45- and 70- base DNAs 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 strategy to design supramolecular DNA structures by rolling circle amplification[11] and by an enzymatic strategy that use alternatively DNA ligase and restriction endonnuclease[13]. Two-dimensional supramolecular DNA structures have been designed by assembly DNA double-crossover molecules14 and DNA sierpinsky triangles[15]. These DNA supramolecular structures are a pathway to many applications, some consider to be unatural: a two-dimensionnal array of DNA triple-crossover molecules has been used to mimic cumulative XOR logical function16 and the two-dimensionnal pattern of DNA Sierpinsky triangles can be used to implementing algorithm for computation tasks15. In this work the possible use of a QCM DNA-biosensor to study dynamics of supramolecular DNA structures synthesis on solid substrate was demonstrated: kinetics during step-by-step polymerizations of 45- and 70- base DNAs were *in situ* monitored.

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 with a 250 Å chromium underlayer. The resonator was connected by a silver conducting paste, through wires, to a BNC adaptator. 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: the crystal was mounted 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 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 H_2SO_4 95%/H_2O_2 30% 10 µL drop for 30 minutes and rinsed with deionized doubly distilled water. The biosensor consists of a monolayer of 20-base disulfide-DNA probe immobilized on the cleaned gold surface of the quartz resonator[11,12], the 20-base DNA sequence of the probe is referred to as A (scheme 1). The frequency decrease Δf observed during the circulation of 10 µg/mL 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%: $\tau = S_{disulfide}/S_{QCM} = |\Delta f|.s.N_A.s_{disulfide}/S_{QCM}.M_{disulfide}$, 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, s = 350 pg/Hz is the experimental microbalance sensitivity17, $N_A = 6.023.10^{23}$ mol^{-1} is the Avogadro constant, $s_{disulfide}$ = 2.2 nm^2 is the average area of one adsorbed disulfide-DNA molecule18 and $M_{disulfide}$ = 6386 g/mol is the molecular weight of the disulfide-DNA. This kind of DNA-biosensor is selective, sensitive and fully renewable12,18: it was hybridized in this study in optimized stingency conditions18, 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, 3M NaCl).

The two 45-base DNA monomers referred to as $\underline{A}$-B and $\underline{B}$-A constit of two 20-base sequences, A, $\underline{A}$, B or $\underline{B}$, spaced by a 5-base DNA sequence (scheme 1). A and $\underline{A}$ are complementary as are B and $\underline{B}$. The frequency changes were recorded during successive circulation of 22.5 µg/mL DNA monomers $\underline{A}$-B and $\underline{B}$-A HEPES solutions (figure 1): successive frequency drops attributed to successive steps of the supramolecular DNA polymerization reaction (scheme 1) where clearly observed. After each polymerization step, HEPES buffer circulation during 10 minutes was performed in order to remove non specific DNA adsorption. The frequency decrease $\Delta f_{\underline{A}\text{-}B}$ of the first step is -166 Hz. The corresponding hybridization ratio r of hybridized DNA $\underline{A}$-B $N_{\underline{A}\text{-}B}$ versus immobilized disulfide-DNA $N_{disulfide}$ is estimated to be 38%: $r = N_{\underline{A}\text{-}B}/N_{disulfide} = \Delta f_{\underline{A}\text{-}B}.M_{disulfide}/\Delta f_{disulfide}.M_{\underline{A}\text{-}B}$, where $M_{\underline{A}\text{-}B}$ = 13779 g/mol is the molecular weight of the $\underline{A}$-B DNA. This hybridization ratio is consistent with values found in the literature for DNA disulfide monolayers which are 48%12 and 47%18. The frequency changes of the successive steps decrease from 201 to 52

† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See http://www.rsc.org/suppdata/xx/b0/b000000x/

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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 extremity 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. Kinetic of the hybridization reaction was estimated by calculating Δτ = τ¾ - τ¼, where τ¾ and τ¼ are respectively the reaction time to hybridized 75% and 25% of successive monolayer. Δτ mean value is 50±12 s, this value is consistent with hybridization kinetic of DNA monolayers which is 80s19. A -585 Hz frequency changes is determined during the five first steps of the polymerization reaction. This value is close to the value found for the hybridization of a 445 bp DNA monolayer on a 10 MHz QCM DNA biosensor, which is -646 Hz reported to a 27 MHz QCM biosensor18. It indicates that the acoustic behavior of the 5 layers 45 base-DNA film is comparable to the accoustic 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 minutes. We do again this experiment and we find the same behavior, there is a progressive decrease of hybridization ratio during the polymerization process.

