Supporting Information

Biocompatible polymer-modified gold nanocomposites of different shapes as radiation sensitizers

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1. Materials

Sodium hydrogen carbonate ($NaHCO_3$), sodium chloride (NaCl), citric acid (anhydrous), magnesium sulfate, anhydrous ($MgSO_4$), 2,2'-bipyridyl (Bpy), copper (II) bromide ($CuBr_2$), trisodium citrate dihydrate, tris(hydroxymethyl)aminomethane, HCl, H_2SO_4, DMEM, and D-PBS were purchased from Nacalai Tesque Co. (Kyoto, Japan). Dichloromethane (DCM), methanol (MeOH), ethyl acetate (EtOAc), hexane, disodium hydrogen phosphate (anhydrous, Na_2HPO_4), sodium dihydrogen phosphate (anhydrous, NaH_2PO_4), L-ascorbic acid, tetrahydrofuran (THF), silver nitrate, $NaBH_4$, and HNO_3 were purchased from Wako Co. Ltd

(Osaka, Japan). N, N-diisopropylethylamine (DIEA), di-tert-butyl dicarbonate ($(Boc)_2O$), cetyltrimethylammonium bromide (CTAB), α-bromoisobutyryl bromide, tris(2-carboxyethyl)phosphine hydrochloride (TCEP), and 5,5'-dithiobis(2-nitrobenzoic acid) were purchased from Tokyo Chemical Industries (Tokyo, Japan). 4*N* HCl/dioxane was purchased from Watanabe Chemical Industries, Ltd. (Hiroshima, Japan). Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4$) and cystamine dihydrochloride were purchased from Sigma-Aldrich (MO, USA). 2-methacryloyloxyethyl phosphorylcholine (MPC) was purchased from NOF Corporation (Tokyo, Japan). Methacryloyloxyethyl thiocarbamoyl rhodamine B (MTRB) was purchased from Polyscience Inc. (PA, USA). Deionized water was obtained from a Millipore Milli-Q purification system.

2. Apparatus

^{1}H-NMR spectra were measured using a JNM-LA300FT NMR SYSTEM and an NMR data processing software ALICE 2 (JEOL Ltd., Tokyo, Japan). The particle size distribution was obtained using a DLS system Zetasizer Nano ZS (Malvern Instruments Ltd., U.K.). Ultraviolet-visible (UV-Vis) spectra were obtained using a V-560 spectrophotometer (JASCO Ltd., Tokyo, Japan). Fluorescence spectra were obtained using a fluorescence spectrophotometer (F-2500, Hitachi, Tokyo, Japan). Cells were cultured in a CO_2 incubator (CO_2 water-jacketed incubator, Thermo Fisher Scientific Inc., MA, USA) and observed under an inverted microscope (CKX31, Olympus, Tokyo, Japan). The cells were collected by centrifugation (KUBOTA2800, Kubota, Osaka, Japan). Absorbance was measured using a microplate reader (Spectrafluor Plus, Tecan, Männedorf, Switzerland). The cellular uptake behaviour of MIP-NGs was observed using CLSM (IX81, Olympus, Tokyo, Japan) at 25 °C using a U-MWIG3 filter (λ_{ex} = 559 nm, λ_{em} = 580 nm) for rhodamine. The objective lens was a UplanSApo (Olympus, 60×, NA = 1.35). The multi-Ar laser was equipped with Optiλ (NTT Electronics, Kanagawa, Japan) and GLS3135 (Showa Optronics, Tokyo, Japan).

3. ATRP Initiator

3.1. Product 1

Cystamine dihydrochloride (4.5 g, 20 mmol) and DIEA (10.2 mL, 60 mmol) were dissolved in MeOH (20 mL). MeOH solution (40 mL) containing $(Boc)_2O$ (2.18 g, 10 mmol) was slowly added to the solution in an ice bath, and the reaction was carried out overnight at room temperature. After removal of MeOH by evaporation, the crude product was dissolved in 1 M NaH_2PO_4 and washed three times with diethyl ether. The pH of the aqueous phase was adjusted to 9.0 using 1 M NaOH. The product was extracted with EtOAc. After drying with $MgSO_4$, the product was dried in vacuo. The product was used in the subsequent procedure without further purification. The yield obtained was 1.04 g (41%).

