Gold Nanoparticle-based Optical Probes for Target-Responsive DNA Structures

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Abstract

In this work, we report the use of unmodified gold nanoparticles (AuNPs) as an optical probe for the detection of target-responsive structural variations of DNA. By employing two DNA structures, i.e., a pH-responsive i-motif oligonucleotide and a mercury-specific oligonucleotide (MSO), we demonstrated that AuNPs could selectively distinguish target-free and target-bound oligonucleotides via the characteristic surface plasmon resonance-associated red-to-blue color change. Based on these observations, we developed a convenient "mix-and-detect" approach that could selectively detect environmentally toxic mercury ions.

Introduction

Gold nanoparticles (AuNPs) are highly attractive nanomaterials featuring unique optical properties. Because the light-induced plasmons, which are collective oscillation of conduction-band electrons of gold, AuNPs feature very high extinction coefficients and show size-dependent brilliant colors. Therefore, AuNPs have found important applications in a variety of areas including biology. Mirkin group [1] and Alivisatos group [2] first reported the construction of AuNPs-based nanoscaffolds by self-assembling thiolated oligonucleotides on the surface of AuNPs. By exploiting the unique optical and electronic properties of these AuNPs-DNA conjugates, Mirkin and coworkers later developed a series of elegant approaches for ultrasensitive detection of DNA and proteins [3-7].

More recently, Li and Rothberg reported a simple method for visual detection of target DNA by using unmodified AuNPs [8, 9]. Their detection strategy relied on the observation that single-stranded (ss-) DNA, while not double-stranded (ds-) DNA, spontaneously adsorbed on the surface of unmodified AuNPs with high affinity [8, 10]. Since the color of Au NPs depends acutely on whether they are isolated or agglomerated, salt-induced aggregation of AuNPs occurring in the dsDNA solution led to the characteristic red-to-blue color change. In contrast, the ssDNA-stabilized AuNPs remained dispersed and red-colored upon salt addition. Inspired by this work, we proposed that unmodified AuNPs could also serve as a visual probe for ligand-induced aptamer structural variations. Aptamers are ligand-specific nucleic acids that are in-vitro selected via SELEX (systematic evolution of ligands by exponential enrichment), which are known to often undergo significant structural variation upon target binding [11, 12]. In a preliminary communication, we demonstrated an AuNPs-based potassium aptamer sensor [13]. We found that potassium (K^+) selectively induced the structural variation of an anti-K^+ aptamer, resulting in the characteristic red-to-blue color change of AuNPs. Here we extended this work to two non-aptamer target-responsive DNA structures, i.e., a pH-sensitive i-motif structure and a mercury-specific oligonucleotide (MSO) probe, and demonstrated that AuNPs were generalizable optical probes for the detection of target-responsive structural variations of DNA.

Experimental methods

Materials

Hydrogen tetrachloroaurate(III) ($HAuCl_4.4H_2O$, 99.99%) was purchased from China National Pharmaceutical Group Corporation and used without further purification. All other chemicals were of analytical grade. Water was purified using a Millipore filtration system. 13-nm AuNPs (3.5 nM) were synthesized by reduction of $HAuCl_4$ as previously reported [14]. DNA oligonucleotides were synthesized and

purified with HPLC in Sangon Biotechnology Inc. (Shanghai, China). The sequences of the involved oligonucleotides are as follows: 5'-CCC TAA CCC TAA CCC TAA CCC-3' (i-motif), 5'-TTC TTT CTT CCCC TTG TTT GTT-3' (MSO), 5'-AGC AAC CTC AAA CAG ACA CCA TGG-3' (control sequence).

Instrument

UV-Vis absorption spectra were performed on a Hitachi U-3010 spectrophotometer, and fluorescence spectra were performed on a Hitachi F-4500 fluorescence spectrophotometer. Photographs were taken with Olympus C-5060 digital camera.

