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研究課題名（和文）Isothermal DNA Sequencing by Diffusion Current in a MoS2 Nanopore

研究課題名（英文）Isothermal DNA Sequencing by Diffusion Current in a MoS2 Nanopore

研究代表者
徐　偉倫（HSU, Wei-Lun）

東京大学・大学院工学系研究科（工学部）・講師

研究者番号：５０７７１５４９

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研究成果の概要（和文）：It was demonstrated that the structural information of ssDNA molecules can be revealed using artificial solid-state nanopores. The proposed isothermal sensing method by diffusion current has simultaneously resolved the excess DNA translocation speed and high thermal noise issues.

研究成果の学術的意義や社会的意義
This project has made a remarkable progress by increasing the nanopore sensing resolution, which paves the way for DNA sequencing using solid-state nanopores. This breakthrough not only opens up new opportunities for molecule sequencing, but provides hopes to high resolution proteomic analysis.

研究成果の概要（英文）：Since the concept of resistive pulse sensing using solid-state nanopore was envisaged in the beginning of this century, there have been tremendous expectations for DNA sequencing by artificial nanopores. However, researchers fail to show DNA structural information using conventional methods based on conduction current and electrophoretic transport of molecules. This project has both experimentally and theoretically investigated an effective approach using diffusion current and diffusiophoretic transport of molecules that enables us to probe structural information of ssDNA molecules. By tracing the diffusive current variation through a monolayer molybdenum disulfide nanopore using an ultra-low current measurement system, we are able to reveal four levels of current signals representing different nucleotide acids. Using theoretical simulations, we conclude that the improved results are due to the reduced DNA translocation speed and elimination of Joule heating.

研究分野：Analytical Chemistry

キーワード：Nanopore　DNA sequencing　Bionanosensing　Diffusiophoresis　2D materials　Joule heating　Diffusion current

１．研究開始当初の背景

The nanopore technology using biological pores embedded in a lipid bilayer has been proven to be an efficient alternative to the conventional DNA sequencing methods. Theoretically, artificial solid-state nanopores possess the advantages of mechanical strength, modifiable geometry and stable chemical properties over the biological nanopores, and therefore they should be more favorable for biomolecule detections. However, in reality solid-state nanopores are confronted by two major obstacles of spatial and temporal resolution limitations hindering them from practical sensing applications. Comparing with the gap between DNA base pairs is merely 0.3 nm, the thickness of conventional silicon nitride membranes is approximately two orders of magnitude larger. To resolve this, two-dimensional materials nanopores are recently selected to enhance spatial resolution whose thicknesses coincide with the distance between each nucleotide (e.g. the thickness of a monolayer molybdenum disulfide is 0.65 nm). Nevertheless, although these ultrathin nanopores conceptually promise single nucleotide resolution, they still suffer from the temporal resolution issue and thus no directly DNA sequencing results have been achieved due to the excessively fast translocation of the molecules through the nanopore. Another daunting issue would be concurrent Joule heating effects when applying an electric potential difference over a short distance, which could result in superheating effects in the nanopore denaturing the DNA molecules.

２．研究の目的

To accelerate the advances of nanopore technology for medical diagnosis and precision medicine, it is of primary importance to have a quick, low-cost and reliable DNA sequencing device based on solid-state nanopores which is yet to be available. Therefore, the objective of this study is to develop a new method achieving the world's first nanopore DNA sequencing using two-dimensional nanopores and two main improvements will be done: (i) enhancing temporal resolution and (ii) removing thermal instability. We propose a novel nanopore sensing method using diffusion current when a salt concentration difference is applied across a nanopore. As illustrated in Figure 1, apart from the conventional conduction current-based nanopore sensing (Figure 1a) that uses a uniform electrolyte, two electrolyte solutions possessing different salt concentrations are filled on each side of the nanopore (Figure 1b). Owing to the charge possesses by the nanopore, ions selectively pass through the nanopore. For positively charged nanopores, anions are able to migrate through the pore freely while cations are blocked due to the electric repulsive force. On the other hand, negatively charged nanopores are cation selective. This selectivity gives rise to a diffusion current through the nanopore due to the non-equal ion fluxes from cations and anions, which can be used for molecule detection. The translocation of molecules in the nanopore is driven by the diffusiophoretic (DP) force that pushes molecules toward the high concentration end. When the DNA molecule translocates from the low salt concentration end toward the high salt concentration end, the presence of the molecule will intervene the diffusion ion transport through the nanopore. As a result, a current blockage (or enhancement) can be detected for DNA sequencing.

