ChemComm

Amplified microgravimetric gene sensor using Au nanoparticle modified oligonucleotides

Xi Chun Zhou,*[a] Sean J. O'Shea[a] and Sam Fong Yau Li*[b]

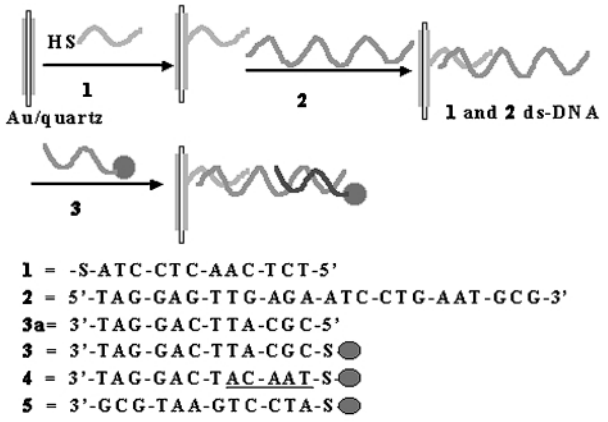
[a] *Institute of Materials Research and Engineering, 3 Research Link, Singapore 117602. E-mail: xc-zhou@imre.org.sg*
[b] *Institute of Materials Research and Engineering and Department of Chemistry, National University of Singapore, Singapore 119260*

Received (in Cambridge, UK) 3rd March 2000, Accepted 26th April 2000

A novel microgravimetric gene sensing system has been developed using an oligonucleotide anchored on the gold electrode of a quartz crystal microbalance and an Au nanoparticle modified oligonucleotide, both of which formed a sandwich-type ternary complex with the target DNA to give an amplified frequency response.

The microgravimetric quartz crystal microbalance (QCM) is a promising candidate for biosensor applications, and its potential for the detection of DNA hybridization has been demonstrated recently.[1–6] Although the QCM has a high inherent sensitivity (capable of measuring sub-nanogram levels of mass changes), methods for improving the detection limit of this device are being sought to enable wide application of the technique for DNA hybridization detection. A detection limit of *ca*. 10^{-18} M, which corresponds to *ca*. 10^{-12} g (depending on the number of DNA base pairs) of target DNA is required for many applications. The sensitivity can in some cases be improved by using QCM crystals of higher frequencies[7] or by amplification of the nucleic acids to increase the concentration of the analyte DNA by polymerase chain reaction (PCR).[8] These two methods, however, have practical limitations, particularly in the development of automated biosensor systems for genetic detection: QCM devices of higher frequencies (> *ca*. 10 MHz) are often difficult to operate in liquids because of frequency stability problems, while use of PCR can be laborious and time consuming and requires a number of manipulations. Amplifications of QCM gene sensors by adsorption of an anti-ds-DNA antibody to the formed ds-DNA complex[9] or using dendritic nucleic acid as sensing probes[10] have been reported, but the sensitivities are not good enough.

Here we describe a novel microgravimetric technique for gene detection in which a sandwich-type ternary complex consisting of an oligodeoxynucleotide (ODN) immobilized on a QCM electrode, the target DNA and a Au nanoparticle modified oligonucleotide is formed to give an amplified frequency signal (Scheme 1). Because the mass of each nanoparticle is relatively large in comparison to the masses of the binding pair members themselves, the mass coupling of the nanoparticles to the oscillator surface effectively amplifies the mass increase. As a result, this method provides an amplified frequency shift and substantially extends the limits of sensitivity of the QCM detection system.



Scheme 1 Schematic illustration of the sensing and amplified system based on the formation of a complementary sandwich-type complex.

All ODNs used in this study were prepared on a fully automated DNA synthesizer (GENSET CA). The 12-mer oligonucleotide **1**, which includes a hexanethiol residue linked to the 3′-terminus, has a sequence that is complementary to part of the target DNA **2**. Oligonucleotide **1** was immobilized *via* self-assembly on a cleaned Au electrode of the QCM (AT-cut, 10 MHz, ICM Co., 0.204 cm^{-2} area Au electrode; the detection limit of the QCM instrument in liquid = 1 Hz) from a 10 μg mL^{-1} solution of **1** (0.05 M HEPES buffer, pH 7.5) for 1 h. The immobilized amount of the probe was estimated to be 7.4 ± 1 pmol cm^{-2} from the frequency change of 6 Hz (in air), which corresponds to *ca*. 11% coverage of single strand nucleotide (area per molecule *ca*. 2.2 nm^2) on the electrode.

