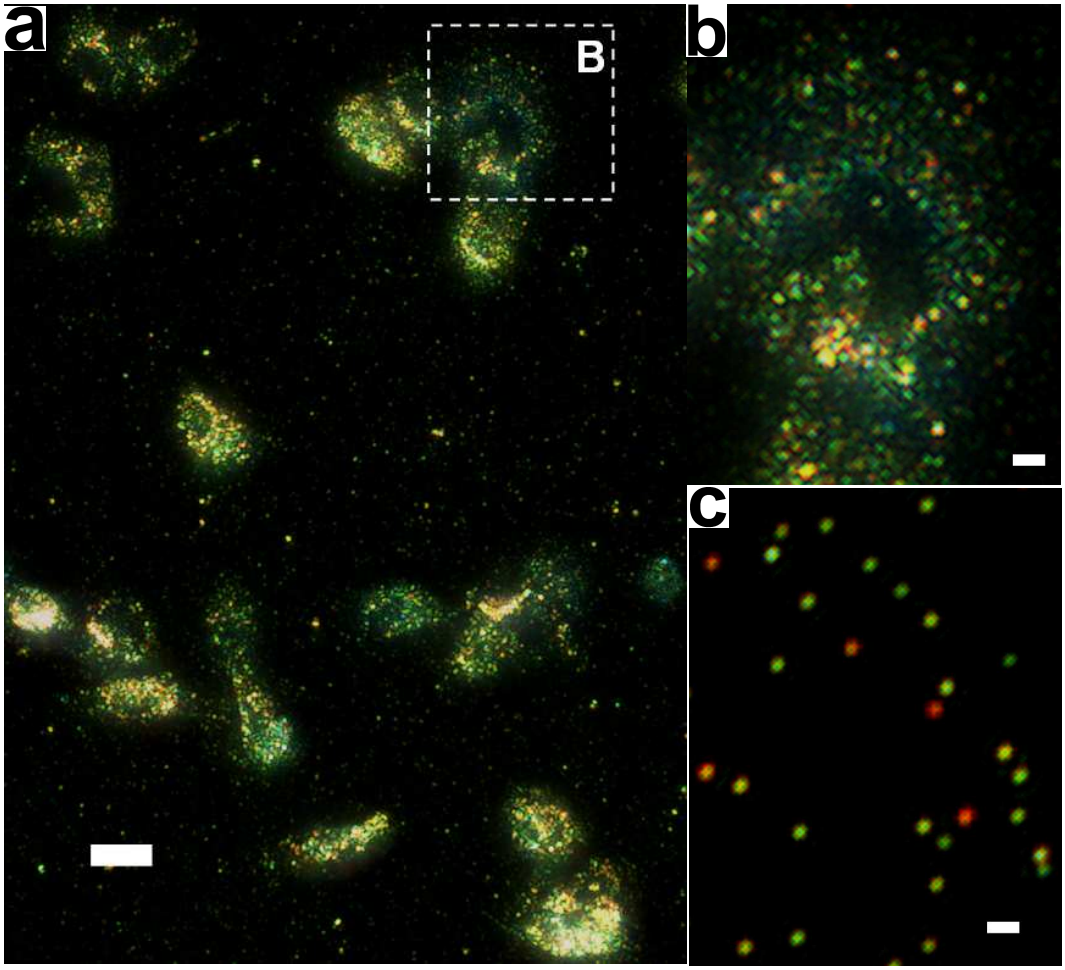
Supplementary Figure S1. The preparation of a carboxylic acid-terminated gold nanoplasmonic probe[1]. Physisorbed polymer can stabilize colloidal gold nanoparticle due to its strong Van der Waals repulsion. To remove unbound chemicals such as anions, polymer, MPA, and reducing agents, centrifugation was conducted prior to internalization.



