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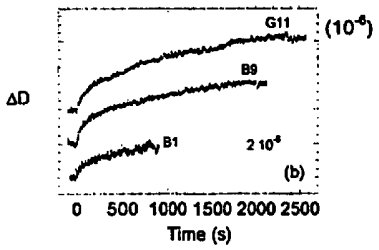
(57) Abstract

The present invention relates to a method for measuring of the interaction between at least one target molecule and at least one receptor molecule using a piezo-electric crystal micro balance, where at least one type of the molecules is immobilised, whereby at least one crystal is brought into oscillation by means of a driving circuit, and measuring the dissipation (D) and/or change in dissipation (ΔD) during driving and/or after switching off the driving circuit.



SENSOR FOR DETECTING BIOLOGICAL MATTER

Description

Field of invention

The present invention relates to a method for the measurement, using a piezo-electric crystal micro balance, of the interaction between at least one target molecule and at least one receptor molecule, of which at least one of the molecules is immobilised. The method includes measurement of dissipation (D) or dissipation (D) and frequency (f).

Background of the invention

Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

Antibodies protect against diseases and infections. When a human being, for instance, is exposed to an antigen, the body produces antibodies with binding sites for the antigen, which thereby is immobilised. Accordingly, the antibodies can be used to decide whether a specific antigen is carried by a human being or not. The detection is performed by presenting the right antigen for a certain antibody and to decide whether binding takes place or not. The most developed analyses using immuno sensors includes antigen radioactive labelling of the antigen/antibody complex or labelling with fluorescence. The most commonly used method using fluorescence is called ELISA (Enzyme Linked Immuno Sorbent Assay). ELISA is used among others to estimate if blood from a subject contains antibodies against a specific disease. Firstly the antigen from a virus or bacteria is bound to a surface. The surface is then exposed to the blood. If the blood contains antibodies specific to the antigen bound to the surface, they will bind to the antigen. If no antibodies are present, the surface will not be affected at all. At last the presence of antibodies are checked for by presenting the surface to a solution containing antibodies, labelled with a fluorescent group, which is specific for the antigen-antibody complex. If the surface contains the antigen-antibody complex, the labelled antibody will bind to the surface which can be detected. ELISA is a commonly used method and commercial systems and reagents are manufactured at a large scale, which renders the method relatively cheap. However, it presents several serious disadvantages. It is very time-consuming and its performance takes normally from a couple of hours up to a day. Monitoring in real time of the

binding of the antibodies is not possible, i.e. no hints or information of the reaction kinetics of the system is being given.

The discovery of the linear relationship between the change in oscillator frequency in a piezo-electric crystal and change in mass on the crystal due to binding or adsorption, makes it possible to gravimetrically supervise the antigen-antibody reactions. The mathematical relationship between the change of frequency in a piezo-electric crystal, Δf, and the change of mass, Δm, at a crystal is given by the following formula:

$$\Delta f = -\text{constant} \cdot \Delta m.$$

It was for the first time reported in 1972, the usage of piezo-electric crystals for analytical purposes for antigen-antibody reactions (A. Shons, F. Dorman, J. Najarian, *J. Biomed. Mater. Res.* **6**:565). A crystal was covered with bovine serum albumine (BSA) and the forming of the BSA-antibody complex on the crystal was monitored by the change in frequency. Since then a number of studies of antigens and antibodies using piezo-electric methods has been performed. The development of this area has been concluded by A. A. Suleiman and G. G. Guilbault, *Analyst.*, **119**:2279 (1994) and M. D. Ward and D. A. Butty, *Science*, **249**:1000 (1990).

Several patents describe how QCM (abbreviation for Quartz Crystal Microbalance, i.e., a method of weighing using a quartz piezo-electric crystal) was used to analyse antigens and antibodies. In the American patent US-A-4,242,096, (1980), it is described how to determine the presence of an antigen in a sample by using adsorption of an antigen to a crystal. The antigen-coated crystal is exposed to a sample with a predetermined amount of antibody, whereupon the amount of antigen can be determined from the frequency shift, by using a standard plot.