Gold nanoparticle modified ODN probes **3**, **4** and **5** were synthesized by derivatizing 1 mL of aqueous 13 nm diameter Au nanoparticle solution (≅17 nM)[11] with 10.5 μg of (alkanethiol) oligonucleotide. After standing for 16 h, the solution was adjusted to buffer conditions (0.1 M NaCl, 0.05 M HEPES buffer, pH 7.5) and allowed to stand for 40 h, followed by centrifugation to remove excess reagents. After removal of the supernatant, the red oily precipitate was washed twice with 0.5 mL of 0.1 M NaCl, 0.05 M HEPES buffer solution, recentrifuged, and redispersed in 0.5 mL of 0.05 M HEPES buffer (containing 0.1 M NaCl, 0.01% azide) as stock solution.

The **1**-functionalized QCM was reacted with target DNA **2** at different concentrations to form **1** and **2** ds-DNA complex. The frequency decrease resulting from the interaction of the **1**-functionalized crystal with **2** is enhanced as the bulk concentration of **2** increases. This is consistent with the increased surface coverage of the sensing interface by **2**. At a bulk concentration of **2** corresponding to 3.2×10^{-8} M, the crystal frequency decreases by 12 Hz and levels off to a constant value after 300 s exposure. Interaction of the monolayer-functionalized crystal with higher concentrations of **2** does not increase the crystal frequency change. This implies that the **1**-monolayer interface is saturated as a result of the formation of the ds-DNA complex. At a bulk concentration of **2** corresponding to 1.7×10^{-9} M, a frequency change of only 3 Hz is observed after 300 s exposure. This seems to be the sensitivity limit according to the IUPAC 3:1 signal to noise guideline.

Fig. 1 illustrates the amplification of the sensing signal by interaction of the resultant **1** and **2** ds-DNA interface (formed by interaction of the **1**-functionalized crystal with **2** at 1.7×10^{-9} M) with the Au nanoparticle modified oligonucleotide **3**. The resulting frequency decrease is enhanced as the bulk concentration of **3** increases, up to 1.3×10^{-7} M where the largest frequency decrease Δf = 67 Hz is observed [Fig. 1(*a*), (*b*) and (*c*)]. Interaction of the **1** and **2** ds-DNA with oligonucleotide **3a** at 3.5×10^{-7} M which is not modified with

DOI: 10.1039/b001737j

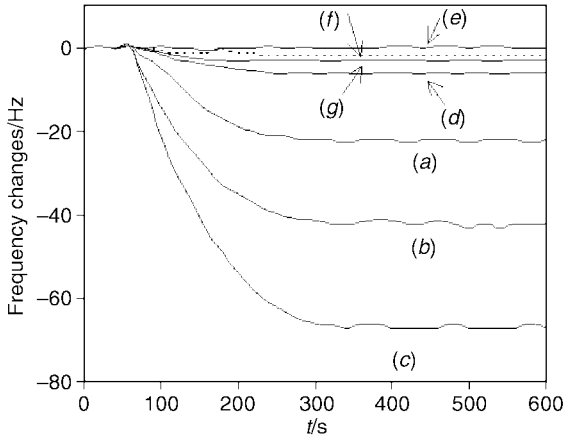


Fig. 1 Response of the **1** and **2** ds-DNA functionalized QCM upon addition of (*a*) Au nanoparticle labeled DNA probe **3,** 2.2×10^{-8} M; (*b*) probe **3,** 6.7×10^{-8} M; (*c*) probe **3,** 1.3×10^{-7} M; (*d*) **3a,** 3.5×10^{-7} M; (*e*) pure Au nanoparticle solution; (*f*) probe **5**, 7.6×10^{-8} M and (*g*) probe **4**, 6.7×10^{-8} M.

Au nanoparticles results in low frequency changes, $\Delta f = -(6 \pm 1)$ Hz, [Fig. 1(*d*)]. Increasing the oligonucleotide **3a** concentration further does not change the frequency further. The frequency change of $\Delta f = -(6 \pm 1)$ Hz at saturated adsorption for oligonucleotide **3a** is consistent with the estimated 100% hybridization of the **1** and **2** ds-DNA interface. The similar time dependency of the frequency changes (*i.e*. the attainment of steady-state signals within 300 s) observed for Au nanoparticle modified oligonucleotide **3** as compared with conventional oligonucleotide **3a** indicates that the Au nanoparticle does not compromise the hybridization kinetics. Control experiments reveal that the **1** and **2** ds-DNA interface is unaffected upon interaction with pure Au nanoparticle solution, $\Delta f = -(1 \pm 1)$ Hz, [Fig. 1(*e*)]. Furthermore, treatment of the **1** and **2** ds-DNA interface with Au nanoparticle modified oligonucleotide **5** at 7.6×10^{-8} M, does not yield any significant change in the frequency response, $\Delta f = -(2 \pm 1)$ Hz, [Fig. 1(*f*)].

