TARGET

Supporting information for

Tunable and Amplified Raman Gold Nanoprobes for Effective Tracking (TARGET): *In Vivo* Sensing and Imaging

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Materials

All materials were used as received without any further purification. Cetyltrimethylammonium bromide (CTAB), gold chloride ($HAuCl_4.4H_2O$) solution, sodium borohydride (NaBH4), ascorbic acid, sodium chloride (NaCl), polyvinylpyrrolidone (PVP (58000 MW)), 1- tetradecanol, cetyltrimethylammonium chloride (CTAC) solution (25 wt% in H_2O) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Silver nitrate ($AgNO_3$, 99.995%) was purchased from Alfa Aesar (Ward Hill, MA). MEBM (Mammary Epithelial Basal Medium) medium and MCF-10A cell line were obtained from the Duke University Cell Culture Facility (Durham, NC). The Formvar®/carbon-coated copper TEM grids were purchased through VWR (Radnor, PA).

Methods

Gold nanoparticles synthesis

Gold nanoparticles were synthesized using a previously reported method.[1] Seed solution was prepared by vigorous mixing of 10 ml of aqueous CTAC solution (0.1 M), and 250 µl of $HAuCl_4$ (10 mM) with 450 µl of ice-cold $NaBH_4$ (10 mM) solution. The seed solution was aged for 2 hrs at room temperature (25^{o}C). In a seperate vial, the growth solution was prepared in 10 ml of aqueous CTAC solution (0. 1M), 250 µl of $HAuCl_4$ (10 mM), and 25 µl of ascorbic acid (0.1 M). To this colorless solution, 25 µl of seed solution was added with vigorous stirring and kept undisturbed overnight to obtain highly uniform spherical nanoparticles that exhibit a Localized Surface Plasmon Resonance (LSPR) at 518 nm. The diameter of the nanoparticles obtained at this stage was ~20 nm.

