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Anharmonic acoustic effects during DNA hybridization on an electrochemical quartz crystal resonator

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ABSTRACT

The paper describes a sensor for single-stranded DNA (ssDNA) biomarker based on anharmonic acoustic signals arising during hybridization with complementary thiolated ssDNA functionalised on the gold electrode of an electrochemical quartz crystal resonator. The steps of sensor preparation and hybridization are carried out in an electrochemical microfluidic flowcell. While the electrochemical impedance spectroscopy does not allow a definitive interpretation, the changes in resonance frequency and third Fourier harmonic current of the resonator on actuation at the fundamental mode indicate formation of a flexibly bound layer. The functionalization and hybridization steps monitored by the anharmonic detection technique (ADT) are described with a simple model based on Duffing nonlinear equation.

1. Introduction

Detection of single-stranded DNA (ssDNA) is of wide interest in biomedical diagnostics and pharmaceutical manufacturing. Optical fluorescence is one of the most widely used methods in the detection of ssDNA. Although fluorescence-based methods have shown unprecedented sensitivity, the need for multiple reagents and steps has restricted these methods from use in the point-of-care or real-time setting [1,2]. In this paper, we investigate the feasibility of an electrochemical quartz crystal microbalance (EQCM) for direct and rapid detection of ssDNA using the nonlinear acoustic response of the quartz resonator. A sensor is developed by functionalizing thiolated ssDNA to one of the gold electrodes of the EQCM. This ssDNA has a complementary sequence to the target ssDNA in solution, and therefore acts as a specific biomolecular receptor for the latter. The binding event is recorded by electrochemical methods, particularly measuring changes of charge transfer resistance R_{ct} or double layer capacitance C_{dl}, which are assessed in electrochemical impedance spectroscopy (EIS) [3,4]. If the gold electrode is part of the EQCM, the change in resonance frequency indicates the hybridization event as a mass change in the simplest case [5,6,19–21].

In practice, these two methods EIS and EQCM have crucial disadvantages when they are applied to detection of DNA hybridization. In EIS, it is not clear how the charge transfer resistance should change when thiol-bound ssDNA is complemented by a matching strand. The interpretation of EQCM resonance frequency shift is also challenging as the frequency shift can either be negative or positive in the case of rigid or flexible binding respectively, depending on the dominance of mass or elastic loading. We observed that thiol-bound ssDNA under the influence of electrode potential resembled the second case, i.e. where elastic loading is dominant over mass loading. As a result, the positive resonance frequency shifts reflects not a variation in mass but rather change in the spring constant of the bonds between the sensor and the attached analyte. Therefore, although the EQCM offers a direct and rapid platform for measurement, there is a need for an improved method for quantification of flexible adsorbate, such as ssDNA. An anharmonic detection technique, or ADT, has been reported recently to overcome the abovementioned issues and measure

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flexible adsorbates, such as microparticles, with a strong quantitative correlation and transduction specificity [7–10]. A QCM is largely linear at modest amplitudes, i.e. when driven by a harmonic (or purely sinusoidal) actuation near its fundamental resonance frequency, it responds predominantly at the same frequency. The response at higher odd Fourier harmonics is significantly low. It may be noted that even Fourier harmonics are not transduced in a thickness-shear mode quartz resonator. However, the interaction forces between the QCM oscillating in thickness shear mode and the surface-bound flexible adsorbate projected along the direction of the quartz oscillation (which is piezoelectrically transduced) are anharmonic (*not harmonic*) and distort the harmonic oscillation of the QCM. As a result, the binding of flexible adsorbates modifies the amplitude of higher odd Fourier harmonic due to piezoelectric transduction (even harmonics are not transduced in a thickness-shear mode quartz). In the anharmonic detection technique (ADT), we consider the *change in amplitude* in the third Fourier harmonic, which is measured at three times the drive frequency, as fifth or higher odd harmonics are considerably low in amplitude. It may be clarified here that although the frequency of the *third Fourier harmonic* (equal to three times the drive frequency) lies close to the *third overtone resonance* (as the drive frequency is set to the fundamental resonance), the two are different conceptually. While the third overtone refers to a higher mode of resonance of an oscillator, a third Fourier harmonic signal (i.e. three times the drive frequency) may be produced in principle even in a nonlinear oscillator with a single resonance mode by driving at the single (fundamental) mode of resonance. It may be noted, however, that a single-mode quartz resonator is practically impossible. This is where ADT differs from an impedance analyzer or a ring-down analyzer, which measures the change in frequency at a chosen resonance mode by driving at or around the same mode, whereas ADT measures the change in *amplitude* of a higher Fourier harmonic (usually third, which is near the third overtone resonance) by *driving near the fundamental resonance frequency*.

