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Articles

Electrical Detection of Oligonucleotide Using an Aggregate of Gold Nanoparticles as a Conductive Tag

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Sequence-specific DNA detection is a routine job in medical diagnostics and genetic screening. Alternative to a fluorescence readout scheme or electrophoresis approach, various kinds of rapid, low-cost, facile, and label-free methods have also been developed in last decades. Among these, direct electrical detection of DNA received increasing attention but more research is desirable. Particularly, enhancement with high discrimination must be employed to selectively amplify the responding signal. A chip-based biosensor was developed in this work to electrically detect 22-mer oligonucleotide DNA at low concentration, from 50 fM to 10 pM. First, a gold nanoparticle (NP) was capped with 3-mercaptopropionic acid through a thiol−gold bond. The derivatized carboxylic acid group showed strong complex interaction with an inorganic linker, Zr^{4+}. As a result, Zr^{4+} could link several hundreds of individual gold NPs together to form an aggregate of nanoparticles (ANP), which was capable of being used as a conductive tag for the electrical detection of DNA. Second, in order to achieve the discriminative localization of ANP to bridge two comb-shaped electrodes (with height of ∼50 nm and interdistance of 300−350 nm) gapped with insulative material of silicon oxide, peptide nucleic acids were covalently bonded to the silicon oxide in the gap as capture sites for DNA. After hybridization with target DNA, the charged phosphate-containing backbone of DNA was introduced into the gap. Phosphate groups also exhibited strong complex interaction with the linker of Zr^{4+} and could react with the residual Zr^{4+} on the ANP surface. As a consequence, the conductive tags were linked to the phosphate groups and localized into the gap, which could modify the conductance between the two comb-shaped electrodes in turn. The degree of modification correlated directly to the amount of hybridized DNA and to the concentration of target DNA in sample solution. Compared with the individual NPs used as the tag, a strong enhancement from the gold ANP was obtained.

Direct detection of DNA is still a challenge. Direct detection means there is no need of any kind of label so that it can provide obvious advantages over the current techniques.[1−3] Not only limited to the label-free approach, methods with easy operation, not relying on polymerase chain reaction (PCR) or any other similar target-amplification systems, were also developed recently.[4−6] Among all these developed technologies, electrochemical sensors offer sensitivity, selectivity, and low cost for the detection of DNA.[7−12] The progress made in nanotechnology provides the possibility to employ an ultramicroelectrode array for the detection. For example, multiplexing and serial readout were realized using a CMOS ASIC module and a computer-controlled multichannel potentiostat.[13] As a simplified version of electrochemical sensor, a microbiosensor based on direct electrical measurement has also attracted more and more attention.[14,15] The directly measured parameters include not only resistance,[14]

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but also impedance,[10] capacitance,[16,17] perturbation current or charge,[18] etc. Due to its being a less expensive instrument than an optical method,[19,20] and owing to its lack of need of a specific electrochemistry theory, direct electrical detection method has become a suitable candidate for the next generation of DNA sensor.[18,21−23] Furthermore, owing to its inherent superiorities of electrical transduction methods, such as excellent compatibility with advanced semiconductor technology, miniaturization, and low cost, nucleic acid biosensors based on electrical detection are capable of behaving at high performance at a low cost with a simple miniaturized readout.[22−24] This kind of detection can also be tailored to be extremely sensitive with a high multiplexing capability.[22,23] In addition, by combining the unique electrical properties of nanoscale materials, electrical detection systems supply excellent prospects for the designing DNA detection devices.

A good example is the use of metal nanoparticle (NP), such as gold [25,26] and silver NPs,[15] as conductive tags for the sensitive electrical transduction of different biomolecular recognition events. The concept was initially demonstrated by Mirkin and his co-workers.[14] After hybridization with target DNA and gold NP-labeled detection probes, gold NPs were localized into an insulative gap. The subsequent silver deposition to enlarge the gold NPs created a "conductive bridge" across the insulative gap terminated with two conductive electrodes. A simple detection of the conductance change resulted in a detection limit of ~500 fM. Unfortunately, the synthesis of the NP labels requires a complicated and tedious process and is time-consuming. Moreover, the non-target-related (nondiscriminative) deposition of silver onto electrodes itself and onto a silicon oxide background in the gap can significantly lift the background response and even lead to false-positive signals. More recently, at the cost of sensitivity, Diessel proposed a modified version of the gold NP enlargement method by introducing online continuously monitoring the autometallographic enhancement process.[25] There is a need for multistep enhancement and all the washing, drying, and measurement cycles in between. An alternative version is to shorten the gap distance, from ~20 μm[14] to less than 150 nm,[27,28] to increase the responding current signal. The signal from the background or control, however, is also increased correspondingly. Several other kinds of enhancement approach were also developed in an effort to get a strengthened response whereas the unspecific metal deposition was intentionally prevented, i.e., to increase the ratio of signal-to-noise, such as enzymatic amplification,[21,24,29] bio bar code-based amplification,[22,23,30] and so on. Nonetheless, the eventual acceptance of electrical detection techniques will depend on how these techniques compare with the current golden standards, i.e., PCR or others, in terms of simplicity, sensitivity, specificity, reliability, and portability.