Detection of i-motif structure

The i-motif oligonucleotide of 100 μM was diluted in either a citrate buffer (0.05 M, pH 5.5) or a Na_2CO_3/$NaHCO_3$ buffer (0.05 M, pH 8.5) to a final concentration of 10 μM. Two microliters of oligonucleotide was added to 100 μL of AuNPs solution and incubated for several minutes at room temperature. Of note, the solution pH of as-prepared AuNPs is 5.5, thus it was first adjusted to pH 8.5 with 0.1 M NaOH prior to being incubated with the i-motif oligonucleotide that was buffered at pH>5.5. Then several microlitres of NaCl (0.35 M) were added to the mix solutions, followed by visual observation and UV-Vis characterization.

Detection of mercury

We first mixed 35 pmol MSO and 350 pmol Hg^{2+} in 7 μL of water for several minutes, then 100 μL AuNPs was added and incubated for 5 min at room temperature. After that, several microlitres of $NaNO_3$ (0.5 M) was introduced to the mixtures, followed by either visual observation or UV-Vis characterization.

Results and discussion

The i-motif structure formed by cytosine-rich oligonucleotides is a naturally occurring sequence widespreaded in human telomeric DNA. It is well known that telomeres are essential for genome integrity and closely associated with cellular aging and cancer, thus the i-motif was regarded as a promising target for chemotherapy [15, 16]. Here we demonstrated that AuNPs could be a selective probe for i-motif. Previous work showed that it was possible to use AuNPs-thiolated i-motif as pH sensors [17-19]. Here we employed as-prepared AuNPs instead to perform pH sensing. It has been well documented that the closely packed four-stranded i-motif structure is formed at slightly acidic while not at alkaline pH, through the protonated cytosine-cytosine base pair formation [20, 21]. We first challenged the i-motif oligonucleotide with AuNPs in a solution of pH 5.5. Interestingly, the solution of AuNPs rapidly changed its color from red to blue (within seconds) (Figure 2, left, a). This color change was characteristic of the aggregation of AuNPs that shifted the surface plasmon resonance (SPR) absorption to the longer wavelength. In contrast, at pH 8.5 (Figure 2, left, b) the solution of AuNPs treated with the i-motif oligonucleotide retained its red color even with the addition of salt. These observations strongly implied that the i-motif oligonucleotide was unstructured at pH 8.5, which protected AuNPs by wrapping at their surfaces; instead the i-motif structured formed at pH 5.5 could not stabilize AuNPs due to its low affinity to AuNPs. This marked difference in affinity to AuNPs between structured and unstructured oligonucleotides might arise from several combined effects. DNA bases are known to strongly interact with gold through Au-N coordination [22]. These bases are largely buried within phosphate backbones in the i-motif structure, while they are much more exposed in the unstructured state. Moreover, the rigidity of

