Supplementary Information for Rapid Structural Analysis of Nanomaterials in Aqueous Solutions

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The supplementary Information includes:

1. Supporting Figures (Figs. S1-S10)

Figure S1. Scanning electron microscopy image of the low aspect ratio nanopore. A 1000 nm-diameter nanopore fabricated on a thin Si₃N₄ membrane for the detection of Pst beads.
Figure S2. Performances for the present ion current measurement system. (a) Rising edges of a square wave (from $-1.0$ V to 1.0 V) generated from a voltage source. The direct signal from the source (blue: signal 1) and the same signal through a high-speed amplifier (amplitude gain: $1.0 \times 10^8$) with a 100 MΩ series resistance (red: signal 2) were detected by a 500 MHz oscilloscope (sampling rate: 5GHz, LeCroy WaveRunner6050A). Green curve corresponds to a filtered signal from the source (signal 1) with FIR (Finite Impulse Response) low-pass filter, of which cutoff frequency is 10 MHz. Since the rise time is defined to be the time between 10% and 90% of the...
edge, both rise times of “signal 1” and “signal 2” are estimated to be 29 ns and 44 ns, respectively, indicating that the rise time of the amplifier is 33.1 ns \[33.1 = (44^2 - 29^2)^{1/2}\]. The bandwidth (BW) for the present measurement system is thus obtained to be 10.5 MHz from the definition, \(BW = 0.35/[\text{rise time}]\). In addition, a rise time of the signal filtered with 10 MHz low-pass filter (green) is comparable to that of “signal 2” via the amplifier (red). It is thus found that the high-speed amplifier can trace 10 MHz-signals accurately.

The high-speed amplifier system was realized by 2 kinds of techniques. The techniques are: (1) Reducing noise from an electric power source for the amplifier and (2) preventing oscillations in an operational amplifier circuit at high gain \((1.0 \times 10^8)\). We first reduced noise from an electrical power source by cutting a feedback circuit for a power (voltage) supply in the source because a feedback circuit causes switching noises with each feedback action. In order to make applied voltage stable, we employed digital photocouplers in place of the feedback circuit because digital photocouplers can physically separate the power supply from a circuit sending out signals about voltage we apply, resulting in that switching noises are completely eliminated from a sample. Indeed, S/N ratio of the present original power source is improved compared to an existing low noise power source (see Fig. S3). Secondly, a 1 GHz FastFET op amp (ADA4817) and chip resistors as a feedback resistance were employed in our amplifier system because floating capacities in chip resistors are very small compared to carbon resistors. In addition, some chip resistors are used in a series trace because the total floating capacitance is decreased with increasing number of the resistors in the series trace (here, total resistance is adjusted to 100 MΩ). Although, reducing the total floating capacitance of the amplifier system improves the measurement bandwidth (BW), this also causes an increment in noise because the op amp with a lower floating capacitance can gain higher-frequency band noise. Thus, we found out the best floating capacitance of the feedback resistance for this study by optimizing the number of chip resistors. Furthermore, soldering conditions and
positioning of all stuffs (op amp, chip resistors, wires, etc) in the amplifier system are optimized in order to prevent oscillations. On top of these, floating capacities between the amplifier and a sample were reduced by shortening the physical distance between the amplifier system and a sample in order to prevent noises and oscillations. A single point ground system and a thick shielding box were also employed for the system. In this study, the 10 MHz ionic current–time data were compressed to 1 MHz to obtain high measurement accuracy by averaging 10 data points.

(b) (c) Peak to peak noise levels of the collected data before and after the amplifier are respectively ca. ±30 mV (b) and ca. ±50 mV (c). Because Johnson noise is estimated to be 28.7 mV when 100 MΩ, 500 MHz, and 300 K, these noises consist of Johnson noise and another noises. (d) Peak to peak noise levels of 38 pA in the case of nanopore devices for the Pst bead detections in this study. Johnson noise in the measurements was obtained to be 4.1 pA [0.41 mV (10.3 MΩ, 1 MHz, 300 K)].
Figure S3. Comparison of S/N ratios between a low noise power source (blue) (ShibaSoku: PA15A1) and our original ultra low noise power source (red). The data were collected using 500 MHz oscilloscope (sampling rate: 5GHz, LeCroy WaveRunner6050A). There are no switching noises in a signal from the original source because digital photocouplers were employed in place of a feedback circuit in the amplifier system.
Figure S4. Calculated ionic current density distribution \( J(r, z) \) for the system, where a dumbbell-like Pst beads is dwelling inside the nanopore. Here the nanopore parameters are the same as shown in the main text. A 2-dimensional axial symmetrical multiphysical model was built up using COMSOL including Navier–Stokes, Nernst–Plank, and Poisson equations as shown in the Method section of the main text. By assuming the radius of the joined part of the double beads to be 190 nm, the calculated electrical current blockade agrees with our experimental results.

