Supporting Information

Molecular Dynamics Simulation Study of Transverse and Longitudinal Ionic Currents in Solid-State Nanopore DNA Sequencing

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System 2: Transverse and longitudinal ionic currents and their correlation. Figure S1a-c demonstrate two transverse and one longitudinal ionic currents along the x, y, and z axis respectively as a function of time during the translocation of four types of homo-oligonucleotide ssDNA calculated from five sets of MD simulations. The applied voltages along the x, y and z axes are 0.5V, and the average diameters of cylindrical longitudinal and transverse pores are 2 nm and 1.4 nm respectively.

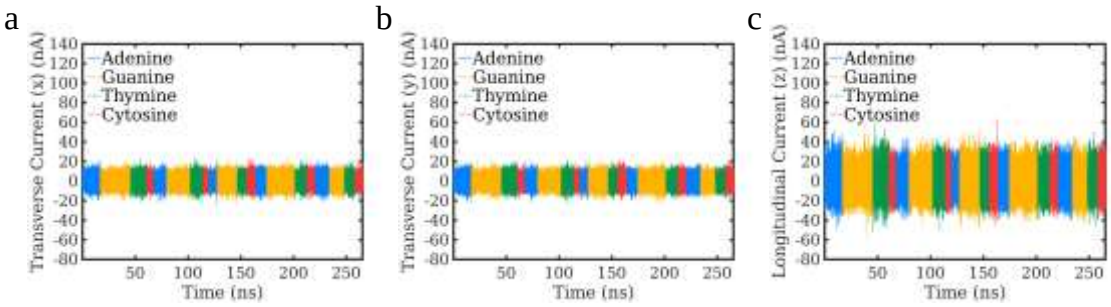

Figure S1. System 2: Instantaneous ionic current versus time **for four types of oligonucleotide ssDNA and five sets of MD simulations. a) Instantaneous transverse ionic current along the x axis. b) Instantaneous transverse ionic current along the y axis. c) Instantaneous transverse ionic current along the z axis.**

In the main text for system 2 in Figure 5b we showed the cross-correlation of two transverse ionic currents along the x and y axis. In Figure S2a-c we present the cross-correlation between the transverse ionic currents along the x and y axes and each of them with the longitudinal ionic current along the z axis.

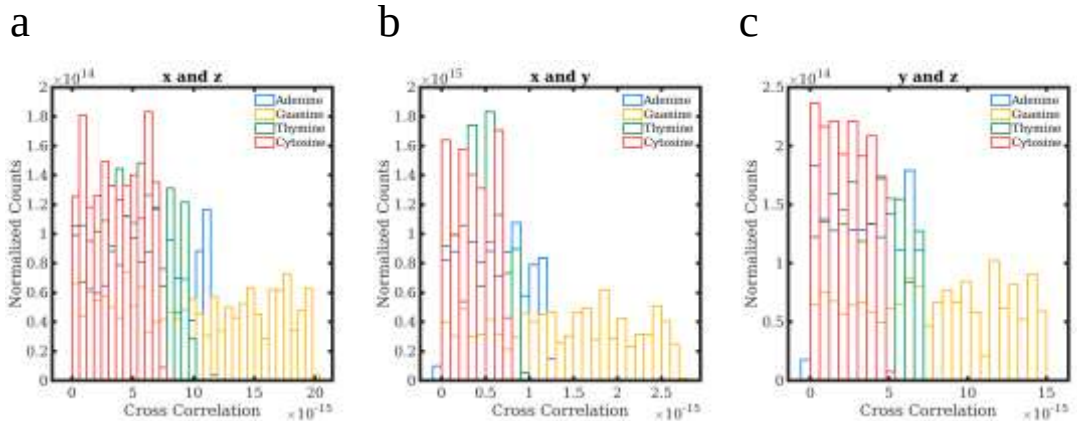

Figure S2. System 2: Cross-correlation of transverse ionic currents with longitudinal ionic currents. a-c) The cross-correlation of transverse ionic current along the x and y axis together and with longitudinal ionic current along the z axis.