Not only were the metal NPs used as the conductive tag to bridge the insulative gap, but semiconductive ITO NPs[6] and polymer nanowires[24] were also employed to modify the gap's conductance. It was found that some fundamental changes could in principle result in a much simpler and more robust system for the detection of both long- and short-stranded DNA, to bring the electrical biosensors one step closer to the market. Due to its stability and simple synthesis process, gold NP is a suitable candidate to conduct the gap sensor. Unfortunately, it has been reported that a monolayer of gold NPs was not enough to bring about an obvious response and a multilayer must be introduced to enhance the response.[31,32] The introduction of a multilayer, however, means a complicated and time-consuming process. In this context, a simplified and sensitive electrical biosensor array, based on the hybridized DNA-guided formation of a conductive network using a gold aggregate of nanoparticles (ANPs), was proposed in this work to detect 22-mer oligonucleotide DNA. The needed enhancement came from the conductive tag of ANP, an aggregate of several hundreds of individual NPs, the preparation of which has been described in our recent report,[33] when compared with the individual NP as the conductive tag. The discriminative association of the gold ANPs with hybridized DNA molecules led to the formation of an electrically conducting network in the gap. The attachment of an ANP to the DNA molecules resulted in the fact that the localized conductive tag into the gap was not just an individual NP but several hundreds of individual NPs in an aggregate, suggesting a simple operation to enhance the responding signal. The enhanced modification of the gap's conductance by the gold ANP could directly signal the presence of target DNA in the gap and the concentration of target DNA in sample solution.

EXPERIMENTAL SECTION

Materials and Reagents. $HAuCl_4$, trisodium citrate, 3-mercaptopropionic acid ($HS\text{-}C_2H_4\text{-}COOH$), zirconyl chloride octahydrate, and all other reagents of analytical grade were purchased from Sigma-Aldrich and used without further purification. The sequence of peptide nucleic acid (PNA) and DNA (all from Biolabs.) are shown in the following. PNA: 5′-NH_2- A ACC ATA

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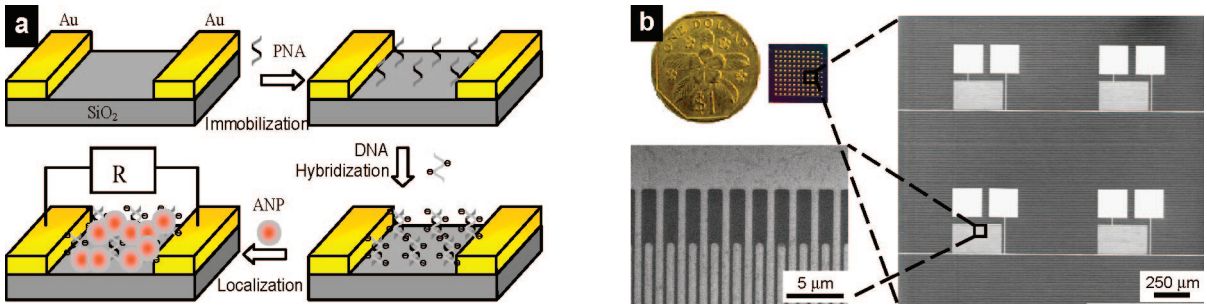

Figure 1. Schematic drawing of the detecting mechanism (a) and the chip's structure (b).

CAA CCT ACT ACC TCA-3′. DNA (i): 5′-TGA GGT AGT AGG TTG TAT GGT T-3′. DNA (ii): 5′-TGA GGT AGT AGG TTG TGT GGT T-3′. DNA (iii): 5′-TGA GCT AGT AGG TTG TGT GGT T-3′.