The mesh size distribution and salt concentrations used here are below,

- Maximum Size: 100 nm
- Minimum Size: 0.401 nm
- Maximum Growing Rate: 1.1
- Resolution in Flexion: 0.1
- Resolution in Minimal Region: 1
- Boundary Layer: set at the interface of SiN Membrane and Solution
  
  - Layer Number: 10
  - Stretching Factor: 1.2
  - Thickness Factor: 1

Salt Concentration: 10 mMTris-HCl
and the surface charge density on the SiN is not taken account in this study because the surface charges on the nanopore wall, as characterized by $\sigma$, gave negligible contribution to the measured open-pore current and blocked current. For imposed salt with concentration $C_{\text{tris}} = 10$ mM, the thickness of electrical double layers (EDL) was about 3 nm. It is trivial compared to the diameter of our fabricated nanopores ($d = 1000$ nm). Here we provide a detailed description why we neglect the influence of SiN nanopore wall surface charges.

1) The ratio of contribution by $\sigma$-induced counterions to the cross-pore ionic current and that by the ordinary ions is estimated as follows:

$$\gamma = \frac{2\pi\sigma R}{(eN_A 2C_0 \pi R^2)} = \frac{\sigma}{(eN_A C_0 R)}.$$ 

In the above, $e$ is the electron charge, $N_A$ is the Avogadro constant. $2\pi\sigma R$ is line density of $\sigma$-induced counterions along the pore axial direction. Given $\sigma \sim 0.01$ C/m$^2$ (Data comes from the reference: "Determination of surface charge density parameters of silicon nitride", Colloids and Surfaces A: Physicochemical and Engineering Aspects, vol. 108, pp. 27), $\gamma \cong 1\%$ for our case. Therefore, our first conclusion is that $\sigma$ made negligible contribution to the measured open-pore electrical current.

2) The extent of overlap between EDL by pore-wall surface charges and by the target particles is negligible. For Pst beads dwelling inside the pore, in principle the Pst bead motion is affected by the $\sigma$-induced electroosmotic flow (EOF). As seen in the following figure, since both the pore-wall and the Pst beads are negatively charged, the Pst beads are assumed to translocate along the central axis of the pore due to the electrostatic repulsion. Moreover, given $C_{\text{tris}} = 10$ mM, the thickness of EDL surrounding the wall and that surrounding the Pst beads is about 3 nm (the light-yellow layers sketched in the figure).
As shown in the above figure, the two layers of EDL do not overlap in a $R = 500$ nm nanopore. From our previous analysis (Fig1C of "Controlling DNA Translocation through Gate Modulation of Nanopore Wall Surface Charges", *ACS Nano* vol. 5, pp. 5509), $\sigma$-induced electroosmotic flow (EOF) has negligible influence on the anionic target particle movement. In other words, the motion of the target Pst bead does not be tuned by the wall surface charges. In conclusion, in our low aspect-ratio nanopore systems, the effects by the pore wall surface charges are negligible compared to that by the bulk ions.
Figure S5. $I_{\text{ion}}$-$t$ profile of the Pst bead translocation events including 454 restive pulses. Figure 2b is a part of this profile, then the histograms shown in Figure 2c and 2d are constructed from this data.
Figure S6. The series resistance model. The ionic current \( I_{\text{ion}} \) obeys Ohm’s law, thus \( I_{\text{ion}} = \frac{V_b}{R_{\text{total}}} \), where \( V_b \) denotes the applied voltage and \( R_{\text{total}} \) is found to be

\[
R_{\text{total}} = R_{\text{pore}} + R_{\text{acc}} = \frac{\rho L}{\pi r^2} + \frac{\rho}{2r},
\]

where \( R_{\text{pore}} \) is the pore resistance and \( R_{\text{acc}} \) is the access resistance, \( \rho \) is the resistivity of the buffer solution, \( L \) is the thickness of the pore, and \( r \) is the radius of pore. For conventional high aspect ratio pores, \( R_{\text{acc}} = \rho/2r \) can be assumed as a constant because the change in \( R_{\text{acc}} \) due to particle translocation is much smaller than that of \( R_{\text{pore}} = \rho L/\pi r^2 \).

