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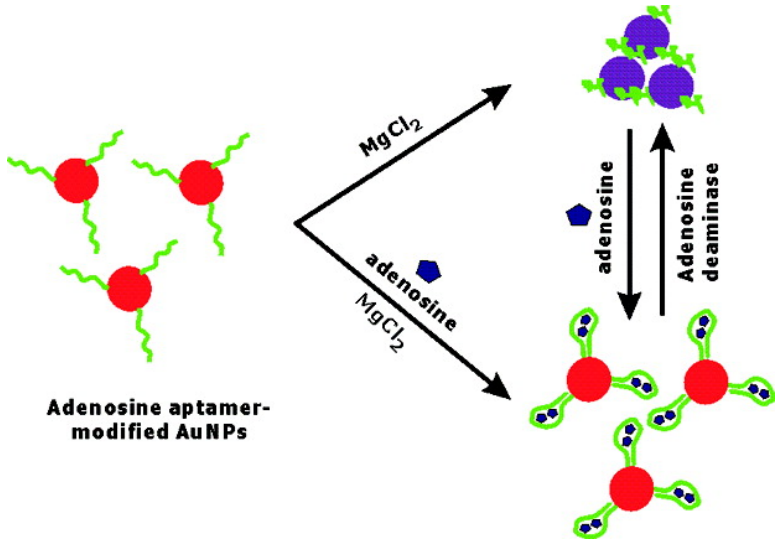

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DNA Aptamer Folding on Gold Nanoparticles: From Colloid Chemistry to Biosensors

Weian Zhao,[†] William Chiuman,[‡] Jeffrey C. F. Lam,[‡] Simon A. McManus,[‡] Wei Chen,[§] Yuguo Cui,[§] Robert Pelton,[§] Michael A. Brook,*[,†] and Yingfu Li*[,†,‡]

Department of Chemistry, Department of Biochemistry and Biomedical Sciences, and Department of Chemical Engineering, McMaster University, 1200 Main Street West, Hamilton, Ontario, Canada, L8N 3Z5

Received November 12, 2007; E-mail: liying@mcmaster.ca; mabrook@mcmaster.ca

Abstract: We have investigated the effect of the folding of DNA aptamers on the colloidal stability of gold nanoparticles (AuNPs) to which an aptamer is tethered. On the basis of the studies of two different aptamers (adenosine aptamer and K^+ aptamer), we discovered a unique colloidal stabilization effect associated with aptamer folding: AuNPs to which folded aptamer structures are attached are more stable toward salt-induced aggregation than those tethered to unfolded aptamers. This colloidal stabilization effect is more significant when a DNA spacer was incorporated between AuNP and the aptamer or when lower aptamer surface graft densities were used. The conformation that aptamers adopt on the surface appears to be a key factor that determines the relative stability of different AuNPs. Dynamic light scattering experiments revealed that the sizes of AuNPs modified with folded aptamers were larger than those of AuNPs modified with unfolded (but largely collapsed) aptamers in salt solution. From both the electrostatic and steric stabilization points of view, the folded aptamers that are more extended from the surface have a higher stabilization effect on AuNP than the unfolded aptamers. On the basis of this unique phenomenon, colorimetric biosensors have been developed for the detection of adenosine, K^+, adenosine deaminase, and its inhibitors. Moreover, distinct AuNP aggregation and redispersion stages can be readily operated by controlling aptamer folding and unfolding states with the addition of adenosine and adenosine deaminase.

1. Introduction

The effect of polymers, particularly charged polymers (or polyelectrolytes), on colloidal stabilization/aggregation has been a subject of extensive study over the last century because of their applications in a large variety of areas such as biological fluids, the paper-making industry, water treatment, pharmaceuticals, paints and coatings, among others.[1] The study of biopolymers such as negatively charged nucleic acids (DNA or RNA) on colloidal systems has recently gained considerable attention, owing to their biological importance and applications.[2]

The nanobiotechnology revolution over the past decade has made nucleic acid/colloid systems one of the most exciting research fields, owing to their potential applications for biosensors, nanomedicine, and nanoelectronics.[2] DNA/gold nanoparticle (AuNP) systems are good examples of such systems. Owing to its unique Watson−Crick hydrogen-bonding nature, DNA ensures the specificity and precision required by biosensors and programmable nanoassemblies.[3] AuNPs, with desirable nanoscaled sizes and unique physical properties (particularly the colors associated with their surface plasmon resonance), are highly suitable signal transducers for biosensors[4] and building blocks in nanoassemblies.[5] Mirkin and co-workers pioneered the study of DNA-modulated AuNP assembly and biosensors.[3d,4a] In their studies, DNA-modified AuNPs are associated into aggregates in the presence of complementary DNA strands that are used as cross-linkers; the aggregation of AuNPs is accompanied by a red-to-purple (or blue) color change. More recently, the redispersion of DNA-cross-linked AuNP aggregates, associated with the inverse purple-to-red color transi-

[†] Department of Chemistry.
[‡] Department of Biochemistry and Biomedical Sciences.
[§] Department of Chemical Engineering.

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tion, has also been developed by Liu and Lu for the detection of metal ions and small molecules.[6]

In addition to interparticle cross-linking (or bridging) mechanisms, colloid stabilization/aggregation can be adjusted by a number of other factors such as surface charge and the use of non-cross-linked surface-grafted polymers.[7] These phenomena, although extensively studied in colloid chemistry, are largely unexplored for biosensing and nanoassembly applications. We[8] and others[9,10] have recently developed biosensing assays that take advantage of AuNP aggregation induced by the loss (or screening) of surface charges.

The colloidal stabilization/aggregation phenomena of DNA-modified AuNPs are rather complicated. Like other polyelectrolytes, both the charge and polymeric nature of DNA molecules must be considered when discussing the stabilization/aggregation of DNA-modified colloids. The unique nature of DNA can complicate normal considerations because of the potential for interparticle forces such as hydrogen-bonding and hydrophobic forces. More strikingly, molecular recognitions between colloidal particle bound ssDNA molecules and their complementary DNA strands (or non-nucleic acid targets such as small molecules) make it more difficult to predict the colloidal stability of these particles. For instance, Maeda and co-workers found that the hybridization of complementary DNA strands with colloid-tethered DNA molecules resulted in a decrease of colloidal stability toward salt-induced aggregation.[9] This is somewhat surprising because, with respect to electrostatic stabilization, the addition of extra negative charges associated with the complementary DNA molecules should lead to more highly stabilized colloids. While the precise mechanism was not fully explained, the authors attributed this to the entropic loss associated with the formation of a rigid DNA duplex. This work clearly suggests that the conformations DNA molecules adopt on a colloid surface may have a significant influence on colloidal stability.

