Nucleic Acid-Regulated Perylene Probe-Induced Gold Nanoparticle Aggregation: A New Strategy for Colorimetric Sensing of Alkaline Phosphatase Activity and Inhibitor Screening

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Ⓢ Supporting Information

ABSTRACT: A positively charged perylene probe (probe 1) could induce aggregation of the gold nanoparticles (Au NPs). As a result, significant assay solution color changes were observed. A duplex DNA (DNA-1) could induce aggregation of the perylene probe. It was observed that DNA-1 could efficiently regulate the probe 1-induced Au NP aggregation. When probe 1 and DNA-1 were first mixed, DNA-1 induced aggregation of the perylene probe. Au NPs were subsequently added, and no induced aggregation of the Au NPs was observed. Thus the color of the assay solution remained to be red. The assay is quite sensitive; 200 pM DNA-1 could cause a clear solution color change. On the basis of this observation, a novel method for the detection of alkaline phosphatase (ALP) activity has been demonstrated. Our method does not require covalent immobilization of the nucleic acid, or the addition of an excess amount of salt. It is sensitive and convenient.

KEYWORDS: gold nanoparticles, perylene probe, nucleic acid, enzyme activity, colorimetric assay
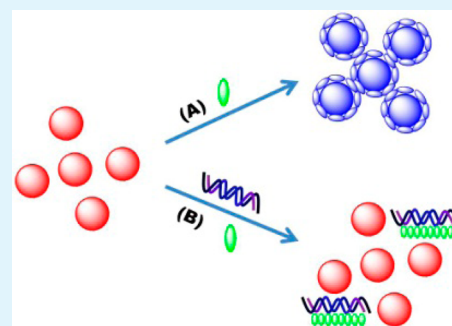
■ INTRODUCTION

Gold nanoparticles (Au NPs) are one of the most extensively studied classes of nanomaterials in biosensing.[1] The aggregation and deaggregation of the nanoparticles can cause significant assay solution color changes, which could be easily observed by the naked eyes.[2] And because the plasmon resonance absorption band in the visible spectrum of the Au NPs has remarkably high extinction coefficient, the sensitivity of the colorimetric assay is usually high,[3] without the use of expensive instrumentation.[4]

Au NPs have been combined with functional nucleic acid (for example, nucleic acid aptamer) for the development of many novel sensing strategies for the selective sensing of nucleic acid,[5] protein,[6] small biomolecule,[7] metal ion,[8] and enzyme activity.[9] There are two basic sensing strategies: (1) The oligonucleotide sequences are first covalently linked onto the surface of the Au NPs. Hybridization with the target sequence causes cross-linking of the Au NPs, and significant assay solution color changes could be observed.[10] (2) High concentrations of salt could induce aggregation of the Au NPs.[11] A single-stranded oligonucleotide could be adsorbed onto the surface of the Au NPs. Because the oligonucleotide is negatively charged, electrostatic repulsive interactions can stabilize the Au NPs colloid, and thus prevent the salt induced Au NP aggregation.[12] A notable benefit of the second approach

is the elimination of covalent functionalization of the Au NPs, and hence it is more simple and convenient.

Our group has reported a new perylene probe.[13] It contains four positive charges (probe 1, Figure 1). Probe 1 could
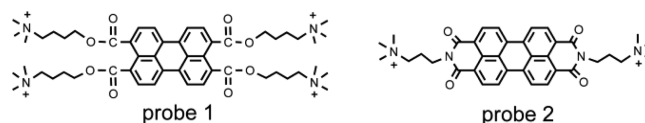

Figure 1. Structures of the perylene probe 1 and probe 2.

efficiently induce aggregation of the Au NPs. It was observed that when probe 1 was first mixed with a duplex DNA (DNA-1), DNA-1 could induce aggregation of probe 1, and thus prevent probe 1-induced Au NP aggregation (Scheme 1). On the basis of this observation, a sensitive colorimetric method for the detection of alkaline phosphatase activity has been developed. A 5′-phosphorylated duplex DNA (DNA-1) was employed. DNA-1 could be degraded by lambda exonuclease (λ exo) along the 5′ →3′ direction, probe 1 was released, and it could induce aggregation of the Au NPs. ALP could remove the

Scheme 1. DNA Inhibited Probe 1-Induced Au NP Aggregation
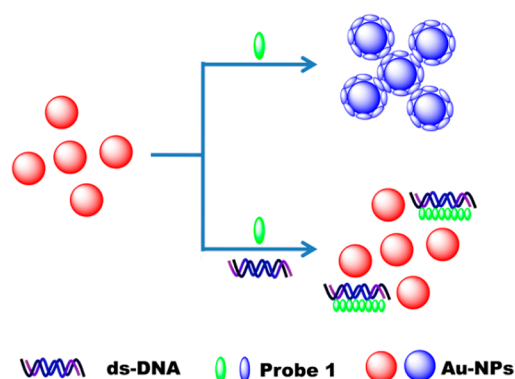
end phosphate groups of DNA-1. DNA-1 could no longer be degraded by λ exo, no probe 1 was released, and no induced aggregation of the Au NPs could be detected. Our method does not require covalent immobilization of the nucleic acid to the Au NPs, and it also does not require the addition of an excessive amount of salt to induce Au NP aggregation. It is sensitive and convenient.

