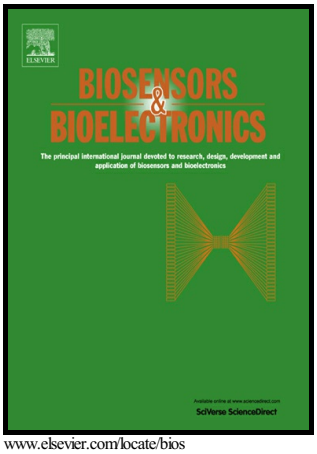

www.elsevier.com/locate/bios

Quantification of Antigen by Digital Domain Analysis of Integrated Nanogap Biosensors

Cho Yeon Lee, Hyung Ju Park, Jimin Park, Dae Keun Park, Hannah Pyo, Seok Cheol Kim, Wan Soo Yun

PII: S0956-5663(17)30386-X
DOI: http://dx.doi.org/10.1016/j.bios.2017.06.012
Reference: BIOS9782

To appear in: *Biosensors and Bioelectronic*

Received date: 28 February 2017
Revised date: 25 May 2017
Accepted date: 7 June 2017

Cite this article as: Cho Yeon Lee, Hyung Ju Park, Jimin Park, Dae Keun Park Hannah Pyo, Seok Cheol Kim and Wan Soo Yun, Quantification of Antigen by Digital Domain Analysis of Integrated Nanogap Biosensors, *Biosensors an Bioelectronic,* http://dx.doi.org/10.1016/j.bios.2017.06.012

Quantification of Antigen by Digital Domain Analysis of Integrated Nanogap Biosensors

Cho Yeon Lee[a,1], Hyung Ju Park[b,1], Jimin Park[a,1], Dae Keun Park[a], Hannah Pyo[a], Seok Cheol Kim[a], Wan Soo Yun[a*]

[a]Department of Chemistry, Sungkyunkwan University, 2066 Seobu-Ro, Jangan-Gu, Suwon, Gyeonggi-Do 440-746, Republic of Korea

[b]Bio-Medical IT Convergence Research Department, Electronics and Telecommunications Research Institute (ETRI), Daejeon 305-700, Republic of Korea

[*]Corresponding author at Department of Chemistry, Sungkyunkwan University, 2066 Seobu-Ro, Jangan-Gu, Suwon, Gyeonggi-Do 440-746, Republic of Korea. wsyun87@skku.edu

Abstract

Nanogap biosensor shows a distinct conduction change upon sandwich-type immobilization of gold nanoparticle probes onto the gap region in the presence of target biomolecules. Although this large conductance change could be advantageous in distinguishing signal on or off devices, since the extent of conductance change is quite irregular even at the same analyte concentrations, it fails to extract quantitative information from its level of conductance change. In other words, the conductance change of a single device does not reflect the concentration of the target molecule. In this study, we introduce an alternative approach of interpreting the concentration of target molecules using digital domain analysis of integrated nanogap devices, where the fraction of signal-on-devices, or on-device-percentage (ODP), was translated into the concentration of the target molecule. The ODP was found to be closely related to the number density of the immobilized probes and, therefore, to be an excellent measure of the analyte concentration, which was demonstrated in the immuno-selective detection and quantification of influenza A hemagglutinin and prostate specific antigen.

Keywords: Digital domain analysis, Integrated nanogap, Nanobiosensor, Immunosensor

[1] Authors contributed equally.

1. Introduction

The quantification of low-concentration biomolecules is of great scientific and clinical importance. In order to quantify biomolecules at very low concentrations, amplification of the target concentration or the transducer signal is required to collect data above the limit of quantification. For gene identification, the target amplifying techniques of polymerase chain reaction (PCR) (Livak and Schmittgen, 2001), rolling circle amplification (Lizardi et al., 1998), and helicase-dependent amplification (Vincent et al., 2004) are usually adopted. These methods have been successfully used to assess the presence of target DNA or RNA. However, it is difficult to quantitatively determine the initial gene concentration based on these amplification methods, because the intensities of amplified signals are not well-correlated to the starting concentrations of the target gene. Therefore, digital PCR (Vogelstein et al., 1991; Belmonte et al., 2016), where separate amplification signals from a large number of divided detection units provide a measure of the analyte concentration, has been developed to overcome this challenge.