In this paper, we investigate by means of EIS and ADT, the steps of developing an ssDNA sensor (surface functionalization) and the hybridization event (sensing). The functionalization and hybridization steps are carried out in a microfluidic flowcell, and the measurements are taken in liquid after each step. The experimental results show satisfactory agreement with the theory, governed by the basic equations of a nonlinear QCM response with flexibly-bound adsorbate. In particular, the third Fourier harmonic signal obtained from the experiment exactly fit to a cubic expression as predicted by the Duffing equation and as elaborated in the paper. The cubic shape holds true both for clean QCM and for QCM with bound analyte, with the only difference being in the value of the pre-exponent.

1.1. *Modelling the anharmonic influence of non-rigid load*

In an earlier paper [10], we treated the problem of anharmonic influence of non-rigid load in its general case by considering a damped driven oscillator. In this paper, we have neglected losses in the theoretical model, and therefore omitted the drive force and treated the problem as free oscillation. This simplified the analytical expression of the result without any considerable impact on the agreement with the experimental results as the change in dissipation due to biomolecular binding at any stage is negligible. The resistance (real part of acoustic impedance) measured at any step in our experiment (with or without DNA) remained the same, i.e. ~220 Ohm, as obtained from Kanazawa equation.

Let us consider the model of a particle bound to the quartz resonator surface with a bond length of l (Fig. 1). As the frequency of oscillation of the resonator (14.3 MHz in our case) is significantly higher than the natural frequency of shear oscillation of the particle-bond system ($\omega_s = (k/m_s)^{1/2}$), the particle remains nearly at the same location. As a result, the bond stretches and changes angle, as shown in Fig. 1.

The oscillations in the system are described by two coupled differential equations (Eq. (1) and Eq. (2)), with mutual motions of the particle and the quartz resonator both parallel to the quartz surface. We model quartz as a nonlinear oscillator using the one-dimensional Duffing equation, neglecting damping and external drive force as mentioned above.

$$\ddot{x} + \omega_0^2 x = -\beta x^3 + \frac{NS}{M_q} F_x \qquad (1)$$

Here, x – displacement of quartz, N – number of particles attached per unit area, S active area of the quartz sensor, ω_0, β – eigen frequency and non-linearity constant for the quartz. The last term in Eq. (1) is the pulling force from NS adsorbed particles, and M_q is the nodal mass of the crystal, which is equal to half of the total mass [6]. Since the particle remains nearly stationary during the oscillation of the resonator, its motion is described by the following differential equation, where F_x is the projection of the total particle-bond force on quartz plane.

$$m_s \ddot{X} = -F_x = k\left(\sqrt{x^2 + l^2} - l\right)\frac{x}{\sqrt{x^2 + l^2}} \approx \frac{kx^3}{2l^2}, \text{ as } x << l \qquad (2)$$

Here, ms is the mass of attached particle, X is the displacement of adsorbed particle placed at the origin and k is force constant of attachment bond. As the particle remains stationary in the laboratory reference frame, the extension of the bond is given by $(\sqrt{x^2 + l^2} - l)$, and $x/\sqrt{x^2 + l^2}$ is the factor that projects the elastic force $k(\sqrt{x^2 + l^2} - l)$ (which is along the bond) onto the direction of oscillation.

Since the displacement of the resonator is small compared to the bond length, i.e. $x << l$, the anharmonicity in the spring constant of the bond can be neglected, and the bond can be treated as a linear spring as in Eq. (2). Other notations are:

Substituting Eq. (2) into Eq. (1) results in the following equation for quartz oscillations:

$$\ddot{x} + \omega_0^2 x = -\beta x^3 - \frac{NS}{M_q}\frac{k}{2l^2} x^3 \qquad (3)$$

The two terms on the right side of Eq. (3) describe the motion of the harmonic oscillator in an anharmonic field of the fourth order.

$$U_4 = \frac{M_q}{4}\left(\beta + \frac{NS}{M_q}\frac{k}{2l^2}\right)x^4 \qquad (4)$$

The first term in parentheses is due to anharmonicity of the quartz itself, and the second is due to the particle-resonator interaction. Therefore harmonic but relatively long bonds generate anharmonic potential for oscillation of the quartz parallel to the surface.

Solution of Eq. (3) is very well known (see for example references [11] or [12]). The fundamental resonance frequency shifts with higher oscillation amplitude due to drive-level dependence. For small oscillation amplitude a, the shift is proportional to square of the oscillation amplitude:

$$\omega = \omega_0 + \frac{3}{8\omega_0}\left(\beta + \frac{NS}{M_q}\frac{k}{2l^2}\right)a^2 \qquad (5)$$

The first Fourier harmonic of the oscillator displacement is given by