Anal. Chem. **XXXX,** *xxx,* 000–000

Inhibitory Effect of Target Binding on Hairpin Aptamer Sticky-End Pairing-Induced Gold Nanoparticle Assembly for Light-up Colorimetric Protein Assay

Zai-Sheng Wu,* Haixia Lu, Xueping Liu, Rong Hu, Hui Zhou, Guoli Shen,* and Ru-Qin Yu

State Key Laboratory for Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, P. R. China

Gold nanoparticles (GNPs) possessing strong distance-dependent optical properties and high extinction coefficients have emerged as important colorimetric materials. Almost all colorimetric studies are based on two working mechanisms: sandwich cross-linking and non-cross-linking systems. In the present study, a new working mechanism, hairpin sticky-end pairing-induced GNP assembly, is introduced based on the discovery of unique aggregation behavior of aptamer-functionalized GNPs. The salt-induced aggregation of oligonucleotide probe-modified GNPs can readily occur due to the sticky-end pairing effect while addition of target molecules favors the formation of the hairpin structure of probe sequences and substantially inhibits the nanoparticle assembly. Along this line, we developed a proof-of-concept colorimetric homogeneous assay using immunoglobulin E (IgE) as an analyte model via transforming a commonly designed "light-down" colorimetric biosensor into a "light-up" one. From the point of view of both conformational transition of aptamer and steric bulk, oligonucleotide−GNPs display an additional stability upon binding to target molecules. The assay showed an extremely high sensitivity from both naked eye observations and absorbance measurements. Compared with almost all existing IgE sensing strategies, the proposed colorimetric system possesses a substantially improved analytical performance. Investigating the assembly behavior of hairpin aptamer-modified GNPs could offer new insight into the dependence of the GNP properties on the structure switching and open a new way to design signaling probes and develop colorimetric assay schemes.

Recent advances in nanoscience and nanotechnology have provided new opportunities for the application of nanomaterials in biological analysis and disease diagnosis.[1,2] Among nanomaterials, gold nanoparticles (GNPs) are especially attractive because of their unique physical and chemical properties.[3−9] Since the aggregation of DNA-modified GNPs and its application in DNA detection were reported,[10,11] GNP-based colorimetric measurement has attracted much attention in the detection of a broad range of target molecules.[5,9,12−22] In addition to its generality, GNP-based colorimetric assays possess several substantial advantages. First, desirable sensitivity and selectivity are comparable to those of fluorescence techniques. Second, the detection can be accomplished at low cost. Third, the colorimetric assay displays a rapid response that results from instant color change. Fourth, operation of the assay is convenient. Additionally, colorimetric systems make on-site real-time detection easier compared with fluorescent measurements.[6,14,23]

In addition to solely relying on the Watson−Crick base pairing, a new type of DNA called functional DNA has been increasingly used for directing the assembly and disassembly of nanomaterials

* To whom correspondence should be addressed. Tel/Fax: (+86) 731-8821355. E-mail: wuzaisheng@163.com; glshen@hnu.cn.

(1) Zhu, M. Q.; Wang, L. Q.; Exarhos, G. J.; Li, A. D. Q. *J. Am. Chem. Soc.* **2004**, *126*, 2656–2657.
(2) Matsui, J.; Akamatsu, K.; Nishiguchi, S.; Miyoshi, D.; Nawafune, H.; Tamaki, K.; Sugimoto, N. *Anal. Chem.* **2004**, *76*, 1310–1315.
(3) Storhoff, J. J.; Elghanian, R.; Mucic, R. C.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1998**, *120*, 1959–1964.
(4) He, H.; Xie, C.; Ren, J. *Anal. Chem.* **2008**, *80*, 5951–5957.
(5) Liu, J.; Lu, Y. *J. Am. Chem. Soc.* **2004**, *126*, 12298–12305.
(6) Huang, C.-C.; Huang, Y.-F.; Cao, Z.; Tan, W. H.; Chang, H.-T. *Anal. Chem.* **2005**, *77*, 5735–5741.
(7) Nath, N.; Chilkoti, A. A. *Anal. Chem.* **2002**, *74*, 504–509.
(8) Lin, S.-Y.; Chen, C.-h.; Lin, M.-C.; Hsu, H.-F. *Anal. Chem.* **2005**, *77*, 4821–4828.
(9) Thanh, n. t. k.; Rosenzweig, Z. *Anal. Chem.* **2002**, *74*, 1624–1628.
(10) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. *Nature* **1996**, *382*, 607–609.
(11) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078–1080.
(12) Aslan, K.; Luhrs, cc; Perez-Luna, vh *J. Phys. Chem. B* **2004**, *108*, 15631–15639.
(13) Pavlov, V.; Xiao, Y.; Shlyahovsky, B.; Willner, I. *J. Am. Chem. Soc.* **2004**, *126*, 11768–11769.
(14) Liu, J.; Lu, Y. *Anal. Chem.* **2004**, *76*, 1627–1632.
(15) Liu, J.; Lu, Y. *Angew. Chem., Int. Ed.* **2006**, *118*, 96–100.
(16) Lin, S.-Y.; Liu, S.-W.; Lin, C.-M.; Chen, C.-h. *Anal. Chem.* **2002**, *74*, 330–335.
(17) Liu, J.; Lu, Y. *J. Am. Chem. Soc.* **2005**, *127*, 12677–12683.
(18) Sato, K.; Hosokawa, K.; Maeda, M. *J. Am. Chem. Soc.* **2003**, *125*, 8102–8103.
(19) Han, M. S.; Lytton-Jean, A. K. R.; Mirkin, C. A. *J. Am. Chem. Soc.* **2006**, *128*, 4954–4955.
(20) Li, J.; Chu, X.; Liu, Y.; Jiang, J. H.; He, Z.; Zhang, Z.; Shen, G.; Yu, R. Q. *Nucleic Acids Res.* **2005**, *33*, e168.
(21) Aslan, K.; Lakowicz, J. R.; Geddes, C. D. *Anal. Biochem.* **2004**, *330*, 145–155.
(22) Aslan, K.; Lakowicz, J. R.; Geddes, C. D. *Anal. Chem.* **2005**, *77*, 2007–2014.
(23) Liu, J.; Lu, Y. *J. Am. Chem. Soc.* **2007**, *129*, 8634–8643.

