Supporting Information

Single-Molecule DNA Detection Using Novel SP1 Protein Nanopore

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Experiment Section

Atomic Force Microscopy (AFM) imaging: The liphytanoyl-phosphatidyl-choline in $CHCl_3$ was obtained from Avanti Polar Lipids Inc (Alabaster, Ala) and used without further purification. The $CHCl_3$ lipid solution was dried under a vacuum for 4 h. The lipid suspension, about 0.5 mg/ml of lipids in 20 mM NaCl solution was centrifuged at 14000 rpm for about 30 min [1]. The supernatant was used. A drop of about 20 μLlipid suspension was dripped onto a piece of freshly cleaved mica and allowed to interact with the support at 4°C overnight. [2] Excel of the lipid suspension was then removed by 0.15 mL Millipore water (18 MΩ·cm) to remove excess lipid suspension and the air-dried lipid was used as the substrate. Subsequently, 10 μL of the SP1 solution (3×10^{-6} M) were dropped out the lipid material. The mica was attached to the 1.6- cm diameter metal disk supplied by Digital Instruments (Santa Barbara, CA).

AFM images were acquired by using Nanoscope IIIa Multimode AFM with an extender electronics module (Veeco, Santa Barbara, CA). Oxide-sharpened silicon nitride cantilevers with a nominal spring constant of ≈ 3 N/m were used for experiments. All imaging experiments were carried out in both regular contact mode and in tapping mode (at oscillation frequencies between 9 and 15 kHz). The scan rates ranged between 1 and 12 Hz. All imaging experiments were performed in 10 mM Tris-HCl solution (pH 8.0) by using AFM lipid cell at room temperature.

Transmission Electron Microscopy (TEM) imaging: A TEM carbon-evaporated grid was laid on the drop for 1 min, withdrawn, dried. This method has been commonly used to acquire 2D arrays for a variety of soluble and non-soluble proteins [3]. TEM imaging revealed a well-defined hexagonal closed pack arrangement of the SP1 proteins.

Materials

The experiments for bilayer forming were performed as described previously [4]. The only exception is SP1 nanopore self-assembled on bilayer membrane with Tris-HCl buffer at pH 4.5. A pore was deemed stable by monitoring its channel capacitance and then titrated to the pH 8.0 by 0.2 M NaOH solution. Once an appropriate single-pore insertion was detected, the experimental and data collection procedure were conducted with previously described [5]. DNA oligonucleotides were synthesized by Shanghai Sangon (Shanghai Sango Corporation, Shanghai, China) and were HPLC pure. The sequence of RD is AAC TAC TGG GTT ATC GTG AC.

The experimental results using α-HL nanopore

The blockade current value was proportional to the length of ssDNA and the applied potentials. The three parameters in analyzing duration time for all ssDNAs using two biological nanopores from at least three independent experiments are listed in Table S1. The blockade current population was obtained by fitting the blockade current distribution with the Gaussian function. The duration time distribution data were decided by fitting with single- exponential function. The SP1 formation on lipid bilayer was observed by transmission electron microscopy (TEM) and Atomic Force Microscopy (AFM) (Figure S1).

Table 1 Three parameter are used in analyzing duration time

Oligonuleotide	t_d (ms)		t_p (ms)		τ_T (ms)	
	α-HL	SP1	α-HL	SP1	α-HL	SP1
$Poly(dT)_{20}$	1.42	4.74	0.38	0.58	0.47	1.07
$Poly(dT)_{45}$	0.76	1.98	0.37	0.48	0.34	1.59

Poly(dA)$_{20}$	0.50	0.88	0.33	0.44	0.10	0.41
Poly(dA)$_{45}$	0.67	9.25	0.52	0.45	0.17	0.78
RD	1.14	1.27	0.45	0.46	0.20	0.47

All data were recorded at 25 ± 0.5°C, applied potential 100 mV.

