Supplementary material (ESI) for Journal of Materials Chemistry

Peptide-Mediated Surface-Immobilized Quantum Dot Hybrid Nanoassemblies with Controlled Photoluminescence**

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Experimental

Materials. All solvents and reagents were purchased from Aldrich and used as received unless otherwise stated. Deionized water for substrate cleaning and sample rinsing was produced with a NANOpure DIamond^{TM} purification unit (Barnstead International, Dubuque, IA) and had a resistivity of ~15 MΩ cm^{-1}. Absolute (200 proof) ethanol (Aaper Alcohol and Chemical Company, Shelbyville, KY) was used for making the thiol solutions. (10-mercaptomethyl-9-anthryl)(4-aldehydephenyl)acetylene (MMAPA) and 11-mercaptoundecyl-tri(ethylene glycol)-alcohol (OEG) were synthesized in our laboratories. Streptavidin functionalized CdSe-ZnS core-shell quantum dots (SA-QDs) were acquired from Invitrogen Corp. (Carlsbad, CA); SA-QDs have an absorption maximum at 595 nm and an emission maximum at 605 nm. Biotinylated three-repeat gold-binding peptides (bio-3RGBP1) were synthesized and purified by United Biochemical Research, Inc. (Seattle, WA).

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Substrate preparation. Polycrystalline gold substrates were prepared by electron-beam evaporation of gold (23 nm thick; 99.999%, Kurt J. Lesker Company) onto Si(100) wafers (Silicon Sense, Nashua, NH; 100 mm in diameter, ~500 μm thick) that had been primed with a layer of titanium (2 nm thick; 99.995%, Kurt J. Lesker Company) to promote adhesion between silicon and gold. Electron-beam evaporation (SEC 6000, CHA Industries) of gold was performed under high vacuum with a pressure of ~1 x 10^{-6} Torr at a rate of 0.1 nm/s. Gold substrates are reproducible with regard to their physical and chemical characteristics, and were characterized to have a root-mean-square (RMS) roughness of 0.6 ± 0.2 nm and an average grain diameter of 20-40 nm. Gold substrates were fractured into slides (~ 1 cm x 1 cm) for μCP of OEG molecules.

Microcontact Printing (μCP). Micropatterned photoresist films, fabricated by photolithography, were used as masters to replicate stamps for μCP. Masters were coated with a self-assembled monolayer of (1, 1, 2, 2-tetrahydroperfluorodecyl)trichlorosilane (Sigma-Aldrich) to ensure a clean release of the cured stamp. Two masters were used: one consisted of a square array of 2 μm circles with interspacing of 2 μm while the other consisted of an array of alternating 10 μm lines. Stamps were made by casting a 10:1 (v/v) mixture of polydimethyl siloxane (PDMS) and curing agent (Sylgard 184, Dow Corning, Midland, MI) against a silanized master for 2 days at room temperature in ambient conditions. Before being used, freshly prepared stamps were washed successively with ethanol, heptane, and ethanol, followed by drying under nitrogen. Stamps were used as cast and the surface chemistry of the stamp was not modified. Inking was done by covering the patterned side of the stamp with OEG solution for 1 min and dried under nitrogen. The inked stamp was brought into a conformal contact with the gold substrate by hand for ~20 sec. The patterned gold substrates were rinsed copiously in ethanol, dried in nitrogen, and were used immediately for the subsequent step in the experiment.

Self-assembly of MMAPA. After immersing the patterned gold substrate into a degassed solution of MMAPA (0.5 mM ethanolic solution), ammonium hydroxide (1.0 μL (28.0-30.0% NH_3) per mL of MMAPA solution) was added to hydrolyze the acetyl protecting group for the chemisorption of MMAPA to take place onto the regions unmodified by the printed OEG SAM. After backfilling MMAPA under nitrogen, the sample was taken out, rinsed thoroughly in ethanol, and incubated immediately in a bio-3RGBP1 solution for the covalent attachment of peptides.

