Supporting Information

Significant Metal Enhanced Fluorescence of Ag_2S Quantum Dots in the Second Near-Infrared Window

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Methods

Materials:

Polystyrene microspheres with diameters of 500 nm and 620 nm (10 wt.%) were purchased from Bangs Laboratories Inc., USA. Silver diethyldithiocarbamate (Ag(DDTC), ≥99%), 1-Dodecanethiol (DT, ≥98%), Dihydrolipoic acid (DHLA, ≥98%), cyclohexane (≥99%), Streptavidin (from *Streptomyces avidinii*, affinity purified), 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS), phosphate buffered saline (PBS, pH 7.4) and biotinylated bovine serum albumin (bBSA) were purchased from Sigma-Aldrich, UK. Glass microscope slides were obtained from VWR International, USA. Nanopure water (>18.2 MΩ), purified using the Millipore Mili-Q gradient system, was used in all the experiments.

Ag_2S QD Synthesis:

Silver sulfide (Ag_2S) quantum dots (QD) of two different sizes (2.7 and 4.1 nm) were synthesized *via* thermal-decomposition of Ag(DDTC) as described in [1]. Briefly, 0.1 mmol of Ag(DDTC) was added to 10 g of DT in a 100 mL three-necked flask at room temperature. Oxygen was removed from the slurry with vigorous magnetic stirring under vacuum for 5 min. The reaction was flask was refilled with Ar and the reaction temperature was raised to 130 °C or 210 °C, at a rate of 10 °C/min, and retained at this temperature for 1 min or 60 min to allow the growth of 2.7 and 4.1 nm Ag_2S QDs, respectively. The solution was cooled to room temperature under ambient atmosphere and subsequently 50 mL of ethanol were poured into the solution. Ag_2S QDs were collected by centrifugation at 16000g for 30 minutes.

In order to render Ag_2S QDs hydrophilic with a carboxylic acid group capping, ligand exchange of DT with DHLA was performed.[2] A mixture of as-prepared Ag_2S QDs (10 mg), cyclohexane (15 mL), ethanol (15 mL), and DHLA (100 g) was stirred at room temperature for 48 h. The hydrophilic Ag_2S QDs were collected by centrifugation at 16000g for 1 h, washed with deionized water twice, redispersed in deionized water and stored at 4 ^{o}C in the dark.

Ag_2S QDs were physicochemically characterized by transmission electron microscopy (TEM). TEM samples were prepared by drop casting Ag_2S QD aliquots on 300 mesh holey carbon film TEM grids (TAAB, UK). The grids were blot-dried with filter paper, dried under vacuum and imaged immediately. Bright field transmission electron microscopy (BFTEM) and high resolution transmission electron microscopy (HRTEM), combined with energy-dispersive X-ray spectroscopy (EDX, Oxford Instruments, UK), were carried out using a JEOL JEM-2100F, under an accelerating voltage of 200 kV. The size distribution of

the Ag_2S QDs was measured using several TEM images and ImageJ software (http://rsb.info.nih.gov/ij/).

The optical properties of the Ag_2S QDs and the Au nanostructured arrays were measured at room temperature using a Cary 5000 UV-Vis-NIR spectrophotometer. Their fluorescence emission spectra were collected using an NS 1 NanoSpectralyzer® (Applied NanoFluorescence, USA), with a 782 nm excitation laser and a 512 element TE-cooled InGaAs array near-IR detector.

Streptavidin conjugation with Ag_2S QDs:

Conjugation of streptavidin with Ag_2S QDs was performed *via* EDC using NHS chemistry. Briefly, Ag2S QDs in MES buffer (50 nM, 1 mL, pH 6.0) were added to EDC (0.4 mg) and NHS (0.6 mg). The reaction mixture was vortexed at room-temperature for 20 min, the QDs were precipitated by centrifugation, and the supernatant was discarded. The QDs were redispersed in PBS (pH 7.2) and streptavidin dissolved in PBS was added to the QDs. The solution was vortexed for 2 h at room temperature. The streptavidin-conjugated Ag_2S QDs were collected by centrifugation, washed with Milli-Q water twice and redispersed in Milli-Q water.

