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Application of a Quartz Crystal Microbalance (QCM) Twin Sensor for Selective Label-free Immunoassay to Simultaneous Antigen-antibody Reactions

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Abstract: In this work a 30 MHz quartz crystal microbalance (QCM) twin sensor system was used for simultaneous monitoring of antigen-antibody reactions. The twin sensor system provided a significant improvement of the conventional mass-sensitive devices, which allows to undertake simultaneous control and analyte measurements under the same experimental conditions. In the given system one channel of the QCM electrode, fixed in a thermostatic flow cell, was modified with chrome pure human IgG (H-IgG) while the second with chrome pure chicken IgY. The antigen-antibody reaction was measured by admitting affinity pure anti-human IgG (Anti-H-IgG) and affinity pure anti-chicken IgG (Anti-C-IgY) into the flow cell and recording the frequency change caused by the selective adsorption of the complementary antigen on the particular antibody immobilized on the surface of the QCM electrode. The system showed high sensitivity with a limit of detection (*LOD*) of 62.1 ng/mL and fast response time within 5 min. *Copyright © 2012 IFSA.*

Keywords: Mass sensitive twin sensor, Quartz crystal microbalance, Immunoassay, antigen-antibody reaction, Label-free.

1. Introduction

The piezoelectric phenomenon – production of electrical charge at the mechanical stress or mechanical deformation at applied electric field in some asymmetric crystals was discovered by Pierre and Jacques

Curie in 1880. The first practical application of this phenomenon was realized in the sonar and since then has been employed extensively in a daily life in electric circuits, mobile phones, watches and etc.

The piezoelectric phenomenon was implemented in sensoric mainly via mass-sensitive devices that include quartz crystal microbalance (QCM) and surface acoustic wave (SAW) sensors. The basic principle of QCM sensors is measurement of the frequency shift as a result of the mass adsorbed on the QCM resonator. The amount of the analyte adsorbed on the QCM sensor can be determined using the Sauerbrey equation (1) [1]:

$$\delta m = -\frac{S \cdot \sqrt{\rho \cdot \mu}}{2F^2} \cdot -(\delta F), \tag{1}$$

where δm is the mass adsorbed on the active electrode surface, S is the surface area of the QCM electrode, ρ is the density of the quartz, and μ is the quartz shear module.

According to this equation the sensitivity to the mass change of the QCM sensor can be increased with the increase of the fundamental oscillation frequency of the piezoelectric crystal. However, practical implementation of this approach is limited by physical dimensions of the piezoelectric crystal and due to the noise increase with frequency increase. Generally, 9 MHz QCM electrodes are widely applied to sensors since they provide high stability, sufficient sensitivity for monitoring mass changes in the nano-gram range with a satisfactory signal-to-noise ratio and robustness of the electrode. Another challenging application of QCM sensors is direct measurements in the liquid phase, where not only loaded mass but also changes in viscoelastic properties of media should be accounted [2].

In the matter of fact, QCM electrodes do not possess any intrinsic selectivity to chemical species; however, when the electrode surface is properly modified they can be applied to various situations that need sensitive, inexpensive and convenient sensors [3-7]. Efforts over the last decades have resulted in the synthesis of huge variety of synthetic molecular receptors, such as crown ethers, cryptands, spherands, cavitands, porphyrins, calixarenes and cyclodextrins, and these substances can be used for the modification of the QCM surface to provide a variety of interactions (electrostatic interaction, hydrogen bonding, van der Waals force, and donor-acceptor binding), thus being able to bind cationic, anionic or neutral molecules in a powerful and selective manner [8].

In this work we introduce a 30 MHz twin electrode modified with two types of antibodies, which was used for simultaneous, on-line measurements of the selective antigen adsorption in the liquid phase. NAPICOS analyzer system (NDK Co, Ltd.) used in the current work was designed by Nihon Dempa Kogyo Co., LTD with the increased stability of the oscillating frequency in the liquid phase with the low noise, high sensitivity and accuracy of 10 mHz that to the best of our knowledge has been achieved for the first time. Additionally, due to the employment of two channels, twin sensor, can be self-calibrated to the external condition changes by simultaneous measurements of the control and data signals [9, 10]. The antigen-antibody reaction has been used to demonstrate capabilities of the twin sensor system. The QCM sensor was fixed in the thermostatic flow cell, one channel was modified with a chrome pure human IgG (H-IgG) while the second channel was modified with a chrome pure chicken IgY (C-IgY). After that, the correspondent antigen solutions of different concentrations were used to check the sensor response on each channel.