■ MATERIALS AND METHODS

Materials. Alkaline phosphatase was purchased from Takara Biotechnology Co., Ltd. (Da Lian, China). Lambda exonuclease (λ exo, 10 units/μL) was purchased from Fermentas (Canada). The oligonucleotides were Ultra-PAGE purified and purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). Probe 1 and probe 2 were synthesized as previously described.[13,14] All other chemicals were of analytical grade and used without further purification.

The 5′-phosphorylated oligonucleotides used in the current investigation are listed as follows:

Oligo-1: 5′-p-CTG CCT AAA TTA CAT GTT GGC GTG AGA ATC GCC ATA TTT AAC AAA TTA AGC CTC GCT GCC GTC GCC A-3′

Oligo-2: 5′-p-TGG CGA CGG CAG CGA GGC TTA ATT TGT TAA ATA TGG CGA TTC TCA CGC CAA CAT GTA ATT TAG GCA G-3′

DNA-1 = oligo-1 + oligo-2

Oligo-1 and oligo-2 are complementary to each other. DNA-1 is the duplex DNA made by mixing of equimolar of oligo-1 and oligo-2.

Oligo-3: 5′-CTG CCT AAA TTA CAT GTT GGC GTG AGA ATC GCC ATA TTT AAC AAA TT-3′

Oligo-4: 5′-AAT TTG TTA AAT ATG GCG ATT CTC ACG CCA ACA TGT AAT TTA GGC AG-3′

DNA-2 = oligo-3 + oligo-4

Oligo-3 and oligo-4 are complementary to each other. DNA-2 is the duplex DNA made by mixing of equimolar of oligo-3 and oligo-4.

Oligo-5: 5′-CTG CCT AAA TTA CAT GTT GGC GTG AGA-3′
Oligo-6: 5′-TCT CAC GCC AAC ATG TAA TTT AGG CAG-3′
DNA-3 = oligo-5 + oligo-6

Oligo-5 and oligo-6 are complementary to each other. DNA-3 is the duplex DNA made by mixing of equimolar of oligo-5 and oligo-6.

The oligonucleotide stock solutions were stored at 4 °C before use.

Instrumentation. UV−vis absorption spectra were taken using a Cary 50 Bio Spectrophotometer (Varian Inc., CA, USA) equipped with a xenon flash lamp. Quartz cuvettes with 10 mm path length and 2 mm window width were used for UV−vis measurements. The photographs were taken using a Panasonic DMC-FX01 camera at the macro mode. Dynamic light scattering results were obtained using Zetasizer Nano ZS. Transmission electron microscopy (TEM) images were taken using a JEM-2100F high-resolution transmission electron microscope (Philips, The Netherlands) operated at 200 kV. Samples

for TEM characterization were prepared by placing a drop of the Au NPs solution onto a carbon-coated copper grid and then dried at room temperature.

Ultrapure water was prepared with a Milli-Q A10 filtration system (Millipore, Billerica, MA, USA). All stock and buffer solutions were prepared using the ultrapure water. Unless otherwise specified, the photographs and the UV−vis absorption spectra were taken at an ambient temperature of 22 °C.

Synthesis of the Au NPs. Thirteen nm Au NPs were prepared according to the reported literature methods.[15] Briefly, 4.3 mL of 1% (w/v) HAuCl$_4$ was added to a two-neck round-bottom flask containing 100 mL of water. The solution was heated to reflux and stirred vigorously. Twelve milliliters of 1% (w/v) sodium citrate was quickly added. The solution color changed from pale yellow to deep red. Reflux was continued for another 20 min. The solution was allowed to gradually cool to room temperature under stirring. The as-prepared Au NPs solution was stored at 4 °C before use.

Comparison of Probe 1- and Probe 2-Induced Au NP Aggregation. 5.28 nM of the Au NPs was mixed with 700 nM of the perylene probes. The sample mixtures were left standing at an ambient temperature for 5 min, and the photographs were taken (total sample volume: 50 μL). 450 μL of water was then added, and the UV−vis absorption spectra were taken.

Probe 1-Induced Au NP Aggregation. Increasing amounts of probe 1 were gradually added to 5.28 nM Au NPs sample solution and mixed with the pipet tip several times; UV/vis absorption spectra were then taken. The UV/vis absorption ratio of A_{650}/A_{520} (the absorption value at 650 nm versus the absorption value at 520 nm) was calculated (total sample volume: 500 μL).

Different amounts of probe 1 were added to 5.28 nM Au NPs sample solution (total sample volume: 50 μL). The mixed samples were left standing for 5 min, and the photographs were taken.