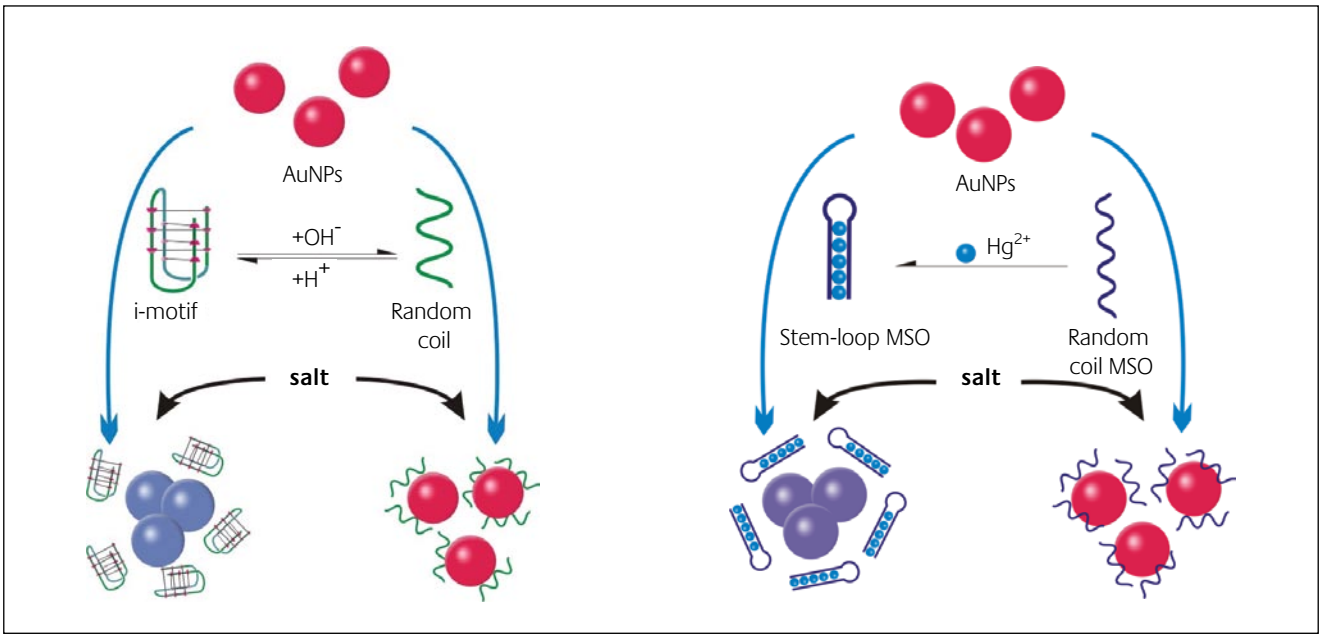

Figure 1

Schemes for optical detection of the pH-responsive i-motif (left) and the mercury-specific (right) oligonucleotides

