45- and 70-Base DNA supramolecular polymerizations on quartz crystal microbalance biosensor

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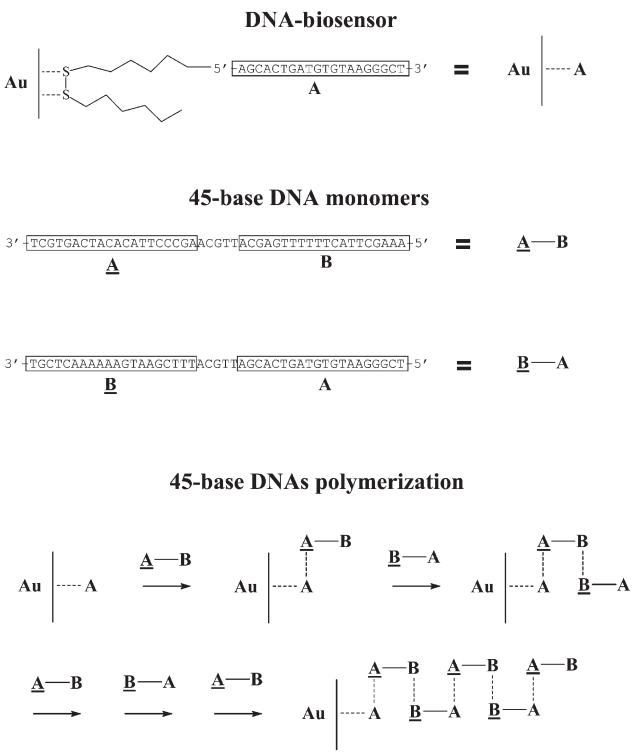
Supramolecular polymerizations of 45- and 70- base DNAs on the surface of an *in-situ* time-resolved 27 MHz quartz crystal microbalance biosensor.

The quartz crystal microbalance (QCM) classically based on a thickness shear mode resonator[1] is an useful tool in the design of sensitive and selective gravimetric *in situ* time-resolved DNA-biosensors[2] in many fields of human interest: genetic diagnosis,[3] detection of genetically modified organisms,[4] bacteria detection[5] and toxicology.[6] Moreover, QCM DNA-biosensors have been successfully used to elucidate various biomolecular mechanisms: DNA surface hybridization kinetics,[7] DNA cleavage reaction,[8] binding of globular proteins to DNA,[9] evaluation of UV-C DNA damage[10] and DNA–drug interactions.[6] Elsewhere, recent works report elegant strategies to design supramolecular DNA structures by rolling circle amplification[11] and by an enzymatic strategy that use alternatively DNA ligase and restriction endonuclease.[12] Two-dimensional supramolecular DNA structures have been designed by assembly of DNA double-crossover molecules[13] and DNA Sierpinsky triangles.[14] These DNA supramolecular structures are a pathway to many applications, some considered to be unnatural: a two-dimensional array of DNA triple-crossover molecules has been used to mimic cumulative XOR logical function[15] and the two-dimensional pattern of DNA Sierpinsky triangles can be used for implementing an algorithm for computation tasks.[14] In this work the possible use of a QCM DNA-biosensor to study dynamics of supramolecular DNA structure synthesis on a solid substrate was demonstrated: the kinetics during step-by-step polymerizations of 45- and 70- base DNAs were monitored *in situ*.

The resonator of the microbalance was an AT-cut planar quartz crystal, 14 mm in diameter with a 9 MHz nominal resonance frequency. Two identical gold electrodes, 2000 Å thick and 5 mm in diameter, were deposited by evaporation techniques on both sides of a quartz crystal with a 250 Å chromium underlayer. The resonator was connected by a silver conducting paste, through wires, to a BNC adaptor. A home-made oscillator was designed to drive the crystal at 27 MHz, which corresponds to the third overtone of the quartz resonator. To improve the stability, all the electronic oscillator components were temperature-controlled by a heater current monitor with a stability better than 0.1 °C. An experimental cell was developed by mounting the crystal between two O-ring seals inserted in a plexiglass cell. Only one face of the quartz was in contact with the solutions. The cell volume was 50 μL. The apparatus included a micropump to assure a 50 μL min^{-1} constant flow of the solutions in the cell. The experiments were performed at 25 °C, the room temperature. The frequency was computer-controlled by home-made software and measured with a frequency counter.

