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Paper-Based Bioassays Using Gold Nanoparticle Colorimetric Probes

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The majority of bioassays utilize thermosensitive reagents (e.g., biomolecules) and laboratory conditions for analysis. The developing world, however, requires inexpensive, simple-to-perform tests that do not require refrigeration or access to highly trained technicians. To address this need, paper-based bioassays using gold nanoparticle (AuNP) colorimetric probes have been developed. In the two prototype DNase I and adenosine-sensing assays, blue (or black)-colored DNA-cross-linked AuNP aggregates were spotted on paper substrates. The addition of target DNase I (or adenosine) solution dissociated the gold aggregates into dispersed AuNPs, which generated an intense red color on paper within one minute. Both hydrophobic and (poly(vinyl alcohol)-coated) hydrophilic paper substrates were suitable for this biosensing platform; by contrast, uncoated hydrophilic paper caused "bleeding" and premature cessation of the assay due to surface drying. The assays are surprisingly thermally stable. During preparation, AuNP aggregate-coated papers can be dried at elevated temperatures (e.g., 90 °C) without significant loss of biosensing performance, which suggests the paper substrate protects AuNP aggregate probes from external nonspecific stimuli (e.g., heat). Moreover, the dried AuNP aggregate-coated papers can be stored for at least several weeks without loss of the biosensing function. The combination of paper substrates and AuNP colorimetric probes makes the final products inexpensive, low-volume, portable, disposable, and easy-to-use. We believe this simple, practical bioassay platform will be of interest for use in areas such as disease diagnostics, pathogen detection, and quality monitoring of food and water.

There is a general need to develop simple, rapid, and inexpensive detection assays for diagnostic applications, monitoring of food and water contamination by bacteria and other pathogens, environmental analysis, among others. Currently, most analyses are performed under laboratory conditions, frequently with the assistance of expensive instruments and trained personnel. Exceptions include home pregnancy tests and glucose sensors that utilize "test strips", but generally speaking, specific bioassays are not suited for quick and on-site tests for average users. Thus, the development of test strip formats for biosensing will be welcome in general and, particularly so in the developing world, which does not have ready access to laboratory facilities. The problem of thermal stability of the assay is particularly acute in hot climates: biomolecules frequently undergo thermal denaturation at elevated temperatures (e.g., above 45 °C), leading to loss of sensitivity in traditional bioassays.

Paper, a cellulose fiber web with high surface area, is abundant and inexpensive; bleached paper also provides a bright, high contrast background for colorimetric assays. The structure and porosity of paper are highly controllable,[1−12] and the surface nature of paper-based materials is readily modified. Furthermore, reagents can be easily coated or patterned on paper by high-speed spotting, coating, and printing. Therefore, paper-based bioassays, which are low-volume (in terms of both biosensing reagents on paper and target analyte solution required), portable, disposable, and easy-to-use, have been applied for separation and bioanalysis applications.[1−12] For example, Whitesides and co-workers have recently developed patterned paper-based microfluidic systems for biodetection.[10−12]

Colorimetric bioassays as dipsticks or in a chromatographic format would be ideal for the developing world: the visual colors (or color changes) can be observed by naked eyes, and therefore no affiliated instruments are required. Gold nanoparticles (AuNPs) are good materials for colorimetric bioassays. Small AuNPs

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Table 1. Names and Sequences of DNA Oligonucleotides Used in This Study

name	sequence
S1	3′-thiol-TTTTTTTTTTCTAGCTGTACTACCGTTCGAACATCACCTAGC-5′
S2	5′-GATCGACATGATGGCAAGCTTGTAGTGGATCGTTTTTTTTTT-thiol-3′
S3	3′- thiol-AAAAAAAAAAAATGAGTAGACACT
S4	5′-thiol-CCCAGGTTCTCT-3′
APT	5′-ACTCATCTGTGAAGAGAACCTGGGGGAGTATTGCGGAGGAAGGT-3′

(normally 10−50 nm in diameter) have an intense red color in water due to the localized surface plasmon resonance.[13−15] Their extinction coefficients are significantly higher than those of common organic dyes.[14,15] The intensive red color has allowed AuNPs to be used as colorimetric labels in immunochromatographic test.[6−9] Importantly, the colors of AuNP solution are highly dependent on the interparticle distance of AuNPs.[14,15] When individual spherical AuNPs come into close proximity (the center-to-center distance is normally smaller than 2.5 times of the diameter of AuNPs), the surface plasmon of individual AuNPs combines (interparticle plasmon coupling), which results the change of colors from red to blue. The interparticle distance-dependent AuNP colors have been extensively applied in solution phase for colorimetric biodetection,[16−24] but have not received much attention in solid-phase applications,[25−27] which presumably are more suited for practical applications. Liu and Lu have demonstrated a dipstick assay where AuNP aggregates immobilized on a lateral flow device are broken into red-colored dispersed AuNPs upon addition of target analytes in flow buffer.[27]