3.2. Product 2

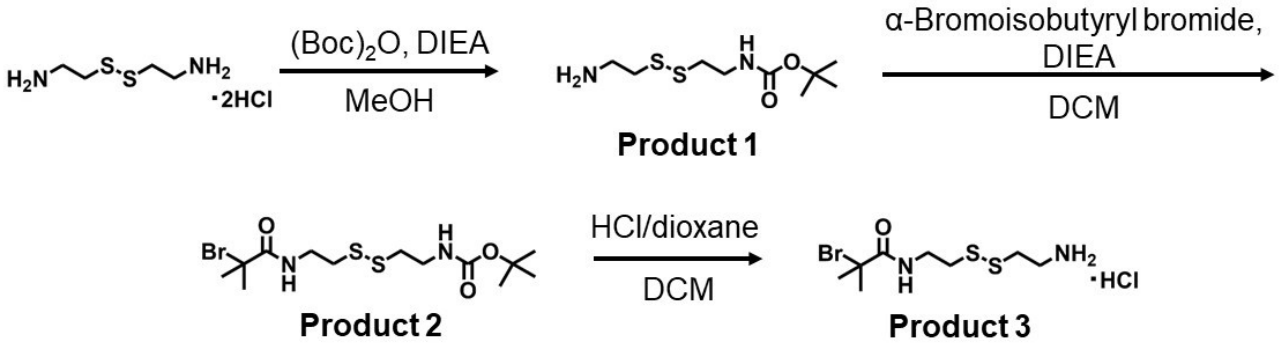
Product 1 (1.04 g, 4.1 mmol) and DIEA (1.42 mL, 8.2 mmol) were dissolved in DCM (40 mL). DCM solution (40 mL) containing α-bromoisobutyryl bromide (0.66 mL, 5.72 mmol) was slowly added to the solution in an ice bath, and the reaction was carried out overnight at room temperature. The crude product was washed thrice with citric acid aqueous solution, $NaHCO_3$ aqueous solution, and NaCl aqueous solution three times. After evaporation of DCM, the crude product was washed using silica column chromatography (Hex:EtOAc: 1:1), and the product was dried in vacuo. The yield obtained was 1.06 g (64%).

^{1}H-NMR (500 MHz, $CDCl_3$): δ=4.984 (s, 2H), 3.650–3.613 (m, 2H), 3.506–3.470 (m, 2H), 2.890–2.823 (m, 4H), 1.994 (s, 9H), 1.472 (s, 6H)

3.3. Product 3

Product 2 (1.06 g, 2.77 mmol) was dissolved in DCM (50 mL). DCM solution (40 mL) containing 4N-HCl/dioxane (2.6 mL, 10.4 mmol) was slowly added to the solution in an ice bath, and the reaction was carried out overnight at room temperature. After evaporation of the solvent, the crude product was dissolved in a small amount of DCM, and the product was precipitated by adding diethyl ether. The product was then dried in vacuo. The obtained yield was 0.49 g (62%).

^{1}H-NMR (500 MHz, D_2O): δ=3.492–3.467 (m, 2H), 3.282–3.256 (m, 2H), 2.899–2.874 (m, 2H), 2.824–2.798 (m, 2H), 1.823 (s, 6H)



Scheme 1. Synthetic procedure of hydrophilic atom transfer radical polymerization initiator containing disulfide linkages.