System 3: Transverse and longitudinal ionic currents through a thin Si_3N_4 nanosheet. In system 3, we calculated the longitudinal and transverse ionic currents during the translocation of four types of homo-oligonucleotide through nanopores in Si_3N_4 sheets at higher transverse applied fields of 4 V and 10 V. The signals were computed from two sets of molecular dynamics (MD) simulations for each oligonucleotide type at each field. We compared the average current and translocation time for each homo-oligonucleotide with each other. Also, we studied the combination and cross-correlation of longitudinal and transverse ionic current signals to obtain more information about each type of homo-oligonucleotide (Described in Methods). In nanopore DNA sequencing, the effect of neighboring nucleotides should be minimized so that only one nucleotide is the cause of a change in ionic current. In this regard, we made a thin nanosheet with a thickness of 1.6 nm to reduce the number of nucleotides that occupy the pore at each time step. The length of a single nucleotide is around 0.8 nm in a straight ssDNA. As a result, approximately two nucleotides contribute to modulation of the longitudinal ionic current during the translocation of ssDNA through a nanopore. We could not model a thinner sheet that retained the smallest size of transverse nanochannel compatible with measuring ion translocation through the channel. The average diameters of cylindrical longitudinal nanopores and elliptical transverse nanochannels are 2 nm and 1 nm, respectively.

a. Applied transverse voltage bias of 4V and longitudinal voltage of 0.5V.

Figure S3a shows system 3 with a very thin Si_3N_4 nanosheet and four reservoirs creating a cross-shaped model. In simulations on system 3, we used 0.5 V longitudinal voltages to drive the ssDNA and ions through longitudinal nanopores, and a voltage of 4 V in the direction of a very

narrow transverse nanochannel (diameter 0.8nm) to drive an adequate number of ions through the nanopore (diameter 2.0nm) and enough force on the ions present in the nanopore to interact with ssDNA. Figures S3b and S3d show the side and top view of the system, respectively.