10.1021/ac100422h © XXXX American Chemical Society

and for the colorimetric assay of non-nucleic acid targets.[23] Functional DNA means DNA with functions beyond the storage of genetic information, exhibiting properties similar to those of proteins, including catalytic DNA molecules (commonly referred to as DNAzymes, or deoxyribozymes),[5,17,24,25] aptamers,[26−28] and aptazymes.[14,29−32] Aptamers are conveniently prepared through combinatorial biology methods such as systematic evolution of ligands by exponential enrichment (SELEX) or in vitro selection. These recognition elements possess numerous advantageous characteristics for developing assay devices.[7,33] Employing these recognition elements for the preparation of nanomaterials can broaden the variety of stimuli to trigger the aggregate assembly process and the range of the colorimetric assay to meet application requirements for such uses as biological analysis and disease diagnosis. Therefore, aptamer−GNPs have attracted considerable attention for the development of colorimetric sensing devices.[6,15,23,34,35]

According to the the molecular conformation and hybridization state of surface-confined oligonucleotides and the role of extraneous cross-linking molecules, almost all colorimetric assays based on the assembly of GNPs fall into two categories: cross-linking sensing systems and non-cross-linking systems. Both sensing systems are used not only to detect large targets (e.g., target DNA sequences and proteins) but also to detect small species, such as metal ions which are recognized by functional DNAs. In a review article,[36] the technologies involved in the two types of colorimetric biosensors were briefly discussed. For the functional DNA-conjugated GNPs, the non-base-pairing regions containing ligand binding sites played a critical role in both activating assembly/disassembly and signaling the target binding events. It is well-known that a key feature of aptamers is the conformational transition upon binding to their targets. This conformational change not only plays an essential role in specific identification of targets but also can become the starting point for biosensing and nanoassembly applications. However, the interactions between GNPs associated with molecular conformation of surface-confined biopolymers are not well understood at present, as the effect of polyelectrolytes on colloidal stability is extremely complicated. Naturally, little is known about how the sticky-end aptamers behave on nanoparticle surfaces and how the sticky-end aptamer-modified GNPs behave (e.g., colloidal stability toward salt-induced assembly). Comprehensive investigations of the aggregation behavior of the colorimetric systems would make important contributions to fundamental principles of biopolymer-conjugated GNP interactions, facilitating the development of nanoscience/nanotechnology and the colorimetric sensors.

Proteins are ubiquitous in nature and essential for life. Among various proteins, the determination of IgE is of substantial importance for human health protection.[37] On the basis of the above considerations, we investigated the dependence of GNP assembly behavior on the sticky-end hairpin probe with a non-base-pairing target-binding region. We proposed a sticky-end pairing effect for GNP aggregation and demonstrated a conceptually novel colorimetric IgE assay. The interaptamer sticky-end pairing in a head-to-tail manner dramatically promotes the aggregation of oligonucleotide−GNPs while the target binding-induced conformational change of aptamers leads to a significant increase in colloidal stability. By presenting experimental data and comparing with the observations reported in previous works (see text for details), we demonstrated in detail the sticky-end pairing-induced assembly to explain the surprising aggregation behavior of GNPs and investigated the analytical performance of this colorimetric assay system. Given the visible color change, simplicity, and potential generality, hairpin aptamer sticky-end pairing-induced assembly has potential applications for nanoparticles in analytical chemistry and for colorimetric assays of biomarkers.

EXPERIMENTAL SECTION

Chemicals and Materials. The oligonucleotides designed were synthesized by Shanghai Generay Biotech Co. (Shanghai, China), and their sequences are shown as the following:

Stabilizing probe: 5′-SH-TCC TCT CTC TCT CTT TTT T-3′

Anti-IgE aptamer: 5′-SH-GGG GCA CGT TTA TCC GTC CCT CCT AGT GGC GTG CCC C-3′.

Immunoglobulin E (IgE) purified from human plasma was purchased from Meridian Life Science, Inc. (Saco, ME) while bovine serum albumin (BSA), human IgG, and human serum albumin (HSA) were obtained from Beijing Dingguo Biotechnology Center (Beijing, China). Unless otherwise indicated, protein samples and aptamer solution were prepared in 138 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, and 10 mM phosphate buffer solution at pH 7.4 (PBS1) while stabilizing probes were dissolved in 1 M KCl and 10 mM phosphate buffer solution at pH 7.4 (PBS2).

All other chemicals were of analytical grade and used without further purification. Throughout this study, deionized, sterilized water (resistance >18 MΩ/cm) was used.

Instrumentation. UV−vis absorption measurements were carried out using a MultiSpec-1501 spectrophotometer (Shimadzu, Japan) with HYPER UV Version 1.50 software. The spectra of samples were recorded at room temperature. Melting curves were acquired on a CFX96 Real-Time System (Bio-Rad Laboratories, Hercules, CA).

Preparation of Stabilizing Probe-Modified GNPs. GNPs were prepared according to the literature method.[38] Although the size of nanoparticles characterized by TEM is about 12 nm in that method, about 15.6-nm diameter particles are obtained in our work.

Prior to modification, GNPs were concentrated by a factor of 2. Stabilizing probe-modified GNPs were prepared according to

(24) Lee, J. H.; Wang, Z. D.; Liu, J. W.; Lu, Y. *J. Am. Chem. Soc.* **2008**, *130*, 14217–14226.
(25) Liu, J. W.; Lu, Y. *J. Am. Chem. Soc.* **2003**, *125*, 6642–6643.
(26) Ellington, A. D.; Szostak, J. W. *Nature* **1990**, *346*, 818–822.
(27) Wang, J.; Wang, L.; Liu, X.; Liang, Z.; Song, S.; Li, W.; Li, G.; Fan, C. *Adv. Mater.* **2007**, *19*, 3943–3946.
(28) Tuerk, C.; Gold, L. *Science* **1990**, *249*, 505–510.
(29) Liu, J.; Lu, Y. *Chem. Commun.* **2007**, *46*, 4872–4874.
(30) Ogawa, A.; Maeda, M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6517–6520.
(31) Jiang, Y.; Zhu, C.; Ling, L.; Wan, L.; Fang, X.; Bai, C. *Anal. Chem.* **2003**, *75*, 2112–2116.
(32) Mendonsa, S. D.; Bowser, M. T. *Anal. Chem.* **2004**, *76*, 5387–5392.
(33) Famulok, M.; Hartig, J. S.; Mayer, G. *Chem. Rev.* **2007**, *107*, 3715–3743.
(34) Medley, C. D.; Smith, J. E.; Tang, Z.; Wu, Y.; Bamrungsap, S.; Tan, W. *Anal. Chem.* **2008**, *80*, 1067–1072.
(35) Zhao, W.; Chiuman, W.; Lam, J. C. F.; McManus, S. A.; Chen, W.; Cui, Y.; Pelton, R.; Brook, M. A.; Li, Y. *J. Am. Chem. Soc.* **2008**, *130*, 3610–3618.
(36) Sato, K.; Hosokawa, K.; Maeda, M. *Anal. Sci.* **2007**, *23*, 17–20.
(37) Maehashi, K.; Katsura, T.; Kerman, K.; Takamura, Y.; Matsumoto, K.; Tamiya, E. *Anal. Chem.* **2007**, *79*, 782–787.
(38) Yin, X.-B.; Qi, B.; Sun, X.; Yang, X.; Wang, E. *Anal. Chem.* **2005**, *77*, 3525–3530.

the procedure described previously[39] with slight modification. The probe-to-nanoparticle ratio was about 88:1. The concentration of nanoparticles was estimated from the optical density at the plasmon resonance frequency using an extinction coefficient of 4.2×10^8 M^{-1} cm^{-1}.[40] In brief, 60 μL of 4.7 μM stabilizing probe solution was gradually added to 1 mL of concentrated GNP solution over 30 min and incubated for 16 h at room temperature; subsequently, 110 μL of 100 mM phosphate buffer solution at pH 7.4 (PBS3) was added drop by drop. During the salt-aging process, 57 μL of aqueous 2 M NaCl (aging solution; also called destabilizing solution, taking into account that this solution was also used to trigger the aggregation of DNA−GNPs) was injected twice at a time interval of 8 h. After incubation overnight, excess reagents were then removed by centrifugation (twice). The stabilizing probe-functionalized GNPs were redispersed in 600 μL of 10 mM phosphate buffer solution and stored in a refrigerator at 4 °C for further use.