Fast and reliable detection of the antigen-antibody reaction kinetics is highly desired along with high sensitivity in biology and medicine because it can be broadly used for prompt disease diagnosis. Antibody is an immune response of the living organism to the presence of the foreign substance that can be called antigen. Consequently, antigen and antibody can be used as markers of various diseases and the design of reliable, sensitive, inexpensive measurement systems is highly important. The quantitative

detection of the antigen-antibody reaction has been mainly conducted using surface plasmon resonance (SPR) and various label-free immunoassays (metal nano-particles or electrochemical probes) with high sensitivity [11-15]. On the other hand, with the use of labels the detection can be down to the sub pg/ml level [16, 17]. In spite of the high sensitivity, the main disadvantage of the SPR and other biosensors using labels such as enzyme-linked immunosorbent assay (ELISA) is time-consuming for sample preparation and labelling procedures. Therefore, the developed QCM twin sensor system can be used as an alternative analytical tool for the antigen-antibody reaction detection.

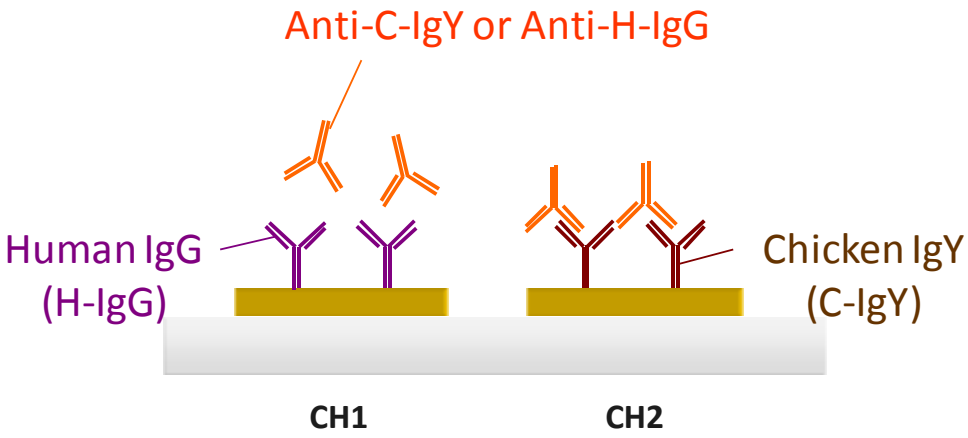

Fig. 1. Schematic illustration of the sensing principle of the QCM twin sensor modified with H-IgG and C-IgY on CH1 and CH2, respectively.

2. Experimental

2.1. Materials

Chrome pure human IgG (H-IgG), chrome pure chicken IgY (C-IgY), affinity pure rabbit anti-human IgG (Anti-H-IgG) and affinity pure rabbit anti chicken IgY (Anti-C-IgY) were purchased from Jackson Immuno Research, West Grove, PA. Phosphate buffer solution (PBS, 10 mM) was obtained from Nippon Gene Co., LTD. 30 MHz QCM twin electrodes were received from Nihon Dempa Kogyo Co., LTD. Chemicals were reagents of analytical grade, and used without further purification. Deionized pure water (18.3 MΩ·cm) was obtained by reverse osmosis followed by ion exchange and filtration (Millipore, Direct-QTM).

2.2. Modification of the QCM Twin Channels

Two types of antibodies were deposited on the gold surface of each channel (Fig. 2a): channels 1 and 2 (CH1 and CH2) were modified with the H-IgG and C-IgY, respectively, as shown in Fig. 2. Prior to deposition of the antibodies the gold surface of both channels was washed using a piranha solution (H_2SO_4:H_2O_2 = 3:1). The deposition of the antibodies was carried out using a casting method: 100 μL of 100 μg/mL of the corresponding antibody solution was placed on the top of the gold surface of each channel for 5 min, as shown in Fig. 2. A specially designed teflon holder was employed and two channels were sealed and separated from each other using a silicon cover that has two modification rooms to prevent leak of the solutions, Fig. 2 b. The adsorption of the antibody to the gold surface of the electrode occurred via hydrophobic interaction.

