Extended Abstracts of the 2012 International Conference on Solid State Devices and Materials, Kyoto, 2012, pp1095-1096

# Single-Molecule Electrical Identification Towards Nucleotide Sequencing by using nano-gap devices.

Takahito Ohshiro, MakusuTsutsui, Masayuki Furuhashi, Soh Ryuzaki, Kazumichi Yokota, Masateru Taniguchi, Tomoji Kawai.

ISIR, Osaka University
8-1 Mihogaoka, Ibaraki City, Osaka 567-0047, Japan
Phone: +81-6-6879-4306 E-mail: toshiro@sanken.osaka-u.ac.jp

## 1. Introduction

There is broad interest in using personal genome sequencing to better understand human genetic variation and genome-related diseases, such as cancer, and to guide discoveries and decisions about the health of individuals [1]-[3] . For the coming "personal-genome" era, a *deno vo* high-speed and low-cost approach to whole-genome sequencer have been required. A use of single-molecule detection for sequencing is a one of the solutions for the "personal-genome sequencer". A traverse electrical conductivity detection of the individual single-nucleotides in the DNA/RNA sequence has been proposed for the single-molecule genome sequencing by several groups [4]-[6]. We have been proposed a tunneling-current based identification (**Figure 1**) as a single-molecule DNA/RNA sequencing. This methodology is based on sequentially reading the tunneling-current across individual single-nucleotides in the sequence, resulting in a high-speed electrical discrimination of the individual nucleotides without chemical probes and PCR amplifications.

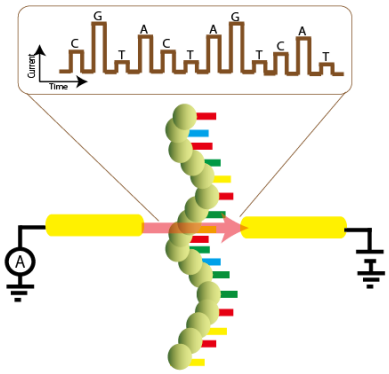


Figure 1: Single-Molecule Tunnel-Current Based Electrical Detection by using Nano-MCBJ. Sequential reading of the tunneling-current across individual single-nucleotides in the sequence.

In this study, we report on a read of DNA / RNA sequence by the transverse electron transport through nanogap-electrode (**Figure 2**). When the molecules passed between the nanoelectrodes separated by a sub-nanometer gap, the tunneling-current through the molecules was increased, relative to that in the absence of molecules (Figure 3). The current intensity is closely related to the indivisual electronic conductance. We measured the extent of the electron-tunneling by using nanofabricated, mechanically controllable break junction (nano-MCBJ).

## 2. Experimental section.

The nano-MCBJ was prepared in the following procedure. First, a gold-wire was fabricated by a standard electron-beam lithography method. The gold-wire was mechanically broken by using a piezo-controller, resulting in forming a pair of gold nanoelectrodes. After the formation of a pair of electrodes, the nanogap-electrode was reconnected, and then the reconnected gold-nanowire was thermally broken under dc 0.1V apply [7]. We found that this gold-nanowire self-breaking phenomena reproducibly allow to form 0.5 nm, which is comparable to the size of one-gold atom. After the formation of nanogap, by using piezo-controller, the gap distance was tuned to be 0.8 nm, which would be comparable to the size of mono-nucleotide molecules, and used in the following electrical measurements [8].

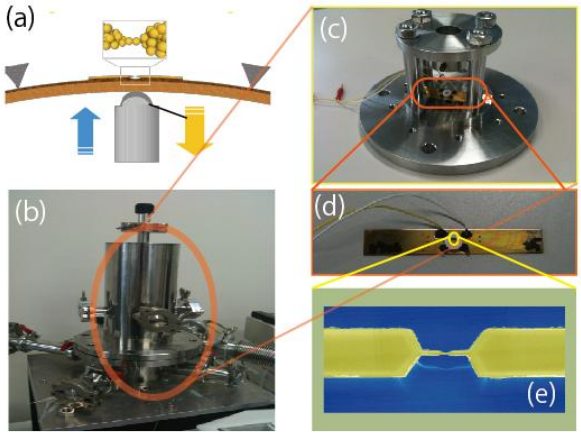


Figure 2: Single-Molecule Electrical Detection System. (a) Schematics of MCBJ system. (b) photo image of Faraday Gage for MCBJ instruments (c) MCBJ holder (d) Fabricated MCBJ. (e)a SEM image of gap-electrode in the MCBJ .

## 3. Results and Discussion

We investigated the conductance values of single base molecules of DNA and RNA, and determined the conductance values for four deoxyribonucleoside monophosphates (dAMP, dCMP, dGMP, dTMP) and four ribonucleoside monophosphates (rAMP, rCMP, rGMP, rUMP). The magnitude of the peak conductance of four nucleotides was found to be in the following order: dGMP > dAMP >

dCMP > dTMP, and rGMP > rAMP > rCMP > rUMP **(Table 1)**.