Procedure of Protein Detection. The protein detection involves only three mixing steps: (a) a 22-μL aliquot of aptamer solution (0.5 μM) was added to 100 μL of IgE sample at a specific concentration and incubated for 40 min; (b) 40 μL of stabilizing probe-modified GNP solution was injected into the resulting solution; (c) after 60 min, a 20-μL droplet of destabilizing solution was added and allowed to stand for 15 min. The amount of IgE in samples, which determined the aggregation degree of GNPs, was valuated from the absorption peaks of UV−vis spectra collected. All the experiments were conducted at room temperature.

To assess the detection selectivity of the present assay system, protein interferents substituting for IgE as analytes were used for the aptamer−protein binding reaction, and detection experiments were performed as described above.

Investigation of the Dependence of the GNP Assembly on the Aptamer. The stabilizing probe-functionalized GNP solution (40 μL) was mixed with PBS1 (100 μL) and incubated for 40 min, and then 22 μL of PBS2, aptamer, or stabilizing probe was added. After 60 min, 20 μL of destabilizing solution at specific concentration was injected, and the mixture was allowed to stand for 15 min prior to UV−vis absorption measurements. The ionic strength mentioned in the corresponding section is that present in destabilizing solution.

RESULTS AND DISCUSSION

Intensive Aggregation of Aptamer-Functionalized GNPs. The intensive aggregation of aptamer-functionalized GNPs was discovered by accident in our work. In the initial experiments, we tried to prepare stable thiolated anti-IgE aptamer-conjugated GNPs. Unexpectedly, during the aging process, aptamer-modified GNPs invariably encountered salt-induced aggregation whereas under identical conditions the stabilizing probe-modified GNP solution almost completely retained its red color (shown in Figure 1). The results are inconsistent with the expected assembly behavior estimated from the relationship between the base number of DNA sequences and the interparticle electrostatic repulsion. To further investigate the aggregation behavior, the aptamer-functionalized GNPs were prepared under other experimental conditions such as a higher concentration of aptamers, a longer incubation time for the interaction between aptamers and GNPs, and a slower addition of salt solution. Note that a high concentration of aptamer was expected to increase the surface charge density of GNPs and enhance electrostatic repulsion between GNPs. However, aptamer−GNP aggregation occurred likewise, which was readily detected with both the naked eye and a UV−vis spectrometer. This distinct change in colloidal color demonstrates that anti-IgE aptamer-functionalized GNPs exhibit unique assembly behavior that is distinctly different from common DNA−GNPs (further supporting data are seen below). To our knowledge, no aptamer−GNP-based colorimetric assay of IgE has been reported though considerable research aims at developing colorimetric assays using aptamer DNAs as recognition probes (classically divided into cross-linking systems mediated by extraneous molecules[8,15] and non-cross-linking systems mediated by DNA aptamer folding).[35,41] The preparation of desirable anti-IgE aptamer-functionalized GNPs is probably the main obstacle to this assay, as we mentioned above. Very likely, the unique aggregation behavior of anti-IgE aptamer-functionalized GNPs represents a new assembly mechanism.

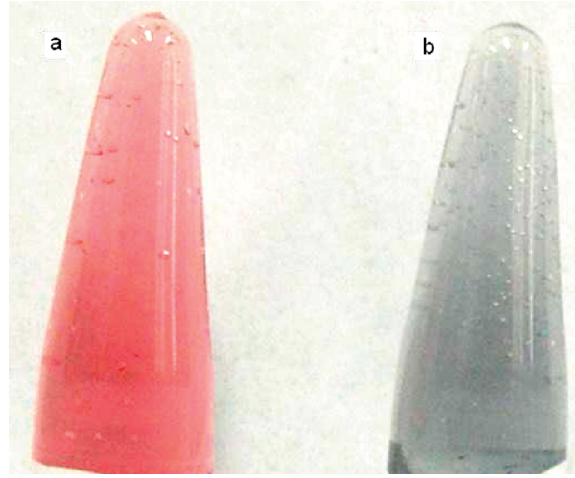


Figure 1. Direct comparison of the stability of the stabilizing probe-functionalized GNPs (a) and that of anti-IgE aptamer-functionalized GNPs (b). Solution a was prepared according to the following procedure: the stabilizing probe solution (60 μL, 4.7 μM) was gradually added to 1 mL of concentrated GNP solution over 30 min, and the self-assembly reaction was allowed to proceed for 16 h at room temperature. Then, 110 μL of PBS3 and 28.5 μL of aging solution were added to the resulting solution. The method used for the preparation of solution b was the same as that for solution a except that anti-IgE aptamer replaced the stabilizing probe.

Possible Mechanisms. The assembly characteristics of GNP modified with DNA strands are, although extensively studied and utilized, extremely complicated, originating from the complex inter- and intramolecular interactions with the aid of normal base pairings,[42] non-Watson−Crick-type base pairings,[43] Hoogsteen hydrogen bonds,[44] and DNA strand folding.[45] It is well-known that the assembly behavior of GNPs depends on the net potential of interparticle attraction and repulsion forces. The attraction force originates from the van der Waals attraction and the intermolecu-

(39) Wu, Z. S.; Jiang, J. H.; Fu, L.; Shen, G. L.; Yu, R. Q. *Anal. Biochem.* **2006**, *353*, 22–29, and the corresponding references.
(40) Demers, L. M.; Mirkin, C. A.; Mucic, R. C.; Reynolds, R. A., III; Letsinger, R. L.; Elghanian, R.; Viswanadham, G. *Anal. Chem.* **2000**, *72*, 5535–5541.
(41) Zhao, W. A.; Chiuman, W.; Brook, M. A.; Li, Y. F. *ChemBioChem* **2007**, *8*, 727–731, and the corresponding references.

