SCIENCE CHINA
Chemistry

• ARTICLES •

June 2016 Vol.59 No.6: 765–769
doi: 10.1007/s11426-016-5572-5

Improving the sensitivity for DNA sensing based on double-anchored DNA modified gold nanoparticles

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Received July 31, 2015; accepted October 23, 2015; published online April 15, 2016

DNA modified nanoparticles (AuNPs) are an established and widely used type of nucleotide sensor. We sought to improve the design by applying short rigid DNA duplexes near the surface of the AuNPs forming a so called double-anchored AuNP sensor, and compared it with other conventional DNA modified AuNPs. The improved design exhibited higher assembly efficiency, and consequently increased its sensitivity to target DNA.

DNA, gold nanoparticles, single nucleotide polymorphisms (SNP), surface plasmon resonance, sensing

Citation: Ma XZ, Wang M, Chen C, Isbell MA, Wang R, Liu DS, Yang ZQ. Improving the sensitivity for DNA sensing based on double-anchored DNA modified gold nanoparticles. *Sci China Chem*, 2016, 59: 765−769, doi: 10.1007/s11426-016-5572-5

1 Introduction

Gold nanoparticles (AuNPs) have played a significant role in nanotechnology notably due to their straightforward synthesis and both their size and surface chemistry customizability [1–3]. Combined with unique localized surface plasmon resonance (SPR), AuNPs have been widely used in the detection of single nucleotide polymorphisms (SNP) [4–15]. This is achieved by designing AuNPs with a layer of outwards branching single stranded DNA (ssDNA) which will hybridize with the target DNA resulting in the aggregation of AuNPs. This leads to a decrease in absorption wavelength that can be monitored by UV-Vis spectroscopy or naked eyes. However, the ssDNA on the AuNPs can fold back and adhere to the surface due to non-specific interactions between the DNA and gold surface, thus decreasing both the assembly and sensing efficiency [16–19]. Though a few previous examples illustrated several strategies to improve the design, such as employing a tetrahedron DNA or adding poly A to absorb on AuNP surface in order to decrease the non-specific absorption between the probe DNA and the surface of gold nanoparticles, a simple and facile method is consistently needed [20–26]. Herein, we developed a DNA-AuNP sensor with improved sensitivity by adding a short duplex proximally to the surface of the AuNP, which prevented the ssDNA from folding back. This strategy can be potentially utilized along with other sensors.

Our design strategy, as shown in Scheme 1, can be summarized as follows. Both 3′ thioctic acid modified 12 bp DNA **a** and 5′ thioctic acid modified 24 bp DNA **b** were synthesized with DNA strand complementarity [27,28].

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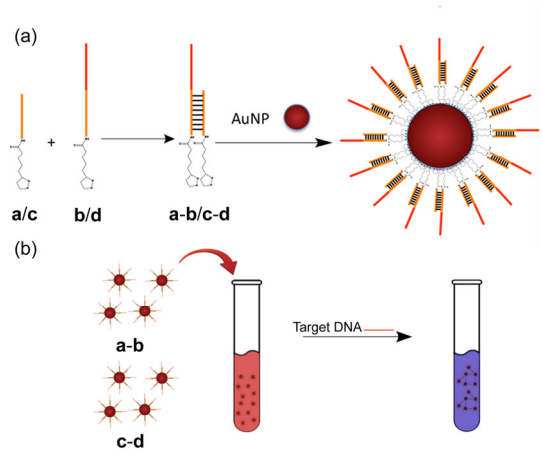

Scheme 1 AuNP sensor design schematic. (a) Preparation of double-anchored AuNP sensor; (b) in the presence of target DNA, the color of the sensor changes from red to purple (color online).

Stoichiometric amounts of **a** and **b** were then annealed to form the duplex structure **ab** with a dual thioctic acid group at one end and a 12 bp sticky end at the other. AuNPs of diameter size 13 nm were prepared according to the literature [5,23,29]. An identical procedure was followed for the geometrically identical but non-complementary **a** and **b** equivalents, **c** and **d**. The duplex **ab** and **cd** structures were bonded to the AuNPs, and the two double-anchored **ab** and **cd** modified AuNP solutions were mixed at 1:1 ratio to form double-anchored AuNP sensors [17,30,31]. In the presence of target DNA, which was complementary to the sticky ends of the duplexes **ab** and **cd**, two types of double-anchored AuNPs were connected by DNA hybridization resulting in the aggregation of AuNPs at the nanoscale and a color shift visible to naked eyes.

