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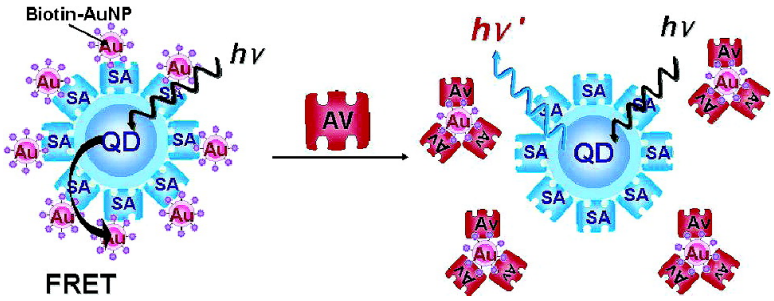


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# Inhibition Assay of Biomolecules based on Fluorescence Resonance Energy Transfer (FRET) between Quantum Dots and Gold Nanoparticles

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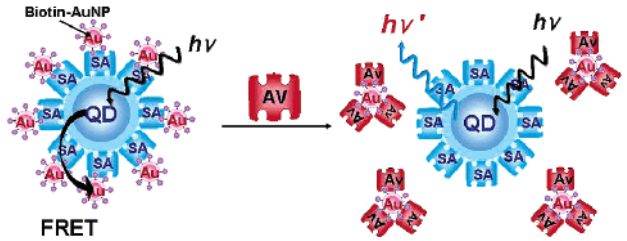
In recent years, inorganic nanoparticles (NPs) have become the subject of intensive research for detection and labeling of biomolecules due to their size-dependent physical and chemical characteristics.[1] As a result, a variety of optical methods using NPs have been developed for sensitive detection of molecular recognitions, such as colorimetric detection,[2] fluorescence resonance energy transfer (FRET)/quenching,[3] surface plasmon resonance analysis,[4] and scattering-based sensing.[5] In these systems, a number of biomolecule-conjugated NPs were employed to detect biospecific interactions. In the past few years, quantum dots (QDs) have been favorably adopted in the FRET-based studies due to their distinct optical characteristics.[3] In addition, gold nanoparticles (AuNPs) have been of great interest because of their high extinction coefficient and a broad absorption spectrum in a visible light that is overlapped with the emission wavelength of usual energy donors. As a photoluminescence (PL) quencher of organic dyes, AuNPs were employed to detect single-mismatched nucleotides based on a molecular beacon technique and other protein interactions.[3] However, despite a number of bioanalytical applications of NPs,[6] to the best of our knowledge, there have been few attempts employing the PL quenching of QDs by AuNPs for studying an inhibition assay in aqueous solutions.

Here, we report an inhibition assay method based on the modulation in FRET efficiency between QDs and AuNPs in the presence of the molecules which inhibit the interactions between QD- and AuNP-conjugated biomolecules. By employing a streptavidin−biotin interaction as a model system, our sensing system enables us to assay the avidin concentration in sample solution by monitoring the changes in the PL quenching of streptavidin-conjugated QDs (SA−QDs) by the biotinylated AuNPs (biotin−NPs) (Scheme 1). As the crucial points in the NP-based detection system, the dispersion stability or coagulation, functionalization, and nonspecific binding of NPs to other molecules have been considered.[7] In this sense, many methods were reported to modify gold colloids by using various materials.[8−10] With the similar viewpoints, we first stabilized AuNPs with *n*-alkanethiols and then further modified them by using amine-ended polyamidoamine (PAMAM) dendrimers to improve the dispersion stability of the modified AuNPs, to alleviate nonspecific adsorption of AuNPs to proteins, and as a linker to functionalize ligand molecules on the surface 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 having eight primary amine groups to functionalize the AuNPs with biotin. The amine functionalization of AuNPs with G1 PAMAM dendrimers was confirmed by using Fourier transform infrared spectroscopy (FTIR).