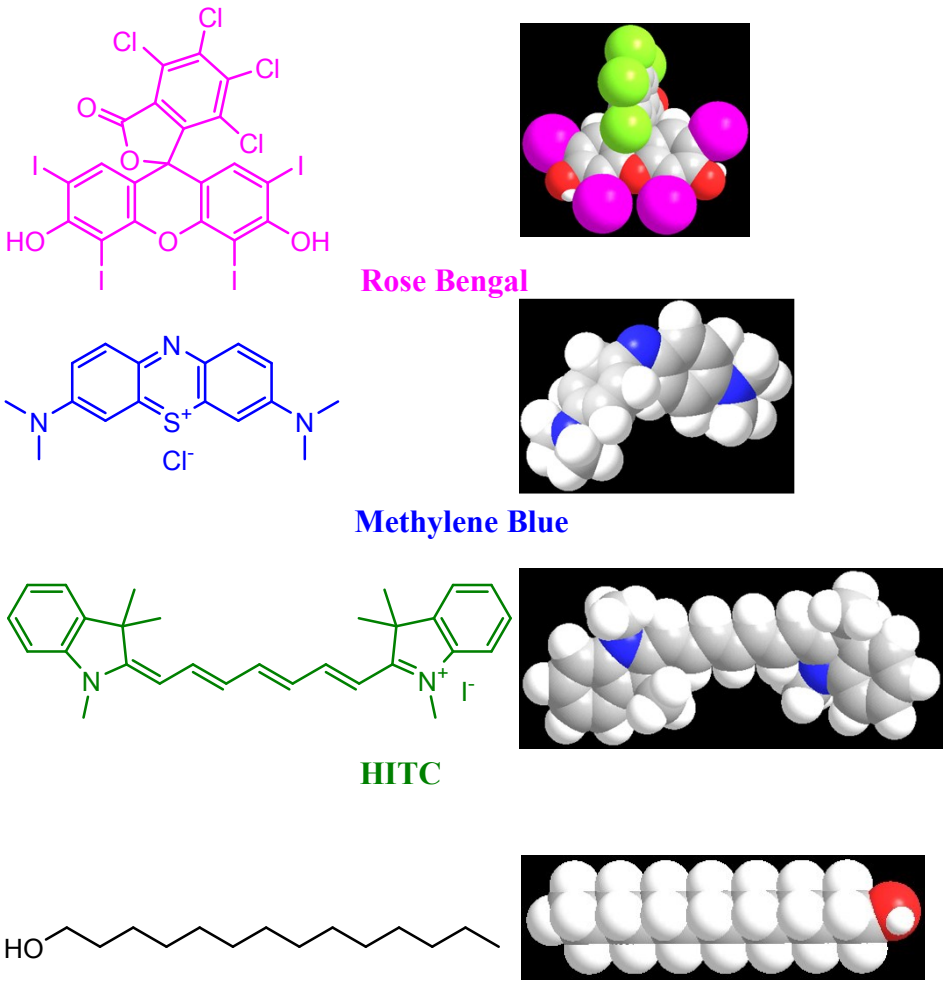
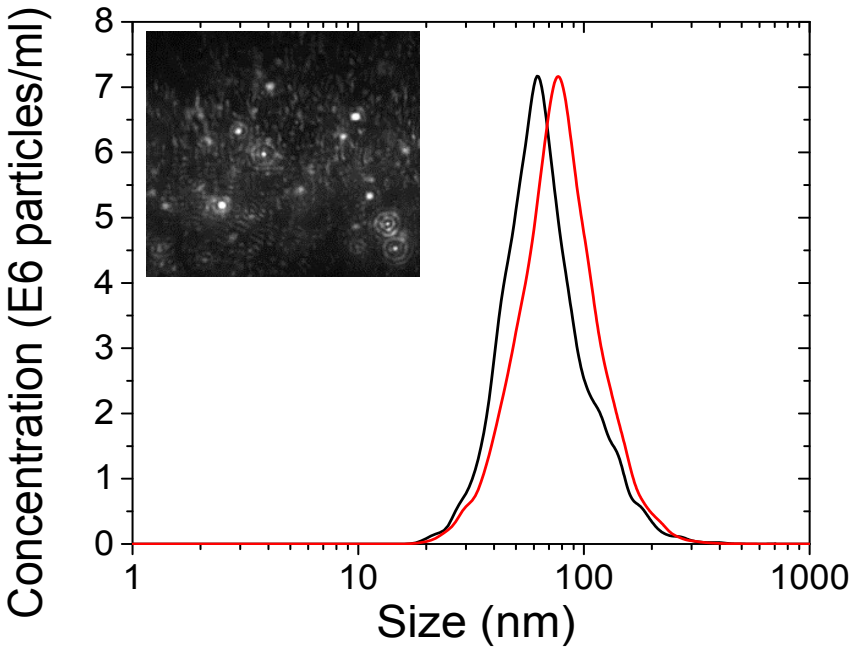


Figure S 1.**Molecular structures and space filling models of Rose Bengal, Methylene Blue, HITC, and 1-Tetradecanol.**

Cell culture

Human epithelial breast cells (MCF-10A) were purchased from CCF at Duke University (Durham, NC) and sub-cultured in MEGM (MEBM mixed with a kit obtained from Lonza/Clonetics Corp. Catalog No. CC-3150, which is optimized to grow the cells in serum free medium obtained from Lonza) as suggested by the American Type Cell Culture protocol (Manassas, VA). Cells were grown in an air-jacketed incubator at 37^0C with 5% CO_2-humidified atmosphere in 25 cm^2 tissue culture flasks. Once the cells reached 80-90% confluence, they were washed with phosphate buffered saline (PBS) and detached with 1 mL of 0.25% trypsin-EDTA solution (Sigma). The cells were dispersed in 10 ml MEGM medium

and centrifuged at 800 RPM to completely remove trypsin. Cells were counted in a Nexcelon biosciences automated cell counter using cellometer cell counting chambers and seeded 4×10^5 cells per well (in three weels) in a 6 well plate.



***In vitro* SERS measurements**

Figure S 2. Size and concentration of all nanoparticles used in this research were measured using the nanoparticle tracking analysis (NTA) option available in the Nanosight NS500 device. The graph shown above is the size and concentration of nanorattles (red) and the sacrificial nanorattle template (black) diluted 1000 folds from the original concentration. The silver-coated gold nanoparticle templates had a hydrodynamic diameter of 62 ± 52 nm, while the nanorattles were found to be 77 ± 47 nm. The zeta potential of the nanorattles was measured to be +16 ± 4 mV.

