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Study on Dynamics of Particles in Extended Solid State Nanopore

August 2021

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Chapter 1. General introduction

Coulter Counter is an apparatus patented in 1953¹ as a sensor useful for counting and sizing vessel in blood vessels.² The principle of operation is based on measurements of temporal changes in the electric current associated with the interference of the electric field by particles passing through a micrometer-scale channel that serves as a sensing zone. The advance in semiconductor technologies drove up this resistive pulse sensing method to an emerging technology called solid-state nanopore sensing capable of detecting a nanometer-scale objects of variable size from viruses to DNA by a simple electric current measurement.

The first idea of nanopore sensing originates from an intriguing concept of using a tiny hole formed in a transmembrane protein. In 1996, Kasianowicz and co-workers demonstrated resistive pulse detections of polynucleotide molecules electrophoretically passed through a few nano-meter diameter pore in α -haemolysin³. Since this first report, tremendous efforts were devoted to realize sequencing by nanopore measurements, *i.e.*, discriminations of adenine, thymine, cytosine, and guanine by the difference in the ionic current flowing through the channel upon a single-stranded DNA translocate through there. After three decades of extensive researches in the field, Cherf et al. finally succeeded in distinguishing DNA bases by leveraging the amazing ratcheting motions of the molecule through phi29 polymerase reactions in vicinity of a bionanopore⁴. Nowadays, the bionanopore sequencers are used as commercialized devices in various practical scenes as a tool for transcriptome and metagenome analyses.

However, the bionanopore sensor was known to become unstable under subtle change in the environment like mechanical stress, salt concentration, temperature, pH and so on. Therefore, a solid state nanopore has attracted much attention as an alternative structure with better stability. In fact, it was as early as 2000s when a nanoscale hole comparable to the diameter of DNA was fabricated by irradiating an electron or ion beam on a several tens of nanometer thick dielectric membrane made of SiN_x or $SiO_2^{5,6,7}$. These solid-state nanopores were also proven useful for detecting DNA at the single molecule level by the Coulter principle, which spurred many studies on the translocation dynamics. Later, much works was performed to enhance the spatial resolution of the sensor so as to enable single-nucleotide discriminations by ionic current,⁸ where two-dimensional materials such as graphene and MoS_2 were employed as ultra-thin membranes of single-atom thick. Recent work by Radenovic et al. proved the usefulness of the 2D nanopores by demonstrating identifications of the four nucleotides by the difference in the resistive pulse heights.⁸

Whereas the sensor concept explained above is based on Coulter principle to discriminate the size of objects via the amount of ionic current blockade, advance in the nanopore technology also led to novel ideas to analyze molecules and particles by their motions. For example, a pore in an ultra-thin membrane with a low depth-to-diameter aspect ratio structure was found to be capable of sensing particle motions not only inside but also at the orifice of the channel due to the predominant roles of the Maxwell-Hall access resistance on the cross-pore ionic current. This unique ability was utilized to estimate not only the size and shape of particles and molecules but also their surface charge and even the dipole moments by the resistive pulse waveforms.

This research aims to deepen the fundamental understanding of the translocation motion of particles in nanofluidic channels to enable a sensor capability beyond the Coulter principle. On Chapter 2, I introduce fundamental basis for understanding the Coulter principle, ion distribution around charged particles and the process of manufacture of nano-architecture.

Chapter 3 presents a nanopore device for accurate measurements of the fast translocation dynamics of objects. Because of the nanoscale depth of the conduit, the analytes pass through the sensing zone quite instantaneously causing critically narrow resistive pulse signals that may not be detectable by common ionic current measurement systems. In order to accomplish reliable nanopore sensing of small molecules and particles, therefore, previous efforts have been devoted to establish a means to slow down the electrophoretic velocity of the objects⁹⁻¹². One may think that such a thing can be easily attained by lowering the cross-membrane voltage. However, since lower voltage also makes the resistive pulse signals smaller, it leads to degraded signal-to-noise ratio. Much of the previous works have thus sought for ways to retard the electrophoretic motions by adding external probes such as light and electric field^{13,14}. More simply, it was also examined to slow down the translocation by increasing the viscosity and lowering the temperature of the solution media^{15,16}. Recently, more complicated yet effective approaches have been reported that uses salt and viscosity gradients across the nanofluidic channels¹⁷. Besides these efforts, it has also been recognized as an important issue to clarify how fast the ionic current responses to the actual translocation motions of the objects. For example, the electrolyte solution-filled nanopore can be seen as resistors and capacitors connected in a complicated fashion. It therefore anticipates signal retarding at certain extents by the finite RC time constant. Despite the importance, however, there was no established model to estimate the response time of the ionic current, which makes it difficult to even discuss the reliability of the nanopore sensing method for tracking the fast translocation events. In this study, therefore systematic experiments were performed to clarify an optimal nanopore device configurations for the accurate motion tracking of small particles and molecules via the ionic current measurements.

After establishing the nanopore sensing method for tracing the fast electrophoresis of objects in nanofluidic channels, it was examined to explore the detailed physical picture of translocation dynamics of analytes. Conventionally, the translocation motions of molecules such as proteins and DNA are considered to be in steady state moving at a constant speed under the balance between the electrophoretic and hydrodynamic drag forces. Nonetheless, the fact that they are accelerated by the focused electric field at the pore entrance and then decelerated by the viscous dragging at the exit brings another picture that there should be a finite effect of inertia on the actual motions. Classical theory treats the problem in terms of Stokes time that certainly predicts weaker inertial effects for smaller objects in liquid. However, one should also keep in mind that the speed of objects inside nanopores is also often high as denoted by the very short translocation time. In order to investigate the roles of inertia in the electrophoretic translocation dynamics of small objects, therefore, a novel nanofluidic system was designed that had multiple nanochannels in series as elaborated in Chapter 4. While the contributions of inertia were very weak for the dimensions of the system tested, it allowed to elucidate its influence in a form of changes in the particle trajectories. More importantly, these findings led to a device concept for analyzing the mass and surface zeta potential of individual particles and molecules such as amyloids and proteins in liquid via the ionic current measurements.

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Chapter 2. Fundamentals and Method

Nanopore sensing measures ionic current through a hole that occurs via electrochemical reactions at the interfaces between the electrode surface and electrolyte solution. The amount of current is usually dominated by the ion transport in the conduit due to its small size. When an object gets through there, it partially blocks the ion migration thereby causing temporal suppression of the ionic current. In this chapter, I first elaborate the theory behind the ionic current in nanofluidic channels followed by descriptions of the actual ways for fabricating the conduits.

2.1. Electrical Resistance of Nanopore and Nanochannel

Ionic current is induced by the bias voltage applied to a nanopores or nanochannels filled with electrolyte solution. The resistance is governed by the largest one at the nanoscale conduit R_{pore} given by the resistivity of the solution ρ and the channel volume under the ohm's law.

$$R_{\rm pore} = \frac{4\rho L}{\pi d^2}$$

where *L* and *d* are the thickness and the diameter of the pore. The rule is valid when the thickness-todiameter aspect ratio is high for the case that a cylindrical channel but the discussion is assumed essentially the same for conduits of different shapes. However, when the aspect ratio becomes lower than 1, the simple model is needed to be modified¹. This is because of two aspects: one is that the strong electric field focused at the nanopore orifice gives rise to additional resistance known as Maxwell's access resistance R_{acc} ; whereas R_{acc} is negligibly small compared to R_{pore} in the high-aspectratio nanopores, it starts to be important in shallow channels for the access resistance changes only with the diameter of the channel as shown below².

$$R_{\text{total,pore}} = R_{\text{accsess}} + R_{\text{pore}}$$

 $R_{\text{accsess}} = \frac{\rho}{2d}$

While both are resistance, their physical meaning is completely different; R_{access} shows the potential variation at the nanopore openings while R_{pore} represents the resistivity of ions inside the pore.



Figure 2.1. 3D schematic of nanopore.

This simple model in electromagnetics was found useful in understanding the ionic conductance of solid-state nanopores of a vast size range from $10 \,\mu\text{m}$ to several nm due to the fact that the ion transport in water is a diffusive process with very short mean free path. Meanwhile, more precise analyses call for estimations of ion distributions depicted by Poisson-Nernst-Planck (PNP) equations^{3,4}.

$$R_{\text{total.channel}} = \frac{\rho}{2} \left(\frac{d}{hw} + \frac{L_1}{H_1 W_1} + \frac{L_3}{H_3 W_3} + 2f_1 + 2f_3 \right)$$

$$f_i = \frac{2}{wh W_i H_i} \sum_{n,m=1}^{\infty} \frac{\sin(\lambda_n^{(i)} h) \sin(\gamma_m^{(i)} w)}{\kappa_{n,m}^{(i)} \lambda_n^{(i)} \gamma_m^{(i)}} \tanh(\kappa_{n,m}^{(i)} L_i)$$

$$+ \frac{1}{h W_i H_i} \sum_{n=1}^{\infty} \frac{\sin(\lambda_n^{(i)} h)}{(\lambda_n^{(i)})^2} \tanh(\lambda_n^{(i)} L_i) + \frac{1}{w W_i H_i} \sum_{m=1}^{\infty} \frac{\sin\left(\gamma_m^{(i)} w\right)}{(\gamma_m^{(i)})^2} \tanh(\gamma_m^{(i)} L_i)$$

$$\lambda_n^{(i)} = \frac{\pi m}{H_i}$$

$$\gamma_m^{(i)} = \sqrt{\left(\lambda_n^{(i)}\right)^2 + \left(\gamma_m^{(i)}\right)^2}$$

where ρ is the resistivity, w is width of narrowed portion, W_i is width of broad portion, l is length of

narrowed portion, L_i is length of broad portion, h is height of narrowed portion, H_i is width of broad portion, f_i shows the focusing of field lines from the broad portion to the narrowed portion.



