Stimuli-Responsive SERS Nanoparticles: Conformational Control of Plasmonic Coupling and Surface Raman Enhancement

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Materials and Methods

Materials. Ultrapure water (18 MΩ cm^{-1}) was used throughout the work. The following chemicals were obtained from commercial sources and were used without further purification: 60-nm citrate-stabilized gold particles at a concentration of 2.6×10^{10} particles per milliliter (Ted Pella Inc., Redding, CA); NH2-PEG5000-PMAA3000 copolymer (MW=8,000) (Polymer Source Inc., Canada); QSY21 quencher dye (Invitrogen Corporation, Carlsbad, CA).; doxorubicin hydrochloride (Polymed Therapeutics, Inc., Houston. TX). All other reagents were obtained from Sigma-Aldrich (St Louis, MO) at the highest purity available.

Synthesis of block copolymer LA-PEG-PMAA. Lipoic acid (LA) was firstly activated by DCC-NHS method as described by Ponpipom et al. (*J. Med. Chem. 1987, 30, 705*). The pure product of NHS-activated lipoic acid was obtained by re-crystallization in toluene and was stored in -20 ^{0}C for further use. Amino copolymer NH2-PEG-PMAA (MW 8000) (100 mg) and NHS-activated thioctic acid (12.5mg, 10 times excess) were dissolved in 4 mL anhydrous DMF. About 0.5 mL triethylamine was added dropwise, and the resulting solution was stirred for 1 day. After dialyzing against water for 3 days, the mixture was lyophilized, re-dissolved in CH_2Cl_2 and isolated via silica gel chromatography using ethylacetate/hexane as an eluent. Final product LA-PEG-PMAA was obtained as a white powder after evaporating the solvent.

Stimuli-responsive SERS nanoparticles. Citrate-stabilized colloidal gold nanoparticles (60 nm diameter) were first encoded with a reporter molecule such as the quencher dye QSY21 or a drug molecule such as thiol-modified doxorubicin, each showing distinct Raman spectroscopic signatures. Then the nanoparticles were encapsulated with a thiolated block copolymers consisting of a pH-responsive ploymethacrylic acid (PMAA) block (MW 3000), an amphiphilic polyethylene glycol (PEG) block (WM 5000), and a terminal lipoic acid (LA) anchoring group. After overnight incubation at room temperature, two rounds of centrifugation were performed to remove any excess polymer.

Characterization and Measurement. UV-Vis absorption spectra were recorded on a Shimadzu (UV-2401) spectrometer using disposable polyacryl cuvettes. Transmission electron micrographs (TEM) were taken by using a Hitachi H7500 high-magnification electron microscope. The nanoparticle sample (5 L) was dropped onto copper 200 mesh grids that were pre-treated with UV light to reduce static electricity. Dynamic light scattering data were obtained by using a Brookhaven 90Plus particle size analyzer instrument. Each sample was measured three times consecutively. SERS spectra were recorded on a compact Raman system using 633 nm (3 mW) or 785 nm (40 mW) laser excitation (DeltaNu, Laramie, WO). SERS intensities were normalized to the Raman spectra of cyclohexane and polystyrene to correct for variations in optical alignment and instrument response. The spectral resolution was approximately 5 cm^{-1} for the Raman spectrometers used.

Supporting Figures

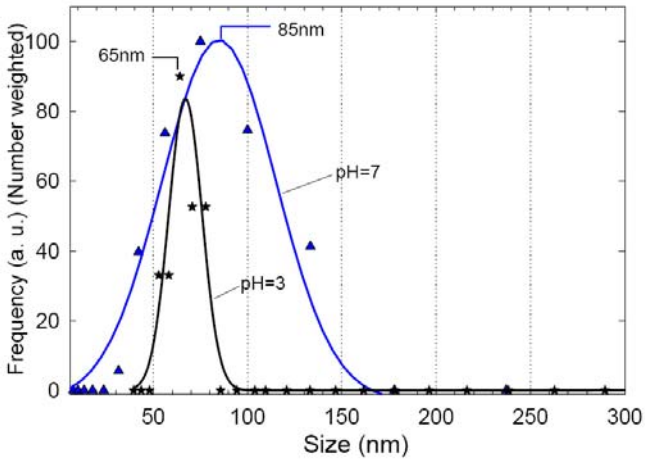

Figure S1. Hydrodynamic size data of stimuli-responsive SERS nanoparticles measured by dynamic light scattering (DLS) at pH 7 and pH 3 (prior to aggregation). The data indicate that the polymer layer's thickness decreases from ~12.5 nm at pH 7 to ~ 2.5 nm at pH 3.

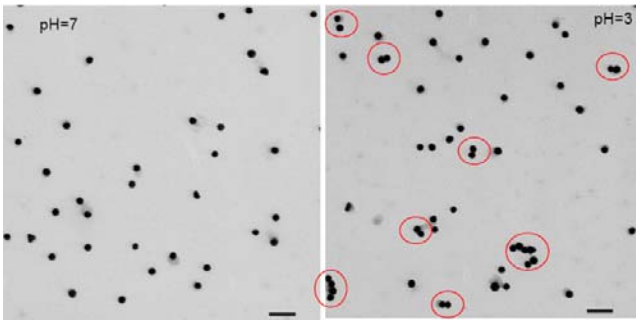

Figure S2. TEM images of stimuli-responsive SERS nanoparticles showing dispersed single particles at pH 7 (left) and small nanoparticle clusters at pH 3 (right). Scale bar is 200 nm. The circles highlight small nanoparticle aggregates that are believed to be efficient for SERS. See the text for discussion.