PNA was used as DNA catching probe in this work. DNA (i) was complementary to PNA and was selected as the target DNA, with DNA (ii) as one-base-mismatch and DNA (iii) as two-base-mismatch for negative control, respectively. Blank control means the step for DNA hybridization was ignored but all the rest of the procedures were kept the same. Hybridization was conducted in a TE buffer solution (10 mM Tris-HCl + 1.0 mM EDTA + 0.15 M sodium chloride, pH ~8.0) at room temperature (~24 °C) for 1−2 h.

Modification of Gap. A schematic drawing of the detecting mechanism is shown in Figure 1. Modification of the chip was carried out by following the previous reports.[6,24,34] First, the chip (fabricated at IME, Singapore) was washed with chloroform, acetone, and methanol, respectively. After being immersed in water overnight to transfer silicon oxide into the silicon-hydroxyl group on the surface, it was activated by dipping into a solution of 97% aminopropyltriethoxysilane (0.5 mL) + deionized water (0.25 mL) + high-purity methanol (24.5 mL) overnight. After being washed with methanol and water, the chip was baked at 120 °C for 20 min. The chip was further modified by immersing into a solution of 25 mL of 10% anhydrous pyridine in dimethylformamide containing 50 mg L, 4-phenylenediisothiocyanate for 2 h. PNA probe was immobilized onto the chip by covering the chip surface with 50 mM Na_2CO_3/$NaHCO_3$ buffer solution (pH ~9.0) containing 1×10^{-6} M PNA. Then it was put in a humid chamber containing a saturated NaCl aqueous solution at 37 °C overnight. The amino-functionized PNA was covalently bonded into the gap by the well-established isothiocyanate/amine-coupling reaction.[34] The rest of the unreacted groups were blocked with a solution of aminoethanol (0.7 mL) in 25 mL of dimethylformamide for 2 h. Then all the modified chips were stored at 4 °C for further application.

Preparation of Gold ANP from NPs. Synthesis of gold NP followed Frens' method[35] and the preparation of ANP from NP was reported in our previous paper.[33] About 100 mL of colloid solution was synthesized, and the diameter of the resulting NP was estimated to be 13 ± 2 nm.[33] At the same time, two commercial colloid solutions of gold NPs (Sigma) with specific diameters of 5 ± 1 and 20 ± 3 nm were also used for comparison in this work. HS-C_2H_4-COOH (5 μL) was added into the gold colloid solution (100 mL) with slight shaking overnight to derivatize the carboxylic acid group onto the gold NP surface.

The capped gold NPs were centrifuged at 4 °C for 10 min, 10 000 rpm. After being washed with water, it was centrifuged once again to get the derivatized NPs. Daily prepared solution of zirconyl chlorides octahydrate in 60% ethanol, from 1 to 25 mM, was used to functionalize the carboxylic acid group on the gold NP surface with Zr^{4+}. After incubation for 20 min, the sample was centrifuged once again and washed with 60% ethanol and water, respectively. In the end, the functionalized NPs were dispersed into 10 mM Tris buffer solution with pH value ~10. When it was used as the conductive tag or prepared for microscopy characterization, sonication was carried out to refresh the solution and to get the ANPs with a uniform size intentionally.[36,37] The copper grid was dipped into the resulting solution and dried in the air for transmission electron microscopy (TEM, Philip, with PW6061/25 EDX). Scanning electron microscopic (SEM) experiments were conducted on FE-SEM (JSM-6700F, Japan) after the conductance measurement.

Measurement Procedure. The modified chip was hybridized in ~5.0 mL of TE buffer solution containing different concentrations of target DNA for 1−2 h with slight shaking. After that, it was washed with SOL I (1 × SSC + 0.1% SDS) (SSC, aqueous solution of 0.3 M sodium citrate + 3 M NaCl, pH ~7.0; SDS, aqueous solution of sodium dodecyl sulfate, pH ~7.0), SOL II (0.1 × SSC + 0.1% SDS), 10 mM Tris, each for 5 min, respectively. Then the ANP dispersing solution of 200 μL was dropped onto the chip surface in an attempt to attach ANPs to the hybridized DNA molecules. Notice the solution should not be dried during this attaching period. In the end, the chip was washed carefully with Tris buffer solution and 60% ethanol, respectively. Conductance changes of at least 30 pieces of array from a chip or from different chips, each array has its own gap (Figure 1b), were recorded for every single experiment to get an average value. The conductance measurements were performed under ambient conditions in the air with an Alessi REL-6100 probe station (Cascade Microtech.) and an Advantest R8340A ultrahigh resistance meter (Advantest Corp., Tokyo, Japan).