Figure 2.2. 3D schematic plot of the nanochannel.

The above model is for the ion transport in the channel with no object inside. When the electric field is disturbed via the particle translocating through the channel, the resistance of the conduit becomes⁵

$$R_{\text{total}} = R_{\text{b}} + R_{\text{s}}$$
$$R_{\text{b}} = \frac{8\rho}{\pi} \int_{0}^{r_{0}} \frac{dz}{d_{\text{p}}^{2} - 4r_{0}^{2} + 4z^{2}}$$
$$R_{\text{s}} = \frac{4\rho(L_{\text{eff}} - 2r_{0})}{\pi d_{\text{p}}^{2}}$$

where R_b is the resistance in the region that the particle blocks the channel, R_s is the other in the channel except for R_b . d_p is the diameter of the pore, r_0 is the radius of the particle, ρ is the resistivity of electrolyte, L_{eff} is the effective transport length of the pore that is defined as equal to the sum of the distances from the nanopore center where the electric field is at its maximum to the points along the central axis where their electric field falls to e^{-1} of this maximum.



Figure 2.3. schematic model of nanopore and particle.

2.2. Ion distribution around charged particles

Around the fine particles in the electrolyte solution, ions having the opposite sign of the surface charge of the fine particles (counter ion) gather, and ions having the same sign (co-ion) move away. Many counter ions and a small number of co-ions are floating around the fine particles like a cloud, and this ion cloud is called an electric double layer. Figure 2.4. shows the diffused electric double layer around the charged spherical particle with radius *a*. The thickness of the electric double layer is represented by $1/\kappa$, and the interfacial electrical phenomenon depends on the ratio κa of the particle size and the thickness k of the electric double layer. The model shown in Fig. 2.4. is called the G model and treats ions as point charges.



Figure 2.4. Schematic model of the diffused electric double layer.

2.3. COMSOL multiphysics simulation

COMSOL uses the finite element method to perform numerical calculations on the initial value and boundary value problems of partial differential equation (PDEs). Two or more types of physical phenomena can be combined and analyzed. In this study, the ion density and the electric field derived from the electric field are represented by Poisson Boltzmann equation, the diffusion of ions and their movement derived from the electric field are addressed by Nernst-Planck equation, and the flow of the aqueous solution by pressure and electroosmosis are expressed by Navier-Stokes equation. The steady-state solution is obtained by using these three equations as iterations in the software.

Poisson Boltzmann equation is the following.

$$\varepsilon_{\rm r}\varepsilon_0\nabla^2\phi = -\rho_{\rm e}$$

 $\rho_{\rm e}$ is charge density, ϕ is potential, $\varepsilon_{\rm r}$ is relative permittivity, ε_0 is permittivity of free space. It shows the relationship between charge and potential distribution in an electrolyte solution, which allows us to determine the potential distribution when a point charge is placed at a certain location. Nernst-Planck equation is the following.

$$\frac{\partial c_{i}}{\partial t} + u \cdot \nabla c_{i} = \nabla \cdot (D_{i} \nabla c_{i} + \mu_{m,i} c_{i} \nabla \phi)$$

 c_i is ionic density, i is ionic species (anion, cation), u is velocity of solution, D is ionic coefficient of diffusion, μ_m is electrophoretic mobility. It describes the motion of charged species in a fluid medium based on the law of conservation of mass.

Navier-Stokes equation is following.

$$\rho\left(\frac{\partial u}{\partial t} + (u \cdot \nabla)u\right) = -\nabla p + \mu \nabla^2 u + \rho_{\rm e} \nabla \phi$$

In the equation, μ is viscosity, ρ is density, and p is pressure. It represents the motion equation of a fluid.

2.4. Device Fabrication

2.4.1 Nanopore Fabrication

A 4-inch silicon wafer both-side coated 50 nm SiN_x layer was ultrasonicated for 15 minutes in *N*,*N*dimethylformamide (DMF) and UV-exposed at 300 degrees Celsius for 30 minutes to remove organic substances on the surface. Subsequently, the wafer was spin-coated in positive type photoresist AZ5260A on one side and photo-lithography and developing in NMD-3 and RF sputtered and lift-off in DMF to print marks for Electron beam (EB) lithography. The printed wafer was cut into 25 mm x 25mm chips. Each chip was spin-coated with positive type photoresist TSMR at the back side and wet-etched to make a SiN_x membrane. Finally, the chips were spin-coated with electron beam resist ZEP520A on the membrane and dry etching to drill hundreds nm size pore. Some of the chips were spin-coated with photoresist PW-1230 on the membrane side and photo-patterned a few µm diameter circle and developed in NMD-3 and baking to make 3 um polyimide layer.





Figure 2.5. Fabrication process of nanopore.

2.4.2. Nanochannel Fabrication

A 4-inch silicon wafer both-side coated 50 nm Si₃N₄ layer was ultrasonicated for 15 minutes in N, N-dimethylformamide (DMF) and UV-exposed at 300 degrees Celsius for 30 minutes to remove the organic substance on the surface. The wafer was cut to 25 mm x 25 mm by hand. The chips were RF sputtered to a few tens Cr layer for a mask and were spin-coated with electron beam resist ZEP520-A on the membrane and dry etching to drill the hundreds nm size channel. Finally, the Cr layer that had served as a mask was removed by etching.





Figure 2.6. Fabrication process of nanochannel.

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Chapter 3. Crucial Role of Out-of-Pore Resistance on Temporal Response of Ionic Current in Nanopore Sensors

3.1. Intoroduction

Resistive pulse analysis is a sensor technology used for the detection and identification of single particles in electrolyte solution.^{1–4} The principle is based on measurements of the ionic current flowing through a nanoscale through-hole (nanopores) opened in a thin solid membrane. Each time when a particle passes through the pore, the in-pore ion flow is hindered, causing a pulse-like change in the current. As the temporal change in the ion transport is highly sensitive to the physical features of the analytes such as the size, shape, and surface charge density, one can discriminate them by analyzing and comparing the fine profiles of the resistive pulse signals.^{5–8}

Meanwhile, in this method, it is a prerequisite to be very sure what actually observed in the ionic current measurements is of transient ion blockage associated with the translocation of single particles since the charging/discharging at the stray capacitance may cause a considerable influence on the current signals.^{9,10} More specifically, it has recently been reported that this effect may significantly retard the temporal response of ionic current against the ion blockade phenomena occurring upon the fast translocation of single particles through a nanopore. An equivalent circuit model^{9,11} has been proposed to describe the signal retardation,^{12,13} wherein the degree of signal smearing is expressed by a time constant given as the product of stray capacitance and resistance. Meanwhile, this poses a question: how come the resistive pulse method work in nanopores with a diameter of several nanometers to detect signals way shorter than 0.1 ms with the large pore resistance usually at a G Ω level, ^{14–16} as it should yield the time constant as long as 1 ms even under an assumption of small capacitance as low as 1 pF. To shed light on the intriguing role of the signal retardation-relevant

resistance, a change in the resistive pulse waveforms for a nanopore of specific geometry but with different out-of-pore resistance conditions was herein investigated to elucidate the origin of resistance components contributing to the current response.

3.2 Results and Discussions

The ionic current between the cis and trans chambers separated by a 50 nm thick Si₃N₄ membrane was measured under a DC applied voltage V_b of 0.1 V. In the membrane, a pore with a diameter d_{pore} was penetrated using nanofabrication technology. Each chamber was filled with phosphate-buffered saline (PBS) diluted to 0.4 × PBS with ultrapure water (Merck Millipore) or a dispersion solution of carboxylated polystyrene particles with a diameter d_{ps} at a concentration of c_{ps} in the same buffer. Unless otherwise stated, d_{pore} , d_{ps} , and c_{ps} are 300 nm, 200 nm, 1.8 × 10⁸ particles/ml. The fluidic channel in the PDMS block had a rectangular shape of 0.4 mm wide and 12.1 mm long with the height ranging from 50 µm to 700 µm. Because of the relatively large volume of the channel compared to the 300 nm-sized nanopore, the ionic conductance remained almost the same irrespective of the range of the channel heights tested. Meanwhile, the Ag/AgCl electrode was installed at one of the three positions with respect to the position of the nanopore: at the top (hereinafter referred to as the short position), 3 mm away (hereinafter referred to as the middle position), or 6 mm away from the pore (hereinafter referred to as the long position) (Fig 3.1). In this way, the resistance outside the pore was further modulated to testify its role on the signal retardation.



Figure 3.1. (a) Schematic model depicting the nanopore device setup and (b) electrophoretic translocation of a 200 nm-sized polystyrene nanoparticle through the 300 nm-sized nanopore. (c) Scanning electron micrograph of a nanopore with diameter 300 nm sculpted in a 50 nm-thick Si₃N₄ membrane. (d) Dimensions of the microfluidic channels formed in PDMS blocks. $L_{ele} = 0$ mm position is located at the top of the nanopore.