In the American patent US-A-4,236,893 (1980) and US-A-4,314,821 (1982) to Rice methods are described wherein antibodies are detected using QCM. In the first mentioned patent, the antigen was immobilised on a crystal coated with a polymer. The frequency change from the binding of antibody was related to the concentration of antibody in the sample, but the analysis was disturbed by unspecific binding to the crystal. In the second patent, the detection of low

molecular compounds is described using a crystal coated with anti-antibodies. All these analyses were performed by treating the crystal in solution and then measure the frequence in open air. This two-step procedure with solution /gas improves the sensibility with oscillating QCM, but causes technical complications and disturbs the hydration/dehydration which affects the frequency parameters.

Liquid phase piezo-electric immunoassay-technique has technical advantages as one can perform stationary analyses and flow analyses of a solution. Thus, the method has had physical limitations due to small frequency changes. Liquid phase piezo-electric-immunoassays has been reported by J. E. Roederer, G. J. Bastiaans, *Anal. Chem.*, **55**: 2333, (1983) and in their American patent US-A-4,735,906. The quartz crystal was here modified with glycidoxypropyl trimethoxysilane (GOPS) and the surface modified crystal was later additionally modified with anti-human IgG antibody and was then used for piezo-electric detection of human IgG. A similar method is described by H. Muramatu, J. M. Dicks, E. Tamiya and I. Karube, *Anal. Chem.*, **59**:2769 (1987), wherein the surface of quartz crystals was modified with γ-aminopropyl triethoxysilane and then with protein A. The surface modified crystal was then used to determine the concentration of human IgG.

In the PCT application WO97/04314 a method is described wherein the binding of e.g. antigen-antibody complex in solution is determined using a piezo-electric crystal. A weakness in this system and in the above mentioned liquid phase system is that separation of two closely related antibodies having e.g. different viscoelastic properties, is not possible since only the change in resonance frequency is measured.

The PCT application WO96/35103 describes an equipment for measurement of the resonance frequency and/or the dissipation factor in a piezo-electric resonance unit, wherein a piezo-electric crystal is connected to an operating unit to achieve oscillation of said crystal, at which oscillation was measured after switching off the crystal operating unit. The device can be used for the measurement of absorption of i.e. organic material to one surface of the crystal.

US-A-5,653,939 describes a method for investigation of a test sample with respect to target nucleic acids, the method comprising the formation of a set of test sites on several locations, each site comprising oligonucleotide probes formed there upon with known binding

characteristics and wherein the probes at each test site differ from the probes at other test sites in a predetermined known way such that the localisation of the test sites of the probes and their binding characteristics also is known, that a test substance is applied on the test sites, and that a change in the electromagnetic properties is detected at the test sites, which arise from binding of target nucleic acid to the probes.

Brief description of the invention

According to one aspect, the present invention provides a method to measure the interaction between at least one target molecule and at least one receptor molecule using a piezo-electric crystal microbalance, where at least one type of molecules is immobilised,

wherein,

a receptor molecule is immobilised to a piezo-electric crystal microbalance,

at least one crystal is brought into oscillation by means of a driving circuit,

a target molecule is presented to a crystal micro balance surface comprising the immobilised receptor molecule, and permitting a reaction between the target molecule and the receptor molecule,

the resonance frequency (f) is measured during the driving and/or switching off of the driving circuit,

the dissipation (D) and/or the change in dissipation (ΔD) is measured during the driving and/or after switching off of the driving circuit,

whereby the quantities resonance frequency and the dissipation factor are measurements or signatures of the process on the surface of the crystal micro balance, which surface affects the resonance frequency and the dissipation factor.

According to another aspect, the present invention provides a method to measure the interaction between at least one target molecule and at least one receptor molecule using a piezo-electric crystal microbalance having a crystal, the method including:

immobilising at least one receptor molecule to the crystal;

providing a driving circuit that is switched on and off such that, when switched on, the circuit brings and maintains the crystal in oscillation;

presenting a target molecule to the immobilised receptor molecule and permitting a reaction between the target molecule and the immobilised receptor molecule;

measuring the resonance frequency (f) and the change in resonance frequency (Δf) of the microbalance when the driving circuit is switched on and/or off; and

measuring the dissipation factor (D) of the microbalance and/or the change in dissipation factor (ΔD) when the driving circuit is switched on and/or off, whereby the resonance frequency and the dissipation factor provide measurements indicative of the reaction.