In this work, we examined the ability of target analytes to change the interparticle distance of AuNPs (and therefore the colors) on paper substrates, and further investigated the thermal stability of the paper supported tests. Two assays were examined in detail, an endonuclease (DNase I) enzyme assay, and an aptamer-based adenosine detection assay. In both cases, DNA-cross-linked AuNP aggregates were first spotted on paper, and a red color signal was "turned on" in the presence of the DNase I or adenosine, respectively, by redispersion of the AuNP aggregates on papers of different hydrophilicity or hydrophobicity.

1. EXPERIMENTAL SECTION

1.1. Materials. Trisodium citrate, $HAuCl_4$, adenosine, ATP, inosine, poly(ethylene glycol) (PEG) (MW ≈ 2000), Tween 20, poly(vinyl alcohol) (PVA; MW = 130,000) were purchased from Sigma and used as received. Sucrose was obtained from BioShop Canada Inc. Thiol-modified and unmodified DNA samples were obtained from Keck Biotechnology Resource Laboratory at Yale University and Integrated DNA Technologies (IDT), respectively. All DNA samples were purified by standard 10% denaturing (8 M urea) polyacrylamide gel electrophoresis (PAGE), and the concentrations were determined spectroscopically. DNase I was purchased from MBI Fermentas. Hydrophilic filter paper (Whatman #1) was purchased from VWR. Hydrophobic paper (#40 Pointflex coated paper) was provided by Stora Enso as a gift.

1.2. Preparation of Citrate-Capped AuNPs. 13-nm citrate-capped AuNPs were prepared according to published protocols.[28−30] Trisodium citrate (25 mL, 38.8 mM) was added to a boiling solution of $HAuCl_4$ (250 mL, 1 mM). Within several minutes, the color of the solution changed from pale yellow to deep red. The mixture was allowed to heat under reflux for another 30 min to ensure complete reduction before it was slowly cooled to room temperature, and stored at 4 °C before use. The concentration of these AuNPs was ∼13.4 nM as determined by UV−visible spectroscopy, based on an extinction coefficient of $2.7 \times 10^8\ M^{-1}\ cm^{-1}$ at $\lambda = 520$ nm for 13-nm AuNPs.[29]

1.3. Preparation of DNA-Modified AuNPs. In a typical protocol for coupling DNA to AuNPs,[29,30] thiol-modified DNA (S1, S2, S3 or S4, see Table 1 for their sequences) (280 μL, 6.6 μM) were added to citrate-modified AuNP solution (600 μL, ∼13.4 nM). Subsequently, Tris-HCl (10 μL, 1 M, pH 8.3) and aqueous NaCl (90 μL, 1M) were added to the mixture and incubated for another 18 h. Additional Tris-HCl (5 μL, 1M, pH 8.3) and aqueous NaCl (50 μL, 5 M) were added and the mixture was further incubated for 18 h at room temperature. The final solution was separated in a centrifuge at 22000*g* for 20 min. The precipitated DNA-modified AuNPs were washed twice with 600 μL of Tris-HCl buffer (50 mM, pH 8.3, NaCl 0.3 M) by centrifugation, and finally redispersed in 700 μL of the same buffer. The final concentration of DNA-modified AuNPs is ∼12 nM, assuming no particles were lost during the preparation process.

1.4. DNase I Assay. 1.4.1. Preparation of DNA-Cross-Linked AuNP Aggregates. Typically, S1 and S2-modified AuNPs (50 μL each, ∼12 nM) were mixed in Tris-HCl buffer (50 mM, pH 8.3, NaCl 0.3 M). The solution was heated at 70 °C for 2 min and allowed to cool at room temperature for 15 min. Then the mixture was stored at 4 °C for 6 h. A blue colored solution was observed, followed by AuNPs sedimentation.

1.4.2. Preparation of Paper Substrates. Hydrophobic paper was used as received. Hydrophilic paper was immersed in

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poly(vinyl alcohol) (PVA) (or PEG, or Tween 20) solution (1% (w/v)) for 10 s and then dried at 60 °C for 10 min.