After 24 hrs, passively targeted MCF-10A cells with TARGETs were detached using 1 ml of 0.25% trypsin-EDTA and centrifuged at 800 RPM to redisperse in fresh complete medium. The detached cells were placed in cellometer cell counting chambers and incubated for 1 hr to attach partially to the surface of the plastic on the cell counting chamber. These cell counting

chambers did not show any fluorescence or Raman background signal, which is important for SERS measurements. Then, Raman mapping with 1-micron spatial resolution was performed from single MCF-10A cells using a confocal InVia Renishaw Raman microscope with a 633-nm (10 mW) laser as the excitation source. We also measured the SERS signal from cells dispersed in medium after 24 hrs using a 785-nm laser to make sure the probes are stable over time.

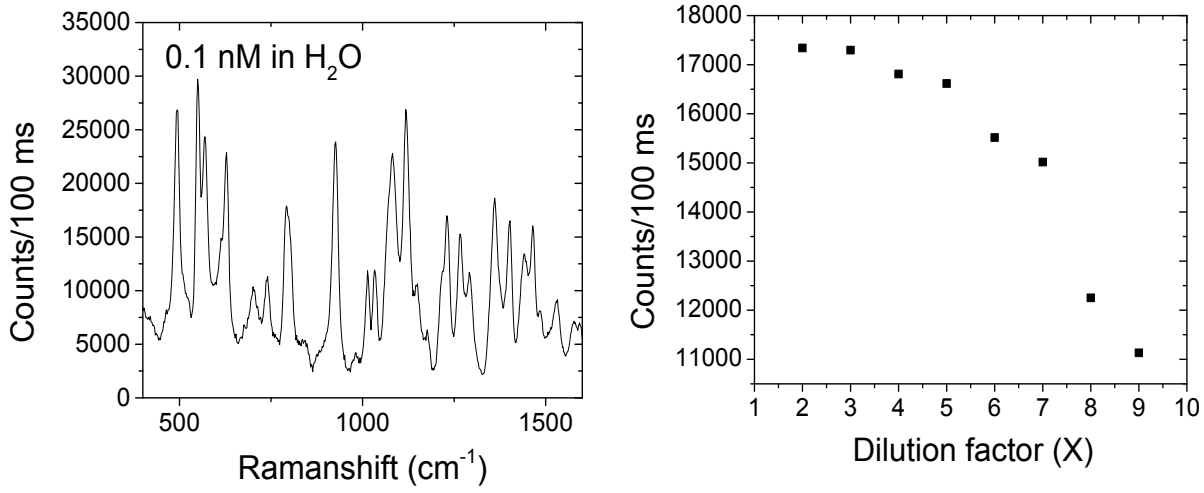


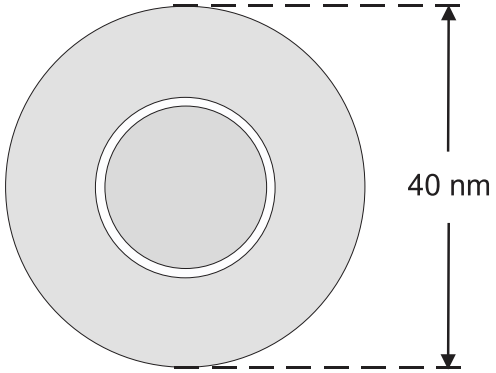
Figure S 3 LEFT: SERS spectrum of 0.1 nM nanorattles in water; RIGHT: SERS signal intensity at different concentrations of nanorattles to make sure the detector is not saturated due to the large signal.