4. ^{1}H-NMR Spectra for the Polymerization Solution

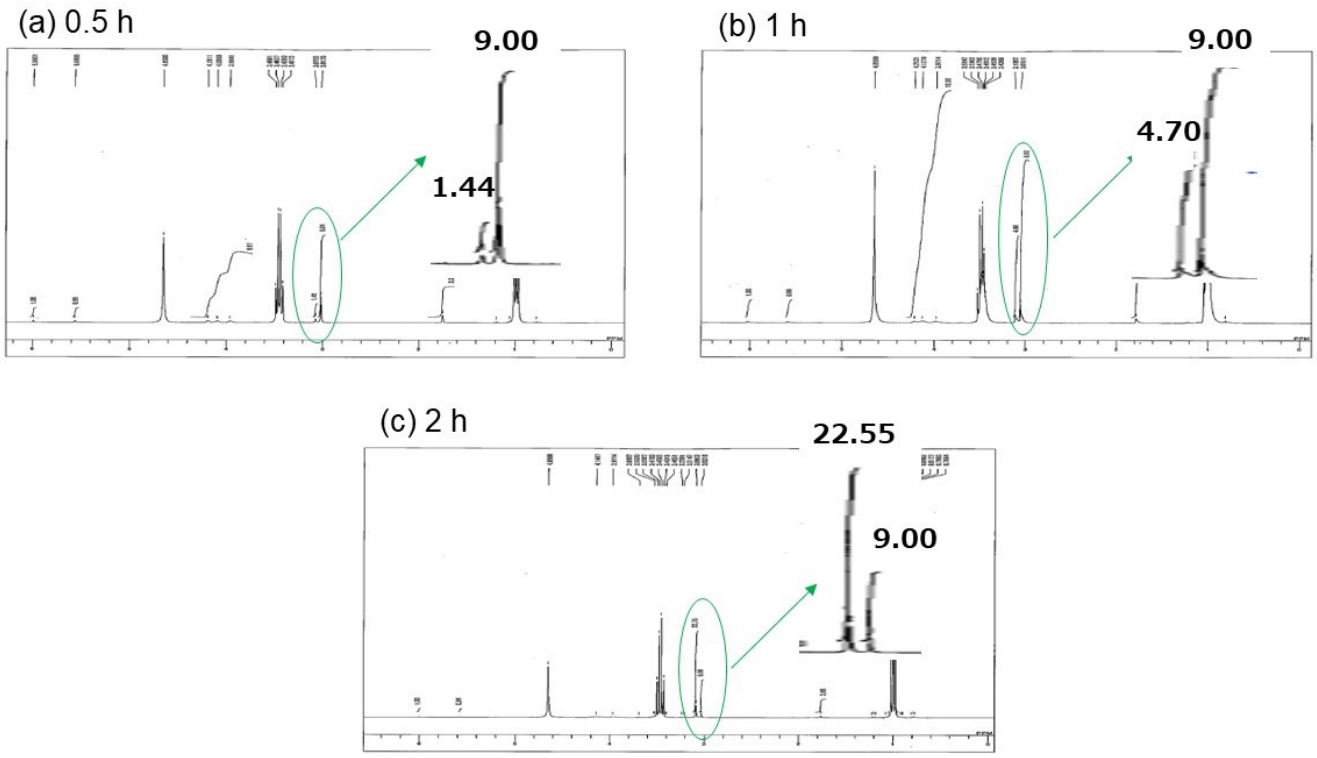


Figure S1. ^{1}H-NMR spectra of polymerized solution after ATRP of MPC for 0.5 (a), 1 (b), 2 h (c).

ATRP of MPC was estimated from the ratio of 1H peak intensities derived from the choline moiety of PMPC (peak at lower magnetic fields shown in the insert in Figure S1) and MPC monomer (peak at higher magnetic fields in the insert in Figure S1). The degree of polymerization of PMPC was calculated from the conversion and target maximum degree of polymerization, which was determined from the initial [monomer]/[initiator] ratio.