DNA-1 Inhibited Probe 1-Induced Au NP Aggregation. Different amounts of DNA-1 were added to a sample solution containing 700 nM probe 1. 5.28 nM Au NPs was then added, and the samples were mixed completely. The samples were left standing at an ambient temperature for 5 min, and the photographs were taken (total sample volume: 50 μL). Finally, 450 μL H$_2$O was added to the samples, mixed completely, the UV/vis absorption spectra were taken, and the A_{650}/A_{520} values were obtained.

Comparison of the Oligonucleotide Inhibited Probe 1-Induced Au NP Aggregation. Different amounts of DNA-1, DNA-2, and DNA-3 were added to a sample solution containing 700 nM probe 1. 5.28 nM of the Au NPs was then added, and the samples were mixed completely. The samples were left standing at an ambient temperature for 5 min, and the photographs were taken (total sample volume: 50 μL).

Probe 1-Induced Aggregation of the Au NPs at a Lower Concentration. Au NPs (2.64 nM) were mixed with different concentrations (20, 40, 80, 120, 160, 200, and 240 nM, respectively) of probe 1 (total sample volume: 50 μL). The samples were left standing at an ambient temperature for 5 min, and the photographs were taken.

Sensitive DNA Detection with Probe 1. Probe 1 (240 nM) was mixed with different concentrations of DNA-1 (0 pM, 200 pM, 500 pM, 1 nM, and 2 nM, respectively). Au NPs (2.64 nM) were added. The samples were left standing at an ambient temperature for 5 min, and the photographs were taken (total sample volume: 50 μL).

ALP Activity Assay. One microliter of DNA-1 (100 nM), 1 μL of ALP 10× buffer [500 mM Tris-HCl (pH 9.0), 10 mM MgCl$_2$], different amounts of ALP (1 μL), and 7 μL of water were mixed. The samples were incubated at 55 °C for 20 min, then heated to 90 °C and incubated for 5 min to inactivate ALP. Samples were cooled to room temperature, 1 μL λ exo (0.1 U) was added. Samples were incubated at 37 °C for 30 min, heated to 90 °C and incubated for 5 min to inactivate λ exo. The samples were cooled to room temperature and centrifuged briefly. Five microliters of the sample solution was mixed with 3.5 μL probe 1, and 41.5 μL Au NPs was then added. The samples were thoroughly mixed, left standing at room temperature for 5 min, and the photographs were taken (total sample volume: 50 μL). Four hundred fifty microliters of water was added to the sample

solution, the UV−vis absorption spectra were recorded, and the A_{650}/A_{520} values were calculated.

Kinetics of the Enzymatic Reaction. Kinetic analysis of the ALP enzymatic reaction was conducted as follows: 1 μL DNA-1, 1 μL ALP 10× buffer [500 mM Tris-HCl (pH 9.0), 10 mM MgCl$_2$], 1 μL ALP (0.1 U), and 7 μL water was mixed, incubated at 55 °C for different times (1−30 min), and then at 90 °C for 5 min to inactivate the enzyme. The samples were allowed to cool to room temperature. One μL (0.1 U) λ exo was added, the digestion reaction was allowed to continue for 30 min at 37 °C. λ exo was inactivated at 90 °C for 5 min. The samples were cooled to room temperature and centrifuged briefly. Five microliters of the products (sample) solution was mixed with 3.5 μL of probe 1; 41.5 μL of the Au NPs was then added. The samples were thoroughly mixed, and allowed to equilibrate for 5 min at room temperature. Four hundred fifty microliters of water was added to the sample solutions, the UV−vis absorption spectra were taken, and the A_{650}/A_{520} values were calculated.

Verification of the Colorimetric Assay by PAGE. Five microliters of the ALP assay solution was mixed with the electrophoresis loading buffer. The samples were loaded onto 1.0 mm thick nondenaturing 10% polyacrylamide gel (acrylamide:bis-acrylamide = 29:1). The electrophoresis buffer contained 1× TBE (100 mM Tris-HCl, 83 mM boric acid, and 1 mM EDTA at pH 8.0). After 1 h of electrophoresis (voltage: 80 V), the gel was visualized via silver staining.

Inhibition Effect of Sodium Orthovanadate (Na$_3$VO$_4$). One microliter of DNA-1, 1 μL of 10× buffer [500 mM Tris-HCl (pH 9.0), 10 mM MgCl$_2$], 1 μL of ALP (0.1 U), 1 μL of Na$_3$VO$_4$ of various concentrations, and 6 μL of water were mixed. And the same assay procedures for ALP activity were exactly followed.

Selectivity of the Assay. One microliter of DNA-1, 1 μL of ALP 10× buffer [500 mM Tris-HCl (pH 9.0), 10 mM MgCl$_2$], 1 μL of ALP (0.1U) [or 1 μL potential interference protein (20 μM)], and 7 μL of water were mixed. And the same assay procedures for ALP activity were exactly followed.