In addition to hybridization with its complementary nucleic acid strand via Watson−Crick hydrogen bonding and base stacking, some DNA (or RNA) molecules known as aptamers[11] can also specifically recognize non-nucleic acid targets such as small molecules, metal ions, and proteins. These aptamers are of great biological importance and find increasing success in applications as biosensors, controlling nanostructure assemblies, drug delivery, and diagnostics.[12] For instance, recent work revealed that RNA aptamers, modulated by the binding of a specific metabolite, played an important role in gene regulation in vivo.[13] Moreover, DNA aptamers, isolated from random ssDNA pools in vitro, have been widely applied as recognition motifs in biosensors.[12a] A key feature associated with the recognition of an aptamer with its target is a conformational transition (or folding) from loose random coil to a compact tertiary structure. This conformational change not only is essential for aptamer function, but also can be exploited as a very useful tool in the design of optical and electronic biosensors.[14] An improved understanding of aptamer conformational transitions upon binding of its target on surfaces will greatly facilitate the understanding of biorecognition mechanisms on surfaces, particularly for the development of surface-based biosensing devices (e.g., microarray and nanoparticles)[12b,c] that can then serve as a guide for improving device performance. While its importance is acknowledged, little experimental and theoretical work has been conducted on understanding aptamer conformational behaviors on surfaces.

We are interested in understanding the relationship between aptamer conformational transitions on colloid surfaces and colloidal stability. Unlike traditional polyelectrolytes, DNA aptamers can fold into compact tertiary structures in the presence of their cognate targets.[11] Little is known about how these rigid structures behave on surfaces and how they affect the colloidal properties (e.g., stability). We speculate that the improved understanding of the behavior of these entities will facilitate the development of applications in biosensors and nanotechnology.

AuNP was chosen as a model colloid system mainly because its aggregation/redispersion is directly observed by a solution color change owing to surface plasmon coupling.[15] The color change can be used as a convenient tool to test the colloidal stability and monitor the aggregation process.[16] If aptamer conformational transitions on AuNP surface, upon binding of its target, lead to changes in AuNP colloidal stability, the system can be directly transformed into a colorimetric biosensor for this target.

In the present study, two different systems (i.e., adenosine and potassium aptamers) were used to study how aptamer folding on AuNP surface affects colloidal stability. A surprising colloidal stabilizing effect associated with the aptamer folding on AuNPs was discovered: AuNPs bearing folded aptamer-target complexes were surprisingly shown to be more stable toward salt-induced aggregation than aptamer-modified AuNPs without targets. In this article, we will first discuss this

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phenomenon and propose mechanisms for the observed stability and then demonstrate its utilities for the design of colorimetric biosensors.

2. Results and Discussion

2.1. Colloidal Stability upon DNA Aptamer Folding on AuNPs. We first investigated the effect on colloidal stability of adenosine aptamer folding on AuNP surfaces. Adenosine aptamers were chemically coupled onto AuNPs using Au−S chemistry[17] (Supporting Information, Experimental Section). Radiolabeled aptamers were used to determine the concentration of aptamers on each AuNP.[3e,8a] By measuring the radioactivity in the supernatant and on the pellets after the coupling and washing steps, it was found that there were ~180 DNA aptamers on each gold nanoparticle. Adenosine aptamer-modified AuNPs (Au-Ado) were briefly treated with 6-mercaptohexan-1-ol (MCH) solution (4 μM) for 30 min at room temperature. This ligand exchange reaction can remove nonspecifically adsorbed and some of the chemically attached DNA from the AuNP surface, which helps to improve subsequent biomolecular recognition (e.g., hybridization) efficiency.[8a,18a,b] On the basis of a similar radioactivity measurement, it was found that ~96 aptamers were attached on each AuNPs after MCH treatment.

Before the addition of adenosine, the ssDNA aptamer on AuNPs adopts a loose random coil structure, since there is no strong intramolecular base pairing, based on an analysis using *mfold* software.[19a] By contrast, the aptamer folds into a well-characterized tertiary structure (Supporting Information, Figure S1)[19b,c] in the presence of adenosine (1 mM) in a buffer containing 4 mM $MgCl_2$, 100 mM NaCl, and 20 mM Tris-HCl (pH = 7.5). We will refer to the AuNPs with folded aptamer/target complex as Au-Ado-Target. At a salt concentration of 4 mM $MgCl_2$ and 100 mM NaCl, both Au-Ado and Au-Ado-Target are colloidally stable and the solutions appeared red in color. The stability of Au-Ado and Au-Ado-Target toward salt-induced aggregation was then determined by gradually adding $MgCl_2$ (1 M) solution (with the NaCl concentration fixed at 100 mM) until a rapid red-to-purple color change was observed over a short period of time (e.g., 1 min). It was determined that Au-Ado was stable only up to $MgCl_2$ concentrations of approximately ≤5 mM: at or above this concentration a rapid red-to-purple color change resulted. By contrast, Au-Ado-Target is much more stable: the particles were colloidally stable up to 30 mM $MgCl_2$. These differences are illustrated in Figure 1. To provide a direct contrast between the colloidal stability of aptamer-modified AuNPs in the presence and absence of adenosine, 30 mM $MgCl_2$ was added to both solutions. As shown in Figure 1B, Au-Ado-Target did not show any significant color change (vial 1) within 10 min. By contrast, Au-Ado turned purple at the same conditions (vial 2): the characteristic red shift was accompanied by broadening of the surface plasmon

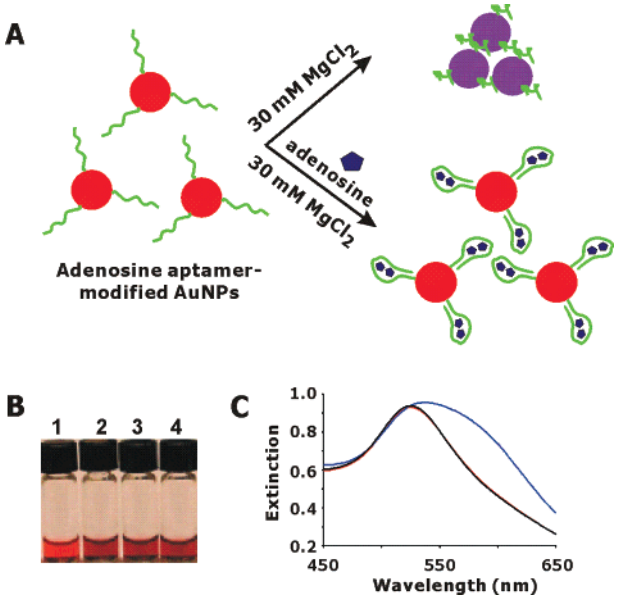

Figure 1. (A) Schematic illustration of the different stability of AuNPs with folded and unfolded adenosine binding DNA aptamer. (B) Photographs of (1) Au-Ado + adenosine, (2) Au-Ado, (3) Au-Ado + inosine, and (4) Au-AdoM + adenosine. 30 mM $MgCl_2$ was used in these solutions, and the photographs were taken 10 min after the addition of $MgCl_2$ to AuNP solutions. (C) UV−visible spectra of Au-Ado before (black curve) and after (blue curve) the addition of 30 mM $MgCl_2$. Red curve is the UV−visible spectrum for Au-Ado-Target with 30 mM $MgCl_2$. Spectra were taken 10 min after the addition of $MgCl_2$.