1.4.3. Spotting DNA-Cross-Linked AuNP Aggregates on Paper. Prior to spotting on various paper surfaces, aggregated AuNPs were spun down at 2000*g* for 1 min, and washed once by DNase I buffer (10 mM Tris-HCl, pH 7.5, 2.5 mM $MgCl_2$ and 0.5 mM $CaCl_2$). The AuNP aggregate pellets were then redispersed in DNase I buffer (20 μL) using a pipet. The colloidal solution of aggregated AuNPs was blue. Note that these aggregates were very unstable in solution: they quickly precipitated to form blue (or even black)-colored pellets in the bottom of test tube. Thus, AuNP aggregate solution was agitated with a pipet immediately before spotting onto paper (typically a 1 μL solution containing AuNP aggregates was spotted). The AuNP aggregate-coated paper was then dried at room temperature for ~10 min unless stated otherwise.

1.4.4. DNase I Detection. Sample solutions (1 μL each) with various amount of DNase I in DNase I buffer were directly applied onto AuNP aggregate spots on paper. The color change was monitored using a HP scanner (ScanJet 5370C).

1.5. Adenosine Assay. The preparation of the adenosine-sensing assay was similar to that of DNase I except: (1) an adenosine aptamer strand (APT, Table 1) (100 nM) was used as a cross-linker to bridge S3 and S4 modified AuNPs to form aggregates; (2) after the preparation and washing steps, AuNP aggregates were dispersed in a buffer containing Tris-HCl (25 mM, pH 8.3), 200 mM NaCl and 10% (w/v) sucrose; (3) hydrophilic paper was used as received without PVA (or PEG, or Tween 20) coating because sucrose used in this case can play the similar roles; 4) for adenosine detection, sample solutions (1 μL each) with various amount of adenosine in a buffer containing (25 mM Tris-HCl, pH 8.3, 100 mM NaCl) were applied.

1.6. Scanning Electron Microscopy (SEM). The samples were coated with a thin platinum layer and SEM photographs were obtained on a JEOL JSM-7000F scanning electron microscope with 5 kV accelerating voltage and a probe distance of 6 mm.

2. RESULTS AND DISCUSSION

2.1. DNase I-Sensing Assay. AuNP aggregation occurs spontaneously upon mixing DNA-modified AuNPs and cDNA-attached AuNPs, a process driven by interparticle DNA hybridization.[31] These intensely blue-colored AuNP aggregates were previously used as colorimetric probes in solution phase for the detection of endonuclease (e.g., DNase I), an enzyme that cleaves double-stranded (ds) DNA cross-linkers and therefore dissociate AuNP aggregates into well-dispersed, red AuNPs.[31] Given that endonucleases play key roles in biological process such as DNA replication, repair and recombination of nucleic acids, there is a general need to develop facile biosensing assays for the detection of endonuclease activity and inhibition.[31] These assays can not only help the diagnosis of endonuclease-associated diseases but also facilitate the discovery of drugs that inhibit these enzymes.[31]

In the present work, DNA-cross-linked AuNP aggregates were prepared by mixing AuNPs modified by S1 and S2 (Figure 1, panels A and B). After annealing, the AuNP aggregates were isolated and washed using centrifugation. Although very brief (2000*g* for 1 min) centrifugation was applied, observable large aggregates with a blue or even black color formed (presumably due to the large size of the resulting gold aggregates, Figure 1C, left tube). While agitation by a pipet can partly break these large aggregates into smaller fragments, resulting temporarily in a more homogeneous solution, the colloidal solution was unstable and the aggregates precipitated rapidly (<1 min). To ensure the DNA molecules in these large-sized aggregates were still accessible by DNase I, we first tested the assay performance in a solution phase. It was found that the smaller aggregate fragments could be readily redispersed upon addition of DNase I (0.1 unit/ μL), which generated a red color in <1 min (Figure 1C, right tube).

