Amplified microgravimetric quartz-crystal-microbalance analyses of oligonucleotide complexes: a route to a Tay–Sachs biosensor device

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An oligonucleotide monolayer acts as an active interface for the microgravimetric, quartz-crystal-microbalance analysis of the complementary oligonucleotide.

The formation of specific double-stranded (ds)-oligonucleotide complexes on solid supports has evoked substantial recent research activities directed to the nanoscale architecture of metal-colloid arrays by means of oligonucleotide bridges[1] and to the tailoring of functionalized surfaces for gene detection.[2,3] Electrochemical DNA-sensors based on the intercalation or interaction of redox-active transition metal complexes,[4,5] *e.g.* $Co(bpy)_3^{3+}$, or dyes[6] such as acridine or Hoechst 33258, were reported. Microgravimetric quartz-crystal-microbalance (QCM) analyses, that use oligonucleotide sensing interfaces, were recently initiated to identify oligonucleotide–protein[7] or oligonucleotide–DNA complexes.[8] Although oligonucleotide–protein interactions were reported to reveal specificity,[7] the sensitivity of the oligonucleotide–oligonucleotide or DNA sensors is low and the specificity of these devices needs to be established. Recently, we applied antigen or antibody monolayer-functionalized Au–quartz crystals for the microgravimetric analyses of the complementary antibodies[9] or antigens.[10]

The assembly of biologically active monolayers on Au-supports *via* thiol bridging units has been extensively developed by our laboratory.[11] Enzyme-electrodes,[12] photoactive enzyme-electrodes,[13] immunosensors[9,10] and reversible immunosensors[14] were developed by this method. Here we wish to report on the development of an active oligonucleotide interface for the microgravimetric, piezoelectrical analysis of the complementary oligonucleotide. The method is exemplified for the analysis of the oligonucleotide residue characterizing the Tay–Sachs genetic disorder.[15] We demonstrate means to confirm and amplify the formation of the oligonucleotide–oligonucleotide complex at the crystal interface.

The 41-mer oligodeoxynucleotide **1** includes ten successive thiophosphate thymine residues linked to the 5′-terminus and acts as thiol-tag for attachment to the Au surface.[16] The remaining 31-mer oligonucleotide includes the complementary characteristic base-order of the normal Tay–Sachs gene. The 31-mer oligodeoxynucleotide **2** includes the base sequence of TS 4I-N in the gene in which mutations lead to the Tay–Sachs genetic disorder.[15] The thiol-tagged oligonucleotide **1** was assembled on a Au–quartz crystal (AT-cut, 9 MHz, EG&G) by interaction of the crystal with a phosphate buffer (0.01 M, pH 7.3) solution of **1**, 50 μg ml for 12 h at 4 °C.

Fig. 1(*b*)–(*e*) show the frequency changes of different crystals exposed to different concentrations of the complementary oligonucleotide **2**. Interaction of the monolayer-functionalized crystal with **2** yields a frequency decrease, implying a mass increase on the crystal surface as a result of the formation of the ds-oligonucleotide between **1** and **2**, Scheme 1. The extent of the crystal frequency decrease is enhanced as the bulk concentration of **2** increases, consistent with the increased surface coverage of the sensing interface by **2**. At a bulk concentration of **2** corresponding to 11.5 μg ml^{-1} [Fig. 1(*e*)], the crystal frequency decreases by 11 Hz and it levels-off to a constant value after *ca.* 200 s. Exposure of the monolayer-functionalized crystal to higher concentrations of **2** does not enhance the crystal frequency change, and the value of $\Delta f = -(11 \pm 1)$ Hz is preserved. This implies that the **1**–monolayer interface is saturated by **2** as a result of the formation of the ds-oligonucleotide. Using the observed frequency change, we estimate the surface coverage of the ds-oligonucleotide or the surface density of the base monolayer of **1** to be *ca.* 4.4×10^{-12}