Table 1. Oligonucleotides Used in the Study[a]

names	sequences
Ado	5′-HS-ACCTGGGGGAGTATTGCGGAGGAAGGT-3′
T10Ado	5′-HS-TTTTTTTTTTACCTGGGGGAGTATTGCG-GAGGAAGGT-3′
AdoM	5′-HS-ACCTGTGGGAGTATTGCGTAGGAAGGT-3′
K	5′-GGTTGGTGTGGTTGG-SH-3′
KM	5′-GGTTGGTGTTTTTGG-SH-3′

[a] Ado and T10Ado refer to adenosine aptamers without and with T10 linker, respectively. AdoM is the adenosine aptamer mutant. K and KM represent K^+ aptamer and its mutant, respectively.

band in UV−visible spectrum (Figure 1C, blue curve). TEM experiments have further confirmed the color change was indeed induced by the aggregation of AuNPs (data not shown).

Although we could not provide the direct evidence for aptamer folding on AuNP surfaces at this stage, it is implicated: when control experiments used AuNPs modified with mutant aptamer (AdoM in Table 1) or used inosine as target, there was no colloidal stabilization effect (Figure 1B, vials 3 and 4). This confirmed that the colloidal stabilization effect was indeed due to specific aptamer folding upon binding its target (i.e., adenosine).

2.2. Effect of DNA Spacer and Aptamer Graft Density. To fully understand this phenomenon, we examined how the incorporation of a DNA spacer between AuNP and aptamer affects the additional colloidal stabilization provided by the aptamer folding on AuNPs. An adenosine aptamer with a T10 spacer (T10Ado, Table 1) was grafted onto AuNPs using the same Au−S chemistry to give a product referred to as Au-T10Ado. A similar MCH exchange reaction was then conducted, and the radioactivity study showed that there were ~160 and ~94 aptamers on each AuNP before and after MCH treatment, respectively. Similar to Au-Ado, Au-T10Ado com-

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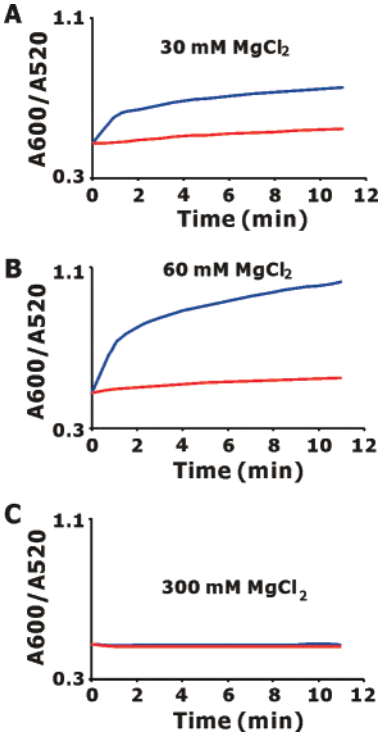

Figure 2. Effects of DNA spacer and aptamer graft density on the colloidal stabilization of AuNP attached with folded aptamers. The samples in (A), (B), and (C) are MCH-treated Au-Ado, MCH-treated Au-T10Ado, and non-MCH-treated Au-T10Ado, respectively. Red and blue curves are in the presence and absence of adenosine (1 mM), respectively. In (A) and (B), a rapid aggregation over the first 2 min was observed, followed by a slower aggregation phase. This may be because the aggregation is characterized by the color change. Once the AuNPs start to aggregate, the surface plasmon peak shifts from 520 nm to the longer wavelength (e.g., 600 nm), which leads to the decrease of A520 and increase of A600 nm. Therefore, A600/A520 showed a sharp increase at the initial aggregation stage. However, as aggregation continues, the surface plasmon peak may further shift to even longer wavelength (e.g., 700 nm). As a result, the increase of A600 (and therefore A600/A520) may not be as aggressive as that at the initial aggregation stage.[16]

plexed with adenosine (referred to as Au-T10Ado-Target) was more stable than Au-T10Ado toward salt-induced aggregation. Specifically, the stabilities of Au-T10Ado and Au-T10Ado-Target are $\leq$10 mM $MgCl_2$ and $\leq$60 mM $MgCl_2$, respectively.

To determine how the T10 spacer affects the colloidal stabilization effect resulting from aptamer folding, the aggregation kinetics of Au-Ado and Au-T10Ado with and without adenosine were investigated. As shown in Figure 2, the AuNP aggregation process was interpreted using the increase of the ratio of extinction at 600 and 520 nm (A600/A520) as a function of time. These experiments were conducted at salt concentrations such that Au-Ado-Target or Au-T10Ado-Target was barely stabilized. Thus, the $MgCl_2$ concentrations used for Au-Ado/Au-Ado-Target and Au-T10Ado/Au-T10Ado-Target were 30 and 60 mM, respectively. At their respective salt concentrations, both Au-Ado-Target and Au-T10Ado-Target showed little aggregation as indicated by insignificant A600/A520 increases (Figure 2A,B). By contrast, Au-Ado and Au-T10Ado both underwent rapid aggregation, and Au-T10Ado had a faster aggregation rate than Au-Ado (Figure 2A,B). This indicates that the stability difference between Au-T10Ado-Target and Au-T10Ado is larger than that of Au-Ado-Target and Au-Ado, which suggests the T10 spacer enhances the colloidal stabilization effect resulting from aptamer folding on AuNPs.