RESULTS AND DISCUSSION

Detection Mechanism. A schematic drawing of the detecting mechanism and the chip's structure are shown in Figure 1.[24]

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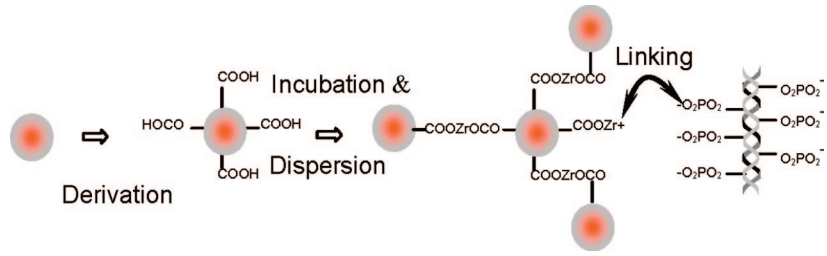

Figure 2. Preparation of gold ANP and its linking interaction with negatively charged phosphate group from the DNA backbone. The linking interaction leads to the attachment and localization of ANP into the gap.

Within the cross-finger gap area, the insulative material of silicon oxide can be modified, and the conductance between two neighboring comb-shaped gold electrodes is correspondingly modified. In this work, PNA was covalently immobilized into the gap by a sequence of steps beginning from the silicon-hydroxyl group.[34] Then it was used as a DNA probe to catch the cDNA by hybridization. After the hybridization, negatively charged phosphate groups from the backbone of target DNA were introduced into the gap. The phosphate groups showed strong complex interaction with the linker of Zr^{4+}. Due to the complex reaction between the residual Zr^{4+} on the ANP surface and the phosphate groups, the as-prepared gold ANP could be selectively attached and localized into the gap, as shown in Figure 2. As a consequence, conductance between the two neighboring gold electrodes was increased, the degree of which was correlated directly to the amount of hybridized DNA into the gap and to the concentration of target DNA in the sample solution. The reason to select PNA as the capture site is that there is no phosphate group on its neutral backbone. The attaching of the conductive tag can be avoided when there is no hybridization with the target DNA, which provides the needed specificity.

Selection of Gold ANP. As pointed out in ref 31, a monolayer of gold NPs deposited in the gap could not lead to a significant modification of the gap's conductance. Therefore, it was not individual NP but ANP that was selected to modify the gap's conductance and enhance the response in this work, as presented in Figure 1 and Figure 2.

The preparation of gold ANP is schematically drawn in Figure 2, and the detailed discussion on the formation mechanism can be found in another paper.[33] The linking chemistry of H-bond or cation complex bond has been reported by Mallouk's and Murray's group, respectively.[38,39] In this work, a cation of Zr^{4+} was selected as a linker. The derivatized COOH group on the gold NP surface exhibits strong reaction affinity to Zr^{4+} by forming a complex bond [37,38] so that the individual NPs can be linked together by this linker. When the concentration of Zr^{4+} is relatively low, a cation of Zr^{4+} has the opportunity to be anchored by at least two COOH groups. If these two groups are from two individual NPs, these two NPs are linked together. The rest of linkers functionized onto these two NPs' surface can further link other particles and so on. In the end, a network of NPs was formed, as presented in Figure 3.

On the one hand, more and more NPs were linked together to form an ANP, an aggregate of NPs, as presented in Figure 3c, d. On the other hand, the linking interaction depends on the portonation/deportonation of the carboxylic acid group and on the concentration of Zr^{4+}.[38a] That is, this kind of interaction can be adjusted by changing the pH value of the dispersion solution and the concentration of linker. By doing so, perhaps the net charge density on the surface of each individual NP has been modified, which can act as an electrostatic repulsive interaction and keep them far away from each other.[33] When the linking interaction was weakened or the repulsive interaction among the neighboring NPs was strengthened, the ANP structure was not stable any more and broke down. As a consequence, nanochain (Figure 3b), even individual NPs (Figure 3a), was obtained. On the contrary, when the linking interaction was strengthened, the ANP with diameter of ~50−200 nm (Figure 3c, d) was formed from the individual NPs with diameter of ~13 ± 2 nm (Figure 3a). The as-prepared ANP was selected as the conductive tag for biosensor, as discussed in the following.