Note that the oligonucleotide **5** is essentially complementary to **3**. Thus, the lack of frequency change upon interaction of the **1** and **2** ds-DNA interface with **5** indicates that non-specific oligonucleotide binding is negligible on the interface. The specificity of the sensing interface with oligonucleotide mutants was also investigated. Fig. 1(*g*) shows the interaction of the **1** and **2** ds-DNA with the Au nanoparticle labeled 12-mer oligonucleotide **4** (6.7×10^{-8} M), which has a five-base mutation in the base order compared to **2**. No detectable frequency decrease is observed, indicating that the removal of the five-base pair recognition between the sensing interface and **5** is sufficient to eliminate significant binding interactions. Note that with a fully Au nanoparticle labeled complementary oligonucleotide **3**, a frequency change of −67 Hz was observed at this bulk concentration. Therefore, the oligonucleotides modified with Au nanoparticles still preserve the specificity as that observed on QCM gene sensors with normal oligonucleotide probes.[9,10]

The results show that Au nanoparticle modified oligonucleotides can be applied to detect target DNA at sub-nanomolar concentration. The frequency changes after treatment with different concentrations of the analyte **2** followed by the Au nanoparticle modified DNA probe **3** were investigated. When the concentration of analyte **2** $> 5 \times 10^{-9}$ M, a constant frequency change was observed, implying saturation of the sensing surface. When the analyte **2** is in the concentration range of 3.2×10^{-11} M to 5×10^{-9} M, the frequency changes show a linear behavior. The crystal frequency changes from $\Delta f = -3$ Hz at 3.2×10^{-11} M of **2** to $\Delta f = -160$ Hz at 5×10^{-9} M. The linear regression line was $\Delta f(\text{Hz}) = 0.33C + 3.1$ (with C in units of 10^{-11} M) with $R^2 = 0.98$. A detection limit of *ca*. 3.2×10^{-11} M of the target DNA **2** has been estimated from the response of the QCM signal according to the IUPAC 3 : 1 signal to noise guideline. The significant sensitivity enhancement achieved with the Au nanoparticle modified oligonucleotides compared to the conventional detection system (detection limit of 1.7×10^{-9} M) is attributed to the high density of the Au nanoparticles which are capped to the DNA probes.

In conclusion, we have demonstrated a novel approach for highly sensitive detection of DNA using Au nanoparticle modified oligonucleotides as amplifying probes. The sensing process also showed specificity. This method exhibits extraordinary sensitivity and provides a simple means for rapid detection of a target oligonucleotide at sub-nanomolar concentration. Although the reported sensitivity is impressive, the sensitivity of the system may be further enhanced by using oligonucleotides modified with much smaller Au nanoparticles.

Notes and references

1 S. Yamaguchi, T. Shimomura, T. Tatsuma and N. Oyama, *Anal. Chem.*, 1993, **65**, 1925.
2 H. Su, K. M. R. Kallury, M. Thompson and A. Roach, *Anal. Chem.*, 1994, **66**, 769.
3 J. Wang, P. E. Nielsen, M. Jiang, X. H. Cai, J. R. Fernandes, D. H. Grant, M. Ozsoz, A. Beglieter and M. Mowat, *Anal. Chem.*, 1997, **69**, 5200.
4 R. C. Ebersole and M. D. Ward, *J. Am. Chem. Soc.*, 1988, **110**, 8623.
5 Y. Okahata, M. Kawase, K. Niikura, F. Ohtake, H. Furusawa and Y. Ebara, *Anal. Chem.*, 1998, **70**, 1288.
6 K. Niikura, H. Matsuno, H. Ohatake, H. Furasama and Y. Ebara, *J. Am. Chem. Soc.*, 1998, **120**, 8537.
7 K. Bizet, C. Gabrielli, H. Perrot and J. Therasse, *Proceedings of The Fourth World Congress on Biosensors*, Bangkok, Thailand, 29–31 May 1996. Elsevier Advanced Technology, Oxford, UK, 1996.
8 H. A. Erlich, D. Gelfand and J. J. Sminsky, *Science*, 1991, **252**, 1643.
9 A. Bardea, A. Dagan, I. Ben-Dov, B. Amit and I. Willner, *Chem. Commun.*, 1998, 839.
10 J. Wang and M. Jiang, *J. Am. Chem. Soc.*, 1998, **120**, 8281.
11 J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin and R. L. Letsinger, *J. Am. Chem. Soc.*, 1998, **120**, 1959.