

## Evaluation of specific binding behavior between proteins using higher-order-mode frequencies measured by RAMNE-Q biosensors

ラムネ Q バイオセンサーによる高次モード周波数応答を用いた蛋白質間特異結合性の評価

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### 1. Introduction

Recently, various biosensors have been developed not only for diagnostics but also for drug discovery. The quartz crystal microbalance (QCM) biosensor is a mass-sensitive biosensor. It detects adsorbed mass on the quartz surface as the resonance-frequency shift of the quartz resonator<sup>(1)</sup>. Therefore, the QCM biosensor can achieve a real-time monitoring of binding reactions of biomolecules. Moreover, it is a label-free biosensor, which allows to evaluate intrinsic interactions among biomolecules and to make the assays more simplified. Thus, the QCM biosensor is a powerful tool for studying interactions among biomolecules.

Viscoelastic properties of protein layers are linked to binding conditions and structure of protein layers. They are thus important in detecting biomarkers and evaluating capability of biosensors. The QCM biosensors are also used for evaluation of viscoelastic properties of protein layers by measuring dissipation along with the frequency, known as the QCM-D technique<sup>(2-3)</sup>.

The conventional QCM biosensors show lower sensitivity than others that use labels. That issue is due to heavy electrodes attached on the quartz surfaces. They have to use Au electrodes to apply effective electric field and immobilize receptor proteins. However, it has been proven that increase of inertia resistance due to those electrodes cause significant decrease in the mass sensitivity<sup>(4)</sup>. The QCM-D biosensor also has problems about low sensitivity due to low frequencies and low accuracy for measuring dissipation. These issues make it difficult to evaluate viscoelastic properties of low molecular weight molecules. A more sophisticated way for evaluating the viscoelastic properties will be using only frequency responses, excluding the unreliable dissipation, but this requires extremely high frequency measurements.

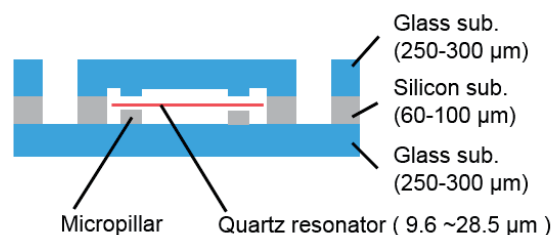


Fig. 1 Schematic of the RAMNE-Q sensor chip.

We then developed a resonance acoustic microbalance with naked-embedded quartz (RAMNE-Q) using the micro-electro-mechanical systems (MEMS) technology. **Fig. 1** shows a schematic of the RAMNE-Q sensor chip. Both and entire surfaces of the naked quartz resonator can be used for biosensing, and the quartz resonator are supported without any fixed parts, achieving higher Q factors<sup>(6-8)</sup>.

In this study, we propose a methodology to evaluate viscoelastic properties of protein layers using higher-order-mode frequencies measured by RAMNE-Q biosensors and demonstrate effectivity of RAMNE-Q biosensors for evaluation of specificity of binding between proteins.

### 2. Experimental

**Fig. 2** shows the schematic of the laboratory-built measurement system. The RAMNE-Q chip is installed in a handmade sensor cell. The sensor cell has two plate antennas to operate the quartz resonator wirelessly with a network analyzer.

In this study, we measured frequency response of the fundamental resonant frequency ( $\sim 58$  MHz) and three (3<sup>rd</sup>, 5<sup>th</sup>, and 9<sup>th</sup>) overtones.

To determine viscoelastic properties of protein layers inversely from the frequency responses, we used a three layers model analysis as illustrated in **Fig. 3**. We used the Voight model<sup>(9)</sup> for the three layers, consisting of the quartz-oscillator layer, the viscoelastic protein layer, and the viscous solution layer. Properties of

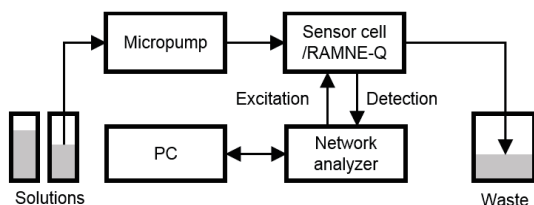


Fig. 2 Schematic of measurement system.