The information of the change in dissipation and resonance frequency of a system can be related to the concentration of the target molecule in the solution and the affinity between the target molecule and the receptor molecule. This is possible as the different target complexes have viscoelastic properties, since the conformations of the complexes

differ. In addition, the target complexes affect the absorbing layer differently, which also affect the result of the measurements. Accordingly, interaction between different target molecule-receptor molecule complexes according to the present invention, can be distinguished from their dissipation, or dissipations and frequency responses for a certain system. For instance, f and D are together said to form some kind of fingerprint of the measured system. The change in dissipation, or frequency and dissipation, when the target molecule interacts with the receptor molecule can then be used as a measurement and/or a signature of the process.

Use of the method according to the invention typically requires:

1. a receptor molecule with a specificity for the target molecule;
2. a method for binding the receptor molecule to the piezo-electric crystal surface;
3. a suitable reaction condition to form a reaction product such as target analyte receptor complex, and
4. an equipment for monitoring the change in dissipation and resonance frequency, or dissipation only.

With the expression interaction are all kinds of interaction appreciated which may be but not necessarily temporary and may comprise several molecules, and also the interaction of reaction products against the surface.

Method for binding the receptor molecule to the surface of the piezo-electric crystal can either be performed directly via adhesion, or via binding surfaces.

The expression target molecule relates to compounds which are to be detected, which includes but are not limited to antigens, antibodies, drugs, nucleic acids, hormones, proteins, enzymes, and carbohydrates. The target molecule has to be able to bind to the receptor molecule, which may also be but are not limited to the above mentioned compounds.

The target molecule and the receptor molecule can form a target complex, e.g. antigen-antibody, ligand-receptor, sugar-lectin, biotin-avidine, enzyme-substrate, oligonucleotide-oligonucleotide with a complementary sequence, oligonucleotide-

protein, oligonucleotide-cell, etc. The forming of these target complexes can be detected, as well as the dissociation of such complexes.

The piezo-electric crystal micro balance is preferably of the QCM type, and are preferably used here by measurements in liquids but the measurements can also be performed in gas phase or in vacuum. The invention is a fast and sensitive technology for e.g. immunoreactions and in particular antibody-antigen reactions. Compared to other techniques for real time measurements of protein adsorption kinetics in liquid phase e.g., ellipsometry this technique is relatively cheap, as quartz crystals are also used as sensor chips in other application areas.

A further aspect of the present invention provides an instrument which takes advantage of the method according to the above mentioned aspect.

Unless the context clearly requires otherwise, throughout the description and the claims, the words 'comprise', 'comprising', and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to".

Description of the drawings

Fig. 1 shows Δf versus time for binding of the antigen gG-2 in concentrations of 1 μg/ml for two separate, but identically treated TiO_2-crystals;

Fig. 2 shows ΔD versus time for binding of the antigen gG-2 in concentrations of 1 μg/ml for two separate, but identically treated TiO_2-crystals;

Fig. 3 shows Δf versus time for three separate measurements of a quartz crystal, which has been identically treated with TiO_2-gG-2-Hb, for binding of 0.5 mg/ml B9, G11, and B1;

Fig. 4 shows ΔD versus time for three separate measurements of a quartz crystal, which has been identically treated with TiO_2-gG-2-Hb, for binding of 0.5 mg/ml B9, G11, and B1;

Fig. 5 shows ΔD versus time for two separate measurements of a quartz crystal, which has been identically treated with TiO_2-gG-2-Hb, for binding of 0.5 mg/ml B9, G11, and B1.

Detailed description of a preferred embodiment of the invention

The present invention relates to a method to measure the interaction between at least one target molecule and at least one receptor molecule, using a piezo-electric crystal micro balance, preferably of the QCM type.

