Supporting Information

Experimental Procedure

Materials

All chemicals, highest grade as far as possible, were used without further purification. Ribonuclease A, chymotrypsin, ovalbumin and BSA were purchased from Sigma (Tokyo, Japan). Catalase, trypsin, and hose radish peroxydase (HRP) were obtained from Tokyo Kasei Kogyo (Tokyo, Japan), Wako (Tokyo, Japan) and Calzyme Laboratories (San Luis Obispo, CA, USA), respectively.

Activation of a Gold Surface of QCM and Protein Immobilization on QCM.

A gold surface of a quartz crystal microbalance (QCM) was cleaned with 1 % SDS aqueous solution and washed with milli-Q water. Then the Au surface was treated with piranha solution (H_2SO_4 : H_2O_2 = 1 : 3) for five minutes. This piranha treatment was repeated three times. The cleaned QCM tip was soaked in the 4 mM solution of 3,3'-dithiopropionic acid for 1 hour and washed with milli-Q water. Dithiopropionic acids were activated with aqueous solution of 100 mM *N*-hydroxysuccinimide and 100 mM 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide for 30 minutes. This activated QCM chip was set on the acryl-made flow cell described in the text.

The flow cell was set in a temperature controlled (25.0 °C) aluminum block. HEPES buffer (20 mM, 200 mM NaCl, pH = 7.4) was used as a running buffer with a diaphragm pomp (a flow rate: 85 μl/min). After stabilizing the frequency of the QCM and reflectivity of the spectrometer (USB-4000; Ocean Optics, Dunedin, FL, USA), 4.2 mM protein funning buffer solution was injected into the flow cell by a valve switching.

After the interval time in 23 seconds, the ΔF_{water} and ΔR changing could be monitored. And at 22 minute, sample solution was switched to the running buffer again.

Calibration of ΔR with ΔF_{air}

After simultaneous measurement of ΔF_{water} and ΔR with the flow system, the QCM sensor chip, on which proteins or polystyrene beads having amino groups on the surface were immobilized, was washed with milli-Q, and then dried in a grove box with dry N_2 flow. The amount of the immobilized substances on the QCM sensor chip was measured as ΔF_{air} by a network analyzer (R3754B; Advantest, Tokyo, Japan).