There are several advantages to this strategy compared to other conventional ssDNA modified AuNPs: (1) the dual thioctic-gold bond both stabilizes and reinforces the linkage between the DNA and AuNPs; (2) the short 12 bp duplex prevents the single stranded DNA from folding back onto the surface of the AuNPs; and (3) the steric hindrance caused by the duplex creates more space between adjacent DNA strands, thus facilitating the hybridization between the AuNPs DNA and the target DNA. Taking all these factors into account, the double-anchored AuNPs performance as a sensor is more effective.

2 Experimental

2.1 General methods

All DNA (including 5′ or 3′ amine-linker modified) were purchased from Invitrogen Corp. (USA). Sodium chloride (NaCl, 99.5%), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI, 98%), 4-(dimethylamino)pyridine (DMAP, 99%) and *N*-hydroxysuccinimide (NHS, 98%) were purchased from Sigma Aldrich (USA). Gold(III) chloride trihydrate was purchased from J&K (China). Thioctic acid was purchased from Alfa Asesar (USA). Acetonitrile (ACN, HPLC grade, meets ACS specification, SK Chemical, Japan), acetic acid (99.8%, for biochemistry, Acros Organics, USA) and triethylamine (99%, pure, Acros Organics, USA) were used in HPLC (1200 Infinity serious, Agilent, USA). Water used in all experiments was ultra-pure MilliQ water (resistance>18.2 MΩ/cm). All the UV-Vis spectra came from a UV-Vis spectrophotometer Cary 100 Bio, Varian's (USA). The transmission electron microscope (TEM) used was a JEM-2010, JOEL (Japan).

2.2 Synthesis of thioctic acid modified DNA molecules

Thioctic acid ester (synthesis method showed in the Supporting Information online) was added in excess (molar ratio of thioctic acid ester and amine linker modified DNA at more than 200:1) to amine linker modified DNA at a molar ratio of more than 200, in 50 mM pH 7 acetic acid-triethylamine (TEAA) buffer containing 70% acetonitrile (ACN) and 30% water. The mixture was incubated at room temperature over 10 h. The thioctic acid-DNA was purified by the 3 K cutoff (14000 rcf, 30 min) and HPLC to get pure thioctic acid modified DNA molecules [30].

2.3 Synthesis of 13 nm AuNPs

The synthesis of 13 nm AuNPs followed published methods [5,23,29]. 48 mL of water and 2 mL of 1% (*w*/*v*) $HAuCl_4$ were placed into a 100 mL flask. The solution was then boiled where 5 mL of 1% (*w*/*v*) sodium citrate was rapidly added. The solution boiled for 10 min until the color of the solution turned to deep red. Then the heating mantle was removed and the stirring was continued until it cooled to room temperature. TEM and dynamic light scattering (DLS) were used to identify the diameter of the product (Figure S1, Supporting Information online).

2.4 Synthesis of double-anchored AuNP sensor

100 μL of DNA **a** and **b** were mixed in 20 mM pH 7.0 phosphate buffer with 50 mM NaCl, quickly heated to 95 °C, and then gradually cooled to room temperature to form the **a-b** or **c-d** molecule. To make the double-anchored AuNP sensor, 16.5 μL of **a-b** (25 μM) or **c-d** was added to the 200 μL AuNP solution (~14 nM), respectively [19]. Then, 20 μL 200 mM pH 7.0 phosphate buffer was added into the mixture and left undisturbed for the first 12 h. 22 μL of 1 M NaCl was added to increase the concentration of NaCl to 100 mM. After mixing at room temperature for 40 h, the DNA-AuNP sensor was purified via centrifugation (14000 r/min, 30 min), and dissolved in 200 μL 20 mM pH 7.0 phosphate buffer with 300 mM NaCl solution. The final sensor solution was a bright red liquid (~9 nM).

2.5 Target DNA detection using double-anchored AuNP sensor

A control UV-Vis spectrum, between 400 and 800 nm at room temperature, of the double-anchored AuNP sensor was recorded. Then 1 μL of 100 μM target DNA (the no-mismatch target DNA, the half sequence mismatches target DNA or the whole sequence mismatches target DNA) was added to 50 μL of double-anchored AuNP sensor solution producing a black precipitate. The mixture was left undisturbed for about 30 min and then vortexed to redisperse the black precipitate. A UV-Vis spectrum, with an identical setup to the control, of the mixture was recorded to compare to the control spectrum.