ALP Activity Detection in A549 Cell Extracts. A549 cells were cultured, and the cell-free extracts were prepared following the procedures described previously.[16] Briefly, the cells were cultured in DMEM containing 10% fetal calf serum. They were harvested in the exponentially growing stage by washing three times with ice cold buffer 1× (137 mM NaCl, 2.7 mM KCl, 10 mM Na$_2$HPO$_4$, 2.0 mM KH$_2$PO$_4$), and resuspended in 10 μL of buffer I (10 mM Tris-HCl, 200 mM KCl, pH 7.8). Ten microliters of buffer II [10 mM Tris-HCl (pH 7.8), 600 mM KCl, 2 mM EDTA, 40% glycerol, 0.2% NP40, 2 mM DTT, 0.5 mM PMSF protease inhibitor] was added to lysis the cells. The mixture was shaken at 4 °C for 1.5 h, and centrifuged for 10 min at 16000 g to remove the cell debrises. The supernatant was recovered. Diluted cell extracts (1%) were added to the assay solutions, and the assay conditions were the same as those described in Figure 5.

■ RESULTS AND DISSCUSSION

Literature reports have shown that the perylene probes have a strong tendency of self-aggregation.[13,14] It was observed that the positively charged perylene probes (probe 1 and probe 2, Figure 1) have much reduced degree of self-aggregation because of the electrostatic repulsive interactions among the positive charges of the probe molecules. They are very soluble in water, and could efficiently induce the aggregation of the Au NPs.[17] Figure 2 shows that 700 nM probe 1 and probe 2 could both induce very significant Au NP aggregation. The assay solution color changed from pink red to dark blue or dark purple. However, the UV−vis absorption spectra show that probe 1-induced more significant spectral changes. The peak maximum shifted from 520 to 680 nm, and the intensity of the maximum absorption of the new peak at 680 nm is comparable to that of the free Au NPs at 520 nm. On the contrary, for probe 2-induced Au NP aggregation, there was only a shoulder
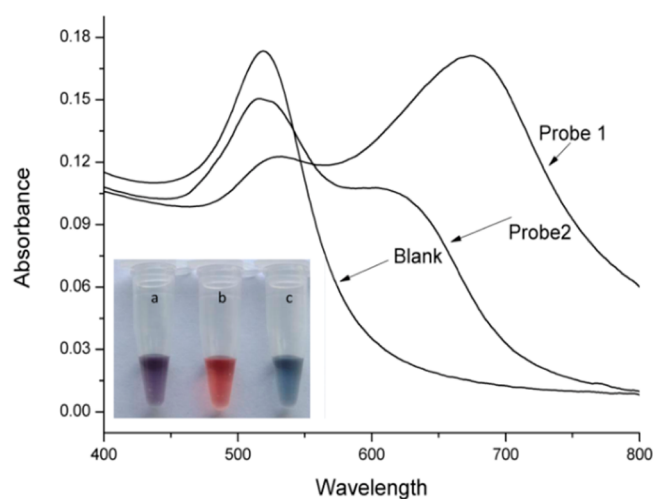

Figure 2. UV−vis absorption spectra of the perylene probe-induced 0.528 nM Au NP aggregation. Inset: comparison of the perylene probe-induced Au NP aggregation. 5.28 nM Au NPs were mixed with (a) 700 nM probe 2, (b) water, or (c) 700 nM probe 1.

peak appeared at 620 nm, and the intensity of the maximum absorption was also much smaller than that of the probe 1-induced Au NP aggregation.

Probe 1 contains four positive charges, and probe 2 contains two positive charges. The surface of the Au NPs is covered with the negatively charged citrate ions. There are strong electrostatic attractive interactions between the perylene probe and the Au NPs. In addition, because the perylene probe contains a large planar aromatic ring structure, it could also interact with the surface of the Au NPs through hydrophobic interactions. The adsorbed perylene probe could neutralize the citrate negative charges on the surface of the Au NPs, and the π−π stacking and hydrophobic interactions among the perylene probe molecules adsorbed on adjacent Au NPs could also facilitate the nanoparticle aggregation. Because probe 1 contains more positive charges, it seems understandable that probe 1 could induce stronger Au NP aggregation.

The probe 1-induced Au NP aggregation was further investigated in details. Figure 3 shows that with the increase in probe 1 concentration, the color of the Au NPs sample solution gradually turned into dark blue. At 100 nM probe 1 concentration, the Au NPs solution color changes could still be clearly observed (see Figure 3 and Figure S1 in the Supporting Information). In addition, Figure S2 in the Supporting Information shows that at a lower Au NPs concentration (2.64 nM), 20 nM probe 1 could induce clear assay solution color changes.

