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High Frequency QCM Flow Cell with Enhanced Accuracy for Liquid and Biochemical Sensing

B.P. Stehrera, H. Gruberb, R. Schwödiauera, I.M. Grazc, S. Bauera

aSoft Matter Physics, Johannes Kepler University, Linz, Austria
bInstitute of Biophysics, Johannes Kepler University, Linz, Austria
cNanoscience Centre, University of Cambridge, Cambridge, United Kingdom

Abstract

Most biosensing systems based on thickness shear mode resonators work with resonance frequencies between 5 and 20 MHz. The utilisation of high frequency fundamental (HFF) quartz resonators could further improve the sensitivity of such systems. We designed an acoustic biosensor flow cell for HFF quartz resonators. For the development of a biosensor we solved important questions like the functionalization of the small and fragile sensor surface, the sensitive and repeatable detection of dissolved analytes and the regeneration of the sensor. The system performance is evaluated by a number of experiments including the step-wise growth of a protein multilayer system.

Keywords: Quartz crystal microbalance, High Frequency Fundamental, Flow cell

1. Introduction

The quartz crystal microbalance (QCM) has proven to be a highly sensitive mass detector which found its way into biomedical sciences. The measuring technique is ideally suited for online label-free detection of biological and chemical analytes.

Systems based on thickness shear mode (TSM) quartz resonators are already established and commercially available. As the mass sensitivity of quartz resonators is directly proportional to the square of the resonance frequency, high frequencies are desirable. High frequency crystal oscillators are due to their minor size and thickness and their fragility difficult to handle, so that it bears a challenge to design an adequate flow cell.

A suitable flow cell for 50 MHz HFF quartz crystals was designed, which is qualified for bioanalytical measurements. Furthermore the important issue of the functionalization of the sensor was resolved.

Instead of immobilizing biomolecules covalently at the quartz crystal surface, a lipidlayer for coupling molecules to the sensor is used. Onto a gold-covered crystal oscillator which is functionalized by a Self-assembled Monolayer (SAM) composed of alkanethiols a monolayer of lipid molecules can be established. At this stage the functionalized quartz crystal surface looks like a lipid membrane from top. Consequently one can simulate the surface of a cell or a cell organelle.

In the present work the effective operation of the system is demonstrated with two kinds of experiments: In a first experiment we have tested the reversible functionalization of an installed sensor by phospholipid monolayer formation from an aqueous buffer suspension of phospholipid vesicles, followed by complete lipid removal by a
detergent. In the second experiment, formation of an alternating protein multilayer composed of streptavidin and biotinylated immunoglobulin G was monitored.

2. Experimental

Most biosensing systems based on quartz crystal oscillators apply crystal oscillators with fundamental oscillations of 5 – 20 MHz. These are more or less large and thick and consequently insensitive to moderate mechanical stress. In that case one can realize conventional flow cells with simple clamp constructions. The requirements on a flow cell for HFF quartz crystal oscillators are more difficult to meet. A general problem is the smallness and fragility of HFF quartz crystals from which arise contradictory general conditions for a functional design. Above all the clamping mechanism has to act on the quartz crystal with a gentle and uniform pressure. Under no circumstances, liquid must leak through the flow cell/sensor interface. Further, all materials which get in contact with the liquid have to be biologically inert.

The constructive solution of all technical requirements was the central challenge in the fabrication of the flow cell. A detailed exploded assembly drawing is presented in Figure 1. The flow cell is made up of two main components: The lower flow-channel unit, which supports the HFF quartz crystal, constitutes the major part of the system. The top structure – the flow cell head – provides the high frequency signal via a planar electrode and simultaneously generates a uniform pressure upon the quartz crystal. The quartz crystal is sandwiched in between these two parts seating on a soft and slightly adhesive polydimethylsiloxane (PDMS) sealant, which seals the interface effectively and is a crucial element.

The main structure of the flow cell system is made of aluminum because of its favorable thermal and electrical properties; stainless steel is used for the electrodes. The lower unit incorporates the flow cell-core, made of poly(etheretherketon) (PEEK).

The HFF-QCM flow cell system is part of a flow injection analysis system (FIA), which consists of three subunits: the active thermal control unit, the fluid transport channel unit and the measurement unit. More detailed information about the FIA system can be found in Sagmeister et al. 2.
3. Results and discussion

A measurement was carried out by alternately adsorbing a lipid monolayer consisting of 1,2-dioleoylphosphatidylcholin (DOPC) and 1,2-dioleoylphosphatidylserin (DOPS) from an aqueous buffer suspension of phospholipid vesicles to the functionalized quartz crystal and desorbing it with the detergent octyl-β-D-glucopyranoside.

The assembled HFF sensor was maintained at 22°C and perfused with a continuous flow of PBS at a rate of 30 μl/min. In this configuration, a base line with a mean resonance frequency of about 50.8549 MHz and a standard deviation of ± 10.2 Hz could be measured. As can be seen in Figure 2 the injection of lipid vesicles resulted in a rapid change of the resonance frequency and after about 7 min a stable level was reached which was almost 700 Hz lower than before. This process is well known to reflect the formation of a phospholipid monolayer on the hydrophobic SAM of octadecanethiol 3. The phospholipid monolayer could perfectly be removed by addition of octyl-β-D-glucopyranoside, as regularly used to regenerate BIAcore chips with octadecanethiol SAMs 4. Thereby the different viscosity and density of the detergent caused a strong response with a frequency shift of almost -2 kHz. After the detergent injection the sensor surface was again superfused with PBS by what the resonance frequency returned to the original value.

The procedure of lipid monolayer adsorption and its desorption with detergent can be repeated many times and the sensor response is always reproducible.

Another experiment was the formation of a multilayer composed of streptavidin and biotinylated immunoglobulin G. Another HFF quartz crystal was coated with 1-octadecanethiol and installed in the flow cell. The sensor was exposed to a continuous flow of PBS at a rate of 30 μl/min and a mean resonance frequency of about 50.8423 MHz and a standard deviation of ± 10.2 Hz could be measured. Then a vesicle suspension consisting of DOPC, DOPS and biotinylated 1,2-dioleoylphosphatidylethanolamin (biotin-cap-DOPE) was injected, resulting in monolayer formation which carried biotin residues on 20% of the phospholipid head groups. The biotin-presenting monolayer allowed for biospecific binding of streptavidin which is a tetramer having two pairs of biotin-binding
sites of which only two are used for docking to the lipid monolayer. The second pair of biotin-binding sites is then available for binding of antibodies (IgG) carrying about 5-6 biotin residues per protein molecule, allowing for protein multilayer formation, as exemplified in Figure 3. In consecutive steps of about 11 min a 2 μM streptavidin solution and a 2 μM biotin-IgG solution were injected whereby each immobilisation caused a strong decrease in the resonance frequency.

**Fig. 3.** Protein multilayer composed of streptomycin and biotinylated immunoglobulin G on a biotinylated phospholipid monolayer.

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