The gold side of the quartz used in the experiments was cleaned with a 1:1 95% H_2SO_4–30% H_2O_2 10 μL solution for 30 min and rinsed with deionized doubly distilled water. The biosensor consists of a monolayer of a 20-base disulfide–DNA probe immobilized on the cleaned gold surface of the quartz resonator;[16,17] with the 20-base DNA sequence of the probe referred to as A (Scheme 1). The frequency decrease Δf observed during the circulation of 10 μg mL^{-1} disulfide–DNA in 0.5 M NaCl solution was −206 Hz indicating adsorption of a DNA–disulfide monolayer on the gold QCM surface. The coverage of the surface τ is estimated to be 73%: $\tau = S_{disulfide}/S_{QCM} = |\Delta f|sN_A s_{disulfide}/S_{QCM}M_{disulfide}$, where S_{QCM} = 0.2 cm^2 is the active surface of the QCM, $S_{disulfide}$ is the active surface of the QCM covered with DNA–disulfide strands,



Scheme 1 DNA biosensor, 45-base DNA monomers and 45-base DNAs polymerization reaction.

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s = 350 pg Hz^{-1} is the experimental microbalance sensitivity,[18] N_A = 6.023 × 10^{23} mol^{-1} is the Avogadro constant, $s_{disulfide}$ = 2.2 nm^2 is the average area of one adsorbed disulfide–DNA molecule[18] and $M_{disulfide}$ = 6386 g mol^{-1} is the molecular weight of the disulfide–DNA. This type of DNA-biosensor is selective, sensitive and fully renewable:[16,17] it was hybridized in this study in optimized stringency conditions,[16] HEPES buffer (0.5 M NaCl, 0.05 M HEPES, pH 7.3) at 25 °C, and successfully dehybridized with a basic saline solution (0.5 M NaOH, 3 M NaCl).

The two 45-base DNA monomers referred to as A–B and B–A consist of two 20-base sequences, A, A, B or B, spaced by a 5-base DNA sequence (Scheme 1). A and A are complementary as are B and B. The frequency changes were recorded during successive circulation of 22.5 μg mL^{-1} DNA monomers A–B and B–A HEPES solutions (Fig. 1): successive frequency drops attributed to successive steps of the supramolecular DNA polymerization reaction (Scheme 1) were clearly observed. After each polymerization step, HEPES buffer circulation during 10 min was performed in order to remove non-specific DNA adsorption. The frequency decrease Δf_{A-B} of the first step is −166 Hz. The corresponding hybridization ratio r of hybridized DNA A–B, N_{A-B}, *vs.* immobilized disulfide–DNA, $N_{disulfide}$ is estimated to be 38%: $r = N_{A-B}/N_{disulfide} = \Delta f_{A-B} M_{disulfide}/\Delta f_{disulfide} M_{A-B}$, where M_{A-B} = 13 779 g mol^{-1} is the molecular weight of the A–B DNA. This hybridization ratio is consistent with values found in the literature for DNA disulfide monolayers which are 47%[16] and 48%.[17] The frequency changes of the successive steps decrease from 201 to 52 Hz, indicating a decrease of hybridized DNA ratio during the polymerization reaction. This decrease can be attributed to steric hindrance between DNA strands in the film: the reactive non-hybridized sequences at the extremities of the DNA strands anchored on the biosensor surface are less accessible to free DNA monomers in solution when the thickness of the DNA film increases. The kinetics of the hybridization reaction was estimated by calculating $\Delta\tau = \tau_{¾} - \tau_{¼}$, where $\tau_{¾}$ and $\tau_{¼}$ are, respectively, the reaction time to hybridize 75 and 25% of successive monolayers; the $\Delta\tau$ mean value is 50 ± 12 s, this value is consistent with hybridization kinetics of DNA monolayers which corresponds to 80 s.[19] A −585 Hz frequency change is determined during the five first steps of the polymerization reaction. This value is close to the value, −646 Hz, found for the hybridization of a 445 bp DNA monolayer on a 10 MHz QCM DNA biosensor, measured on a 27 MHz QCM biosensor.[16] This indicates that the acoustic behavior of the five-layer 45 base-DNA film is comparable to the acoustic behavior of a 445 base DNA hybridized monolayer. After this step-by-step polymerization, the disulfide–DNA probes were dehybridized by circulating a 0.5 M NaOH, 3 M NaCl solution for 30 min. We repeated this experiment and we find the same behavior, there is a progressive decrease of hybridization ratio during the polymerization process.