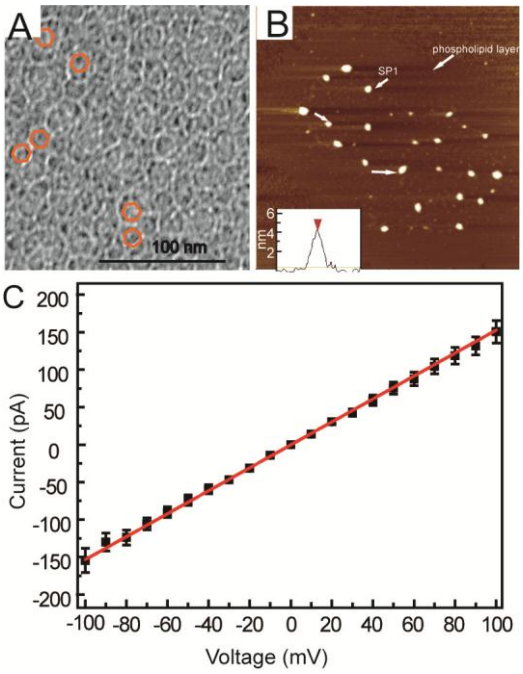

Figure S1 A. TEM image of the wild-type SP1 self-assembled on a lipid bilayer. (invaginated in lipid membrane and indicated as orange hexagons) B. AFM imaging of the wild-type SP1 self-assembled on a lipid bilayer. Arrows indicate locations where a single self-assembled pore structure can be observed clearly. C. I-V curve: the current as function of voltage (measured in 1M KCl Tris-HCl buffer) yields a locally linear dependence.

In contrast to SP1 nanopore, we analyzed the same 20-mer and 45-mer polydeoxyadenines and polydeoxythymine using α-HL. The blockade current level of poly(dA)$_{20}$ and poly(dA)$_{45}$ in α-HL distribute from 50% to 98% of the unblocked level, while the duration time is shorter than that of SP1 (Figure S2). And the translocation events of poly(dT)$_{20}$ and poly(dT)$_{45}$ upon using SP1 nanopore respectively were also analyzed in scatter plot distribution in Figure S3. Poly(dT)$_{20}$ and poly(dT)$_{45}$ exhibited the same blockade current level and the fitted duration time are shorter than that detected by SP1 nanopore. All of those results are showed that the geometry of SP1

could slow down the translocating velocity of ssDNA.