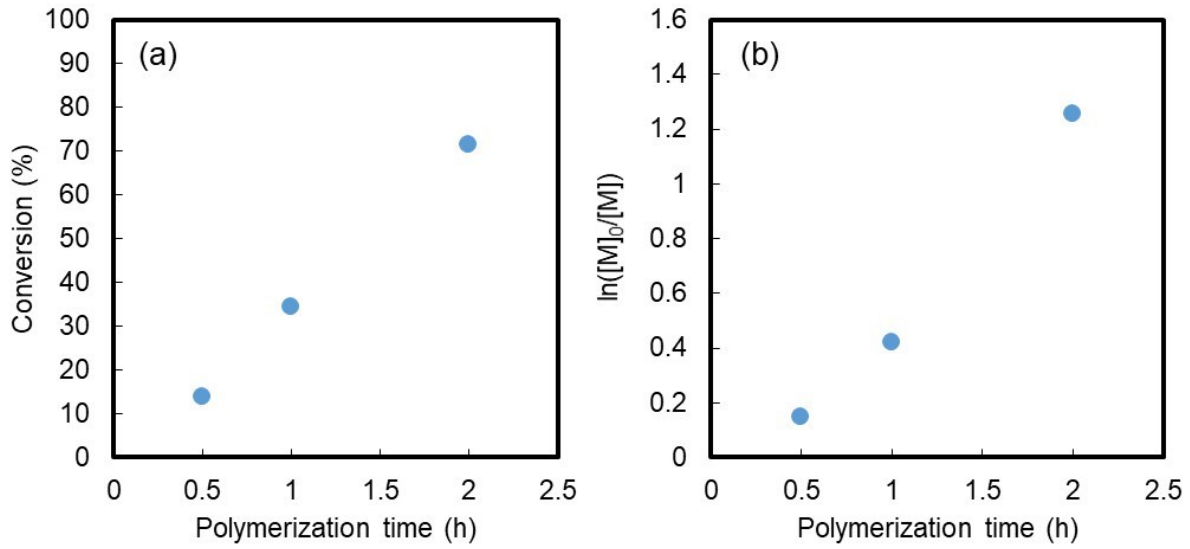


Figure S2. Conversion vs. time (a) and first-order plot (b) of ATRP of MPC.

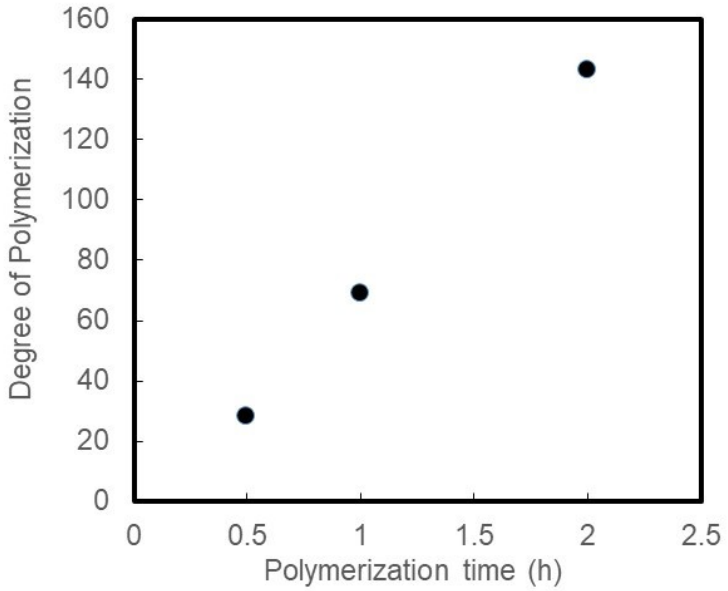


Figure S3. Degree of polymerization of PMPC estimated from ^{1}H-NMR at various polymerization periods.

5. DTNB Assay

An aqueous solution of PMPC before and after TCEP treatment (20 mg/mL, 500 μL) was mixed with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) aqueous solution (500 μL) at room temperature for 1 h. The UV-Vis spectra of these aqueous solutions were then measured.