This approach becomes useless when the target molecule is impossible to be amplified. For instance, since methods for protein amplification are not available, other signal amplification techniques need to be developed for the analysis of low-concentration proteins. Various innovative approaches such as optical (Tong et al., 2013; Liu et al., 2012; Liang et al., 2015; Guo et al., 2016), mass (Liu et al., 2011; Liu et al., 2010), electrochemical (Han et al., 2014; Zhu et al., 2015; Chikkaveeraiah et al., 2012; Miao et al., 2016), and electric methods (Kim et al., 2013; Jun et al., 2014; Chen et al., 2007; Roy et al., 2009; Kim et al., 2016) have been reported for this purpose including nanogap-based detection of proteins (Chen et al., 2007; Roy et al., 2009).

Nanogap devices, where the electric conductance change between two electrodes is mediated

by the immobilization of biomolecule-attached metallic nanoparticles in the gap region, exhibit a high signal amplification ratio, meaning that their conductance increases dramatically upon the particle immobilization. However, the extent of conductance change is quite irregular or variable. Although this high signal amplification ratio can allow us to clearly distinguish signal-on (conducting) from the signal-off (non-conducting) devices, the rather unpredictable and random nature of the conductance change makes it difficult to correlate with the analyte concentration. In other words, the conductance change of nanogap devices is not useful in determining the concentration of analytes (Marcon et al., 2008; Park et al., 2010).

In this study, we demonstrate that nanogap devices, an example of a high amplification technique with a random nature, can be used for the quantification of a low-concentration biomolecule. This was possible simply by applying the digital domain analysis: the analyte concentration is correlated with the number of signal-on-devices over the total number of devices, which is defined as ODP (on-device-percentage). The fraction of conducting devices among all integrated nanogap devices is found to be an excellent measure of the analyte concentration. Protein biomarkers were selected as the target molecules: influenza hemagglutinin (HA), a major biomarker for the diagnosis of influenza viral infection, and prostate specific antigen (PSA), critical for the diagnosis of prostate cancer.

2. Experimental

Nanogap devices were fabricated using conventional lithography techniques. The SiO_2 surfaces of the devices were hydroxylated with O_2 plasma and treated with 1% (3-aminopropyl) trimethoxysilane for 1 h to form an amine-terminated molecular layer. The immobilization of the primary antibody was performed by dropping its solution on the device. After rinsing with buffer solution, the unreacted amine groups were blocked with bovine serum albumin to reduce non-specific binding. After the target antigen solution was dropped on the device, it was maintained for 1 h. After rinsing, secondary antibody functionalized gold nanoparticle (Au NP) probes were dropped on the antigen immobilized surface and it was maintained for 30 min. After water rinsing and N_2 blowing, the conductance was measured by applying DC voltage, and the ODP was then obtained from the ratio of conducting devices to the total number of devices with a preset threshold value of 100 pS (see Supplementary Information for more details).

3. Results and Discussion

3.1 Analysis of ODP vs. analyte concentration

The nanogap device in our study was designed to measure the electrical conductance change derived by the selective immobilization of bio-functionalized Au NP probes. When based on a sandwich immunoassay, the nanogap device can function as an immunosensor [Fig. 1(a)]. Fig. 1(b) shows images of a 2×2 cm nanogap device for the detection of H1N1 HA using 42 interdigitated electrodes (IDEs) with a 500 nm gap. Distinct changes in the electric conductance upon bio-selective processes can provide an excellent indication of the existence of a target molecule.

However, by using a single unit of a nanogap device, one cannot escape from several cases

that restrict the interpretation of data such as (1) no current change even with the selectively-immobilized Au NP probes, (2) detectable current change upon the non-specific binding of the probes, and (3) no immobilization of Au NP probes despite the presence of target antigens.