Another DNA polymerization reaction was investigated by using two 70-base DNA monomers referred to as B–C–C and C–B–B which consist of three 20-base DNA sequences, B, B, C or C, spaced by two 5-base DNA sequences (Scheme 2). C and C are complementary. The QCM frequency changes are measured during circulation of 22.5 mg mL^{-1} DNA A–B HEPES solution and successive circulation of 35 μg mL^{-1} DNA monomers B–C–C and C–B–B HEPES solutions (Fig. 2). There is a first frequency drop corresponding to A–B DNA hybridization and successive frequency drops attributed to successive steps of the supramolecular DNA polymerization reaction of B–C–C and C–B–B DNAs (Scheme 2). The frequency decrease Δf_{A-B} of the first step is −160 Hz and the corresponding hybridization ratio r of hybridized DNA A–B *vs.* immobilized disulfide–DNA is estimated to be 37%. This value is close to 38% calculated previously for the 45-base DNA polymerization: the first step of the two polymerization reactions, hybridization of A–B DNA strands, is the same. The frequency change of successive frequency shifts is constant during the five first steps of B–C–C and C–B–B hybridization, between 190 and 210 Hz, and there is a decrease from 149 to 60 Hz during the four last steps. These results indicate that there is regular

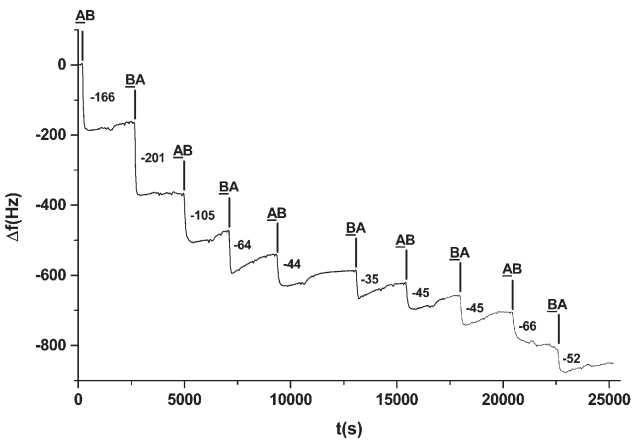
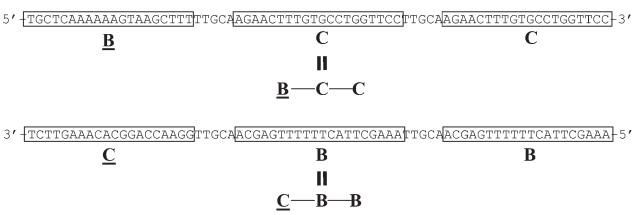
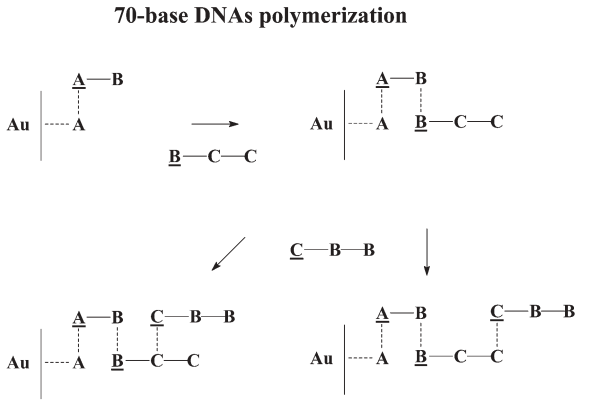


Fig. 1 QCM frequency variation during step-by-step 45-base DNAs polymerization.