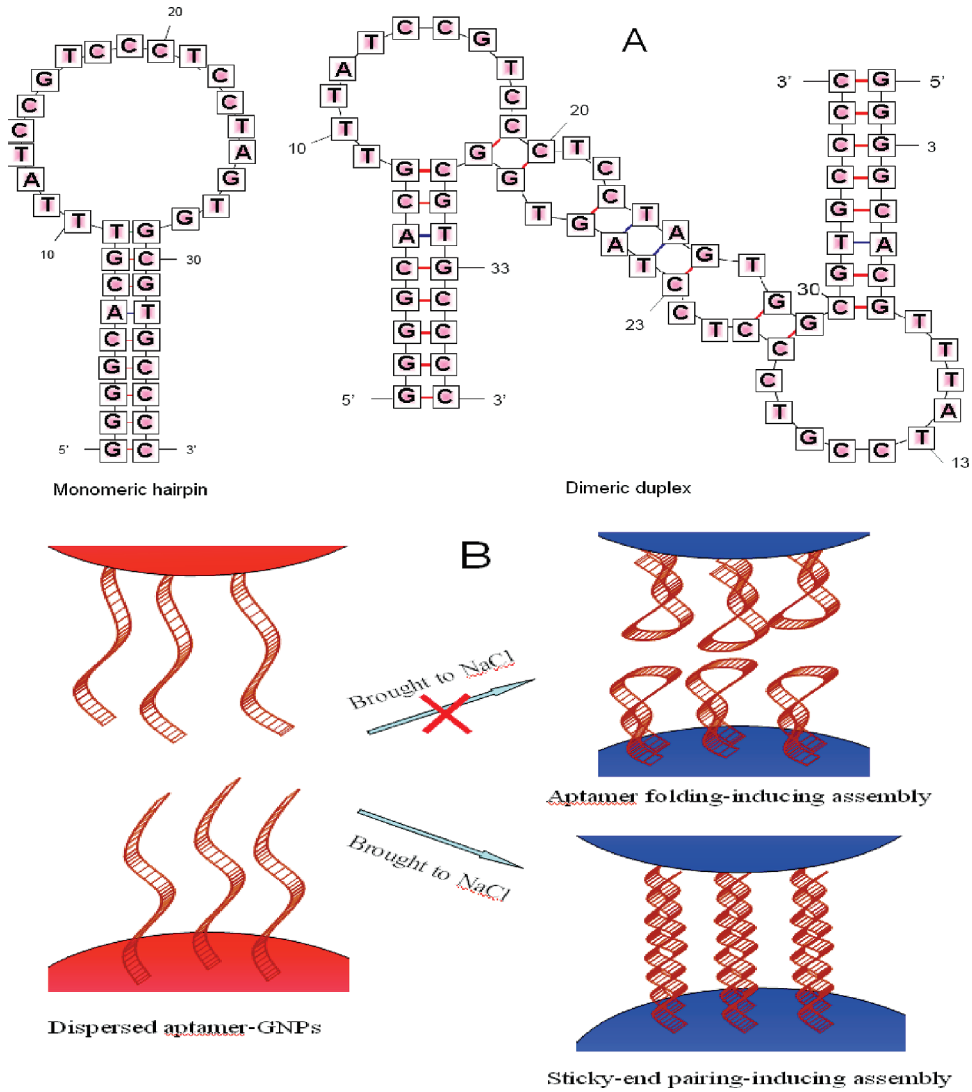


Figure 2. (A) Secondary structure of the anti-IgEg aptamer predicted by the program "mfold". The monomeric hairpin contains a non-Watson–Crick base pairing between T and G. The intermolecular hybridization triggering the formation of the dimeric duplex is called the hairpin DNA sticky-end pairing effect. (B) The assembly mechanisms for the intensive aggregation of the anti-IgE aptamer-conjugated GNPs. The impracticable GNP aggregation is marked with a red cross.

lar interaction between biopolymers chemically attached onto the surfaces of GNPs. The repulsion force originates from the two major repulsion effects: electrostatic repulsion and steric hindrance. To ascertain the assembly mechanism for the unique aggregation phenomenon observed for the present aptamer-functionalized GNPs, the possible structures of anti-IgE aptamer under the experimental conditions without GNPs were evaluated with the help of DNA folding software (mfold) based on previous works.[46,47] The calculated data demonstrate that the anti-IgE aptamer can adopt two distinct molecular configurations under certain conditions: the monomeric hairpin resulting from intramolecular folding, and the dimeric duplex via intermolecular hybridization with a head-to-tail format as shown in Figure 2A. Compared with the latter (T_m: about 40 °C), the former exhibits a higher melting temperature (T_m: about 70 °C) because the presence of the loop stabilizes the hairpin structure via stacking between the bases in the loop and the stem.[48] However, the predominant species are dimeric duplexes under selected conditions where the free energy is favorable for intermolecular hybridization. The melting curve was analyzed by monitoring the level of SYBR Green fluorescence as a function of temperature according to the literature method.[49] As shown in Figure 3, the melting temperature of this aptamer is about 40.5 °C, confirming the formation of the dimeric duplex. Moreover, the anchoring of aptamers onto GNP surfaces causes an increase of local concentration and further promotes the intermolecular hybridization to

(42) Li, Z.; Jin, R.; Mirkin, C. A.; Letsinger, R. L. *Nucleic Acids Res.* **2002**, *30*, 1558–1562.
(43) Hill, H. D.; Hurst, S. J.; Mirkin, C. A. *Nano Lett.* **2009**, *9*, 317–321.
(44) Wu, Z. S.; Guo, M. M.; Shen, G. L.; Yu, R. Q. *Anal. Bioanal. Chem.* **2007**, *387*, 2623–2626.
(45) Chen, S. J.; Huang, Y. F.; Huang, C. C.; Lee, K. H.; Lin, Z. H.; Chang, H. T. *Biosens. Bioelectron.* **2008**, *23*, 1749–1753.
(46) Zuker, M. *Nucleic Acids Res.* **2003**, *31*, 3406–3415.
(47) SantaLucia, J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 1460–1465.
(48) Wabuyele, M. B.; Farquar, H.; Stryjewski, W.; Hammer, R. P.; Soper, S. A.; Cheng, Y. W.; Barany, F. *J. Am. Chem. Soc.* **2003**, *125*, 6937–6945.
(49) Hamalainen, H. K.; Tubman, J. C.; Vikman, S.; Kyrola, T.; Ylikoski, E.; Warrington, J. A.; Lahesmaa, R. *Anal. Biochem.* **2001**, *299*, 63–70.

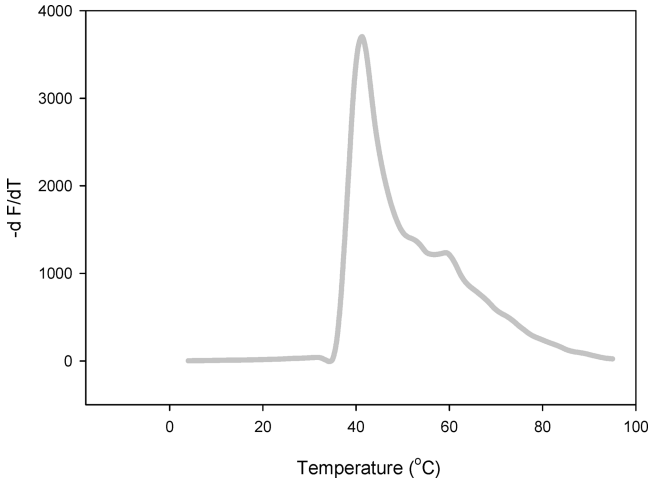


Figure 3. Melting curve of anti-IgE aptamer under the same conditions as those in the final state reached in Figure 1 but without GNPs. The dilution ratio of SYBR Green I: 1:1000. *Y*-axis: first negative derivative of fluorescence over temperature. *X*-axis: temperature (°C).

