

## Monitoring of Deposition Reaction of A $\beta$ Peptides on Heterogenous Nuclei by TIRF-QCM

全反射蛍光顕微鏡 QCM による異種核への A $\beta$ ペプチド凝集過程のモニタリング

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### 1. Introduction

Alzheimer's disease (AD), a principal cause of dementia, is deeply associated with assembly of amyloidogenic peptides, as well as Parkinson's disease and Prion disease. Amyloid  $\beta$  (A $\beta$ ) peptides accumulate in the human brain, leading to these depositions for AD. However, little is known about the specific mechanism of the A $\beta$  assembly state. Lack of an appropriate method for monitoring the aggregate formation is one of the factors making the mechanism uncovered. Here, we has developed the wireless-electrodeless quartz crystal microbalance (WE-QCM) combined with total internal reflection fluorescence (TIRF) microscopy as a possible tool for making the aggregation mechanism of A $\beta$  peptides clear.

The QCM biosensor is a mass-sensitive biosensor. When the receptor immobilized on quartz-crystal resonator captures the receptor-specific molecules, the effective mass of the resonator increases, which yields a decrease in the resonance frequency of the resonator. Hence, we can measure interactions between biomolecules quantitatively in real time. However, conventional QCM biosensor has a lower sensitivity than that of other biosensors using labels such as the ELISA method. To measure interactions between biomolecules precisely, a higher-sensitive QCM is necessary. Here, to accomplish this purpose, we has developed WE-QCM, by removing thick gold electrodes and wires attached to generate and detect oscillation of quartz crystal oscillator on the surface of the oscillator in such a way as to make the oscillator thinner[1].

Furthermore, we propose the flow-injection WE-QCM combined with total internal reflection fluorescence (TIRF) microscopy as a new research device for life science studies. TIRF is a microscopy method with high spatial resolution due to the evanescent-light field generated by the total internal reflection of the excitation light on the cover glass surface only for the fluorescence

excitation source. Not the conventional QCM but only the WE-QCM (without electrodes) allows its combination with the TIRF microscopy.

By performing the TIRF-QCM measurement, we can evaluate the location and quantity of interacted biomolecules simultaneously. In this study, we originally develop a flow-injection TIRF-QCM system and examine the aggregation reaction of A $\beta_{1-40}$  peptide on the heterogeneous nuclei of A $\beta_{1-42}$  peptide, which is suggested to be a candidate mechanism of Alzheimer's disease (AD) [2].

### 2. TIRF-QCM

*In vivo*, the first step in the aggregation process of A $\beta$  peptide is deposition of amyloid nuclei on the surface of nerve cell. Then, A $\beta$  peptides aggregate on them to form fibrils and neurotoxic oligomers [3]. In order to duplicate this condition, A $\beta$  monomers were flowed on the surface of the quartz-crystal oscillator, on which A $\beta$  nuclei were immobilized.

**Figure 1** shows the developed TIRF-QCM sensor cell in order to flow A $\beta$  monomeric peptide solutions along the electrodeless oscillator surface. Excitation light must satisfy the total reflection condition on upper surface of the oscillator where A $\beta$  peptides aggregate. Hence, the oscillator is placed on a cover glass and held lightly by a silicon-rubber sheet, where the flow path is composed. Two wires are located on the upper side as the transmitting and receiving antennas for generation and detection of the oscillation through electromagnetic wave. This sensor cell enables us to measure aggregation process by WE-QCM and to observe the deposition progress by TIRF simultaneously.

For observing A $\beta$ -peptides aggregations with TIRF, we used Thioflavin T (ThT) and Nail Red as fluorescent molecules, ThT has been adopted for evaluating formation of protofibrils and their elongation. The benzothiol-dye ThT specifically binds to the  $\beta$ -sheets constructing

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amyloid fibrils and produces enhanced light emission. Nail Red can identify both oligomeric aggregates and amyloid fibril. Therefore, these fluorescent molecules permit the observation of a variety of amyloid aggregates.