***Scheme 1.*** Schematic Illustration of Inhibition Assay Method Based on the PL Quenching of SA−QDs by Biotin−AuNPs[a]




[a] SA denotes the streptavidin immobilized on the surface of QDs, and Av is the externally added avidin.

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 dendrimers (Figure S1 in the Supporting Information). We found that the capping with G1 PAMAM dendrimers significantly reduced the nonspecific binding of *n*-alkanethiol-stabilized AuNPs to streptavidin on the QDs in an aqueous solution. Dendrimer-capped AuNPs were stable even after 2 weeks in water without significant aggregation or changes of optical characteristics. It is anticipated that the hydrophobic interaction and the electrostatic force between G1 PAMAM dendrimers and *n*-alkanethiols lead to stabilization of AuNPs.[11]

The number of free amines per dendrimer−AuNP was estimated to be 54 by titration of free amines at the surface of dendrimer−AuNPs using 4-nitrobenzaldehyde (4-NB) (see Experimental Section of Supporting Information).[12] We used sulfo-NHS-biotin (biotinamidohexanoic acid 3-sulfo-*N*-hydroxysuccinimide ester) to biotinylate the dendrimer−AuNPs. Given that the free amines are fully functionalized when reacted with an excess amount of sulfo-NHS-biotin, each AuNP is supposed to have the maximum biotin ligands of 54. SA−QDs showing an emission of red color were purchased from Quantum Dot Corporation. Each QD contains approximately 15−25 streptavidin molecules on its surface according to the data provided by the manufacturer.

To examine whether FRET between SA−QDs and biotin−AuNPs is specific and applicable to the inhibition assay, we monitored the changes in the PL spectra of SA−QDs at different conditions by using a photoluminescence spectrometer (SLM-AMINCO). As a result, the PL intensity of SA−QDs was quenched over 80% by the presence of biotin−AuNPs. On the other hand, addition of biotin−AuNPs, which had been presaturated with avidin, resulted in a full recovery of the PL intensity at 620 nm compared to that of free SA−QDs (Figure S2 in the Supporting Information).

To obtain some insights into the phenomenon of PL quenching, electron microscopy analysis of NPs was conducted. It was revealed that biotin−AuNPs have a spherical shape with a diameter of 2−3 nm, and SA−QDs show a rod shape with a length of 10−15 nm and a diameter of ∼5 nm (Figure S3 in the Supporting Information).

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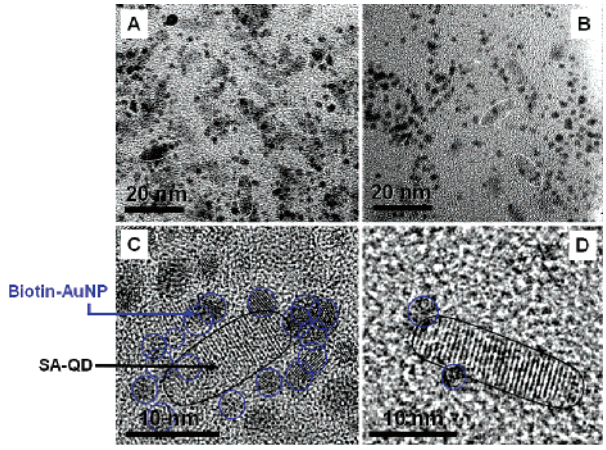


***Figure 1.*** High-resolution TEM images of NPs. (A) SA−QDs in the presence of biotin−AuNPs. (B) SA−QDs in the presence of avidin-saturated biotin−AuNPs. (C and D) Magnified images from A and B, respectively. Lattice structures of crystalline NPs are clearly shown. Some NPs are outlined with guide lines.

