

Microcantilever resonance-based DNA detection with nanoparticle probes

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Microcantilevers are used to detect DNA strands with a specific sequence using gold nanoparticle modified DNA. The hybridization reactions lead to the attachment of gold nanoparticles. After the amplification process by catalyzing the nucleation of silver, the shift of the cantilever frequency signals the binding events. The method can detect target DNA at a concentration of 0.05 nM or lower. Combined with stringency washing, a single base pair mismatched DNA strand can be discriminated. The cantilever is 1/100 times smaller than its macroscopic counterpart (quartz crystal microbalance), and can be mass produced as miniaturized sensor arrays by current processing technology. Multiple DNA detection is possible by coating multiple cantilevers with various capture DNA strands and monitoring the change in their resonance frequencies. © 2003 American Institute of Physics. [DOI: 10.1063/1.1576915]

Highly sensitive and selective DNA detection has attracted extensive attention for its importance in clinical diagnostics, treatment, and various genome projects. Recently there has been a flurry of activities with the use of nanoparticle labels to detect DNA and proteins.¹⁻¹⁰ These detection methodologies strongly depend on the availability of a mechanism that transduce and amplify specific DNA binding events to detectable signals. DNA strands with a complementary sequence to that of target DNA are chemically linked on gold, magnetic, or semiconductor nanoparticles.¹⁻³ After the hybridization with target DNA, the unique optical,⁴⁻⁶ electric,⁷ or magnetic^{8,9} properties of nanoparticle probes or the presence of nanoparticle are used to detect the existence of certain DNA strand.

The “mechanics-based” detection of certain chemical or biological materials has been realized in several systems including DNA^{10,11} and protein.^{12,13} One approach is to form a compact molecular film on one side of a microcantilever that leads to a detectable asymmetric stress within the film with appropriate binding event.¹⁴ The stress is mostly determined by the physical properties of the molecule such as its length, electrostatic interaction between each molecule, and the formation of hydrogen bonding. Generally, short molecules produce less stress in the film and are thus difficult to detect. Another way is to use such cantilever as a minibalace to weigh microbead¹⁵ or solid chemical materials¹⁶ by mechanical displacement or frequency shift. Practically, however, the mass change of most molecular process is too small to be weighed directly and accurately.

In this report, we demonstrate a microcantilever based mechanical resonance DNA detection using gold nanoparticle-modified probes. We believe that the method to transduce and amplify signal has much wider implications for many binding assays in chemistry and biology, such as that in immunoassay. The core idea is to measure the mass change of a microfabricated cantilever induced by DNA hybridization through the shift of the resonance frequency of

the cantilever. The hybridization is reflected by the attachment of gold nanoparticles on the cantilever and then chemically amplified by gold nanoparticle-catalyzed nucleation of silver in a developing solution.

As shown in Fig. 1, capture DNA strands are linked on the cantilever by gold-thiol covalent bonding; after that the cantilever is dipped into the target DNA solution for hybridization. Next, gold nanoparticle labeled DNA strands are hybridized on the other end of target DNA through complementary interactions. Gold nanoparticles then act as a nucleating agent for the growth of silver when exposed to photographic developing solution. The growth of silver particles leads to a detectable frequency shift by increasing the effective mass of the microcantilever, which can be readily detected.

The sequence of thiol modified capture ssDNA is 3′-HS-(CH₂)₃-A₂₀-CTCCCTAATAACAAT-5′. The sequence of thiol modified probe ssDNA is 3′-TTATAACTATTCCTA-A₂₀-(CH₂)₂-SH-5′. Target ssDNA has a sequence of 5′-GAGGGATTATTGTTAAATATTGATAAGGAT-3′. Gold