We reasoned that the efficacy of the effect of aptamer folding on colloidal stability could be affected by the available space on the AuNP surface, and therefore we examined how aptamer graft density influences colloidal stabilization. To tune aptamer graft density, aptamer-modified AuNPs were treated with MCH, a ligand exchange reaction where some thiol-modified aptamer strands are displaced by MCH molecules.[18a,b] Negatively charged DNA polymers are mainly responsible for the colloidal stability of DNA-modified AuNPs via both electrostatic and steric effects (see section 2.3 for details). Thus, MCH treatment affects AuNP colloidal stability by reducing the number of DNA molecules on each AuNP: these uncharged small molecules themselves have little (if any) effect on colloidal stability; however, the loss of stabilizing DNA molecules from the surface can facilitate aggregation of DNA-modified AuNPs.[8a,18c−f]

The highest aptamer graft densities were observed with AuNPs before the MCH exchange reaction. Stability studies showed that these non-MCH-treated AuNPs were much more stabilized toward salt-induced aggregation than the MCH-treated AuNPs. For instance, the stability of AuNP-T10Ado before MCH treatment is larger than 300 mM $MgCl_2$, evidenced by the fact that no color change or A600/A520 increase (Figure 2C) was observed at salt concentrations up to 300 mM $MgCl_2$. Non-MCH-treated Au-T10Ado showed similar aggregation kinetics with and without adenosine (1 mM) in all investigated salt concentrations (300 mM $MgCl_2$ in Figure 2C as an example), suggesting that the addition of adenosine did not significantly increase the colloidal stability of Au-T10Ado with high aptamer graft density ($\sim$160 aptamers/AuNP). By contrast, as demonstrated in Figure 2B, Au-T10Ado with lower graft density ($\sim$94 aptamers/AuNP) obtained by MCH treatment showed significant colloidal stability difference before and after the addition of adenosine.

In summary, AuNPs modified with aptamers with a DNA spacer or with a lower surface graft density showed higher stability contrast in the presence and absence of adenosine. This may be because the incorporation of spacers[20] or the decrease of aptamer graft density[8a,18a,b] provides more available space for biorecognition and therefore improves the aptamer folding or binding efficiency to adenosine. As demonstrated in section 2.1, AuNPs with folded aptamer-target complex appeared to be more stable than AuNPs with unfolded aptamers. Therefore, parameters (e.g., spacers and aptamer graft density) that can help the aptamer folding will enhance the AuNP colloidal stabilization.

2.3. Proposed Mechanisms. The effect of polyelectrolytes on colloidal stabilization/aggregation is, although extensively studied, highly complicated.[1] Colloids with charged biopolymers such as proteins and nucleic acids are more complex systems than most because of the unique inter- and intrabiomolecular interactions such as hydrogen bonding and hydrophobic interaction.

It was initially a surprise that AuNPs with folded aptamer structures were more stable than those with unfolded structures toward salt-induced aggregation. Maeda and co-workers found that AuNPs with rigid double-stranded (ds) DNA duplexes were less stable than AuNPs with flexible single-stranded (ss) DNA, presumably because of the entropic loss upon formation of the

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Table 2. Particle Sizes Obtained from DLS Measurement[a]

		non-MCH-treated Au-T10Ado		MCH-treated Au-T10 Ado			MCH-treated Au-Ado		
particles	bare AuNP	H_2O	$MgCl_2$	H_2O	$MgCl_2$	$MgCl_2$ + adenosine	H_2O	$MgCl_2$	$MgCl_2$ + adenosine
diameter (nm)	19.6 ± 0.1	29.4 ± 1.1	28.5 ± 0.3	29.3 ± 0.6	24.9 ± 0.8	27.3 ± 0.9	27.3 ± 1.1	24.2 ± 0.3	25.6 ± 0.5

[a] The concentrations of $MgCl_2$ and adenosine are 4 mM and 1 mM, respectively.

rigid DNA duplexes.[9] Guided by this finding, we expected to observe a similar phenomenon with AuNPs bearing rigid folded aptamer structures. As noted, this expectation was completely opposite to our experimental results.

Whether colloids are stabilized or undergo aggregation depends on the net potential of interparticle attraction and repulsion forces.[7] In the present work, the interparticle attraction force is van der Waals force, which is responsible for the AuNP aggregation.[7,21] The two major repulsion forces that contribute to AuNP stabilization are electrostatic and steric repulsion forces.[1,7,21]

Electrostatic repulsion results from the negatively charged phosphate groups along the DNA backbone. These negative charges, together with the counterions in the medium, form a repulsive electric double layer that stabilizes colloids against van der Waals attraction.[7] The thickness of the electric double layer is a measure of how far the repulsive potential extends from the colloid surface. A characteristic feature of the electrostatic repulsion force is that it is highly sensitive to the bulk ionic strength: the electrostatic repulsion force diminishes significantly at high salt concentration where electric double layer is highly suppressed.[7] This explains why AuNPs modified with aptamers (folded or unfolded) are stable at low salt concentrations but undergo aggregation at high salt concentrations. With respect to the AuNPs with folded and unfolded aptamers, one conceivable difference between these two systems might be the charge distribution. Like other polyelectrolyte-grafted colloids, the surface charges in the current system are extended along the DNA backbone from the surface.[1,21] Therefore, the conformation of DNA (such as folded and unfolded structures) on AuNP surface, which directly contributes to the surface charge distribution, could be a key factor determining their relative colloidal stability.

Steric stabilization (or polymeric stabilization)[1,21] is another key contribution to the repulsion forces in the current system. Macromolecules grafted on colloid surfaces impart a polymeric barrier that prevents colloids from coming close enough such that van der Waals attractive forces can dominate.[1,21] Steric stabilization is highly dependent on the thickness of polymer layer and surface graft density.[1,21] In general, thicker polymer layers and higher graft densities lead to more effective steric stabilization effect. This is exactly the case for the non-MCH-treated aptamer-modified AuNPs: they are stabilized in solutions up to 300 mM $MgCl_2$. Although it would be expected that electrostatic repulsion is significantly reduced under these conditions,[7] their stability appeared not to be sensitive to salt concentration. The stabilities of the MCH-treated aptamer-modified AuNPs are much lower, whether with folded or unfolded aptamers ($\leq$60 mM $MgCl_2$), presumably because of the decreased surface density of DNA on AuNPs produced by MCH exchange reactions.[18a,b] In principle, AuNPs bearing folded and unfolded aptamers share the same aptamer chain length and graft density. Nevertheless, they may adopt different conformations: the folded aptamer has a well-defined compact structure, whereas the unfolded aptamer chain may be more randomly configured. These conformational factors, which highly impact on the stabilization/aggregation of steric-stabilized colloids,[1,21] may also contribute to the different stability observed for AuNPs with folded and unfolded aptamers.

