Supporting Information

Inhibition Assay of Biomolecules based on Fluorescence Resonance Energy Transfer (FRET) between Quantum Dots and Gold Nanoparticles

Experimental

Preparation and functionalization of AuNPs

AuNPs were first synthesized in the presence of *n*-alkanethiols by using two-phase (water-toluene) reduction of $HAuCl_4$ as described elsewhere[4, 8] and then capped with the first generation polyamidoamine (G1 PAMAM) dendrimers to functionalize the AuNPs with biotin. Briefly, 0.1 g of a $HAuCl_4 \cdot 3H_2O$ solution (1% (w/w), Aldrich) was dissolved in 10 mL distilled water and added to 10 mL toluene containing 2% tetraoctylammonium bromide. The solution was completely mixed by stirring, and the transfer of gold to the organic phase was confirmed by color changes of the two phases, from yellow in water to red in toluene. A mixture of 7 mg 11-MUA (11-mercaptoundecanoic acid) and 15 mg 1-OT (1-octanethiol) dissolved in 3 mL toluene (11-MUA : 1-OT = 1 : 3 reaction ratio) was added to the separated toluene layer containing the transferred gold, and stirred again. For the reduction and formation of gold colloids, 10 mL of distilled water containing 0.09 g $NaBH_4$ was added slowly, and stirred for 4 hr. The toluene layer containing *n*-alkanethiols-stabilized AuNPs (*n*-alkanethiol-AuNPs) was saved and stored at – 10 ^oC. The resulting precipitate of AuNPs was separated and resuspended in ethanol.

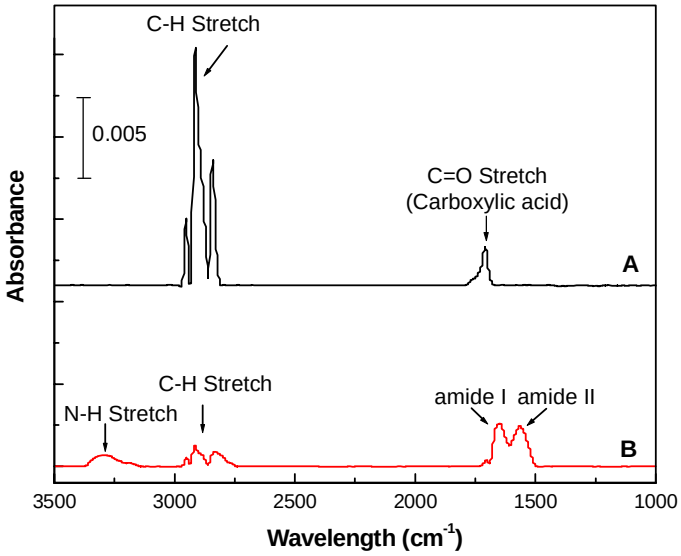
For functionalization of the *n*-alkanethiol -AuNPs with amine groups, we used the G1 PAMAM dendrimer having 8 primary surface amine groups. G1 PAMAM dendrimers (14 mM) dissolved in methanol were added to 9 μM of *n*-alkanethiol - AuNPs and incubated at room temperature overnight. To prevent aggregation of AuNPs during capping process, excess amount of G1 PAMAM dendrimers were added to the gold colloids. The reaction mixture was filtered through a microfilter with the molecular weight cut-off of 30 kD (Microcon YM30, Millipore Corp.) in order to remove the free dendrimers, and the resulting amine-functionalized AuNPs (dendrimer-AuNPs) were resuspended in water. For covalent modification with biotin, sulfo-NHS-biotin (Biotinamidohexanoic acid 3-sulfo-N-hydroxysuccinimide ester, Sigma) was added to the dendrimer-AuNPs colloids and incubated at 37 ^oC for 1 hr. The biotin-AuNPs were collected by filtration for further use. We also tested PAMAM dendrimers with different generation numbers (namely G2, G3, and G4), and observed that AuNPs capped with G1 PAMAM dendrimers are most effective (data not shown). In this work, G1 PAMAM dendrimers were used for further modification of *n*-alkanethiol - AuNPs.