Electric field enhancement calculations in nanorattle gap:

The electric field enhancement (E/E_o)is calculated using the following equation:

$$\frac{E}{E_0} = 9\left(\frac{\varepsilon_0\varepsilon_1\varepsilon_3}{\varepsilon_2}\right)\frac{1}{(2\varepsilon_0+\varepsilon_1)(2\varepsilon_1+\varepsilon_3)+2(\varepsilon_0-\varepsilon_1)(\varepsilon_1-\varepsilon_3)(R_2/R_1)^3}$$

R_1 = outer radius of shell
R_2 = inner radius of shell
ε_0 = dielectric constant of evironment
ε_1 = dielectric constant of shell
ε_2 = dielectric constant in gap
ε_3 = dielectric constant of core



$$\varepsilon(\omega) = \varepsilon_{\infty} - \frac{\omega_p^2}{\omega(\omega + i\gamma)}$$ (Drude model of dielectric constant)

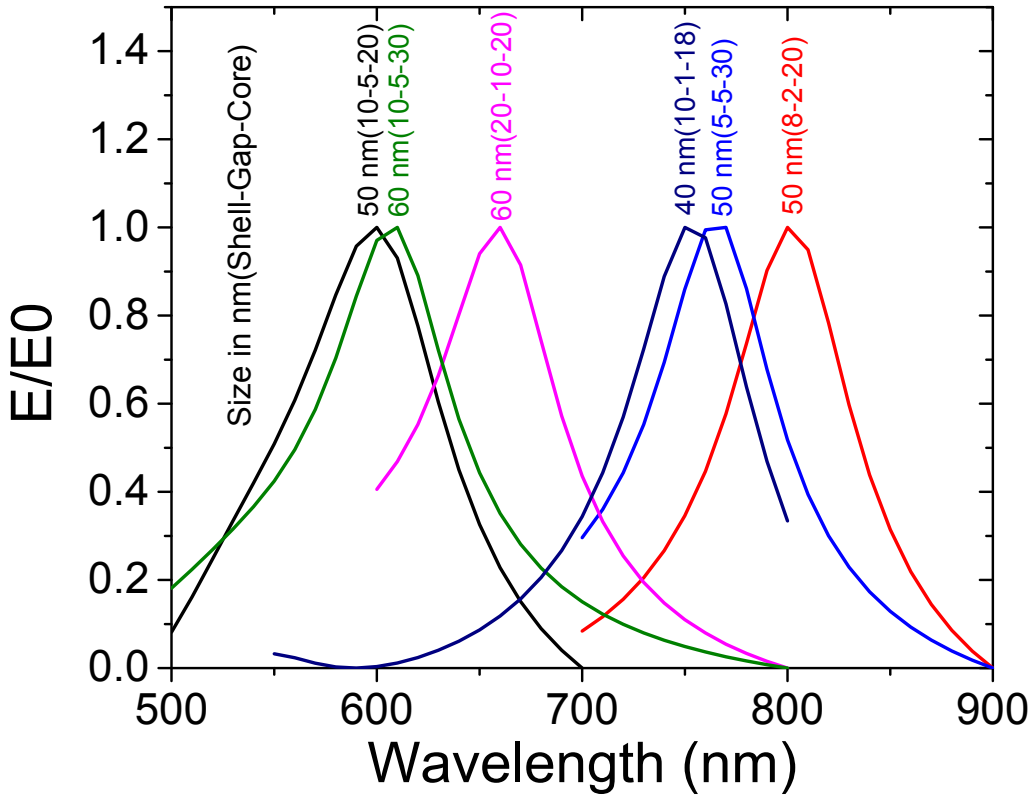


Figure S4 Possibility of tuning the plasmon band of the gold nanorattles: Finite element method (FEM) calculations of TARGETs with varying shell thickness, core size, and gap size (COMSOL Multiphysics v4.3 software). The E-field distribution inside the gap significantly changes with shell, core, and gap dimensions of the TARGET.

