Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Large Enhancement of Single Molecule Fluorescence Coupling to Hollow Silver Nanoshells

Yi Fu, Jian Zhang, and Joseph R. Lakowicz

S1. Preparation of hollow Silver Nanoshells

Amine-functionalized silica nanoparticles with a low polydispersity were obtained according to the slightly modified Stöber procedure described in the literature in a multistep reaction[1,2]. All reagents were purchased and used as received from Sigma. Briefly, ammonium hydroxide (14 M) was added to 100 ml absolute ethanol in a 250 ml round bottomed flask and stirred at room temperature for 30 min. An aliquot of tetraethylorthosilicate (TEOS) (10 mM) was quickly added to the mixture, which was stirred overnight. After centrifuge, the obtained silica particles were dispersed in ethanol and further functionalized with aminopropyltrimethoxysilane (APTMS). Excess APTMS of 0.5 ml was added to the silica particle suspension; the solution was vigorously stirred for 8 h and refluxed for 1 h to promote covalent boning of APTMS moieties to the surface of the silica particles.

The solution of small gold seed was prepared by reduction of hydrogen tetrachloroaurate (III) hydrate with tetrakis(hydroxymethyl) phosphonium chloride (THPC) as described by Duff et al[3]. The amine-functionalized silica spheres in ethanol (5 ml) as prepared were added dropwise to the aqueous gold nanocluster solution (5ml) within 5 min under stirring and the mixture was stirred for another 12 h. The small gold nanoclusters were removed by centrifuging at 2000 rpm, the remaining light brown pellet was redispersed in water. The washing procedure was repeated twice to remove loosely bound nanoclusters. The coating of silver nanoshells was prepared by using the similar procedures as described elsewhere. [1,4] 8ml (0.25 mM) $AgNO_3$ solution was mixed with the THPC gold seeded silica nanoparticles (2ml), after stirring the mixture for 10 min, 0.02 ml of formaldehyde was added followed by addition of 0.05 ml of ammonium hydroxide to reduce the silver. The procedure was repeated twice to obtain smooth silver shells. The obtained nanoshells were centrifuged and re-dispersed to remove any

unreacted chemicals and small seed particles. The hollow nanoshells was prepared by etching out the silica core[5]: 2ml Hydrofluoric acid (4 vol %) was added to a solution of silver shells (4 ml) and left standing for overnight. The sedimentation was diluted to 1 ml water for further experiments, after centrifuge and washing with copious of water.

S2. DNA sample immobilization

Complementary single stranded DNA oligomers used in the experiment:

HS-5'-TCC ACA CAC CAC TGG CCA TCT TC-3'

3'-AGG TGT GTG GTG ACC GGT AGA AG-5'-Cy5

All oliogonucleotides were obtained from the Biopolymer Core Facility at the University Of Maryland School Of Medicine. Nanopure water purified using Millipore Milli-Q gradient system, was used for all experiments. All other compounds were purchased from Sigma-Aldrich and used as received.. The coverslips used in the experiments were first soaked in a 10:1 (v:v) mixture of concentrated H_2SO_4 and 30% H_2O_2 overnight, extensively rinsed with water, sonicated in absolute ethanol for 2 min and dried with air stream. The purity was checked by fluorescence measurements at single molecule levels.

The glass slides were first coated with amino groups by dipping the slides in 5% aqueous solution of 3-aminopropyltriethoxysilane (APS) for 30 minutes at room temperature. The slides were thoroughly rinsed with water and air-dried prior to monolayer silver nanoshell formation. The deposition of silver nanoshells on glass coverslips was carried out by incubating the slides into 5ml solution containing 200 µl hollow silver nanoshells as prepared for 4 hours. Only one side of each slide was deposited with silver nanoshells. The slides were rinsed thoroughly with purified water to remove loosely bound nanoparticles. The free amino groups remaining on the quartz surface were blocked with succinic anhydride by dipping in a freshly prepared solution of 0.111g succinic anhydride in 7 ml of 1-methyl-2-pyrrolidone and 0.77 ml 0.2 M sodium borate buffer, pH 8. After 15-min-incubation at room temperature slides were washed in three change of water.