Although the status and the level of conductance of a single nanogap device is not predictable, the number of on-devices out of the total number of devices can provide a rather stable value when all the devices are examined under the same experimental conditions. Because the occurrence of the on-signal is a function of the number density of Au NP probes immobilized in the gap region, the fraction of on-devices will increase with increasing immobilized Au NP probe number. Therefore, one may expect a concentration-dependence of the number of on-devices, as shown in Fig. 1(c). Surprisingly, this expectation showed a very good match with actual experimental results shown in Fig. 1(d). In other words, the ODP, which is the percent ratio between the number of on-devices (N_{on}) and the total number of nanogap devices (N_{tot}) was found to reflect the analyte concentration very well.

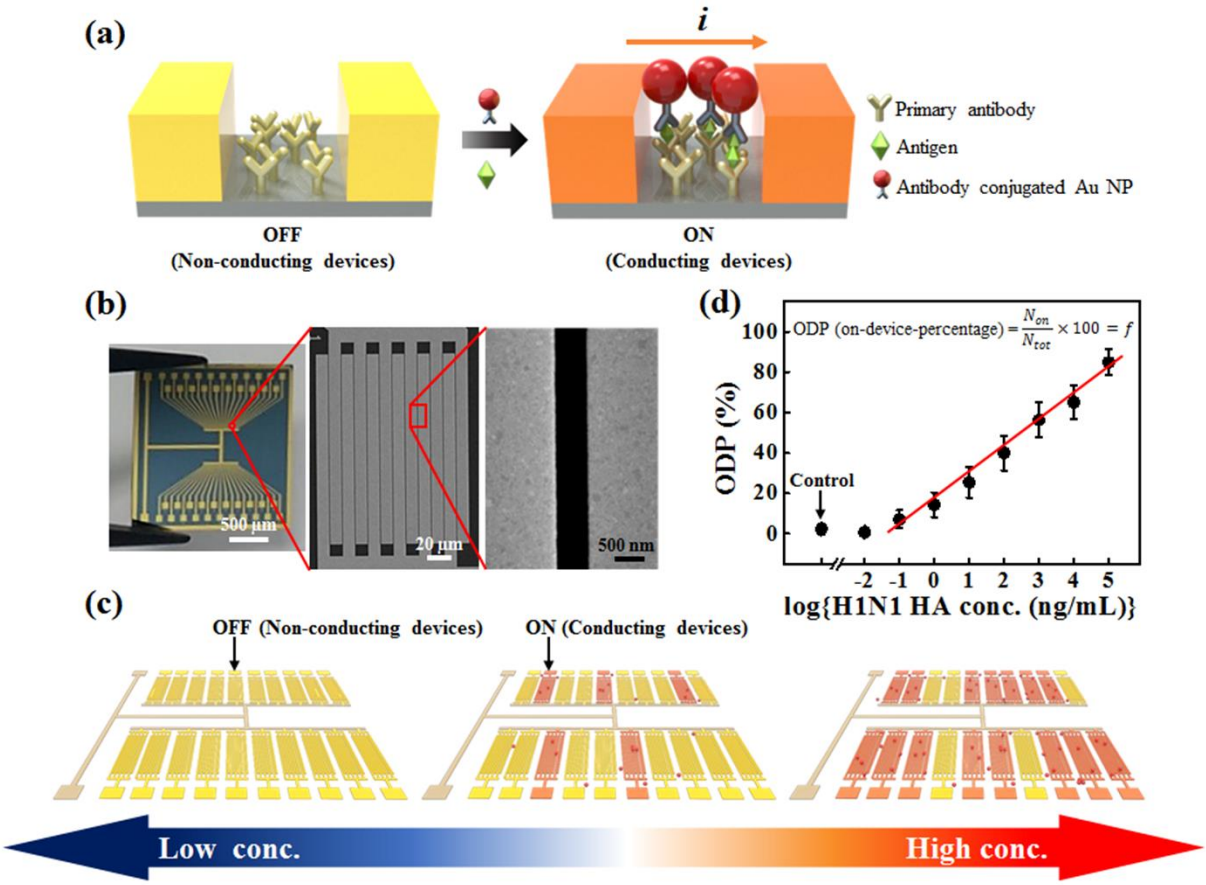

Fig. 1 (a) Schematic illustration of sandwich-immunoassay-based antigen detection with a nanogap device. (b) Photographs of an integrated nanogap device having 42 nanogap units. (c) Conceptual illustration of digital domain analysis. ODP (on-device-percentage) increases along with an increase in target concentration. (d) ODP vs. HA concentration curve. Error bars represent the 95% confidence intervals of measured counts.