An other DNA polymerization reaction was investigated by using two 70-base DNA monomers referred to as <u>B</u>-C-C and <u>C</u>-B-B consist of three 20-base DNA sequences, B, <u>B</u>, C or <u>C</u>, spaced by two 5-base DNA sequences (scheme 2). C and <u>C</u> are complementary. The QCM frequency changes are measured during circulation of 22.5 mg/mL DNA <u>A</u>-B HEPES solution and successive circulation of 35 μg/mL DNA monomers <u>B</u>-C-C and <u>C</u>-B-B HEPES solutions (figure 2). There is a first frequency drop corresponding to <u>A</u>-B DNA hybridization and successive frequency drops attributed to successive steps of the supramolecular DNA polymerization reaction of <u>B</u>-C-C and <u>C</u>-B-B DNAs (scheme 2). The frequency decrease $\Delta f_{\underline{A}\text{-B}}$ of the first step is -160 Hz and the corresponding hybridization ratio r of hybridized DNA <u>A</u>-B versus 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 reaction, hybridization of <u>A</u>-B DNA strands, is the same. The frequency change of successive frequency shifts is constant during the five first steps of <u>B</u>-C-C and <u>C</u>-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 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 <u>B</u>-C-C and <u>C</u>-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 <u>A</u>-B DNA monolayer which is hybridized at 39% with the first DNA-disulfide monolayer. Kinetic of the hybridization reaction was estimated by calculating Δτ defined previously: mean value and standard deviation are 188±19 s. The hybridization kinetic is slower than the 45-base DNA hybridization kinetic, indicating that the increase of the length strand decrease 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 more 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. An other 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 will be controlled by using electroacoustic analysis and 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 minutes. We do again this experiment and 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 caracterize multilayer biostructures on solid substrate. Indeed, it permits to follow *in situ* kinetic of successive reactions and its subleties.

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Notes and references

† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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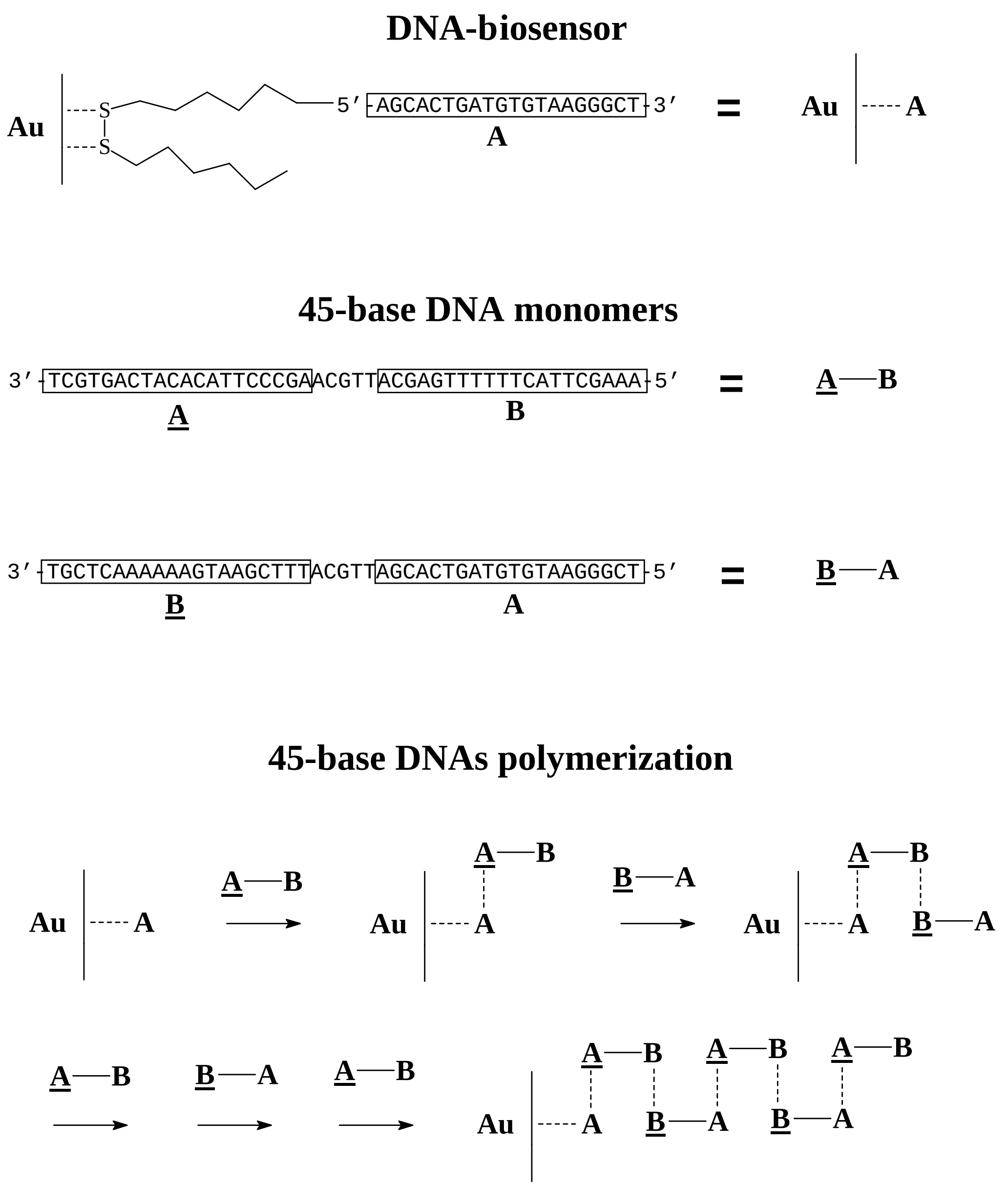
Single column figure/scheme (below)