In our previous reports, we have shown that an oligonucleotide (a negatively charged polyanion) could very efficiently induce the aggregation of the positively charged perylene probe.[15] Our results show that when probe 1 was first mixed with DNA-1 (a duplex DNA), DNA-1 could induce aggregation of probe 1. When the Au NPs were subsequently added, because the concentration of the free probe 1 molecule was dramatically reduced, probe 1-induced Au NP aggregation was also greatly reduced (see Figure S3 in the Supporting Information). The degree of the Au NP aggregation and the color change of the sample solution were greatly dependent on the concentration of DNA-1 added to the assay solution. Figure 4 shows that with the increase of DNA-1 concentration, the color of the sample solution gradually turned into pink red. The
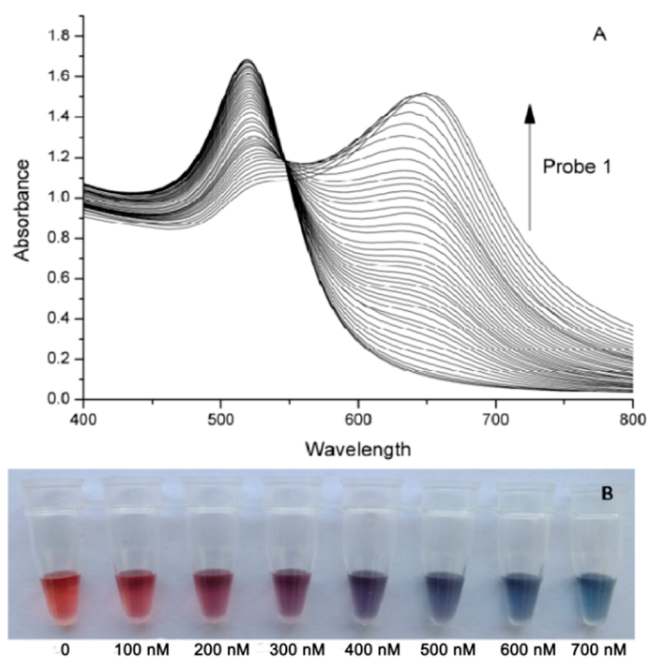
Figure 3. Probe 1-induced Au NP aggregation. (A) Changes in UV−vis absorption spectrum upon the addition of increasing concentrations of probe 1 from 100 to 700 nM (Au NPs, 5.28 nM). (B) The corresponding photograph (Au NPs, 5.28 nM).
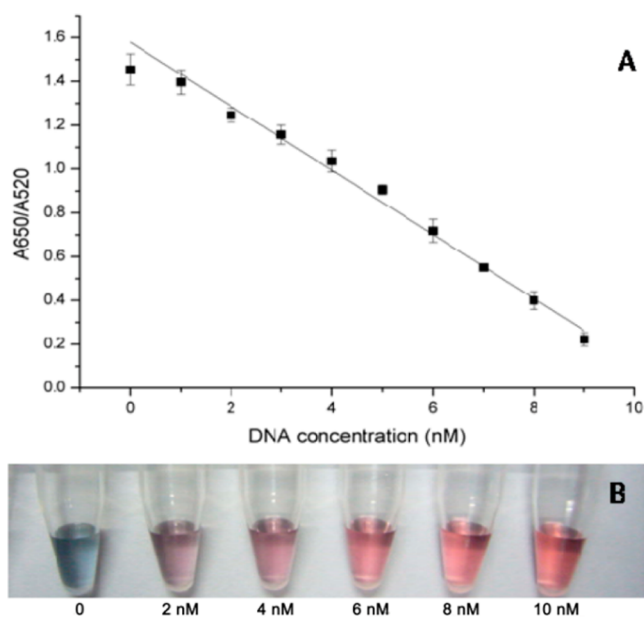

Figure 4. DNA-1 inhibited probe 1-induced Au NP aggregation. (A) Changes in the A_{650}/A_{520} value as a function of DNA-1 concentration. Final concentrations: 0.528 nM Au NPs; 70 nM probe 1. (B) Sample solution color changes as a function of DNA-1 concentration. Final concentration: 5.28 nM Au NPs; 700 nM probe 1.

intensity ratio of the UV−vis absorption at 650 and 520 nm (A_{650}/A_{520}) was plotted against the concentration of DNA-1, and a linear relationship was obtained. This DNA assay is very sensitive. Two nM DNA-1 could induce very clear assay solution color changes. We have also tested oligonucleotides of different length, and the results are shown in Figure S4 in the Supporting Information. The sensitivity decreased slightly with the reduced of the length of the DNA. And upon further

optimization of the sample concentrations, the amount of DNA-1 needed to induce assay solution color changes was further reduced. Our results show that as low as 200 pM DNA-1 could induce clear assay solution color changes (see Figure S5 in the Supporting Information), which is about 20 times more sensitive than the traditional unmodified Au NP-based DNA detection.[18]

Dynamic light scattering (DLS) results also supported our conclusions. Figure S6 in the Supporting Information shows that without the addition of probe 1, the mean diameter of the particles/aggregates observed in the sample solution was about 13 nm, which is the mean diameter of the Au NPs (see Figure S7 in the Supporting Information). Upon the addition of probe 1, the mean diameter of the particles/aggregates drastically increased to about 1000 nm, which strongly indicates aggregation of the Au NPs induced by probe 1. However, when DNA-1 was first added, the mean diameter of the particles/aggregates shifted back to about 13 nm. The results clearly suggest that DNA-1 could prevent the probe 1-induced Au NP aggregation. It is also worth noting that the DNA-1-induced probe 1 aggregation was not observed, presumably because the light scattering by the Au NPs was very strong, and the concentrations of DNA-1 and probe 1 were relatively low.

