SERS immunosensor for detecting Influenza A

Kullavadee Karn-orachai 1,2, Kenji Sakamoto 1, Rawiwian Laocharoensuk 3, Suwussa Bamrungsap 3, Tararaj Dharakul 3,4, Kazushi Miki 1

1 National Institute for Material Science, 2 University of Tsukuba, 3 National Nanotechnology Center, 4 Mahidol University
E-mail: KARNORACHAI.Kullavadee@nims.go.jp

1. Introduction
Surface-enhanced Raman spectroscopy (SERS) has great promise as a technique for detecting trace amounts of biological and chemical samples, because of its advantages, e.g. nondestructive, no photobleaching, sensitive and fast detection. Our SERS immunosensor is composed of an Au@Ag two-dimensional (2D) array substrate and SERS probes, which are 4, 4′-thiobisbenzenethiol (TBBT)-labeled AuNPs conjugated with antibodies. The detection of influenza A nucleoproteins is achieved by observing the Raman signal of TBBT. We have succeeded in selective detection of influenza A nucleoproteins. We found that the detection sensitivity is increased by using the Au@Ag2D array substrate, instead of a SERS inactive substrate (Au film). This improvement is attributed to the plasmonic coupling between the SERS substrate and SERS probes.

2. Experimental
The SERS probes were prepared by immobilizing Influenza A antibody on PEGylated 25nm AuNPs labeled with TBBT. The SERS substrate was fabricated by arraying 55 nm Au@Ag coreshell nanoparticles using a hybrid method proposed by our group, and then it was treated with Mercaptohexadecanoic acid (MHA) solution to obtain hydrophilic 2D array. After COOH activation with 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC)/ N-Hydroxysuccinimide (NHS), nucleoprotein was immobilized on the 2D arrays. In the final step, the SERS probe solution was dropped on the substrate to detect amount of nucleoprotein.

3. Result and discussion
Two kinds of substrate were used to clarify the enhancement effect of the SERS substrate. Hydrophilic 2D arrays of Au@Ag coreshell nanoparticles were used as SERS active substrates, whereas, Au film substrates were utilized as SERS inactive substrates. The TBBT characteristic Raman peak intensity for both SERS and Au film substrates increased with increasing concentration of recombinant nucleoprotein. Figure 1 shows the calibration curves, which was obtained by plotting the characteristic peak intensity of TBBT at 1065 and 1565 cm⁻¹ [1, 2] as a function of concentration of recombinant nucleoprotein. Comparing the slopes of the two calibration curves, we found that the sensitivity of the immunoassay using the SERS substrate is 43 times higher than that using the Au film substrate. This higher sensitivity may be caused by the electromagnetic enhancement effect of the SERS substrate. The detection limits (signal to noise ratio of 3:1) of immunosensors using SERS and Au film substrates were 3.2 and 60 ng/mL, respectively.

4. Conclusions
SERS immunoassay with high sensitivity and selectivity for Influenza A detection was demonstrated with TBBT-labeled AuNPs and SERS substrate (hydrophilic Au@Ag 2D arrays). The nucleoprotein-antibody reaction was detected by Raman signal of TBBT (Raman reporter). Immunoassay taken place on the SERS substrate shows 43 times higher sensitivity than immunoassay on an Au film substrate. The higher sensitivity is attributed to the amplification of electromagnetic field by the SERS substrate. This immunoassay shows good performance with a limit of detection (LOD) of ~3 ng/mL, and a limit of qualification (LOQ) of 26 ng/mL.

Acknowledgements
We would like to thank Japan Science and Technology Agency (JST) for financial support of e-ASIA IRP. K.K. thanks the Ministry of Education, culture, Sports, Science and Technology of Japan (MEXT) for financial support by Japanese Government Scholarship Program. This study was supported by NANOTEC, NSTDA, Thailand for biomaterial including both the influenza A nucleoproteins and the Influenza A antibody, and equipment support.

References