In the case of open pore with \( L = 35 \) nm, \( r = 500 \) nm, \( R_{\text{pore}} \) and \( R_{\text{acc}} \) are respectively estimated to be \( 4.42 \times 10^5 \) \( \Omega \) and \( 9.92 \times 10^6 \) \( \Omega \) when \( \rho = 9.92 \) \( \Omega m. \) Thus, base line ionic current is obtained to be 9.65 nA when \( V = 100 \) mV. On the other hand, \( R_{\text{pore}} \) is estimated to be \( 1.13 \times 10^6 \) \( \Omega \) when pore is occupied by a 780 nm-diameter Pst bead. Here, effective \( r \) when the occupation is defined as the quotient of a difference between a volume of a nanopore \( (L \times \pi r^2) \) and a Pst bead inside a nanopore by pore thickness. Here, volume of Pst bead inside a nanopore is calculated by

\[
\int_{-L/2}^{L/2} x^2 dy, \quad r^2 = x^2 + y^2.
\]
Figure S7. Simulations for the off-axis translocation effect on $I_p$ and $I_p$ distribution [1]. (a) The size distribution of the Pst beads used in this simulation. It is assumed to be a symmetrical Gaussian distribution in a range of $D = 741 - 819$ nm. (b) Definition of the off-axis distance $b$, where $2b < d$ (pore diameter) $- D$ (beads diameter) for each bead size. (c) Dependences of estimated $I_p$ distributions on $b$. The probability of the Pst bead translocations on the central axis inside a pore was assumed to be the same to that of any off-axis translocations although there were driving forces toward center of a pore for the beads such as coulomb interactions between beads and a pore, and electro-osmotic flows (EOF). Thus, the probability of translocation with $b = 0$ “$P_{b=0}$” (i.e. the central axis translocation) is equal to $P_{b=2.5}$, $P_{b=5}$, $P_{b=7.5}$, … $P_{b=87.5}$ in the case of $b = 0 - 87.5$ nm (resolution of $b = 2.5$ nm). In this simplified calculation, all-size-beads were also assumed to translocate on all axes with the same probability. However, $2b < d$ (pore diameter) $- D$ (bead diameter) in this calculation. Thus, when off-axis translocation with $b = 127.5$, only Pst beads with $741 - 745$ nm diameter can
pass through a pore on the off-axis. The distribution centered at 599 pA corresponds to due to only the central axis translocations, i.e. \( b = 0 \) nm (blue), and distributions considering \( b = 0 – 87.5 \) and \( 0 – 127.5 \) nm show the center at 610 pA (red) and 630 pA (green), respectively. Since the present \( I_p \) histogram shown in Figure 2c shows the center of the symmetrical distribution at approximately 610 pA, which is slight larger than the estimated center (599 pA) of the \( I_p \) distribution due to only the axis translocation, the present \( I_p \) histogram should be considered to be slightly affected by the off-axis translocations of the beads. Although this calculation should take account for the coulomb interactions and EOF for more strict quantitative discussion, the simplified simulation indicates that a range of the off-axis distance of the present Pst bead translocations could be \( b = 0.0 – 87.5 \) nm because a simulation with \( b = 0.0 – 87.5 \) nm reproduces an \( I_p \) distribution centered at 610 pA. (d) The breakdowns of the bead size resulting in \( I_p = 590, 600, 610, 620, \) and 630 pA in the simulated histogram (Figure S7c, red). As shown in Figure S7d, it is found that 780±3 nm-diameter beads account for 71% of all beads casing the resistive pulses with 610 pA. The smallest Pst bead of 770 nm is responsible for only 4.7% of the translocation events with \( I_p = 610 \) pA. Thus, most of the Pst beads resulting in \( I_p = 610 \) pA have a diameter around 780 nm. Furthermore, the calculation reveals that the abundance ratio of 780±3 nm-diameter Pst beads shows the highest value 71% when \( I_p = 610 \) pA. The ratios are estimated to be 27, 56, 40, and 32% in the case of \( I_p = 590, 600, 620, \) and 630 pA, respectively.