To examine the effect of AuNP surface-bound aptamer conformations on colloidal stability, dynamic light scattering (DLS) studies, a technique commonly used for characterizing colloidal properties such as size, shape, polydispersity, and thickness of surface coatings,[22] were conducted. The results are summarized in Table 2. Bare AuNP (before the modification of aptamer) has a diameter of 19.6 nm. The size of bare AuNP measured by DLS is larger than that obtained from TEM study (13.5 nm), which is mainly because DLS measures the hydrodynamic radius while TEM provides a more precise measurement of the hard AuNP core.[23] After modification with aptamer-T10Ado (aptamer-Ado behaved similarly with T10Ado in DLS studies and therefore will not be discussed here in detail) and MCH treatment, Au-T10Ado is 29.3 nm in diameter in ddH_2O, which corresponds to a thickness of DNA layer of 4.8 nm. By contrast, Au-T10Ado has a diameter of 24.9 nm in salt solution (4 mM $MgCl_2$, 20 mM Tris-HCl, pH 7.5), with a DNA coating thickness of 2.6 nm. The reduced thickness of the DNA layer of Au-T10Ado in the salt solution can be explained as follows: in ddH_2O, negative charges distributed along the DNA backbones create a significant electrostatic repulsive force between DNA chains.[21] This repulsion facilitates DNA chain extension, which results in a thicker DNA layer.[21] In contrast, the addition of salt largely suppresses the electric double layer and reduces the electrostatic repulsion between DNA chains.[21] DNA molecules therefore tend to collapse back toward the AuNP surface, leading to smaller particle size and DNA layer thickness.[21] This size shrinkage appears to be significant only for the MCH-treated Au-T10Ado. For non-MCH-treated ones, the particle diameters are 29.4 and 28.5 nm in ddH_2O and salt solution, respectively. This is presumably due to insufficient room for the DNA layer to collapse in the case of non-MCH-treated Au-T10Ado with high surface graft density. Importantly, the AuNP with folded aptamers, obtained by mixing MCH-treated Au-T10Ado and adenosine (1 mM) in 4 mM $MgCl_2$, 20 mM Tris-HCl, pH 7.5, has a diameter of 27.3 nm. The height of folded aptamer structure was therefore determined to be 3.8 nm, which is in good agreement with the theoretical value obtained from the previous NMR study.[19b] These results indicate that the height (or thickness) of folded aptamer structure is larger than that of unfolded (but largely collapsed) aptamers in a given

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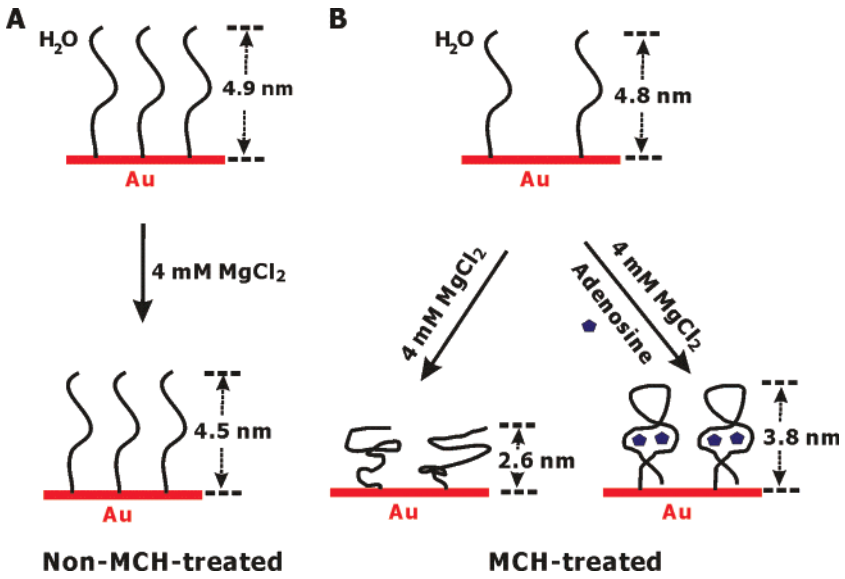

Figure 3. Schematic illustration of aptamer conformational behaviors on (A) non-MCH-treated Au-T10Ado in H_2O and 4 mM $MgCl_2$, and (B) MCH-treated Au-T10Ado in H_2O and 4 mM $MgCl_2$ (with and without adenosine).

salt solution. To assess the size of individual particles, the salt concentration (4 mM $MgCl_2$, 20 mM Tris-HCl) used for DLS experiments was relatively low (but sufficient for the aptamer folding) and AuNPs with folded and unfolded aptamers are both stabilized. Higher salt concentration may initiate the aggregation process for AuNP attached with unfolded aptamers, which makes the DLS measurement of individual particle size difficult. However, because of the nature of unfolded aptamer structures, it is reasonable to predict that the size of AuNPs with unfolded aptamers (no target bound) may undergo further shrinkage at higher salt concentrations. By contrast, the size of AuNP with folded aptamer structures would not be significantly affected by higher salt concentrations (e.g., 30 mM $MgCl_2$) because of their rigid and compact structures. DLS experiments support this theory: the size of Au-Ado (or Au-AdoT10) with folded aptamer in the presence of adenosine at 30 mM $MgCl_2$ (or 60 mM $MgCl_2$) is 25.3 $\pm$ 0.3 nm (or 27.6 $\pm$ 0.6 nm), which is comparable to their sizes at lower $MgCl_2$ concentrations (i.e., 4 mM) (Table 2).

The heights (or thickness) of aptamer structures in different AuNP samples are illustrated in Figure 3. From the electrostatic stabilization point of view, the repulsive force is dependent on the thickness of electric double layer.[7] For AuNP with folded aptamers, the negative charges (and thus formed electric double layer) may be more extended from the surface than those of AuNP with unfolded aptamers at higher salt concentrations owing to their different heights on surface (Figure 3B). This could be why AuNPs with folded aptamer structures are more stable toward salt-induced aggregation.

With respect to the steric stabilization, it is known that thicker coated polymer layers and higher surface graft densities yield more effective repulsive forces.[1,21] This is certainly the case for non-MCH-treated Au-T10Ado that can be stabilized even in solutions up to 300 mM $MgCl_2$, where electrostatic repulsion is significantly diminished. The size measurements obtained from DLS support this theory. Only a small decrease in thickness was found for non-MCH-treated Au-T10Ado in the salt solution (Figure 3A), which is presumably due to the limited space available for the aptamer to collapse. Therefore, AuNPs are always coated by a relatively thick, dense DNA layer regardless of bulk salt concentration. This DNA layer serves as an effective polymeric stabilizer to prevent AuNP aggregation. This steric stabilization should play a dominating role at high salt concentration where electrostatic repulsion is negligible. For MCH-treated AuNPs (with folded or unfolded aptamers), their stability dropped significantly, as a result of the decrease of aptamer graft density and thickness (Figure 3B). MCH-treated AuNPs with unfolded aptamers showed a lower graft thickness in salt solution than AuNPs with folded aptamers (Figure 3B). Therefore, compared to the AuNPs with folded aptamers, the AuNPs with unfolded (but largely collapsed) aptamers can more readily come close to an interparticle distance such that van der Waals forces will give a net attractive potential. This might be another reason the AuNP with folded aptamer is more stable than that with unfolded aptamers.