2.6 The selectivity of double-anchored AuNP sensor

To test the selectivity of double-anchored AuNP sensor, 1 μL of 100 μM different mismatched DNA sequences was individually added into 50 μL of 8 nM double-anchored AuNP sensor solution respectively, producing a black precipitate. The mixture was left undisturbed for 30 min until the black precipitate appeared, then the solution was vortexed to redisperse the precipitate. A UV-Vis absorption spectrum at 260 nm of the solution was performed over a temperature range of 5 to 95 °C, at a rate of 1 °C/min.

2.7 LOD and sensitivity of double-anchored AuNP sensor

To test the limit of detection (LOD) and sensitivity of double-anchored AuNP sensor, stock solutions of target DNA with different concentrations were prepared. 1 μL of target DNA solution was added into 50 μL double-anchored AuNP sensor to make final target DNA concentrations at 5, 10, 15, 20 or 40 nM. Each mixture was vortexed and was left undisturbed for 30 min, and their UV-Vis spectrums recorded respectively. The SPR wavelengths of the sensor batches before and after the addition of the target molecule were compared.

3 Results and discussion

3.1 Colorimetric property of double-anchored AuNP sensor

We investigated the optical properties of the sensor by designing three DNA sequences [5,32]: a no-mismatch target DNA, a half sequence mismatches target DNA, and a whole sequence mismatches target DNA, listed in Table S1 (Supporting Information online). The SPR wavelength of the double-anchored AuNP sensor (8 nM, 50 μL) was around 520 nm, and shifted to approximately 570 nm in the presence of 2 μM target DNA (Figure 1(a)). A color change noticeable from the naked eye occurred from bright red to purple within 5 min of mixing at room temperature (Figure 1(b)). This phenomenon was attributed to the aggregation of AuNPs linked by the target DNA through hybridization. In contrast, in the presence of half sequence mismatches of target DNA or whole sequence mismatches of target DNA at concentration 2 μM, there was no visible color change with the solution remaining red (Figure 1(c)). This was attributed to the mismatches in target DNA preventing the hybridization between the AuNPs. Aggregation of the AuNPs was also verified by TEM, Figure S3.

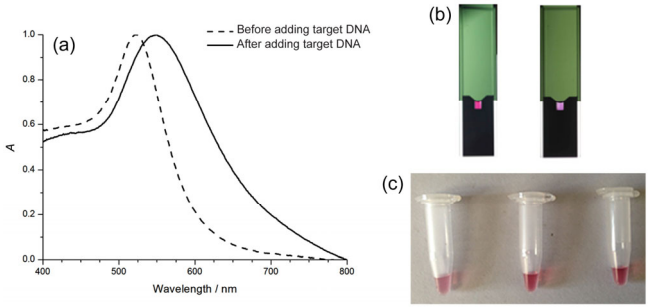

Figure 1 (a) The red shift of the double-anchored AuNP sensor before and after adding target DNA; (b) the color of the double-anchored AuNP sensor before (left) and after (right) adding target DNA for 5 min at room temperature, changing from bright red to purple; (c) the pictures of double-anchored AuNP sensor after adding no-mismatch (left), half sequence mismatches (middle), and whole sequence mismatches (right) target DNA for 5 min at room temperature, respectively (color online).

3.2 Selectivity of double-anchored AuNP sensor

We additionally sought to test the specificity of the double-anchored AuNP sensor by designing four target DNA sequences with different single mismatches, deleting the 1st or 9th base of the target DNA, or changing the 1st or 9th base of the target DNA from G to T, Table S1.

It is known that mismatches in DNA duplexes will decrease its stability and lower its melting temperature (T_m), therefore, it was possible to detect SNP by examining the T_m [5]. A stated solution containing 2 μM target analytes, target DNA or DNA with a variety of mismatches, was added into the double-anchored AuNP sensor solution (8 nM, 50 μL) respectively, left at room temperature for more than 2 h until a precipitate appeared. This was attributed to the DNA hybridization between the double-anchored AuNP sensor and the target DNA, forming the target-sensor complex. The T_m of the target-sensor complex was then measured by UV-Vis spectroscopy at 260 nm, at a temperature range of 5 to 95 °C at 1 °C/min. Figure 2 illustrates that the T_m of target-sensor complex with no-mismatch target DNA (Line 1) was higher than that of the others. It concludes that by heating the sample and observing the T_m, the double-anchored AuNP sensor can be used to distinguish target DNA accurately to within one mismatch.