[1] Z. Qin, J. Zhe and G.-X. Meas Sci Technol. 2011, 22, 045804.
1) Diffusion coefficient ($D$) of the 780nm-diameter particle:

$$\frac{kT}{6\pi R\eta}, \quad (1)$$

where $kT$ is 0.026 eV, $R$ is the particle radius and $\eta$ is the viscosity.

2) Deviation [Displacement ($L$)] by Brownian motion:

$$L = \sqrt{2Dt} \ (for \ z \ axis), \quad L = \sqrt{4Dt} \ (for \ xy \ plane), \quad (2)$$

where $t$ is the translocation time.

**Figure S8. Influences of the Brownian motion on $I_p$ and $t_d$.** The diffusion coefficient is obtained to be $6.1792e-013$ from eq.(1). Thus, Brownian motion displacements ($L$) of the beads per translocation time 1.01 ms in xy-plane and z-axis are 49.9 and 35 nm, respectively. If a Pst bead moves through a distance of 49.9 nm in xy-plane direction during a translocation, the off-axis translocation effects on $I_p$ would be induced. However, the displacement is too short to affect $I_p$ because the present determining factor ($2L/d$) for the effects is maximally only ca. 0.1 (= $2 \times 49.9 \text{ nm} / 1000 \text{ nm}$). As the value less than 0.1 contributes little to $I_p$ [1], the influence of the Brownian motion on $I_p$ in this study is almost negligible. On the other hand, in a case of influence of Brownian motion on $t_d$, although temporal displacements were found to be ca. 1 nm in 1 $\mu$s, the change in the particle positions due to Brownian motion in the course of translocation events taking about 1 ms is 35 nm on average. Therefore, Brownian motion has the potential to give non-negligible influence on the ionic current spike patterns; in case for the present experimental conditions, it may blur the particle shape acquired by the nanopore tomography by several tens of nanometers. As the diffusion coefficient scales inversely with their size, the effects on the spatial resolution would be more (less) significant for larger (smaller) particles.

In addition, The Pst beads transverse movement caused by Brownian motion has discussed in following theoretical study. We establish a 3-dimensional multiphysical
model for evaluating the related ionic current blockage. The electrostatic and ion transport equations are the same as those shown in the main context, while the geometry has been constructed as follows:

![Figure A: The geometry for 3-dimensional modeling of the particle moving through the nanopore system. The radii of the cis chamber, trans one and the pore are characterized by $R_{cis}$, $R_{trans}$ and $R_{pore}$ in the figure. The transverse deviation of the particle center from the nanopore axis is denoted by $\Delta x$.](image)

In the above, $R_{cis}$ and $R_{trans}$ characterize the radii of cis and trans hemispheres, $\Delta x$ is the distance of the particle center from the pore axis, and $R_{pore}$ is radius of the nanopore. The analytic expression for resistance of system without the nanoparticle shown in the above figure should be

$$R_{model} = \left[\left(\frac{1}{4} - \frac{1}{2\pi R_{cis}}\right) + \frac{L}{\pi R_{pore}^2} + \left(\frac{1}{4} - \frac{1}{2\pi R_{trans}}\right)\right] \cdot \frac{1}{e \sum \mu_i n_i |z_i|}$$

while the resistance for the space outside of the area which is neglected in the above figure is

$$R_{outer} = \left(\frac{1}{2\pi R_{cis}} + \frac{1}{2\pi R_{trans}}\right) \cdot \frac{1}{e \sum \mu_i n_i |z_i|}$$

In our numerical simulation, the voltage difference $\Delta V$ on the two arc ends of the hemispheres in the above figure is initially guessed as:

$$\Delta V = V_z \cdot \frac{R_{model}}{R_{model} + R_{outer}}$$

where $V_z$ is the applied voltage to the nanopore system.

