Sensors and Actuators B 272 (2018) 175–184

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Characterisation of particle-surface interactions via anharmonic acoustic transduction

Carlos da Silva Granja[a], Niklas Sandström[b], Igor Efimov[a], Victor P. Ostanin[c], Wouter van der Wijngaart[b], David Klenerman[c], Sourav K. Ghosh[a,*]

[a] Centre for Biological Engineering, Loughborough University, UK
[b] Micro and Nanosystems, KTH Royal Institute of Technology, Sweden
[c] Department of Chemistry, University of Cambridge, UK

ARTICLE INFO

Article history:
Received 6 January 2018
Received in revised form 13 April 2018
Accepted 4 May 2018
Available online 29 May 2018

Keywords:
Quartz crystal microbalance
Nonlinear acoustic transduction
Label-free biosensor
Realtime immunosensor
Cell-surface profiling
Immunophenotyping

ABSTRACT

Most transduction methods for measuring particle-surface interactions are unable to differentiate the strength of interaction and thus rely largely on extensive washing to reduce ubiquitous non-specific background. Label-based methods, in particular, are limited in wide applicability due to their inherent operational complexity. On the other hand, label-free force-spectroscopic methods that can differentiate particle-surface interaction strength are skill-demanding and time-consuming. Here, we present a label-free anharmonic (nonlinear) acoustic transduction method employing the quartz crystal resonator that reads out ligand-receptor binding based on the interaction strength. We show that while stronger specific interactions are transduced more strongly, and in linear proportionality to the ligand concentration on microparticles, non-specific interactions are significantly attenuated. This allows ligand quantification with high specificity and sensitivity in realtime under flow without separate washing steps. Constructing an analytical model of a quartz resonator, we can relate the number and type (specific vs. non-specific) of ligand-receptor interactions with the change in characteristic nonlinearity coefficient of the resonator. The entirely-electronic and microfluidic-integrable transduction method could potentially allow a simple, fast and reliable approach for characterising particle-surface interactions with economy of scale.

1. Introduction

Characterising biomolecular interactions is pivotal to the development of novel therapeutic and diagnostic platforms. Although significant progress has been made in the development of analytical methods for these applications, there still lies some fundamental limitations [1,2]. Labelled optical transduction often requires skill-demanding and time-consuming procedures involving multiple reagents and steps, which restrict its use to controlled environments. Moreover, biochemical sensor interfaces (i.e. receptor-ligand) are prone to nonspecific interactions and fouling caused by other sample constituents. Most transduction methods, labelled or label-free, lack the intrinsic ability to differentiate strength of interactions. As a result, extensive washing and assay development are employed to overcome the problem of non-specific binding [3,4]. On the other hand, while force spectroscopic methods, such as atomic force microscopy and optical tweezers, allow label-free differentiation of particle-surface interaction strength, they are complex and slow, and require a laboratory infrastructure. Thus, there exists an unmet need for a label-free transduction method that is easy-to-use and scalable, and can characterise ligand-receptor interactions in realtime, providing reliable differentiation from non-specific interactions with minimal or no washing.

Quartz crystal resonators have been widely used for label-free quantification of biomolecular binding [5]. Quartz crystals are commonly driven close to their fundamental or higher modes of resonance. The thickness (mass) and softness (viscosity) are conventionally estimated from the shifts in resonance frequency and dissipation measured *at the driven mode*. This transduction method is referred to as the Quartz Crystal Microbalance (QCM) [6]. However, it should be noted that inertial (mass) loading, which causes decrease in resonance frequency, is dominant only in the case of thin and rigid adsorbent layer. When relatively large ($d > 1\,\mu m$) and more flexible adsorbates bind to the quartz crystal via small contact points, they form a coupled oscillator with the quartz crystal resonator. The binding of colloidal particles [7] and biological particles, such as bacterial spores [8], to the quartz crystal res-

* Corresponding author.
E-mail address: S.Ghosh2@lboro.ac.uk (S.K. Ghosh).

https://doi.org/10.1016/j.snb.2018.05.016
0925-4005/

onator via ligand-receptor bonds are examples of such coupled resonators. The mass coupling is weak for these particles. On the other hand, the drive frequency, which is typically in the MHz range, is significantly higher than the resonance frequency of the particle-bonds system. As a result, governed by the principle of vibration, the particle is unable to follow the thickness-shear oscillation of the resonator and remains almost stationary in space. The resulting stretch in the bonds contributes to a restoring force, which increases the effective stiffness of the resonator. Thus, elastic loading is dominant over mass loading, and causes an increase in resonance frequency that is proportional to the increase in stiffness of the particle-resonator contact [9]. Several strategies have been employed to study this phenomenon, including subjecting the particle-QCM contact to a range of centrifugation forces [10], ionic strengths [11] and dehydration levels [12]. In this study, we functionalised polystyrene microparticles with different concentrations of human immunoglobulin-E (IgE, ligand) and the quartz crystal resonator with anti-IgE aptamer (receptor), and investigated the particle-resonator interactions for the various ligand concentrations comparatively using changes in resonance frequency, dissipation and a novel acoustic parameter, the third Fourier harmonic current, which we explain below.