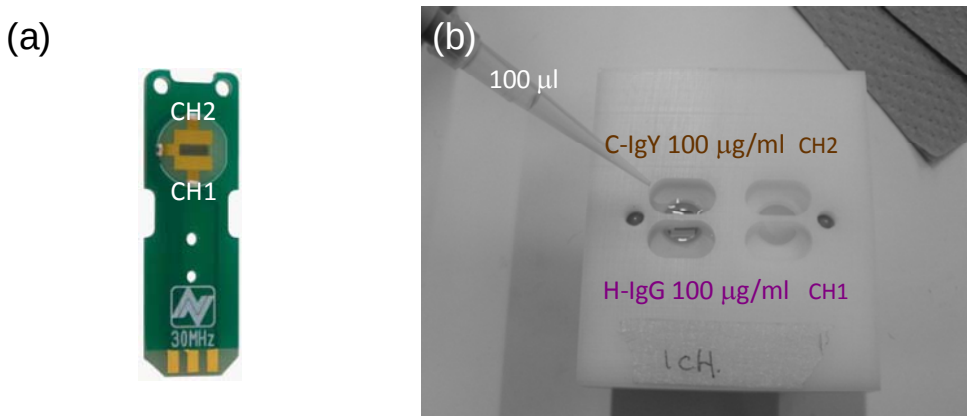

Fig. 2. Photographs of (a) a 30 MHz QCM twin electrode used in this study, and (b) a teflon holder with two modification rooms.

After 5 min the antibody solutions were removed from the electrode surface using a suction pump followed by washing using 200 µl of PBS (10 mM, pH 7.2). The washing procedure was performed for two times followed by drying the surface of the twin electrode with nitrogen gas. The modified electrode was fixed in a thermostatic chamber for antigen-antibody reaction measurements.

2.3. Measurement Procedure

The adsorption response of the antibodies or antigens was investigated using a Napicos QCM system (Nihon Demba Kogyo Co., LTD), as shown in Fig. 3. The system consists of syringe pump (Harvard apparatus Co., LTD), and injector (Upchurch Scientific), thermostatic chamber (PSA-CA-3002, Nihon Demba Kogyo Co., LTD) and frequency counter designed for the 30 MHz QCM electrode connected to the computer via USB interface.

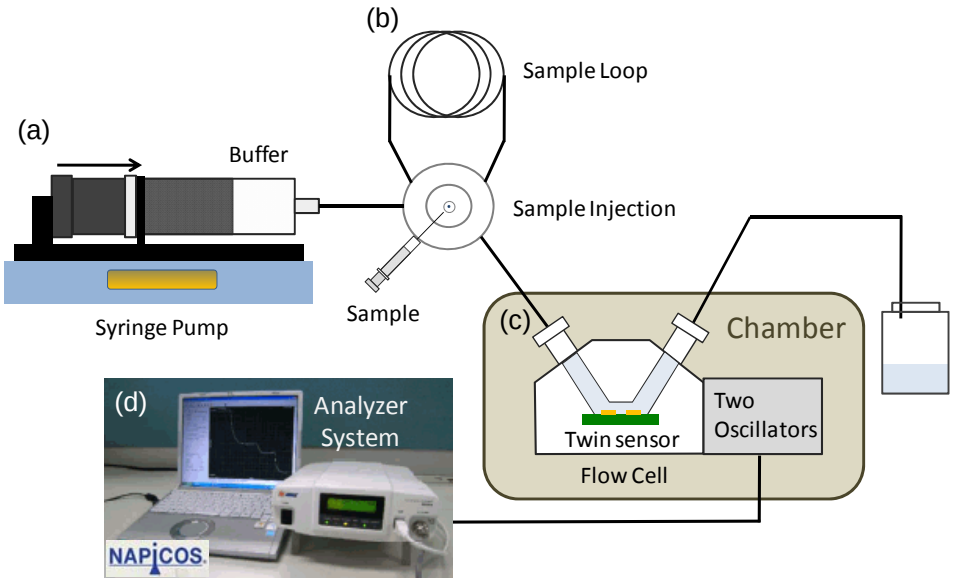

Fig. 3. Measurement set-up used to measure antigen-antibody reactions: (a) Flow system used to deliver solution into the measurement chamber consisting of syringe pump and injector, (b) detailed view of the sample loop and injection port, (c) schematic view of the measurement chamber including a QCM twin electrode in the flow cell, and (d) data acquisition system consisting of a frequency counter connected to the personal computer used for data processing.