AFM characterizations. The AFM characterizations were carried out in air under ambient conditions (*ca.* 40%-50% relative humidity, 25^{O}C temperature) on a Digital Instruments Nanoscope IIIa Multimode AFM (Veeco Inc., Santa Barbara, CA) using tapping mode. Unlike contact mode, tapping mode minimizes shear force and is more suitable for imaging peptide-QD hybrid nanostructures. During data acquisition, the feedback loop was constantly adjusted to ensure a minimum tip-sample interaction force – just enough to obtain a stable image – was applied (usually in the range of 0.2 – 0.4 nN) to avoid damaging the QD arrays. The samples were scanned at a rate of 0.5-1.0 Hz using silicon probes (Veeco Inc., Santa Barbara, CA) having spring constant of ~42 N/m and resonant frequency of ~320 kHz. These tips are conical in shape with a cone angle of 20^{O} and an effective radius of curvature of ~10 nm. The sharp features of these tips were necessary to reduce tip convolution and to decrease the effect of capillary forces with the surface. The images included 512 x 512 data points. Measurements and analysis were performed using the algorithms contained in the software. For reliable AFM characterizations, three random areas in each sample were analyzed under the same conditions (scan size, scan rate, scan angle, and gain controls) using the same tip. For complete experiments, the same area was recorded at the beginning and at the end to verify the consistency in the resolution.

The heights of peptide linkers and MMAPA-grafted linkers were experimentally determined relative to the height of OEG self-assembled monolayers. This is accomplished by backfilling of

linkers into the 2-μm hole arrays generated by μCP of OEG molecules, followed by cross-sectional profile analysis to measure the relative difference in heights between patterned OEG regions and linker regions (Figure SI-1). The height of OEG self-assembled monolayer was measured to be 2.90 ± 0.30 nm (Figure SI-1a). This is in good agreement with the literature values. After immobilizing a monolayer of bio-3RGBP1, the difference in height between OEG regions and peptide regions was measured to be 1.75 ± 0.35 nm (Figure SI-1b). Therefore, it was inferred that the height of peptide linker was ~ 1.15 nm. Similarly, based on the height difference of 0.45 ± 0.35 nm relative to OEG regions (Figure SI-1c), the height of MMAPA-grafted peptide was inferred to be ~ 2.45 nm.

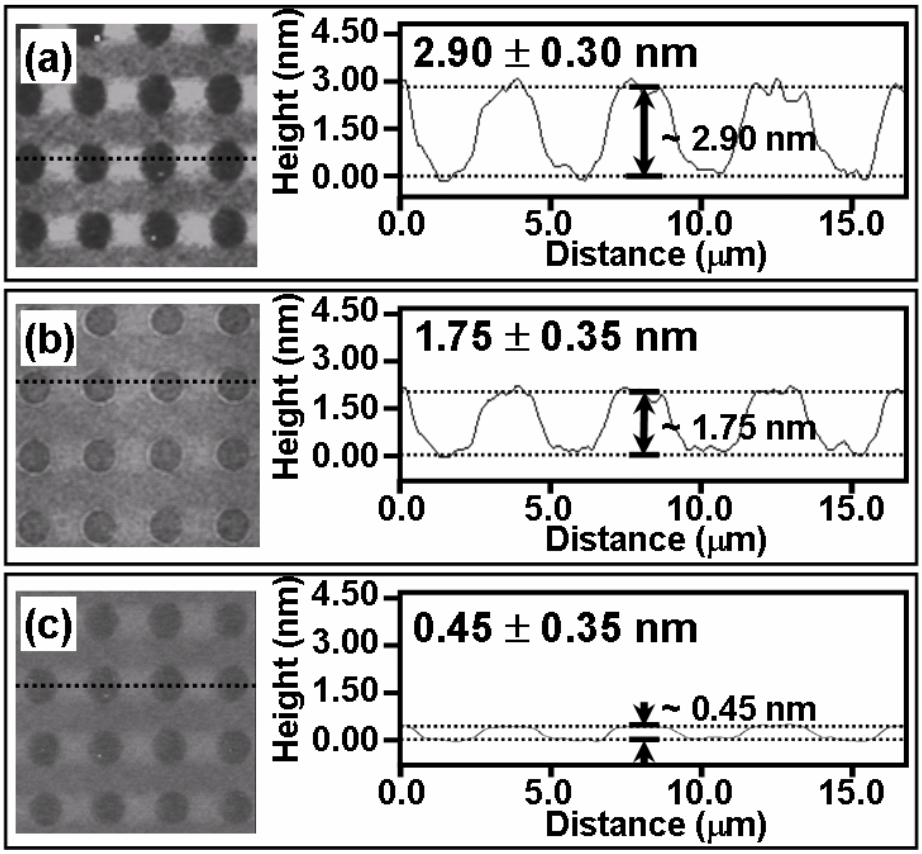

Figure SI-1. Experimental determination of the heights of linkers. Linkers were backfilled into the 2-μm hole arrays generated by μCP of OEG molecules and cross-sectional profile analysis was performed to measure the relative difference in heights between patterned OEG regions and linker regions.