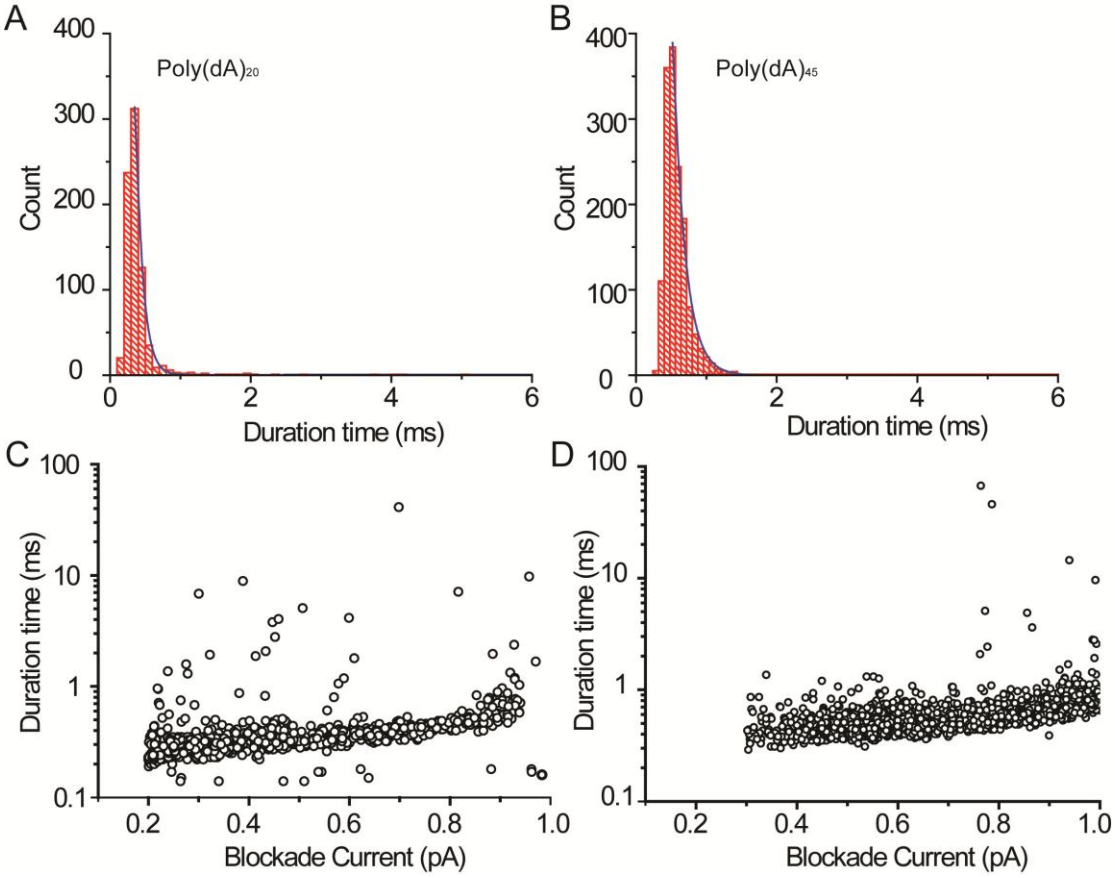

Figure S2. Detection of poly(dA)$_{20}$ and poly (dA)$_{45}$ transits through an α-HL pore in 1.0M KCl at pH=8.0 Tris-HCl. A) The histogram distribution of the duration time of poly(dA)$_{20}$. B) The histogram distribution of the duration time of poly(dA)$_{45}$. All the distribution is fitted by single exponential. C) The scatter plots of current transit of the blockages level produced by poly(dA)$_{20}$. D) The scatter plots of current transit of the blockages level produced by poly (dA)$_{45}$.

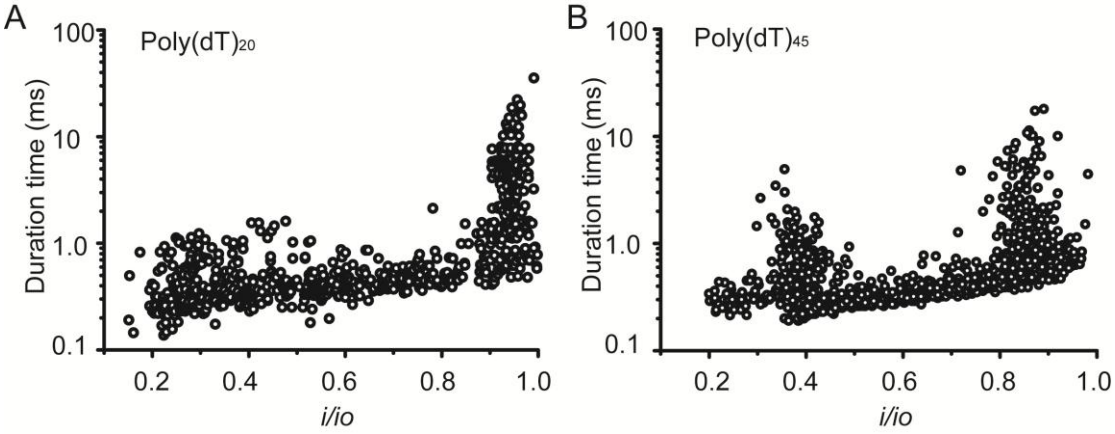

Figure S3 A) The scatter plot of poly(dT)$_{20}$ blockade events. B) The scatter plot of poly(dT)$_{45}$ blockade events using α-HL nanopore.

Reference

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