H_2O_2 etching studies

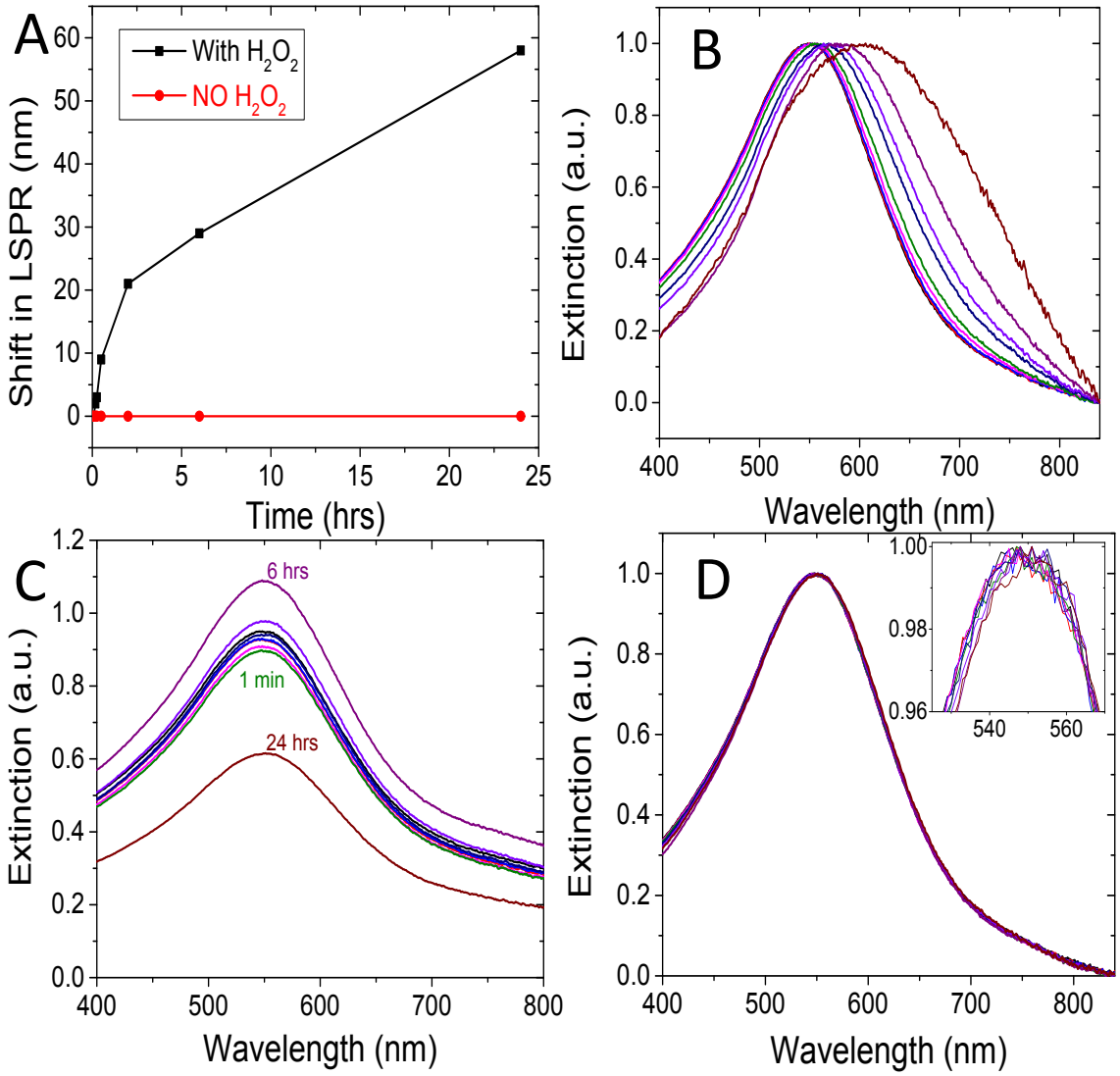


Figure S 5 H_2O_2 etching studies were performed to monitor the effect of nanorattles shell thickness and formation of pores on the SERS intensity overtime. (A-B) Significant red shift in localized surface plasmon resonance (LSPR) was observed in the presence of H_2O_2 due to the change in shell thickness and formation of pores over time. UV/Vis absorption spectra of nanorattles in the presence (C) and absence (D) of H_2O_2, which clearly indicates there is significant drop in intensity of absorption of nanorattles in H_2O_2 due to shell rupture whereas in the absence of H_2O_2 there is no change in absorption spectra.

In vivo two-photon luminescence (TPL) imaging:

TPL imaging of nanorattles was performed using an Olympus FV1000 multiphoton system containing a tunable femtosecond Ti-Sapphire laser (680-1080 nm). The focal volume of the objective lens (40×, 0.75NA, water emersion) was used to scan along the Z-axis using a pair of gold-coated scanning mirrors. The mouse was anesthetized using a ketamine/xylazine solution (as described in the Methods section) and placed on a heated stage (Warner instuments). The TPL from nanorattles was collected using 800-nm excitation wavelength and Blue, Green and Red standard fluorescence cubes.