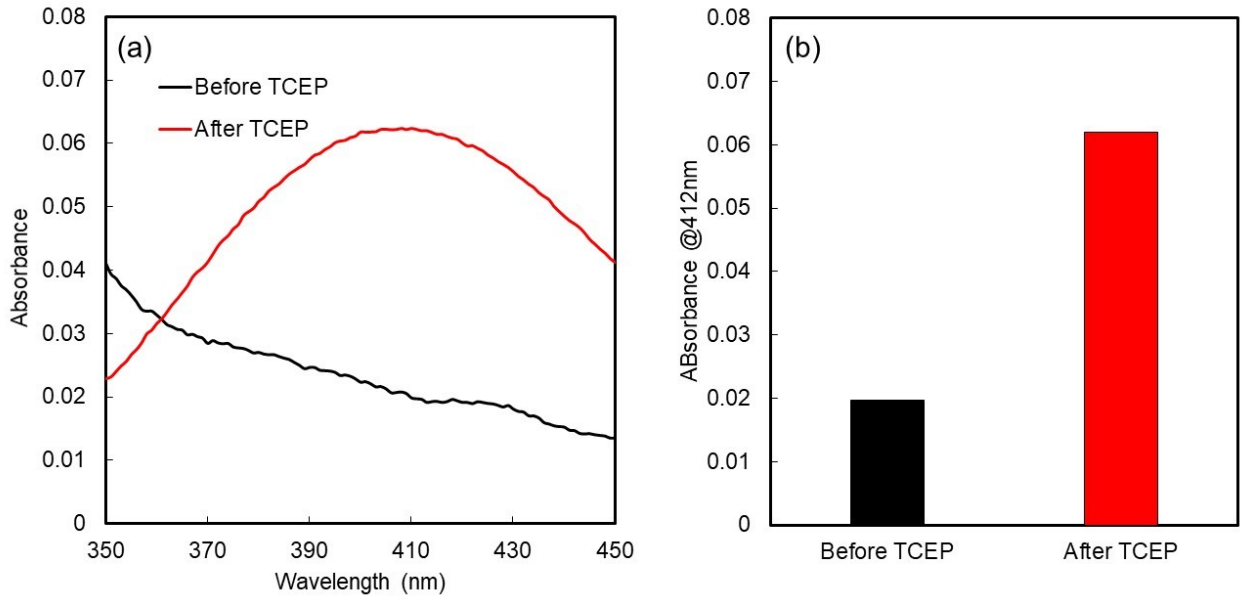


Figure S4. UV-Vis spectra (a) and absorbance at 412 nm (b) for DTNB assay of PMPC prepared using ATRP before (black) and after (red) TECP treatment.