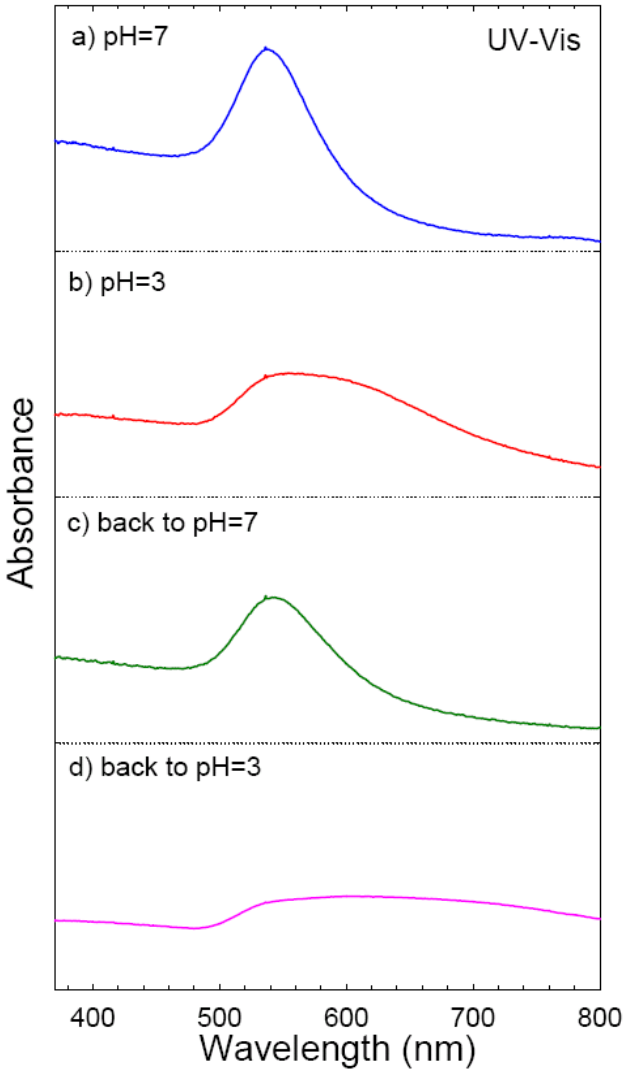

Figure S3. UV-Vis absorbance of stimuli-responsive gold nanoparticles obtained at (a) pH 7, (b) pH changed to 3, (c) pH changed back to 7, and (d) pH changed back to 3.

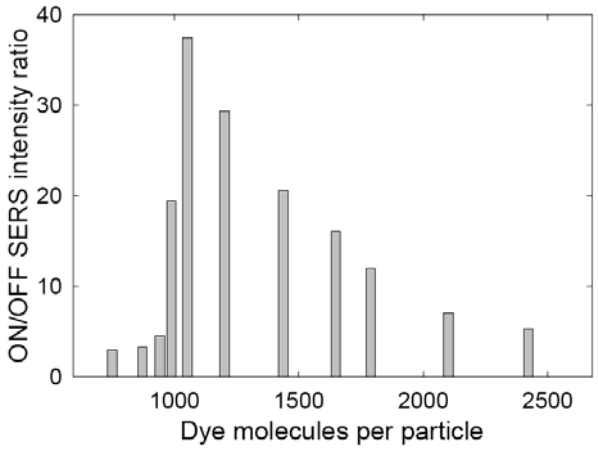

Figure S4. On/off SERS intensity ratio obtained at various reporter dye (QSY) densities on the particle surface. The SERS signal was measured at the Raman peak 1500 cm^{-1} (with baseline subtraction). The dye coverage was calculated as the average number of dye molecules per particle. The maximum signal ratios (30-35) were achieved at about 1000 dye molecules per particle.

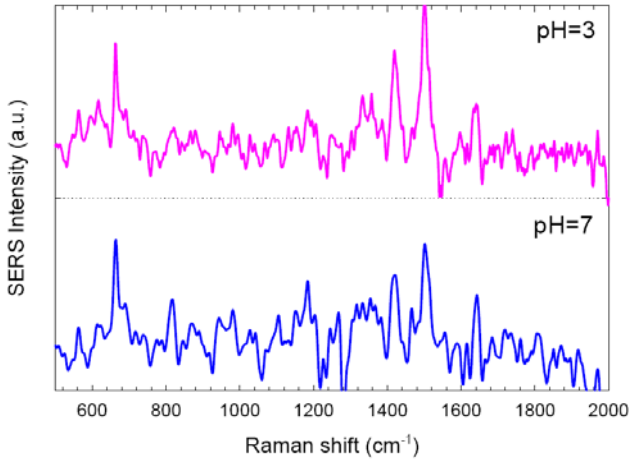

Figure S5. Effect of polymer surface densities on the pH response of SERS nanoparticles. The nanoparticles were coated with the copolymer at a low surface density by reducing the particle/polymer incubation time from 24 hours (overnight) to 2 hours.