Preparation of Au nanostructured arrays:

Polystyrene monolayer templates were prepared using a modified colloidal lithography method, as described in [3]. Briefly, monodisperse polystyrene microspheres with diameters of 500 nm or 620 nm (referred to as PS500 and PS620, respectively) were diluted with ethanol at a 1:1 ratio. Glass substrates (10 mm × 10 mm) were cleaned by immersion in

piranha solution (3:1 concentrated H_2SO_4:30% H_2O_2) at 80 °C for 1 h. Once cool, the substrates were repeatedly rinsed with deionized (DI) water and sonicated for 60 min in a 5:1:1 H_2O:NH_4OH:30%H_2O_2 solution. Following sonication, they were again rinsed thoroughly with DI water and used immediately. Approximately 3 to 5 μL of the prepared PS solutions were applied onto the surface of a clean silicon wafer (~ 30 mm × 20 mm), which had been previously kept in a 10% sodium dodecyl sulfate solution for 24 h. The wafer was then slowly immersed in a 15 cm glass vessel filled with 150 mL of Milli-Q water, causing the PS particles to form a disordered monolayer on the water surface. To consolidate the particles, the water surface tension was changed by adding 4 μL of a 2% sodium dodecyl sulfate solution, allowing a large monolayer with highly ordered areas to be obtained. Such monolayers were then lifted off from the water surface using the prepared glass substrates.

Following formation of the two-dimensional (2D) colloidal crystal templates, the substrates, without (0 s; referred to as Ox0) or with (5 or 15 s; referred to as Ox5 or Ox15) O_2 plasma etching, were mounted into the chamber of a Mantis e-beam evaporation system, equipped with a deposition monitor quartz crystal microbalance, for Au deposition with a fixed thickness of 100 nm or 50 nm. The nanosphere mask was removed by sonicating the entire substrate in either CH_2Cl_2 or absolute ethanol for 2 min, following which an array of triangularly shaped particles remained on the substrate.

The Au nanostructures after template removal were imaged by scanning electron microscopy (SEM) using a LEO Gemini 1525 field emission gun (FEG) SEM (Carl Zeiss Microscopy GmbH, UK). The SEM was operated in secondary electron mode at an accelerating voltage of 5 kV, using the InLens detector.

Immobilization of Ag_2S QD–protein conjugation monolayers:

Both the Au nanostructured arrays and clean glass substrates were covered by Ag_2S QD monolayers *via* biotin-streptavidin interaction, as previously demonstrated in [3]. First, a biotinylated-BSA (bBSA) solution of 100 mg/mL in PBS (pH 7.2) was added to the substrate surface, incubated for 1 h, and rinsed with PBS to remove unbound proteins. This step allowed the formation of a monolayer of bBSA on both Au array and glass surfaces. Clean glass substrates incubated with bBSA only, were used to establish the fluorescence background used as reference. Binding of the streptavidin–Ag_2S QD conjugations was carried out by adding 25 μg/mL onto the substrate surfaces and incubating for 2 h. The substrates were washed with PBS to remove any unbound Ag_2S QDs. The formed streptavidin– Ag_2S QD monolayers on both glass and nanostructured arrays, makes it possible to quantitatively compare the fluorescence intensity of Ag_2S QD –protein conjugates in the absence and presence of Au nanostructures on glass (after background signal subtraction and correction for differences in surface coverage). The averaged fluorescence enhancement factor from Au samples was calculated as described in [3].

Sample	PS620-Ox0	PS620-Ox5	PS620-Ox15
a (nm)	133 ± 16	182 ± 20	218 ± 18
s (nm)	166 ± 30	96 ± 21	23 ± 20
λ_{max} (nm)	564, 1195, 1594	572, 1102, 1532	550

Table S1. Structural parameters of nanoparticles in Au nanostructured arrays fabricated with different oxygen plasma etching times (0, 5 or 15 s). *a* is the in-plane width (tip to tip dimensions), *s* is the interparticle distance and λ_{max} is the surface plasmon resonance peak position.

References

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