We then numerically calculate the electrical current $I_0$. In this way we estimate the
resistance of blocked simulation region to be

$$R_{\text{block}} = \frac{\Delta V}{I_0}$$

Therefore, the actual voltage falls between the two hemispheres is

$$\Delta V_{\text{block}} = V_z \cdot \frac{R_{\text{block}}}{R_{\text{block}} + R_{\text{outer}}}$$

And the electrical current is

$$I_{\text{block}} = \frac{V_z}{R_{\text{block}} + R_{\text{outer}}}$$

By setting $\Delta r \approx 0$ nm and 49.9 nm separately, we obtain the ionic current variation during the $R = 390$ nm beads translocation through the pore. Results are just as that shown in Fig.4c of our previous work (“Particle Trajectory-Dependent Ionic Current Blockade in Low-Aspect-Ratio Pores”, ACS Nano, vol. 10, pp. 803). The two traces $I(z)$ almost overlap. So we conclude that the transverse deviation caused by the Brownian motion in our low aspect-ratio nanopore system contributes negligibly to the tuning of ionic current blockage.

[1] Z. Qin, J. Zhe and G.-X. Meas Sci Technol. 2011, 22, 045804.
Figure S9. SEM images of Pst beads prepared by drop casting on a SiO$_2$ substrate. TE buffer solution containing 780 nm-sized beads at a concentration of 0.1 pM was dropped on a SiO$_2$ substrate. After approximately a second, the drop was subsequently blown by a N$_2$ blower. (a) Distributed single-Pst and double-Pst beads on the substrate at a scale of 20 µm. (b) Dumbbell-like Pst beads at a scale of 500 nm.
Figure S10. Discrimination and structural analysis of bioparticles using a low-aspect-ratio pore. We evaluated usefulness of the shape-sensitive nature of low-aspect-ratio pore sensors for discriminating bioparticles. E. coli was employed as a test system, which has a cylindrical motif of diameter and length of about 400 nm and 1500 nm, respectively. Ionic current measurements across a Si$_3$N$_4$ pore with an aspect ratio of 0.042 ($L = 50$ nm, $d = 1200$ nm) in a PBS buffer including E. coli yielded resistive pulses of various $t_d$ revealing diverse range of electrophoretic speed involved in the experiment. (a) Typical resistive pulses due to Pst bead (blue, green, and pink), E. coli (red), and pollen allergen (black) passing through a pore with an aspect ratio of 0.042 ($L = 50$ nm, $d = 1200$ nm) in a PBS buffer. Insets are SEM images of each analyte. (b) Profile of the various resistive pulses of E. coli translocations indicating...
diverse peak current $I_p$ and duration time $t_d$. (c) Comparison between larger (red) and smaller (blue) current–time traces of E. coli translocations. Larger and smaller spikes are ascribed to angled and perpendicular translocation events, respectively. (d) $I_p$ and $t_d$ scatter plots of 780 nm- and 900 nm-diameter Pst beads (green and pink), E. coli (red), and pollen allergen (black). (e) Calculated current–time profile of E. coli by the multiphysics simulation (red) with the experimental result (blue). The translated $I_{ion}$ data into tomograms revealed two kinds of units of main body (410 × 1400 nm) and flagella of E. coli (200 × 2000 nm).

Overall difference in the properties eventually enabled unique discrimination of the cylindrical cells among other spherical particles of comparable sizes by virtue of the peculiar geometry on the ionic current responses in low-aspect-ratio pores.

The simulation parameters used in this fitting are below,

\begin{align*}
V &= 0.2 \text{ V} \\
\varepsilon &= 7.08 \times 10^{-10} \text{ F/m}, \\
\eta &= 8.91 \times 10^{-4} \text{ Pa·S}, \\
\mu_{\text{positive}} &= 7.616 \times 10^{-8} \text{ m}^2/\text{s·V}, \\
\mu_{\text{negative}} &= 1.0388*\mu_{\text{positive}} \\
\text{Salt Concentration: } 0.1 \text{ M PBS}
\end{align*}

The fitted surface charge densities of the E. coli $\sigma_{\text{ecoli}}$ were $-1.00 \times 10^{-5} \text{ C/m}^2$. Mesh condition was Extremely Fine.

The reason why we choose the data with short duration time such as $t_d = 0.002 \text{ s}$ is that these short events are due to straight transits of E. coli. For E. coli measurements, we observed larger $I_{ion}$ spikes for slower translocation events as shown in Figure S10b, S10c, and S10d. These slower translocations are caused by E. coli entering at angles. Because the present multiphysics simulation is available for only axial symmetrical models, we thus choose the events caused by straight transits for the fitting. Indeed, the fitting result shown in Figure S10e is consistent to another short time events shown in the scalar map in Figure S10b.