There are two primary benefits of the proposed diffusion current method worth highlighting: (i) due to a different driving force (diffusiophoresis), the translocation speed of the DNA molecules will be significantly reduced enhancing temporal resolution; (ii) Joule heating effects will be completely eliminated reducing thermal instability and thus much low thermal noise is expected.

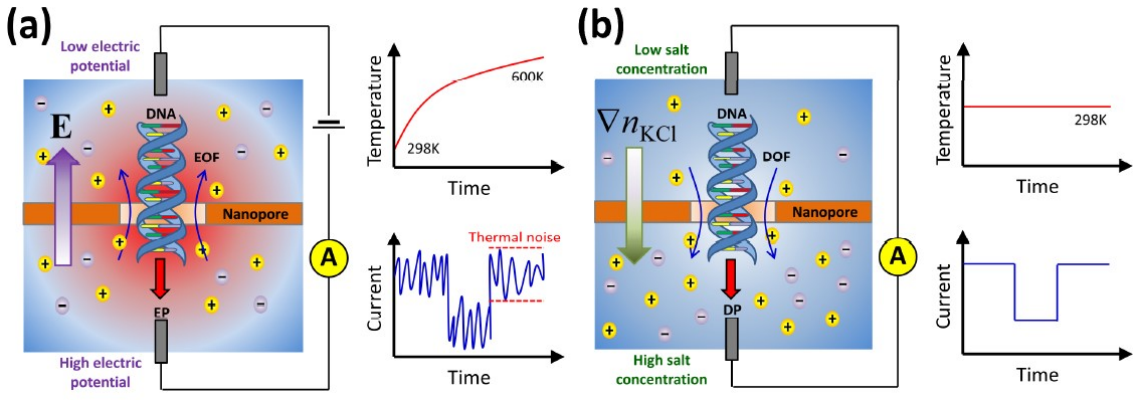

Figure 1. (a) Conventional conduction current DNA sensing using an electric field. (b) Diffusion current DNA sequencing using a salt concentration gradient.

３．研究の方法

[Theoretical]

For both cases, the electrokinetic behavior of the electrolyte solution and DNA molecule can be described by a modified Poisson equation (including the charge from the DNA molecule), Nernst–Planck equations, continuity, and a modified Navier–Stokes equation (including a drag force term considering the friction between the solution and DNA molecule) as follows:

$$\nabla^2\psi = -\frac{\rho_e + \rho_{DNA}}{\varepsilon_0\varepsilon} \tag{1}$$

$$\nabla\cdot\mathbf{J}_\alpha = \nabla\cdot\left[-D_\alpha(\nabla n_\alpha + \frac{z_\alpha e}{k_B T} n_\alpha \nabla\psi) + n_\alpha \mathbf{v}\right] = 0 \tag{2}$$

$$-\rho\nabla\cdot\mathbf{v}\mathbf{v} - \nabla p + \eta\nabla^2\mathbf{v} - \rho_e\nabla\psi - \gamma(\mathbf{v} - v_{DNA}) = 0 \tag{3}$$

$$\nabla\cdot\mathbf{v} = 0 \tag{4}$$

In these expressions, ψ is the electric potential, ρ_e is the solution space charge density, ρ_{DNA} is the volume charge density of the DNA molecule, ε_0 is the vacuum permittivity, ε is the dielectric constant of water, e is the element charge, k_B is the Boltzmann constant, T is the temperature, $\mathbf{v}$ is the flow velocity vector, v_{DNA} is the DNA translocation velocity, ρ is the density, p is the pressure, γ is the friction coefficient between the solution and DNA molecule and η is the viscosity. In addition, $\mathbf{J}_a$, D_a, n_a, z_a are the ionic flux vector, ionic diffusivity, ionic concentration and valence of the cations or anions.