After the formation of ANP, there are still some residual Zr^{4+} ions on the gold ANP's surface.[33] As shown in Figure 2, those residual linkers are further employed to react with the phosphate group from backbone of the hybridized DNA because this group also exhibits strong complex interaction with Zr^{4+}.[37−39] As a consequence, ANP was linked and attached onto the phosphate groups by forming the complex bond and localized into the gap after removing the dispersion buffer solution. The gap's conductance was modified in turn, the degree of which provided the needed information about the concentration of target DNA.

When used as the conductive tag, the responses of individual NPs with various diameters (some obtained commercially) were studied and compared with that of as-prepared ANP. The corresponding results are presented in Figure 4. As for the individual NP, the small one (5 ± 1 nm) shows a stronger response than the large one (13 ± 2 or 20 ± 3 nm). Although the detailed reason is not clear so far, it may be related to the fluffy structure of the large particles stacked into the gap, or due to the difficulty to localize the large particle into the gap. In ref 40, a higher density of the attached NPs was obtained from the smaller gold NPs, which seems to support the first assumption. On the other hand, however, it is well-known that the small diameter of NP can lead to an increased specificity and reactivity. The possible reason is

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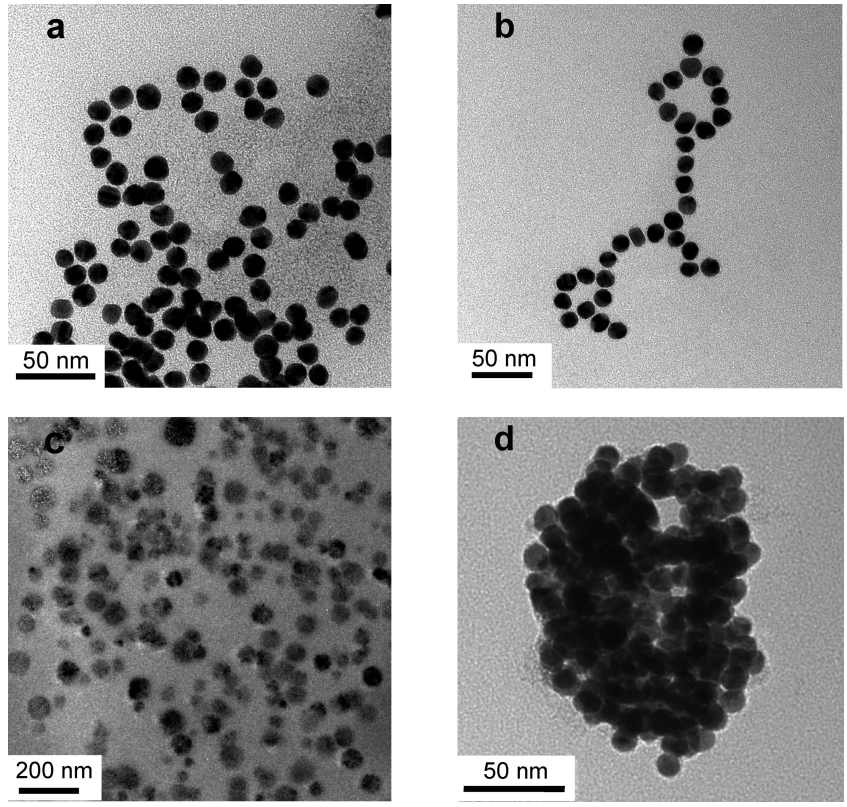

Figure 3. TEM pictures of gold NPs (a), nanochain (b), and ANP (c, d, under different magnification). The concentration of Zr^{4+} in incubation solution and the pH value of dispersing buffer solution are as following: (a) 25 mM, pH 10.4; (b) 5 mM, pH 10.4; (c and d) 1 mM, pH 10, respectively. More details in text.

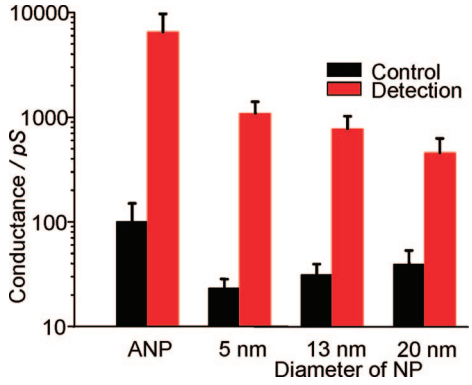

Figure 4. Response comparison among gold ANP and NP with different diameters. The concentration of DNA for hybridization was 1×10^{-11} M for DNA (i) (for detection) and DNA (ii) (for one-base-mismatch control). More details in text.

that the van Der Waals interaction is typically at or below the thermal energies of a chemical bond when the diameter of NP is small, such as less than 10 or 5 nm.[20,41] That is, the chemical bond dominates the attaching interaction when the small NPs are used as the conductive tags, which means increased localization specificity. Compared with the "control" from 5 ± 1 nm NP, the increased "control" response from 13 ± 2 or 20 ± 3 nm NPs may be due to the nondiscriminative localization of large NPs. When a further big individual NP (~100 nm) was used as the tag, an even worse response was observed (not shown here). The diameter of the big NP is similar with the as-prepared ANP, as discussed in the following.