Immobilization of DNA samples on silver nanoshells was accomplished by placing each slide in 3 ml of a solution of thio-derivatized single-stranded DNA (5 nM) for 48 h at 5 °C. Hybridization of DNA samples was carried out by immersing in a buffer solution containing complementary Cy5 tagged single stranded oligonucleotides (1 nM), 5 mM Hepes (pH 7.5), 0.1 M KCl, and 0.25 mM EDTA. The sample

was cooling very slowly after incubation at 70 °C for 2 minutes. A final washing step largely removed the unbound probe DNA from the substrate.

S3. Single-molecule experiments

All single molecule studies were performed with a time-resolved confocal microscopy (MicroTime 200, PicoQuant). A single mode pulsed laser diode (635 nm, 100ps, 40 MHz) (PDL800, PicoQuant) was used as excitation light. The collimated laser beam was spectrally filtered by an excitation filter (D637/10, Chroma) before directing into an inverted microscope (Olympus, IX 71). An oil immersion objective (Olympus, 100×, 1.3NA) was used both for focusing laser light onto sample and collecting fluorescence emission from the sample. The fluorescence that passed a dichroic mirror (Q655LP, Chroma) was focused onto a 75 µm pinhole for spatial filtering to reject out-of-focus signals and then reached the single photon avalanche diode (SPAD). Images were recorded by raster scanning (in a bidirectional fashion) the sample over the focused spot of the incident laser with a pixel integration of 0.6 ms. The excitation power into the microscope was maintained at around 40 nW. Time-dependent fluorescence data were collected with a dwell time of 50 ms. The fluorescence lifetime of single molecules was measured by time-correlated single photon counting with time-tagged-time-resolved (TTTR) mode (TimeHarp 200, PicoQuant). The data was stored in a time-tagged-time-resolved (TTTR) mode, which allows recording every detected photon with its individual timing information. Instrument Response Function (IRF) widths of about 300 ps FWHM can be obtained in combination with a pulsed diode laser, which permits the recording of sub-nanosecond fluorescence lifetimes extendable to less than 100ps with reconvolution. Lifetimes were estimated by fitting to a χ^2 value of less than 1.2 and with a residuals trace that was fully symmetrical about the zero axis. All measurements were performed in a dark compartment at room temperature.

S4. Additional single-molecule images

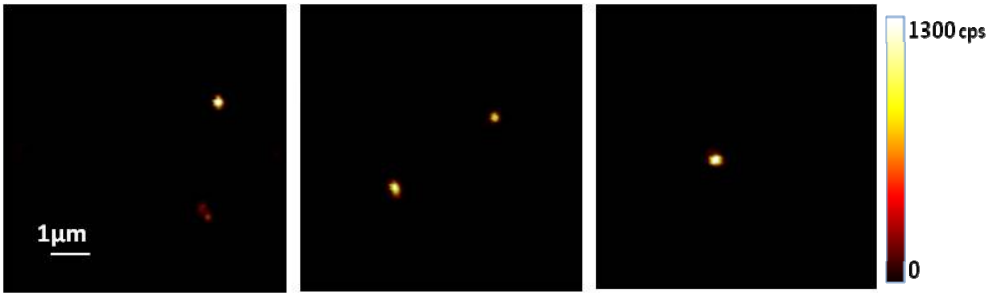

Figure S1. Additional confocal images (10x10 µm) recorded in the experiments. Images were recorded by raster scanning (in a bidirectional fashion) the sample over the focused spot of the incident laser with a pixel integration of 0.6 ms. Many images were recorded to obtain desired number of single molecules for further time trajectories and lifetime analysis.

S4. TEM of nanoshells

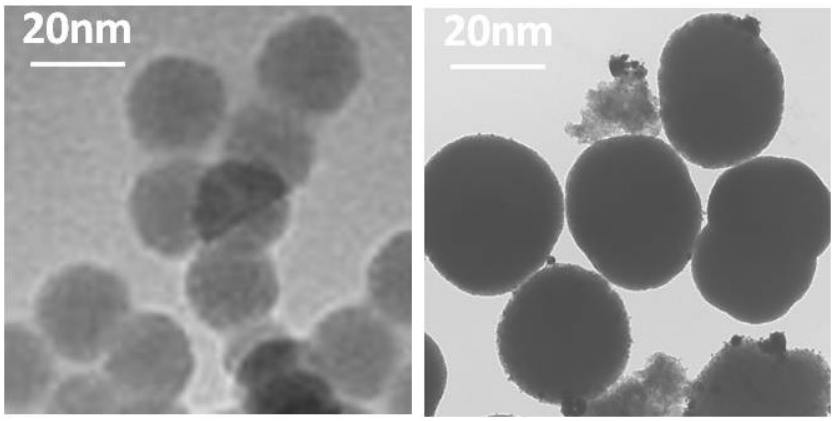

Figure S2. TEM images of silica particles before (left) and after (right) silver deposition process. The measured silica particles (d: 36 ± 5 nm) are with less than 15% polydispersity, and the averaged size of silver coated silica particles is 48 ± 6 nm, the approximate thickness of silver shells is estimated by subtraction the size of silica particles from that of the silver-coated particles, which is about 10 ± 2 nm.