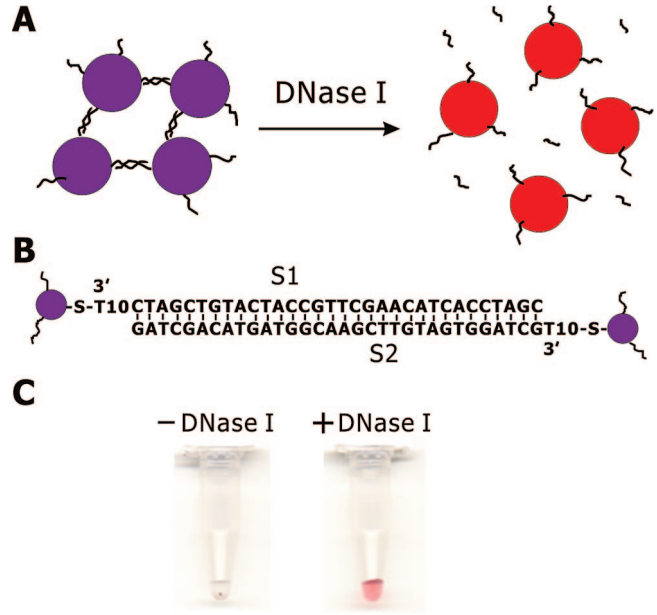

Figure 1. Schematic presentation of a DNase I-sensing system. (A) AuNPs modified with S1 and S2 are cross-linked via DNA hybridization to form blue-colored aggregates. The addition of DNase I that cleaves DNA cross-linkers breaks aggregates into well-dispersed AuNPs, which appear a red color. (B) The sequences of S1 and S2. (C) Solution-phase DNase I assay. Left tube: a clump of AuNP aggregates in DNase I buffer without DNase I. Right tube: DNase I (0.1 unit/μL) was added to the left tube. The right tube was scanned at 20 s after the addition of DNase I.

2.1.1. Hydrophobic Paper. The dispersed AuNP aggregates were spotted onto a variety of paper substrates with distinct surface hydrophilicity. An AuNP aggregate solution was first agitated by a pipet and then 1 μL of this solution was spotted onto hydrophobic paper: the liquid drop did not spread on the hydrophobic surface, and dried at room temperature over ~10 min (see section 2.1.3 for drying condition studies). The black, DNA-modified AuNP aggregate fragments on the paper surface (Figure 2A) adhere strongly to hydrophobic paper surface, presumably due to the physical adsorption forces (e.g., van der Waals forces).

The addition of DNase I solution (1 μL, 0.1 unit DNase I, 10 mM Tris-HCl, pH 7.5, 2.5 mM $MgCl_2$ and 0.5 mM $CaCl_2$) to the dried AuNP aggregate spots led to the formation of a liquid drop that did not spread on the hydrophobic paper surface. The color of the liquid drop rapidly turned to red (a few seconds, Figure 2B). This suggests that DNase I can successfully process the AuNP aggregates on a paper substrate, and the dispersed AuNPs

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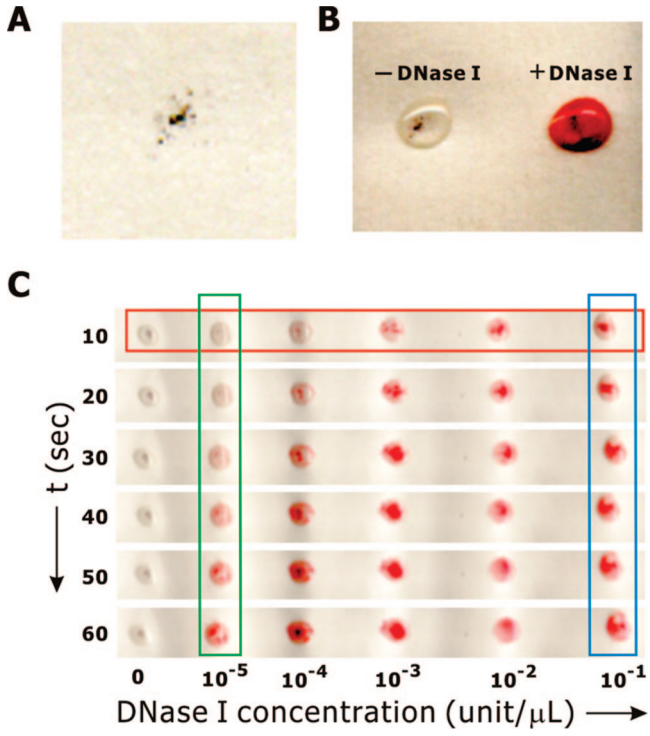

Figure 2. DNase I assay on hydrophobic paper. (A) Black AuNP aggregates were left on hydrophobic paper after drying. (B) 1 μL of DNase I buffer solutions with (0.1 unit/μL) and without DNase I were applied to dried AuNP aggregates on hydrophobic paper. Images were taken at 20 s after the addition of a relevant solution. (C) DNase I-sensing assays on hydrophobic paper as functions of assay time and DNase I concentration. As expected, more red color is observed when more target analyte is added (red box) or longer assay time was utilized (green box). Note that the color intensity eventually reaches a plateau. For example, when a target analyte at a high concentration (10^{-1} unit/μL) was applied (blue box), a signal maximum was reached very quickly (~10−20 s), after which time no further color change was observed. Note that there is a technique-related issue that will have to be addressed in future work. The amounts of AuNP aggregates (starting material) spotted on paper strips for each assay in Figure2C are not exactly the same due to the heterogeneous nature of the AuNP aggregates. This explains why the assay at 10^{-4} unit/μL DNase I appeared with the maximum color intensity: it is simply because there were initially more AuNP aggregates than in the other samples.