Two antigens (Anti-H-IgG and Anti-C-IgY) were adjusted with PBS to give the concentrations of 0.1, 1, 10, and 100 μg/ml and admitted into the flow cell which was placed in the contact with the antibodies modified twin sensor. In the beginning the baseline was recorded using PBS admitted into the flow cell with a flow rate of 150 μl/min, until the stable baseline (change of 10 Hz per min of recording) was achieved. Then, the respective antigen was injected into the flow cell at a flow rate of 50 μl/min. Through series of experiments the 50 μl/min flow rate was found to be optimal, providing the lowest noise. In addition this flow rate gives enough time to complete the binding between the antigens and antibodies.

The amount of the adsorbed antibodies during the modification of the QCM sensor surface and antigens during the binding experiments was calculated using the Sauerbrey Eq. 1. Using the parameters of the system such as surface area 0.088 cm^2, density of the quartz 2.65 g/cm^3, and shear module $2.95 \times 10^{11} g/cm \cdot sec^2$, 1 Hz in the given system corresponds to the mass change of ca. 40 pg adsorbed on the QCM sensor.

2.4. Surface Morphology

The surface morphology of the QCM electrode before and after adsorption of the antigens and antibodies was studied with a JEOL JSPM-5200 atomic force microscope (AFM) working in non-contact mode using a MicroMash NSC12/Ti-Pt/15 silicon cantilever (curvature tip radius <40 nm, tip length 15–20 μm). Additionally, the particle size distribution of all antigens and antibodies in the phosphate buffer solution were determined using dynamic light scattering (DLS) equipment (Zetasizer Nano-ZS, Sysmex) at 25° C.

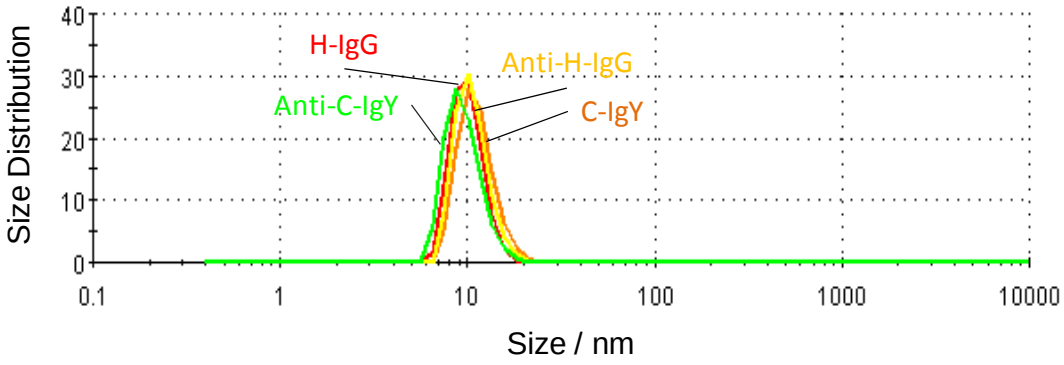

Fig. 4. Hydrodynamic diameters of the antigens and antibodies: all compounds (each 100 μg/ml) were dissolved in the phosphate buffer.

