Chapter 18

Label-Free, Real-Time Interaction and Adsorption Analysis 2: Quartz Crystal Microbalance

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Abstract

In this chapter, a second biosensor technique is described: the quartz crystal microbalance (QCM). The quartz crystal microbalance is a physical technique that detects changes in the resonance frequency of an electrically driven quartz crystal with changes in mass. Unlike surface plasmon resonance (SPR), QCM is affected by both the water that may be associated with the adsorbed layer and by conformational changes in the adsorbed species, while SPR is insensitive to both effects. Thus QCM can both corroborate the findings of an SPR experiment and provide some complementary information. Also, the QCM surface is highly versatile and can range from plain quartz, through gold and other metal surfaces (e.g., titanium or stainless steel) to polymeric materials. Thus, the QCM technique has wide utility in tracking interactions with a variety of materials.

Key word Quartz crystal microbalance

1 Introduction: Quartz Crystal Microbalance

The quartz crystal microbalance (QCM) allows the real-time measurement of mass associated with adsorption of solution-phase analytes either directly on material surfaces or onto ligands immobilized on a surface, forming a so-called adlayer. The underlying principle of operation is that the resonant frequency of a quartz crystal excited by an oscillating electrical current (Fig. 1a) is related to the total mass of the crystal, which, to a first approximation, includes bound coatings and adsorbed materials as well as local solvent molecules, particularly water. More particularly, such a crystal can generally be coated with various materials, including gold, silver, or titanium, and a number of polymers so can be used, for example in biomedicine applications (1). A further refinement of the technique is made possible if the resonant frequency is followed immediately after ceasing the electrical excitement in order to measure the rate of decay in the signal or, indirectly, the dissipation of the energy in the oscillating system (Fig. 1b). This approach

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Fig. 1 (**a**) Cross-sectional representation of the strain of a quartz crystal driven by an alternating electric current when the circuit is closed. (**b**) Decaying resonant frequency when the circuit is opened

is known as QCM with dissipation (QCM-D), where the rate of decay in the signal is related in part to the viscoelasticity of the adlayer, such that a loosely bound, soft adlayer will tend to dampen the signal more than a tightly bound rigid adlayer. The 2001–2005 literature on protein interaction studies by QCM was reviewed by Cooper and Singleton, providing an introduction to the QCM technique, protein interactions, and describing a wide range of applications (2).

2 Principles of QCM-D

A quartz crystal, or more specifically the α-quartz form of SiO_2, is a piezoelectric material, i.e., one in which a mechanical strain (for example in response to compression or shear) will induce an electric field within the material. Conversely, the application of an electric field will induce a strain in the material. Thus, a thin slice (0.1–0.3 mm) of quartz crystal can be made to distort laterally through the application of an electric potential across electrodes coated onto two opposite faces of the slice. An oscillating current will cause the crystal to deform in alternate directions at a resonant frequency, f, given by

$$f = n\frac{\nu_q}{2t_q} = nf_0 \tag{1}$$

where n is an odd integer describing the fundamental frequency ($n = 1$, $f_0 = \nu_q/2t_q$) or harmonic overtones ($n = 3, 5, 7\ldots$), ν_q is the speed of sound in the quartz slice, and t_q is the thickness of the slice. The mass per area of crystal, m_q, is related to the thickness by $m_q = t_q\rho_q\,(\mathrm{kg/m^3})$, where ρ_q is the density of the quartz (3). Substituting for m_q in Eq. 1 and differentiating with respect to m_q gives

$$\mathrm{d}f = -\frac{f}{m_q}\mathrm{d}m_q \tag{2}$$

If we can assume that an added mass is small compared to the total weight of the crystal; that an adlayer is adsorbed tightly with no slip; and that it is evenly distributed over the entire surface, then

by letting $d \rightarrow \Delta$, we can obtain the Sauerbrey relation, which states that the frequency change is linearly related to mass change (4):