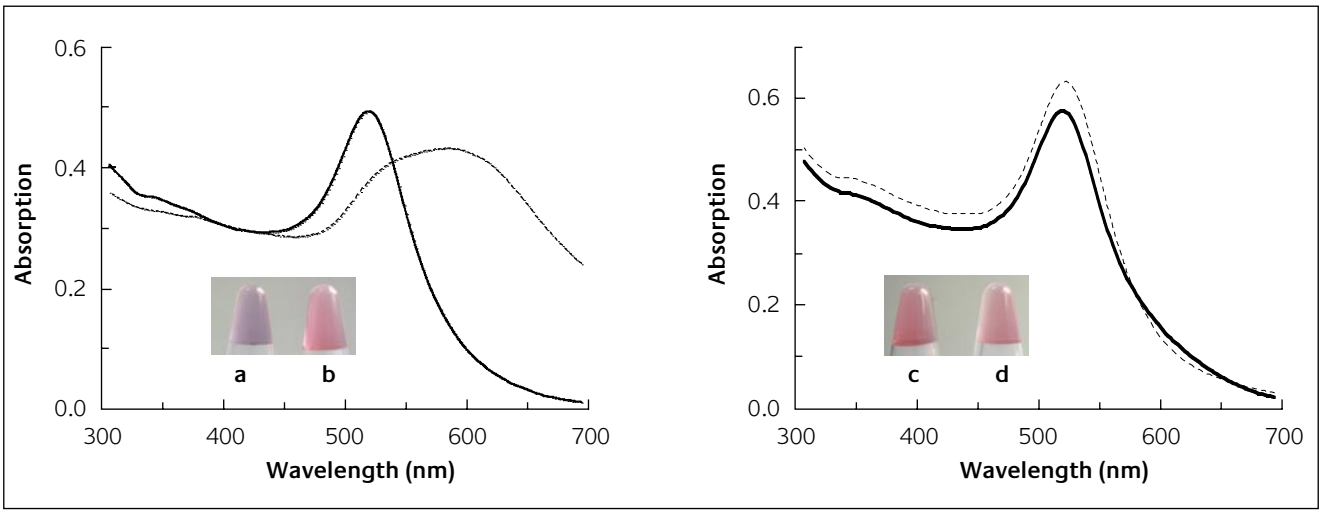

Figure 2

UV-Vis spectra for the AuNPs solutions treated with i-motif (left) at 5.5 (solid line) and pH 8.5 (dashed line). Inset: Visual detection of the structural variation of i-motif, pH 5.5 (a); pH 8.5 (b). Control experiments (right) showed the AuNPs were well stabilized with a random sequence at both pH 5.5 (solid line) and 8.5 (dashed line). Inset: Visual detection for pH 8.5 (c); pH 5.5 (d)

structures does not favor the absorption while the "soft", random coil-like oligonucleotides offer much more freedom for being wrapped at the surface of AuNPs [23].

UV-Vis spectroscopy was further employed to characterize this system. Consistently, when the i-motif structure was not formed at pH 8.5, it protected AuNPs and the SPR absorption located at 522 nm was unchanged (red color); when the i-motif structure was formed at pH 5.5, the SPR at 522 nm significantly decreased and a new absorption band at 650 nm increased, corresponding to a red-to-blue color change (Figure 2, left). In addition, we also confirmed that such color change was specific for the structural variation of i-motif. As shown in Figure 2 (right), in the presence of a control sequence that does not form the structure, solutions of AuNPs at both pH 8.5 and 5.5 retained their red color upon salt addition, suggesting that AuNPs were well stabilized irrespective of solution pH.

It is interesting to study the pH-induced i-motif transition profile. We employed solutions with a series of pH values and tested them following the above-mentioned protocol. These solutions were red at pH > 6.5 and blue at pH < 5.5, and purple-like at pH 6.0. Plotting of the absorption ratio (A650/A522) vs. solution pH showed an S-shape curve, with the sharp slope in the region of pH 5.5~6.5, and two plateaus at regions of pH > 6.5 and pH < 5.5 (Figure 3). Of note, the half-transition point was pH 6.3, which coincided well with that obtained in previous circular dichroism (CD) experiments (half-transition at pH 6.2) [24]. This clearly suggested that AuNPs might be used as a convenient and effective nanoprobe for DNA structures.