Resistive pulses were observed when measuring I_{ion} in the nanoparticle dispersion solution with 300 nm-sized nanopores regardless of the channel height as well as the electrode position conditions (Fig 3.2a). Here, the electrodes were first placed at the top of the nanopore ($L_{ele} = 0$ mm). Subsequently, after recording more than 100 resistive pulses, they were moved to $L_{ele} = 3$ mm. It should be noted here that the base current is around 18 nA, which is approximately equal to the bias voltage divided by $R_{acc} + R_{pore}$ where $R_{acc} = \rho/d = 4.1$ M Ω is the access resistance with ρ and d being, respectively, the resistivity of the electrolyte buffer and the diameter of nanopore and $R_{pore} = 4\rho L/\pi d^2 = 0.9$ M Ω is the resistance in the pore of length L,¹⁷⁻²⁰ irrespective of L_{ele} due to that the resistance at the channel is sufficiently smaller than the resistance at the nanopore (The slightly lower current (about 6 %) at $L_{ele} = 3$ mm than that at $L_{ele} = 0$ mm in Fig. 3.2a is presumably a cause of the resistance at the 50 µm fluidic

channel). In contrast to the little contribution to the net cross-membrane resistance, L_{ele} led to a marked change in the pulse heights I_p to become much smaller when moving the electrodes from $L_{ele} = 0$ mm to 3 mm (by more than a factor of 3 as displayed in Fig. 3.2b). It was also confirmed that when returning the electrodes to $L_{ele} = 0$ mm, the pulses again became larger (see Fig. S3.1). This is surprising as, according to the Coulter principle^{21,22}, the voltage division at the small PDMS channel resistance is anticipated to give less than 10 % change in the pulse heights for the conditions in Fig. 3.2a. It is therefore likely that a non-Coulter-principle mechanism is playing an important role in the resistive pulse measurements. Similarly, the resistive pulses were observed to become weaker with decreasing the channel height (Figure 3..3a). These results consistently show the significant influence of the out-of-pore resistance on the pulse waveforms. Signal retardation by RC effects can be a possible explanation for the anomalous feature as the larger external resistance is expected to give smaller pulses, which in turn suggests that the resistance component contributing to the temporal response of ionic current was actually not the resistance at the pore but the access resistance outside the channel.^{9,10,23,24}



Figure 3.2. Ionic current traces recorded in a dispersion solution of 200 nm-sized polymeric beads in 0.4 x PBS under the applied DC voltage of 0.1 V using PDMS channel with $d_c = 50 \ \mu m$. (a) Ionic current curves obtained at $L_{ele} = 0 \ mm$ (blue) and $L_{ele} = 3 \ mm$ (red). (b) The difference in ionic current pulse heights. The open pore current is offset to zero. (c) A magnified view of a resistive pulse. (d) The pulse tail. Orange curve if a fit with the exponential function with the time constant τ .



Figure 3.3. (a) Pulse height and (b) time constant plotted as a function of the channel height d_c (blue: $L_{ele} = 0$ mm; red: $L_{ele} = 3$ mm).

In order to investigate the intriguing observations in more detail, the RC time constant τ was extracted from the resistive pulses measured by the following procedure (Fig 3.2d). More than 100 pulses were first merged into one pulse by arithmetic averaging, and the tail portion of the averaged signal (defined as the region where the ionic current falls in a window between 15 % to 1 % from the pulse top) was fitted exponentially by $I_{ion} = I_0 \exp(-t/\tau)$. The time constant obtained by this was indeed found to change with L_{ele} as well as d_e (Fig 3.3b). As for the channel height, it provided longer RC in shallower channels. On the other hand, the time constant was found to become longer as the position was changed from $L_{ele} = 0$ mm to 3 mm. Both of these tendencies can be interpreted as positive contributions of the resistance outside the pore on the time constant as the shallower channels and the longer distance from the electrodes to the nanopore both lead to larger out-of-pore resistance. More quantitatively, this resistance is given as $R_{ch} = \rho L/S$ where $\rho = 2 \Omega m$ is the resistivity of the electrolyte buffer while *S* and *L* denote the cross-sectional area and the length of the fluidic channel, respectively. Tentatively defining the time constant τ as $R_{ch}C_m$ with $C_m = 4.55$ nF estimated from a parallel –plate model ($C_m = \epsilon_{0}\epsilon_{SDN4}S/L$ where ϵ_0 is the dielectric constant of vacuum, $\epsilon_{SDN4} = 7.3$ is the relative permittivity of Si₃N₄, L = 50 nm is the thickness of the Si₃N₄ layer, $S = 14 \mu m^2$ is the area of the PDMS channel), Values that agree with the experimental τ within an order of magnitude was obtained. Here, it is noticeable that R_{ch} is amounting only several tens of k Ω , which is about two orders of magnitude smaller than R_{acc} and R_{pore} . These results clearly demonstrate that contrary to *a priori* anticipation, the temporal response of the ionic current is in fact affected only little by the resistance at the nanopore and predominantly that at the remote regions.



Figure 3.4. (a) Pulse height and (b) time constant obtained with polyimide-coated (green) and noncoated (red) nanopore chips. The data at $L_{ele} = 6$ mm were not shown because the signal-to-noise ratio was too low to reliably extract the pulses from raw data curve.

In contrast to the time constant, the pulse height revealed a slightly more complicated dependence on dc. Specifically, while there is an overall trend that Ip decreases upon moving the electrodes from Lele = 0 mm to 3 mm (Fig. 3.3a), it was shown to increase with increasing channel height in some cases such as those at dc = 100 μ m. This counterintuitive result is ascribed to a cause of a variation in the translocation speed of the nanoparticles. When RC time constant is much longer than the translocation time of the nanoparticles, the resistive pulse height become sensitive to the corresponding translocation motion of the particles resulting in higher (lower) I_p when they pass through the channel slower (faster), which can be in fact found in the pulse height versus pulse width scatter plots (Fig. S3.2). On the other hand, as the translocation velocity would affected by many factors including van der Waals interactions at the pore wall surface, it shows slightly different variations on different experiments thus led to the peculiar dependence of the pulse height on d_c . Meanwhile, it is emphasized that this feature causes negligible influence on the tail shapes of the resistive pulses (Figs. S3.3-S3.4) due presumably to the much larger RC compared to the actual translocation time of the nanoparticles. This in fact manifests physical significance of τ on the resistive pulse analyses that may pose a difficulty for estimating the size of analytes from I_p .

How about a role of capacitance? Being RC effects, the capacitance outside the nanopore should also affect the ionic current response. For this, the resistive pulse measurements were examined using the same nanopore size but with thick polyimide layer coated on the chip surface²⁵⁻²⁷ that served to reduce the net capacitance (Fig 3.4**Figure 3.**, see also Fig. S3.5). As expected, larger and narrower resistive pulses were obtained compared to those acquired with the uncoated nanopores due to the two orders of magnitude decrease in Cm. Moreover, as the electrodes were moved away from the nanopore, the pulses became slightly smaller and blunter. This result unambiguously shows the equivalent role of C_m to R_{ch} on retarding the ionic current response against the actual ion blockage phenomena occurring upon the fast electrophoretic trans-location of the nanoparticles through the pore.

Based on the above experimental results, the equivalent circuit shown in Fig. 3.5 to explain the temporal response of I_{ion} is proposed, where R'_i and C'_i are the resistance and capacitance components outside the nanopore, respectively, R_p is the resistance inside the nanopore, and R_{com} is the constant resistance in series. From this circuit diagram, the current I_p flowing through R_p is as follows,



Figure 3.5. Equivalent circuit model of nanopore device structure. R'_i (i = 1, 2, 3, \cdots n) is the resistance outside the nanopore, C'_i (i = 1, 2, 3, \cdots n) is the capacitance outside the nanopore, R_{pore} and R_{acc} are the resistance inside the nanopore, and R_{com} is the resistance other than the R'_i and R_p (= $R_{\text{pore}} + R_{\text{acc}}$). V_1 is the applied bias voltage.

$$I_{\rm p} = \frac{V_1}{R_{\rm p}} \left(1 - e^{-\frac{1}{(\sum C_i')\{(\sum R_i') + R_{\rm com}\}}t} \right)$$

where the time constant τ is defined as,

$$\tau = (\sum C_i')\{(\sum R_i') + R_{\rm com}\}\$$

In fact, this model agrees well with the experimental results shown in the previous section. For instance, when the channel depth was constant, increase in the distance between the electrode and nanopore led to longer τ (Fig. 3.3b), which can be ascribed to larger $\Sigma C'_i$ and $\Sigma R'_i$. On the other hand, for the effects of channel depth, τ was observed to become shorter with increasing d_c for the case of

 $L_{ele} = 3 \text{ mm}$ that corresponds to $\Sigma R'_i$ increase with decreasing channel depth. The only exception is the RC effect for the case of $L_{ele} = 0 \text{ mm}$ demonstrating τ increase with d_c . This is presumably because of the fact that $\Sigma R'_i$ changes little with the channel depth as the electrodes are placed at right above the nanopore.



Figure 3.6. Ionic current traces recorded in a dispersion solution of 200 nm-sized polymeric beads in 0.4 x PBS under the applied DC voltage of 0.1 V (red: non-coated device using PDMS channel with $d_c = 50$, 100, 400, 700 µm, green: polyimide-coated device using PDMS channel with $d_c = 50$ µm) and the approximation line of red circles data.

More quantitatively, the theoretical time constant τ_{theo} is calculated by $\Sigma R'_i = \rho L/S$ and $\Sigma C'_i = 4.55 \text{ nF}$. The results fairly agreed with the experimental values within 20 % error for the range of d_c tested even for the data obtained with the polyimide-coated nanopores, wherein a constant serial resistance $R_{\text{com}} = 190 \text{ k}\Omega$ (Figure 3.6) was considered. This not only manifested the importance of the external resistance on the signal retardation but also the existence of the additional resistance amounting 190 k Ω that may stem in part from $R_{\text{acc.}}$

Although showing good agreement with the experimental results, a care should be taken since the equivalent circuit only explains the signal retardation upon the nanoparticles escaping the nanopore and not during the entrance. This is because of the fact that whereas the longer tails can be interpreted as a cause of larger RC effects, we also observed steep onsets of the pulses irrespective of τ that seems to contradict with the expected outcome of the model circuit. The asymmetric feature in the signal waveforms may in fact stem from the actual ion migrations taking place in response to the ion blockage by the nanoparticles. As a particle is captured by the strong electric field at the orifice, it tends to move swiftly into the nanopore. In the meantime, the cations and anions accumulated on the thin membrane tends to move so as to compensate for the sudden decrease in the ionic current (discharging at the membrane capacitance). However, this process would be relatively slow thereby allowing I_{ion} to drop rapidly via the ion blockage by the particle. On the other hand, after the particle passes through the nanopore, the ionic current through the open pore takes long time to get back to the original level because of the long charging time of the capacitance characterized by τ . Future work is expected to provide a refined model circuit that can take the pre-translocation regime into account.