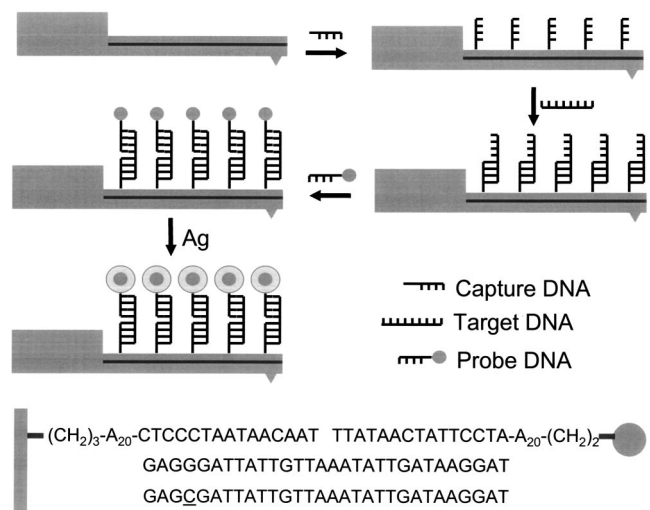


FIG. 1. Scheme of microcantilever based DNA detection using nanoparticle probe and the sequences of capture DNA, target DNA, probe DNA, and single base pair mismatched DNA.

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nanoparticles (with the diameter of 13 nm) are prepared by the reduction of HAuCl_4 by citrate. The probe ssDNA strands are loaded on gold nanoparticles by mixing the aqueous solutions and incubating overnight, followed by a salt aging process. The mixture is then centrifuged at 15 000 rpm for 30 min to remove excess probe ssDNA. The resulting precipitate is washed with 0.3 M NaCl and 10 mM phosphate buffer solution (PBS) at pH 7, centrifuged and dispersed in 0.01% azide and 0.3 M PBS solution.

Microfabricated silicon nitride cantilevers (Digital Instrument) are coated with a thin gold layer on top of a titanium layer on one side. The length, width, and thickness of the triangle cantilever are 150, 90, and $0.6 \mu\text{m}$, respectively. The other side (bare silicon nitride) is passivated to avoid the nonspecific adsorption gold nanoparticles by forming a hydrophobic layer at 150°C for 2 h in the vapor of hexamethyldisilazane (Aldrich). Although the hydrophobic surfaces might have a high binding energy to DNA chains, the randomly physical adsorption prevents the further hybridization of the flatly adsorbed DNA chains. The resonance frequency of each cantilever is measured in air on an atomic force microscope (AFM) (Nanoscope IIIa, Digital Instrument) using the same operative procedure as that used in tapping mode AFM. The controller provides the power for the cantilever vibration, laser detector, and preamp electronics. The same cantilever is used in one experiment to avoid variations in the resonance frequency. A similar frequency-based method has been used previously to measure the spring constant of single cantilever.¹⁶

Capture ssDNA strands are attached on the cantilever through gold-thiol covalent bond by dipping into a 1 mM solution of the alkylthiol-modified ssDNA in 0.3 M PBS (pH 7) for 24 h. After the cantilever is equilibrated in 0.3 M PBS solution of target ssDNA (concentration from 10 to 0.05 nM) for 4 h, it is treated with 0.3 M PBS solution of 2 nM probes ssDNA for 4 h. The cantilever is then rinsed by 0.3 M PBS to remove physically adsorbed probe ssDNA, followed by developing in silver enhance solution (hydroquinone and AgNO_3) (Sigma) for certain times. The enhance solution is changed every 3 min to avoid the formation of silver particles in the solution. The frequency is measured after the cantilever is rinsed with 0.3 M PBS and dried with N_2 .

In a typical experiment, the resonance frequency of a cantilever after sequential attachments of capture ssDNA, target ssDNA, and probe ssDNA is 115.61 kHz, which remains stable for several days and constitutes the basis for the frequency-based DNA detection. The cantilever is dipped in the silver enhance solution for specific times. After every 3 min, the cantilever is taken out of the enhance solution, rinsed with water, dried with N_2 , and the resonance frequency is recorded. It is found that the frequency decreases continuously as enhance time increases [Fig. 2(a)]. After an accumulated 18 min, the frequency is stable at 113.21 kHz, which reflects the saturated mass change [Fig. 2(b)]. Following control experiments are performed to confirm the relationship between the observed frequency shift and ssDNA hybridization events. (1) No resonance frequency shift is observed when a clean cantilever without capture ssDNA is immersed in the enhance solution for 18 min. This suggests that the gold film on a clean cantilever cannot nucleate silver