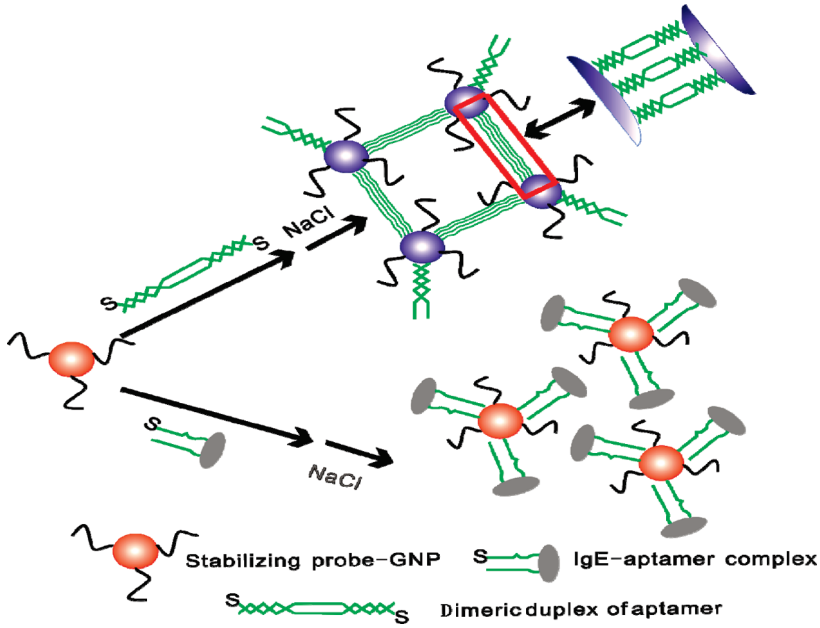
some extent as indicated in the literature.[50,51] For example, the melting temperature of hybrids formed between thiolated oligonucleotide-functionalized GNPs was typically higher than that predicted for the particle-free DNAs.[10,11]

On the basis of the above factors, in this contribution, we proposed a new working mechanism, sticky-end pairing-induced GNP assembly (shown in Figure 2B), for the unique aggregation behavior of anti-IgE aptamer-functionalized GNPs, which is closely related to the sticky-end pairing effect described by Li and Tan.[52] Aptamer-functionalized GNPs can maintain a stable, dispersed state in a low ionic strength solution because efficient intermolecular hybridization cannot be achieved, whereas a red-to-blue change is observed when the colloidal solution is added to a salt solution due to the sticky-end paring-induced nanoparticle assembly. The postulated assembly mechanism is not only based on the melting temperatures of the aptamer estimated from fluorescence measurements and the "mfold" program but is also consistent with previous reports.[35,53] After systematically studying the effect of the folding of surface-confined DNA aptamers on the colloidal stability, Zhao and his co-workers[35] pointed out that the GNPs with folded aptamers are more stable toward salt-induced aggregation than those modified with unfolded aptamers because the former displayed a stronger electrostatic repulsion and larger steric hindrance. In the present work, the folded configuration of the surface-confined aptamer with a 9-mer stem possesses the basic structural rigidity responsible for the unique stability of GNPs.[35] Therefore, even if the intramolecular folded structure of the present aptamer could be formed, the salt-induced aggregation could not occur under the chosen conditions. Additionally, hairpin DNA-modified GNPs have been successfully prepared and are used as a powerful assay tool for single-mismatch discrimination.[53] Because high-salt buffer (1 M NaCl) was involved,[53] the folded DNA−GNPs should exhibit a relatively high stability toward the salt-induced aggregation. When being estimated by the same "mfold" program, the hairpin DNA used in that work may adopt a loop−stem structure but cannot hybridize with each other. Clearly, the results should be attributed to the instability of the sticky-end paring associated with the two short complementary arm sequences. Given that there is a close relationship between the aggregation of GNPs and the hairpin DNA sticky-end pairing, the sticky-end pairing-induced assembly for the GNP aggregation proposed in our work is reasonable. The effect of anchoring of the anti-IgE aptamer on the aggregation behavior of GNPs is detailed in the Supporting Information.

Design of the Colorimetric Biosensor and Optical Response to IgE. Given that the aptamer has a stem−loop secondary structure upon addition of target IgE as predicted by us[54] and other groups,[55] the target binding should disassociate the sticky-end pairing dimeric duplex and ultimately lead to the increased stability of GNPs, offering a potential colorimetric assay tool. However, it is very challenging to transform a conceptual signaling system into a colorimetric biosensor because the assay system must maintain a stable, dispersed state before or occasionally after addition of the target species, especially for the present system that is faced with intensive aggregation. To demonstrate the proof-of-concept of the colorimetric assay system based on the sticky-end pairing-induced GNP assembly, a light-up sensing strategy was proposed in the present work as represented in Scheme 1. Generally, a traditional light-up colorimetric assay system[15] requires the preparation of a solution of nanoparticle aggregates, which is a relatively difficult process, as these solutions are less stable than those consisting of dispersed GNPs. In addition, as shown in the literature,[41] the resuspension of aggregates to dispersed particles was rather difficult because of steric hindrance or poor accessibility to the aggregates even for the small biomolecules. Sometimes the assay required a relatively high temperature.[41] Therefore, taking into account the thermal instability of target protein IgE involved in the present assay system, a novel light-up sensing scheme was developed to circumvent the various problems associated with high-temperature conditions without sacrificing the assay performance, where the destabilizing solution was added after the anchoring of thiolated biomolecules onto GNPs. Moreover, for the colorimetric assay system[6] in which thiolated aptamer is pretethered to the GNP surfaces, the order of detection sensitivities achieved does not correlate with the stabilities of the aptamer−ligand complexes in free solutions because of the changes in the structure of aptamers and protein analytes on the GNP surfaces and differences in the nature of the nonspecific interactions between the proteins and surface-confined aptamers. To maintain an intrinsic binding capability, free anti-IgE aptamers were used to recognize target IgE, followed by their anchoring onto the GNP surfaces to signal the target binding event (the method was called target binding-followed anchoring protocol). Note that the hairpin aptamer mentioned here refers to the anti-IgE aptamer though the dimeric duplex rather than the monomeric folded structure was formed in the absence of ligand. In brief, a stable colloidal solution

(50) Heyduk, E.; Dummit, B.; Chang, Y. H.; Heyduk, T. *Anal. Chem.* **2008**, *80*, 5152–5159.
(51) Gullberg, M.; Gustafsdottir, S. M.; Schallmeiner, E.; Jarvius, J.; Bjarnegard, M.; Betsholtz, C.; Landegren, U.; Fredriksson, S. *Proc. Natl Acad. Sci. U.S.A.* **2004**, *101*, 8420–8424.
(52) Li, J. J.; Tan, W. *Anal. Biochem.* **2003**, *312*, 251–254.
(53) Dubertret, B.; Calame, M.; Libchaber, A. *Nat. Biotechnol.* **2001**, *19*, 365–370.
(54) Wu, Z. S.; Zheng, F.; Shen, G. L.; Yu, R. Q. *Biomaterials* **2009**, *30*, 2950–2955.
(55) Jiang, Y.; Fang, X.; Bai, C. *Anal. Chem.* **2004**, *76*, 5230–5235.