3.2 Analysis on the conductance vs. analyte concentration

The conductance value itself may behave as a measure of the analyte concentration. However, the change in electric conductance upon Au NPs immobilization is quite irregular, and it cannot be used to determine the analyte concentration. This becomes evident from the results shown in Fig. 2, where the I-V (current-voltage) curves and conductance values after the Au NPs immobilization step are depicted.

As shown in Fig. 2(a), the device showing a conductance value less than a few pS (noise level; written as '~0' in the figure for simplicity) was set as an off-device. After the immobilization of Au NP probes, the electric conductance may be different in each specific case of the device. Even after exactly same process, the surface distribution of the Au NP probes in the gap region was not exactly same. Au NP probes could contribute to the conduction change (signal-on) in one case, whereas in the other cases the electric conductance did not change at all (signal-off). Therefore, misinterpreting the presence of the target molecule is always possible when only one nanogap unit is used for detection. Furthermore, the measurement of analyte concentrations is not possible with a single unit if the onset of the conductance change is the only information attainable from a single device.

Fig. 2(b) shows that all the I-V curves were different, although the antigen concentration (H1N1 HA, 10 μg/mL) was same in the three different devices. This randomness was observed in all concentration ranges. As shown in Fig. 2(c), the conductance values of different devices were quite scattered, irrespective of the HA concentration. At this point, one may think that the average conductance may be an indicator of the quantitative value. However, quantitative information could not be obtained from the average value of electrical conductivity [Fig. 2(d)]. This result indicates that the conductance value itself is not a useful parameter to extract information about the analyte concentration. This irregularity in the electric conductance may be attributed to the rather ill-defined nature of the conduction process. The Au NPs, which are the most critical conduction mediator between two electrodes of a nanogap device, were not bare but covered with non-conducting protein molecules, such as the H1N1 HA antibody and BSA. Moreover, the electrode surface might be contaminated with non-conducting chemicals during the surface chemical reactions for the antibody immobilization.

Another important reason for the randomness of the conductance values may be the non-uniform and random distribution of the Au NP probes. It is expected that a minimum three or four of Au NPs lined up together can form a conduction bridge between electrodes because the size of nanogap was about 500 nm, and the average size of Au NP was about 150 nm in this case of H1N1 HA measurement. Actually, the number of Au NP probes in a conduction bridge was not constant, but rather variable, and might also give rise to fluctuation of the electric conductance.