6. TEM Images of PMPC-modified Gold Nanocomposites

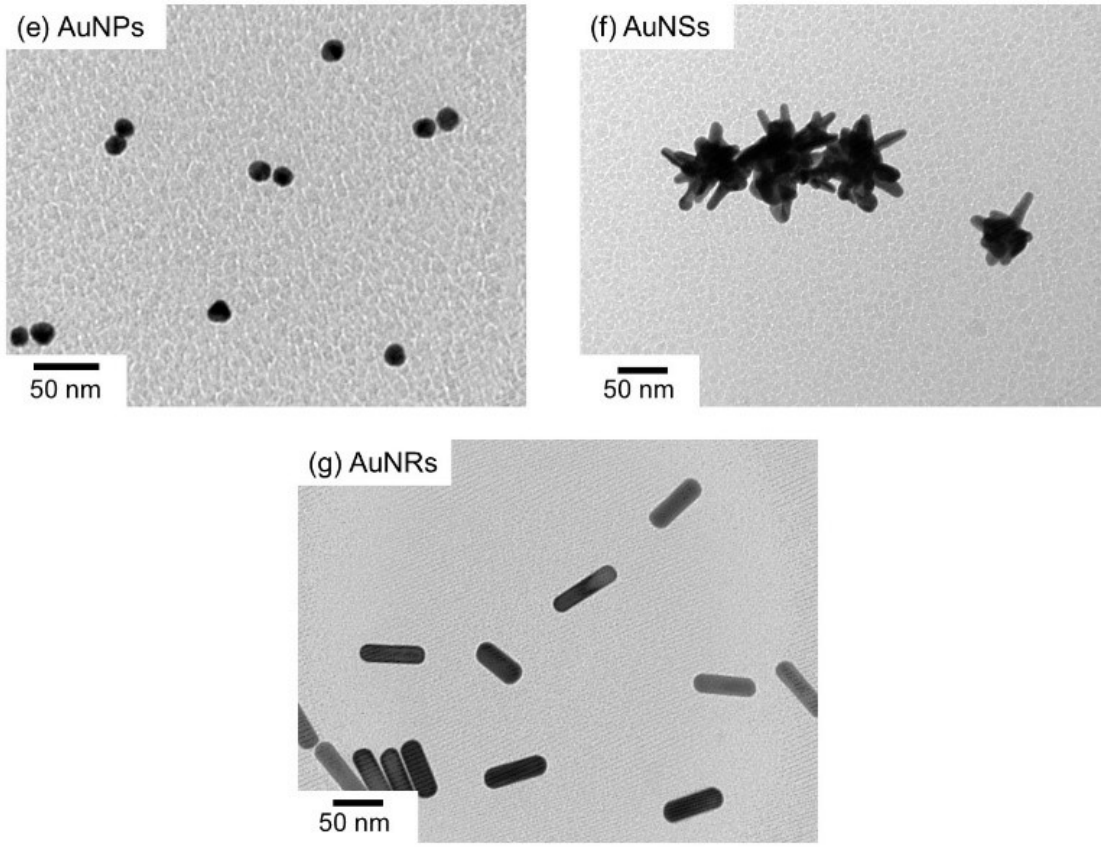


Figure S5. TEM images of AuNPs (a), AuNSs (b), and AuNRs (c) after PMPC modification.

7. UV-Vis and Fluorescent Spectra of Rhodamine-labelled PMPC

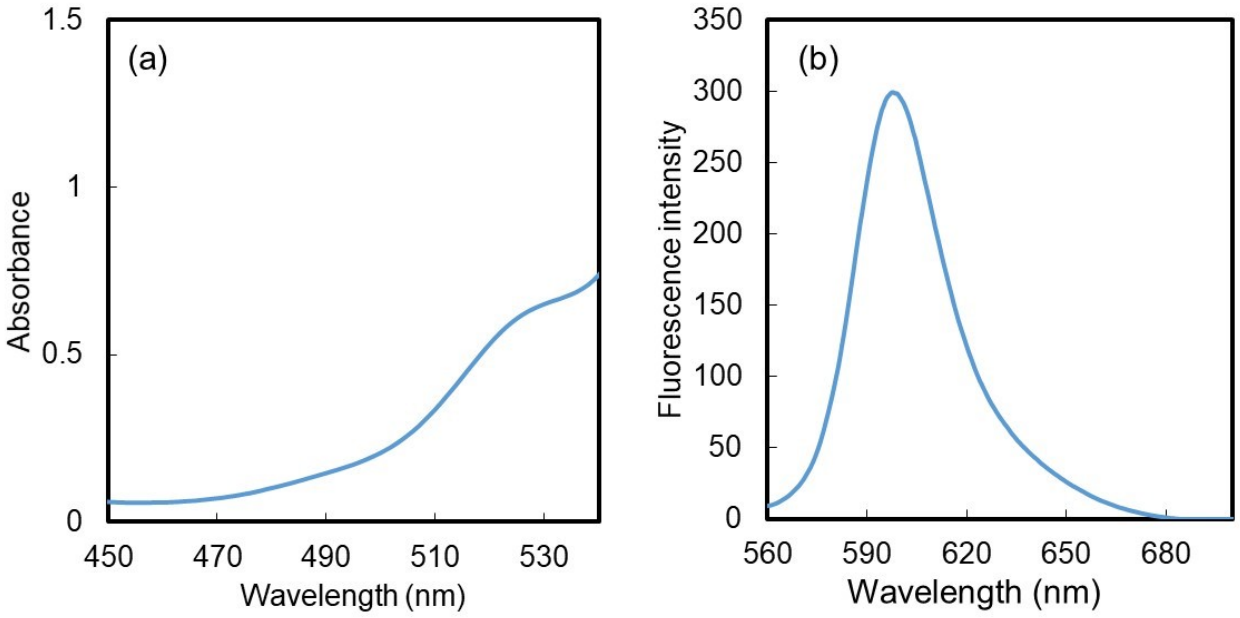


Figure S6. UV-Vis (a) and fluorescent (b) spectra for rhodamine labeled PMPC. The excitation wavelength was set at 550 nm to obtain the fluorescent spectrum.