diffuse into the solution phase to generate a red color. By contrast, the control experiment where the same buffer was applied without DNase I did not show any observable red color (Figure 2B). The AuNP aggregation/dispersion states (before and after the addition of DNase I) were investigated by SEM experiments (Figure S1, Supporting Information) which confirmed that the appearance of a red color is indeed due to the redispersion of AuNPs on paper substrates. Importantly, the red color continued to develop as enzymatic cleavage proceeds (Figure 2C) until the liquid drop was completely dry. Furthermore, the rate of color development (or amount at a specific time point) was dependent on the DNase I concentration used in the experiment (Figure 2C). Using the conditions described in the experimental section, 10^{-5} unit/μL DNase I was detectable in 1 min. This detection limit is in the low nM range based on the calculation using the information provided from manufacturer, and this concentration is significantly

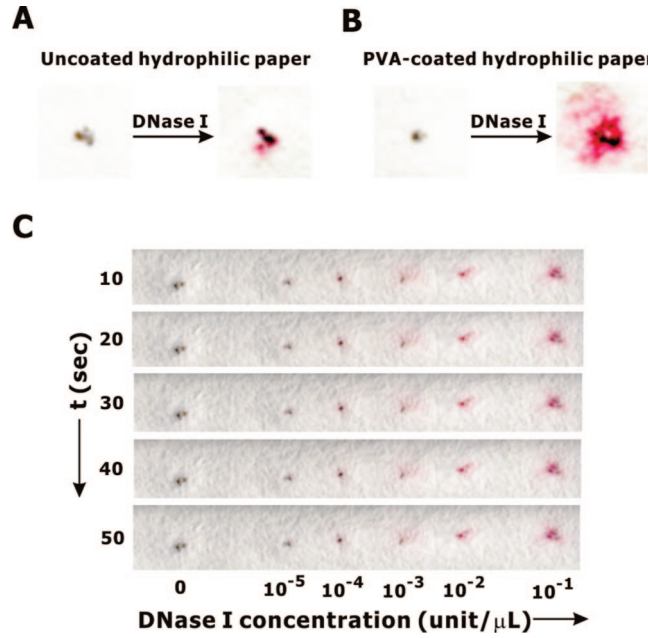

Figure 3. DNase I assay on (A) uncoated hydrophilic paper and (B) PVA-coated hydrophilic paper. One microliter of DNase I solution was applied in (A) and (B). Images were obtained at 20 s after adding DNase I solution. (C) DNase I assay on PVA-coated hydrophilic paper as functions of assay time and DNase I concentration.

lower than the intracellular DNase I concentration (~6−7 μM[32]). This suggests the current assay could potentially be applied to the testing of intracellular DNase I activity in terms of the assay sensitivity, although the assay capability for testing complex samples (e.g., blood) needs first to be conducted.

While this assay is mainly designed to provide rapid yes/no results or semiquantitative results (i.e., more red color indicates that more target analyte is present), it is possible to obtain quantitative analysis by using color image managing tools and software. This has been demonstrated in a recent work reported by Whitesides and co-workers[11] where they used camera phones to take images of colorimetric assays and then conducted off-site laboratory analysis. To demonstrate our assay can be quantitative, a standard color management software called ImageJ (http://rsb.info.nih.gov/ij/) was used to obtain the color intensity of the photos obtained in the assay. This data permitted the assembly of a calibration curve (Figure S5, Supporting Information), which is a normal requirement for quantitative analysis. We also compared the assay sensitivities on paper and in solution (see Figure S6, Supporting Information) and it was found that the assay behaves similarly on paper and in solution in terms of sensitivity.