Alkaline phosphatase (ALP, EC 3.1.3.1) is one of the most commonly assayed enzymes (especially in clinical diagnosis).[19] It can catalyze the hydrolysis of a phosphomonoester (dephosphorylation) into inorganic phosphate and the corresponding alcohol under alkaline conditions with abroad substrate specificity. In mammalian, ALP is a group of isoenzymes that are distributed in many different tissues (liver, intestine, bone, kidney, placenta, etc.).[20] It is a homodimeric enzyme. Each monomer unit contains five cysteine residues and three metal ions (two Zn^{2+} and one Mg^{2+}) that are essential for its activity.[21] The two active sites (one on each monomer) located 32 Å apart.[22] The ALP concentration is at an abnormal level in many diseases such as liver dysfunction,[23] bone disease,[24] breast and prostatic cancer,[25] and diabetes.[26] Recently, it was also found that ALP plays an important role in resolution of inflammation through LPS detoxification.[27]

A number of fluorometric,[28−30] electrochemical,[31,32] surface-enhanced Raman scattering,[33,34] and colorimetric methods[35] for ALP activity assay have been developed in recent years. However, some of the methods offer low detection sensitivity and some are time-consuming, expensive, and heavy-instrument-dependent. Therefore, a convenient, rapid, sensitive, and inexpensive assay method for ALP activity would have a great value in basic biochemical research and clinical diagnosis.

Scheme 2 shows the principle of the probe 1-regulated Au NP aggregatio-based alkaline phosphatase (ALP) activity detection. The 5′ ends of DNA-1 were phosphorylated. Without the addition of ALP, lambda exonuclease (λ exo) could very efficiently degrade DNA-1. When probe 1 and the Au NPs were sequentially added to the assay solution, probe 1 induced Au NP aggregation, and a dark blue color solution could be obtained. However, in the presence of ALP, ALP could very efficiently remove the 5′ end phosphate functional groups. DNA-1 could no longer be degraded by λ exo. When probe 1 was added, DNA-1 induced strong probe 1 aggregation. And when the Au NPs were added, no induced aggregation of the Au NPs took place, and the assay solution remained to be red. The changes in assay solution color and the

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Scheme 2. Assay Strategy for the Colorimetric ALP Activity Detection
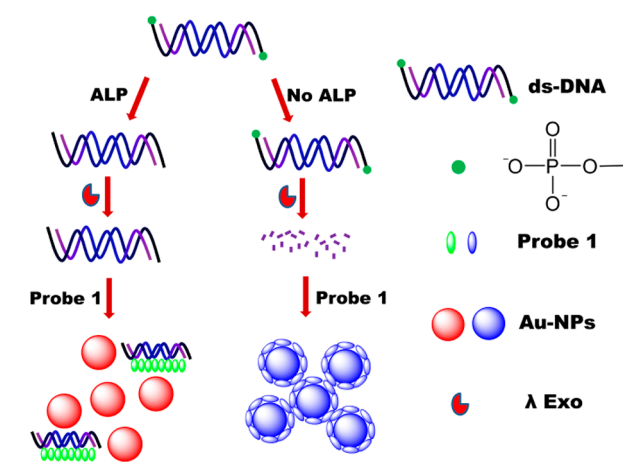
A_{650}/A_{520} value could be directly used for the quantification of ALP concentration in the assay solution.

Figure 5 shows that with the increase in ALP concentration, the color of the solution gradually turned into pink-red. The
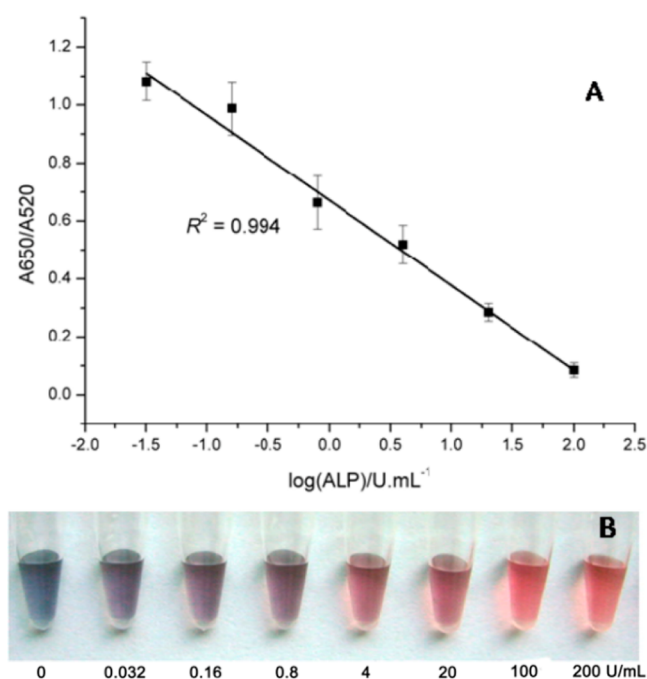