70-base DNA monomers



70-base DNAs polymerization



Scheme 2 70-Base DNA monomer and 70-base DNA polymerization reaction.

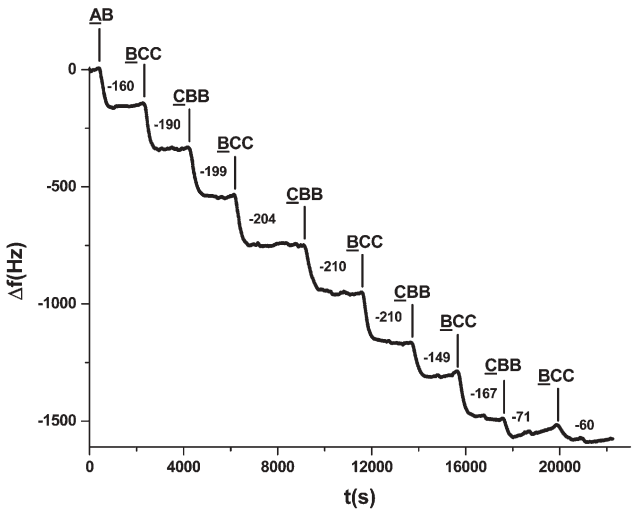


Fig. 2 QCM frequency variation during the step-by-step 70-base DNA polymerization.

hybridization of successive DNA layers during the five first steps. By comparison with the previous 45-base DNAs polymerization, there is no decrease of the hybridization reaction during the five first polymerization steps. We attribute this to the possible hybridization of two DNA sequences, C or B, on each monomer B–C–C and C–B–B, as shown on Scheme 2, that enhances reactivity of the polymerization reaction. The hybridization ratio during the five first steps is close to 100%. This is consistent with hybridization of a diluted DNA monolayer which is 95%.[19] The designed DNA film is diluted taking into account that it is supported by the first A–B DNA monolayer which is hybridized at a level of 39% with the first DNA–disulfide monolayer. Kinetics of the hybridization reaction was estimated by calculating $\Delta\tau$ defined previously: the mean value and standard deviation are 188 ± 19 s. The hybridization kinetics is slower than the 45-base DNA hybridization kinetics, indicating that the increase of the length strand decreases the surface DNA diffusion rate. A -1013 Hz frequency change is calculated during the five first steps of the polymerization reaction. The amount of hybridized DNA is twice that compared with the 45-base DNA polymerization. The frequency changes decrease during the last four steps and this effect can be attributed to a decrease of DNA polymerization reactivity. Another possibility might be the effect of the length of DNA polymer which can induce viscoelastic changes and by this way affect the QCM response. This hypothesis can be controlled by using electroacoustic analysis and results will be presented in a subsequent paper, and is beyond the scope of this communication. After this step-by-step polymerization, the disulfide–DNA probes were dehybridized by circulating a 0.5 M NaOH, 3 M NaCl solution for 30 min. On performing this experiment we find the same behavior, there is a regular formation of a DNA polymer during the five first steps of the reaction and a progressive decrease of hybridization ratio during the four last steps. A -1029 Hz frequency change is calculated during the five first steps of the polymerization reaction, this value is close to -1013 Hz calculated for the first 70-base DNA polymerization.

In summary, two DNA polymers were synthesized on the surface of a QCM DNA-biosensor. A decrease of reactivity was observed during successive steps of the 45-base DNA polymerization reaction. This effect is correlated to steric hindrance in the DNA film. To prove and overcome this loss of reactivity, two 70-base DNA monomers that include two reactive sequences were used. In this case, regular formation of a DNA polymer was obtained during the five first steps of the reaction. This work demonstrates that the QCM biosensor is a sensitive tool to design and to characterize multilayer biostructures on solid substrates. Indeed, it permits one to follow *in situ* kinetics of successive reactions and its subleties.

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