Dendritic amplification of DNA analysis by oligonucleotide-functionalized Au-nanoparticles

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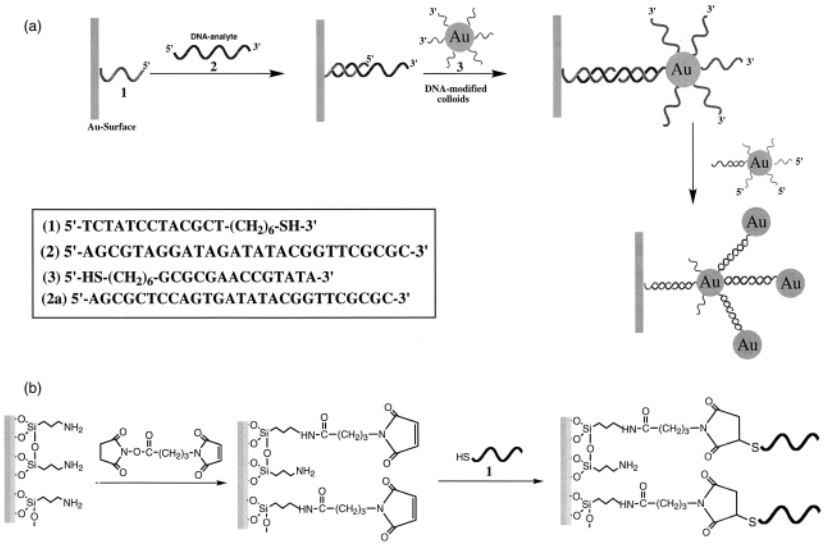
Dendritic amplification of DNA analysis is accomplished by the application of 5′- and 3′-terminated oligonucleotide-functionalized Au-colloids complementary to the analyte DNA.

The development of DNA sensors attracts recent research efforts directed to gene analysis, identification of genetic disorders, tissue matching, and forensic applications.[1,2] Photonic detection of DNA was accomplished by the use of fluorescence-labeled oligonucleotides,[3] the use of surface plasmon resonance, SPR,[4] and through the application of oligonucleotide-modified Au-nanoparticles which exhibit plasmon absorbance.[5,6] Electronic transduction of oligonucleotide–DNA recognition events, and the amplified transduction of DNA sensing, are major challenges in DNA-based bioelectronics.[7] Electrostatic attraction of transition metal complexes[8] or dyes[9] was used for the voltammetric probing of the formation of double-stranded assemblies. Amplified electronic transduction of DNA sensing events was accomplished by the application of enzyme labels that bind to the double-stranded (ds) assembly,[10] the use of protein labels,[11] or the use of branched oligonucleotides.[12] Other amplification routes of DNA sensing events included the application of enzymes that biocatalyze the precipitation of an insoluble product on the transducer,[13] or the use of liposomes that bind to the ds-DNA assembly.[14,15] Electrochemical[12–15] or microgravimetric, quartz-crystal-microbalance,[11,16] methods have been applied as electronic transduction signals for the sensing events. Here, we report on a novel method to amplify DNA sensing events by the application of oligonucleotide-functionalized Au-nanoparticles for the amplified sensing of DNA by a dendritic-type amplification route. Microgravimetric, quartz-crystal-microbalance (QCM) experiments are used to follow the amplified DNA-sensing processes.

Scheme 1(a) shows the method of the amplified sensing of a target DNA. A primer thiol-functionalized oligonucleotide **1** is assembled on the Au-electrode, and the target analyte DNA **2**, hybridizes with the sensing interface. The primary amplification of the sensing process is performed by the interaction of the surface with the **3**-functionalized Au-particles. The secondary, dendritic-type amplification is performed by the interaction of the resulting interface with the analyte sample **2** that is pretreated with the **1**-functionalized Au-nanoparticle. The mass associated with the Au-nanoparticles linked to the crystal in the primary hybridization step, and the dendritic structure formed in the secondary hybridization process, amplify the sensing of **2**. Note that the amplifying Au-nanoparticles that are used in the two modification steps have different modification layers, and are functionalized with the 3′-terminated and the 5′-terminated thiolated oligonucleotides, **3** and **1**, respectively. Both oligonucleotides, **3** and **1**, are complementary to the two ends of the analyte **2**. The **1**- and **3**-functionalized Au-nanoparticles were prepared according to the literature[6] by the reaction of citrate-stabilized Au-nanoparticles (12 ± 1 nm, 5 mL) with the thiolated oligonucleotides **1** or **3** for 20 h.

Fig. 1(A), curve (a), shows the frequency changes of the Au-quartz crystal (AT-cut, 9 MHz) functionalized with **1** (surface coverage 1.4×10^{-11} mol cm^{-2}) as a result of interaction with the analyte **2** (2×10^{-8} M). A frequency decrease of *ca*. 9 Hz is observed. Treatment of the resulting interface with the **3**-functionalized Au-nanoparticles results in an additional frequency change of *ca*. 60 Hz [Fig. 1(A), curve (b)], indicating that the binding of the Au-nanoparticles indeed amplified the primary sensing of **2**. The amplified sensing of **2** is specific and the interaction of the sensing interface with the non-complementary DNA **2a**, at a high concentration (5×10^{-6} M), does not yield any change in the crystal frequency (*ca*. −1 Hz) even after its interaction with the **3**-functionalized Au-nano-



Scheme 1 (a) Dendritic amplified DNA-sensing using oligonucleotide-functionalized Au-nanoparticles. (b) Immobilization of the thiolated oligonucleotide **1** on a glass support.

