

# Logic Gate Operation using Three-way Junction DNA and Biological Nanopore

M. Ohara\* and R. Kawano

Tokyo University of Agriculture and Technology, JAPAN

## ABSTRACT

This paper describes AND operation using a biological nanopore and a three-way junction DNA (TWJ) which can be cleaved by two different restriction enzymes. TWJ has branched DNA structures that is similar to Y-shaped [1]. TWJ we designed has two specific sites at which a restriction enzyme cleaves. The initial structure of TWJ cannot translocate through a nanopore without enzymes. When two restriction enzymes cleave the both stems of TWJ, the structure is changed and it can pass through the nanopore, resulting to produce DNA output. We constructed AND operation using this mechanism. This operation is promising to develop for biological computer at the single molecule level.

## KEYWORDS

Nanopore, DNA computing, Three-way junction DNA, Lipid bilayer, Restriction enzymes

## INTRODUCTION

DNA computing has attracted attentions for large-scale parallel computing or a computer integrated with living cells. Recently, the study of DNA-based computer has reached a turning point because the output sequence of DNA and structure is limited, and DNA-based calculation takes long time to obtain the output [2]. To rapidly obtain a calculation result, we previously proposed DNA computing using  $\alpha$ -hemolysin ( $\alpha$ HL) [3]. An  $\alpha$ HL is well known as a biological nanopore for chemical sensors and next generation of DNA sequencing [4]. The computing using biological nanopores has advantages, which can be reducing the time of obtaining the output signals. Also, the nanopores are able to detect single molecules electrically without the label and amplification of targets. We have been developing the droplet contact method (DCM) for reconstituting the biological nanopore, as shown in Fig. 1a. The method is able to prepare bilayer lipid membranes (BLMs) with high stability and reproducibility. In this study, we tried to construct of the AND operation using: TWJ as a calculation molecule, restriction enzyme as input molecules and an  $\alpha$ HL nanopore as an output gate. TWJ has branched structure that is similar to Y-shaped [1]. The branched structures can adopt enzyme reaction sites to each stem. A restriction enzyme has used in most gene studies.

In the operation, TWJ (Y-shaped DNA) cannot translocate through the nanopore before cleaving the one of the stem by enzymes because of the larger molecule size. When restriction enzymes cleave the two stems, the TWJ structure is changed to single-stranded state, and then it can pass through the nanopore.

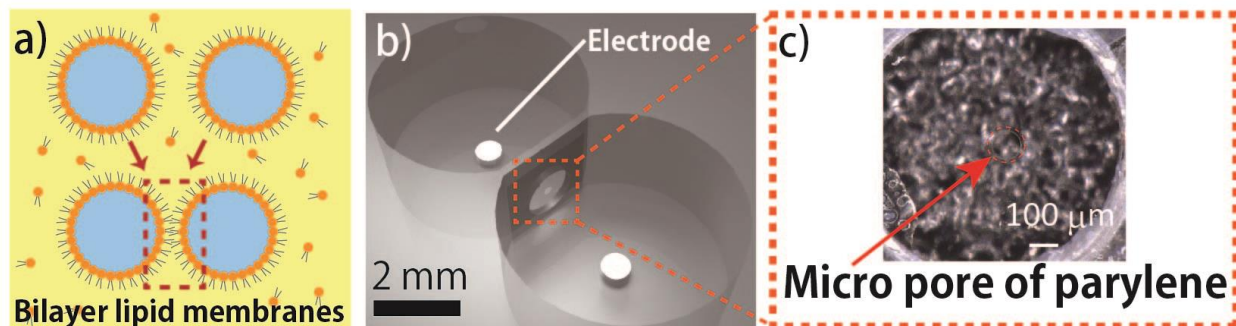


Figure 1: a) BLMs can be formed using droplets contacting method. b) An image of double well chip. Artificial bilayer membranes are made to contact via a hole of parylene film. c) The parylene film were drilled a hole of 100  $\mu$ m in diameter.

We defined the threshold on the output signals as “1” and “0” using the duration of current blocking in this operation. We tried to judge output from the mean value in the duration times. As a result, we found that this system can work as AND operation. This system will develop for single molecule computing which is used more enzymes and a secondary structure molecule.