### 3. Experimental Procedure

A naked quartz crystal oscillator was cleaned in the piranha solution (98%  $\text{H}_2\text{SO}_4$ : 33%  $\text{H}_2\text{O}_2 = 3:7$ ), and then rinsed with ultrapure water.  $\text{A}\beta_{1-42}$  peptide were dissolved in a dimethyl sulfoxide (DMSO) solution and diluted for 10  $\mu\text{M}$  by phosphate buffer solution (pH 7.4) containing 100 mM NaCl. The nuclei were formed by stirring the peptide solution at 1200 rpm. The oscillator incubated with the solution containing the nuclei for 6 h at 4  $^\circ\text{C}$  to immobilize the nuclei on the surface of oscillator in a non-specific manner.

The sensor cell was incorporated in the flow-injection system. The  $\text{A}\beta_{1-40}$  monomeric peptide solution diluted with ultrapure water was mixed with the ThT solution for the TIRF observation. The final concentration of  $\text{A}\beta_{1-40}$  peptide was 10  $\mu\text{M}$ . The flow rate of the monomer solution was 200  $\mu\text{l}/\text{min}$ .

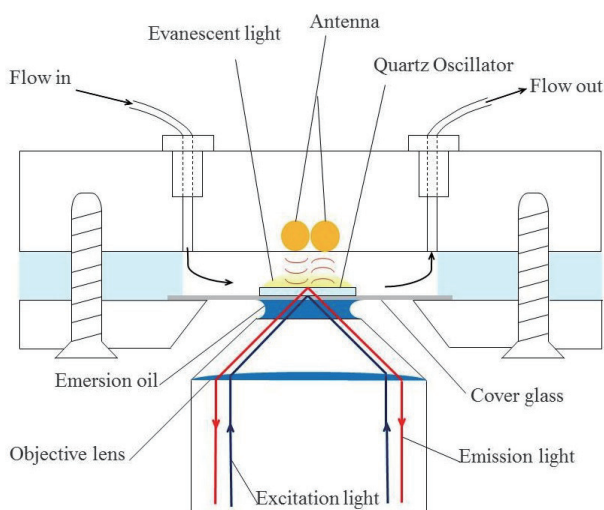


Fig. 1 Cross-section view of developed TIRF-QCM sensor cell. One antenna oscillates the quartz oscillator and the other antenna receives the oscillation during the flow of the  $\text{A}\beta$  monomer solution.

### 4. Results

**Figure 2** shows an example of the resonance frequency change, reflecting aggregation reaction, and TIRF images at representative points. The decrement of resonance frequency indicates that  $\text{A}\beta$  monomeric peptide was deposited on the nuclei. Though obvious amyloid fibril was absent in this condition, TIRF images increased

brightness with the flow time. Thus, we have succeeded in monitoring the aggregation process of  $\text{A}\beta$  peptides with WE-QCM and TIRF microscopy.

### 5. Conclusion

Flow-injection TIRF-QCM system we have developed enables us to evaluate quantitatively the aggregation of  $\text{A}\beta$  peptides and observe the fibril formation at the same time. Using this system, it was suggested that  $\text{A}\beta_{1-40}$  peptide aggregate for  $\text{A}\beta_{1-42}$  nuclei from decreasing in resonance frequency of WE-QCM and TIRF images.

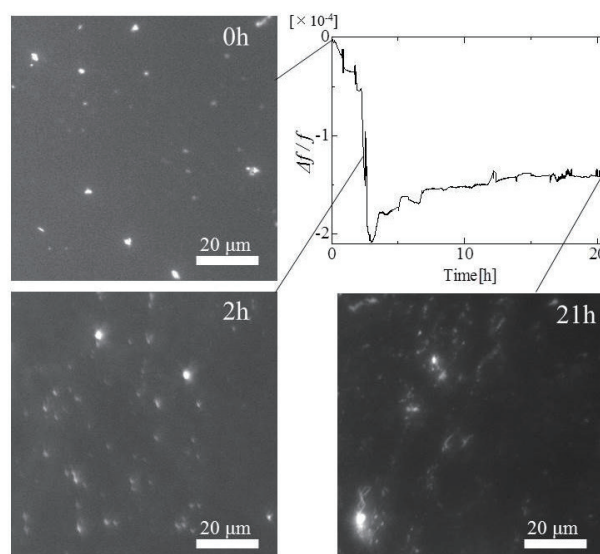


Fig. 2 Change ratio of resonance frequency from WE-QCM caused by deposition of  $\text{A}\beta_{1-40}$  monomer on  $\text{A}\beta_{1-42}$  nuclei and TIRF images from the ThT fluorescence.

### References

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