The Swedish patent of the applicant with publication number 504 199 describes a device and a technique for the measurement of resonance frequency and/or dissipation factor in a piezo-electric crystal micro balance, preferably of the QCM (Quartz Crystal Micro Balance) type. The present invention is a further development of the technique for a method to measure biological target complexes.

QCM is an extremely sensitive balance designed to weigh small quantities of material which are added or removed from the electrode. QCM according to the present invention consists of a crystal disc of a piezo-electric material, preferably quartz, more preferably a AT- or BT-cut quartz-crystal. Such a crystal can oscillate in shear mode with the amplitude of approximately 1-10 nm and with the basic tone frequency

$$f = \text{constant} \cdot d^{-1}$$

wherein d is the thickness of the crystal. With $d \approx 0.17$ is $f \approx 10$ MHz. The oscillation occurs when a AC field is brought perpendicular to the surface of the crystal. The frequency of the AC-field should be centred around the natural frequency of the crystal. In practice, the electrodes are applied by e.g. vaporisation on each side of the crystal. The electrodes are then in electric contact with an external oscillation circuit. This device can measure very small changes in mass on the quartz electrodes, in the range of 1 ng/cm^2, or even less, under advantageous conditions.

Under ideal conditions, as has been mentioned above, the change in resonance frequency, Δf, is proportional against the change in mass, Δm, i.e.

$$\Delta f = -\text{constant} \cdot \Delta m$$

The size of the constant depends on the choice of crystal and cut thereof. The equation presumes that the mass, which is represented as Δm, is rigidly located, is evenly

distributed on the electrode and follows the oscillation of QCM without dissipative losses. The equation is not generally valid when the coated mass consists of a viscous material and/or is not equally distributed over the mass sensitive area of the crystal. In that case a given amount of coated mass can also give rise to different frequency shifts, which is the same as that a certain frequency shift can correspond to different amounts of mass. Thus in these cases, it can be of limited value only to measure the resonance frequency.

An object of the present invention, at least in its preferred embodiments, is to construct a sensitive method for the detection of biological target complexes, using an improved QCM-technique. This can be achieved by an improved characterisation of the system being measured by the sensor. By simultaneously measuring f and the dissipation factor D, (the dissipation being the inverse of the so called Q-factor) of the QCM, the amount and the value of the information in the studied system is significantly increased. A typical measuring method according to the present invention is to both measure f and D, as a function of time. The quantities f and D measure different features of the system. Together f and D provide a kind of finger print of the studied system. However, in certain systems, the measurement of the dissipation, D, is enough.

The method according to the present invention allows system-specific finger prints. Processes/system are being characterised by plotting each measured Δf versus ΔD in a XY-graph, or in any coordinate system, with Δf on one axis and ΔD on the other. The obtained plot, the so called Df-plot, is not explicit, but implicit, time dependent since each pair of Δf-ΔD can be put in the plot. The shape of the Df plot works as a finger print for a given process.

Further finger prints can be obtained by exciting the crystal in its harmonic tones (3rd, 5th etc.) and monitor the Df-plot for every harmonic tone. It is also possible to use different cuts of the crystal simultaneously, not only the AT-cut, and monitor the Df-plot for its different cuts. Further Df plot can be measured at different oscillation amplitudes. That which is called the Df-finger print or Df-signature of a system, according to the invention includes all the Df-plots, which can be measured for the studied/measured

system at different oscillation frequencies, different oscillation modes and different amplitudes.

The shape of the Df-plot can immediately reveal that the interaction between the different types of the target molecule-receptor molecule complex is different, without a detailed interpretation of the nature of the interaction having to be known. Each shift or layer, for e.g., non-rigid and viscous layers on the surface of the crystal has accordingly an Df-signature each. If the signature of two systems at one frequency and/or amplitude are too similar, the difference may increase at other frequencies and amplitudes.

Typical requirements for using the method according to the present invention.