Figure SI-2 shows the schematic representation of QD attachment at different distances from a metal surface based on the linker height as determined by AFM cross-sectional profile analysis. QDs used in this work are CdSe nanocrystals encapsulated by following additional layers: (i) ~ 1 nm ZnS shell, (ii) ~ 1.5 nm polymer coating and (iii) a layer of streptavidin molecules that are ~ 5 nm in width. Therefore, the emissive CdSe core is ~ 7.5 nm away from the surface of the entire QD structure. As such, peptide linkers and MMAPA-grafted peptide linkers place the QDs at ~ 8.65 nm (Figure SI-2b) and ~ 9.95 nm (Figure SI-2a) away from a metal surface respectively.

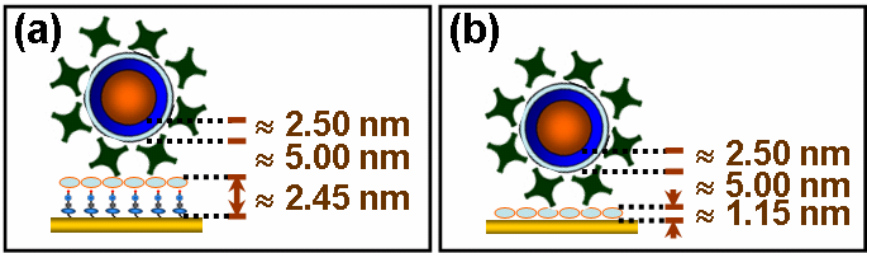

Figure SI-2. Schematic representation of QD attachment at different distances from a metal surface based on the linker height as determined by AFM cross-sectional profile analysis.

Fluorescence microscopy. Fluorescence images were taken in 16-bit capture mode using an inverted Nikon microscope (Eclipse TE2000-U) equipped with tungsten halogen lamp as an excitation source. The filter set included a 460 nm short pass exciter, a 475 nm dichroic, and a 605/20 nm band pass emitter.

Photoluminescence spectroscopy. Absorbance and emission of SA-QDs in solution were obtained using a UV/Vis spectrometer (Beckman Coulter, DU 800). Emission of surface-immobilized QD arrays was obtained using a custom set-up shown in Figure SI-3, which enables the local excitation and measurement of patterned QD ensembles. This apparatus is fitted with a piezo stage for a precise X-Y movement of the sample. Spatially resolved spectral measurements, conducted within patterns of

QD ensembles, were collected on an inverted Nikon microscope (Eclipse TE2000-U, using a 60X objective with numerical aperture of 0.7) and passed through a dichroic beam splitter and a 532 nm notch filter before being dispersed through an Acton 2300i spectrometer and collected by a Princeton-Roper Spec10 CCD.

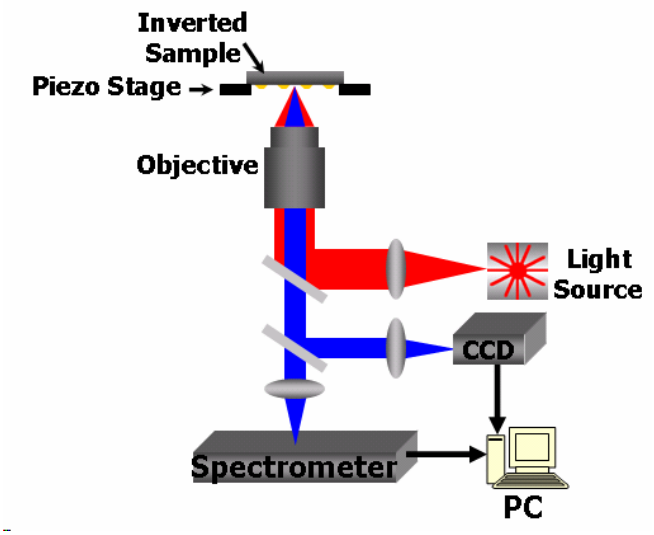

Figure SI-3. Custom set-up for spatially resolved spectral measurements of QD arrays.