We now discuss the temporal resolution of the ionic current in single-nanometer pores for genome sequencing. The nanopore diameter and the depth are considered to be 2.8 nm and 0.7 nm, respectively, for single-base resolution.^{28,29} Since the above results demonstrate that the G Ω -level ionic resistance inside the ultra-small nanopore plays only minor role on τ , we can obtain a small RC-relevant

resistance of $(\sum R'_i) + R_{com} = 23 \text{ k}\Omega$ from the conductivity $\sigma = 10.5 \text{ S/m}$ of 1 M KCl usually used for measuring DNA³⁰ (Here, R_{com} is considered to be proportional to the resistivity of the buffer). On the other hand, the ultra-thin dielectrics brings a huge capacitance amounting 325 nF. It can be, however, reduced by the 5 µm-thick polyimide coating to be 19 pF, or even lower down to 0.5 pF as reported in a previous literature.³¹ Eventually, τ is calculated to be about 437 ns (or 12 ns for the latter case), which promises high-speed single-molecule electrical sequencing of DNA moving through the sensing zone at a technically affordable translocation velocity of 36 base/µs (1 base/µs)³² under an assumption of 10 current data to identify each nucleotide and a sampling rate of 10 MHz.³³

Resistive pulse sensing also holds promise for peptide sequencing.^{14,34,35} There are several issues to overcome for achieving the great task such as denaturation, aggregation, and less amount of surface charges compared to polynucleotides. Besides the challenges, the protein sensing poses a difficult situation in terms of the temporal resolution of the ionic current. This is because of the low stability of the molecular structures requiring physiological ionic strength of salt concentration around 137 mM. As a consequence, it leads to one order of magnitude decrease in the time constant than the one with the high KCl concentration applicable for DNA that needs to be compromised by the translocation speed, or equivalently the sequencing throughput.

3.3. Conclusion

The present study demonstrated for the first time that the resistance outside a nanopore plays a significant role on the signal retardation in resistive pulse analyses rather than the resistance at the pore. Charging at the cross-membrane net capacitance was also found to contribute equally to retard the signal along with the RC-relevant resistance. More importantly, the present findings preclude the anticipated cause of the inevitably large in-pore resistance of single-nanometer scale nanopores thereby paving a way for ultrafast genome sequencing and single-protein analyses.



Figure S3.1. Resistive pulses observed under two-different electrode positions. The open pore current is offset to zero. When the electrodes were placed right above a 300 nm-sized nanopore ($L_{ele} = 0 \text{ mm}$), the resistive pulses of height around several hundreds of pA were observed (blue, 1st), which reflected temporal ion blockage by electrophoretic translocation of negatively-charged carboxylated polystyrene beads of 200 nm diameter under the applied 0.1 V. Subsequently, the electrodes were placed at 3 mm away from the nanopore ($L_{ele} = 3 \text{ mm}$). As a result, the pulses became smaller to be around 100 pA in heights (red). Meanwhile, when the electrodes were returned back to $L_{ele} = 0 \text{ mm}$, the pulse signals became again as large as several hundreds of nA (blue, 3rd).



Figure S3.2. a, Plots of the resistive pulse height I_p as a function of the pulse width t_d obtained for 200 nm-sized carboxylated polystyrene nanoparticles using a non-coated 300 nm-sized SiN_x nanopore under the same channel height condition ($d_c = 400 \,\mu\text{m}$) but with different positions L_{ele} of the Ag/AgCl electrode of $L_{ele} = 0 \text{ mm}$ (blue) and 3 mm (red). It is noted that I_p tends to be larger with t_d due to the *RC* effect that tends to make the signals weaker for the particles with shorter translocation. **b**, $I_{\rm p}$ versus t_d scatter plots for non-coated (blue and red) and polyimide-coated (green) nanopores. As there was little difference in the size of nanoparticles measured, the difference in the pulse height and width is attributed to the difference in the RC effects. For polyimide-coated nanopores, the time constant is shorter than the actual translocation time of the nanoparticles. Therefore, they provided large pulses reflecting the physical size of the nanospheres. While the pulses became much smaller for the non-coated SiNx nanopores due to the large net capacitance, some pulses with relatively long $t_{\rm d}$ were observed to be as large as those acquired with the polymer-coated sensors. This can be interpreted as cases where slow-enough translocation motions of the nanoparticles led to little influence of the signal retardation.



Figure S3.3. Resistive pulses obtained with a 300 nm-sized non-coated SiN_x nanopore with the electrode position $L_{ele} = 3$ mm and the channel height $d_c = 50 \mu m$ under the applied cross-membrane voltage of 0.1 V. White curve is the average of the 280 pulses overplotted.


Figure S3.4. Resistive pulses obtained with a 300 nm-sized polyimide-coated nanopore with the electrode position $L_{ele} = 3$ mm and the channel height $d_c = 50 \mu m$ under the applied cross-membrane voltage of 0.1 V. Yellow curve is the average of the 94 pulses overplotted.



Figure S3.5. Scanning electron micrograph of a nanopore chip coated with a 5 μ m-thick polyimide layer. Photo-sensitive polyimide was spin-coated on a nanopore chip. After that, the polyimide layer of about 50 μ m around the nanopore was removed by a photolithography process. The diameter of the nanopore in the image is 300 nm.



Figure S3.6. Averaged resistive pulses recorded in electrolyte solutions of various ion concentrations.



Figure S3.7. A simplified equivalent circuit model of a nanopore device structure.

The equivalent circuit shown in Fig. 3.5 of the main text can be simplified as the one displayed in Fig. S3.7. The amount of charge Q flowing through this circuit can be described as follows. From Kirchhoff's law,

$$(R_{com} + \Sigma R'_{i})I + \frac{Q}{\Sigma C'_{i}} = V_{1} \quad (1)$$
$$\frac{Q}{(\Sigma C'_{i})R_{p}} = I_{2} \quad (2)$$
$$I = I_{1} + I_{2} \quad (3)$$

$$I_1 = \frac{dQ}{dt} \tag{4}$$

By substituting (3) into (1),

$$(R_{\rm com} + \Sigma R'_{\rm i})(I_1 + I_2) + \frac{Q}{\Sigma C'_{\rm i}} = V_1$$

Also, by substituting (2) into (4),

$$(R_{\rm com} + \Sigma R'_{\rm i}) \left(\frac{dQ}{dt} + \frac{Q}{(\Sigma C'_{\rm i})R_{\rm p}}\right) + \frac{Q}{\Sigma C'_{\rm i}} = V_1$$
$$\frac{dQ}{dt} + \frac{Q}{\Sigma C'_{\rm i}} \left(\frac{1}{R_{\rm com} + \Sigma R'_{\rm i}} + \frac{1}{R_{\rm p}}\right) = \frac{V_1}{R_{\rm com} + \Sigma R'_{\rm i}}$$

By defining $A = \left(\frac{1}{R_{\text{com}} + \Sigma R'_{\text{i}}} + \frac{1}{R_{\text{p}}}\right)$, the following formula is obtained.

$$\frac{dQ}{dt} + \frac{Q}{\Sigma C'_{i}}A = \frac{V_{1}}{R_{\rm com} + \Sigma R'_{i}}$$

$$dQ + \left(\frac{Q}{\Sigma C'_{1}}A\right)dt = \frac{V_{1}}{(R_{com} + \Sigma R'_{1}}dt$$

$$dQ = \left(\frac{V_{1}}{R_{com} + \Sigma R'_{1}} - \frac{Q}{\Sigma C'_{1}}A\right)dt$$

$$dQ = \frac{V_{1}\Sigma C'_{1} - (R_{com} + \Sigma R'_{1})QA}{(\Sigma C'_{1})(R_{com} + \Sigma R'_{1})}dt$$

$$\frac{1}{V_{1}\Sigma C'_{1} - (R_{com} + \Sigma R'_{1})QA}dQ = \frac{1}{(\Sigma C'_{1})(R_{com} + \Sigma R'_{1})}dt$$

$$\int \frac{1}{V_{1}\Sigma C'_{1} - (R_{com} + \Sigma R'_{1})QA}dQ = \int \frac{1}{(\Sigma C'_{1})(R_{i} + \Sigma R'_{1})}dt$$

$$-\frac{\ln\{V_{1}\Sigma C'_{1} - (R_{com} + \Sigma R'_{1})AQ\}}{(R_{com} + \Sigma R'_{1})A} = \frac{1}{(\Sigma C_{i})(R_{com} + \Sigma R'_{1})}t + K$$

$$\ln\{V_{1}\Sigma C'_{1} - (R_{com} + \Sigma R'_{1})AQ\} = -\frac{A}{\Sigma C'_{1}}t - \ln K'$$

$$\ln K'(V_{1}\Sigma C'_{i} - (R_{com} + \Sigma R'_{i})AQ) = -\frac{A}{\Sigma C'_{1}}t$$

$$V_{1}\Sigma C'_{i} - (R_{com} + \Sigma R'_{i})AQ = \frac{1}{K'}e^{-\frac{A}{\Sigma C'_{i}}t}$$

Furthermore, since $K' = \frac{1}{(\Sigma C'_i)V_1}$ at t = 0,

$$Q = \frac{(\Sigma C'_{i})V_{1}}{(R_{\rm com} + \Sigma R'_{i})A} (1 - e^{-\frac{A}{(\Sigma C'_{i})}t})$$

Finally, $A \approx \frac{1}{(R_{\rm com} + \Sigma R'_i)}$ since $(R_{\rm com} + \Sigma R'_i) \ll R_p$ in the present work, and therefore,

$$Q = (\Sigma C'_i) V_1 (1 - e^{-\frac{1}{(\Sigma C'_i)(R_{\rm com} + \Sigma R'_i)}t})$$

Table S3.1. Table of the device and resistive pulse parameters. L_{ele} is the distance between the nanopore and the Ag/AgCl electrodes, d_c is the height of the microchannel, R_{ch} and C_m are the resistance and the capacitance in the channel, τ_{exp} is the time constant calculated from the resistive pulse tails, and τ_{theo} is the time constant calculated using the equivalent circuit model.