1. A receptor molecule having a specificity for the target molecule

The receptor can be any of the following compounds: antibodies, synthetic antibodies, antibody fragment, antigens, synthetic antigens, haptenes, nucleic acids, synthetic nucleic acids, cor, receptors, hormones, proteins, prions, drugs, enzymes, carbohydrates, biotins, lectins, bacteria, virus and saccharides. The only requirement present on the receptor molecule is it must be able to bind to the target molecule, which can be any of the above-mentioned compounds as well. The target molecule and the receptor molecule form a target complex, e.g., antigen-antibody, ligand-receptor, sugar-lectin, biotin-avidine, enzyme-substrate, oligonucleotide-oligonucleotide with a complementary sequence, oligonucleotide-protein, oligonucleotide-cell, etc. The receptor molecule can also be selected in such a way that it is smaller than the target molecule and/or that

the receptor molecule binds close to one end at the target molecule to create a large lever.

2. Method for binding the receptor molecule to the piezo-electric crystal surface

The receptor molecule can be attached directly to the piezo-electric crystal surface through adhesion or via another surface with or without yet another layer, together called the binding surface, through adhesion or a chemical reaction. The binding surface may include oxide, metal oxide, such as TiO_2 and SiO_2, semi-conductors, inorganic compound, organic compound, lipid layer, polymer, avidine or streptavidine, biotin, protein, and self organised layers. The choice of binding surface may affect the finger print, that is, some surfaces, e.g., TiO_2 may amplify the signal of the analysis.

3. Suitable reaction conditions to form a reaction product, such as the target analyte receptor complex

These are all dependant on which system to study, and it is known in the art which reaction conditions are suitable for a certain system. Examples of suitable reaction conditions are, but are not limited to, from 4 °C till 80 °C, pH 4 - 9.0, a salt concentration between 1 µM - 1 M.

4. Equipment to monitor the change in dissipation and resonance frequency, or dissipation only.

The measurement are performed using equipment including at least one crystal with at least one set of electrodes (applied directly on the crystal or in close connection to this) connected to a drive circuit, to put the crystal/crystals in oscillation, which is arranged to perform a manual or automatised measurement in real time of

(i) the dissipation factor (D), and/or the change of this, (ΔD), or

(ii) the dissipation factor(D), and/or the change of this, (ΔD), and the resonance frequency (f) and/or the change of this (Δf),

by driving and/or by disconnecting the drive circuit, and the measurement of the dissipation, or the dissipation and the frequency is measured as a function of time.

The measurements may be performed in gas phase, vacuum, but preferably in liquid phase. The measurements can be performed at basic tone frequency as well as harmonic tones, and/or the oscillation amplitude and it can be performed automatically as well as manually.

To reduce unspecific binding of the target analyte a blocking agent can be used. Then it covers

the areas or parts of areas, on the crystal surface or at the binding surface which are not coated with receptor molecules. Several proteins or other molecules can be used as blocking agent. However, they should have a low, or lowest possible affinity to the target molecule.

Measurement on a sample flow can also be performed, which is brought in contact with the detector, so that the content of target molecule is determined as a function of time.

It need not have to be the binding course itself, which is the main object, which is being studied, but the product, which is the result of the binding. Measurements can also be performed in which way the target complex affects the binding surface and how viscous and/or viscoelastic molecules interact. In addition, measurements of the dissociation of a target complex can also be performed, e.g., an antigen-antibody complex, which dissociates due to effects from the outside.

The method according to the present invention can also be used to detect and/or determine the amount of nucleic acids of a certain sequence present in a sample, using a receptor molecule, which is a single-stranded nucleic acid having a complementary sequence to the target molecule, which are to be detected. Additionally, point mutations in the nucleic acid can be detected by bringing a sample into simultaneous contact with two, or more sensor surfaces, equipped with receptor nucleotide strands, which are complementary to the mutated and the normal sequence, respectively, and measure the difference in their responses.

According to the present invention a device can be designed, which utilises a method according to above mentioned.

Below a system is given as an example, wherein the target molecule is an antibody and the receptor molecule is an antigen. The QCM technology, according to the present invention hereby allows a rapid (less than one hour) real time measurement of the antibody-antigen reaction. It is done by measurement of the dissipation and the frequency shift during time, as the antibodies binds to the antigen which covers the crystal surface. In addition, the kinetics of the antibody-antigen reaction can be measured.