3. Results and Discussion

When the channels CH1 and CH2 were immobilized with H-IgG and C-IgY, the QCM frequency change in each channel was 1087±187 and 1286±223 Hz, respectively. Using Eq. 1, the amount of the adsorbed antibodies on CH1 and CH2 is calculated to be 43.5±7.5 and 51.4±8.9 ng, respectively (data not shown). As revealed from DLS measurements (Fig. 4), the hydrodynamic diameter of the IgG and IgY antibodies is ca. 10 nm and 11.3 nm, respectively. These values are approximately in accordance with the literature data [18]. Based on the DLS data, we can calculate the surface coverage of both antibodies. When the antibodies are assumed to be packed on the QCM surface (0.088 cm^2), maximum occupied density of each antibody is estimated to be 0.0127 and 0.0099 molecules/nm^2 for H-IgG (78.5 nm^2) and C-IgY

(100 nm^2), respectively. On the other hand, the practical density calculated from the QCM frequency shifts are 0.0027 and 0.0028 molecule/nm^2 for H-IgG and C-IgY, respectively, where molecular weights of both antibodies are 147,680 for H-IgG (composed of 66% IgG1, 23% IgG2, 7% IgG3, and 4% IgG4) [19] and 168,000 for C-IgY [20]. From these results, the surface coverage of 21.3% and 28.3% on the electrode surface can be estimated for H-IgG and C-IgY, respectively. These results indicate that both antibodies can be similarly immobilized on the QCM electrode.

Fig. 5 compares AFM images of the QCM electrode surface before and after antibody modification. The surface roughness decreased when the antibodies were assembled on the QCM electrode. The roughness of the non-modified electrode surface was 4.11 nm, while after modification with H-IgG and C-IgY the surface roughness decreased to 0.98 and 1.52 nm, respectively, indicating that the relatively irregular QCM gold surface was uniformly covered with the antibodies.

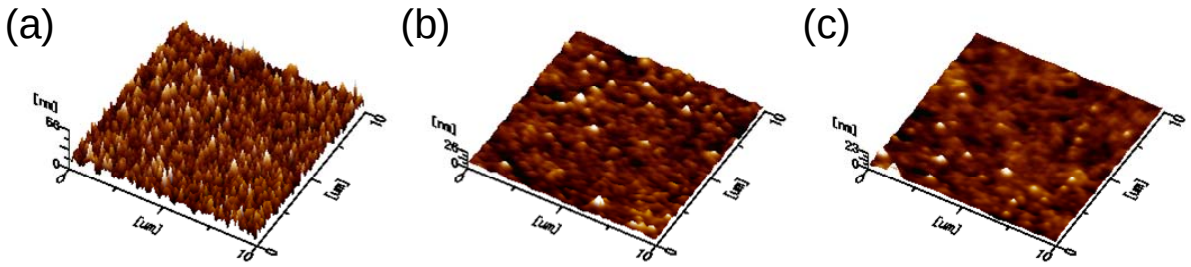

Fig. 5. Atomic force microscopic images of the QCM electrode surface: (a) bare gold electrode before deposition, (b) after C-IgY deposition, and (c) after H-IgG deposition.

Fig. 6 shows dynamic sensor responses of CH1 and CH2 when 100 μg/ml of Anti-C-IgY (Fig. 6a) or Anti-H-IgG (Fig. 6b) were introduced into the flow cell. The frequency decrease of the CH2 modified with C-IgY was 13 times larger than that of the CH1 modified with H-IgG when Anti-C-IgY was admitted, revealing higher specificity of the C-IgY modified QCM channel to Anti-C-IgY (Fig. 6a). Similar effect was obtained when Anti-H-IgG was delivered into the flow cell: the change of the CH1 modified with H-IgG was 6.3 times larger than that recorded for the CH2 modified with C-IgY (Fig. 6b). The amount of the bound Anti-H-IgG and Anti-C-IgY antigens on the CH1 and CH2 was estimated to be 85.5 and 81.2 ng, respectively.