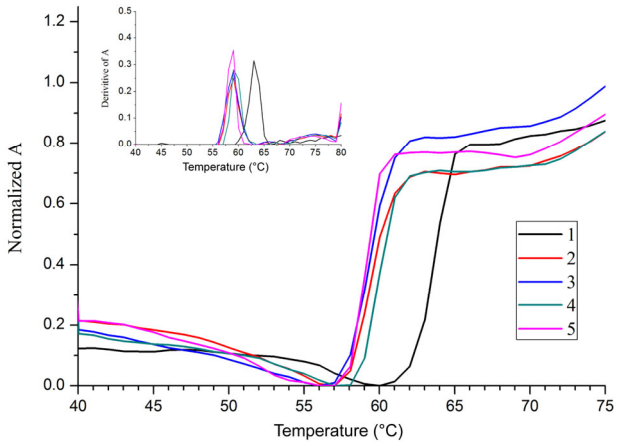

Figure 2 T_m of sensor-target complex under a mixture of target DNA and DNA with different mismatches. Absorbance values at 260 nm were recorded at 1 interval with a holding time of 1 min/°C from 5 to 95 °C. Line 1 represents no-mismatch target DNA; Line 2 represents target DNA whose 1st base was deleted; Line 3 represents target DNA whose 1st base was changed from G to T; Line 4 represents target DNA whose 9th base was deleted; Line 5 represents target DNA whose 9th base was changed from G to T. The insert figure shows the derivative of each line (color online).

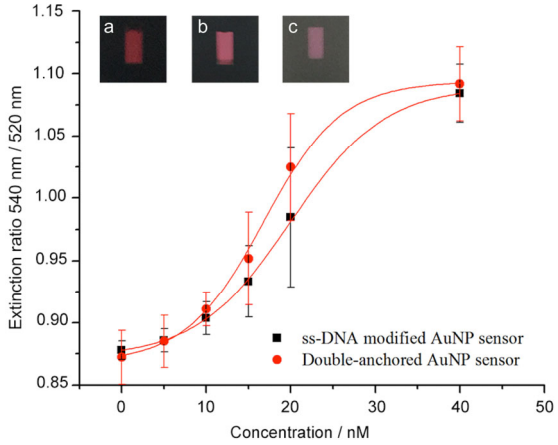

Figure 3 The extinction ratio of 540 nm/520 nm with target DNA concentration for double-anchored AuNP sensor and ssDNA modified AuNP sensor. The insert photos are the solution color change when the concentration of target molecule is 20 nM. Insets: (a) control sensor solution (double-anchored AuNP sensor without target molecule); (b) ssDNA modified AuNP sensor with 20 nM target molecule; (c) double-anchored AuNP sensor with 20 nM target molecule (color online).

3.3 Sensitivity and LOD of double-anchored AuNP sensor

Finally, we compared the performances of double-anchored AuNP and conventional ssDNA modified AuNP as sensors (the methods of making ssDNA modified AuNP sensor please see Scheme S1, Supporting Information online).

It is known that for AuNPs sensor if the concentration of target DNA is lowered, that less of them will aggregate, thus resulting in a smaller decrease in wavenumber [19]. In order to compare the LOD and sensitivity of both sensors, the concentration of target DNA was lowered from the μM to the nM scale. As a result, the SPR wavelength changed from 520 nm to approximately 540 nm, which was lower than 570 nm when the concentration of target DNA was at 2 μM (Figure 1(a)). Considering the low signal strength of the sensor when the target molecule concentration was very low, the extinction ratio for 540 nm/520 nm for both sensors at identical concentrations of target DNA could be used to evaluate the sensitivity and LOD of the sensor [19].