Using the method according to the present invention the reaction kinetics can be directly related

to

(i) the antibody concentration in the solution, and

(ii) the affinity between antigen and antibody.

These two parameters is of great importance for, e.g., medical analyses.

Example 1

The receptor molecule was the antigen glycoprotein G (gG-2) from Herpes Simplex virus type 2 (HSV-2). This protein, having a molecular weight of 120 kD, was purified from virus infected cell membranes using helix promatia lectin affinity chromatography. The invention was demonstrated using three different target molecules, the monoclonal γ-globulin (IgG) the antibodies B9, G11, and B1. It is known that the antibodies B9 and G11 are type-specific and do not bind to other Herpes Simplex virus proteins than HSV-2. Using the ELISA technique it has been proved that these antibodies are specific to two different epitopes on gG-2 (Liljequist, J. Å., Tribala, E., Svennerholm, B., Jansson, S., Sjögren-Jansson, E., Bergström, T., submitted to Journal of General Virology 1997). The binding affinity to the antigen is approximately 100 times larger for B9 than for G 11. The monoclonal antibody B1 which is type-specific for glycoprotein C from Herpes Simplex type 1,were used as control, as it is known that it does not bind to gG-2.

Titan oxide (TiO_2) was used as a substrate surface. Between the reactions the substrate surface was washed several times. It could therefore be used up to 10 times with a high reproducibility.

At the binding of the antigen to the surface, a 50 mM TRIS/ 200 mM KCl buffer with pH 9.6 was used, and for the binding of antibody a 50 mM HEPES/ 200 mM KCl buffer with pH 7.0 was used.

Figure 1 shows Δf versus time and Figure 2 shows ΔD versus the time for binding of the antigen gG-2 at a concentration 1 μg / ml for two separate, but identically treated TiO_2-crystals. The antigen was hereby immobilised before the detection of the antibody. The adsorption did not reach saturation during the measurements, but the binding of the antigen was interrupted when the frequency reached approximately 20 Hz. Measurements were performed at the third harmonic tone. The binding of antigen to different crystals were performed with a high reproducibility, which is illustrated in Figure 1 and 2.

To avoid unspecific binding of antibodies to free surface 30 µg /ml hemoglobin (Hb) was used, as a blocking agent (bovine hemoglobin, Sigma). Thereafter the gG-2-Hb surface was exposed to the antibody solution to be examined.

Figure 3 and 4 show Δf versus time and ΔD versus time of three separate measurements with identically treated TiO_2-gG-2-Hb crystals for binding of 0.5 mg/ml B9, G11 and B1.

The control antibody, B1, gave a very small change in f and D, which shows that the unspecific binding was low. Corresponding shifts in f and D for B9 and G11 are significantly higher. It can also be seen that the antibody B9 having a higher affinity than G11, binds at a higher rate and gives a two times higher frequency change than for G11, but without any greater change in D-shift.

The results shown in Figure 5, wherein ΔD versus Δf is given of the binding of B9 and G11. Both B9 and G11 exhibit linearity between Δf and ΔD, but the slope for G11 is approximately 1.7 times bigger than for B9. It should also be noted that the slopes, i.e. ΔD/Δf, were identical for respective antibody at both low and high concentrations. This shows that antibodies specific for different epitopes on the same antigen can be distinguished from each other, not from velocity or absolute frequency shift for the reaction alone, but also from induced ΔD per Δf. The finger print obtained from Df-signature makes it possible to distinguish between antibodies specific for different epitopes, but having similar affinities. This depends on the fact that the observed changes in dissipation are caused by the different conformations, created by different antibody-epitope reactions by the antigen-antibody complexes. In addition, the different antigen-antibody complexes affect the absorbed layer differently.