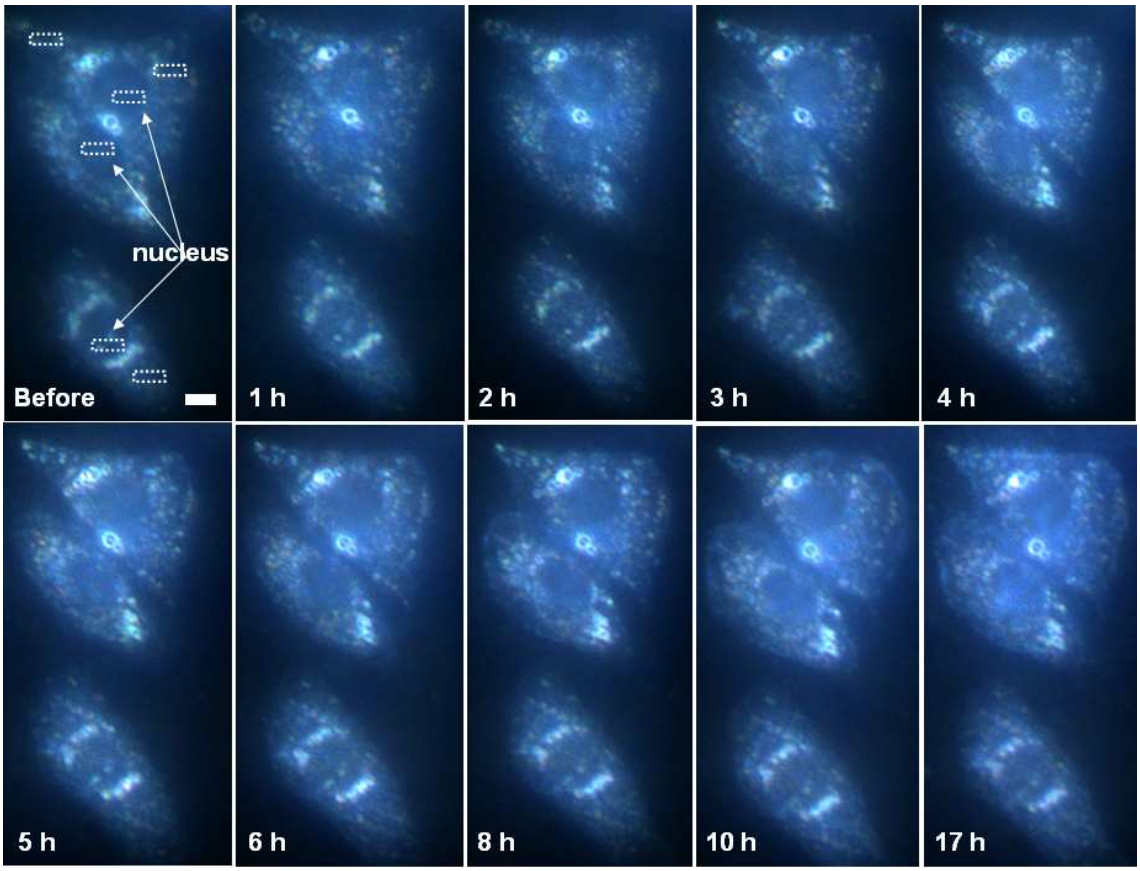
Supplementary Figure S2. Equilibrium differential quenching dip change as a function of Cyt *c* concentrations. The solid line (red) is the calculated value of $\Delta I_{550\ nm}$ using equation (see inset). K_a is the surface-confined thermodynamic affinity constant.



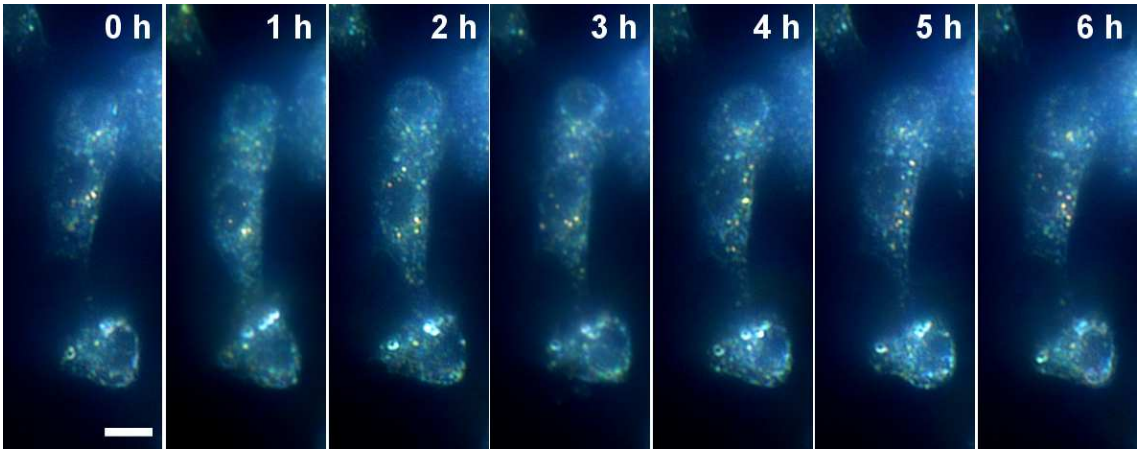
Supplementary Figure S3. The experimental procedures for collecting scattering spectra with live HepG2 cells. (**a**) B/W dark-field scattering image from spectrometer. (**b**) ROI (region of interest, red horizontal lines) selection. (**c**) Scattering spectra of each 7 μm X 3 μm region were measured by an entrance slit (vertical) in front of detector and by defining a ROI in the software.