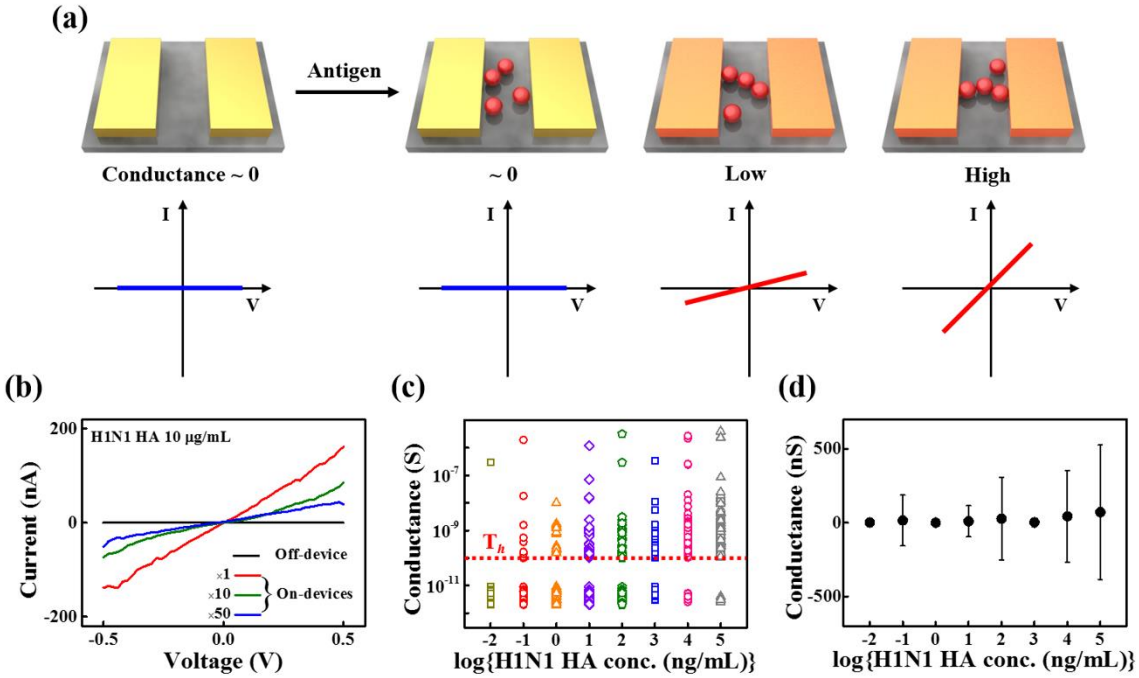

Fig. 2 (a) Conceptual illustration of the particle immobilization and I-V curves before and after the whole sensing process. Yellow and orange colors represent non-conducting and conducting electrodes, respectively. (b) I-V curves obtained from three different devices at the same concentration of 10 μg/mL H1N1 HA, indicating the inconsistencies of the I-V characteristics. (c) Electric conductance values after the selective immobilization of Au NP probes and (d) average electrical conductance at various H1N1 HA concentrations. Error bars in (d) indicate the standard

deviation.

3.3 Comparison between ODP and conductance: Correlation with particle number density

Interestingly, the ODP values in our study exhibited a close correlation with the number density of Au NPs immobilized on the nanogap device, the number density of Au NPs depends on the analyte concentration. Fig. S2 shows the scanning electron microscope images of the immobilized Au NP probes via sandwich-type immunoassay at different antigen concentrations and plots of Au NP number density and ODP with respect to the H1N1 HA concentration. As the antigen concentration in the sample increased, the number density of Au NP probes and ODP values increased synchronously. In other words, the ODP values follow changes in the number density of the probes well and can therefore serve as a good parameter in correlating the analyte concentration. Now, one can estimate the analyte concentration by simply counting the signal-on-devices; in the case of H1N1 HA detection, the response range of measurements was 10^2-10^8 pg/mL, as shown in Fig. 1(d).

3.4 Application to different cases

The ODP approach, or the digital domain analysis of integrated nanogap devices, was also applied to different target molecules: H3N2 HA, which is a different subtype of H1N1 HA, and PSA, which is a biomarker of prostate cancer. The results are summarized in Fig. 3. In both cases, the ODP value is an excellent measure of the antigen concentration. The two datasets in Figs. 3(a) and 3(b) were obtained with variation of the gap distance or particle size, respectively. As shown in the figures, the limit of detection (LOD) and the response range were critically dependent on the gap distance or particle size.

One of the major factors affecting the sensing performance is the gap distance to particle size

ratio (GPR). A smaller GPR implies a decrease in the minimum number of Au NPs necessary to form a conduction bridge between the two electrodes of the nanogap device. Therefore, as the GPR decreases, the probability of forming the conduction bridge will increase at relatively low target concentrations, such that the ODP curve shows a detectable value at that low concentration (increase of LOD). When the GPR was decreased from 4 to 3 in the cases of H3N2 HA, the resulting ODP curves exhibited an increase in the LOD from 1 ng/mL to 100 pg/mL [Fig. 3(a)]. In the case of PSA sensing, a decrease in the GPR from 1.5 to 0.9 also improved the LOD from 5 ng/mL to 150 pg/mL [Fig. 3(b)].

Although the actual values of the LOD of H3N2 HA and PSA were similar, the response ranges were quite different: the response range for the H3N2 HA detection was wider than that for PSA detection. Since those values are strongly dependent on other parameters like antigen-antibody interaction, Au NP probe concentration, surface chemical treatment, and so on, the discrepancy between the response ranges is not incomprehensible. Consequently, similarities or differences in the values of LOD and response range in PSA and HA detection cases cannot be simply described using only GPR variation.