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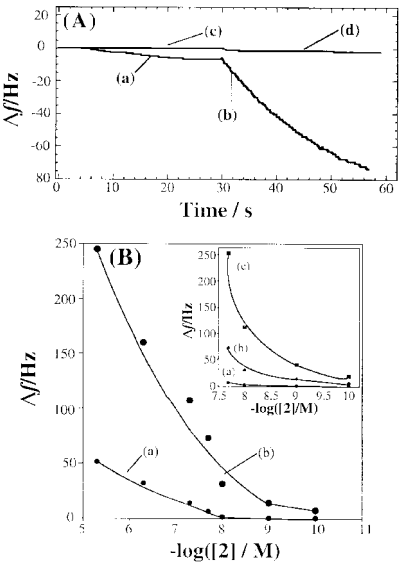

Fig. 1 (A) Time dependent frequency changes of a **1**-functionalized Au-quartz crystal upon interaction with: (a) the analyte DNA **2** (2×10^{-8} M). (b) After treatment of the double-stranded assembly of **1** and **2** with the **3**-functionalized Au-nanoparticles. (c) After treatment of the sensing interface with **2a** (5×10^{-6} M). (d) After treatment of the resulting surface with the **3**-functionalized Au-nanoparticles. All measurements were performed in $2 \times$ SSC buffer, pH = 7. (B) Frequency changes of the **1**-functionalized Au-quartz crystal upon the dendritic amplified sensing of different concentrations of the analyte DNA **2**: (a) upon the association of the analyte **2** with the sensing interface. (b) Upon the amplification of the primary double-stranded assembly of **1** and **2** with **3**-functionalized Au-nanoparticles. (c) Upon the dendritic amplification of the primary **1**–**2**–**3**–Au-nanoparticle array with the **2**–**1**–Au-nanoparticle probe. The Δf values reported in curves (a) and (b) were measured in solution ($2 \times$ SSC, pH = 7.0). The Δf values reported in curve (c) were measured in air. The double-stranded oligonucleotide–DNA assemblies in the first or second amplification steps were interacted for 25 min with the **3**-functionalized Au-nanoparticles or the **1**-functionalized Au-colloid. (SSC = 10 mM sodium citrate and 150 mM NaCl.)

particles [Fig. 1(A), curves (c) and (d), respectively]. The specificity of the sensing process is observed for at least three-base mismatches between the analyte and the probe monolayer interface. The crystal frequency changes resulting upon the primary amplified sensing of different concentrations of **2**, and those originated upon the secondary dendritic-type amplification, are shown in Fig. 1(B) and its inset, respectively. Note that the frequencies of the crystals resulting in the secondary amplification process are measured in air. The primary amplification path enables the sensing of the analyte **2** in the concentration range 2×10^{-8}–1×10^{-10} M, where no noticeable frequency changes can be observed as a result of the hybridization of **2** with the sensing interface itself. The secondary amplification step results in a dendritic type, non-linear amplified transduction of the initial sensing process [Fig. 1(B), inset]. For example, while the primary binding of the **3**-functionalized Au-nanoparticle to the sensing interface treated with 2×10^{-8}, 1×10^{-8}, 1×10^{-9} or 1×10^{-10} M of **2** results in frequency changes of −70, −40, −12 and −8 Hz, respectively, the secondary amplification process introduces a net decrease in the crystal frequencies corresponding to −250, −115, −45 and −20 Hz at these concentrations of **2**. The non-complementary DNA **2a** (1×10^{-6} M) does not show any frequency change of the crystal upon an attempt to follow the two-step amplification route. At a concentration of 2×10^{-8} M of the analyte **2**, the degree of amplification of the primary hybridization process by the two-step Au-nanoparticle array is *ca.* 30-fold within a probing time-frame of 50 min (Δf changes from 8 to 245 Hz). The dendritic-type amplification of the sensing of **2** by the two Au-nanoparticles probes is further supported by optical measurements (Fig. 2). The primer

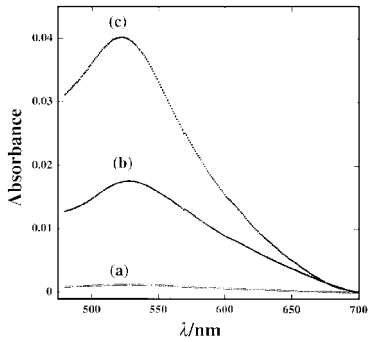

Fig. 2 Absorption spectra of a glass support: (a) after modification with **1** according to Scheme 1(B). (b) After interaction of the **1**-functionalized glass with **2** and then with the **3**-functionalized Au-nanoparticles. (c) After amplifying the primary **1**–**2**–**3**–Au-nanoparticle array with the **2**–**1**–Au-nanoparticle probe solution.

oligonucleotide **1** was immobilized on a glass support as shown in Scheme 1(B). Treatment of the **1**-functionalized glass with the analyte **2** and then with the **3**-functionalized Au-nanoparticles, results in the spectrum shown in Fig. 2, curve (b), consisting of the characteristic plasmon absorbance of Au-nanoparticles. Upon treatment of the sensing interface with the second amplification step, the Au-nanoparticle absorbance band is enhanced *ca*. 2.5-fold.

In conclusion, we have demonstrated the use of oligonucleotide-functionalized Au-nanoparticles as amplifying probes for microgravimetric QCM DNA-sensing. By the use of two different Au-nanoparticle probes consisting of oligonucleotide-functionalized Au-nanoparticles, complementary to the two ends of the analyte DNA, non-linear amplification of the sensing event was observed.

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