8. Fluorescent Spectra of Rhodamine-labelled PMPC Modified Gold Nanocomposites

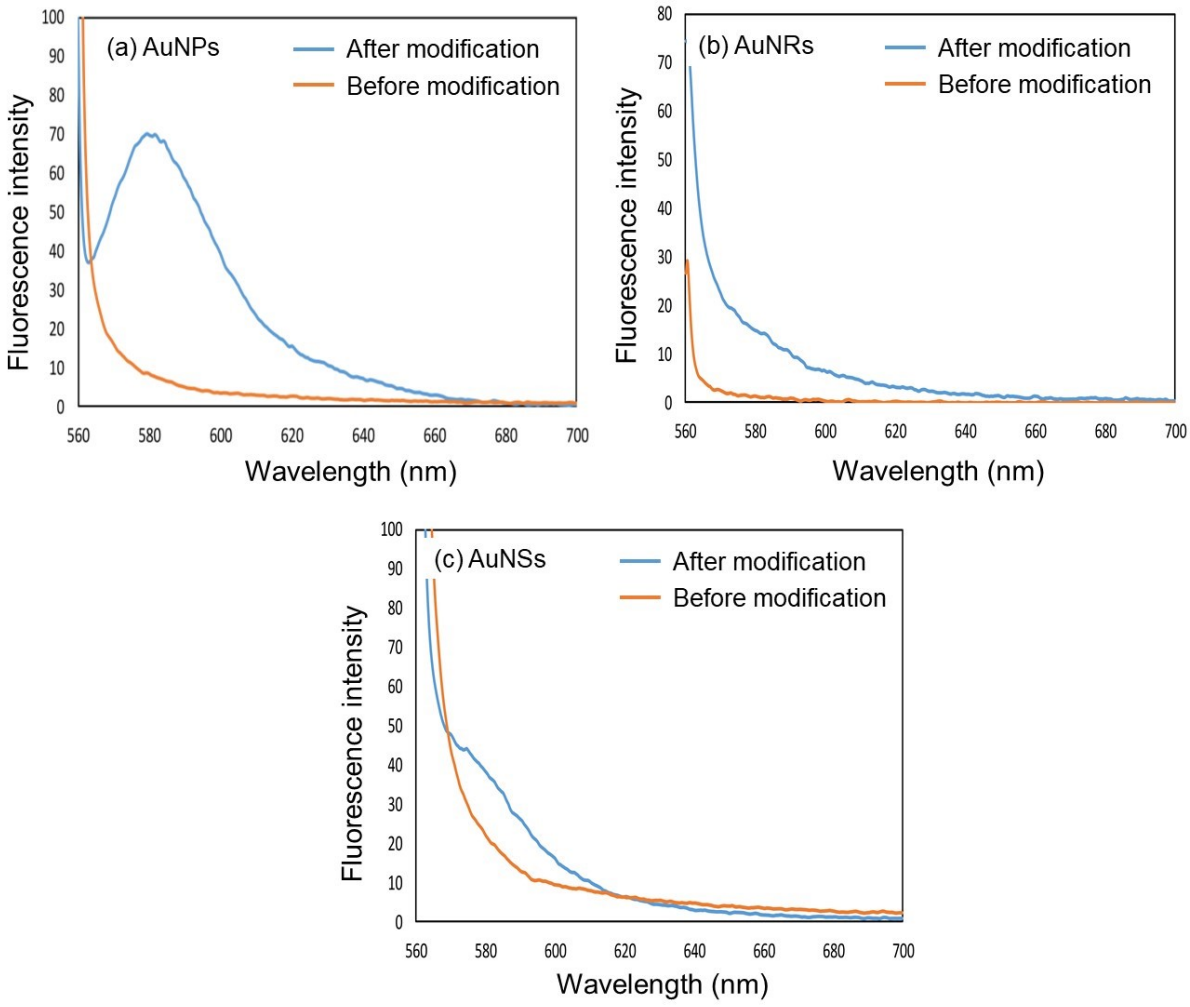


Figure S7. Fluorescence spectra of rhodamine-labelled PMPC-AuNPs (a), PMPC-AuNRs (b), and PMPC-AuNSs (c). The excitation wavelength was set at 550 nm.