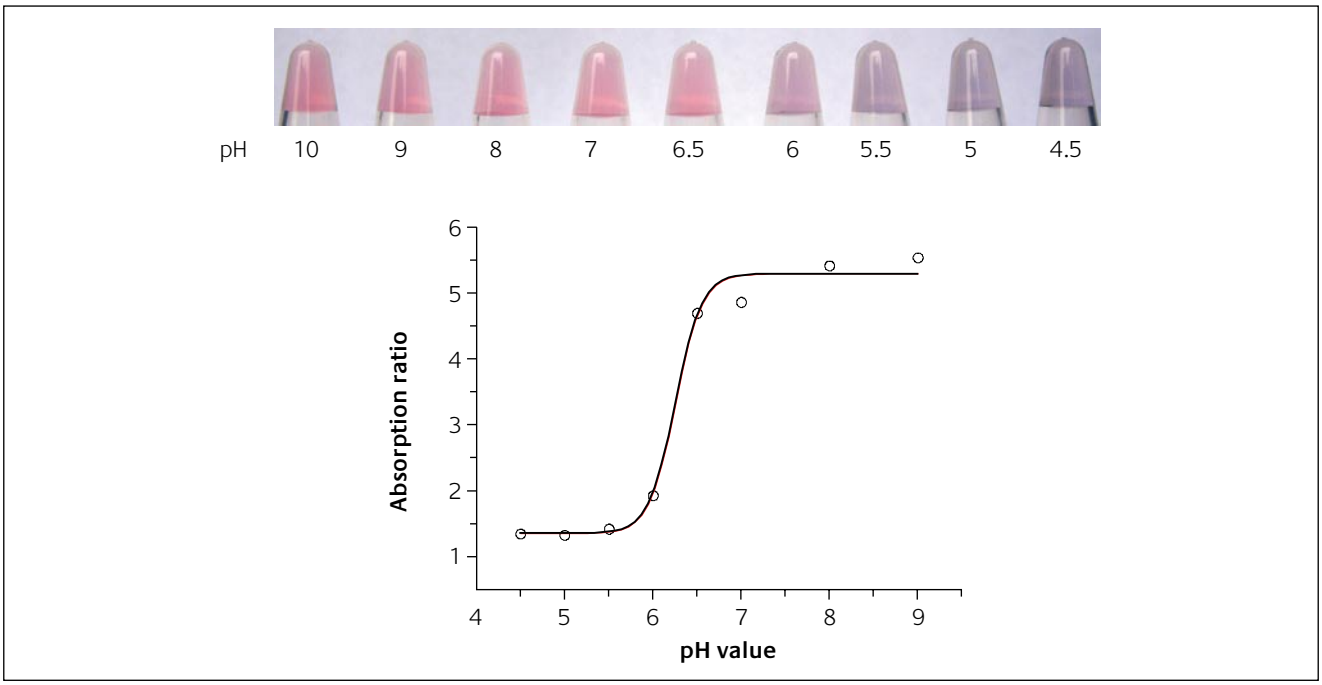

Figure 3

The pH-induced i-motif transition profile as recorded by (a) visualization and (b) absorption ratio (A650/A522) in UV-Vis absorption spectroscopy

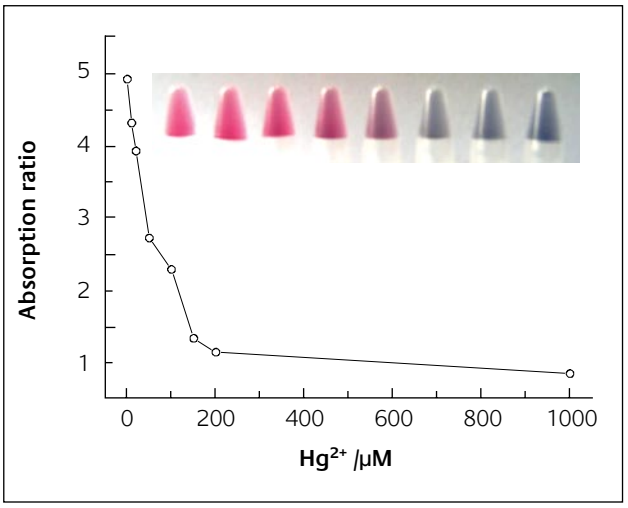

Figure 4

Plot for UV absorption ratio (A522/A650) vs. Hg^{2+}concentration. Inset: colorimetric response of AuNPs to Hg^{2+} (concentrations in test samples: 0, 10, 20, 50, 100, 150, 200, 1000 μM from left to right)