## EXPERIMENTAL

In this experiment, we designed the secondary structure of TWJ and cleavage site of a restriction enzyme. To design the appropriate TWJ structure, we used hydrodynamic simulation (Nupack, <http://www.nupack.org/>). This DNA was synthesized in FASMAC Co., Ltd. (Japan). To operate AND calculation with the enzyme reactions, we conducted following experiments. 10  $\mu\text{M}$  TWJ of the calculation molecule were contained in buffer solution (20 mM Tris-HCl pH 8.5, 10 mM  $\text{MgCl}_2$ , 1 mM Dithiothreitol, 100 mM KCl). Two types of restriction enzyme (*Bci*T130 I and *Dde* I) were added to the solution. The solution were incubated at 37°C in the tube for 60 minutes. The lipid bilayer membranes (BLMs) with  $\alpha\text{HL}$  nanopore were prepared using DCM. BLMs was formed by a micropore of parylene using 1, 2-diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPC) within *n*-decane (Fig. 1c). The incubated solution and the  $\alpha\text{HL}$  solution were dropped at the same side of double well chip (DWC) (Fig. 1b). The electrodes were set in each droplet and the channel current was measured at 120 mV. This electric measurement was used Pico Patch Clamp Amplifier (Tecella, USA). The sampling rate: 40 kHz, low pass filter: 10 kHz, and the final concentration of  $\alpha\text{HL}$  was 0.03  $\mu\text{M}$  in the buffer.

## RESULTS AND DISCUSSION

From the simulation, we found that the suitable DNA sequences which forms a hammerhead structure with the two enzyme reacting points at the stems. In addition, the free energy of TWJ was gradually decreased with the two steps of the enzyme reactions. After cleaving the two stems, the hammerhead structure of TWJ was changed to the partial double-stranded structure and it should be pass through the nanopore, as predicted by the simulation. In the channel current measurements, long blocking current duration of TWJ itself was observed in the solution without restriction enzymes, this system was as input (0, 0). This result indicates that TWJ cannot pass through  $\alpha\text{HL}$  pore, it was just clogging the pore. When the weather *Dde* I or *Bci*T130 I was in the solution, this system was as input (1, 0) or (0, 1), the long duration events were still observed. When both restriction enzymes existed at same time, this system is as input (1, 1), the most of duration time became short. This result would be caused by that the secondary structure of TWJ was cleaved by two restriction enzymes, and it started to pass through  $\alpha\text{HL}$  pore. Fig 2a shows the mean of duration time as a function of the simulated free energy. The duration time was increased exponentially with increasing the free energy. Next, we determined the threshold for the definition of the output signals. The threshold was set at the point of  $2\sigma$  of the mean duration time (Fig. 2b). Using this threshold, we can constructed AND operation with TWJ and nanopore.

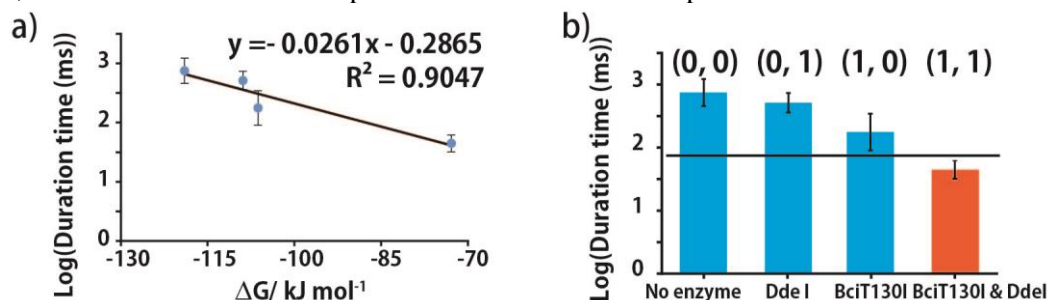


Figure 2: The result of electric measurements. a) The result showed there is correlation in the Gibbs free energy and means of duration time. b) The result of duration time means. Error bar is standard error ( $2\sigma$ ). Threshold line was defined from between error bar of means.

## CONCLUSION

In this study, we constructed AND operation with TWJ and biological nanopore. TWJ structure was gradually changed by the stepwise reaction with two different enzymes as the input molecules, and the reacted TWJ pass through the  $\alpha$ HL nanopore as output molecule. This study will be a first step in constructing the single molecule DNA logic gate.

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## CONTACT

\* M. Ohara ; phone: +81-042-388-7187 ; 50015641105@st.tuat.ac.jp