9. Fluorescent Microscope Images of Rhodamine-labelled PMPC-modified Gold Nanocomposites

An automatic fluorescent microscope system with a pipette tip handling system was used. The automatic analyser consisted of an automatic pipetting device, tip rack, reagent rack, incubation port, detector, and computer. The plastic pipette tip can settle a small gold-coated glass substrate (reaction plate, 5 × 10 mm), and the tip was set in the tip rack. The device automatically demonstrates the pipetting reagents, incubation, washing, and fluorescent detection, as programmed by the computer. After the suction of rhodamine-labelled gold nanocomposites in pure water (100 μg/mL, 100 μL) into the pipette tip, a fluorescent image was obtained in the detection port before and after introducing the fluorescent molecules. Three different ROIs were obtained to calculate the fluorescence intensity for each substrate. Fluorescent microscope: Zyla SCMOS, Andor Technology, UK. Objective lens: ×5. Light quantity: 25%. Exposure time: 0.1 sec. Temperature: 2 5°C. A band-pass filter was used for Cy.

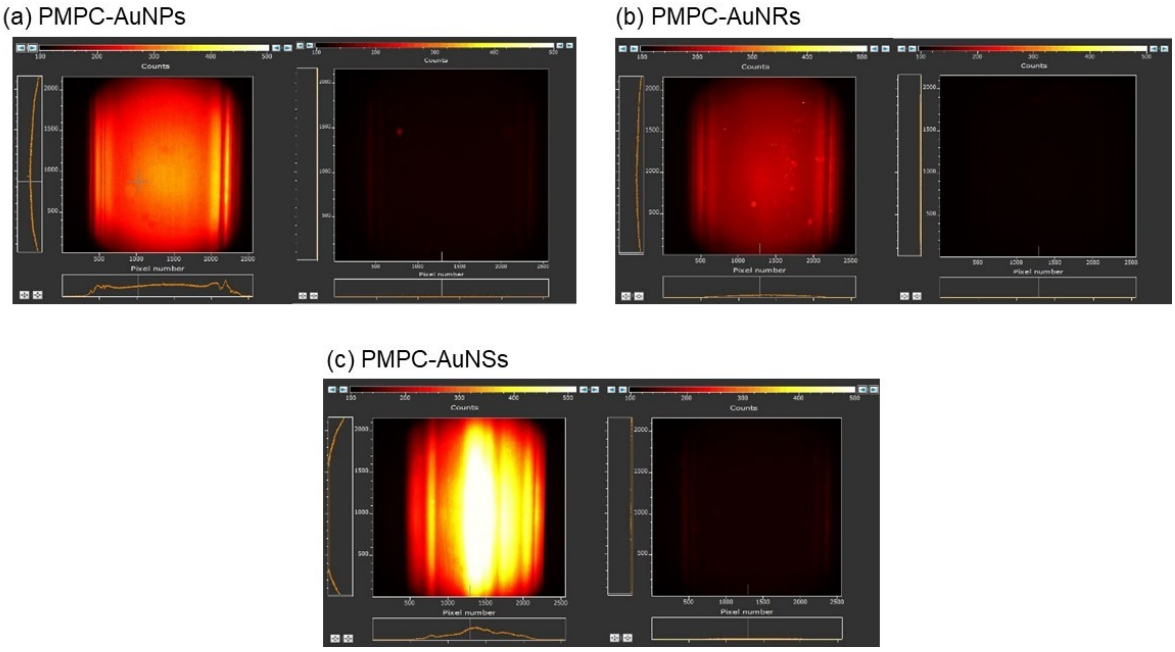


Figure S8. Fluorescent microscope images of AuNPs (a), AuNRs (b), and AuNSs (c), in which the left and right windows show the nanocomposites before and after modification with rhodamine-labeled PMPC, respectively.