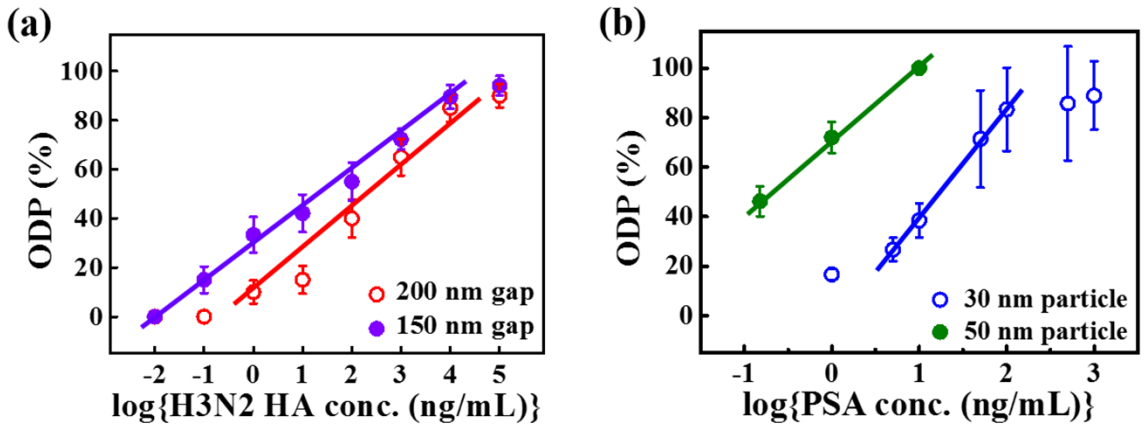

Fig. 3 ODP vs. concentration curves obtained in the cases of (a) H3N2 HA and (b) PSA. The particle size used in (a) and the gap distance used in (b) were about 50 nm and 45 nm,

respectively. Error bars represent the 50% confidence intervals.

The nanogap devices shown in Fig. 1(b) have a gap distance of 500 nm, and the size of Au NPs was about 150 nm. With this combination, our digital approach produced an ODP curve spanning antigen concentrations across seven orders of magnitude. At the target (H1N1) concentration of 10 μg/mL, the ODP value was 65.1 (±8.4)%, which was clearly distinguishable from the control value (without the target) of 2.4 (±2.7)%, strongly supporting the presence of the target. To confirm the selectivity of this sensor, the ODP value on application of the non-target (H3N2) at the same concentration was estimated. It was 7.1 (±4.5)%, which is a much smaller value than that of the target [Fig. S3(a)]. The selectivity was also examined with a different combination of gap distance (~200 nm), particle size (~50 nm), and target molecule (H3N2). In this experimental case, the ODP values were estimated to be 85 (±5.6)% for the target and 5 (±3.4)% for the non-target (H1N1) at a concentration of 10 μg/mL [Fig. S3(a)]. The selectivity can be examined even further using the ODP curves. The two ODP curves obtained using the target and non-target exhibited distinct differences, again supporting the good selectivity of this approach [Fig. S3(b)].

4. Conclusions

Quantitative information regarding target antigens were obtained using digital domain analysis of integrated nanogap devices, where the ODP, or the percent faction of electrically-conducting devices among the total number of devices examined, was used as a good measure of the analyte concentration. Because the nanogap devices exhibited fluctuating conductance values, the conductance change of a single unit of the device failed to reflect the concentration of the analyte. In this study, the ODP curves were used for the quantification of different antigens of H1N1 HA,

H3N2 HA, and PSA. The parameters affecting the quantization and the selectivity of the sensing were also examined.

The main focus of our work was demonstrating that quantification of a target biomolecule is possible with integrated nanogap devices, which inherently have a signal fluctuation that is too high to be directly correlated to the analyte concentration, through a digital domain analysis. Therefore, when compared with other techniques for biosensing [Table S1], we can find some points that must be improved. However, because nanogap sensors are very simple, sensitive, and robust, we believe that this suggestion of an alternative approach to nanogap-based quantification should contribute to paving the way to biosensor-based quantitative analysis of low-concentration biomolecules.