In summary, the conformations adopted by aptamers on the AuNP surface seem to be a key factor in determining their relative colloidal stability. Although the suggested mechanisms can explain the obtained experimental results, we acknowledge that we are still far from having a precise understanding for this unique phenomenon because of its complex nature.

We have only considered thus far the effect of Mg^{2+} on aptamer conformational change through screening the charges associated with phosphate groups on DNA backbone. Meanwhile, the Mg^{2+} may target other binding sites associated with nucleobases, which may or may not affect the secondary (or tertiary) structures of unbound and bound aptamers. For unbound aptamers in the absence of adenosine, the prediction of aptamer structure at different Mg^{2+} concentrations (0−60 mM) using *mfold* software[19a] did not show any specific secondary structures. This suggests that, without a target, the aptamer adopts a rather randomly distributed form, and the addition of $MgCl_2$ does not contribute to any specific aptamer secondary structure formation other than collapsing the aptamer by screening charges associated with aptamer. A certain amount of Mg^{2+} (e.g., 4 mM) is required for the folding of the aptamer in the presence of adenosine. Once folded into a rigid, compact structure, further addition of Mg^{2+}, under studied conditions (Mg^{2+} concentra-

tion: 4−60 mM), seems to have little (if any) effect on the secondary (or tertiary) structure of the folded aptamer/target complex. This assessment is supported by the present work where we showed above that Mg^{2+} concentration has little effect on the size of AuNPs with folded aptamers, as well as by a previous study[12j] in which the adenosine aptamer functions similarly at Mg^{2+} concentrations ranging from 4−60 mM. Nevertheless, there is no quantitative data or precise model available on how Mg^{2+} interacts with the adenosine aptamer before and after folding with adenosine, or how that affects aptamer secondary (or tertiary) structures. These questions represent our future research interests.

Since the amount of counterions (e.g., Mg^{2+}) and H_2O molecules associated with aptamers before and after folding with adenosine might be different,[24] the electrophoretic properties of aptamers in folded and unfolded states could be another factor that affects the colloidal stability. Unfortunately, our preliminary results obtained from ζ potential measurements and agarose gel electrophoresis[25] could not differentiate the AuNPs with folded and unfolded aptamers (data not shown). Quantitative calculations[24] of the amount of charges associated with aptamers before and after folding will be conducted in future work. Furthermore, we could not provide the quantitative data for the entropic factors associated with target-induced specific aptamer folding and salt-induced nonspecific aptamer collapse. In general, the formation of rigid polymer structures or the loss of polymer flexibility on colloid surface results in an entropic penalty that can destabilize the colloids.[9] In the present work, it seems likely that even if there is more entropic loss in the aptamer folding process than that associated with aptamer collapse induced by salt, this entropic penalty can be overcome by the electrostatic and steric contributions mentioned above.

The interparticle attraction forces that cause AuNP aggregation have thus far been attributed to van der Waals attraction. However, because of the particular nature of DNA, one may argue that other interparticle bridging forces (e.g., hydrogen bonding, hydrophobic force) may also exist. We think these forces are less likely than key factors that dominate the AuNP aggregation in this study for the following reasons. First, for the AuNP aggregation induced by the complementary DNA bridging, at least 12 base pairs are required to provide a sufficient hybridization.[4a] There is no such designed base pairing in the present study. More importantly, the AuNP aggregation induced by interparticle DNA bridging is known as a relatively slow process:[4a] it generally requires hours to observe a red-to-purple color change, and an annealing process controlled by temperature is normally applied. In the present work, an instant color change associated with AuNP aggregation was observed at room temperature right after the addition of salt. This rapid aggregation is a characteristic feature of van der Waals attraction induced colloidal aggregation.[7] Furthermore, we studied the aggregation process at an elevated temperature (Figure 4). If the aggregation were indeed induced by interparticle DNA base pairing, it would be expected that the aggregation process would be diminished as the interparticle bonds (e.g., hydrogen bonds) are broken at higher temperatures. In contrast, we found that

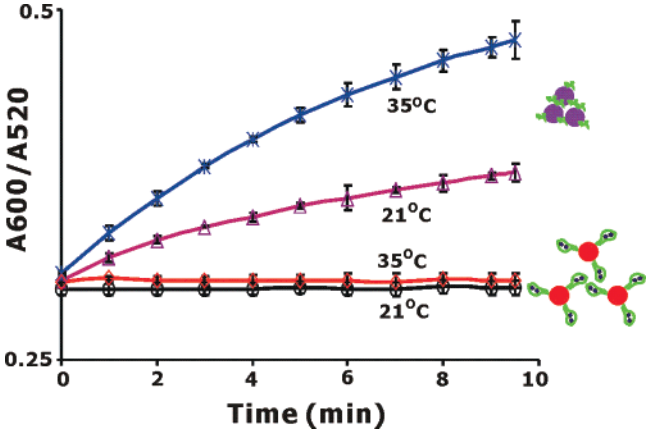

Figure 4. Temperature effect on aggregation kinetics of Au-T10Ado with and without adenosine.

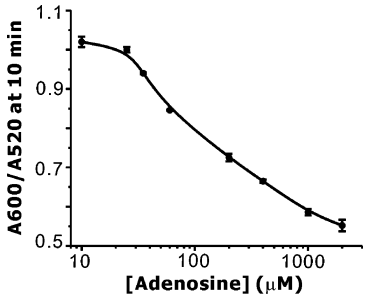

Figure 5. Inhibition of red-to-purple color change (A600/A520) as a function of the concentration of adenosine.

the AuNP aggregation is more rapid at elevated temperatures, a typical phenomenon found in van der Waals force controlled aggregation: higher temperature causes a faster colloid collision rate and therefore more rapid aggregation.[7] This suggests that AuNP aggregation in the present study is less likely due to interparticle bridging forces.

2.4. Biosensors for Adenosine, Adenosine Deaminase (ADA), and ADA Inhibitor. One of the advantages of using AuNP as a model colloid is that aggregation (or redispersion) processes are associated with a visible red-to-purple (or purple-to-red) color change, which allows the system to be directly transformed into colorimetric biosensors. As shown in Figure 5, A600/A520 at 10 min after the addition of adenosine was plotted as a function of adenosine concentration. Since aptamer folding in the presence of adenosine stabilizes AuNPs (decreases the aggregation rate), the addition of adenosine inhibited the color change (or red shift in the UV−vis spectra). Importantly, the degree of the inhibition of color change is directly related to the amount of adenosine used, and a standard target concentration titration curve was established (Figure 5). The detection range of adenosine in the current assay is ~20−2000 μM. Furthermore, the dissociation constant (K_d), which is 263 μM, was obtained on the basis of the binding curves in Figure 5 (Figure S2 in Supporting Information for the K_d calculation). This K_d value, which reflects the aptamer affinity on the AuNP surface, is larger than that (~6 μM[19c]) obtained from free aptamers in solution. This is presumably because aptamers on surfaces are less accessible to their targets than those in solution.