Figure 5. ALP activity detection. (A) A_{650}/A_{520} value was plotted against the ALP concentration. Concentrations of ALP: 0.032, 0.16, 0.8, 4, 20, 100 mU/mL. ALP reaction time: 20 min. Final concentrations: 70 nM probe 1, 0.528 nM Au NPs, 1 nM DNA-1. (B) Assay solution color changes as a function of ALP concentration. Concentrations of ALP: 0, 0.032, 0.16, 0.8, 4, 20, 100, 200 mU/mL. ALP reaction time: 20 min. Final concentrations: 700 nM probe 1, 5.28 nM Au NPs, 10 nM DNA-1.

results indicate that the 5′ end phosphate functional groups were gradually removed by ALP, and more DNA-1 remained intact after the λ exo digestion reaction. The PAGE results also verified the conclusions (see Figure S8 in the Supporting Information). DNA-1 induced aggregation of probe 1, and thus inhibited the probe 1-induced Au NP aggregation. The $A_{650}/$

A_{520} value was plotted against the ALP concentration, and a linear relationship was obtained. The linear regression equation is $A = -0.29\log C + 0.67$ ($R^2 = 0.994$), where "A" is the A_{650}/A_{520} value and "C" is the concentration of ALP in U/mL. Our assay is highly sensitive; 0.032 U/mL ALP could cause very clear assay solution color changes, which is more than 100 times more sensitive than the traditional Au NP-based colorimetric ALP assay.[36] The results suggest that the better sensitivity could be originated from the strong binding of probe 1 to the Au NPs.

Kinetics of the ALP enzymatic reaction was also studied (Figure 6). The results show that with the increase of the
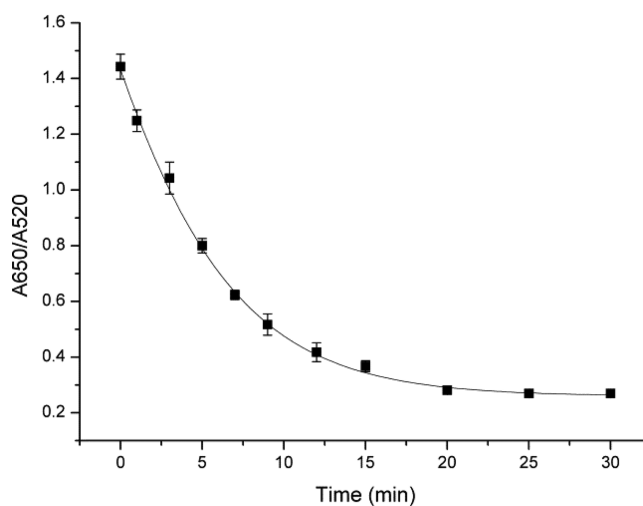

Figure 6. Changes in the A_{650}/A_{520} value as a function of the dephosphorylation reaction time. Final concentrations: 10 mU/mL ALP, 70 nM probe 1, 0.528 nM Au NPs, 1 nM DNA-1. ALP reaction time: 1−30 min.

reaction time, the A_{650}/A_{520} value gradually decreased. And after about 20 min of the reaction, the A_{650}/A_{520} value reached the minimum value. Further increase of the reaction time caused no further decrease of the A_{650}/A_{520} value. The results indicate that after 20 min of the reaction, almost all the 5′-end phosphate functional groups were removed by ALP. λ exo could no longer degrade DNA-1. And DNA-1-induced complete aggregation of probe 1. When the Au NPs were added to the assay solution, because there were few probe 1 free molecules available, no induced Au NP aggregation could be observed.

Selectivity of the assay was also explored. Three nonrelated proteins were tested. They were BSA, trypsin, and lysozyme. The A_{520}/A_{650} values of the UV−vis absorption show that at 20 μM concentration, none of these proteins interfered with the assay (Figure 7). In addition, a mixture of the three proteins also did not show any noticeable interference. The color changes of the assay solutions also confirm that these proteins did not interfere with the assay. The sample solution containing ALP shows red color, whereas all other samples show deep blue color.