Consequently different antibody-antigen reactions can be separated, according to the present invention, in a unique way differentiated from their dissipation and frequency answer. This is of great importance since antibodies from different bacteria and virus infections can be detected with a high sensibility and specificity.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. A method to measure the interaction between at least one target molecule and at least one receptor molecule using a piezo-electric crystal microbalance, where at least one type of molecules is immobilised,

wherein,

a receptor molecule is immobilised to a piezo-electric crystal microbalance,

at least one crystal is brought into oscillation by means of a driving circuit,

a target molecule is presented to a crystal micro balance surface comprising the immobilised receptor molecule, and permitting a reaction between the target molecule and the receptor molecule,

the resonance frequency (f) is measured during the driving and/or switching off of the driving circuit,

the dissipation (D) and/or the change in dissipation (ΔD) is measured during the driving and/or after switching off of the driving circuit,

whereby the quantities resonance frequency and the dissipation factor are measurements or signatures of the process on the surface of the crystal micro balance, which surface affects the resonance frequency and the dissipation factor.

2. The method according to claim 1,

wherein,

the change in resonance frequency (Δf) is also measured during driving and/or after switching off of the driving circuit.

3. The method according to any one of the preceding claims, including the steps of:

providing one or more XY-diagrams, having the resonance frequency on one axis and the dissipation factor in the piezo-electric crystal micro balance on the other axis.

4. The method according to any one of the preceding claims,

wherein,

the target molecule and the receptor molecule are selected from one or more of the following groups comprising antibodies, synthetic antibodies, antibody fragment, antigens, synthetic antigens, haptenes, nucleic acids, synthetic nucleic acids, cells, receptors, hormones, proteins, prions, drugs, enzymes, carbohydrates, biotins, lectins, bacteria, virus and saccharides.

5. The method according to claim 4,

wherein,

the target molecule is an antibody and the receptor molecule is an antigen.

6. The method according to any one of the preceding claims,

wherein,

the receptor molecule is selected in such manner that it is smaller than the target molecule and/or that the receptor molecule binds close to one end of the target molecule, so as to create a large lever.

7. The method according to any one of the preceding claims, including the steps of:

providing equipment to perform manual or automated measurements in real time of:

(i) the dissipation factor and/or the change dissipation factor, or

(ii) the dissipation factor and/or the change in the dissipation factor, and the resonance frequency and/or the change in the resonance frequency,

by selectively driving and disconnecting the drive circuit and measuring the dissipation or the dissipation and the frequency as a function of time.

8. The method according to any one of the preceding claims,

wherein,

the piezo-electric crystal micro balance is of the QCM (Quartz Crystal Microbalance) type.

9. The method according to any one of the preceding claims,

wherein,

the piezo-electric crystal micro balance is coated with a binding surface, which may include oxide, metal oxide, semi-conductor, inorganic compound, organic compound, lipid layer, polymer, avidine or strepavidine, biotin, protein and self organising layers.

10. The method according to any one of the preceding claims,

wherein,

the measurements are performed in gas, vacuum or liquid phase.

11. The method according to any one of the preceding claims,

wherein,

the measurements are performed at basic tone frequency as well as at a harmonic frequency and/or different oscillation amplitudes.

12. The method according to claim 7,

wherein,

the equipment performs automated and manual measurements.

13. The method according to any one of the preceding claims,

wherein,

the measurements are performed with different kinds of piezo-electric crystals and different crystallographic cuts.

14. The method according to any one of the preceding claims,

wherein,

the measurements of the resonance frequency and the dissipation of the piezo-electric crystal are performed as a function of time.

15. The method according to any one of the preceding claims,

wherein,

the measurement is used to obtain a measure on the interaction of viscous and/or viscoelastic molecules, and/or their influence on the binding surface.

16. The method according to any one of the preceding claims,

wherein,

the amount of a certain target molecule, such as antibodies, antibody fragment, antigens, haptenes, nucleic acids, synthetic nucleic acids, cells, bacteria, receptors, hormones, proteins, prions, drugs, enzymes, carbohydrates, biotins, lectins, virus, metabolite (-s), vitamins and saccharides also consisting of other components, is determined, by equipping the surface with a receptor molecule.

17. The method according to any one of the preceding claims,

wherein,

a sample flow is brought into contact with a detector, so that the amount of target molecule is determined as a function of time.

18. The method according to any one of the preceding claims,

wherein,

the amount of nucleic acids, containing a certain sequence, is detected and/or determined in a sample, using a receptor molecule, which is a single-stranded nucleic acid having a sequence complementary to the molecule to be detected.