Nanopore	L _{ele} (mm)	<i>d</i> _c (μm)	τ _{exp} (μs)	R _{ch} (kΩ)	C _m (nF)	τ _{theo} (μs)	τ _{exp} / τ _{theo}
Non-coated Si ₃ N ₄	0	50 100 400 700	310 330 750 840	NA	NA	NA	NA
	3	50 100 400 700 50	1800 1300 1200 780 3500	490 340 228 211 790	4.6 9.2	2230 1550 1040 960 7300	0.81 0.84 1.2 0.81 0.48
Polyimide-coated	0 3 6	50 50 50	170 160 200	NA 490 790	NA 0.023 0.023	NA 11 18	NA 1.7 1.3

For τ_{exp} of polyimide-coated nanopores, the time constant is expected to be much shorter than time scale of the temporal ion blockage by the single-nanoparticle translocation. In this table, we tentatively assumed a time constant attributed to the particle motions to be 140.9 µs and used the value to estimate τ_{exp}/τ_{theo} .

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Chapter 4. Inertial focusing and zeta potential measurements of single-nanoparticles using octetnanochannels

4.1. Intoroduction

Nanofluidic channel is a versatile platform for sensing nanoscale analytes.¹⁻⁵ Resistive pulse analysis is one of its promising applications⁶ that allows to count and discriminate single-particles and molecules in electrolyte solution⁷⁻¹¹. It measures temporal drops in ionic current flowing through a fluidic channel upon translocation of individual objects. Pattern analyses of the detected pulse-like signals were reported to allow assessments of multiple physical properties of the analytes such as the size, shape, zeta potential, and even mass¹²⁻¹⁷. Meanwhile, the ionic current waveforms were known to reflect their dynamic motions¹⁸ that are random in nature due to the three-dimensional motion degrees of freedom. Eventually, it crucially degrades the sensor accuracy especially for the estimations of the zeta potential since the resulting uncertainty in the actual passages of the objects in the conduits causes profound influence on the resistive pulse profiles.¹⁹⁻²¹

On the other hand, whereas significant efforts have been devoted in the past to realize a fine control of translocation dynamics, most of the methods were developed only to slow-down the fast electrophoresis, and hence not for regulating the capture-to-translocation motions.²²⁻²⁵ Perhaps the only effective concepts proven effective are to increase the number of resistive pulse measurements either by repetitively threading an object through a nanochannel by an electrophoretic control^{26,27} or preparing a fluidic channel consisting of several sensing zones in series.^{28,29} The latter method is particularly useful as it involves no additional apparatus and external control for the sensing. In fact, pioneering works by Jacobson *et al.*²⁹ demonstrated up to eight times detections of single-nanoparticles by using an octet-channel architecture that enabled more reliable resistive analyses for

discriminating viruses.²⁹ Nonetheless, the space between the nanochannels in these studies was made to be relatively wide making the capture-to-translocation dynamics of the nanoparticles random due to the pronounced influence of Brownian motion each time after escaping from the sensing zone as signified by the stochastic advent of eight resistive pulses upon the single-particle translocation. Here we report on regulation of single-particle translocation dynamics in a fine-spaced octet-nanochannel. The concept is similar to inertial focusing in microfluidics that uses inertial lift forces in pressurized flow to move particles away from the center³⁰⁻³² but based on an electrokinetic control instead of the fluid dynamics in multi-channels³³⁻³⁵ designed to leverage inertial effects for confining the particle motions, which enabled a protocol for assessing single-nanoparticle zeta potential by resistive pulse pattern analyses.

4.2 Results and discussion

The sensor structure comprises eight channels of dimensions 550 nm × 600 nm × 600 nm (width (w) × depth (t) × length (L)) each spaced by 600 nm (Figs. 4.1a-b and S4.1), which was connected to outer microchannels of the same depth. The conduit was filled with dispersion solution of either amino-modified or carboxylated polystyrene particles of diameter d_{ps} at a concentration of $c_{ps} = 3.6 \times 10^{11}$ particles/ml in phosphate-buffered saline (PBS: NaCl 137



Figure 4.1. Nanoparticle detection using an octet-nanochannel. a, Schematic model depicting the channel structure. Polystyrene nanoparticles translocated through the channel by electrophoresis under the applied voltage of 1 V while measuring the associated ionic current blockade by using a pair of Ag/AgCl electrodes (red poles). The inset at lower right displays the channel dimensions. b, SEM image of the octet-channel viewed from the top. m and n are the indices for the inter-channel and sensor zone regions, respectively. c, A magnified view of a resistive pulse. The curves are represented to be taken at 10 kHz by 100-point averaging of the raw data. d, A fast-Fourier-transformed resistive pulse signal used for data processing. tpp and td represent the time between pulses and a full width at half maximum of the pulse, respectively.

mM). The ionic current I_i through the channel was measured under the DC applied voltage of 1.0 V. This fluidic channel design was chosen among various shapes to enable electrophoretic translocation of the polymeric beads under negligible influence of Brownian motions at the open space between the eight constrictions (for detail see Fig. S4.2). The ionic current Ii through the channel was measured under the DC applied voltage of 1.0 V (Figs. S4.3 and S4.4).

The open-channel ionic current was about 43 nA, which was in accordance to the model calculation within 3 % error where the access resistance stemming from the field focusing at the entrance and exit of the octet-channel was taken into account.²⁴⁻²⁶ Meanwhile, a resistive pulse was observed at each time when a particle (here the amino-modified polystyrene particles of $d_{ps} = 460$ nm) went through the channel. A closer look into the waveform revealed that the signal is composed of eight sub-pulses (Figs. 4.1c-d) that can be naturally attributed to subsequent transits of the eight sensing zones.^{22,23} The pulse signals were found only when the potential at the downstream was set to positive indicating the electrophoretic translocation of the negatively-charged amino-modified polystyrene nanobeads of zeta-potential -34 mV as measured by a zeta sizer.

Here it is noted that the first and last sub-pulses were relatively small compared to the others. This distinct feature existed in all the ionic current blockade events detected. To explore the underlying mechanism, a finite element analysis of the cross-channel ion transport that solved Poisson-Nernst-Plank and Navier-Stokes equations in a self-consistent manner using COMSOL software was carried out. By positioning a 460 nm-sized sphere along the axial direction at x, the ionic current under steady states was assessed. The acquired I_i versus x trace reproduced the characteristic eight resistive pulse waveforms with the first and the last being smaller than the others. It anticipates larger access

resistance at the entrance and exit that would cause the ion blockage effect to be weaker than those occur at the other six channels.^{30,31}

It is noticeable that the ionic current did not return to the base level upon passing through the interchannel regions as signified by the relatively non-zero I_i between the sub-pulses in Fig. 4.1d. This manifests the existence of non-negligible electric field in the gaps between the 550 nm-sized



Figure 4.2. a-b, The inter-channel tpp (a) and cross-channel translocation time td (b) at the sections spp and sd, respectively. Insets are schematic models explaining the definitions of tpp and td. The plots and error bars denote the average and standard deviation, respectively. c-d, The average speed of particles moving through the inter-channel vpp (c) and intra-channel space vd (d).

constrictions that suggests continuous electrophoretic motions of particles through the eight channels. Meanwhile, it should be pointed out that while the fine-spaced multi-channel is expected to allow more regular motions of particles, the overlapping electric field would also hinder straightforward interpretation of the resistive pulse heights. For instance, though the present structure still can discriminate amino-modified 460 nm and carboxylated 520 nm-sized polystyrenes by the signal heights Ip (Fig. S4.4), their actual particle sizes cannot be analytically derived by Coulter principle due to the electric field interference.³² This is not the case when the sensing zones are made far apart with each other so that they work independently to sense the size of analytes.^{22,23}

The note that there is no notable influence of ion concentration polarization on the ion transport nor the particle translocation dynamics in the octet nanochannel is added. This can be found in the numerically-simulated ion density profile (Figs. S4.6-S4.7) displaying only 2 % variation in the ion concentrations in the conduit. This is not surprising considering the fact that ion concentration polarization generally occurs when electric double layer overlap in a fluidic channel,³⁴ which is not the present case where the Debye length in 1 x PBS containing 137 mM NaCl is as short as 0.3 nm that is more than two orders of magnitude smaller than the characteristic size of the octet nanochannel.