2.1.2. Hydrophilic Paper. The AuNP aggregate solution (1 μL) was alternatively spotted on hydrophilic paper (Whatman #1). The liquid drop spread to a greater degree on hydrophilic paper surface and dried more rapidly (≤3 min). The addition of DNase I buffer solution (0.1 unit/μL) to the dried paper resulted in significantly less evolution of red coloration (Figure 3A). The AuNP aggregation/dispersion states were further confirmed by SEM studies (Figure S2, Supporting Information) which showed that AuNP aggregates largely remained in the aggregated form after the addition of DNase I. This was initially a surprise,

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particularly when compared with the rapid and effective color development on hydrophobic paper. However, the simple expedient of premodifying the hydrophilic paper with 1% (w/v) PVA (see Experimental Section) led to a significantly improved assay performance which was as good as that on hydrophobic paper (Figure 3A). Here too, the AuNP aggregation/dispersion states were investigated by SEM (Figure S3, Supporting Information), which showed that AuNP aggregates are largely dispersed after the addition of DNase I. Similar results were obtained when hydrophilic paper precoated with 1% (w/v) PEG (or Tween 20) was used (data not shown).

Among other speculation (see below), we initially thought that the AuNP aggregates on untreated hydrophilic paper would be entrapped inside the large pores in the paper matrix, which restricts both the accessibility of dsDNA to DNase I and the redispersion of aggregates. By contrast, the PVA coating on hydrophilic paper surface can partly block (or fill) these pores so that AuNP aggregates will stay on the top of the paper. This would increase the accessibility of AuNP aggregates to DNase I and also facilitate the redispersion of AuNPs in water droplets on paper surface.

To test this hypothesis, SEM experiments were undertaken on hydrophilic paper with and without a PVA coating (Figure S3, Supporting Information), and of AuNP aggregates on different types of paper materials before and after the addition of target analyte (Figures S2 and S3, Supporting Information). Interestingly, it was found that the PVA coating had little (if any) effect on the morphology and porosity of hydrophilic paper matrix. Therefore, the role of PVA, consistent with previously established theories is more likely to be associated with the facts that (1) PVA (or other surfactants tested in the present study) can retain water that would otherwise spread and dry too rapidly on hydrophilic paper (particularly compared to the good assay performance on hydrophobic paper where water drop does not spread well); (2) these hydrophilic polymers or surfactants help biomolecules to maintain their proper function, which is well-known in the literature (see, for example, ref 27); and (3) hydrophilic polymers or surfactants can reduce the nonspecific interactions between DNA-modified AuNPs and solid materials,[35] which therefore facilitates the redispersion of AuNPs after DNase I treatment.

In the case of PVA coated hydrophilic paper, the initial color development on hydrophilic paper is very rapid: an intense red color appears in ~10 s after the addition of DNase I, and the color intensity directly corresponds to DNase I concentrations used in the assay (Figure 3B). Unlike hydrophobic paper assays, however, where the color continues to develop over a longer period of time (~3 min), the color development on hydrophilic paper stops after ~20 s (Figure 3B) because the enzyme solution spread across the paper surface and dried rapidly. Note that the addition of new portions of sample solution on AuNP aggregate spots can facilitate the continuous color development, which eventually reaches a similar color amount that is typically observed on hydrophobic paper after adding one portion of analyte solution (Figure 2). Thus, the main advantage provided by hydrophobic paper is the development of more highly colored spots over extended periods

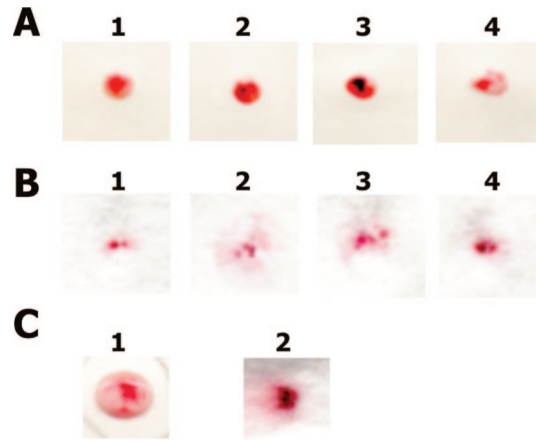

Figure 4. Assay performance of AuNP aggregate-coated (A) hydrophobic paper and (B) PVA-coated hydrophilic paper that were dried at (1) room temperature, (2) 37 °C, (3) 60 °C, and (4) 90 °C for 10 min before adding DNase I solution. (C) Assay performance of AuNP aggregate-coated hydrophobic paper (1) and PVA-coated hydrophilic paper (2) that were dried at room temperature for 12 days. While the data shown here were obtained from a test after 12 days storage, tests conducted after several months storage similarly functioned well. In all assays in this figure 1 μL of DNase I solution (0.1 unit/μL) was applied onto dried AuNP aggregate spots on paper. All images were obtained at 20 s after applying DNase I solution.

of time because competitive drying (facilitated on hydrophilic paper), which suppresses further enzymatic cleavage, is suppressed.