In our earlier work, the quartz crystal was *driven close to its fundamental resonance mode* and the electrical current was measured *exactly* at *three times the drive frequency (3f)*, referred to as the third Fourier harmonic [5,8]. It should be noted that the third Fourier harmonic is a result of nonlinear response of an oscillator and may be present even in an oscillator with a single-mode of resonance, such as a spring-mass oscillatory system. Hence, the third Fourier harmonic is conceptually different from the third overtone resonance (a higher resonance mode), although they may be in the vicinity of each other depending on the crystal curvature. The third Fourier harmonic current is usually low as the quartz crystal is largely a linear oscillator at modest amplitudes. It was shown through modelling and experiments that when streptavidin-coated microparticles bound to the quartz resonator, the microparticle-resonator interaction forces distorted the pure sinusoidal oscillation, contributing to a considerable change in the third Fourier harmonic (3*f*) current. The quantitative change in the 3*f* current was proportional to the number of bound microparticles, and interestingly, significantly smaller for physisorbed microparticles, where the particle-resonator interaction was weaker. The transduction method based on the measurement of the change in the third Fourier harmonic current was referred to as the Anharmonic Detection Technique (ADT) [5,8].

In this study, we employed the ADT transduction method to investigate how the change in the 3*f* current varied for different particle-resonator interactions when the number of resonator-bound particles was similar. A range of specific interactions (IgE-antiIgE) were modelled using various concentrations of IgE functionalised on polystyrene microparticles and anti-IgE aptamer functionalised on the quartz crystal resonator. Non-specific interactions were modelled using goat immunoglobulin-G (IgG) functionalised on the microparticles and the same anti-IgE aptamer on the resonator. We observed that when similar number of microparticles was bound specifically to the resonator, the change in the 3*f* current was greater for stronger specific interactions, and linearly proportional to the ligand (IgE) concentration on the microparticles. We also noted that when similar number of microparticles was bound non-specifically to the resonator, functionalised with IgG, in the absence of adequate washing, the change in the 3*f* current was significantly lower. Interestingly, this differentiation between the various interaction strengths was much more pronounced from the change in the 3*f* current than from the change in the resonance frequency. In particular, the relative 'attenuation' in the shift of 3*f* current for non-specific interactions with respect to the specific interactions was more than one order of magnitude greater than similar attenuation in the change in resonance frequency measured from the same assay. The quantitative correlation between the change in 3*f* current and the particle-bound ligand concentration, together with the ability to differentiate between specific and non-specific interactions, makes it suitable to be developed as a reliable, fast and easy-to-use method for characterisation of particle-surface interactions. This may be applied to relative quantification of affinity of interaction for various ligand-receptor pairs, and relative quantification of ligand concentration on a particle for a given receptor, e.g. cellular immunophenotyping. It needs to be noted that in order to cause a nonlinear distortion of the acoustic response of the quartz crystal resonator, the particle needs to be flexibly attached to the resonator, such as in the case of colloidal particles or bacteria, which have a cell wall that offers the cells some rigidity of form. Here we employed polystyrene microparticles so that we could controllably functionalise a range of concentrations of the ligand (IgE) on them and investigate how their binding to the quartz crystal resonator correlated quantitatively with the change in the acoustic signal.

It may be noted that, in this paper we referred to the quartz crystal resonator as QCR to treat the 'transducer' with distinction from QCM, which generally refers to the 'transduction method' based on the shift in resonance frequency of a QCR.

Below, we present an analytical model by describing the quartz resonator using the one-dimensional Duffing equation. Analysing the experimental data using this model, we discuss how we can directly relate the type (specific vs. non-specific) and strength of ligand-receptor interaction (quantified by the protein concentration on the microparticles) with the change in the nonlinearity coefficient of the resonator, which underpins the change in 3*f* current.

2. Theoretical modelling

To understand the theory underpinning the specificity and quantitative nature of the 3*f* current measurement, we modelled the QCR analytically as a nonlinear oscillator. It is well established that at relatively high drive amplitude, the thickness-shear mode the quartz crystal oscillator (QCR) behaves as a nonlinear oscillator [13]. Modelled as an infinite slab of parallel planes, the oscillation of a QCR can be described by the one-dimensional Duffing equation as

$$\ddot{x} + 2\lambda\dot{x} + \frac{k_q}{m_q}\left(x - \delta_0\cos(\omega t)\right) + \beta_q x^3 = 0 \tag{1}$$

where 2λ is the mass-proportional damping coefficient, primarily due to the liquid-QCR interface, k_q, m_q and β_q are the stiffness, mass and nonlinearity coefficient of the QCR respectively, and $\delta_0\cos(\omega t)$ models the oscillatory drive of amplitude δ_0 and frequency $\omega = 2\pi f$. Only odd orders of x are considered in Eq. (1) due to symmetricity of motion of QCR. 5th and higher orders are neglected.

When microparticles bind to the QCR through protein-receptor interactions, they form a spring-mass system coupled to the QCR. If k_b and N_b are respectively the stiffness of each bond and the number of bonds per microparticle of mass m_p, then $\sqrt{(k_b N_b)/m_p} \ll \omega$, i.e. the resonance frequency of the microparticle spring-mass system is much smaller compared to the drive frequency, which in our experiment was close to 14.24 MHz. As a result, the microparticle remains almost stationary in the laboratory reference frame with weak mass coupling with the QCR while the latter oscillates. This stretches the bonds, applying elastic loading on the resonator, which increases the effective stiffness of the QCR. The elastic loading also distorts the pure sinusoidal oscillation, consequently modifying the nonlinearity coefficient of the resonator. We model this change in