Acknowledgements

This study was supported by the National Research Foundation of Korea through the following projects: NRF-2015M3C1A3002152, NRF-2016M3A7B4910538, and NRF-2013M3A6B2078944.

References

Belmonte, F. R., Martin, J. L., Frescura, K., Damas, J., Pereira, F., Tarnopolsky, M. A., Kaufman, B. A., 2016. Sci. Rep. 6, 25186.

Chen, C.-C., Ko, F.-H., Chen, C.-T., Liu, T.-F., Chang, E. Y., Yang, Y.-H., Yan, S.-J., Chu, T.-C., 2007. Appl. Phys. Lett. 91, 253103.

Chikkaveeraiah, B. V., Bhirde, A. A., Morgan, N. Y., Eden, H. S., Chen, X., 2012. ACS Nano 6, 6546-6561.

Guo, T., Deng, Q., Fang, G., Yun, Y., Hu, Y., Wang, S., 2016. Biosens. Bioelectron. 85, 596-602.

Han, D., Kim,Y.-R., Kang, C. M., Chung, T. D. 2014. Anal. Chem. 86, 5991-5998.

Jun, J., Lee, J. S., Shin, D. H., Jang, J., 2014. Appl. Mater. Interfaces 6, 13859-13865.

Kim, D.-J., Park, H.-C., Sohn, I. Y., Jung, J.-H., Yoon, O. J., Park, J.-S., Yoon, M.-Y., Lee, N.-E., 2013. Small 9, 3352-3360.

Kim, H. J., Kim, J., Yoo, Y. K., Lee, J. H., Park, J. H., Hwang, K. S., 2016. Biosems. Bioelectron. 85, 977-985.

Liang, J., Yao, C., Li, X., Wu, Z., Huang, C., Fu, Q., Lan, C., Cao, D., Tang, Y., 2015. Biosens. Bioelectron. 69, 128-134.

Liu, H., Wu, X., Zhang, X., Burda, C., Zhu, J.-J., 2012. J. Phys. Chem. C 116, 2548-2554.

Liu, J.-M., Li, Y., Jiang, Y., Yan, X.-P., 2010. J. Proteome Res. 9, 3545-3550.

Liu, R., Liu, X., Tang, Y., Wu, L., Hou, X., Lv, Y., 2011. Anal. Chem. 83,2330-2336.

Livak, K. J., Schmittgen, T. D., 2001. Methods, 25, 402-408.

Lizardi, P. M., Huang, X., Zhu, Z., Ward, P. B., Thomas, D. C., Ward,D. C., 1998. Nat. Genet. 19, 225-232.

Marcon, L., Melnyk, O., Stievenard, D., 2008. Biosens. Bioelectron. 23, 1185-1188.

Miao, X., Li, Z., Zhu, A., Feng, Z., Tial, J., Peng, X., 2016. Biosens. Bioelectron. 83, 39-44.

Park, H. J., Chi, Y. S., Choi, I. S., Yun, W. S., 2010. Appl. Phys. Lett. 97, 033701.

Roy, S., Chen, X., Li, M.-H., Peng, Y., Anariba, F., Gao, Z., 2009. J. Am. Chem. Soc. 131, 12211-12217.

Tong, S., Ren, B., Zheng, Z., Shen, H., Bao, G., 2013. ACS Nano 7, 5142-5150.

Vincent, M., Xu, Y., Kong, H., 2004. EMBO Rep. 5, 795-800.

Vogelstein, B., Kinzler, K. W., 1991. Proc. Natl. Acad. Sci. USA 96, 9236-9241.

Zhu, Q., Chai, Y., Zhuo, Y., Yuan, R., 2015. Biosens. Bioelectron. 68, 42-48.

Highlights

- Quantitative analysis became possible from digital domain analysis of integrated nanogap biosensors inherently having an irregular conductance values at the same analyte concentrations.
- On-device-percentage (ODP), or the ratio between the number of conducting devices and that of the whole devices, was found to be a good measure of the antigen concentration.
- By this approach, quantitative measurement of the concentrations of hemagglutinin and prostate specific antigen was successfully implemented, showing a few hundred pg/mL level detection limit.