$$\Delta f = -\frac{n}{C}\Delta m \tag{3}$$

where $C = \frac{\nu_q \rho_q}{2 f_0^2}$ is the mass sensitivity. For the quartz crystals used in QCM-D, ρ_q is typically 2,650 kg/m^3 and ν_q = 3,440 m/s, so the mass sensitivity for a crystal oscillating at a resonant frequency of 5 MHz (n=1) is about 17.7 ng/(cm^2 Hz) (3). In aqueous solutions, the frequency can be resolved to within 0.2 Hz, so that mass changes of the order 3–4 ng can be tracked by following changes in the fundamental frequency on a typical quartz crystal sensor of diameter 1 cm. In water, the QCM operating with a resonant frequency of 5 MHz will probe a region near the crystal surface about 250 nm deep (5). Clearly, the frequency change is amplified by the use of overtones so that sensitivity is increased three-fold by tracking the third overtone (n=3) and a crystal with a higher fundamental frequency will increase sensitivity by f_0^2, although, ultimately, sensitivity will be limited by the signal to noise ratio of the instrument and its ability to track higher frequencies.

Höök gives the useful examples that a monolayer of water on a 5 MHz crystal oscillating at its fundamental frequency gives a –1.4 Hz shift, while a monolayer of protein will (depending upon the molecular weight) result in a frequency shift of –20 to –80 Hz, easily followed by the QCM by a frequency counter (6).

One must also be careful, however, to control experimental conditions carefully, to incorporate suitable control experiments, and to analyze results thoroughly, as the assumption that the adlayer is tightly and rigidly adhered to the surface is highly questionable under many circumstances. Indeed, it is the recognition of the potential for violation of this underlying assumption that can lead to useful insights into adsorption and interaction behaviors through proper use of dissipation measurements.

Dissipation of energy can occur through viscous damping of the surrounding fluid, water bound to the adsorbed protein species or trapped between bound molecules on the crystal surface coating, and/or differential movement between the underlying oscillating quartz and a soft adsorbed layer (which may exhibit viscoelastic behaviors, especially adsorbed cells). In cases where viscoelastic behavior occurs, the Sauerbrey Eq. 3 will underestimate the true mass of the adlayer. The dissipation, D, is the inverse of the Q factor that describes the ability of a system to retain energy, and is directly related to the ratio of energy dissipated, $E_{\text{dissipated}}$, to that stored in the oscillating system, E_{stored}:

$$D = \frac{1}{Q} = \frac{E_{\text{dissipated}}}{2\pi E_{\text{stored}}} \tag{4}$$

Höök realized that when the driving power is switched off, the voltage or current decays as an exponentially damped sinusoidal

$$A(t) = A_{\downarrow}0e^{\uparrow}(t/\tau)\sin(2\pi ft + \varphi) \quad (5)$$

where ϕ is the phase angle of the signal, A_0 is a constant, and τ is the decay time constant of the signal. As dissipation is related to the decay time constant by $D = 1/\pi f\tau$, both f and D can be determined simultaneously by numerically fitting the measured voltage or current during the decay to Eq. 5.

An early example of the information that can be obtained by QCM-D is that of mussel adhesive protein, first adhering loosely to a gold surface (tracked by a negative Δf and a positive ΔD) and then being cross-linked to increase surface rigidity (tracked by a positive Δf and a negative ΔD), as shown in Fig. 2 (7).

Figure 3 shows the adsorption of β-casein on stainless steel before (a) and after (b) protection of the surface by grafting of poly(ethylene glycol) chains (8), showing that protein adsorption could be reduced 40% when using a combination of native poly(ethylene glycol) and polyethyleneimine.

3 QCM-D Experiments

Commercial suppliers offer a range of QCM sensors with various surface coatings. Gold is a common coating material, where the gold serves as the electrodes for the crystal. Plain quartz, silver, copper, chromium, titanium, stainless steel, various polymeric surfaces such as polystyrene or polycarbonate, and functionalized surfaces such as carboxylated, streptavidin, or nitrotriacetate are available. It is also possible to spin coat or vacuum sputter various materials onto a crystal, making QCM/QCM-D an extremely versatile technique for studying the adsorption of proteins, DNA, cells, viruses, etc., on many materials. Its use in biomaterial research is thus an obvious application, as is the development of biosensors or study of fouling of process equipment in the food, beverage, fermentation, and pharmaceutical industries.