Sodium orthovanadate (Na_3VO_4) is a well-known ALP inhibitor.[37] Its inhibition effect was studied. Figure 8 shows that with the increase of assay solution VO_4^{3-} concentration, the A_{650}/A_{520} value gradually increased. At about 200 μM VO_4^{3-} concentration, the maximum A_{650}/A_{520} value was reached, further increase of the VO_4^{3-} concentration caused no further
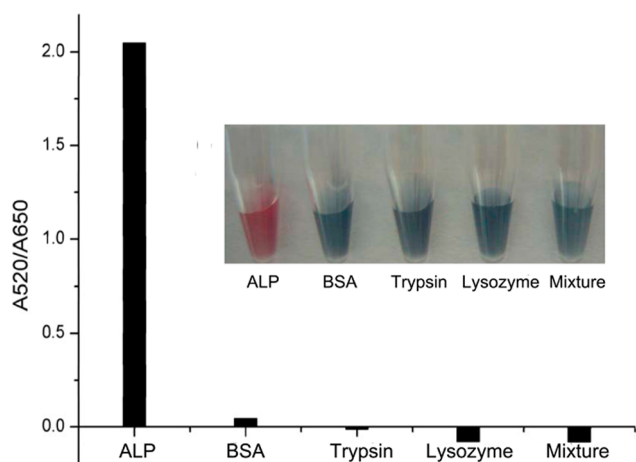
Figure 7. Specificity of the assay. ALP, 10 U/mL; BSA, trypsin, and lysozyme each at 20 μM concentration. The mixed sample contained BSA, trypsin, and lysozyme each at 20 μM concentration. Blank was subtracted for clarity. Final concentrations: 70 nM probe 1, 0.528 nM Au NPs, 1 nM DNA-1. Inset: the corresponding photograph. Final concentrations: 700 nM probe 1, 5.28 nM Au NPs, 10 nM DNA-1.
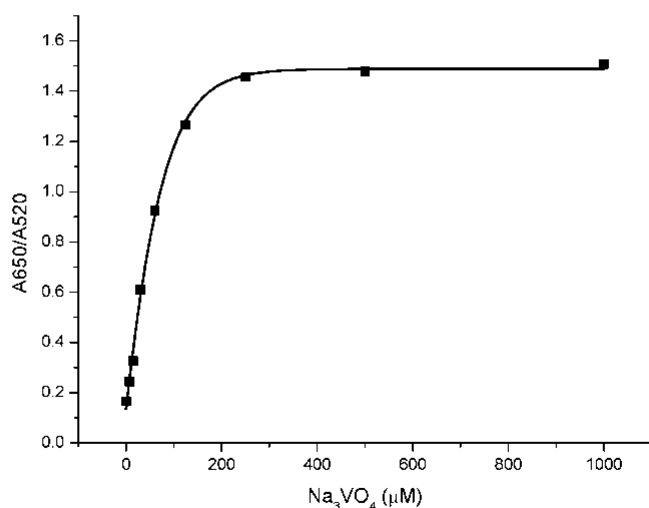

Figure 8. Inhibition effect of sodium orthovanadate. Final concentrations: 10 mU/mL ALP, 70 nM probe 1, 0.528 nM Au NPs, 1 nM DNA-1. ALP reaction time: 20 min.

increase in A_{650}/A_{520} value. The results strongly suggest that VO_4^{3-} could inhibit the activity of ALP. With the increase of VO_4^{3-} concentration, the activity of ALP decreased, less amount of the 5'-end phosphate functional groups of DNA-1 was removed. And the decreased amount of DNA-1 could induce less probe 1 aggregation, which effectively increased the degree of probe 1-induced Au NP aggregation. Our ALP assay was also tested in 1% A549 cell extracts. The results show that with the addition of increasing amounts of ALP, the A_{520}/A_{650} value increased significantly (see Figure S9 in the Supporting Information).

CONCLUSION

In summary, we have developed a new Au NP-based colorimetric method for the quantification of DNA concentration and ALP activity. Our results show that the perylene probe 1 could induce aggregation of the Au NPs. DNA-1 could induce aggregation of probe 1. Thus DNA-1 could efficiently

regulate the probe 1-induced Au NP aggregation. Our assay is highly sensitive; without the use of any signal amplification, 200 pM DNA-1 could cause clear assay solution color change. When the 5'-end phosphorylated DNA-1 was mixed with ALP, the phosphate groups of DNA-1 were efficiently removed by ALP. λ exo could not degrade DNA-1, and DNA-1 induced strong probe 1 aggregation. No aggregation of the Au NPs could be observed, because there were few free probe 1 molecules available in the assay solution. On the contrary, without the addition of ALP, λ exo could efficiently degrade DNA-1, the free probe 1 molecules could induce strong aggregation of the Au NPs, and dramatic assay solution color changes from pink red to dark blue could be easily observed. Our assay does not need nucleic acid covalent immobilization, or the addition of excess amount of salt. It is sensitive and convenient. We envision it could be used for the development of novel perylene probe and Au NP-based colorimetric assays for related biological, biochemical, and pharmaceutical applications.

ASSOCIATED CONTENT

S **Supporting Information**
Figures as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes
The authors declare no competing financial interest.

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