■ Double-strand DNA formation & Protein binding

The binding reaction between single-strand DNA and complementary DNA and that between single-strand DNA and single-strand binding protein (SSB) were observed.

● 1.Protocol

Sensor:30MHz twin electrodes sensor
Flow rate:20 µL/min
Sample amount:100 µL
Running buffer:TNE (10mM Tris-HCl, pH8.0, 150mM NaCl, 1mM EDTA)
DNA:Biotinylated Oligo DNA (20mer)5 µg/mL
biotin-5'-AGGGACTTTCCTGACGTGT-3'
Complementary DNA (20mer)10 µg/mL
3'-TCCCCTGAAAGGACTGCACA-5'
SSB:2.5 µg/mL

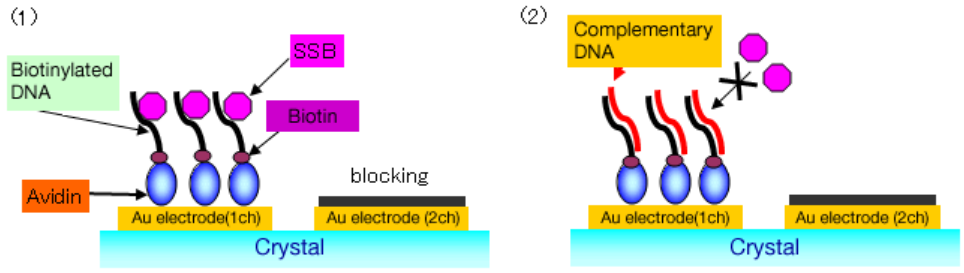

Fig.1:Diagram
(1)First, avidin was immobilized on the reaction electrode and the reference electrode was blocked. Next, biotinylated single-strand DNA was injected and immobilized on the reference electrode, and the binding of SSB was observed.

(2) After removing SSB with NaCl solution, complementary DNA was injected. Then SSB was injected again.

● 2.Reaction waveform

2-1:Biotinylated Oligo DNA was injected and immobilized on the electrode 2-1. where avidin had been immobilized. After that, SSB was injected. It was confirmed that SSB bound to the single-strand DNA.

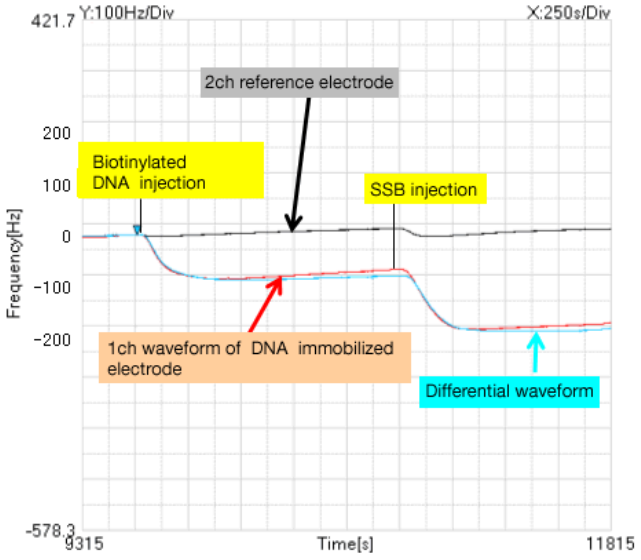

Fig.2:Biotinylated DNA immobilization & SSB biding waveform

2-2: After removing SSB with NaCl, the complementary DNA was injected, and the formation of double-strand DNA was observed. Then, SSB was injected. It was confirmed that SSB did not bind to the double-strand DNA.

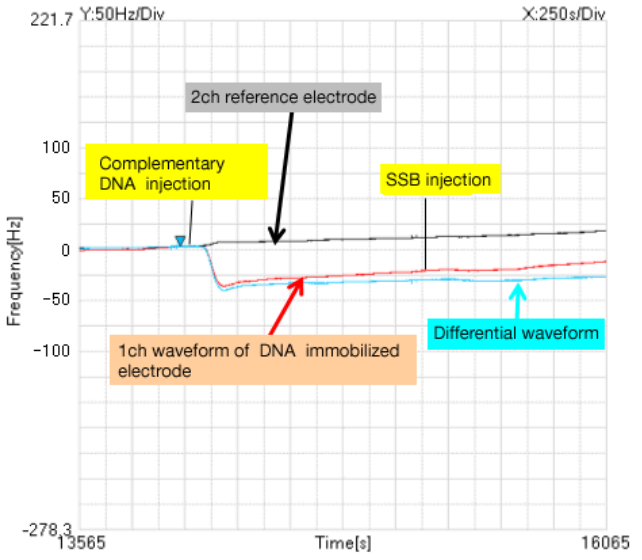

Fig.3:Double-strand DNA formation & waveform acquired after SSB injection

■ Double-strand DNA formation & Protein binding

● 3.Reference

It is possible to observe the reaction of immobilized DNA sequence (Random DNA) that does not bind to the complementary DNA.