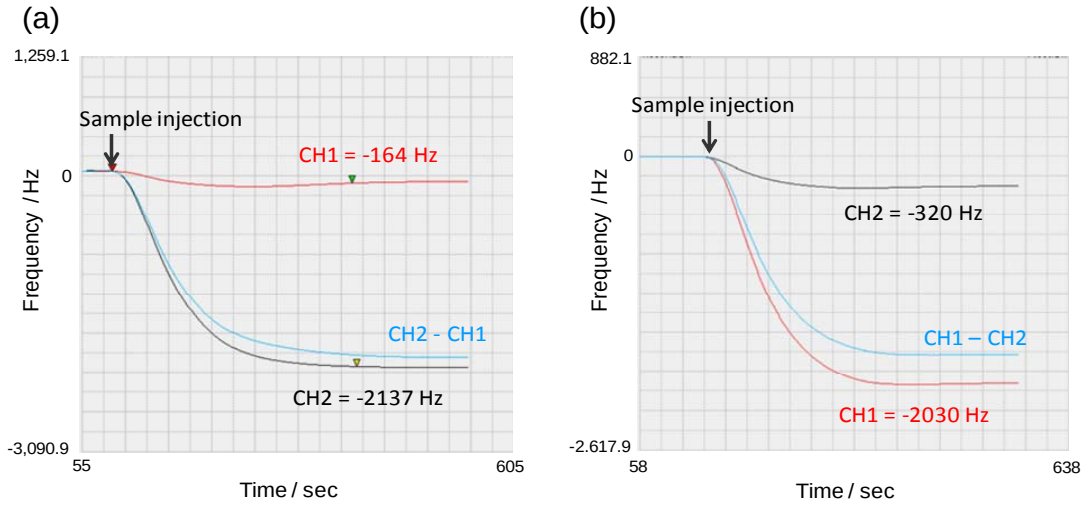

Fig. 6. Dynamic sensor responses at the delivery of the 100 μg/ml antigen solutions: (a) Anti-C-IgY, and (b) Anti-H-IgG.

The saturation time for both antigen-antibody reactions was achieved within approximately 5 min. An additional advantage that the QCM twin sensor system provides is the ability to conduct simultaneous measurements under the same experimental conditions. Highly specific recognition for each analyte is confirmed from the difference between the frequency shifts of both channels (blue lines in Figs. 6a and 6b). The size of the antigen and antibody molecules is estimated to be about 10 nm as revealed from the DLS measurements (Fig. 4). However, since the molecular weights of the antigens are unknown, it is impossible to calculate their coverage on the electrode from the QCM frequency shifts, as conducted for the antibodies. Anyway, the QCM twin sensor enables a specific antigen-antibody reaction on only one channel among both channels. Consequently, the difference between the signals obtained on both channels becomes a real response for a given immune reaction.

To calculate the limit of detection (LOD) of both antigen-antibody reactions, concentration dependence tests for the antigens were conducted (Fig. 7). LOD was defined according to LOD = 3σ, where σ is the standard deviation (noise value of the baseline measured for CH1 and CH2 at flow of the PBS solution) [21]. The antigen concentrations used for this purpose were 0.01, 0.1, 1, 10 and 100 μg/ml. The response time for all concentrations reached saturation within 5 min, while the specificity (ratio of the amount of bound antigens to a specific antibody channel over a non-specific antibody channel) decreased to 2.1 when 10 ng/ml Anti-C-IgY was admitted. Using the concentration dependence (Fig. 7d) and noise values of the baseline measured for twin QCM at flow of the PBS solution (the average signal noise: 3.81 Hz/5 min) the limit of detection of the Anti-C-IgY antigen was estimated to be 62.1 ng/ml. On the other hand, the QCM frequency change for Anti-H–IgG (1 μg/ml) was 13.4 and 5.1 Hz on the CH1 (H-IgG modified) and CH2 (C-IgY modified) channels, respectively. The amount of the antigen recognition in the case of Anti-H-IgG is relatively small compared to the case of Anti-C-IgY. The LOD of the Anti-H-IgG binding is estimated to be 1.31 μg/ml.

5. Conclusions

In this work a new sensor system based on the QCM twin sensor was demonstrated for measuring simultaneous antigen-antibody reactions. The limit of detection of the current sensor system is slightly lower as compared with that of other immunoassay techniques; however, it is believed that this problem can be improved further if the deposition of the antibodies on the electrode surface can be optimized. In addition, the QCM twin sensor enabled quick analysis within 5 min and label-free detection. The main advantage of the proposed system is that control experiments can be undertaken simultaneously with the target analyte measurement under the same conditions providing reliable information about the analyte binding. The developed QCM sensor system offers fast and reliable information on reaction kinetics in real time course. Based on the obtained results we believe that the twin sensor system would be applied to immunological disease diagnostics and discovery of new biomarkers.