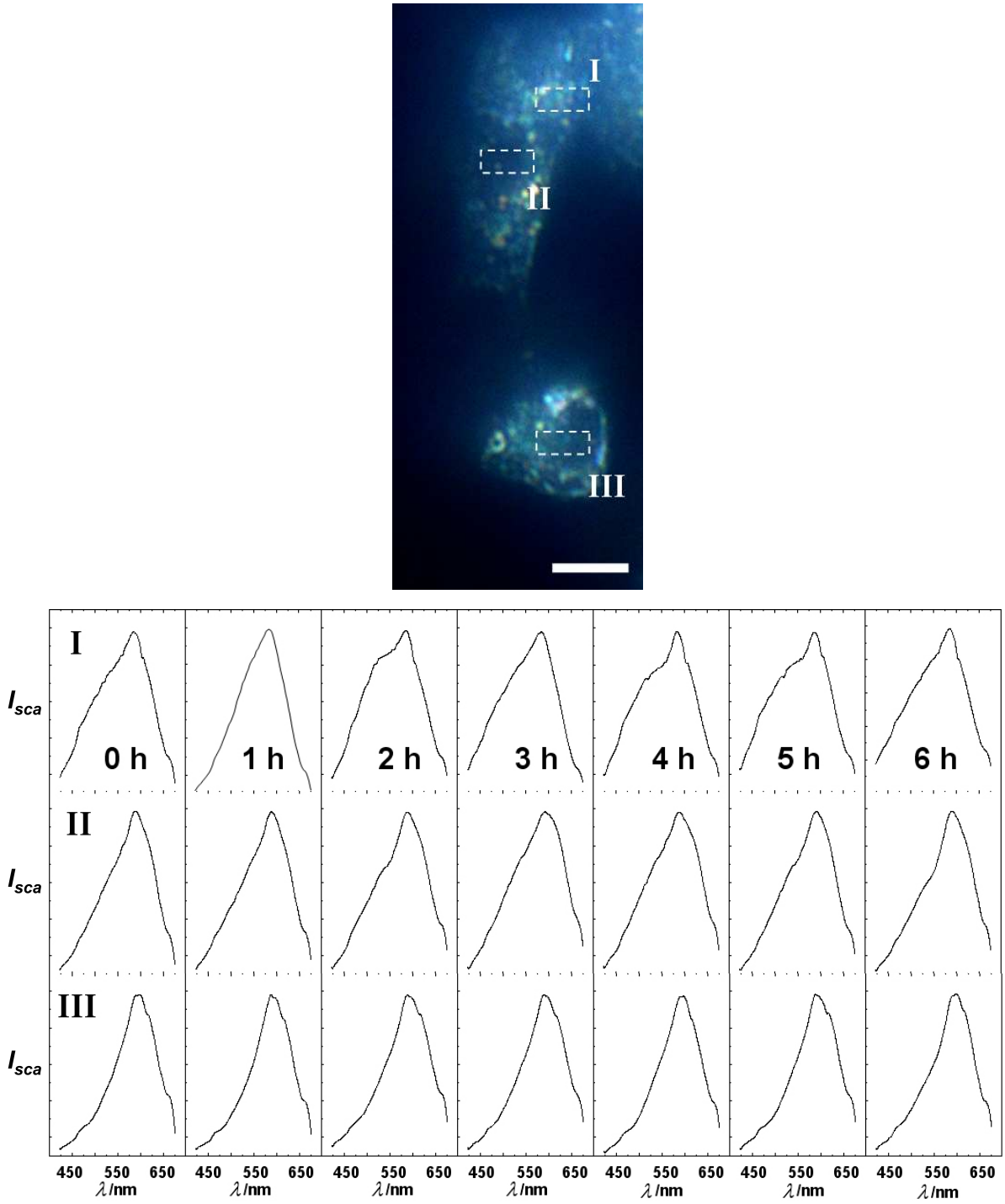
Supplementary Figure S4. (**a**) Dark-field reflectance images of live HepG2 cells labeled with the probes (10^4 particles per one cell, which does not represent the cellular uptake). Scale bar corresponds to 10 μm. (**b**) Magnified image of B (dotted square box in supplementary Fig. 3a) showing that a single gold plasmonic probe can be visualized by its color. The bar corresponds to 2 μm. (**c**) Dark-field image of carboxylic-acid functionalized 50 nm gold particles on a glass slide. The bar corresponds to 2 μm.



Supplementary Figure S5. Dark-field reflectance images of live HepG2 cells labeled with the carboxylic acid-terminated gold nanoplasmonic probes at various time points after exposure with 100 mM ethanol. The bar corresponds to 10 µm.



Supplementary Figure S6. Dark-field reflectance images of live HepG2 cells labeled with the probes at various time points (o ~ 6 h) without exposure with ethanol. The bar corresponds to 10 μm.



Supplementary Figure S7. (top panel) Dark-field image of HepG2 cells at 0 h. The bar corresponds to 10 μm. (bottom panel) Representative scattering spectra from the numbered positions in the top panel without ethanol.

Reference

1. K. Aslan, V. H. Perez-Luna, *Langmuir* **18**, 6059 (2002).