Scheme 1. Hairpin Aptamer Sticky-End Pairing-Based Colorimetric Sensing System for the IgE Assay[a]



[a] A stable colloidal solution was prepared by conjugating stabilizing probes to GNPs, and the target binding-followed anchoring protocol was adopted to maintain the high biological activity of aptamer and ensure the easy accessibility of target protein to the specific binding sites (see text for details).

exhibiting a red color was prepared by conjugating stabilizing probes to GNPs; in the presence of analyte IgE, the intramolecular folding of aptamer was triggered by target binding, disassociating the dimeric duplex via disturbance of the sticky-end pairing. Mixing of the target–aptamer complex solution with the preprepared GNP solution caused a substantial increase in the stability of colloidal solution because of steric hindrance and electrostatic repulsion originating from the anchoring of complexes onto the GNP surfaces. In this case, addition of destabilizing solution did not induce GNP aggregation. The colloidal solution maintained the red color. In contrast, for the blank, the dimeric duplexes of aptamers that were not disentangled brought the nanoparticles together (under chosen conditions, the electrostatic repulsion can be screened). The aggregation of GNPs resulted in a distinct color change from red to purple and to blue. Via this interrogating scheme, target protein was detected with either the naked eye or UV–vis absorption measurements.

To test the feasibility of the present colorimetric system for the target protein assay, we prepared two solutions according to the method given in Procedure of Protein Detection. Sample a contained 1.89×10^{-9} M IgE while sample b was the blank. The two samples exhibited a distinct color. Namely, no detectable color change was observed for sample a while the color of sample b changed from red to blue when the destabilizing solution was introduced. Eventually, the aggregates precipitated from sample b, and the reaction medium was colorless. Their UV–vis absorption spectra are shown in Figure 4A. In general, citrate-stabilized GNPs exhibit a characteristic plasmon resonance band at about 520 nm[18] while aggregated GNPs not only result in the change of the short-wavelength absorption band but also develop a new absorption peak in long-wavelength region depending on the nature and extent of the nanoparticle aggregation.[56]

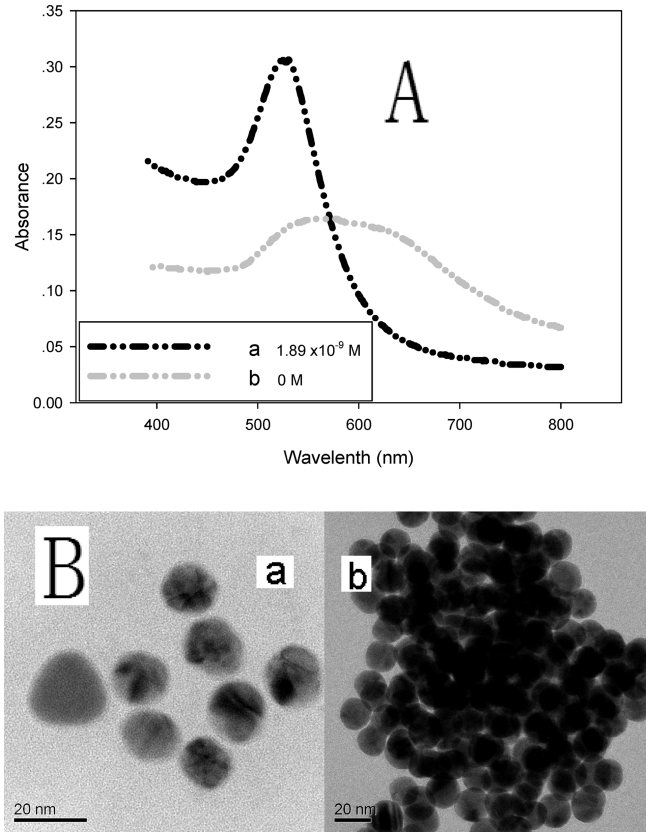


Figure 4. (A) Typical UV–vis absorption spectra of the present colorimetric assay system in the presence (line a) and absence (line b) of target IgE. The measurements were carried out as described in Experimental Section. A distinct color change could be readily observed by the naked eye. (B) HRTEM images of the two samples.

There is substantial difference in the plasmon resonance peak intensity, shape, and position between samples a and b. The

(56) Chowdhury, M. H.; Julian, A. M.; Coates, C. J.; Cote, G. L. *J. Biomed. Opt.* **2004**, *9*, 1347–1357.

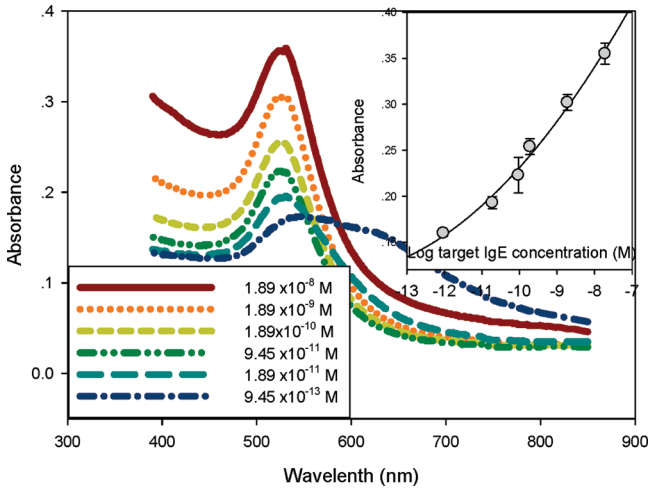


Figure 5. UV−vis absorption spectra of the colorimetric assay system in the presence of target protein at different concentrations. Inset: quantitative analysis of the target concentration using UV−vis absorption at 522 nm. The experiment conditions are described in Experimental Section. The calibration curve obeys a second-order polynomial equation: $Y = 4.452X^2 + 0.1348X + 1.134$ with a correlation coefficient of 0.9904, where Y and X represented the peak intensity and the logarithm of target concentration, respectively. The error bars denote the standard deviation of three measurements.

UV−visible spectrum of sample a (shown in Figure 4A, line a) possesses the characteristic absorption band of dispersed GNPs. In contrast, not only a shift in the absorption peak from 525 to 550 nm accompanied by a substantial loss in intensity but also a new peak in the long-wavelength region, characteristic of aggregated nanoparticles, appear for sample b (Figure 4A, line b). Figure 4B shows the high-resolution transmission electron microscopy (HRTEM) images of the two samples. Clearly, nanoparticles in sample a are nanodisperse while particle aggregates are formed in sample b. The observations by the naked eye, UV−vis absorption measurements, and HRTEM images demonstrate that addition of IgE can efficiently inhibit the assembly of DNA−GNPs, verifying that the proposed sensing system is a promising colorimetric assay platform.

Analytical Performance of Colorimetric IgE Biosensor. The anchoring of aptamer−target complexes onto GNPs can make the colloidal solution more stable via efficient inhibition of nanoparticle aggregation, which is directly related to the target concentration. Therefore, the content of IgE in samples can be estimated from the extent and rapidity of color change without any instrument. The experimental results show that the naked eye assay may achieve semiquantitative detection of target protein over a wide concentration range from 10 nM to 1 pM.