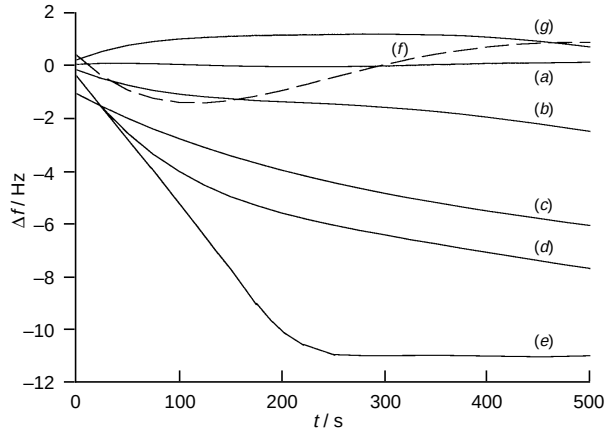
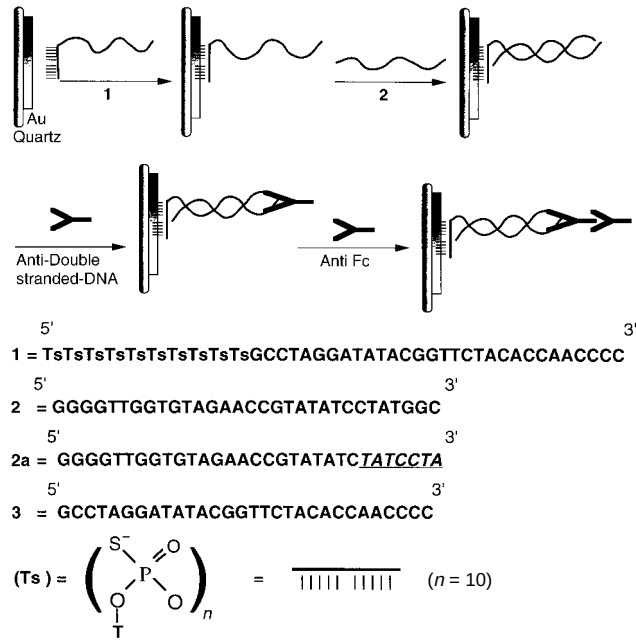


Fig. 1 Time-dependent frequency changes of a **1**-functionalized Au–quartz crystal upon addition of: (*a*) SSC buffer solution, pH = 7.0; (*b*) **2**, 0.6 μg ml^{-1}; (*c*) **2**, 2.4 μg ml^{-1}; (*d*) **2**, 3.5 μg ml^{-1}; (*e*) **2**, 11.5 μg ml^{-1}; (*f*) **3**, 11.5 μg ml^{-1}, (*g*) **2a** 2.4 μg ml^{-1}. Oligonucleotides are dissolved in SSC buffer, pH = 7.0, SSC = 30 mM Na–citrate and 300 mM NaCl.



Scheme 1 Schematic amplified microgravimetric analysis of an oligonucleotide

mol cm^{-2}. This corresponds to *ca*. 50% of a densely packed monolayer of the ds-oligonucleotide. Control experiments reveal that the frequency of a bare Au–quartz crystal is unaffected upon interaction with **2**, (0.6 μg ml^{-1}), $\Delta f = -(1 \pm 1)$ Hz. Also, treatment of the **1**-functionalized crystal with a pure buffer solution [Fig. 1(*a*)] or with the 31-mer oligonucleotide solution **3**, (11.5 μg ml^{-1}), [Fig. 1(*f*)], does not yield any significant change in the crystal frequency. Note that the oligonucleotide **3** is essentially complementary to **2**. Thus, the lack of frequency changes upon interaction of the **1**-functionalized crystal with **3** indicates that non-specific oligonucleotide binding interactions are not operative on the interface. The frequency change observed upon interactions of the **1**-modified crystal with **2** originates from specific complementary interactions that generate the ds-assembly. A major concern in the development of the microgravimetric DNA-sensor relates to the specificity of interactions of the sensing interface with oligonucleotide mutants. Accordingly the quartz-crystal functionalized with the oligonucleotide **1** was interacted with the 31-mer oligonucleotide **2a**. The latter oligonucleotide includes the characteristic mutation differentiating the normal gene and 75% of the Tay–Sachs genetic disorder carriers. Note, that a seven-base mutation occurs in **2** as compared to **2a**. Fig. 1(*g*) shows the crystal frequency response upon interaction of the sensing interface with **2a** (2.4 μg ml^{-1}). No detectable crystal frequency decrease is observed, indicating that the removal of the seven-base pair recognition between the sensing interface and **2a** is sufficient to eliminate significant binding interactions. Thus, the sensing interface reveals sufficient specificity to distinguish between the normal and mutated DNA-sequences involved with Tay–Sachs genetic disorder.