As demonstrated previously, the stabilization effect associated with aptamer folding is specific to adenosine, but not to other control molecules such as inosine. This provided a simple colorimetric assay for the detection of ADA, an enzyme that

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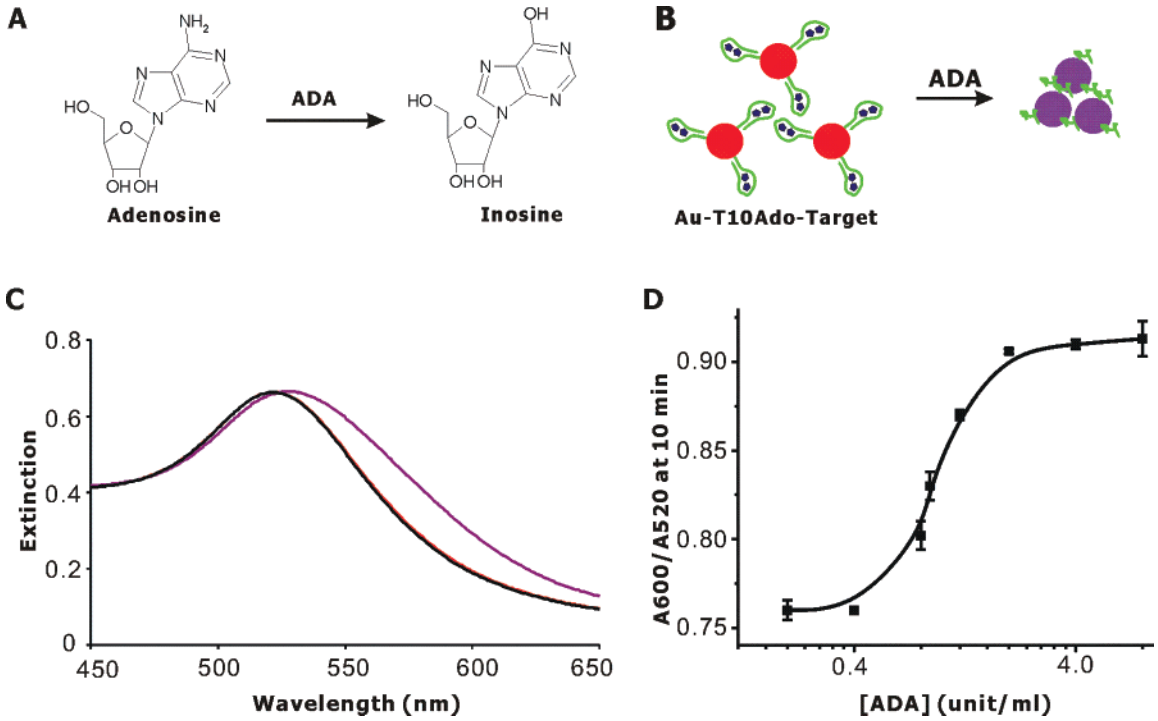

Figure 6. (A) Adenosine can be converted into inosine by ADA. (B) Aggregation of Au-T10Ado-Target can be induced by ADA that converts adenosine to inosine. (C) UV−visible spectra of Au-T10Ado-Target (black curve), Au-T10Ado-Target + ADA (purple curve), and Au-T10Ado-Target + ADA + inhibitor (red curve). Spectra were taken 10 min after mixing all the ingredients in each sample. (D) A600/A520 at 10 min after the addition of ADA is plotted as a function of the ADA concentration.

converts adenosine to inosine (Figure 6A) and is of great biological and clinical importance.[26] In the ADA detection assay, AuNPs with folded aptamer/adenosine complex were used as starting materials. They are stabilized at a relatively high salt concentration, and these well-dispersed AuNPs exhibit a red color. The addition of ADA (1 unit/mL) that converts adenosine into inosine destabilized AuNPs and resulted in a red-to-purple color change and a red shift on UV−vis spectra (Figure 6B,C). The degree of color change is directly related to the ADA concentration used in the assay (Figure 6D). The detection range of ADA under investigated conditions ranges from 0.4 to 4 units/mL. Meanwhile, the addition of *erythro*-9-(2-hydroxy-3-nonyl)-adenine (400 μM), a known inhibitor of ADA,[26a] significantly inhibited the color change (Figure 6C, red curve). This colorimetric assay can therefore find potential applications in the diagnostic of ADA-related diseases and drug discovery.

2.5. Aggregation Reversibility. One fascinating feature of the current system is that the aggregation process of aptamer-modified AuNPs at high salt can be halted by the addition of adenosine, and aggregation can even be partially reversed using adenosine. This process, depicted in Figure 7A, was monitored by A600 in UV−visible spectrum (Figure 7B). The addition of $MgCl_2$ (60 mM) to aptamer-modified AuNPs (3 nM) led to aggregation and a gradual increase of A600. Adenosine (1 mM) was added during the aggregation process, and it resulted in a partial (60−70%) redispersion of aggregated AuNPs as indicated by the drop in A600.

The reversibility was further illustrated by introducing multiple aggregation/redispersion cycles that were achieved by incorporating ADA in the system. As illustrated in Figure 7C, AuNPs with folded aptamer/adenosine complex (Au-T10Ado-Target; 3 nM) were initially stabilized at 60 mM $MgCl_2$. The addition of ADA (4 units/mL) that converted adenosine to inosine led to AuNP aggregation. The addition of adenosine (1 mM) to the system partially redispersed the aggregates to stabilized AuNPs again. Since ADA was always present in the system, adenosine was being continuously consumed, which eventually resulted in AuNP aggregation. This process was monitored at A600 (Figure 7D). Note that, under the current investigated conditions, this aggregation/redispersion process can only operate for a few cycles and it took longer time for ADA to consume adenosine to reach the same aggregation level than it required in the previous cycle. This is presumably due to the denaturation of ADA during the process or the inhibition effect from accumulated inosine product.[26a]

2.6. Generality. To test the generality of the colloidal stabilization effect observed for AuNP with folded aptamers, we investigated another DNA aptamer that specifically binds to K^+ and folds into a well-characterized tertiary structure known as G-quartet.[27] Similarly with the adenosine aptamer/AuNP system described above, AuNPs with the K^+ folded aptamer were more stable than those with its unfolded structure at high salt concentration (Figure 8). Using a similar strategy as described in the adenosine-sensing assay, we obtained a quantification curve where A600/A520 at 10 min after the addition of K^+ was plotted as a function of K^+ concentration (Figure 8B). The K_d for K^+ aptamer on AuNP was then estimated to be ~15 mM (Figure S3 in Supporting Information). Note that the control experiments where Na^+, Li^+, or aptamer mutant sequence (KM, Table 1) was used did not show