Symmetry boundary conditions are applied on the centerline and the top boundary of each reservoir. The salt concentration and electric potential are set as the bulk values at the left end of the *cis* reservoir and right end of the *trans* reservoir, where the flow is assumed to be fully developed. At the interface between the DNA molecule and the solution, the ionic concentrations, electric potential, flow velocity and their gradients are considered to be continuous. On the nanopore surface, the no-slip and zero ionic fluxes boundary conditions are employed, and the surface charge density σ (= -10 mC/m^2) is given based on the following equation:

$$\mathbf{n}\cdot\nabla\psi = -\frac{\sigma}{\varepsilon_0\varepsilon} \tag{5}$$

where $\mathbf{n}$ is the normal vector on the nanopore surface pointing toward the solution. A pseudo steady-state model is used to predict the DNA translocation velocity. Namely, the DNA velocity is derived as the sum of the electric force and hydrodynamic force becomes zero.

A hybrid mesh is constructed for computational simulation using open source software Gmsh as demonstrated in Figure 2, where the blue mesh area indicates the electrolyte solution and the yellow area refers to the ion penetrable DNA molecule. Structured rectangular cells are used in the vicinity of the nanopore surface and within the DNA molecule due to a steeper change of variables in these areas. On the other hand, unstructured mesh arrangement composed of triangular cells is made for the rest area of the simulation domain to minimize the total number of cells. The coupled governing equations with the above boundary conditions are resolved by an implicit finite volume method using an open source package, *arb*.

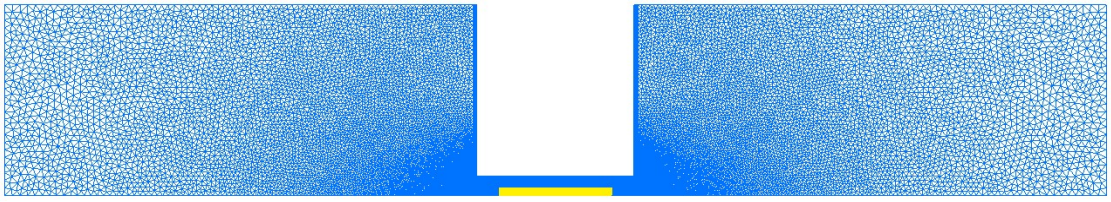

Figure 2. A hybrid mesh used in the simulation. The yellow region indicates the ion-permeable DNA molecule.

[Experimental]

Nanpore experiments are conducted according to the following steps as summarized in Figure 3. (I) We first drilled a nanopore (~ 500 nm in diameter) using focused ion beam (SMI3050: SII Nanotechnology) on a SiNx membrane on top of a Si substrate with a square window of 100μm at its center. (II) A MoS_2 thin layer (~ 10 micron × 10 micron) was synthesized on an O_2 plasma-treated SiO_2/Si substrate and then was mounted onto the SiNx slab covering the opening by a chemical transport method. (III) Following that, a nanopore was sculptured by electron (e-beam) irradiation under transmission electron microscopy (TEM). (IV) The two-dimensional

nanopore chip was attached onto a cell (solution tank) made by a 3D printer using epoxy resin adhesive and then sealed by another reservoir cell. (V) The solution tanks were poured with different concentrations of electrolyte solutions and analytes were added into the high concentration reservoir. After inserting Ag/AgCl electrodes into each reservoir, an ultra-low noise current measurement system was employed to detect ultra-low noise current signals. Given that it took tremendous time and effort to fabricate MoS_2 nanopores, a nanopore on a 20 nm thick SiNx nanopore prepared by dielectric breakdown was also used for preliminary tests, which can be made after applying a high electric potential difference (8V) across the membrane for 1-2 hours.