In this work, the homemade NP with diameter of 13 ± 2 nm was selected to produce ANP, as shown in Figure 3. It is interesting to observe that the gold ANP exhibits a higher response than the individual NPs of diameter from 5 ± 1 to 20 ± 3 nm (Figure 4), suggesting a significant enhancement from the ANP. Since an ANP was composed with several hundreds of individual NPs, one ANP localized into the gap could lead to an easier formation process of the conductive network than an individual NP. As a result, the enhancement of the conductive response is expected, as shown in Figure 2. In addition, the ANP structure means not only the increased stability in the dispersion solution but also the high surface area and numerous attaching sites for the complex reaction. In the end, the ANP localized into the gap collapsed when the buffer solution was removed, which could also enhance the modification of the gap conductance. Perhaps it is the reason why the response of "control" is also relatively increased from the ANP, as shown in Figure 4.

The above assumption can be confirmed from the SEM pictures listed in Figure 5. The terminate structures of the two gold electrodes are shown in Figure 5a, e, where all the conductive electrodes are connected together on one side, just like a comb. Another comb-shaped electrode with a symmetry structure is arranged face-to-face. The two combed-shaped electrodes are gapped with a cross-finger array (100 fingers with length of 200 μm are connected in series), the bottom of which is insulative material of silicon oxide. This kind of structure was designed to average any possible variation from the fabrication process, from the chemical modification, and from the measurement process.

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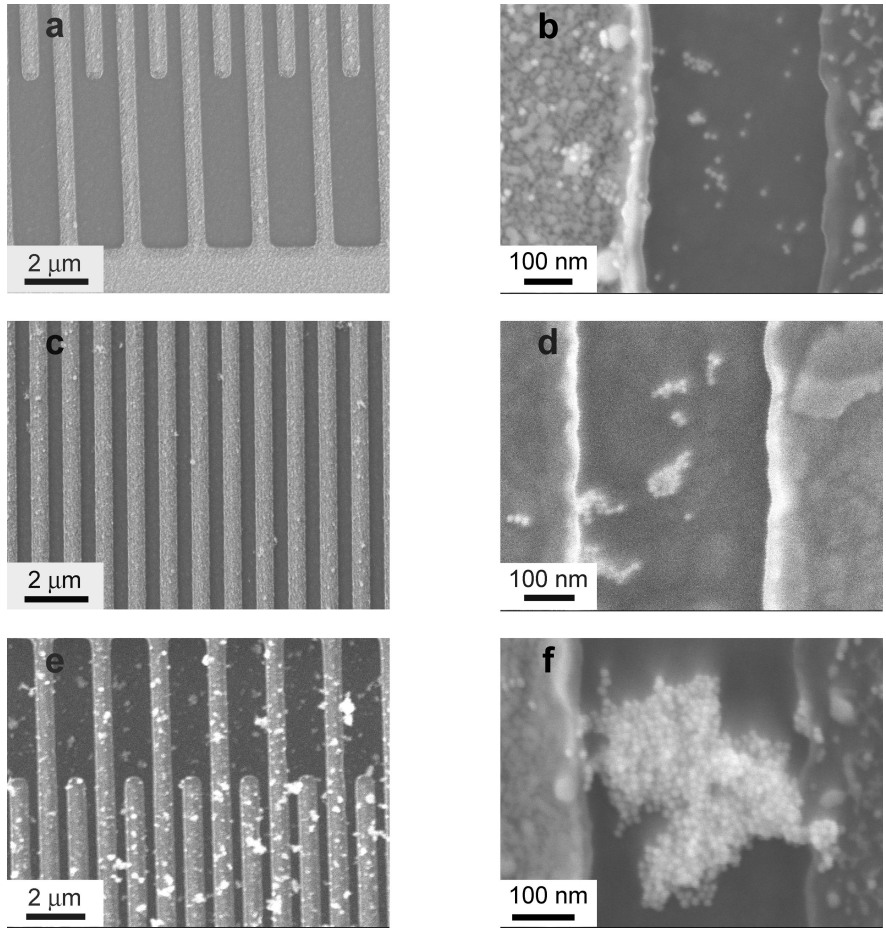

Figure 5. SEM pictures of modified gaps with individual NPs of diameter 13 ± 2 nm (a, b), with as-prepared ANPs (c−f), respectively. In all cases, the concentration of DNA (i) (a, b, e, f) or DNA (ii) (c, d) used for hybridization was 1×10^{-11} M. More details in text.