Assuming that a suitable instrument is available for driving the crystal and monitoring the frequency/voltage/current changes, the experimental set-up for QCM-D is relatively straightforward. Tight temperature control is essential. A flow chamber is typically supplied through inert microtubing by a peristaltic pump at a flow rate of approximately 100 μL/min, although the actual rate will depend upon the dimensions of the crystal and flow chamber concerned. A low-pulse pump type is preferable to minimize pressure pulsations affecting the signals and while in principle the flow can

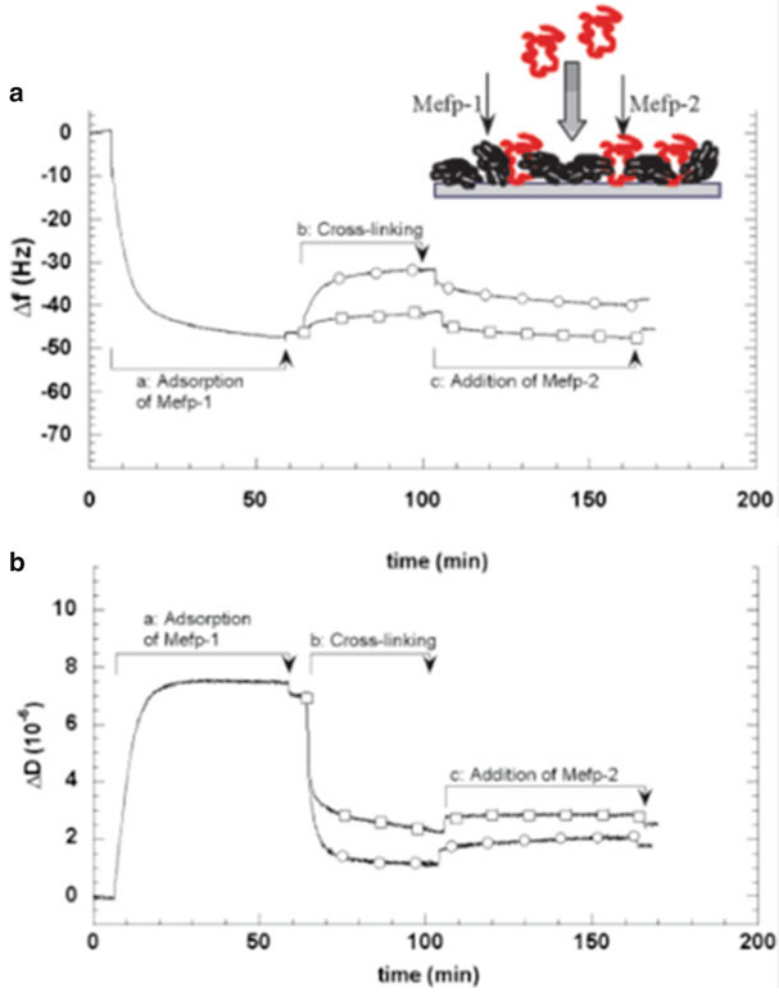

Fig. 2 (**a**) Changes in frequency, Δf, versus time upon adsorption of *Mytilus edulis* foot protein 1 (Mefp-1) (*solid line*) to the CH3-terminated (thiolated), nonpolar gold surface (*arrow* **a**). After rinsing, cross-linking was chemically induced (*arrow* **b**) using 1 mM $NaIO_4$ (*open circles*, *solid line*) or 10 mM Cu^{2+} (*open diamonds*, *solid line*). This was followed by rinsing and subsequent addition of 25 mg/mL Mefp-2 (*arrow* **c**) in the presence of 1 mM $NaIO_4$ (*open circles*, *solid line*) or 10 mM Cu^{2+} (*open diamonds*, *solid line*). (**b**) Changes in energy dissipation, ΔD, versus time for the experiments shown in (**a**). *Inset*: Schematic picture of the binding of Mefp-2 to free-surface patches in the adsorbed and cross-linked Mefp-1 layer (reproduced from ref. 7)

be either injected into the flow chamber from the upstream side or drawn through the chamber from the downstream side, the latter tends to result in smaller pressure pulsations.