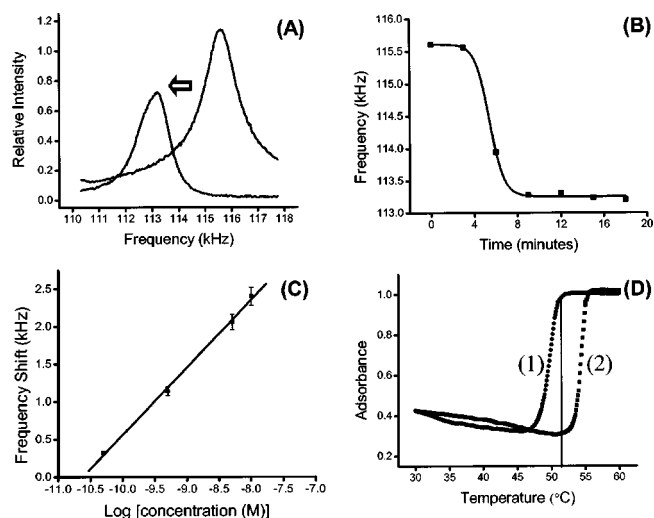


FIG. 2. The resonance curves of a cantilever before and after silver developing (a) and the frequency as a function of the developing time (b). The cantilever was modified with capture, target and probe ssDNA before dipping in the enhance solution. A linear relation exists between the magnitude of frequency shift and the concentration of target ssDNA (c). The hybridization and the enhance time are fixed at 4 h and 18 min. The thermal melting curves (d) of DNA duplex formed by complementary sequence (curve 2) and DNA duplex formed by ssDNA strands with single base pair mismatched sequences (curve 1).

coating by itself. (2) Without target ssDNA, there is no detectable frequency shift even if a cantilever with capture ssDNA is dipped in the solution of probe ssDNA for 4 h and then developed in the enhance solution for 18 min. This means no hybridization occurs between the noncomplementary capture ssDNA and probe ssDNA. (3) A similar frequency shift is observed when a chemical linker 3-aminopropyltrimethoxysilane is used to attach 5 nm gold nanoparticles (sigma) and developed in the enhance solution for 20 min. The linker forms NH_2 terminated self-assembled monolayer on silicon nitride surface (the other side of the cantilever). The positively charged film has strong affinity to sodium azide coated gold nanoparticle in solution.

We studied the dependence of frequency shift as a function of the concentration of target ssDNA by fixing hybridization time and enhance time at 4 h and 18 min. The concentration of target ssDNA as low as 0.05 nM is detected. A linear relation exists between the frequency shift and the concentration of target ssDNA over the range of 10–0.05 nM [Fig. 2(c)]. From the figure, the detection limit of the microcantilever resonance based method is 23 pM without appreciable effort to optimize the sensitivity, which is comparable to the detection limit of indodicarbocyanine labeled DNA using confocal fluorescence microscopy (above 5 pM).⁵ The target selectivity of the current method can be improved by stringency wash as in other nanoparticle-based detections.⁷ From the thermal melting curves of DNA duplex [Fig. 2(d)], we found DNA duplex formed by single mismatched ssDNA dissociates under stringency condition (0.3 M PBS at 52°C), while duplex formed by perfect matched ssDNA does not. A single base-pair mismatched target ssDNA with sequence of $5' - \text{GAGCGATTATTGTTAAATATTGATAAGGAT} - 3'$ is tested at a concentration of 10 nM and hybridization time of 4 h. After treated with the stringency solution for 10 min, the cantilever is dipped into the enhance solution for 18 min.