The individual NPs are also capable of being localized into the gap after the surface modification with linker of Zr^{4+}, as shown in Figure 5a, b. This experiment was carried out for the comparison with the ANP as the conductive tag (Figure 5c−f). The hybridized items were DNA (i) in Figure 5a, b, e, f, whereas for DNA (ii) in Figure 5c, d, the latter was used as one-base-mismatch control. Some deposited individual NPs are visible in the gap (Figure 5b), which could lead to the modification of gap's conductance, but the response was weak due to its low density.[31] ANP, however, could enhance the response by localizing much more NPs into the gap, no matter the hybridized DNA was DNA (ii) (Figure 5c, d) or DNA (i) (Figure 5e, f). When DNA (ii) was used for the hybridization as control, the low density of localized conductive tags is observed in Figure 5c, d, suggesting the coverage of caught DNA is low. The gap was bridged in Figure 5f when the concentration of target DNA (i) was high (10^{-11} M). The nonuniform distribution of the conductive tags along the gap is also observed in Figure 5e. Fortunately, plenty of gaps (100 fingers) were connected in series to average this kind of random effect, as mentioned above. Furthermore, at least 30 pieces of different arrays were tested to further diminish the random factors.

The collapsed ANPs are shown in Figure 5d, f. After collapse, the individual NPs aggregated together and the conductance was further increased. The transportation of electrons among the neighboring metal NPs is not yet completely understood.[2,5,14,38] There are reports about the two-dimensional[40] or three-dimensional[31] network of the individual NP that transports the electron through alkanethiolate chains or by hopping.[38c] Even the participation of ds-DNA into the conductive process has also been reported.[42,43] Detailed discussion is beyond the scope of this work. Some NPs also deposited onto the gold microcomb electrode surface (Figure 5b, d, f); fortunately it exhibited almost no effect on the conductance of the gap.

Detection Range. The results in Figure 6a show the influence of pH value of the buffer solution that was used to disperse the as-prepared gold ANP. The pH can not only modify the size and stability of the ANP but also exhibit strong influence on the complex interaction of linker with carboxylic acid or with phosphate group.[38,39] A low pH value can easily lead to the precipitation of the as-prepared ANP.[33] The unstable ANP can be localized into the gap with low discrimination, which results in the high response from background or from "control". On the other hand, a high pH value can bring about the serious hydrolysis of Zr^{4+}, which is used as the linker to produce the ANP. After hydrolysis, linker may be removed from the conductive tag, which should be avoided. Therefore, a Tris buffer solution of pH ~10 was selected according to the results.

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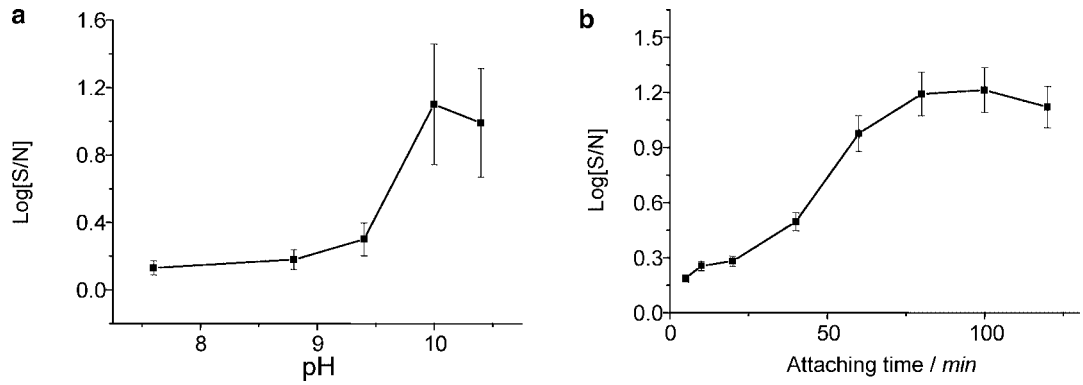

Figure 6. Effect of pH value of the buffer solution employed to disperse ANPs (a) and effect of the attaching time of ANP into the gap (b). Log[S/N] means the logarithm of the ratio between detection signal (from target DNA (i)) and "control" (from one-base-mismatch DNA (ii)). The concentration of DNA (i) or (ii) was 1×10^{-11} M in all cases. More details in text.

