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# DNA hybridization mechanism in an interfacial environment: What hides beneath first order $k$ ($s^{-1}$) kinetic constant?

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ABSTRACT

The scientific question addressed in this work is: what hides beneath first order kinetic constant $k$ ($s^{-1}$) measured for hybridization of a DNA target on a biosensor surface. Kinetics hybridization curves were established with a 27 MHz quartz microbalance (9 MHz, third harmonic) biosensor, constituted of a 20-base probe monolayer deposited on a gold covered quartz surface. Kinetics analysis, by a known two-step adsorption–hybridization mechanism, is well appropriate to fit properly hybridization kinetics curves, for complementary 20-base to 40-base targets over two concentration decades. It was found that the $K_1$ ($M^{-1}$) adsorption constant, relevant to the first step, concerns an equilibrium between non hybridized targets and hybridized pre-complex and increases with DNA target length. It was established that $k_2$ ($s^{-1}$), relevant to irreversible formation of a stable duplex, varies in an opposite way to $K_1$ with DNA target length.



## 1. Introduction

Nucleic acid hybridization mechanism was investigated on nitrocellulose membrane using an acoustic biosensor [1]. The integral hybridization kinetics is equal to:

$$\ln(1-\eta) = -k \cdot t \quad \text{(E1)}$$

where $\eta$ is the hybridization efficiency ratio and $k$, the first order rate kinetics constant, was reported equal to $8.0 \times 10^{-5}\ s^{-1}$ (65 °C, 4000 bases target, $3 \times 10^{-9}$ M). Secondary structure effects on hybridization kinetics were investigated recently with a fluorescent biosensor, designed by deposit of a thiol-labeled probe self-assembled monolayer onto a gold surface [2]. The kinetics law, obtained for DNA targets including secondary structure or not, is equal to:

$$\ln(1-\eta) = -k_{\text{eff}} \cdot C \cdot t \quad \text{(E2)}$$

$C$ (M) is DNA target bulk concentration, and $k_{\text{eff}}$ ($M^{-1}\ s^{-1}$) is the effective hybridization first order kinetics constant. $k_{\text{eff}}$ is equal to $1.2 \times 10^6\ M^{-1}\ s^{-1}$ for a target without secondary structure (20 °C, 25 bases), and $2 \times 10^5\ M^{-1}\ s^{-1}$ for a target including an airpin-like structure (20 °C, 25 bases). The kinetics law (E2) yield to (E1) for a constant bulk target concentration. The $k_{\text{eff}}$ value found for hybridization on nitrocellulose membrane [1], equal to $2.7 \times 10^4\ M^{-1}\ s^{-1}$ (65 °C, 4000 bases), is lower due to the largest size of the target. Kinetics law (E2), taking into account both kinetics constant and bulk solution concentration, is relevant to a one-step surface hybridization mechanism (Fig. 1A). $T$ are target diffusing in bulk solution, $P_\eta$ are single stranded probes grafted onto the biosensor surface and $TP_\eta$ are hybridized probes. Most of the hybridization kinetics monitored on biosensors are well fitted with a first order kinetics (E2). A mechanism including hybridization competition between two targets quoted 1 and 2 was reported [3]:

$$\ln(1-\eta) = -(k1_{\text{eff}} + k2_{\text{eff}}.(1-\alpha)) \cdot C \cdot t \quad \text{(E3)}$$

where $\alpha$ is a competition parameter of hybridization between the two targets. Nevertheless, one study report a second order rate, on a biosensor designed by chemical grafting (i.e. chemical adsorption) of probes on fused silica optical fibers [4]. This difference was attributed to lateral interactions due to high probe surface density. The kinetics integral law is then equal to:

$$\eta = \frac{t}{t + k_{\text{eff}} \cdot C} \quad \text{(E4)}$$

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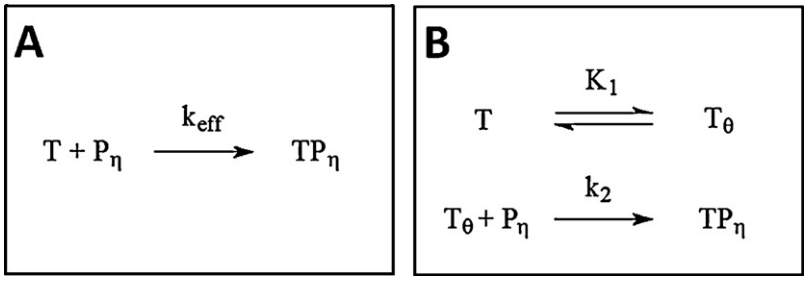


**Fig. 1.** One-step (A) and two-step (B) surface hybridization mechanism.

$k_{eff}$ is the effective hybridization second order kinetics constant. Trends to study effects on hybridization kinetics in an interfacial environment are to use first order kinetics laws similar to (E2) (Fig. 1A). This equation is convenient to enlighten kinetics-target structure dependence. A two-step mechanism was reported to describe hybridization of nucleic acid on the surface of single-walled dispersed carbon nanotubes (Fig. 1B) [5]. A first rapid Langmuir adsorption step, consisting of adsorption of target ($T$) onto the biosensor surface ($T_\theta$). A second irreversible rate determining step, consisting on hybridization of a target adsorbed on the sensor surface ($T_\theta$) with a grafted complementary probe ($P_\eta$), yielding to a stable duplex formation ($TP_\eta$). The kinetics integral law is given by (Appendix):

$$\ln(1-\eta) = -\frac{K_1 \cdot k_2 \cdot C}{1 + K_1 \cdot C} t \quad \text{(E5)}$$