To verify that the present colorimetric assay system can be used to accurately quantify target protein, the UV−vis absorption spectra for the as-prepared solutions containing target analyte at different concentrations were collected, and the absorption peaks at 522 nm were used to estimate the target protein content in samples. As shown in Figure 5, absorption peak increases monotonically with an increasing in target concentration ranging from 9.45×10^{-13} to 1.89×10^{-8} M, indicating a light-up signaling scheme. Separation of the absorption spectrum corresponding to 1.89×10^{-8} M target protein from other spectra in the long-wavelength region is seen though no aggregation occurs in this case. The observation should be attributed to the refractive index change at the surface of individual gold nanoparticles due to the anchor of biomolecules especially at high concentration. This phenomenon was also reported by other groups.[57] The inset of Figure 5 clearly represents the relationship between optical signal and the logarithm of target concentration over ~4 orders of magnitude with a satisfactory regression coefficient of 0.9904. No significant change in absorption peak was observed when the target concentration increased or decreased further, and the response points are not within the range of the dose−response curve. The present colorimetric sensing system has a detection limit of 1.89×10^{-13} M, which is defined as the target concentration generating a absorption peak that is close to, yet still apparently higher than, the peak induced by the blank. This detection limit is about 3 orders of magnitude lower than the literature values obtained by fluorescence anisotropy,[58] molecular luminescence spectrometry,[55] and field-effect transistors,[37] assuming that all analyte samples at the concentration between the bottom limit and the upper limit were accurately quantified in those works. Moreover, the dynamic concentration range is improved by a factor of about 50−2000. The present screening system has more than 200-fold improvement in detection capability and a ~30-fold wider dynamic response range when compared with a robust biosensor, very recently developed based on a delicately designed aptamer[54] by an electrochemical technique, which has shown to be a particularly efficient analytical tool. To our knowledge, the present light-up colorimetric protein assay based on the hairpin aptamer sticky-end pairing-induced nanoparticle assembly is the most effective tool for IgE analysis at present in term of its excellent analytical characteristics and intrinsic advantages, such as simplicity, low cost, and visible detection.

To demonstrate the repeatability of the present colorimetric assay system, a series of three repetitive measurements of target samples were carried out. The relative standard deviations achieved for the samples containing 9.45×10^{-13}, 1.89×10^{-11}, 9.45×10^{-11}, 1.89×10^{-10}, 1.89×10^{-9}, and 1.89×10^{-8} M IgE were 2.1%, 3.4%, 8.7%, 3.4%, 2.9%, and 3.2%, respectively. The recovery test is given in Supporting Information.

Selectivity and Implications for the Assembly Mechanism. To confirm that the optical signal is generated from the specific aptamer-target binding, the degree of nonspecific interaction of the assay system with nontarget proteins such as IgG, BSA, and HSA was assessed under the same conditions as those in the case of IgE. Among these proteins, IgG has a molecular structure very similar to that of target IgE and serves as a good test protein for the aptamer binding specificity.[58] The experimental results are shown in Figure 6. Except for IgE sample, the colloidal solutions inevitably aggregate even though the concentration of nontarget proteins is much higher. Figure 6A inset depicts the accurate quantification of the detection selectivity achieved by the present colorimetric assay system. Excitingly, even though the corresponding colloidal solutions were diluted five times, the differences in color between IgE and the other samples were easily detected by the naked eye as shown in Figure 6B. These results confirm that the aptamer can maintain its binding properties including

(57) Nath, N.; Chilkoti, A. *Anal. Chem.* **2002**, *74*, 504–509, and the corresponding references.
(58) Gokulrangan, G.; Unruh, J. R.; Holub, D. F.; Ingram, B.; Johnson, C. K.; Wilson, G. S. *Anal. Chem.* **2005**, *77*, 1963–1970.

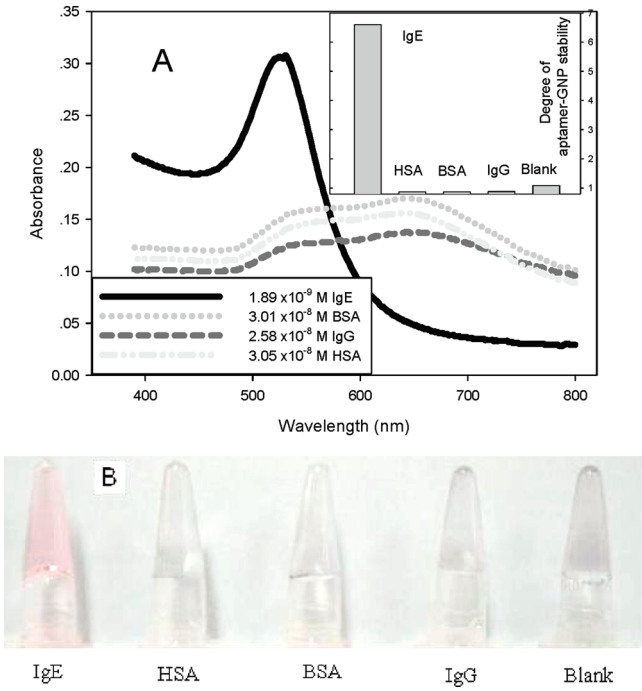


Figure 6. Selectivity of the proposed colorimetric assay platform. (A) UV−vis absorption spectra of the colorimetric assay system in the presence of target and nontarget proteins. Inset: stability of different mixtures quantitatively evaluated by the ratio of absorption intensity at 522 nm to that at 655 nm (A_{522}/A_{655}). (B) Photographs of solutions obtained by diluting the mixtures described in A.

selectivity. This is reasonable because the formation of aptamer−target complex is not disrupted when chemical modification with the external labeling group is far away from the recognition motifs.[59]

Minimal nonspecific binding is most likely a characteristic of the sticky-end pairing-induced nanoparticle assembly because the molecular conformation of the aptamers was preserved when anchored onto the nanoparticles, even upon addition of nontarget proteins, leading to inevitable GNP aggregation. Although we could not provide direct evidence of the sticky-end pairing between oligonucleotide-modified GNPs at this stage, a lack of colloidal stabilization in the control experiments indicated that the increase of colloidal stability observed was indeed due to specific aptamer−target binding which causes the conformational transition of the aptamer, indirectly validating the mechanism of sticky-end pairing-induced nanoparticle assembly.

