Biodegradable Hollow Silica Nanospheres Containing Gold Nanoparticle Arrays

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Experimental Section

Materials

AlexaFluor-680 was purchased by Invitrogen. Poly(ethylene glycol) silane-NH$_2$ (PEG-Silane-NH$_2$) was obtained from Nanocs. All the other chemicals were provided by Sigma-Aldrich. Every reactive was used as it was without further purification.

Synthesis of dye-modified polylsine

1.5 mg of poly(L-lysine) hydrobromide 15-30 kDa were dissolved in 480 \textmu l of PBS and 200 \textmu g of AlexaFluor-680 (10 mg/mL DMSO solution) were added to the solution. The solution was kept under stirring overnight at RT, and it was used without further purification.

Synthesis of gold nanoparticles

Gold seeds with a diameter of approximately 3 nm were prepared according to the following procedure. To 20 ml of milliQ water were added 10 \textmu l of poly(sodium 4-styrene sulfonate) (30% aqueous solution) and 200 \textmu l of HAuCl$_4$ aqueous solution (10 mg/ml). During vigorously stirring, 200 \textmu l of sodium borohydride (4 mg/ml in milliQ water) was added quickly, and the mixture was stirred vigorously for other 2 minutes. After the addition of NaBH$_4$, the solution changed a number of color until becoming brilliant orange. Before its use the solution was generally aged for at least 30 minutes and employed without further purification.

Synthesis of gold nanoparticles arrays

1 ml of gold nanoparticles solution was added to a 2 ml plastic vial followed by 40 \textmu l of poly(L-lysine) hydrobromide (1-5 kDa or 4-15 kDa or 15-30 kDa or 70-150 kDa, 5 mg/ml in milliQ water) or dye-modified poly(L-lysine) hydrobromide (5 mg/ml in PBS) and the solution was allowed to stir for 30 minutes at room temperature. The as synthesized gold aggregates were collected by centrifugation (13400 rpm for 3'), suspended in 100 \textmu l of milliQ water and sonicated for maximum 4 minutes.

Synthesis of AuSi nanoparticles

In a 100 ml round bottomed flask were added 70 ml of absolute ethanol followed by 2.4 ml of ammonium hydroxide solution (30% in water), and 40 \textmu l of tetraethyl orthosilicate (TEOS, 98%). The solution was allowed to stir for 20 minutes at RT. 2 ml of the gold nanoparticles arrays previously prepared were added to the reaction flask and the solution was allowed to stir for further 3 h. The as-synthesized AuSi nanoparticles were collected by 30 minutes centrifugation at 4000 rpm, washed twice with ethanol to remove unreacted precursors and suspended in 1 ml of ethanol. A short spin centrifugation was employed in order to separate the structure over 150 nm from the supernatant, that was recovered such as a pink-iridescent solution. This step can lead to the loss of up to 20% in gold. The colloid was centrifuged at 13400
rpm for 5 minutes, suspended in 500 μl milliQ water, sonicated for 5 minutes and freeze-dried overnight. The pink powder remains stable for at least 1 year if stored in the dark at 10 °C.

**Synthesis of calcinated AuSi nanospheres**

The pink powder of AuSi was calcinated following the sequence: 200 °C for 2h, 400 °C for 1h and 600 °C for 2h. The resulting powder shows a violet color depending on the formation of a single gold nanostructure inside the hollow silica shell.

**AuSi surface functionalization with poly(ethylene glycol) Silane-NH₂ (PEG-Silane-NH₂)**

500 μl of an ethanol solution of AuSi was added to a 10 ml round bottomed flask followed by 2 ml of ethanol and 300 μl of PEG-Silane-NH₂ solution (2 mg/ml in DMF), and the solution was stirred vigorously for 2 hours at room temperature. Subsequently, the temperature was increased up to 50 °C, and the reaction mixture was kept at this temperature for a further 1 hour. In the first step hydrogen bonds were formed between the hydroxyl groups of the silica nanoparticles and the silane moieties of PEG. Covalent binding was then induced by increasing the temperature. After cooling at room temperature, the PEG-functionalized silica nanoparticles were purified by repeated cycles of centrifugation and dispersion in ethanol.

**Electron microscopy observations**

TEM observations were carried out with a ZEISS Libra 120 TEM operating at an accelerating voltage of 120 kV. The samples were deposited on 300-mesh copper TEM grids coated with a carbon film. The depositions were performed by immersion of the grid into the colloidal solution. Imaging was carried out after the evaporation of the solvent. All the reported histograms were made on diameter measurements of at least 100 nanoparticles observed by TEM.

SEM imaging was performed with a ZEISS Merlin SEM operating at an accelerating voltage of 5 or 10 kV. A droplet of the colloid was put on silicon an imaged after few hours.

**Silica shell dissolution test**

Dissolution experiments were conducted as follows: briefly, 100 μl of AuSi nanostructures were precipitated by 5 minutes centrifugation at 13400 rpm and suspended in 800 μl of PBS (1X) buffer and 200 μl of human serum. The resulting solution was kept under stirring at 550 rpm at 37°C. After certain time (0, 1, 6, 12, 24 and 48h) a droplet of the solution was put on silicon and imaged at SEM after oxygen plasma treatment.

**SERS measurements**

Dye-tagged AuSi were obtained following the usual synthesis protocol but using AlexaFluor-680 modified poly(L-lysine). SERS spectra of the so obtained AuSi3A were collected exciting with a 561 CW Oxyus PS-003 laser operating at 561 nm with 150 mW output power; the non-elastic scattered light was collected through a Nikon objective coupled with a bundle of optical fibers to a Horiba iHR550 spectrometer equipped with 1200 g/mm gratings and with a nitrogen-cooled CCD for multichannel detection. The resulting SERS spectrum was compared to the Alexa680 Fluor Raman spectrum collected using a inVia Renishaw Raman Microscope exciting at 532 nm.

**UV/Vis spectrophotometry**
Extinction spectra were collected by means of a double beam spectrophotometer Jasco V-550 UV/VIS equipped with quartz cuvettes of 1.5 mm path length and normalized to the maximum absorbance. Depending on the measurement, MilliQ water, PBS (1X) buffer or ethanol were employed as solvents.
Figure S1

Figure S1: TEM images of AuSi at 0 (left) and 30 (right) degrees. Scalebar 50 nm.
Figure S2: SEM images of AuSi samples. Scalebars 200 nm.
Figure S3: TEM images of gold arrays. Scalebar 100 nm.
Figure S4: SEM images of AuSi (A) and calcinated AuSi (B, C). Scalebars 100 nm.
Figure S5: TEM images of AuSi (A) and calcinated AuSi (B, C, D). Scalebars 100 nm (A, C), and 50 nm (B, D).
Figure S6: compared Raman shifts between AlexaFluor680 (red) and AuSi3A (black).
Figure S7: zeta potential measurements in PBS before (upper panel) and after (lower panel) the coating of AuSi with silane-PEG-NH$_2$.