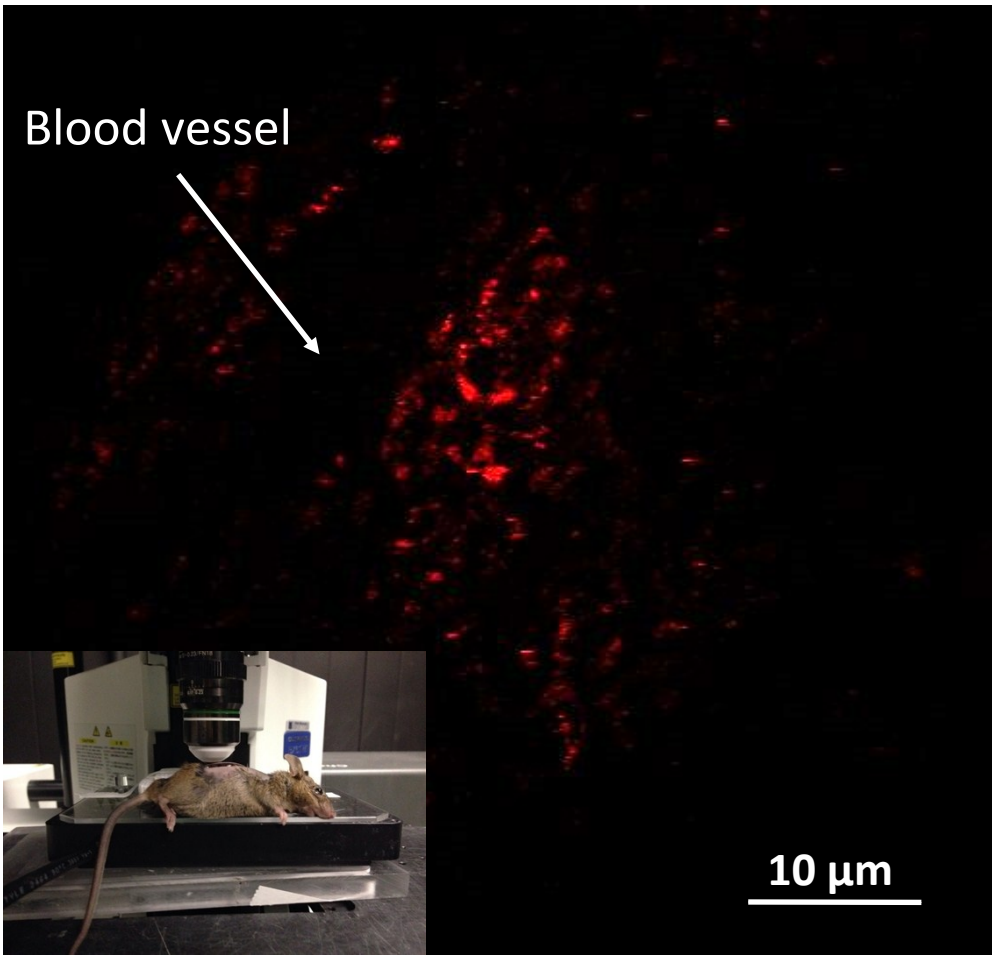


Figure S 6 *In vivo* two-photon luminescence (TPL) of nanorattles and the corresponding experimental TPL set up (inset)

In vivo SERS sensing:

In vivo SERS spectra were collected from the tumor region in the window chamber after nanorattle injection (100 ul of 1 nM). A 785-nm (100 mW) diode laser coupled with an

InPhotonics fiber optic RamanProbe (Norwood, MA) was used for excitation. SERS spectra was recorded using a PIXIS:100BReX CCD mounted to a LS-785 spectrograph (1200 grooves mm–1 grating), controlled by LightField software, from Princeton Instruments (Trenton, NJ). The collection fiber of the Raman probe was coupled to the entrance slit of the LS-785 spectrograph.

1. N. Gandra, A. Abbas, L. Tian and S. Singamaneni, *Nano Letters*, 2012, **12**, 2645-2651.