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

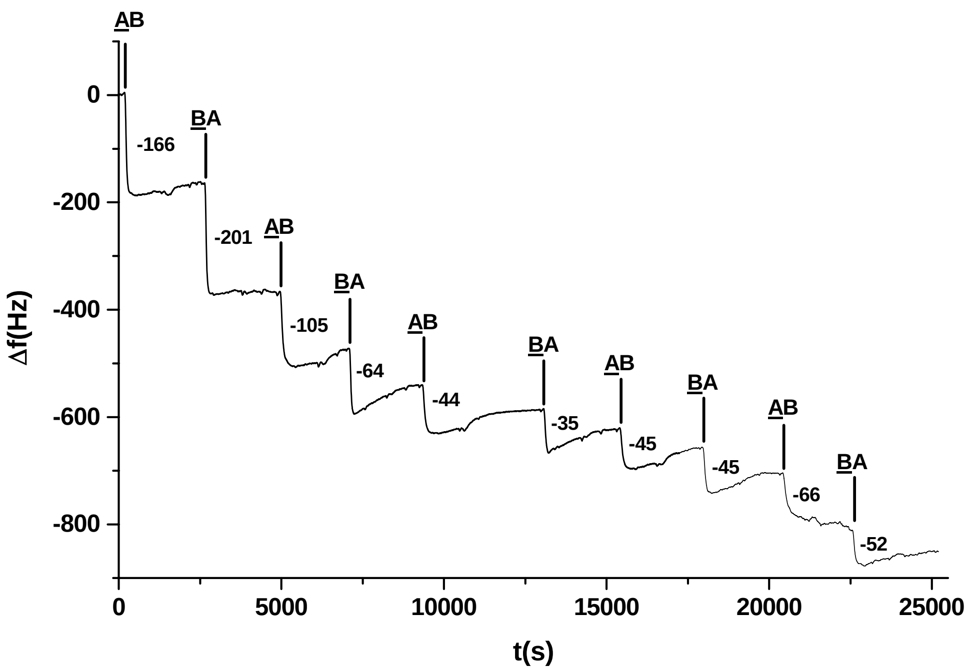
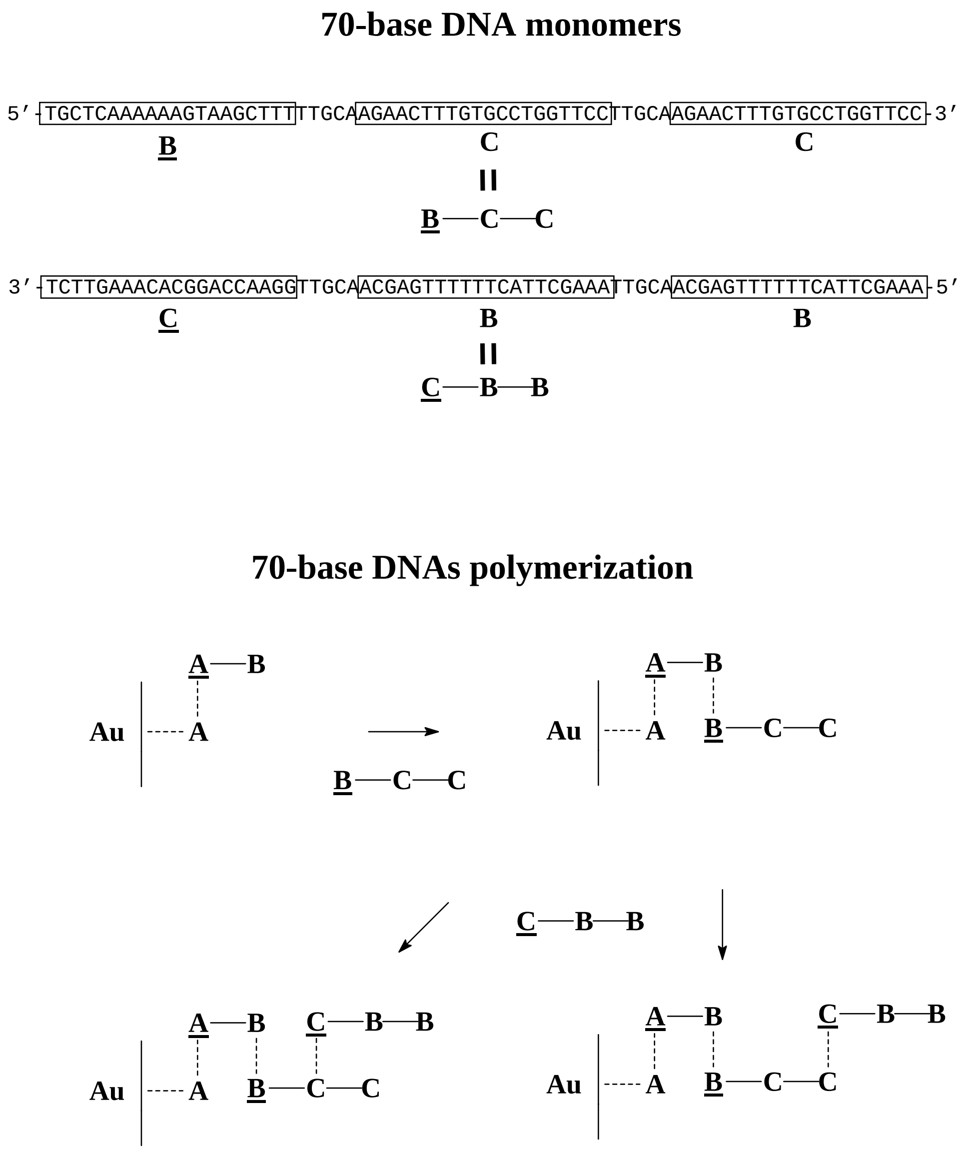