2.1.3. Assay Stability upon Drying and Storage. From the standpoint of practicality, it would be ideal if the AuNP-coated paper products could be completely dried and stored, and remain functional upon rehydration for use. Therefore, we investigated the assay performances of AuNP aggregate-coated papers that were dried under various conditions. AuNP aggregate-coated paper (both hydrophobic and hydrophilic) were dried at different temperatures (i.e., 25, 37, 60, and 90 °C, respectively) for 10 min. It was found that all AuNP aggregates on paper functioned well, even for the paper strips that were dried at 90 °C (Figure 4, panels A, B). Note that the same AuNP aggregates are destroyed in solution phase at elevated temperatures (e.g., 90 °C) due to the denaturation of DNA hybridization.[16,31] This suggests paper substrates (both hydrophobic and hydrophilic) protect AuNP aggregate probes from thermal degradation, which provides a significant advantage of using paper-based platform for biosensing assays. We also investigated the assay performance for dried paper coated with AuNP aggregates after long-term storage. We found that the assay still functioned well for at least several months (Figure 4C).

2.2. Adenosine-Sensing Assay. To test the generality of the biosensing platform, an adenosine-sensing paper assay was developed using DNA-cross-linked AuNP aggregates as color signal probes. Liu and Lu have previously reported colorimetric assays using AuNP aggregates formed by interparticle cross-linking of cDNA-modified AuNPs with an adenosine DNA aptamer cross-linker (Figure 5A, B).[27,30,33] The addition of adenosine, which binds and dissociates DNA aptamer from its cDNA strands,[34] resulted in the redispersion of AuNP aggregates into well-dispersed AuNPs (Figure 5A). Liu and Lu have applied this construction for adenosine detection in a solution-phase and a lateral flow device.[27]

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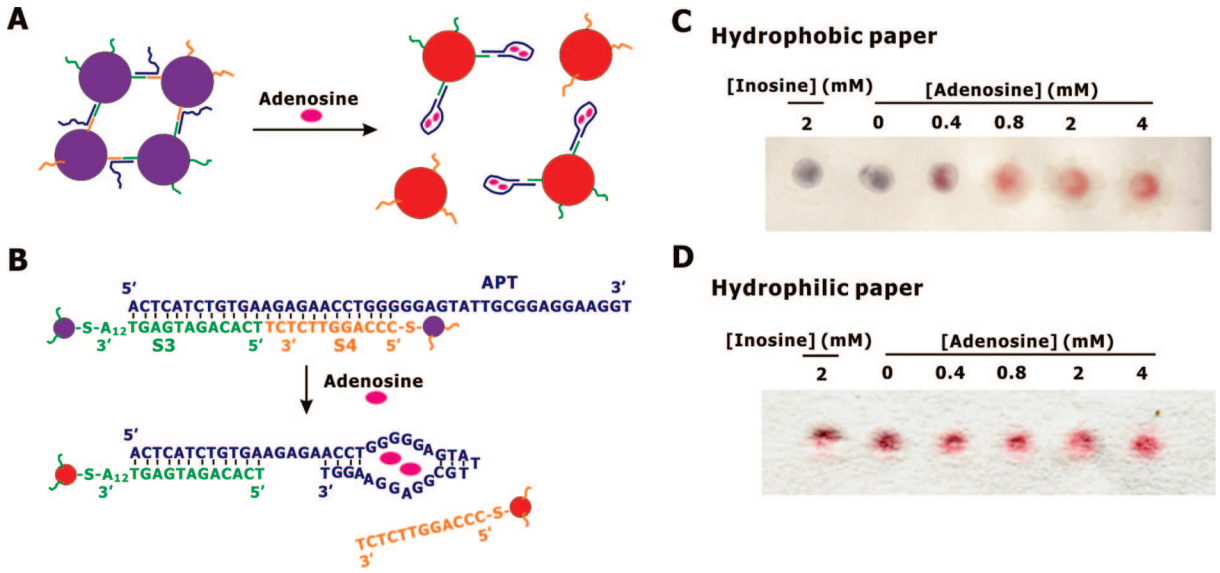

Figure 5. (A) Schematic illustration of an adenosine colorimetric sensor using AuNP aggregates. Adenosine-binding DNA aptamer (APT) is used as a cross-linker to bridge AuNPs attached with cDNA strands (S3 and S4). (B) The sequences of the DNA molecules used. The addition of adenosine which binds to and dissociates the aptamer molecule from the cDNA strand leads to the redispersion of AuNP aggregates. (C) and (D) are adenosine-sensing assays on hydrophobic and hydrophilic paper, respectively. One microliter of analyte solution was applied, and all images were obtained after 1 min.