The translocation motions of the particles were deduced by analyzing the subpulse spacing t_{pp} that corresponds to the time they took to move from one channel to the other. As shown in Fig. 4.2a, t_{pp} tends to decrease with increasing *m*. The tendency was similar for the resistive pulse width t_d that represents the translocation time of the particles through each channel (Fig. 4.2b). The particle velocity v_{pp} at the inter-channel regions from t_{pp} through $v_{pp} = L_{pp}/t_{pp}$ with $L_{pp} = 1.2 \ \mu m$ being the channel-to-channel distance is further able to calculate (Fig. 4.2c).

Similarly, the intra-channel translocation speed v_d can also be deduced by $v_d = L_{ch}/t_d$ with the channel length $L_{ch} = 0.55 \ \mu m$ (Fig. 4.2d). Interestingly, both v_{pp} and v_d suggested faster electrophoresis of the nanoparticles at the downstream side of the octet-channel.

It is worth discussing whether the nanobeads were really being accelerated in the octet-channel. Electrokinetics of the field-accelerated nanoparticle motions can be described as,

$$m_{\rm ps}\frac{d\nu}{dt}=qE-3\pi\eta d_{\rm ps}\nu$$

, where v is the speed of the particle, q is the surface charge of the particle, E is the electric field strength, η is the viscosity of water, and m_{PS} is the particle mass. Considering the focused electric field at the narrowed channels, the particles are anticipated to undergo repetitive field-acceleration to inertial motions as they get into and jet out of the eight channels. Here, it should be pointed out that the nanoparticles were decelerated by the viscous drag every each time after exiting the 550 nm constrictions, whose motions can be described as,

$$v(t) = v_0 \exp(-t/\tau_{\rm es})$$

under an assumption of E = 0 at the inter-channel space, where $\tau_{es} = m_{ps}/R$ is the time constant with the Stokes drag $R = 3\pi\eta d_{ps}$. Using $m_{ps} = 5.5 \times 10^{-16}$ kg and $\eta = 1.0$ mPas, τ_{es} is calculated to be 0.13 µs. This rough analysis therefore predicts that the nanobead motions are instantaneously settled into a steady state by the weak yet finite electric field existed thereat.³⁵ The envisaged accelerated translocation dynamics is thus unlikely to take place in the inter-channel space under the conditions tested.

To shed further light on the translocation dynamics of single-nanoparticles in the octet-channel, the variations in the resistive pulse waveforms was investigated. The standard deviation of t_{pp} (σ_{tpp}) and t_d (σ_{td}), for instance, revealed monotonic decrease with, respectively, s_{pp} and s_d elucidating regulated

electrophoretic motions at the downstream (Figs. 4.3a-b). In addition, the diminished scattering in $I_p(\sigma_{lp})$ with s_d (Figs. 4.3c) was found. This feature cannot be ascribed to variations of neither v_{pp} nor v_d for the pulse height should be the same regardless the speed of objects according to Coulter principle³² (unless it is not too fast to be detected under the given temporal resolution of the ionic current measurement system³⁶). It is thus more rational to consider a possible influence of off-axis effects;¹³⁻¹⁵ i.e. particle trajectories are less (more) scattered at the channel downstream (upstream). To verify this possibility, finite element simulations of the particle paths in the octet-nanochannel was exhibited.³³ Surprisingly, the asymmetric translocation dynamics in the symmetric fluidic channel was found: the scattering in the incident angle at the entrance of the channel is conspicuously broader compared to that at the exit (Figs. 4.3d, see also Figs. S4.5 and S4.6). Closer look into the trace map in the eight nano-constrictions elucidated more confined particle passages with increasing s_d suggesting weaker off-axis effects that qualitatively explained the s_{pp} dependence of I_p shown in Fig. 4.3c. Meanwhile, Fig. 4.3d also illustrated diminished variation in the nanoparticle trajectories at the inter-channel regions with increasing spp, which is seen more clearly in the plots of standard deviations $\sigma_{\rm L}$ of the lateral particle positions (Fig. 4.3e). Here, it is noted that the particles move longer distance when they go along the outer passages (Fig. 4.3d), which would give rise to shorter t_{pp} with s_{pp} even under a constant v_{pp} . To be more quantitative, the average distance L_m the nanoparticles moved in the $s_{\rm pp}$ th inter-channel section (Fig. 4.3f) was estimated. The results indeed revealed steady decrease in $L_{\rm m}$ with s_{pp} at a rate close to that for t_{pp} (Fig. 4.3g). These results consistently imply that it is the particle trajectories and not the change in the translocation speed that led to the $t_{pp} - s_{pp}$ dependence shown in Fig. 4.2a.

The above results indicated the existence of forces acting to restrict the radial motions of particles at the downstream. In order to investigate the physics involved, therefore, the finite



Figure 4.3. Nanoparticle focusing in the octet nanochannel. a-c, Plots of standard deviations σ_{tpp} (a), σ_{td} (b), and σ_{lp}/I_{ave} (c) of t_{pp} , t_d , and σ_{lp} , respectively, at s_{pp} th inter-channel regions. I_{ave} is the average of the eight I_p . d, Map of particle trajectories. Red line points at y = 0. e, Plots of standard deviations σ_L along the lateral directions at s_{pp} th regions. Grey squares and red circles are, respectively, σ_L for the particles moved above and below y = 0 in (f). f, Schematic model depicting a particle motion in an inter-channel region. L_{pp} is defined as the average distance the particle moved from s_{pp} to s_{pp} + 1 calculated from (d). g, Plots of L_{pp} , at s_{pp} th regions normalized by that at $s_{pp} = 1$ (L_1). Grey squares and red circles are L_{pp}/L_1 for the particles moved above and below y = 0 in (f), respectively.

element simulations to virtual buffers having viscosities lower than $\eta = 1$ mPas was extended (Fig. 4.4). The calculations revealed prominent influence of η on the particle trajectories showing more ballistic motions between the channels in the less viscous media (Figs. 4.4a-f). It can be interpreted as a consequence of inertial effects during the field-acceleration and



Figure 4.4. Inertial effects on particle trajectories. a-b, Nanoparticle trajectories (a) and the electric potential contour map (b) in liquid of viscosity 0.8 mPas simulated by finite element analyses using COMSOL. c-f, The same plots under the viscosity of 0.4 mPas (c,d) and 0.08 mPas (e,f). g, Comparison of three particle trajectories under different viscosities: 0.8 (black), 0.4 (red), and 0.08 mPas (blue). Initial positions of the particles are set to be the same. Arrow shows the direction of the electrophoretic particle motions. Light grey area shows the regions inside the eight channels. The particle positions were calculated at every 100 ns. h, Close up view of the trajectories at the entrance of the octet channel in (g). i, The particle velocities v_y along the direction orthogonal to the channel axis. Color coding is the same as that in (g).

viscous deceleration of the particles upon entering and escaping each of the eight channels, respectively (Fig. S4.7). For instance, when $\eta = 0.08$ mPas, τ_{es} becomes as long as 1 µs. The field-accelerated nanobeads are thus expected to move straightly by inertia for longer distance due to the relatively weak electric field strength in the inter-channel space. This is seen in Figs. 4.4g-i where the particle continues to move toward the center even when it gets inside the channel manifesting the less efficient viscous dragging to settle the motion into steady state because of the short translocation time compared to τ_{es} . On contrary, increase in η lowers the nanoparticle velocity via viscous dragging that offers enough time for it to be in steady state (for instance, the particles travel through the first channel in 3.8 µs and 2.0 µs under $\eta = 0.8$ and 0.4 mPas, respectively). Their motions would then tend to follow the direction of the electric field thereby led to the wavy trajectories in the inter-channel space. It was thus the electric field-induced inertial effects that enabled regulation of the translocation motions at the downstream side of the octet nanochannel, which is akin to the mechanism of particle focusing via hydrodynamic inertial forces in microfluidic channels.^{30,32}

The well-defined single-nanoparticle dynamics is expected to allow accurate estimation of its surface charge density. The resistive pulse analysis for carboxylated polystyrene nanobeads of $d_{ps} = 520$ nm possessing average zeta potential of – 48.5 mV as measured using the zeta sizer was thus examined. Theses polymeric nanoparticles also yielded eight consecutive sub-pulses resembling those observed in the amino-modified beads. Yet, the widths of the pulses as well as the pulse-to-pulse durations are elucidated to be much shorter as depicted in the plots of t_{pp} and t_d (Figs. 4.5a and S4.8) reflecting the faster translocation motions of the carboxylated polystyrenes having larger zeta potential than the amino-modified counterparts. Here, when presuming steady state translocation motions of the nanoparticles in the inter-channel spaces,



Figure 4.5. Single-nanoparticle ζ -potential estimations. a, t_{pp} plotted as a function of s_{pp} for the aminomodified (orange) and carboxylated polystyrenes (skyblue), respectively. Solid curves are exponential fits to the plots. b, $t_{pp} - s_{pp}$ characteristics of two resistive pulses obtained for the amino-modified nanoparticles. Solid curves are exponential fitting to the plots. Dashed lines denote t_c . c, ζ -potential deduced from t_{pp} of the amino-modified polystyrenes. Solid line denotes the ζ -potential of -33.5mV measured by a zeta sizer. d, Schematic illustration depicting polymeric nanobeads approaching the octet nanochannel by the electrophoretic forces under weak electric field at the orifice. Size of arrows indicate the strength of the electrophoretic force on the particles. Since particles with higher zeta potential move faster by electrophoresis, they are expected to pass through the nanochannel at earlier time.

the velocity v_{pp} can be approximated as $v_{pp} = \varepsilon_0 \varepsilon_w E(\zeta_{ps} - \zeta_s)/\eta$ where ζ_{ps} and ζ_s are the zeta potentials at the polystyrene nanobeads and the channel wall surface, respectively, ε_0 and ε_w are, respectively, the vacuum permittivity and the relative dielectric constant of water, and *E* is the electric field.³⁷ As $t_{pp} = L_{pp}/v_{pp}$, therefore, the zeta potential of the single nano-particles is anticipated to be deduced from the measured inter-channel translocation time. For this, the trajectory-dependent t_{pp} characteristics was empirically described by an exponential function of $t_{pp} = t_0 \exp(-\alpha m) + t_c$, where the coefficient α depicts the space confinement effect and t_c corresponds to the inter-channel translocation time of the nanoparticle at $s_{pp} = \infty$. Exponential fitting in Fig. 4.4a gave $t_{c_{COOH}} = 1.1$ ms and $t_{c_{NH2}} = 1.7$ ms s for the carboxylated and amino-modified nanobeads, respectively. The ratio between these values is equivalent to ζ_{COOH}/ζ_{NH2} when assuming $\zeta_s = 0$, *i.e.* negligible influence of the electroosmotic flow on the translocation dynamics, which agrees with the bulk measurement result ($\zeta_{COOH} = -48.5$ mV, $\zeta_{NH2} = -33.5$ mV) within 7.6 % error (see Fig. S4.9 for simulated electroosmotic flow speed).