Estimation of free amines and biotin ligands per dendrimer-AuNP

The number of free amines at the surface of a dendrimer-AuNP was estimated by using the titration method with slight modification as described elsewhere.[11] Briefly, 4-nitrobenzaldehyde (4-NB) was reacted with the functional amines of dendrimer-AuNPs through imine formation between aldehyde and amine groups. About 1.4 μM of dendrimer-AuNPs was reacted with 0.5 mg 4-NB and 2 μL acetic acid in 1 mL of anhydrous ethanol at 50 □ for 6 hr under argon atmosphere. Following imine formation, the reaction mixture was filtered through a microfilter with molecular weight cut-off of 30 kD (Microcon YM30, Millipore Corp.). The concentration of non-reacted free 4-NB was determined by measuring the absorbance of filtrate with the extinction coefficient (ε_{max} = 14,500 $M^{-1} \cdot cm^{-1}$). Consequently, the amount of amine groups of dendrimer-AuNPs was estimated from the difference between the initial concentration of 4-NB for reaction with dendrimer-AuNPs and that of non-reacted free 4-NB in the filtrate. As a result, maximum number of free amine groups per dendrimer-AuNP was estimated to be 54 in triplicate experiments. This value is within a reasonable range when compared with the theoretical estimate as shown below. Thus, given that the free amines are fully functionalized when reacted with excess amount of sulfo-NHS-biotin, the maximum number of biotin ligands per dendrimer-AuNP is approximated to be 54.

For comparison, the number of free amines per dendrimer-AuNP was theoretically calculated. It was provided that about 15 molecules of G1 PAMAM dendrimer could adsorb onto the surface of an *n*-alkanethiol-AuNP from the estimated surface area of *n*-alkanethiol-AuNP and the size of G1 PAMAM dendrimer. The average diameter of Au-NP was found to be about 2.5 nm from the TEM image, and the diameter of G1 PAMAM dendrimer is reported to be 2 nm, respectively. From our previous observation, we reasoned that about 50% of amine groups of adsorbed G1 dendrimers on *n*-alkanethiol-AuNP might be freely accessible. G1 PAMAM dendrimer has 8 free amine groups. As a result, the maximum number of free amine groups per dendrimer-AuNP is calculated to be about 60.

Figure S1. FT-IR spectrum of AuNPs



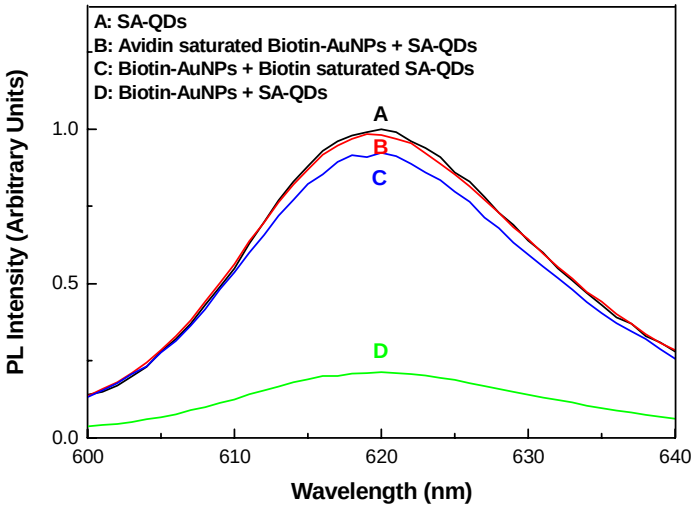
(A) FT-IR spectrum of AuNPs which were stabilized with the mixture of *n*-alkanethiols (11-MUA and 1-OT) having terminal carboxylic acid and alkyl groups. *n*-Alkanethiol-stabilized AuNPs exhibited the C=O stretching peak (~ 1710 cm^{-1}) and C-H stretching mode (~ 2840, 2910, and 2950 cm^{-1}) coming from 11-MUA and 1-OT on the surface of AuNPs