19. The method according to any one of the preceding claims,

wherein,

point mutations are detected in a nucleic acid, by bringing a sample in simultaneous contact with two or more crystal microbalance surfaces equipped with receptor nucleic acid-strands, which are complementary to the mutated and the normal sequence respectively, and the difference of their response is determined.

20. A method to measure the interaction between at least one target molecule and at least one receptor substantially as herein described with reference to any one of the embodiments of the invention illustrated in the accompanying drawings and/or examples.

21. A method to measure the interaction between at least one target molecule and at least one receptor molecule using a piezo-electric crystal microbalance having a crystal, the method including:

immobilising at least one receptor molecule to the crystal;

providing a driving circuit that is switched on and off such that, when switched on, the circuit brings and maintains the crystal in oscillation;

presenting a target molecule to the immobilised receptor molecule and permitting a reaction between the target molecule and the immobilised receptor molecule;

measuring the resonance frequency (f) and the change in resonance frequency (Δf) of the microbalance when the driving circuit is switched on and/or off; and

measuring the dissipation factor (D) of the microbalance and/or the change in dissipation factor (ΔD) when the driving circuit is switched on and/or off, whereby the resonance frequency and the dissipation factor provide measurements indicative of the reaction.

22. A method according to claim 21 including more than one balance driven by at least one driving circuit, wherein the at least one driving circuits selectively bring the respective crystals into oscillation.

DATED this 3rd Day of July, 2002.

Q-SENSE

AUSTRALIAN PATENT OFFICE

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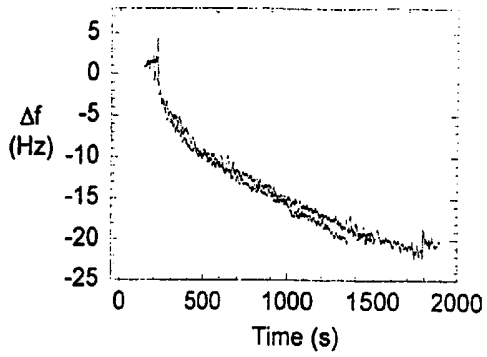
5
0
-5
-10
-15
-20
-25
Δf
(Hz)
0
500
1000
1500
2000
Time (s)

Fig. 1

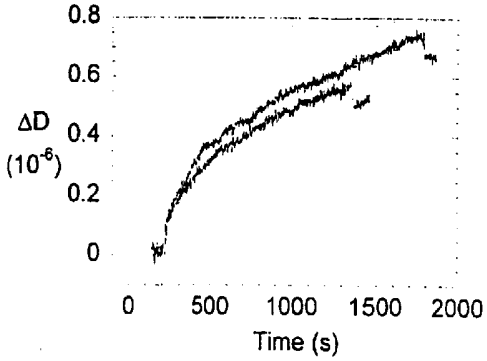
0.8
0.6
0.4
0.2
0
ΔD
(10-6)
0
500
1000
1500
2000
Time (s)

Fig. 2

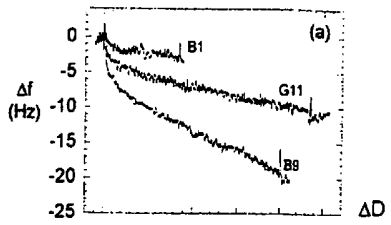

Fig. 3

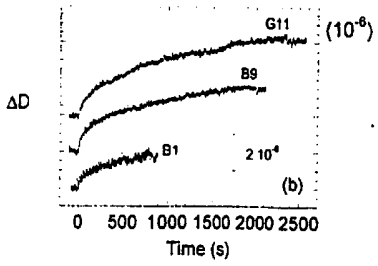

Fig. 4

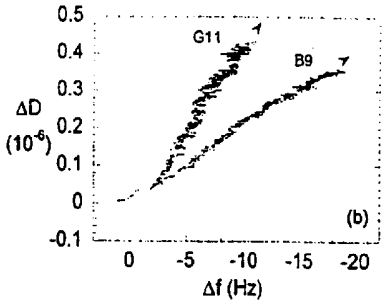

Fig. 5