Figure 1 QCM frequency variation during step by step 45-base DNAs polymerization.



Scheme 2 70-base DNA monomers and 70-base DNAs polymerization reaction.

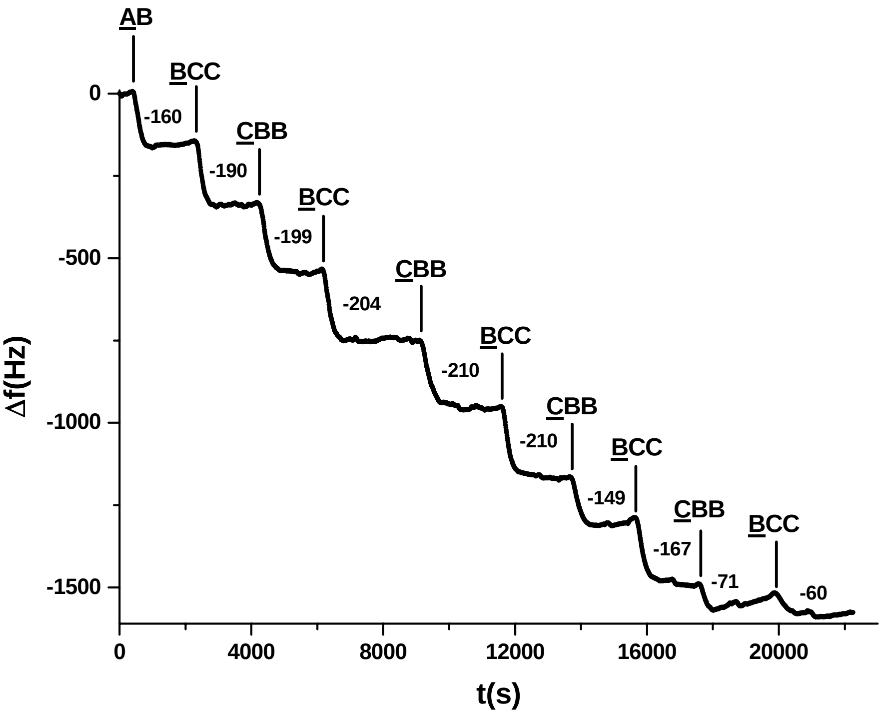


Figure 2 QCM frequency variation during step by step 70-base DNAs polymerization.