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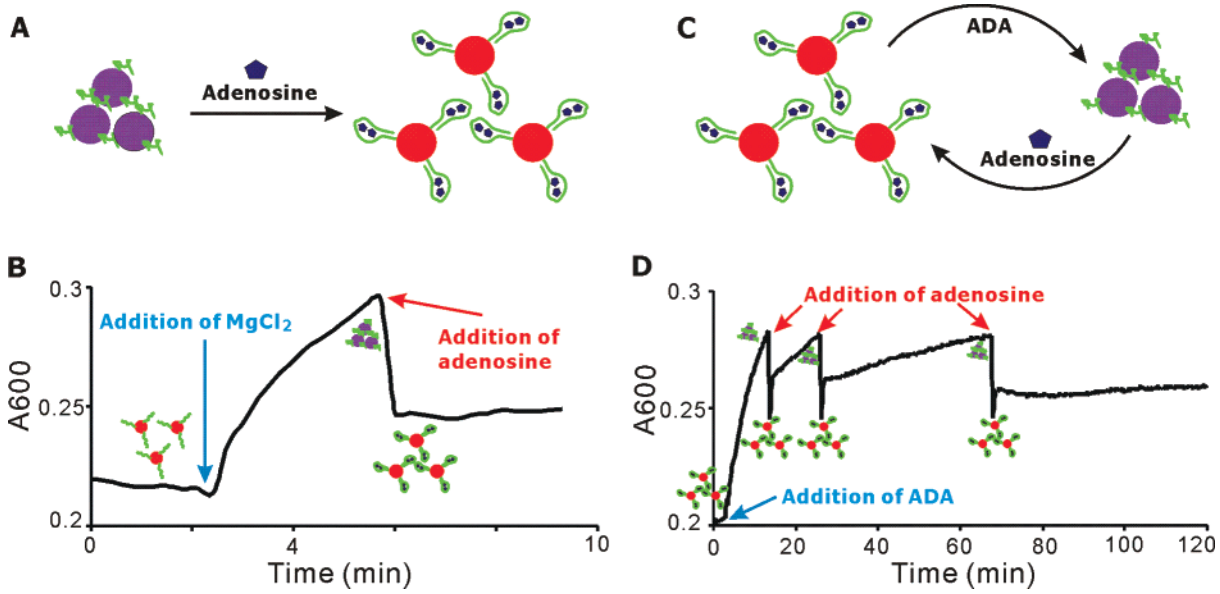

Figure 7. (A) Addition of adenosine during the aggregation process of aptamer-modified AuNPs can halt the aggregation and partially redisperse some of the aggregates. This process was monitored by the change of A600 in UV-visible spectroscopy in (B). (C) Schematic illustration of the operation of multiple aggregation/redispersion cycles: ADA converts adenosine to inosine, which causes the aggregation of Au-T10Ado-Target at high salt concentration (60 mM $MgCl_2$). The addition of adenosine redisperses the formed aggregates. Because ADA is always present in the system, the newly added adenosine will be gradually converted into inosine again, resulting in AuNP aggregation. This aggregation/redispersion process is monitored by A600 in UV−visible spectra (D).

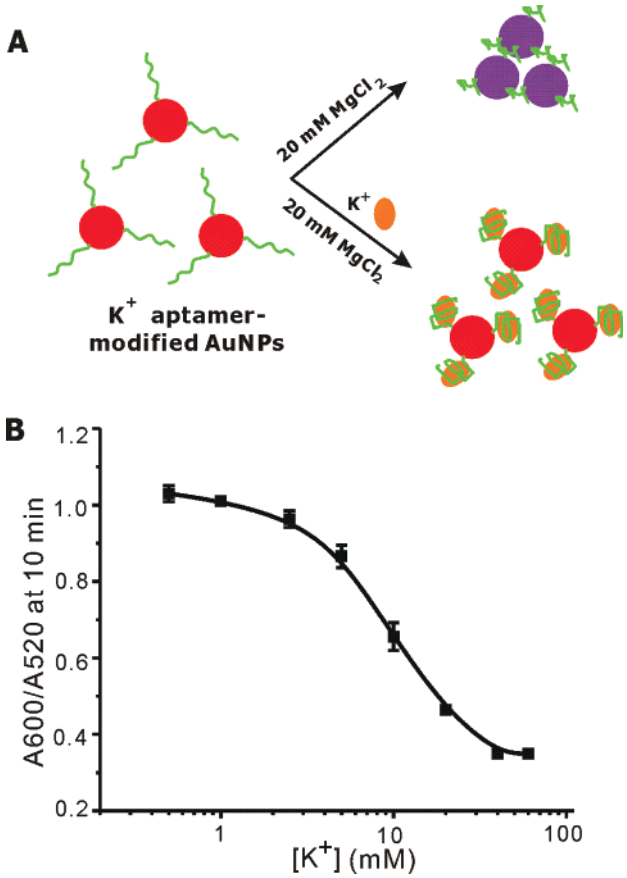

Figure 8. (A) Schematic illustration of the different stability for AuNPs with folded and unfolded K^+ binding aptamer. (B) To quantify K^+, A600/A520 at 10 min after the addition of K^+ is plotted as a function of the K^+ concentration. 20 mM $MgCl_2$ was used in these assays.

significant stabilization effect (data not shown), indicating that the stabilization effect is indeed due to the specific K^+ aptamer folding.

3. Conclusions

This work serves as a first step toward understanding the nature of DNA aptamer folding on AuNP surfaces and its influence on colloid properties. The discoveries in this exploration would not only complement traditional polymer/colloid theories, but also could directly lead to a number of applications in nanobiotechnology, including biosensors and nanoassemblies. In addition to the two different aptamers investigated in the present work, DNA and RNA can have other functions (e.g., catalytic capability) and can adopt a number of other structures such as hairpin and i-motif.[12a] The study of the behaviors of these functions and structures on surfaces should provide more insight to the complex nature of nucleic acid/colloid systems, which could lead to broader applications. Meanwhile, given the difficulties associated with the prediction of nucleic acid secondary and tertiary structures, the facile colorimetric AuNP-based assays may be applied for the study of nucleic acid folding and interpretation of their structural information.[28]

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Supporting Information Available: Experimental section, 3D model for folded DNA aptamer structure, and calculation of K_d for adenosine and K^+ aptamers on AuNP surface. This material is available free of charge via the Internet at http://pubs.acs.org.

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