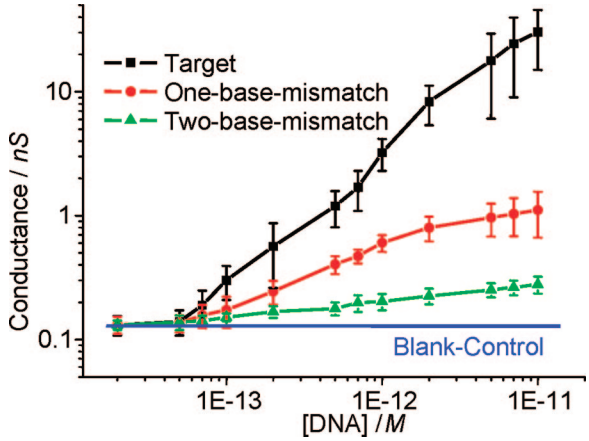

Figure 7. Dependence of gap conductance on the concentration of DNA. "Blank control" was carried out by ignoring the step of DNA hybridization; all the rest of the procedures were kept the same after hybridization with DNA (i) (target), DNA (ii) (one-base-mismatch), or DNA (iii) (two-base-mismatch). More details in text.

The attaching time of the gold ANP is also critical for the detection, as shown in Figure 6b. The short attaching time leads to a low response, indicating the attaching process of ANP onto DNA backbone by linking is slow. Usually, after ~60 min, the response is significant. However, too long an attaching time can result in a high possibility for the ANP to precipitate onto the blank bottom with low discrimination, which leads to a high response from "control". Therefore, 60−90 min was selected as the attaching time for detection.

The calibration curve is presented in Figure 7. There is a linear relationship between the logarithm of target DNA concentration and the logarithm of gap conductance with a detection limit of 5×10^{-14} M. If ignoring the semiplateau response at the high concentration range, which may be related with saturation state of the hybridized DNA, an equation is obtained.

$$\text{conductance/(nS)} = 10^{12.97}[\text{DNA/(M)}]^{1.05}, \quad r = 0.997 \quad (1)$$

The exponent of 1.05 is shifted from 1.0, indicating the complicated conductive process from the collapsed ANP, as discussed above. In other words, the calibrating equation is dependent on the employed approach to bridge the gapped electrodes.[21−30] The "control" experiments from one-base-mismatch and from two-base-mismatch are also listed, suggesting the needed specificity of this detection approach. As for the detection limit of 5×10^{-14} M, since ~5.0 mL of sample solution was used for hybridization and the hybridization efficiency was less than 30%,[44−46] each gap array could catch a maximum of 5×10^5 target molecules (one chip consisted of 100 arrays). From Figure 1b, it can be seen that the gap area inside the cross-finger is smaller than the blank area outside. Taking the ratio as ~1:100, there could be at most 5×10^3 target DNA molecules fixed into a gap array to modify the conductance after attaching ANP. That is the reason why ANP must be selected to enhance the response. The density of target DNA in the gap is relatively low. If the individual NP, not ANP, is localized into the gap as the conductive tag, the modification of gap conductance is correspondingly weak, as shown in Figure 5b. If the hybridization kinetics of DNA can be improved to increase the hybridization efficiency, the volume of target DNA sample solution is enlarged during the hybridization process,[47] and the whole area on the chip is passivated except the gap area exposed to focus the hybridization into the gap;[48] a further low detection limit is expected in turn.

CONCLUSIONS

A biosensor based on gapped electrodes was developed in this work to detect a low concentration of DNA with a significant enhancement from an aggregate of gold NPs when compared with individual NP used as the conductive tag. PNA with neutral backbone was immobilized into the gap as the capture site and provided the discriminative localization of conductive tag. The hybridized DNA offered negatively charged phosphate group from its backbone as the attaching site to localize ANP into the insulative gap for the electric detection of DNA. If a longer stranded DNA is caught and fixed into the gap by hybridization, there will be more attaching site to react with linker. As a consequence, more conductive tag can be localized into the gap and a higher sensitivity is expected in turn. The present approach

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can be generalized for the detection of other DNA sequences using appropriate and complementary PNA sequences.

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