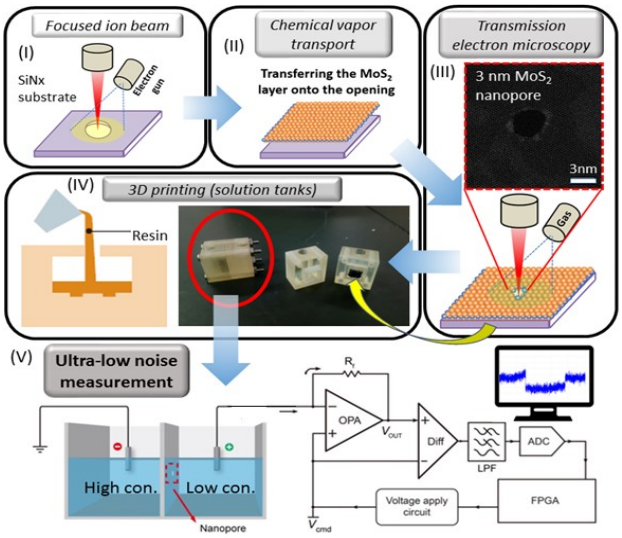

Figure 3. Flowchart of the experimental steps.

4．研究成果

The theoretical distributions of concentration of potassium ions, concentration of sodium ions, electric potential and the flow fields for both electrophoretic and diffusiophoretic cases are reported in Figure 4. Due to the negative charges on the DNA molecule, the cation concentration within the DNA molecule is higher than the bulk solute concentration. In contrast, the chloride ions are repelled from the DNA molecule resulting in a lower concentration than the bulk value.

Regarding the electrophoretic transport system, an electric field exerted between two reservoirs on an ion selective nanopore yields considerable ionic concentration polarization effects. More ions accumulate close to the cis end of the nanopore while the ion depletion occurs at the other end. The external electric field drives the negatively charged DNA molecule toward the *trans* reservoir. In the diffusiophretic case that a concentration gradient is applied, the nonuniform accumulation (along the axis) of cations inside the DNA molecule induces a polarization electric field pushing the negatively charged molecule to the *trans* reservoir (high concentration end). Similar phenomenon occurs in the vicinity of the nanopore surface ending up a higher electric potential when approaching to the *trans* end. However, a notable electric polarization potential (positive) is induced at the junction between the cis reservoir (low concentration) and the nanopore pushing the positively charged solution to the *trans* reservoir. The underlying mechanism of this behavior is due to the polarization of co-ions (type II polarization) happening at the outer region within the electric double layer (diffusion layer).

