Enzymatic preparation of biotinylated naturally-occurring sialylglycan and its molecular recognition on a quartz-crystal microbalance

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Supplemental Text

27 MHz QCM setup and its calibration

An *AffinixQ*[4] was used as a QCM instrument (Initium Co. Ltd, Tokyo, http://www.initium 2000.com) having four 500 µl cells equipped with a 27 MHz QCM plate (8 mm diameter of a quartz plate and an area of 4.9 mm^2 of Au electrode) at the bottom of the cell and the stirring bar with the temperature controlling system. The Sauerbrey's equation was obtained for the AT-cut shear mode QCM in the air phase,

$$\Delta F = -\frac{2F_0{}^2}{A\sqrt{\rho_q \mu_q}} \Delta m \qquad (1)$$

where ΔF is the measured frequency change [Hz], F_0 the fundamental frequency of the quartz crystal prior to a mass change [27 x 10^6 Hz], Δm the mass change [g], A the electrode area [0.049 cm^2], ρ_q the density of quartz [2.65 g cm^{-3}], and μ_q the shear modulus of quartz [2.95 x 10^{11} dyn cm^{-2}].

When the QCM is employed in an aqueous solution, eq. 1 cannot be simply applied due to interfacial liquid properties (i.e., density, viscosity, elasticity, conductivity, and dielectric constant), thin film viscoelasticity, electrode morphology, and the mechanism of acoustic coupling impact on the QCM oscillation behavior. Therefore, we calibrated our 27 MHz QCM in the aqueous solution same as reaction buffers (10 mM phosphate, pH 7.2, 150 mM NaCl, 25 °C).

A respective amount of NeutrAvidin was immobilized through an amine coupling with dithiopropionic acids on the Au electrode of the QCM (5-30 ng) or biotinylated sialylglycan (11-mer) was immobilized on the NeutrAvidin on the QCM (3-10 ng). A linear relationship was observed between the deposited amount of mass (NeutrAvidin or saccharides) and the frequency decrease of the QCM both in the air phase and in the aqueous solution. The slope showed that the frequency decrease of 1 Hz corresponded to a mass increase of 0.62 ± 0.1 ng

cm^{-2} on the QCM electrode. This means that the Sauerbrey's equation can be applicable in the aqueous solution limited to the immobilization of a globular protein such as NeutrAvidin and a short oligosaccharide such as biotinylated sialylglycan. When the long polysaccharides (1000 – 2000 mer) was immobilized on the QCM, however, the slope was different between in the air and water phases due to the hydration or viscoelastic long polysaccharides chains on the QCM.