(B) FT-IR spectrum of AuNPs after further modification with G1 PAMAM dendrimers. Dendrimer-AuNPs showed a broad N-H stretching mode (~ 3290 cm^{-1}), amide I band (~ 1650 cm^{-1}), and amide II band (~ 1560 cm^{-1}) caused by immobilization of G1 PAMAM dendrimers

The spectra were obtained in a single electron mode using a FT-IR spectrophotometer (dry N_2-purged Thermo Nicolet Nexus FT-IR, Thermo Electron Corp.) equipped with the SAGA (Smart Apertured Grazing Angle) accessory. The p-polarized light was incident at 80 o relative to the surface normal of the substrate. A narrow band mercury-cadmium-telluride (MCT) detector cooled with liquid nitrogen was used to detect the reflected light. We averaged about 2,000 scans to yield the spectrum at a resolution of 2 cm-1, and all spectra were reported in the absorption mode relative to a clean gold plate.

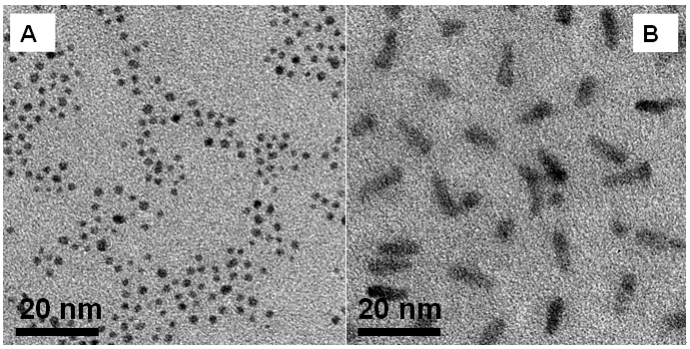
These FT-IR spectra show the effective amine-functionalization through dendrimer immobilization. It is anticipated that the hydrophobic interaction between G1 PAMAM dendrimers and alkyl groups on *n*-alkanethiol-stabilized AuNPs[14] as well as the electrostatic force between amine groups of G1 PAMAM dendrimers and carboxylic groups of 11-MUA on AuNPs[15] would make a significant contribution to the formation of stable, amine-functionalized AuNPs. In addition, we found that the capping with G1 PAMAM dendrimers significantly reduced the non-specific binding of *n*-alkanethiols-stabilized AuNPs to streptavidin on the QDs in an aqueous solution (data not shown).

Figure S2. Photoluminescence (PL) quenching of SA-QDs by biotin-AuNPs.



Trace A shows the PL spectrum of SA-QDs only in double distilled water containing 63 nM of BSA. Maximum emission was observed at 620 nm. Trace B represents the PL spectrum of SA-QDs in the presence of biotin-AuNPs which had been pre-saturated with 2 μM avidin. Addition of biotin-AuNPs which had been pre-saturated with avidin resulted in a full recovery of the PL intensity at 620 nm compared to that of free SA-QDs. Trace C shows the PL spectrum of SA-QDs when biotin-AuNPs were reacted with SA-QDs which had been preincubated with 30 μM biotin. Trace D represents the PL spectrum of SA-QDs in the presence of biotin-AuNPs. The PL intensity of SA-QDs was quenched over 80 % by the presence of biotin-AuNPs. The experimental conditions were : 70 nM AuNPs, 70 nM biotin-AuNPs, 63 nM BSA, and 300 pM SA-QDs in DDW.

Figure S3. Energy-filtering transmission electron microscope (EF-TEM) images of free NPs.



(A) Free biotin-AuNPs (B) Free SA-QDs. The images were obtained by using EF-TEM (EM912 Omega, Carl Zeiss, Germany). Due to organic materials on the surface of NPs, relatively low focused images were observed. SA-QDs resulted in more blurred images compared to the AuNPs.