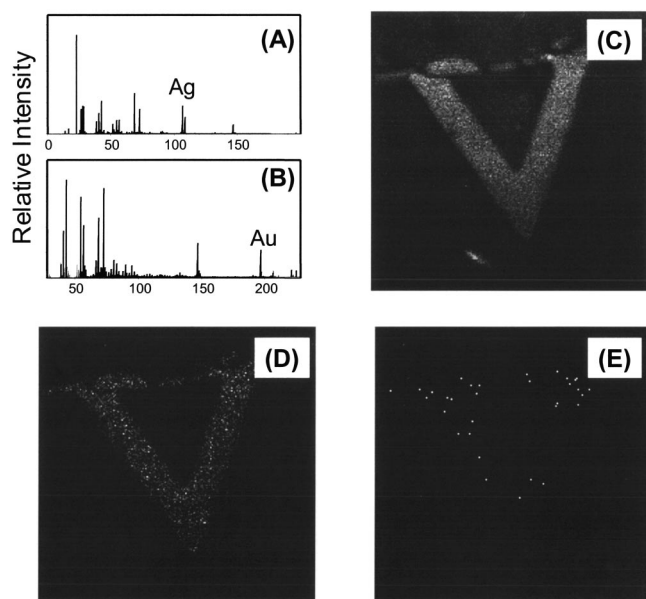


FIG. 3. SIMS of a silver coated cantilever (a). A spectrum from a new cantilever (b) is shown as comparison. The elemental distributions are reflected in total ion image (c), silver image (d), and gold image (e). These images are collected at the same time from the silver coated cantilever. For the SIMS experiment, the cantilever is removed from its support and glued on a conductive carbon tape.

The frequency shift is less than 0.1 kHz. As a comparison, in the control experiment with complementary target ssDNA, the stringency wash does not influence the frequency shift.

The nucleation of silver particle by gold nanoparticle attached on the cantilever is further confirmed by secondary ion mass spectrometry (SIMS). Figure 3(a) is a mass spectrum taken on a silver coated cantilever (hybridized in 10 nM target ssDNA for 4 h and then silver-enhanced for 18 min); two silver peaks at 106.90 and 108.90 can be seen clearly. Figure 3(b) is obtained from a clean cantilever under the same operation conditions as those of Fig. 3(a), which shows the gold peak at 196.97 and no silver signal is detected. Further evidence on the distribution of elements on the cantilever comes from elemental images. Figure 3(c) shows the image of total secondary ion of the silver coated cantilever. Figures 3(d) and 3(e) are the elemental images collected from one silver peaks (106.90) and gold peak, respectively. The uniform distribution of silver strongly suggests the nucleation of silver by gold nanoparticles, which is in dramatic contrast to the weak gold signal collected from the same cantilever.

The method described here is analogous to quartz crystal microbalance (QCM) in the vibration-working mode.^{17,18} However, there are several major differences between the two. (1) QCM sensor element is more than 100 times bigger than the microcantilever and requires large amount of DNA to give out a detectable signal. This is the reason why microfabrication methods such as microfluids are used to create miniaturized devices to reduce the amount of DNA. (2) The microcantilever enables the construction of high-density sen-

sor array to detect multiple species simultaneously at high efficiency. QCM is difficult to be integrated for its relative complex structure and means of detection: each operation unit is coated with conductive film on both sides, wired out, and excited individually. On the other hand, the AFM laser beam works remotely and can shift easily from one cantilever to another. Improved design should be able to increase cantilever density and reduce the price of individual cantilever, as has occurred in microelectronics industry. (3) The state-of-the-art AFM, with its versatile ability to work in liquid and continuously flow, and highly sensitive detection system, constitute the platform for future application and development. For example, a stiffer cantilever makes vibrating peaks sharp and the direct detection of DNA in liquid is possible; an optimized design in cantilever geometry, the using of alternate cantilever materials such as nanotube and nanoribbon, and the application of nanoparticle with different size will improve the detection limit. Most importantly, parallel detection of multiple species at the same time can be made possible by patterning different capture DNA strands on many cantilevers.¹⁹

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