In the present work, the same system was adopted on paper substrates. The adenosine assays were performed using a protocol similar to that of the DNase I assays with the following two modifications: (1) 10% (w/t) sucrose, a reagent that can maintain DNA aptamer function upon drying,[27] was incorporated in the buffer used to store AuNP aggregates before spotting onto paper, and (2) PVA coating on hydrophilic paper is not required in this case, presumably because sucrose can play a similar protective role to PVA.

As shown in Figure 5C and D, the red color "lighted up" when adenosine solutions were applied to dried AuNP aggregate spots on either hydrophobic or hydrophilic paper substrates. The amount of color that developed increased with higher adenosine concentrations. In control experiments where adenosine was absent or inosine was used, no significant red color was observed (Figure 5C, D).

The use of a DNA aptamer in this system will allow the paper-based assay to be generalized to many other targets, given that aptamers for a broad range of analytes are available. It should be noted, however, that the adenosine-sensing assay was less robust than the DNase I assay under the investigated conditions. While this is partly due to the different biomolecular processabilities associated with DNase I cleavage versus adenosine aptamer binding, it may also be associated with the geometric requirements of the two assays. The aptamer assay requires that the target molecule penetrates into the core of an aggregate,[27,30,33] where it can bind to the aptamer. If the AuNP aggregates are too large, it may not be possible to redisperse the AuNP even in the presence of adenosine: careful optimizations on the annealing conditions when preparing AuNP aggregates and centrifugation conditions, which determine the sizes of resultant aggregates (in the present work, the annealing and centrifugation conditions are 4 °C, 2 h and 2000*g*, 1 min, respectively) have to be conducted.[30] This process of aggregation/redispersion is highly dependent on the assay salt concentration.[30] If the salt concentration is too high, AuNP aggregates are not readily redispersed because interparticle DNA hybridization forces hold particles too tightly. On the other hand, if the salt concentration is too low, AuNP aggregates are insufficiently stable in the absence of the target because interparticle repulsive forces (e.g., electrostatic repulsion) dominate. One therefore has to choose an appropriate salt concentration to achieve the best assay performance. The assay salt conditions used in the present work are 25 mM Tris-HCl, pH 8.3, and 100 mM NaCl.

While these parameters constrain formulation, they are not particularly limiting to the strategy of using paper for bioassays. The AuNP, because of their very high extinction coefficients, are extremely sensitive analyte enhancers that can readily be qualitatively characterized even by the naked eye and within a minute: quantitation is similarly facile with the use of spectrophotometry/image analysis. In addition to the increasing range of aptamers that are available, we have demonstrated that enzymatic degradation of surface bound DNA is facile: related assays using different enzymes have been described in solution.[20−23] Paper provides a beneficial substrate for these assays. Besides low cost and availability in variety of formats and surface energies, cellulose has been shown to be protective against thermal degradation of DNA stabilizers on the AuNP.

The concept of using AuNP colorimetric sensors on paper strips is general and versatile. In addition, to the on-spot tests described above, we have also be able to control AuNP aggregation/dispersion states on paper in a flowing fluid flow driven by the natural capillary forces due to the porous nature of paper (i.e., a process related to paper chromatography). The target analytes, which are mixed in the developing buffer, flow along the paper and interact with the AuNP biosensors already on the paper. The presence of the analytes eventually changes the AuNP aggregation/dispersion states (and therefore the colors) on paper. We have demonstrated a proof-of-concept of such a system, based on

an adenosine-5′-triphosphate ATP biosensor (see Figure S7 in Supporting Information for details).

3. CONCLUSIONS

Paper substrates were shown to have excellent properties for use in cost-effective, easy-to-use colorimetric bioassays with AuNPs as the signal transducer. In addition to the bright background provided by paper, which facilitates the colorimetric determination, paper substrates were surprisingly found to protect DNA−AuNP aggregates from external nonspecific stimuli (e.g., heat), which is ideal for long-term storage. Aggregation and deaggregation of AuNPs using controllable interparticle distances give visual readouts of the presence of various biological entities, which makes them ideal signal transducers for paper-based assays. Due to the extremely high extinction coefficient of AuNPs, only small volumes (1 μL in the present work) of both DNA−AuNP aggregate probes and target sample solutions are required. Furthermore, given that both paper and AuNP-based colorimetric sensing systems can be readily modified, the AuNP-coated paper bioassay platform exhibits considerable versatility that will facilitate modification for a variety of other targets.

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SUPPORTING INFORMATION AVAILABLE

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

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