Supplementary Material (ESI) for Chemical Communications

Supplementary information

Photoluminescence enhancement in CdSe/ZnS- DNA linked -Au nanoparticle dimers probed by single molecule spectroscopy

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1.Detailed protocol for nanoparticle functionalization

Gold nanoparticles (AuNP) with diameters of 60nm where either purchased commercially (Nanopartz Inc., Ted Pella Inc.) or synthesized via a seeded growth method (S1). The 60nm AuNP concentrations were calculated via UV-vis absorption methods using known extinction coefficients (3.1×10^{10} cm^{-1} M^{-1}). Linker functionalized single stranded oligonucleotides (ssDNA) were purchased from Integrated DNA Technologies Inc. with disulfide- ,biotin-, and amine-modifications, Table S1. Before *A*- and *B*-type ssDNA functionalization of the AuNP functionalization, the disulfide oligonucleotides were first reduced by dissolving the lyophilized samples (100~300 nmoles) in 0.3 ml of a 100 mM dithiothreitol (DTT) solution in purified water or buffer. The reduced DNA was loaded onto a freshly purified sephadex column (G-25, Amersham Bioscience) and eluted with 2.5 ml 10 mM phosphate buffer (pH = 7.4). The DNA was quantified using UV-Vis analysis with the specific DNA's extinction coefficient. Streptavidin-functionalized paramagnetic beads (1-4 mm) were purchased from Pierce Biotechnology Inc., and modified using biotin-modified ssDNA following manufacturers directions.

The AuNP were then functionalized with *A*- and *B*-type ssDNA following methods for high DNA coverage reported by Mirkin and co-workers.(*S2*) In a typical experiment, an excess of ssDNA ([ssDNA]/[AuNP] = 10,000) was added via an aliquot (1-50 ml) of a purified DNA 50-300 mM solution to a 1 ml solution of gold particles (~1-3 nM). The ssDNA and particle solutions were incubated at room temperature in a non-buffered solution for at least 3 hr before adding phosphate buffer to bring its concentration to 10mM (pH = 7.4). The solution was left to anneal at 25 ^{o}C for 4 hr before the addition of NaCl (0.025M). The salt concentration was then increased gradually from 0.025 to 0.3 M NaCl over 24 hr, and left to anneal for an additional 24 hr at 0.3M. The excess DNA next was removed from the solutions by centrifugation for 30 minutes at 4,500*g*. The final *AB*-AuNP were stored in PBS (10mM PB, 100 mM NaCl, pH=7.4).

The CdSe/ZnS quantum dots (QDs) with emission maxima of ~600nm where purchased from NN-Labs LLC. The QD organic octadecylamine (ODA) shells were first replaced with mercaptopropionic acid (MPA), to facilitate phase transfer. Briefly, the ODA-capped QD were precipitated from toluene twice using acetone to remove excess ODA. Next, an excess of 10,000 fold MPA was added to the QD in chloroform ([QD] = 1.5 mM), followed by heating at 60 ^{o}C for 2 hrs. Next, to the chloroform solution, an equal volume aliquot of ultrapure water (18.2 MΩ) added, and the pH was adjusted to 10 using NaOH. The mixture was then vortexed vigorously, causing a biphasic mixture and emulsion. Next, the solution was centrifuged at 500 rpm for 1-5 minutes, revealing QD phase transfer to the aqueous solution. The MPA-capped QDs were then purified via two acetone precipitation steps to remove excess MPA. The QD were dispersed in 10 mM Phosphate Buffer (PB, pH=8.2), and filtered via a 100 nm centrifugation filter, then used immediately for DNA-conjugation.

The QD ssDNA functionalization with *C*-type ssDNA was achieved using an EDC/NHS cross-linking strategy. The MPA-capped QD's where first diluted in MES saline buffer (pH = 6.2, 50 mM NaCl). Next, a 50x ratio of sulfo-NHS was added, and a 100-fold ratio of EDC was added slowly, and the solution was mixed rapidly for 2-min. Next, the buffer was exchanged rapidly using a 5 kDa molecular weight cut-off centrifuge filter (Millipore). This was followed by diluting the NHS activated QD in PBS buffer (10 mM Phosphate Buffer, 10 mM NaCl, pH = 8.2). Next, a 20x ratio of NH_2-ssDNA (*C*-type) was added, then stored in an ice bath for 2 hr. Due to the instability of the NHS complex, the buffer exchange and amine modification should take place in <30 min. The ssDNA-QD was then purified of excess ssDNA with two freshly purified sephadex columns. The final *C*-QD was concentrated using a 5 kDa MW-cutoff filter, stored in 10 mM PB buffer (pH = 8.2) at QD concentrations of ~500 nM, and quantified using UV-vis absorption methods using known extinction coefficients (S3).