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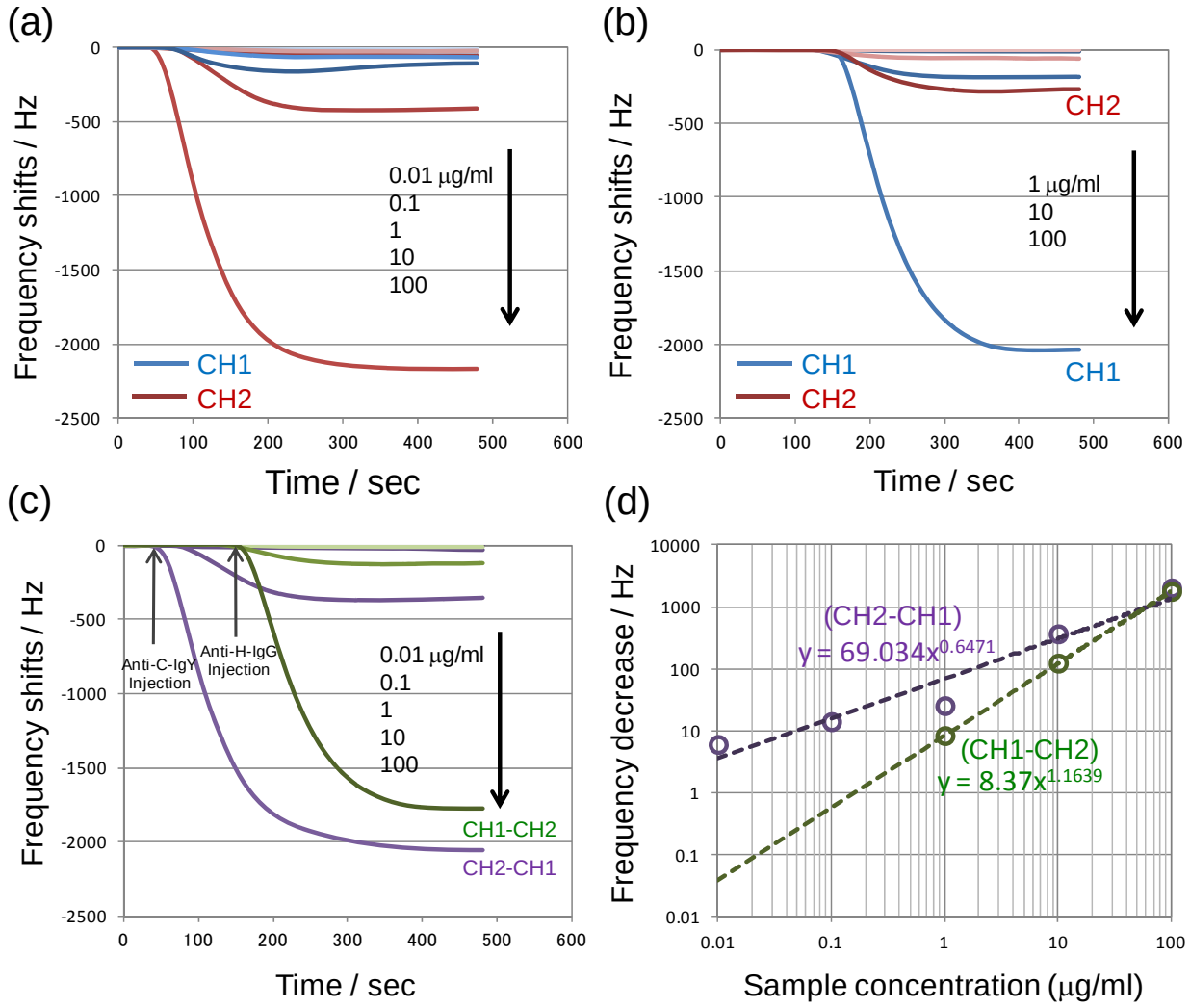

Fig. 7. Dynamic sensor responses on CH1 and CH2 channels: (a) Anti-C-IgY, and (b) Anti-H-IgG; (c) Real sensor responses obtained by subtracting the frequency shifts of both channels (CH1-CH2 and CH2-CH1) measured at different antigen concentrations; (d) Calibration curves obtained using the data measured after 360 sec from antigen injection.

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