At a bulk concentration of **2** corresponding to 0.6 μg ml^{-1}, a frequency change of only 2 Hz is observed after 500 s. This seems to be the sensitivity limit. In order to confirm that the frequency change observed in Fig. 1(*b*) originates from specific complementary interactions, and to amplify the transduced sensing signal, the resulting ds-assembly was interacted with a mouse anti-ds-DNA antibody (Ab). Fig. 2(*a*) shows the crystal frequency changes upon the interaction of **1**-functionalized crystal with **2** (0.6 μg ml^{-1}). A frequency change of $\Delta f = -(2 \pm 1)$ Hz is observed. Fig. 2(*b*) shows the crystal frequency changes upon interaction of the ds-assembly with the anti-ds-DNA antibody. Note that although the coupling of **2** results in a frequency change of only $\Delta f = -(2 \pm 1)$ Hz, the association of the Ab results in a frequency change of $\Delta f = -(14 \pm 1)$Hz. This is consistent with the fact that the mass ratio of **2** : Ab is 1 : 15. Challenging of the supramolecular ds-oligonucleotide/anti-ds-DNA antibody with the goat anti-mouse Fc-antibody results in a further decrease in the crystal frequency of $\Delta f = -(8 \pm 1)$ Hz, Fig. 2(*c*). This originates from the association of the secondary antibody to the Fc-part of the anti-ds-DNA antibody, Scheme 1. The frequency decrease in the presence of the secondary antibody is only half of the frequency decrease observed with the primary anti-ds-DNA antibody. This originates from the fact that the secondary antibody includes two binding sites for the primary antibody. Control experiments reveal that the anti-ds-DNA antibody or the anti-mouse-Fc-antibody do not bind to the **1**-functionalized interface [$\Delta f = -(2 \pm 1)$ Hz]. Thus, the ds-assembly formed by the interaction between the functionalized crystal and **2** specifically associates the anti-ds-DNA antibody. The latter complex with the mouse antibody binds the secondary goat anti-mouse antibody.

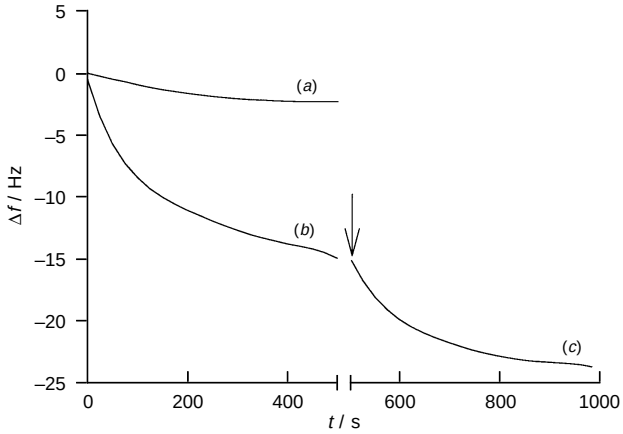


Fig. 2 Time-dependent frequency changes of the **1**-functionalized Au–quartz crystal upon addition of: (*a*) **2**, 0.6 μg ml^{-1}; (*b*) addition of anti-ds-DNA-Ab, 1.2 μg ml^{-1}, to the **1** and **2** double stranded complex on the surface; (*c*) addition of anti-Fc-Ab, 21 ng ml^{-1} to the **1** and **2** ds-complex with linked anti-ds-DNA-Ab. Arrow indicates the time of anti-Fc-Ab addition. Prior to addition of the anti-Fc-Ab, the cell was rinsed with PBS buffer.

Thus, we have developed a novel means to assemble an interface for the specific microgravimetric analysis of complementary oligonucleotides. The interaction of the resulting surface-bound ds-oligonucleotide assembly with the anti-ds-DNA antibody and with the respective anti-antibody provides means to confirm and amplify the primary formation of the complementary oligonucleotide complexes. Further attempts to regenerate the sensing interface by unfolding of the ds-oligonucleotide complex by thermal or chemical treatments are underway in our laboratory.

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Notes and References

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