On-demand DNA sensor with ligand fixing method

Graduate School of Engineering, Osaka Univ.[1] National Defense Medical College (NDMC)[2]

○Huanwen Han[1], Kazuyuki Nobusawa[1], Fumie Takei[2], Ichiro Yamashita[1]

E-mail: huanwen@pmdp.arl.eng.osaka-u.ac.jp

Introduction It is well known that the DNA sequence carries the genetic information. The DNA sequencing and detection is now the central issues of clinical research and a DNA sensor chip is indispensable device. There are many sensing methods, the EIS is a highly sensitive, label free and small ions-strength dependence method. [1] The surface charge transfer resistance (Rct), calculated using the Randles equivalent circuit with Warburg corresponds to the DNA concentration. [2] For the selectivity, probe-DNA which has complimentary sequence to the target DNA is usually fixed on the sensor surface. Once surface is modified, the device is defined for the specific target DNA. To avoid such inflexibility, fresh probe-DNA modification at point of care is needed. To achieve such on-demand DNA chip, we propose a new probe-DNA fixation (Fig. 1).

Experimental Sensor surface was modified with ligands, 2, 7-diamino-1, 8-naphthyridine (DANP). [3] DANP modification was confirmed by XPS and AFM. Then, we designed the probe DNA with cytosine bulge structure at the one end, which could anchor the probe-DNA. EIS measurement was carried out with 10 mM Tris pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$, 1 mM $K_3[Fe(CN)_6]$ / $K_4[Fe(CN)_6]$, frequency with 1M Hz to 0.1 Hz, and applied voltage 5 mVms.

Result and Discussion Figure 2 shows the Nyquist plots. The diameter of the semicircles roughly corresponded to the Rct. [4] The Rct of DNAP surface was as small as 2 kOhm and charge transfer was smooth. After addition of 1 μM probe-DNA carrying 20nt hybridization tail, the semicircle became larger and the Rct increased to 6 times larger (See “probe-DNA” curve). This result indicated that the probe-DNA binds to the DANP on the surface and hindered the electrons transfer. Furthermore, we added a 60nt length ssDNA as the target DNA carrying 20nt complementary sequence to probe-DNA tail. After 1 μM ssDNA interacted with DANP-anchored probe, the Rct increase to 20 times higher than initial Rct (See “Hybridization” curve). These results clearly indicated that C-bulge could anchor the probe-DNA on the surface through the interaction with the DANP, and that the target DNA hybridized the anchored probe-DNA, both of which were detected by EIS. The on-demand sensor was successfully demonstrated.

Reference

1. Bahner, N. et. al., Anal. Bioanal. Chem. 410(5):1453-1462, 2018.
2. Silambarasan, K. et. al., Phys. Chem. Chem. Phys. 18(18):7468–7474, 2016.
3. Suda, H. et. al., Bioorg. Med. Chem. 13(14): 4507-4512, 2005.
4. Ahmed, A. et. al., Clin. Microbiol. Rev. 27(3):631-646, 2014.

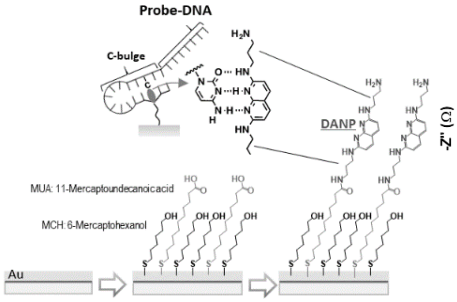

Figure 1. The concept of ligand senso and modification process.

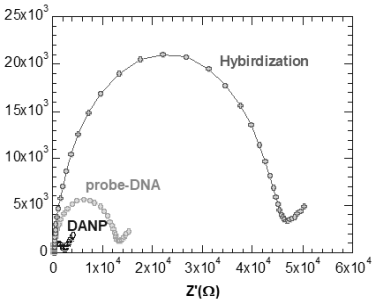

Figure 2. EIS measurement resluts in Nyquist Plot.