A simple but effective approach is one in which the equilibration buffer is first run through the chamber until a steady state is obtained, then the pump stopped briefly and the upstream side of

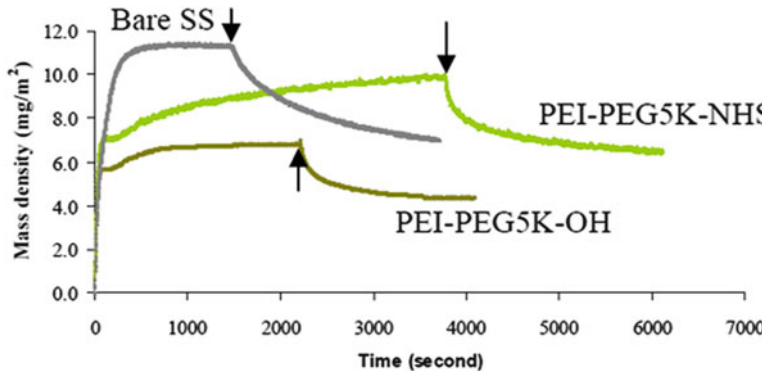

Fig. 3 Surface mass density of β-casein on bare stainless steel (SS) and SS treated with polyethyleneimine and either native 5 kDa polyethylene glycol (PEG) or PEG modified with *N*-hydroxysuccinimide (reproduced from ref. 8)

the flow tubing is swapped from equilibration buffer to the sample of interest, and the pump restarted. Outlet flow can be directed to waste, collected separately or recirculated to the inlet reservoir (beaker). Given that protein adsorption on the crystal surface is generally of the order of nanograms and will thus have little effect on the bulk inlet sample concentration for all but the most dilute of samples, recirculation is an effective means to minimize the amount of sample required. The process of briefly stopping the pump while swapping the inlet tubing to alternate sample reservoirs can be repeated as many times as desired to build up multiple layers or subject the crystal surface to various treatments. At the end of the experimental run or between steps, as desired, one should swap the inlet tubing back to the original buffer to check for reversibility of binding and to adjust for drift that may have occurred during the experiment. This also allows for the changes in signal that occur due to viscosity or conductivity rather than adsorption/desorption to be determined. This experimental approach, although it involves manual intervention to change samples, allows the user to decide when a change of inlet source might be useful by following the behaviors in real time, rather than preprogramming a series of steps through an automated liquid-handling system. For example, it may be difficult to decide in advance how long it might take to reach saturation of the surface, whereas it is a simple matter to determine this by observation.

Figure 4 shows the Q-Sense E4 system, with four parallel flow chambers (channels). Judicious use of a second channel through which the equilibration buffer and/or other buffers that are essentially the same as the samples but without the presence of adsorbing species are flowed is almost always important. A simple experiment in which one equilibrates the crystal, swaps to an adsorbing species

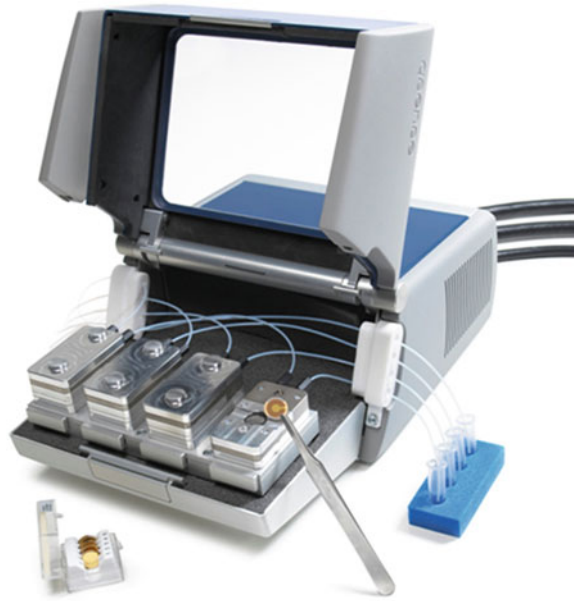

Fig. 4 The Q-Sense E4 system, showing four flow cells in a temperature-control chamber. The *right-hand* flow cell is *open* and a gold-coated quartz crystal ship is shown held in a pair of tweezers, ready for placement onto the "o"-ring-sealed flow chamber (reproduced from Q-Sense E4 manual)

and then back again to the original buffer may not strictly require use of a second control (or reference) channel, particularly for fast, completely reversible adsorption but without it, one cannot easily adjust for drift over the course of an experiment. It is advisable even in such simple cases but absolutely essential in many others for a reference channel to be used so that drift and buffer effects (changes in signal due to viscosity and conductivity changes between buffers) can be taken into account.