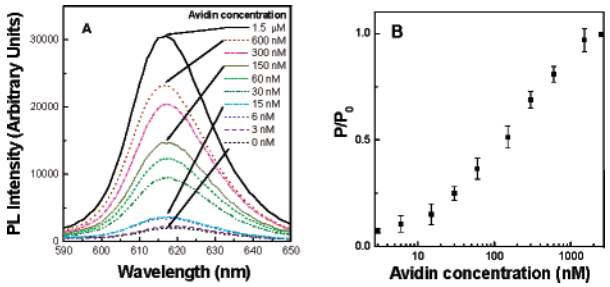


***Figure 2.*** FRET-based inhibition assay of avidin. (A) PL spectra of SA−QDs in the presence of biotin−AuNPs which had been preincubated with different concentrations of avidin. (B) Relative PL intensity ($P/P_0$) of the sensing system under the same experimental conditions as in A. Standard deviations in triplicate experiments are shown at data points.

Free NPs were observed to separate well with each other. As can be seen in high-resolution TEM (HR-TEM, JEM-2100F, JEOL Co.) images, the addition of biotin−AuNPs to the solution of SA−QDs led to the formation of clusters (Figure 1A,C). From these images, it is evident that QDs and AuNPs are within a short distance by specific interaction between biotin and streptavidin, which induces an efficient energy transfer from the donor (SA−QDs) to the acceptor (biotin−AuNPs). In contrast, when avidin-saturated biotin−AuNPs were incubated with SA−QDs, the formation of clusters between QDs and AuNPs was found to be negligible (Figure 1B,D). This clearly supports that externally added avidin molecules bound to the surface of biotin−AuNPs and blocked the PL quenching of QDs by AuNPs.

On the basis of the above results, we attempted an assay for avidin as a model protein which inhibits streptavidin−biotin interactions. Different concentrations of avidin were added to the biotin−AuNPs at a fixed concentration of 70 nM. Then, the SA−QDs were added to the reaction mixtures at a final concentration of 300 pM, and the PL spectra were measured as a function of the concentration of added avidin. As shown in Figure 2A, the PL intensity gradually increased as the concentration of avidin was increased. Figure 2B represents the changes in the relative PL intensity ($P/P_0$) of the sensing system with respect to the concentration of avidin in each sample. $P_0$ denotes the maximum PL intensity of SA−QDs in the presence of avidin (2 $\mu$M)-saturated biotin−AuNPs, and $P$ is the observed PL intensity of SA−QDs when reacted with biotin−AuNPs which have been preincubated with respective avidin concentrations. The PL intensity increased logarithmically with the increase of the concentration of added avidin. From these results, it is obvious that the externally added avidin specifically bound to the biotin−AuNPs, decreasing the binding of AuNPs to SA−QDs, which resulted in reduction of the PL quenching of SA−QDs. The detection limit of avidin was found around 10 nM under our experimental condition. Dynamic range of detection for avidin extended up to 2 $\mu$M.

In conclusion, we have demonstrated that FRET between QDs and AuNPs can be used for assay of a molecule which inhibits the biomolecular interactions. It is anticipated that the sensing system based on the PL quenching of QDs by AuNPs can be applied to the quantitative analysis and high throughput screening of molecules which inhibit the specific interactions between biomolecules.