Detailed protocol for heterodimer fabrication

In a typical heterodimer nano-cluster fabrication experiment, a 0.1M PBS solution (300-1000 ml, 10 mM phosphate buffer, pH = 7.4, 0.1M NaCl) consisting of ~ *AB*-AuNP is quantified using UV-vis. Next, ~75 ml of *A*'-type ssDNA functionalized magnetic colloid support, *A'-s* ([*A'-s*] ~ 0.2 mg/ml) is added, and the mixture is allowed to incubate for 3-6h with stirring (Step 1). During this process, the gradual decrease in solution color from ruby-red (AuNP) to optically transparent, was monitored using UV-vis, demonstrating assembly of *AB*-AuNP to the solid support via 15 bp ssDNA *A*- to *A'*-ssDNA hybridization. Upon separation via a magnetic field, the supernatant is removed containing any un-assembled *AB*-AuNP or impurities, and the sample is redispersed with fresh buffer. This "rinsing" or purification step is repeated at least three times. In the next step, step 2, the *B'C'-l* ssDNA linker is added

at a 3x molar ratio to the assembled *AB*-AuNP ([*B'C'-l*]/[*AB*-AuNP] = 3), via *B'* to *B*-ssDNA 15bp hybridization, and allowed to incubate under mixing for 6-12h, upon which the system is rinsed/purified as described above. Next, (step 3a) the *C*-QD is added at a 1:1 ratio with the assembled concentration of *AB*-AuNP (i.e., first layer), and assembled via *C*- to *C'* 18 bp hybridization for 6-12h at 4 ^{o}C. The second layer assembly was monitored via steady state PL measurements, which showed an uptake of *C*-QD to the *AB*-AuNP. The assembled heterodimers at the support were then separated and rinsed. The final assembled heterodimer product was redispersed in fresh buffer and a 1000x excess of *A''* fuel strand was added, which preferentially binds to *A'-s* under mixing, and incubated for 3-6 h at room temperature with mixing. This process was again followed by UV-vis which monitored the release of dimers via rise in AuNP absorption, and also steady state PL, which revealed an increase in emission intensity at 600nm. After disassembly, a 15b *C''*-ssDNA strand was added in a 1:1 ratio of assembled *B'C'-l*, which passivated any free C' ends in the system (Step 5a). The released heterodimers were separated from *A-s* via magnetic field, concentrated in a 5kDa molecular weight cut-off filter, and stored at 4 ^{0}C before sampling for measurements.

Table S1. The ssDNA used in this study.

ssDNA	*Sequence (5' to 3')*
A	**ATT GGA TTG GAA GTA** TTT TTT TTT TTT TTT-C_3H_6-SH
B	**TTC TCT ACA CTG TCT** TTT TTT TTT TTT TTT-C_3H_6-SH
B'C'-l	**AGA CAG TGT AGA GAA**-GAT AGG TCG GTT GCT GAT AGG TCG GTT GCT GAT AGG TCG GTT GCT-**AAT ATT GAT AAG GAT AGC** + 3'-CTA TCC AGC CAA CGA CTA TCC AGC CAA CGA CTA TCC AGC CAA CTA-5'
C	NH_2-C_6H_{12}-TTT TTT TTT TTT **GCT ATC CTT ATC AAT ATT**
A'	**CTT GTG TCT ACT TCC AAT CCA AT**T TTT TTT TTT TTT TT-Biotin
A''	**ATT GGA TTG GAA GTA GAC ACA AG**

2. Fluorescence correlation spectroscopy Fluorescence correlation spectroscopy (FCS) was performed using a home-built confocal fluorescence microscope based on an Olympus IX 81 microscope (1.2 NA 60x water immersion lens) and the 457nm laser line from a tunable Ar-ion CW

laser (Melles-Griot) 10μW average power at the sample). The photoluminescence emitted by freely diffusing Qdots and Qdot-AuNP dimers was collected by the same lens, spectrally filtered from excitation by a dichroic mirror (DRLP455, Omega Filters) and a bandpass filter (HQ605/40, Omega Filters) and imaged, via a 75 μm pinhole and a 50/50 beam splitter, onto two single photon counting avalanche photodiodes (MPD, Picoquant GmbH, Germany). FCS (intensity correlation) curves were acquired in cross-correlation mode using a real-time hardware correlator (PicoHarp300, Picoquant GmbH, Germany) and analyzed using IgorPro 6 (Wavemetrics, Inc). FCS curves were recorded for 1 minute. Diffusion coefficients and hydrodynamic size (radius) were estimated by fitting FCS curves with a simple model accounting for 3D diffusion and by the Stokes-Einstein equation. Calibration of the FCS probe volume was performed using rhodamine 110 in water.

3. Single molecule spectroscopy (SMS). Single molecule photoluminescence experiments were performed on a home built instrument based on an Olympus IX81 microscope (1.4 NA, 100x oil immersion lens) equipped with a closed-loop piezo-scanner (Physics Instrumente, Germany) by using either the 457nm light from an Ar-ion laser (Melles-Griot, 1 μW average power at the sample) or the 543nm light from a He Ne laser (Melles-Griot, 1,4 μW average power). Fluorescence was collected by the same lens, spectrally filtered from excitation by a dichroic mirror (DRLP455, Omega Filters) and a bandpass filter(HQ605/40, Omega Filters) and imaged, via a 75 μm pinhole onto a single photon counting avalanche photodiodes (MPD, Picoquant GmbH, Germany). Fluorescence images of individual Qdots and Qdot-GNP heterodimers as well time traces of photoluminescence intensity were collected with a time-correlated single photon counting analyzer (PicoHarp300, Picoquant GmbH) using the Symphotime software (Picoquant GmbH, Germany). The same software was used for intensity data rebinning (1 ms for analysis of the on and off events, 10 ms for trace representation).

References:

S1: Park, Y.-K.; Park, S. *Chem. Mater.* **2008**, *20*, 2388.

S2: (a) Lytton-Jean, A. K. R.; Mirkin, C. A. *J. Am. Chem. Soc.* **2005**, *127*, 12754. (b) Hurst, S. J.; Lytton-Jean, A. K. R.; Mirkin, C. A. *Anal. Chem.* **2006**, *78*, 8313.

S3: Yu, W.W.; Qu, L.; Guo, W.; Peng, W. *Chem. Mater.* **2003**, *15*, 2854-2860.