The ssDNA modified AuNP sensor did not contain the short ssDNA to form 12 bp duplex near the particle surface, other than that, however, the sensor was modified with the same 24 bp ssDNA as that of the double-anchored AuNP sensor and prepared in the same conditions. A stated amount of target DNA was added to both sensors respectively forming black precipitate after being left at room temperature for more than 2 h. The solution was vortexed to confirm that the target-sensor complex was well dispersed before being examined by UV-Vis spectroscopy. In Figure 3 we plotted the dose-response curve giving us the LOD of both the double-anchored AuNP and ssDNA modified AuNP sensors at about 10 nM, which was in agreement with previously reported data [4,5]. It is worth mentioning that for the double-anchored AuNP sensor, the extinction ratio for 540 nm/520 nm increased very sharply with the concentration increase of target DNA from 15 to 30 nM, and plateaued when higher than 30 nM. In a parallel experiment, the ssDNA modified AuNP sensor produced a smaller extinction ratio (i.e., weaker signal) than that of the double-anchored AuNP sensor. As the color shift could be detected by naked eyes when the red shift is above 540 nm [32], our result shows that the double-anchored AuNP sensor though have the same LOD as ssDNA modified AuNP sensor, could be observed color change under lower concentration of target molecule. We concluded that when the concentrations of target DNA were between 10 and 30 nM, the double-anchored AuNP sensor exhibited greater sensitivity compared to its ssDNA modified AuNP counterpart.

Furthermore, the accurate value of LOD of the double-anchored AuNP sensor should be calculated. As the signal change of the sensor under low concentration of target molecule is proximately linear, the LOD of the double could be approximately calculated. Firstly, we tested the red shift (absorption peak wavelength after added target molecules minus the peak wavelength before detection) of the sensor under different target molecule concentrations from 0 to 10 nM (Figure 4). From the figure we can find that the change of red shift is approximately linear. So we fitted the data linear, the equation of the fitted line is:

$$y=A+Bx$$

where y is the amount of red shift, and x is the concentration of the target molecule. After fitting, we got the value of A is 0.52, B is 0.92 and R^2 is 0.95 (the strategy table could be see in Table S1). This equation could be used to calculate the

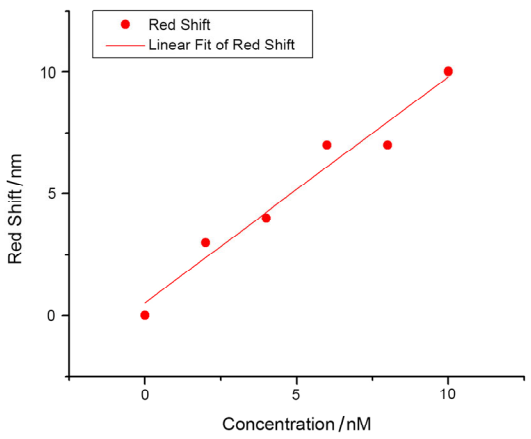

Figure 4 The LOD of the double-anchored AuNP sensor. The data was linear fitted showed as red line. The equation of the fitting is $y=A+Bx$, where A is 0.52, B is 0.92, y is red shift of the sensor and x is the concentration of target molecule. The calculated LOD of the sensor is 0.803 nM.

LOD of the double-anchored AuNP sensor. Generally, the LOD of the sensor is the lowest concentration of the target molecule that the sensor could detect, that is, the signal of the sensor after adding target molecule is higher than the noise. The wavelength of the sensor without target molecule is 523.25±1.25831, so that the LOD of the sensor is when the red shift of the sensor should be above 1.25831 nm after adding the target molecule, that is, 0.803 nM.

4 Conclusions

This study introduced a new strategy for improving the sensitivity of DNA-AuNP sensors. By extending the DNA sequence with a short duplex, proximal to the AuNPs we prevented the DNA strands from folding onto the gold surface, therefore eliminates the unwanted non-specific interactions between the DNA and gold surface. It was observed that the sensitivity of this altered AuNP sensor increased compared to other conventional ssDNA modified AuNP sensors. This design is simple and more importantly, other signal amplification methods [6,8,22,33] can be utilized alongside this sensor. This strategy could be applied to other DNA-AuNPs based sensors, including those for detecting ions [34] or biomolecules [35], and medical diagnoses [36–39].

Acknowledgments This work was supported by the National Basic Research Program of China (2013CB932803), the National Natural Science Foundation of China (91427302, 21421064), and the National Natural Science Foundation of China-Deutsche Forschungsgemeinschaft (NSFC-DFG) joint project TRR61.

Conflict of interest The authors declare that they have no conflict of interest.

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