The adsorption equilibrium constant $K_1$ was found equal to $5 \times 10^7\ M^{-1}$, and first order kinetics constant $k_2$, related to irreversible surface hybridization, was found equal to $5.57 \times 10^{-5}\ M^{-1}$ (25 °C, 25 bases target). This law is more general than (E2), and is equal to this last one for feeble target concentrations. $k_{eff}$ was in this case equal to $2.8 \times 10^3\ M^{-1}\ s^{-1}$. This value of $k_{eff}$, lower than those reported on biosensor constituted of probe self-assembled monolayers deposited onto solid substrate, was attributed to high activation hybridization barrier of nanostructures. The question addressed in this work is to establish if the two-step mechanism observed for hybridization on nanoparticles is general, and can be used to describe hybridization in the case of a biosensor designed by self-assembled monolayer formation onto a gold surface. This problematic is investigated by analysis of hybridization kinetics, of DNA-targets of different length (10–45 bases) over 2 target concentration decades, monitored with a 27 MHz quartz crystal microbalance biosensor (9 MHz, third harmonic).

## 2. Experimental

### 2.1. Chemical and biochemical reagents

Water is deionized and double distillated. $H_2SO_4$ 95%, $H_2O_2$ 30%, NaOH, HCl, NaCl and 1 M HEPES are from Sigma Aldrich (biochemical grade). DNA probe and targets synthesized by Eurogentec [6] are purified by chromatography, checked by MALDI-TOF analysis and dosed by UV optical density measurements. Sequences of disulfide labeled probe (P) and targets (T1 to T6) are presented in Table 1. The sequence of the probe ($P$) is a partial sequence of the gene encoding for the large ribosomal RNA sub-unit of *Alexandrium minutum* [7,8], a toxic algae responsible of paralytic shellfish poisoning on American, Asian and European coastal waters [9].

### 2.2. Buffers and solutions

The solution for DNA grafting consists in 0.5 M NaCl. Hybridization experiments are performed in optimized stringency condition [10]: the hybridization buffer is composed of 0.5 M NaCl, 50 mM HEPES, adjusted to pH 7.2 with 1 M NaOH. The regeneration step is carried out with 0.5 M NaOH, 3 M NaCl solution.

### 2.3. QCM apparatus

The resonator of the microbalance is a Matel-Fordhal France 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, are deposited by evaporation techniques on both sides of piezoelectric quartz, with a 250 Å chromium underlayer. The resonator is connected by a silver conducting paste, through wires, to a BNC connector. A home-made oscillator is 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 are temperature-controlled by a Watlow heater monitor with stability better than 0.1 °C. The crystal is mounted between two O-ring seals inserted in a home-made plexiglass cell. Only one face of the quartz is in contact with the solutions. The cell volume is 50 μL. The apparatus includes a Pharmacia micropump to assure a 50 μL/min constant flow of the solutions in the quartz cell. The frequency is measured with a PM 6685 frequency counter and recorded with a home-made C language software. The experiments are performed at 25 ± 2 °C.

## 3. Results

### 3.1. Biosensor design, hybridization efficiency and regeneration

The biosensor is designed by grafting the thiol-labeled probe P on the gold quartz surface of the microbalance via Au—S bonds. Probe grafting, selective target hybridization and biosensor regeneration are monitored by frequency measurements (Fig. 2A). A −205 Hz frequency decrease is recorded during circulation of a probe P saline solution (3.2 μM, NaCl 0.5 M). No frequency variations are observed during circulation of a saline solution (NaCl 0.5 M), indicating that DNA probes are irreversibly grafted to the sensor surface. The biosensor surface coverage $\tau$(%) with grafted probes is equal to:

$$\tau = 100 \cdot |\Delta f_P| \cdot S_{QCM} \cdot N_A \cdot A_P / A_{QCM} \cdot M_P \quad \text{(E6)}$$

**Table 1**
Structure of DNA probe (P), random DNA (T) and complementary DNA targets (T). Complementary probe-target sequences are underlined.

| Name | DNA structure |
|---|---|
| P | 5′ disulfide C6 AGCAC TGATG TGTAA GGGCT 3′ |
| T1 | 3′ CCTTG GTCTG TGTTT CAAGA 5′ |
| T2 | 3′ ACATT CCCGA 5′ |
| T3 | 3′ TCGTG ACTAC ACATT CCCGA 5′ |
| T4 | 3′ TCGTG ACTAC ACATT CCCGA T1 5′ |
| T5 | 3′ ACGTT ACGAG TTTTT TCATT CGAAA 5′ |
| T6 | 3′ TCGTG ACTAC ACATT CCCGA T5 5′ |