We now estimate the single-nanoparticle zeta potential. t_{c_NH2} was extracted from each resistive pulse recorded for the amino-modified nanobeads by the exponential fitting (Fig. 4.5b). ζ_{NH2} was then calculated as $\zeta_{NH2} = \zeta_{COOH}t_{c_NH2}/t_{c_COOH}$ using the bulk $\zeta_{COOH} = -48.5$ mV as a reference. The results manifested a small variation in the surface potential by ± 1.5 mV from the average value of -32.4 mV among the amino-modified nanobeads detected (Fig. 4.5c). On the other hand, the statistical accuracy of the zeta potential assessment using zeta sizer was evaluated to be -33.5 mV ± 2.6 mV by performing ten measurements. Since the result in Fig. 4.5c falls within the error range of the zeta sizer analysis, it validates the accuracy of the octet nanochannel approach for estimating the single-particle zeta potential.

It is worth discussing whether the present method is more reliable than the other techniques. In this regard, it should be pointed out that conventional zeta sizers cannot address singleparticle properties. On the other hand, solid-state nanopores are capable of assessing zeta potential of individual objects.⁴⁷⁻⁴⁹ The single-channel measurements, however, involve variations in the particle trajectories,²¹ which in general leads to an order of magnitude scattering in the translocation time,⁴⁹ and hence deteriorated sensor accuracy in estimating zeta potential.⁴⁷⁻⁴⁹ In contrast, the multi-channel strategy allows strong suppression of the translocation time variation to less than 4 % at the downstream (from Fig. 4.3a showing $\sigma_{tpp} = 0.06$ ms at $s_{pp} = 7$) via the inertial effects that render better detection accuracy of the single-particle zeta potential.

Interestingly, $\zeta_{\rm NH2}$ versus the signal number $N_{\rm ps}$ (sequential number of the resistive pulses detected) demonstrated smaller zeta potential of the particles detected at later time (Fig. 4.5c; see also Fig. S4.13). This tendency can be attributed to their electrophoretic motions outside the channel. Since no pressure was added, nanobeads were moved toward the octet channel mainly by the electrophoretic forces under the weak electric field at the orifice. In this circumstance, nanoparticles with larger (smaller) zeta potential tended to move faster (slower) and became detected at earlier (later) time (Fig. 4.5d) thereby led to the temporal dependence of $\zeta_{\rm NH2}$ (Fig. 4.5c).

We add to note that the present technique is not suitable for analysing particle suspensions of too-high concentrations. This is because of the fact that such conditions would lead to a higher chance to have more than one particle captured at the same time in the octet nanochannel. When this happens, signal features are anticipated to become too complicated to analyze their translocation motions. For example, when two particles were too close to each other, it will not be possible to discriminate them in the ionic current signal,⁵⁰ *i.e.*, they are anticipated to show up as a large single particle with 8 sub-pulses in the resistive pulse signal instead of two small particles with 16 sub-pulses. The consequence is similar when they pass through different nanochannels concurrently. These situations become even more difficult to resolve when the particles have different zeta potentials. It is thus of critical importance to avoid

having more than two particles captured in the nanochannel by arranging the particle density so that the capture rate becomes much lower than their translocation time.

The above results indicate that the translocation dynamics can be focused more efficiently by incorporating a higher number of sensing zones to reduce the uncertainty in the electrophoretic passages. Nevertheless, a care should be taken that such a channel design with higher s_{pp} is a trade-off between the signal intensity since the increase in the number of channels makes the relative change in the resistance upon passing through the sensing zone smaller. More specifically, the octet nanochannel can be roughly depicted as serially-connected eight resistors each possessing the resistance R at the 600 nm-sized constrictions. Each time when a particle passes through the constrictions, it raises one of the resistance to $R + \Delta R$. This is seen as temporal drops in the ionic current by $\Delta I = V_b(-\Delta R/(8R+\Delta R)8R) \sim -V_b\Delta R/64R^2$ under the applied voltage V_b where it is assumed $\Delta R \ll R$. In case of a single 600 nm-sized nanochannel of resistance R, translocation of the same particle also causes the resistance change by ΔR . The resulting blockade current, however, is predicted to be a factor of 64 larger than that in the octet nanochannel amounting $\Delta I = V_b(-\Delta R/(R+\Delta R)) \sim -V_b\Delta R/R^2$. On the other hand, the ionic current noise in nanofluidic channels is expected to change little by the number of channels since what affects the characteristics relevant to the signal-to-noise ratio is anticipated to be the capacitance of the nanochannel chip⁵¹ that may not change appreciably by the structure of the conduits. Overall, the signal-to-noise ratio in the multichannel is anticipated to become lower with increasing the number of channels by the associated weaker signal heights under the channel-number-insensitive noise floor. Alternatively, it would be more effective to compromise the number of channels and implement the fitting on the exponential $t_{pp} - s_{pp}$ relation for each pulse. In this way, the multi-channel approach can be used for accurate zeta potential measurements of single-particles and molecules.

4.3 Conclusion

The electrophoretic motions of single-nanoparticles in a closely-spaced octet-nanochannel were investigated. The resistive pulses revealed almost equi-positioned sub-pulses indicating negligible contributions of Brownian motions on the translocation dynamics. The average translocation duration was found to become shorter at the downstream side of the conduit due to the inertial effects that restricted the radial motions degrees of freedom during the field-acceleration and viscous deceleration processes upon passing through each of the eight nanochannels. Most importantly, the inertial focusing allowed estimations of nanoparticle surface zeta potentials at the single-particle level by the pulse-to-pulse time analyses.

Appendix

Finite element simulations. Electrophoretic motions of the polystyrene nanoparticles in an octet nanochannel were simulated on a two-dimensional Cartesian coordinate system. The model structure was defined by taking the dimensions of the nanochannel from scanning electron micrographs. Channel material was assumed as bulk SiO₂ with known electrical conductivity and relative permittivity. The polystyrene particles were also considered to have bulk properties except the surface charge density σ set to -20 mC/m^2 estimated from the zeta potential measured with a zeta sizer. The electric field *E* and the fluid flow field *U* for the constructed model using a finite element mothed (FEM) by simultaneously solving Poisson equation, continuity equation at steady-state current, Nernst-Planck equation, and Naiver-Stokes equation were evaluated. All simulations were conducted by a software package of COMSOL multiphysics 5.4 using AC/DC, Chemical Reaction Engineering, and Computational Fluid Dynamics (CFD) modules. The particle trajectories were then obtained from the equation of motion

$$m_{\rm i} \frac{dv_{\rm i}}{dt} = \sum F_{\rm i}$$

For the *i*-th particle. Here, m_i , v_i and F_i are the mass $(= 4\pi (d_p/2)^3 \rho/3 \text{ with } d_p = 780 \text{ nm and } \rho = 1040 \text{ kg/m}^3$ for PS), the velocity, and the force acting on the ith particle. This force was acquired from the electrostatic force $q_i E$ and the Stokes drag force $3\pi \eta d_p(v_i - U)$ where q_i is the amount of surface charge on the particle calculated from σ . Meanwhile, the particles were initially placed at the channel upstream with random radial positions and zero velocity. Thereafter, the one ordinary differential equation (ODE) was solved at the condition to deduce the particle displacements at every 10 µs.

Data analysis: Raw data were analyzed by a program coded in Python to extract the resistive pulses. The derivative of ionic current was taken as a reference to define a pulse region using a current threshold. The moving base level current was offset to zero by subtracting the linearly fitted component from the data. The extracted pulse waves were further digitally processed by fast Fourier transform (FFT) and inverse Fourier transform (IFT) for the post resistive pulse analyses to extract t_{pp} , t_d , and I_p .

Supplementary Figures



Figure S4.1. SEM image of an octet nanochannel. a, Scanning electron micrograph taken from the top. b, A magnified view of the red rectangle region in (a).



Figure S4.2. a-c, Octet nanochannels having channel length of 1 μ m with inter-channel gaps of size 1 μ m (a), 2 μ m (b), and 3 μ m (c). Typical resistive pulse signals were shown below each image. The signals demonstrate larger current up/downs upon particle translocation in the octet channels with wider inter-channel gap space. This can be explained by the decrease in the resistance at the gaps between the channels that indicates weaker electric field there. Meanwhile, the heights of sub-pulses were observed to scatter more with increasing the channel distance, which is presumably due to the more random incident angles of the particles at the capture stages that led to varying off-axis effects on the resistive pulse heights.^{S1} Since negligible influence of Brownian motions at the gap space for the sake of more regular translocation dynamics to accurately estimate the particle zeta potential was wanted to have, the inter-channel distance to be as short as the length of each channel was chosen. d, Meanwhile, since the longer channels led to lower signal-to-noise ratio (channel length is 3 μ m in this case that led to the weaker resistive pulse signals), the relatively short channel structures was selected.