In practice, one must be careful to avoid microbubbles forming on the crystal sensor surface, which can occur due to changes in temperature, for example. The crystals themselves can develop microcracks and the fundamental frequency of the crystal in situ within the instrument is very sensitive to orientation and the distribution of forces when sealed into the flow chamber. Thus, it is important to degas buffers and check for imperfections on the crystal surface before use and it is generally not possible to obtain quantitatively comparable results after a crystal flow chamber has been opened or even loosened/tightened between runs.

Crystals should be thoroughly cleaned prior to use. The cleaning protocol should be developed and confirmed experimentally, particularly for unique surfaces, but for plain gold, the surface can usually be stripped of adsorbed species by soaking in Pirhana solution or a mixture of hydrogen peroxide (30%), ammonia (25%), and water at a ratio of 1:1:5 by volume for 5 min at 75°C

and then rinsed consecutively with hexane, ethanol, and deionized water. Finally, prior to use, gold-coated crystals should be exposed to UV/ozone for 10–15 min. A minimum of 12 mW/cm^2 at 25 mm from a 185/254 nm lamp is recommended. The latter treatment may not be suitable for other materials, particularly polymeric coatings, on which free radicals may be generated during UV irradiation.

Signal processing (removal of artifacts, alignment of injections, subtraction of reference curves) and analysis of binding kinetics follow essentially the same principles as already described in Chapter 17 for SPR so will not be repeated here. It is essential though, just as was stressed for SPR experiments, that statistical reproducibility and goodness of modelling fits are shown for QCM/QCM-D experiments. Sauerbrey (non-viscoelastic) modelling may be possible if the dissipation value remains close to zero or $\Delta D/\Delta f < 0.05$ (where ΔD is expressed in terms of 1×10^{-6} and Δf is expressed in Hz) throughout the experiment. Viscoelastic behavior will induce an overtone-dependent Sauerbrey thickness (5), so deviations of D from zero or significant differences between the frequency shifts of different overtones indicate that viscoelastic modelling should be undertaken, using either the Voigt or the Maxwell model. The Maxwell model treats the behaviour of a material placed under strain as a spring and dashpot in series, which results in a model that responds immediately to stress through the spring element and then more slowly takes on a viscous component with permanent displacement through the dashpot. The Voigt model models behavior as a spring and dashpot in parallel, which results in a reversible strain with a good approximation of creep behavior. These and other models are reviewed in more detail by Ferreira et al. (9). The final model should be able to fit a wide range of overtones well, although the fundamental frequency signal ($n = 1$) is not generally very useful. Deviations of the model from some overtones may indicate nonoptimal parameters or a poor choice of model.

In addition to time series for f and D, it is possible to plot ΔD versus Δf and obtain potentially useful information on the changes in dissipation as a function of deposited mass. For example, it is possible to see differences in viscoelasticity with adsorbed mass either within a single run, for example as a result of multiple layer formation, water ejection, or cross-linking, or between adsorption runs made at, say, different pH values or temperatures. An example of this is shown in Fig. 5, where changes in slope are indicative of distinctly different processes occurring during adsorption (10). Figure 6 shows a transition from “mushroom” or “pancake” conformation to extended “brush” formation of polymer chains on a gold surface (11).