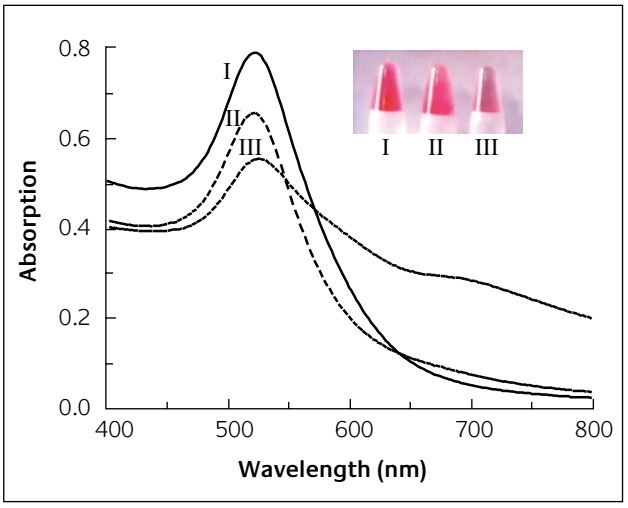

Figure 5

*Selectivity for Hg^{2+} detection. UV spectrum of three groups. **I**: Ca^{2+} and Mg^{2+} (25 mM each), **II**: a mixture of metal ions (Fe^{2+}, Cu^{2+}, Co^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+} and Cd^{2+}, (0.5 mM each)) **III**: Hg^{2+} (0.1 mM). Solutions contain 35 pmol MSO, 100 μL AuNPs and 1 μL of samples **I/II/III**, to which 13 μL of 0.5 M $NaNO_3$ was added. Inset: visualization of the assay selectivity*

We further proposed a novel strategy to detect environmentally toxic mercury (Hg^{2+}) by coupling the unique optical properties of AuNPs with the Hg^{2+}-binding specificity of a MSO probe. MSO is a thymine-rich ssDNA and exists as a random-coil in aqueous solution [25, 26], thus the unstructured MSO could protect AuNPs from being salt-aggregated (solution stayed in red). Upon binding to Hg^{2+}, MSO folded into a hairpin structure through T-Hg-T pairs. Analogous to the potassium aptamer and the i-motif, MSO only weakly interacted with AuNPs in its hairpin-structured state, and the solution of AuNPs turned to blue upon salt addition. UV-Vis characterization confirmed that the SPR absorption at 522 nm decreased, along with the increase in absorption at longer wavelength, suggesting that large-sized aggregates formed in this condition. Of note, the color of AuNPs became purple-like when reacted with as little as ~50 μM Hg^{2+}. Figure 4 showed the plot of absorption ratio (A650/A522) vs. concentrations of Hg^{2+}, the trend of which was consistent with visual observations.

As demonstrated in Figure 5, the AuNPs/MSO-based Hg^{2+} detection was also highly selective. We prepared two control solutions containing various divalent metal ions, that is, solution I (Ca^{2+} and Mg^{2+}, 25 mM each) and solution II (Fe^{2+}, Cu^{2+}, Co^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+} and Cd^{2+}, 0.5 mM each). We found that both solutions did not significantly alter the MSO structure and solutions of AuNPs stayed in red, which was in sharp contrast to the specific ion Hg^{2+}. UV-Vis spectroscopy also correlated well with these visual observations, where significant change in absorption was only observed for Hg^{2+} while not for non-specific metal ions.

Accumulation of mercury in organisms is well known to damage DNA, disrupt the immune system homeostasis, and even lead to death [27, 28]. Our proposed detection strategy for Hg^{2+} has several advantages compared to previously reported approaches. For example, it is rapid and cost-effective compared to instruments analysis techniques such as atomic fluorescence spectrophotometer (AFS), inductively coupled argon plasma mass spectrophotometer (ICP-MS) and X-ray fluorescence (XRF). Compared to previously reported fluorescent sensors for Hg^{2+} using small chelating organic molecules [29-32] the AuNPs/MSO based approach avoids the poor solubility problem and thus is applicable to detection in aqueous solution.

Conclusions

In summary, we have demonstrated that unmodified AuNPs could serve as a promising optical probe for target-responsive DNA structures by using two non-aptamer structures; i-motif and MSO. In both cases, unstructured oligonucleotides showed high affinity to AuNPs and prevented salt-induced nanoparticle aggregation, while target-bound structured oligonucleotides only weakly interacted with AuNPs and could not stabilize them against agglomeration. This difference was clearly demonstrated by the color change of AuNPs, that is, solution stayed in red for the former and turned to blue for the latter. We also demonstrated the use of this strategy to specifically detect Hg^{2+}, offering a convenient "mix-and-detect" approach to detect environmentally toxic mercury ions.

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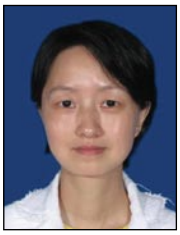

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