■ Calculation of the dissociation constant of glycoprotein

The calculation of the dissociation constant of glycoprotein and lectin is explained as follows. First, asialofetuin (glycoprotein) was immobilized as ligand on a sensor. Then, concanavalin A (lectin) was injected and the reaction speed and reaction amount were measured. By using "NAPiCOS analysis (analysis software attached to NAPiCOS)", the dissociation constant was calculated.

● 1.Protocol

Sensor: 30MHz twin sensor
Flow rate:50 μL/min
Sample amount:100 μL
Running buffer:PBS
Sample:Concanavalin A
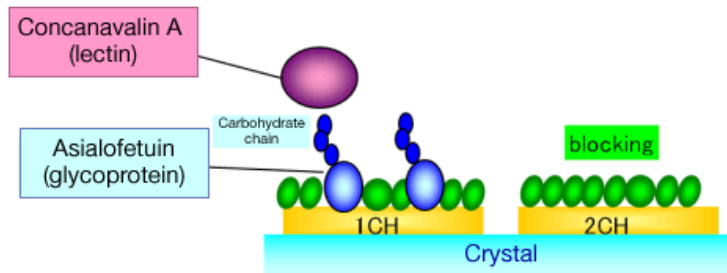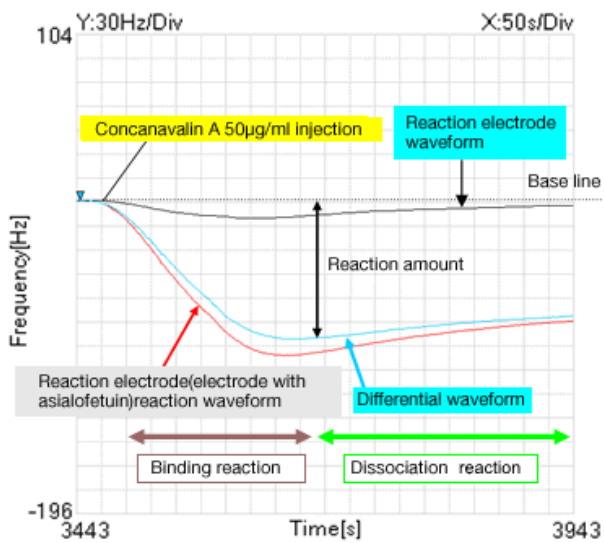

Fig.1 Diagram

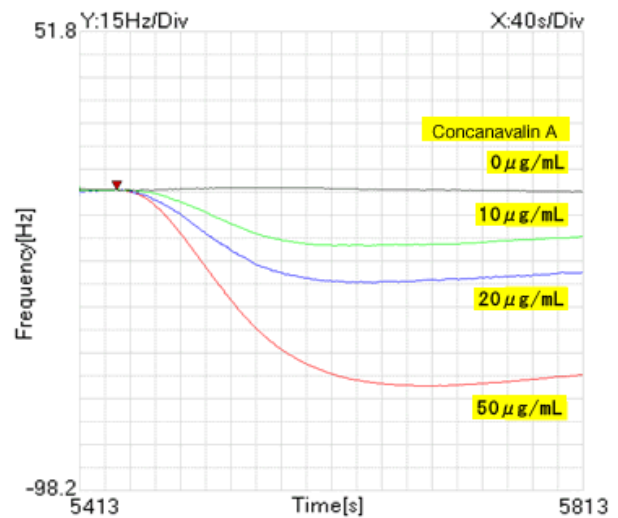### ● 2.Reaction waveform

Fig.2:Binding & dissociation waveform

Fig.3:Differential waveform after concanavalin A injection

■ Calculation of the dissociation constant of glycoprotein

● 3. Calculation of dissociation constant

By using our analysis application "NAPiCOS analysis", the dissociation constant and the rate constant for reaction were calculated based on the reaction amount of concanavalin A (concentration: 10 μg/mL, 20 μg/mL, 50 μg/mL). The table below shows the dissociation constant and rate constant for reaction calculated with 3 kinds of analysis methods. The rate constant for reaction can be calculated only when the Kinetics method is used.

Analysis	Kon $M^{-1}sec^{-1}$	Koff sec^{-1}	KD M
Affinity method	–	–	1.06E–06
Scatchard method	–	–	1.03E–06
Kinetics method	1.38E+03	1.10E–03	7.94E–07

● 4.Glossary

Asialofetuin:	A protein obtained by removing sialic acid from a glycoprotein called fetuin. The molecular weight is 40,000.
Concanavalin A:	A lectin (sugar chain-binding protein) that agglutinates red blood cells by binding to the sugar chain on the surface of red blood cells.The molecular weight is 100,000.
Dissociation constant:	The ratio between the concentration of the reacting substances and product in equilibrium. This is used as the gauge of the affinity between substances.
Rate constant for reaction:	The rate of increase or decrease of reacting substances or products.