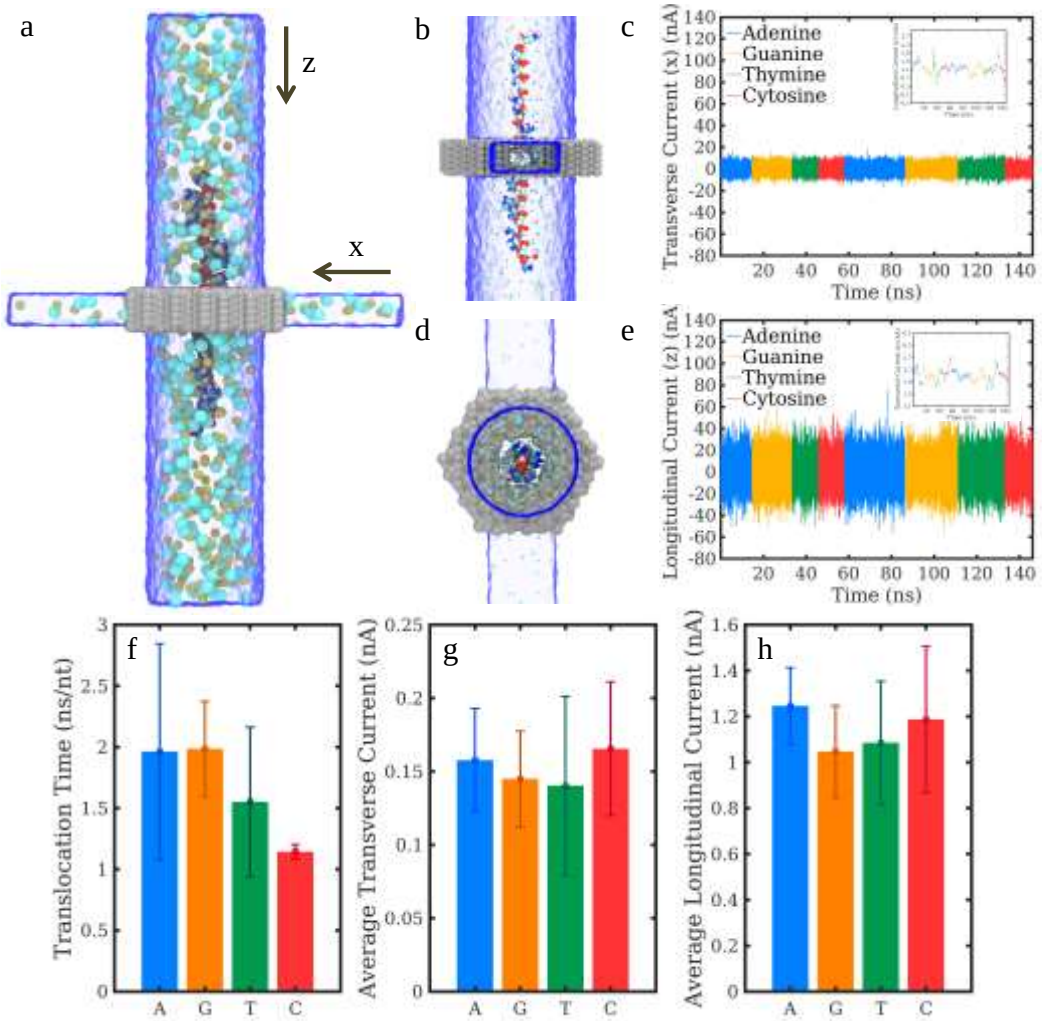

Figure S3. System 3: Translocation of four types of homo-oligonucleotides through longitudinal Si_3N_4 nanopore and calculated transverse and longitudinal ionic currents. (a) A perspective of the system 2 along the y axis. Blue continuum color represents water, 1.8 Å diameter cyan color and 1.3 Å diameter brown color spheres represent Cl^- and K^+ ions in water respectively. The grey sheet in the middle is Si_3N_4 nanosheet in the presence of an adenine homo-oligonucleotide ssDNA with the sequence of poly(A)16. The Si_3N_4 ions, and ssDNA are shown as van der Waals (vdW) representations.

(b,d) Perspectives along the x and z axis respectively. The colors in 4b,d represent the same species as in Figure 5a with the difference that ions are shown as scaled vdW representations. (c,e) Instantaneous transverse and longitudinal ionic currents (nA) versus time (s) for four types of oligonucleotide ssDNA from two sets of MD simulations. The smooth line over a 5 ns window of transverse ionic current is shown in the small boxes (insets). (f) Average translocation time (ns/nt) of each homo-oligonucleotide. (g,h) Average transverse and longitudinal ionic currents (nA) for four types of homo-oligonucleotide.

Figures S3c and S3e sequentially show the noisy transverse and longitudinal ionic currents during the translocation of homo-oligonucleotides. The longitudinal nanopore has a larger diameter than the transverse nanochannel, which results in more ions traversing in the longitudinal direction regardless of the large transverse voltage of 4 V. Consequently, the longitudinal ionic current fluctuates more than transverse ionic current. Figure S3f is a bar graph of the average translocation time for each type of homo-oligonucleotide. The order of translocation of time (ns/nt) is G > A > T > C corresponds to the order of the total size of atoms in each nucleotide G > A > T > C. Figures S3g and S3h show that the order of the average transverse ionic current is C > A > G > T and for the average longitudinal ionic current is A > C > T > G.

Figure S4a is a 2D scatter plot of filtered ionic current signals (nA) over a window of 5 ns generated from currents through transverse and longitudinal nanochannels and nanopores. Figure S4a illustrates the individual Kernel probability distribution of averaged signals in each transverse and longitudinal direction. The scatter plot combines two signals measured simultaneously from two different directions with two different orientations of nucleotides in each homo-

oligonucleotide ssDNA. This increases partial differentiation between signals of homo-oligonucleotide types in comparison to overlapped distributions of individual signals (Figure S4a).

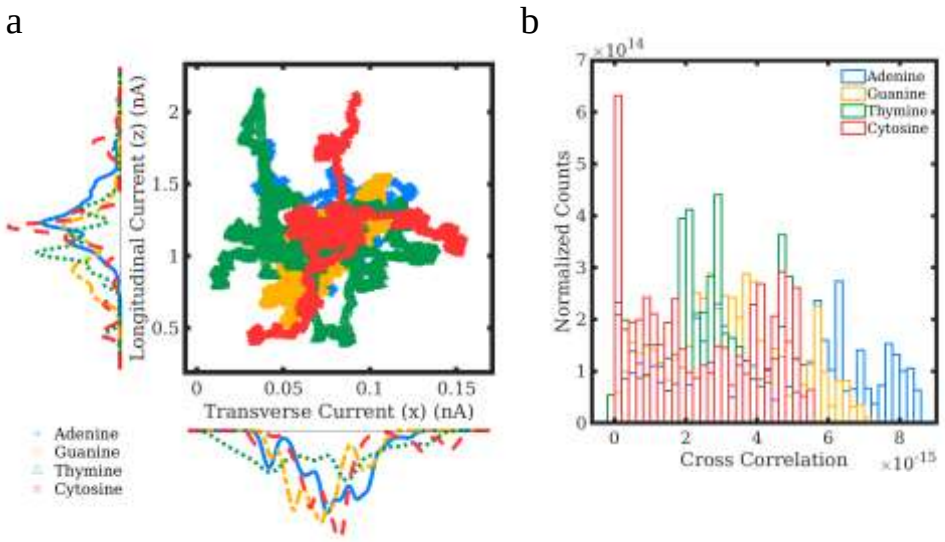

Figure S4. System 3: Combination of transverse and longitudinal ionic currents. a) A scatter plot with longitudinal ionic current (nA) in the z direction and transverse ionic current (nA) along the x axis. Also, the Kernel fitted histogram of each ionic current is represented individually on each axis. b) A Cross-correlation histogram of the transverse and longitudinal ionic currents.

b. Applied transverse voltage bias of 10 V and longitudinal voltage bias of 0.5 V.