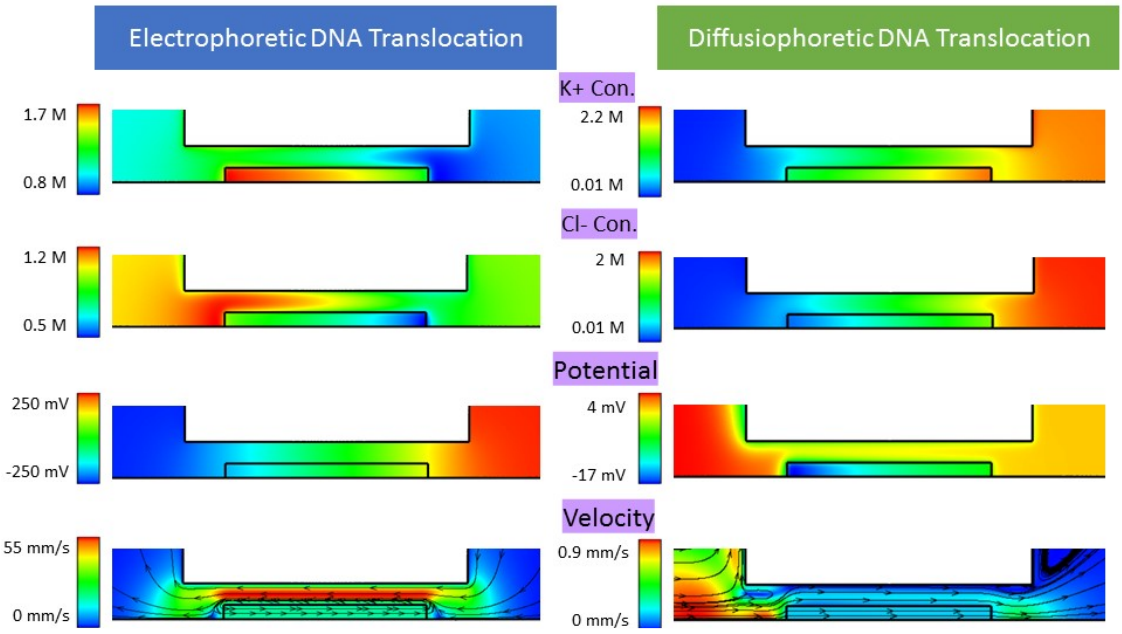

Figure 4. Contours of simulation potassium ion concentration, chloride ion concentration, electric potential and flow field in the cases of electrophoretic DNA translocation (left) and diffusiophoretic DNA translocation (right).

The experimental results are shown in Figure 5. Note that, consistent with the literature, no blockage signals were obtained for 20-mer ssDNAs passing a monolayer molybdenum disulfide nanopore (4 nm in diameter) using the conventional conduction current-based resistive pulse sensing. It was primarily due to the excess translocation speed. Figure 5a shows typical DNA translocation signals in a 20 nm thick silicon nitride nanopore. No structural information had been revealed as expected. On the contrary, as indicated in Figure 5b, the proposed isothermal diffusion current sensing of 20-mer ssDNAs showed unambiguous structural information using the 4 nm (diameter) monolayer molybdenum disulfide nanopore. The histogram of the sensing results are plotted in Figure 5c, four peaks indicating different nucleotide acids can be clearly observed, evidencing the proposed diffusion current method can be a simple but powerful tool for real time nanopore DNA sequencing using artificial materials.