Forward-Looking Colorimetric Assay Scheme and Significance in Molecular Configuration Screening. According to the literature,[18] as compared to the cross-linking sensing system in which a single linking molecule can pull two nanoparticles together, a conceivable disadvantage of the non-cross-linking system is the consumption of target molecules. Thus, GNP aggregation does not occur in the non-cross-linking colorimetric system unless target DNA at a high concentration is involved. Naturally, it is difficult to obtain a low detection limit[35,41] for the non-cross-linking colorimetric assay though this sensing protocol is useful for the direct analysis of single nucleotide polymorphisms.[36,60] In fact, the consumption of target molecules cannot be completely avoided even for the cross-linking colorimetric system because a single sandwich hybridization event often is not strong enough to overcome the electrostatic repulsion between two GNPs. Moreover, the subsequent hybridization of cDNAs to DNA molecules tethered onto GNPs previously linked by cross-linking reactions might occur more easily than that to dispersed DNA−GNPs because the electrostatic repulsion between linked GNPs was substantially diminished. As a result, the aptamer-based cross-liking system for the colorimetric protein assay often offered only a moderate detection capability in terms of the detection limit,[6] that is, at least 3 orders of magnitude less sensitivity than that achieved by a fluorescent aptamer probe.[61] Sometimes the opposite is true. For example, Zhao and his co-workers have reported versatile non-cross-linking colorimetric sensing systems that can give much higher sensitivity compared with the cross-linking colorimetric assay based on the same aptamer, and the benefits achieved were demonstrated in detail.[41] In sharp contrast to the two types of colorimetric assay, the consumption of target molecules should be considerably diminished in the present hairpin aptamer sticky-end pairing assembly system. Using 34 Å/10 bases as the length,[62] the molecular length of the 37-mer aptamer probe theoretically is 12.6 nm even at an almost full extension. Additionally, 12-nm-diameter GNPs and IgE molecules with a size of ~10 nm[37] are used as optical reporters and target analytes, respectively. On a nanometer scale, where the sizes of nanoparticles and biopolymers are comparable, the size change upon the anchoring of aptamer−IgE complexes can significantly affect the assembly performance of the GNP system.[22] Presumably, target binding not only changes the configuration of aptamers disassociating sticky-end pairing complexes but also creates a large steric hindrance after anchoring that substantially prevents GNPs from "linking" to each other. Perhaps the increased steric hindrance also prevents the latter aptamer−IgE complexes from anchoring to the same GNPs. Additionally, the anchoring of aptamer−IgE complexes can slow the Brownian motion of GNPs, reducing the corresponding collision probability. Thus, the GNPs without aptamer−IgE complexes became the preferential sites of anchorage, and aptamer−IgE complexes could be evenly distributed on the GNP surfaces. As a result, although target protein forces the aptamer sequence to form a stem−loop structure rather than bringing together two aptamers to form a sandwich complex that often is the foundation of a cross-linking colorimetric assay,[6] a very small amount target protein could efficiently inhibit the GNP assembly, resulting in an extremely low detection limit. Furthermore, free aptamer replacing the surface-confined aptamer might contribute to the improved sensitivity of our assay scheme to some extent due to the easy target accessibility to binding sites.[41] The desirable assay capability achieved is attributed to the joint action of the intensive aggregation associated with sticky-end pairing-induced GNP assembly, the desirable stabilizing effect originating from the anchoring of nanoscale target−aptamer complexes onto nanoparticles, and the high target binding activity of the aptamer.

The proper sticky end sequence and high target-binding activity is of critical importance to the construction of a colorimetric sensing system based on the hairpin aptamer sticky-end pairing assembly mechanism. As illustrated in the previous

(59) Fang, X.; Cao, Z.; Beck, T.; Tan, W. *Anal. Chem.* **2001**, *73*, 5752–5757.
(60) Sato, K.; Onoguchi, M.; Sato, Y.; Hosokawa, K.; Maeda, M. *Anal. Biochem.* **2006**, *350*, 162–164.
(61) Yang, C. J.; Jockusch, S.; Vicens, M.; Turro, N. J.; Tan, W. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 17278–17283.
(62) Steel, A. B.; Herne, T. M.; Tarlov, M. J. *Anal. Chem.* **1998**, *70*, 4670–4677.

works,[61,63] the stems can be regulated by extending or shortening hairpin aptamer sequences at both ends to meet the requirement of efficiently transducing target-binding events into a measurable mechanical output signal. Furthermore, in a recent electrochemical biosensing platform,[54] we introduced a long stem into the original aptamer to obtain a large hairpin structure in the absence of target protein, leading to an electrochemical sensing probe. Therefore, future work will be aimed at optimizing the incubation times, nanoparticle size, the number of stabilizing probes on each GNP, aptamer concentration and buffered working solution, and particularly at understanding the effect of sticky end nature on the aggregation behavior of GNPs by the careful design of aptamer sequences. Further optimization and exploration of this concept should lead to colorimetric aptamers with sticky ends and could be extended to some nonhairpin aptamers via elegantly incorporating stem regions into aptamer sequences to trigger the sticky-end pairing-induced nanoparticle assembly. Namely, the proposed design strategy could be generalized not only to hairpin aptamers but also to nonhairpin aptamers to develop aptamer-based colorimetric assays for cognate analytes of interest. Moreover, this new concept should avoid the deleterious situation in the fluorescent molecular beacon strategy in which the two ends of the hairpin aptamer do not move away from each other regardless of target binding[64] and also circumvent the drawbacks stemming from the splitting of an intact aptamer.[65] Clearly, the new aggregation mechanism described herein and future systematic explorations should provide a potential colorimetric sensing platform as a versatile sensing tool.

On the basis of the relationship between the distinct changes in optical characteristics and the conformational transition induced by aptamer−target binding, the proposed mechanism of sticky-end pairing assembly should also be a useful tool for roughly screening the molecular configuration of aptamers by UV−vis measurements or even by naked-eye detection. Understanding the function of aptamers and their conformational transition upon binding target molecules is of central importance in developing useful aptamer probes. Sometimes this is a very challenging task. For example, the anti-IgE aptamer cannot be investigated at present by the fluorescence resonance energy transfer signaling scheme, reagentless electrochemical method, or colorimetric readout strategies because of its complicated conformational change unless additional DNA strands are involved. The present preliminary study may lead to a new detection platform for screening the conformational change and function of such aptamers.

CONCLUSIONS

This report describes the intensive aggregation of hairpin aptamer-conjugated GNPs by a new GNP aggregation mechanism, hairpin aptamer sticky-end pairing-induced GNP assembly, and furthers the understanding of GNP aggregation behavior regarding the configuration of surface-confined hairpin aptamers. We demonstrated a designed thiolated aptamer for the highly sensitive colorimetric detection of target protein by exploiting the unique spectral properties of GNPs and the target binding-induced conformational transition of aptamers. By both colorimetric and spectroscopic detection, we have proven that GNPs can be assembled in the absence of target via sticky-end pairing assembly while dispersed in the presence of target species because of the anchoring of aptamer−target complexes, accomplishing a direct visualization and accurate quantification of analyte samples. This is the first colorimetric assay for the highly efficient sensing of IgE molecules and possesses excellent analytical performance such as wide linear response range, low detection limit, low cost, considerable time saving, and high selectivity. Additionally, this sensing strategy possesses a generalized scope, and other colorimetric aptamer probes could be created for in-field and point-of-care quantitative testing of numerous disease-related biomarkers and other targets of interest.

ACKNOWLEDGMENT

Financial assistance is gratefully acknowledged from the National Natural Science Foundation of China (Grants No. 90817101, 20905022, 20775023) and "973" National Basic Research Program of China (No. 2007CB310500).

SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review February 16, 2010. Accepted April 2, 2010.

AC100422H

(63) Liss, M.; Petersen, B.; Wolf, H.; Prohaska, E. *Anal. Chem.* **2002**, *74*, 4488–4495.
(64) Nutiu, R.; Li, Y. *J. Am. Chem. Soc.* **2003**, *125*, 4771–4778.
(65) Wu, Z. S.; Guo, M. M.; Zhang, S. B.; Chen, C. R.; Jiang, J. H.; Shen, G. L.; Yu, R. Q. *Anal. Chem.* **2007**, *79*, 2933–2939.