We assumed that increasing the transverse voltage bias from 4V to 10 V would increase the nucleotide-ion interaction as the number of voltage-driven ions translocating through the narrow nanochannel increases at the same voltage. Figures S5a and S5b show that the fluctuation of transverse and longitudinal ionic currents diminishes in the presence of an applied voltage bias of 10 V compared with 4 V. Figure S5c indicates a lower translocation rate of oligonucleotide ssDNA through longitudinal nanopore (2 nm diameter) when we applied a transverse voltage of 10 V. The

order of translocation time (ns/nt) remains the same for applied transverse voltage biases of 4 V to 10 V.

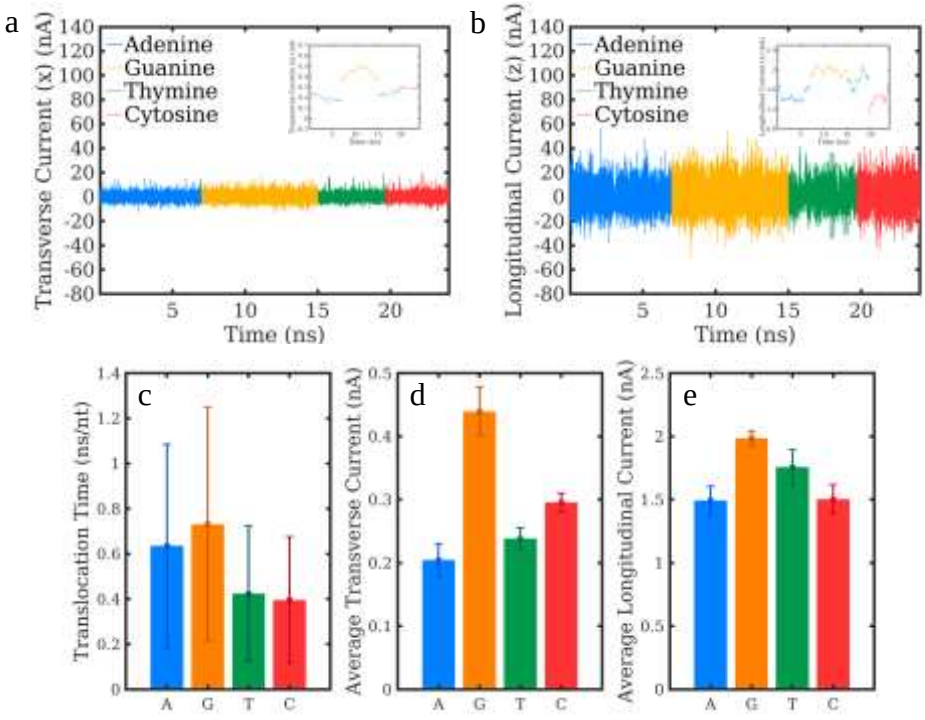

Figure S5. System2: Transverse voltage bias of 10 V and longitudinal voltage bias of 0.5 V. a,b) Instantaneous transverse and longitudinal ionic current respectively. The smooth lines over a 5 ns window of transverse and individual currents are shown in small boxes. c) Average translocation time (ns/nt) of each homo-oligonucleotide. b,c) Average transverse and longitudinal ionic currents in nano Amperes (nA) for four types of homo-oligonucleotide.

Figures S5d and S5e show an increase in average transverse ionic current and, unexpectedly, in the longitudinal ionic current, despite a decrease in the magnitude of fluctuations as shown in Figures S5a and S5b. Looking at the recorded trajectories and frames, we found that a turbulent flow of ionic currents carrying water molecules is produced through the nanopore under a high transverse voltage bias e.g. 10 V. As the nanopore diameter is significantly larger than the

nanochannel diameter and considering that they are interconnected, the ions and water molecules that enter the transverse nanochannel prefer to exit from the longitudinal nanopore and create a fountain of water flowing through both sides of Si_3N_4 nanopore. Consequently, for the homo-oligonucleotide ssDNA to enter the nanopore, more time is required to overcome energy barriers induced by ions and waters coming out of the nanopore. The reverse driving force induced by ions and waters lead to a faster translocation rate once ssDNA enters the nanopore. This interpretation of ions and water molecule behavior in our MD simulations justifies the high translocation rate of homo-oligonucleotide ssDNA through the nanopore in the presence of a transversely applied electric field with a voltage bias of 10 V compared to a voltage bias of 4 V. Furthermore, the average ionic current signals for the four types of oligonucleotides do not show a noticeable enhancement of separation based on their ionic current modulation.