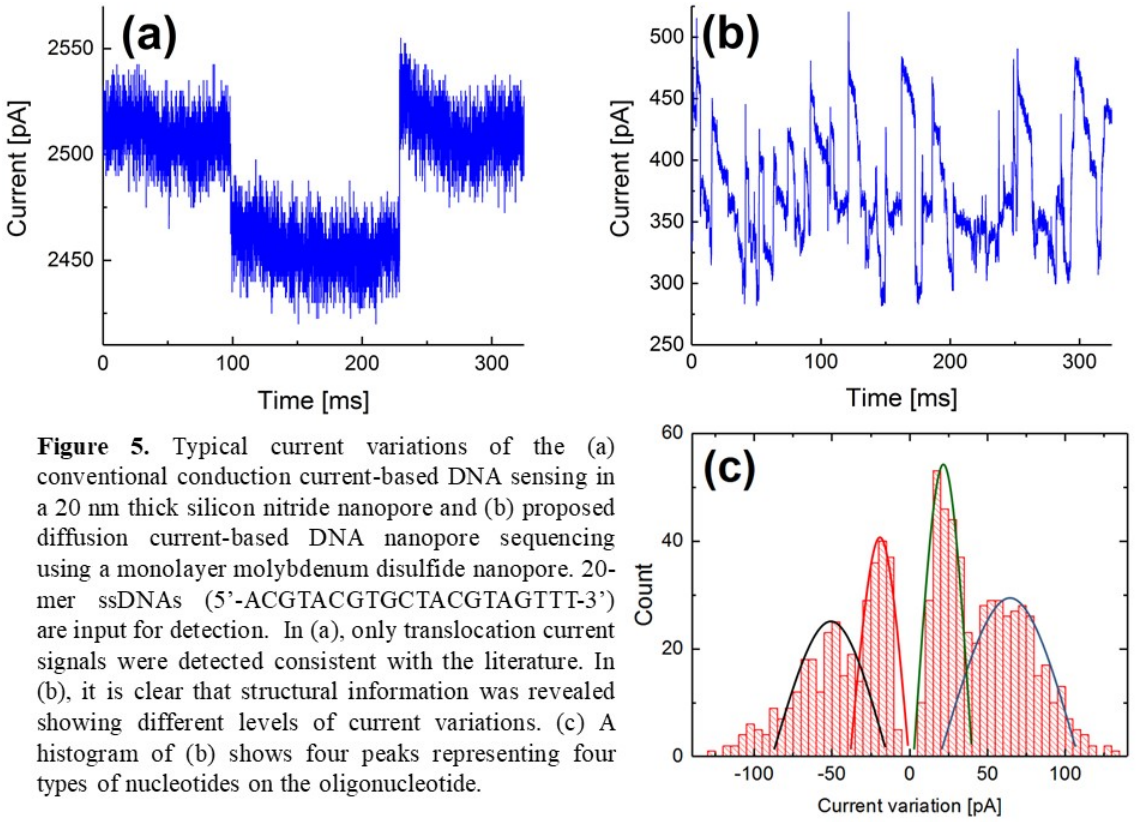

Figure 5. Typical current variations of the (a) conventional conduction current-based DNA sensing in a 20 nm thick silicon nitride nanopore and (b) proposed diffusion current-based DNA nanopore sequencing using a monolayer molybdenum disulfide nanopore. 20-mer ssDNAs (5'-ACGTACGTGCTACGTAGTTT-3') are input for detection. In (a), only translocation current signals were detected consistent with the literature. In (b), it is clear that structural information was revealed showing different levels of current variations. (c) A histogram of (b) shows four peaks representing four types of nucleotides on the oligonucleotide.

In summary, we have invented an effective method to detect structural information of short oligonucleotides using solid-state nanopores (for the first time in history). Supported by theoretical analysis, the experimental results indicate that the slow diffusiophoretic behavior plays a key role of the success of this novel method. Several advantages of the proposed isothermal nanopore sensing using diffusion current exist over the conventional resistive pulse sensing using an external electric field across a nanopore, including (i) improvement of the capture rate, (ii) extension of the translocation time and elimination of thermal instability. These results not only improve the development of molecule sequencing technology, but, with the superb flexibility in the pore size, open up new opportunities to high resolution structure analysis for a wide range of biological entities, leading to a new era of nanopore biosensing.

５．主な発表論文等

〔雑誌論文〕（計　1　件）

〔学会発表〕（計　4　件）

〔図書〕（計　0　件）

○出願状況（計　1　件）

名称：固体ナノポア分子シーケンサー
発明者：徐偉倫（Wei-Lun Hsu）

権利者：東京大学
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名称：
発明者：
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６．研究組織

(1)研究分担者
研究分担者氏名：
ローマ字氏名：
所属研究機関名：
部局名：
職名：
研究者番号（8 桁）：

(2)研究協力者
研究協力者氏名：大宮司　啓文
ローマ字氏名：Hirofumi DAIGUJI
研究協力者氏名：岩佐 義宏
ローマ字氏名：Yoshihiro IWASA
研究協力者氏名：阿部 英司
ローマ字氏名：Eiji ABE
研究協力者氏名：Ying Yi-Lun
ローマ字氏名：Yi-Lun YING
研究協力者氏名：Long Yi-Tao
ローマ字氏名：Yi-Tao LONG