S5. SEM image of Nanoshell assembly

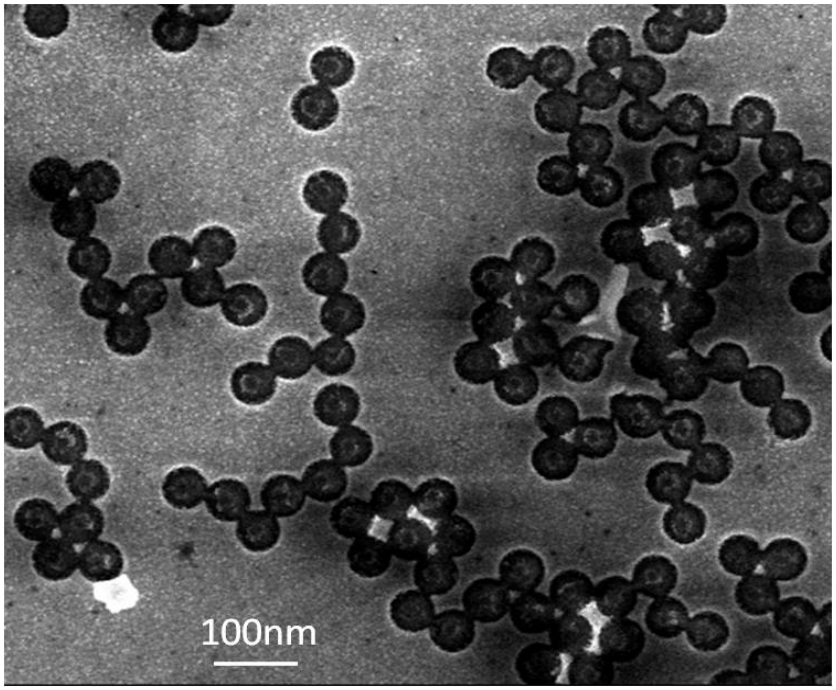

Figure S3. A SEM image of hollow silver nanoshells deposited on a cover glass, indicating the formation of nanoshell monolayer assembly deposited on a glass coverslip.

S6. Extinction spectra of nanoshells

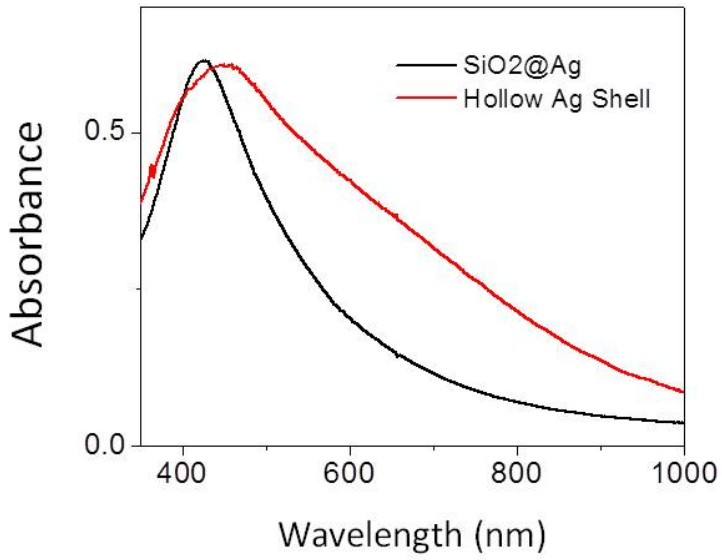

Figure S4. Extinction spectra of silver coated silica and the hollow Ag nanoshells. The plasmon resonance of hollow silver nanoshells occurs at longer wavelengths than the corresponding solid nanoshells, and shifts the resonance across more of the NIR spectra range.

References

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