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**Supporting Information Available:** Preparation and functionalization of AuNPs, FTIR spectrum of AuNPs, HR-TEM images of AuNPs and SA−QDs, quantification of free amine groups at dendrimer−AuNPs, and PL intensity of SA−QDs with biotin−AuNPs at different conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) (a) Alivisatos, A. P. *Nat. Biotechnol.* **2004**, *22*, 47. (b) Murphy, C. J. *Anal. Chem.* **2002**, *74*, 520A. (c) Bruchez, M., Jr.; Moronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P. *Science* **1998**, *281*, 2013. (d) Zheng, J.; Zhang, C.; Dickson, R. M. J. *Phys. Rev. Lett.* **2004**, *93*, 077402.
(2) (a) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078. (b) Li, H.; Rothberg, L. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 14036.
(3) (a) Clapp, A. R.; Medintz, I. L.; Mauro, J. M.; Fisher, B. R.; Bawendi, M. G.; Mattoussi, H. *J. Am. Chem. Soc.* **2004**, *126*, 301. (b) Willard, D. M.; Carillo, L. L.; Jung, J.; Van Orden, A. *Nano Lett.* **2001**, *1*, 469. (c) Nagasaki, Y.; Ishii, T.; Sunaga, Y.; Watanabe, Y.; Otsuka, H.; Kataoka, K. *Langmuir* **2004**, *20*, 6396. (d) Dubertret, B.; Calame, M.; Libchaber, A. J. *Nat. Biotechnol.* **2001**, *29*, 365. (e) Maxwell, D. J.; Taylor, J. R.; Nie, S. *J. Am. Chem. Soc.* **2002**, *124*, 9606.
(4) (a) Haes, A. J.; Van Duyne, R. P. *J. Am. Chem. Soc.* **2002**, *124*, 10596. (b) Aslan, K.; Lakowicz, J. R.; Geddes, C. D. *Anal. Biochem.* **2004**, *330*, 145.
(5) (a) Perez, J. M.; Simeone, F. J.; Saeki, Y.; Josephson, L.; Weissleder, R. *J. Am. Chem. Soc.* **2003**, *125*, 10192. (b) Roll, D.; Malicka, J.; Gryczynski, I.; Gryczynski, Z.; Lakowicz, J. R. *Anal. Chem.* **2003**, *75*, 3440. (c) Aslan, K.; Luhrs, C. C.; Pérez-Luna, V. H. *J. Phys. Chem. B* **2004**, *108*, 15631.
(6) (a) Dulkeith, E.; Morteani, A. C.; Niedereichholz, T. A.; Klar, T. A.; Feldmann, J. *Phys. Rev. Lett.* **2002**, *89*, 203002. (b) Wargnier, R.; Baranow, A. V.; Maslov, V. G.; Stsiapura, V.; Artemyev, M.; Pluot, M.; Sukhanova, A.; Nabiev, I. *Nano Lett.* **2004**, *4*, 451.
(7) Danial, M. C.; Astruc, D. *Chem. Rev.* **2004**, *104*, 293.
(8) (a) Aslan, K.; Pérez-Luna, V. H. *Langmuir* **2002**, *18*, 6059. (b) Leff, D. V.; Brandt, L.; Heath, J. R. *Langmuir* **1996**, *12*, 4723. (c) Mayer, A. B. R.; Mark, J. E. *J. Mater. Sci.* **1997**, *11*, 2151. (d) Otsuka, H.; Akiyama, Y.; Nagasaki, Y.; Kataoka, K. *J. Am. Chem. Soc.* **2001**, *123*, 8226.
(9) (a) Garcia, M. E.; Baker, L. A.; Crooks, R. M. *Anal. Chem.* **1999**, *71*, 256. (b) Chechik, V.; Crooks, R. M. *Langmuir* **1999**, *15*, 6364. (c) Esumi, K.; Houdatsu, H.; Yoshimura, T. *Langmuir* **2004**, *20*, 2536.
(10) (a) Fréchet, J. M.; Tomalia, D. A. *Dendrimers and Other Dendritic Polymers*; John Wiley & Sons: Chichester, U.K., 2001. (b) Hong, M. Y.; Yoon, H. C.; Kim, H. S. *Langmuir* **2003**, *19*, 416.
(11) (a) Khopade, A. J.; Caruso, F. *Langmuir* **2003**, *19*, 6219. (b) Tsukruk, V. V.; Rinderspacher, F.; Bliznyuk, V. N. *Langmuir* **1997**, *13*, 217
(12) Moon, J. H.; Shin, J. W.; Kim, S. Y.; Park, J. W. *Langmuir* **1996**, *12*, 4621.

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