Fluctuations in transverse and longitudinal ionic currents are caused by thermal energy. The magnitude of fluctuation in the transverse ionic current is less than that of the longitudinal ionic current. The ionic current decreases linearly with the length of the pore. In system 2, with equal transverse and longitudinal applied voltages of 0.5 V, we showed in Figure S1 that the fluctuation of the transverse ionic current through nanochannel with a diameter of 0.8nm and length of 60 nm is seemingly more than twice the fluctuation of the longitudinal ionic current through the nanopore (2 nm diameter) with a length of 24 nm. Furthermore, in system 3 we showed that thermal fluctuations decrease with increase in an applied electric field to 10 V (Supporting Information Figures S3 and S5). We hypothesized, that at higher voltage the number of ions traversing the pores per unit time increases and as a result the fluctuation of ionic current decreases. However, both the average transverse and longitudinal ionic currents increase at higher transversely applied voltages. The ions entering transverse nanochannel add to the ionic current in longitudinal

direction as they prefer to exit through the wider nanopore due to the very narrow diameter of transverse nanochannel (0.8 nm) in comparison to diameter of the longitudinal nanopore (2 nm).

Figure 6Sa sequentially present the scatter plot of transverse and longitudinal ionic currents with their individual Kernel probability distribution along with the x and y axis. Figure 6Sb shows the cross-correlation of transverse and longitudinal ionic currents.

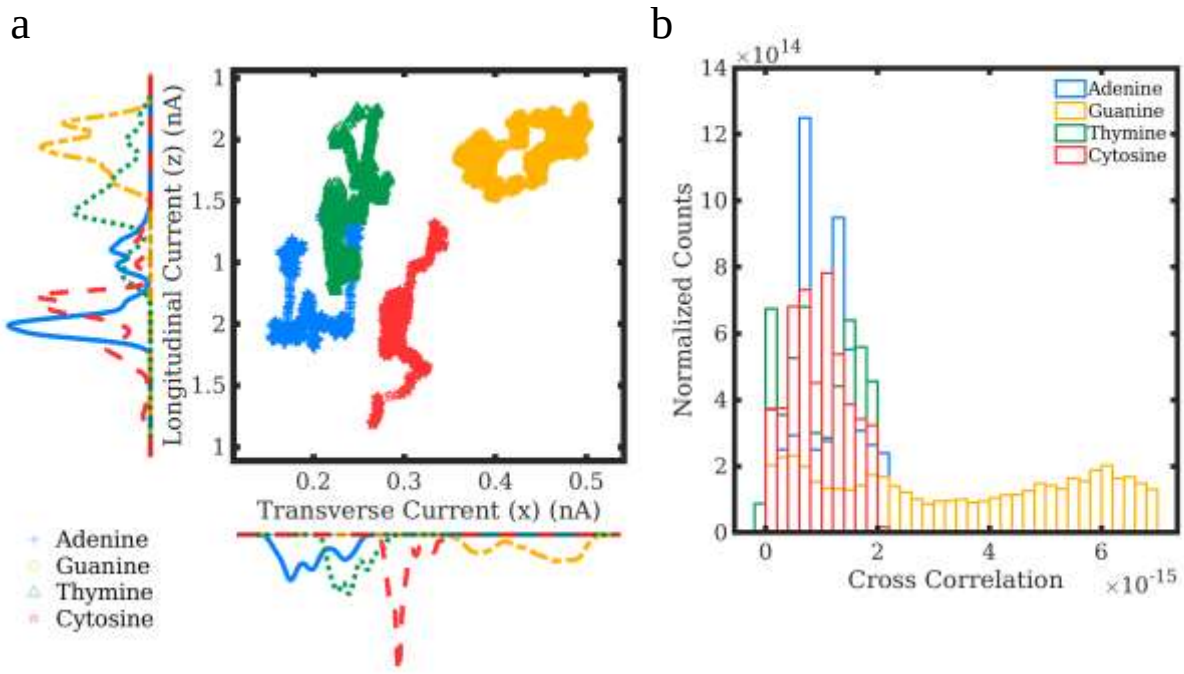

Figure S6. System 3: Combination of transverse and longitudinal ionic currents under applied voltage base of 10 V. a) A scatter plot with longitudinal ionic current (nA) in the z direction and transverse ionic current (nA) in x axis. Also the Kernel fitted histogram of each ionic current is represented individually on each axis. b) A cross-correlation histogram of the transverse and longitudinal ionic currents