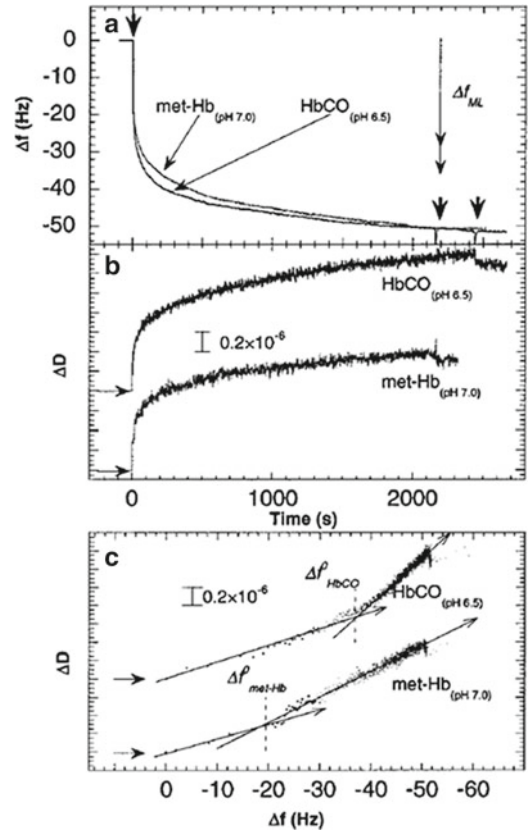

Fig. 5 Changes in frequency (**a**) and dissipation (**b**) as a function of time during adsorption of met-hemoglobin (met-Hb) and hemoglobin-CO (HbCO) at pH 7.0 and 6.5, respectively, to gold covered by a hydrophobic methyl-terminated thiol monolayer (10 mM Hepes). The proteins were introduced at $t=0$. The two *right arrows* in (**a**) indicate the times at which the two protein solutions were exchanged for pure buffer solutions. The *vertical double arrow* in (**a**) shows the predicted frequency change for a monolayer of Hb. Two limiting values are given ($29\ \text{Hz} < \Delta f_{ML} < 35\ \text{Hz}$) depending on the orientation of the Hb molecules on the surface. (**c**) *D–f* plots using the data from (**a**) and (**b**). Note that the density of data points (equispaced in time) becomes smaller the faster the kinetics, in this type of plot. This explains the small number of data points near the origin, where the kinetics is fast. The *horizontal arrows* in (**b**) and (**c**) denote the starting points of the experiments, i.e., $\Delta D = \Delta f 5 t = 0$. The *arrows* drawn through the *D–f* graphs in (**c**) indicate the direction of time. Δf_0 denotes the break point of the *D–f* graph into two regimes (reproduced from ref. 10)

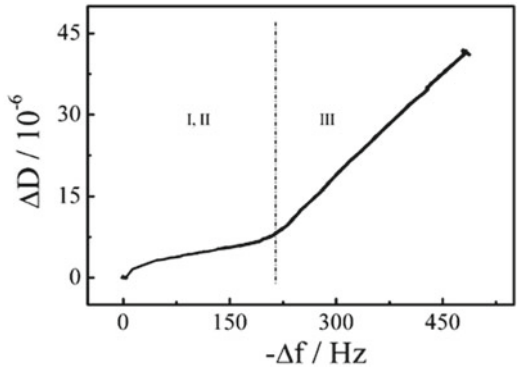

Fig. 6 ΔD versus Δf for the transition between various configurations of polymer chains on a gold surface, going from "mushroom" or "pancake" conformations in regions I and II to extended "brush" conformations in region III (11)

4 Summary

The QCM technique is able to track changes in mass on the surface of a quartz crystal, including on a thin surface coating, making it useful not only for studying molecular interactions but adsorption/desorption on a range of surface materials. Because the measured variable, resonant frequency, is related to mass, unlike optical techniques QCM can indicate the presence of bound and loosely associated water. For thin, rigid, evenly distributed adlayers, the Sauerbrey relation shows that a change in the resonant frequency of a quartz crystal is directly related to the change in mass. However, the assumption of a rigid layer is highly likely to be violated during protein adsorption and even more so for cell adsorption on surfaces because of the inherent viscoelasticity of the adsorbed species and the association of solvent (water) with the adsorbed species or trapped within the adlayer. In this case, viscous damping of the crystal oscillations will occur and it is necessary to allow for this by including a measurement of the energy dissipation of the crystal-adlayer system. QCM-D is a powerful technique that indicates when deviations from the Sauerbrey relation occur and provides additional information on the viscoelastic properties of the adlayer and thus insights into the nature of association during adsorption/desorption.

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