Figure S4.3. Measurement set up for resistive pulse detections of single nanoparticles. a, Photograph displaying the octet nanochannel chip sealed by a PDMS block from the top. The six holes punched in the PDMS were used as inlets and outlets to flow nanoparticle dispersion solution into the nanochannel. The other two holes were utilized for placing Ag/AgCl rods for the cross-channel ionic current measurements. b, A close view showing the microchannels in the PDMS and the SiO₂/Si chip. Through the holes in the PDMS, PBS containing polystyrene nanoparticles was flown at one side of the nanochannel, while only PBS was injected at the other side. c, Schematic model of the device set up. d, A partial ionic current (I_i) versus time (t) curve obtained for the 460 nm-sized aminomodified polystyrenes.



Figure S4.4. The ionic current I_i through the octet nanochannel in 1 x PBS plotted as a function of the applied voltage V_b .



Figure S4.5. a, Resistive pulse heights I_p of 460 nm-sized amino-modified (orange) and 520 nm-sized carboxylated polystyrene beads (skyblue) plotted as a function of the number of pulses N_{sp} . I_p is defined as the height of the fifth sub-pulse as shown in the inset. b, Variations in I_p of the carboxylated particles among the five trials of measurements.



Figure S4.6. a-d, A schematic model of the octet nanochannel with no voltage applied (a) and the simulated densities of anions (b), cations (c), and both ions (d) along the axial direction x. The cation density c_c was slightly higher in the eight constrictions due to the electrostatic attraction to the negatively charged SiO₂ channel wall surface. The anion density c_a is, on the other hand, almost homogeneous throughout the conduit. e-l, The ion distributions under the applied voltage of + 1V (e-h) and -1 V (i-l). Under the positive voltage, local density of anions become higher and lower at the left and right sides of the channel (g). In contrast, the effect is reversed under the negative voltage where c_a becomes lower and higher at the left and the right sides (k), which is interpreted as ion concentration polarization induced by the slightly cation selective nature of the SiO₂ nanochannel.^{S2} As a result, the ion concentration at the left and right sides is enriched (depleted) and depleted (enriched) under the positive (negative) voltages (h,i) although the change is quite small amounting less than 2 % of the bulk concentration.



Figure S4.7. Electric potential profile along the axial direction of the octet nanochannel. No clear signs of ion concentration polarization effect were observed due to the relatively large size of the conduit compared to the Debye length.



Figure S4.8. Coarse view of particle trajectories. Inset: Averaged trajectories of the particles moved above and below y = 0.



Figure S4.9. Grid dependence of the finite element analyses. There were no notable difference in the results obtained with the three different mesh sizes. The 'Fine' grids to run the simulations was used.


Figure S4.10. Inertial focusing of nanoparticle electrophoresis in the octet nanochannel. a, Particle capture by electric field focused at the narrowed constriction. Yellow lines denote a contour map of the electric potential. The particle speed is low letting the inertial effects to be ineffective to change the direction from that of the electric field. b, The particle is accelerated in the strong electric field at the nanoconstriction. When the viscosity of the media is high, however, the motion is rapidly retarded by the viscous drag after getting out the narrowed region since the electric field is relatively weak at there. In this case, the particle moves along the potential gradient that results in the wavy trajectories depicted in Figs. 4.3d and S4.4. c, On the other hand, the particle moves much faster under a low viscosity condition (c). Therefore, the viscous dragging is insufficient to settle the motion into steady-state due to the short relaxation time. As a result, the particle moves more to the channel where the inertial force, now directing toward the channel axis, brings the particle more to that direction. As these processes repetitively occur eight times in the octet nanochannel, it gives rise to focusing of the nanoparticles at the downstream.



Figure S4.11. Average Translocation time of amino-modified and carboxylated nanoparticles. a, t_{pp} plotted as a function of *m*. b, t_d plotted as a function of *n*. The anomalous 6th t_{pp} of the carboxylated polystyrenes is presumably due to fabrication error in the size of the 6th nano-constriction.



Figure S4.12. Electroosmotic flow velocity v_{EOF} in the octet nanochannel. a, v_{EOF} is around 3 mm/s, which is more than an order of magnitude lower than the electrophoretic speed of the particles estimated by the finite element calculations (Fig. 4.4i). Meanwhile, that this flow velocity is faster than the translocation speed of the particles deduced from the channel length and the resistive pulse widths, which is around 0.8 mm/s is noted. This is presumably a consequence of a discrepancy between the bulk properties assumed in the numerical simulations and the actual conditions in the experiments, such as viscosity for example. In fact, the particle motions were also overestimated in the simulations (Fig. 4.4i). Therefore, vEOF is only valid within the framework of the numerical analyses and should not be used to compare with the experimental results. b, Color map of v_{EOF} . The flow speed is high at the nanochannel wall surfaces. c, The model used for the electroosmotic flow speed calculations. The meshes were made finer at the wall surface to accurately simulate the counterion motions.



Figure S4.13. Resistive pulse waveforms. a, Comparison of two resistive pulse signals obtained at the beginning (gray) and after several hours of the ionic current measurement. Δt denote the time between the k_{th} ionic current dips between the two pulses. b, Plots of Δt as a function of k. The linear increase in Δt indicates the difference in the electrophoretic mobility of the two particles in (a).

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Chapter 5. Conclusions and remarks

Nanopores and nanochannels are subjected to large electric fields, and particles passing through them are accelerated by electrophoretic forces and subsequently decelerated by viscous forces. In this thesis, this series of dynamics involving inertial effects is being studied. The new findings in this study are summarized as follows.

The acceleration and deceleration of particles in the electrolyte solution converge instantly due to viscosity, and the particles move at a constant speed. In order to measure the acceleration and deceleration of particles which pass through a nanopore, it is necessary to measure phenomena that occur in a small amount of time, and it is important to understand what determines the time response of the pulse waveform. The degree of retardation of the pulse waveform is an indicator of the time response of the pulse waveform. It is known that reducing the capacitance of the membrane results in a smaller retardation in the pulse waveform. This phenomenon implies that ions are adsorbed and desorbed on the membrane, which may interfere with the transfer of ions between the electrode and the membrane. However, the full extent of the problem is not yet clear. In this study, we investigated the mechanism of how ions moving between the electrode and the film are involved in the retardation of the pulse waveform. In chapter 3, it was clarified that the resistance out-of-pore plays dominantly a role in the translocation of the signal, that resistance contains the access resistance partially, and the shorter the distance between the electrode and the pore the smaller that resistance becomes. The results showed that the resistance outside the pores and the capacitance of the membrane contribute to the responsiveness of the pulse waveform when particles pass through the pores, and the smaller the resistance, the better the responsiveness. This finding overturned the conventional idea that it is difficult to improve the pulse waveform response in DNA measurement, which requires a pore diameter of several nanometers, because the resistance of the pore contributes to the responsiveness of pulse waveform response. If the results of this study are applied to DNA measurement using nanopores, the problematic signal responsiveness will be improved and base identification will become possible.

It turns out that the resistance of the outside of the pore and the capacitance of the membrane contribute to the responsiveness of the pulse waveform, and optimizing them opens the way to measuring the transient movement of particles in solution. Therefore, in Chapter 4, we attempted to observe the dynamics of particles just before constant velocity motion, when electrophoretic and viscous forces are in equilibrium. We devised a channel structure called an "octet nanochannel" with vertically aligned pores, and assumed that the acceleration of particles at the pore entrance and deceleration of particles at the pore exit are repeated. The experimental results showed that the particles clearly passed between the pores. The results of the experiment showed that the velocity of the particles passing through the channel increased as they went downstream. A fine-designed simulation showed that the particles passed toward the central axis of the channel, and the further downstream they went, the shorter the distance they passed. As a result, it was found that the particles were moving in the direction of movement was toward the central axis of the channel due to inertial effects, resulting in a shorter distance traveled. In addition, the single-particle zetasizer was invented to take advantage of the fact that particles reach an almost constant velocity as they move downstream in the flow path.

Experiments with octet nanochannels show that inertial effects affect the trajectories of particles. Since the inertia force depends on the mass, the mass difference can be measured by observing the particle trajectories. Furthermore, before the convergence of the time for particles to move between the pores of the octet nanochannel, the trajectory of the particles is expected to change significantly for different particle masses, so the nanostructure proposed in this study may serve as a bridge for future mass measurements of particles in solution. Because of the Stokes time, the smaller the object being measured, the faster the ion current response needs to be. To measure the mass of a globular protein with a size of 20 nm and a mass density of 1 g/cm³, the ion current response must be at least about 0.18 ns or less, which is feasible with the findings already reported. More importantly, the clarification of the mechanism of ion current response and the relationship between the mass of a single particle in solution and the trajectory of the particle has shed light on the realization of mass spectrometry for objects on the scale of one nanometer, such as DNA and proteins.

List of publications

 Shohei Kishimoto, Sanae Murayama, Makusu Tsutsui, Masateru Taniguchi., Crucial Role of Outof-Pore Resistance on Temporal Response of Ionic Current in Nanopore Sensors. ACS Sensors. 2020 5 (6), 1597-1603

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2. Shohei Kishimotoa, Makusu Tsutsuia, Kazumichi Yokotab, Masateru Taniguchi.; Inertial focusing and zeta potential measurements of single-nanoparticles using octet-nanochannels. Lab Chip. 2021, ID: LC-COM-03-2021-000239.R2

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