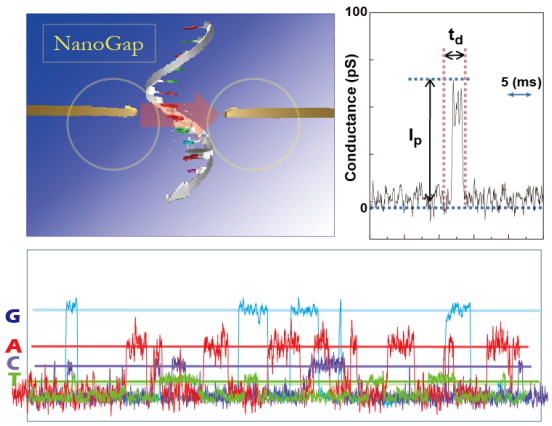


Figure 3. Homobase-Oligoucleotide Molecular Signals by Using Nano-MCBJ. (a) Schematics of Mono-Nucleotide Electrical Measurements by using a gap-electrode. (b) Typical current-time profiles of dGMP aqueous solution. When the nucleotide molecule passed between the nanoelectrodes, the positive-signals were observed. The mono-nucleotide molecules were stochastically trapped between the nano-electrodes because of the interaction of amino functional group of nucleotide with gold electodes. After a few miliseconds, releas Brownian motion. (c) Enlarged molecular signal. The signal was characterized by the retention time ($t_d$), and peak current ($I_p$)..

Table 1. Conductance value for Single DNA/RNA Nucleotide (pS)

| | G | A | C | T | mC | oxoG |
|---|---|---|---|---|---|---|
| DNA | 123 | 93 | 64 | 50 | 105 | 98 |
| RNA | 87 | 67 | 60 | 39 | - | - |

This conductance values is due to the individual molecular energy level. Calculations based on density functional theory indicated that the order based on the highest occupied molecular orbital (HOMO) energy was similar to our experimental results. Note that this order corresponds to the order of the relative G values, suggesting that our single-molecule electrical detection method can identify molecular species based on characteristic energy levels, particularly the HOMO energy level.

.

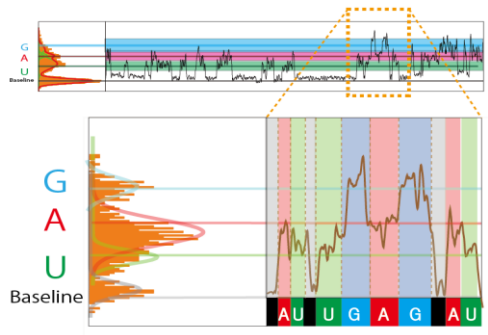


Figure 4. Nucleotide-base Assignments for the RNA oligonucleotides (UGAGGUA). The base-assignment procedures are the following; In first step, a conductance histogram was constructed from the whole data-point in the I-t profiles. In second step, from the lowest data-point peaks, we determined the baseline level of the I-t profile. In third step, we determined the components of the nucleotide-base species in the I-t profiles, compared to the single-nucleotide conductance value (Table 1). In the fourth step, compared to single-nucleotide conductance values, the plateaus regions were sequentially assigned to each of base-species. Finally, we sequentially connected the assigned base signal-region, resulting in reading the signal sequences

We also applied this single-molecule electrical identification method to base-typing in oligonucleotides. Based on the electrical conductivity for single-nucleotides, we identified the base-type in the sample oligonucleotides. We can read the fragment of sample nucleotide passing through the sensing electrode (Figure 4). On the basis of a reconstruction of the read fragment sequences, we successfully determined a sample RNA sequence

## 3. Conclusions

This single-molecule electrical sequencing using nano-MCBJ can be used to randomly identify sequences of single base DNA molecules. This method could be one of the promising whole-genome sequencing strategies for personalized medicine.


## Acknowledgements

This research is partially supported by the Japan Society for the Promotion of Science (JSPS) through its Funding Program for World-Leading Innovative R&D on Science and Technology.


## References


[1] Branton, D., Deamer, D. W., Marziali, A., Bayley, H., Benner, S. A., Butler, T., Di Ventra, M., Garaj, S., Hibbs, A., Huang, X., Jovanovich, S. B., Krstic, P. S., Lindsay, S., Ling, X. S., Mastrangelo, C. H., Meller, A., Oliver, J. S., Pershin, Y. V., Ramsey, J. M., Riehn, R., Soni, G. V., Tabard-Cossa, V., Wanunu, M., Wiggin, M. & Schloss, J. A. The potential and challenges of nanopore sequencing. Nat. Biotech. 26, 1146-1153 (2008).

[2] Schloss J. A. How to get genomes at one ten-thousandth the cost. Nat. Biotech. 26, 1113-1115 (2008).

[3] Service, R. F.The race for the $1000 genome. Science 311, 1544-1546 (2006)

[4] Tsutsui, M., Taniguchi, M., Yokota, K. & Kawai, T. Identifying single nucleotides by tunnelling current. Nat. Nanotechnol. 5, 286-290 (2010).

[5] Zwolak, M. & Di Ventra, M. Colloquium: Physical approaches to DNA sequencing and detection. Rev. Mod. Phys. 80, 141 (2008)

[6] Shuai Chang, Jin He, Ashley Kibel, Myeong Lee, Otto Sankey, Peiming Zhang and Stuart Lindsay. Tunnelling readout of hydrogen-bonding-based recognition Nat.Nanotech., 4, 297-301 (2009)

[7] Tsutsui, M., Shoji, K., Taniguchi, M. & Kawai, T. Formation and self-breaking mechanism of stable atom-sized junctions. Nano Lett. 8, 345-349 (2007).

[8] Tsutsui, M., Ohshiro, T., Matsubara, K., Furuhashi, M., Taniguchi, M., Kawai, T., J.Appl.Phys., 108, 064312, (2010).