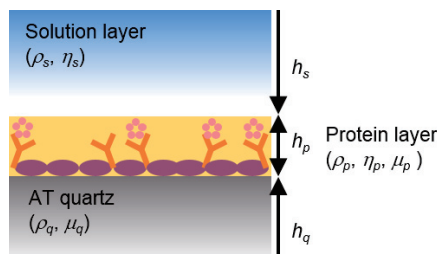


Fig. 3 A three layer model composed of quartz, viscoelastic protein layer, and viscous solution layer.  $\rho$ ,  $\eta$ ,  $\mu$  and  $h$  denote the mass density, viscosity, shear modulus, and thickness, respectively. Subscripts q, p, s denote the quartz layer, protein layer, solution layer respectively.

quartz and solutions are known. Thus, frequency shifts depend on the viscoelasticity of the protein layer. We then developed the inverse-calculation method to determine these properties.

### 3. Results and Discussions

**Fig. 4** compares frequency responses calculated the three layer model for two QCMs with different fundamental resonant frequencies ( $f_1$ ). In the QCM with  $f_1 = 58$  MHz, larger frequency shifts occur with the change in the viscosity, while they are hardly affected in the QCM with  $f_1 = 5$  MHz. Moreover, the frequency response of higher  $f_1$  shows significant change when the viscosity slightly changes. These results demonstrate that a high-frequency QCM shows high sensitivity not only to absorbed mass but also to viscoelastic properties of protein layers. Then we applied these analysis in experimental results: nonspecific immobilization and specific binding of proteins. Results of inverse calculation show that specific binding showed lower viscosity than nonspecific immobilization.

### 4. Conclusion

The RAMNE-Q biosensor is an ultra-high frequency QCM with MEMS process. The calculation results of the three layer analysis demonstrate that high frequency QCM has the high sensitivity to viscoelastic properties of protein layers. These results indicates the capability of RAMNE-Q biosensors as a powerful tool of evaluating specificity of the binding among proteins.

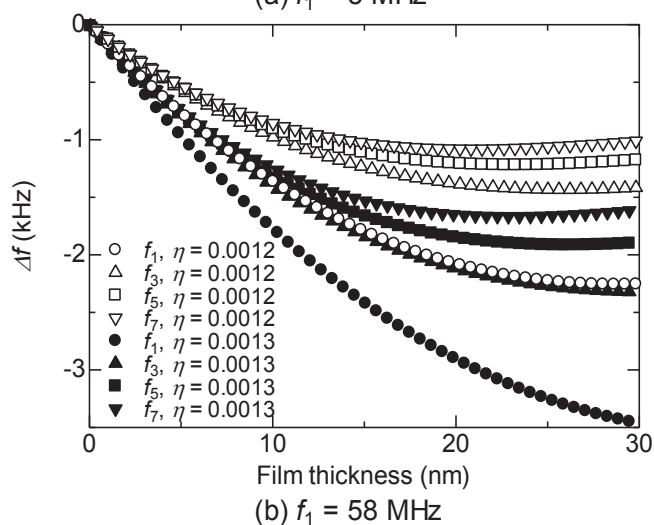
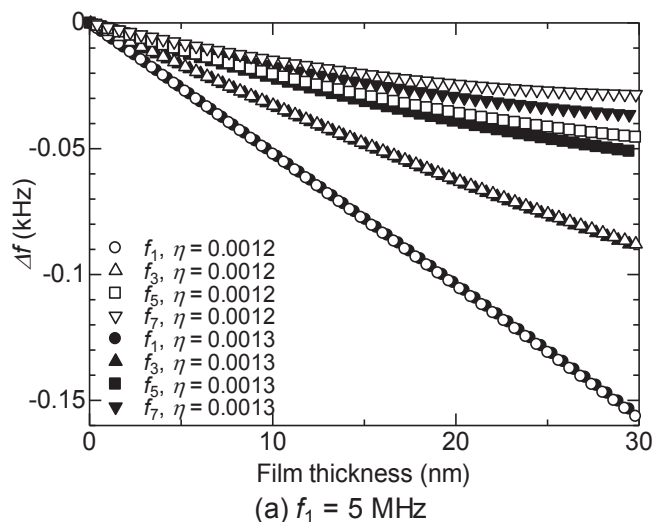


Fig. 4 Calculated frequency responses.  $\eta$  denotes the viscosity of protein layers.

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