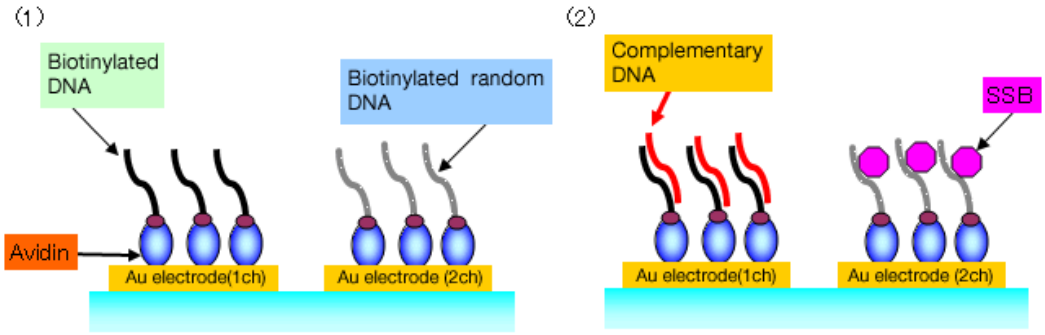

Fig.4:Diagram
(1)Avidin was immobilized on both electrodes, after which Biotinylated DNA was immobilized on each electrode.
(2)After immobilizing complementary DNA, SSB was injected.

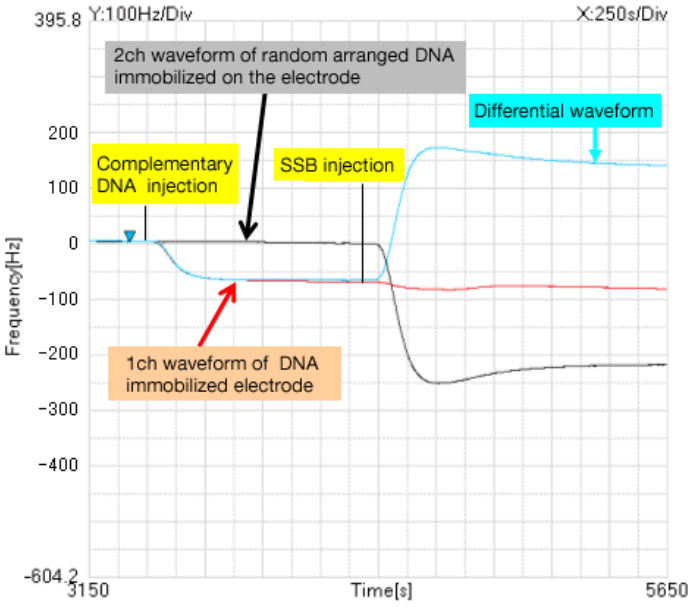

Fig.5:Reaction waveform

The complementary DNA bound to ch1 only. When SSB was injected, no reaction was observed because of the double-strand DNA formation. On the other hand, the complementary DNA did not bind to ch2 but SSB bound to it because of the single-strand DNA.

● 4.Glossary

DNA (deoxribonucleic acid):	DNA consists of base (adenine (A),guanine (G), cytosine (C) and thymine (T)), sugar and phosphoric acid. Two DNAs bind to each other inversely with the base at the center. A huge variety of genetic information is kept in the base sequence.
Complementary DNA:	The single-strand DNA on the side opposite to another single-strand DNA. Since a base binds to a specific base only (A to T and G to C), there is only one sequence for the complementary DNA.
SSB (Single strand binding protein):	Protein that specifically binds to single-strand DNA. It prevents the formation of double-strand DNA.
Oligo DNA:	Artificially created single-strand DNA
Random DNA:	DNA that has randomly arranged bases. It does not have the target DNA sequence