Enhanced Translocation of Poly $(dT)_{45}$ Through an α -Hemolysin Nanopore by Binding with Antibody[†]

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Supporting Information

1. Experimental Section

 α -HL was purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without purification. Diphytanoyl-phosphatidyl-choline was purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA). poly(dT)₄₅ (0.1 nmol) pre-incubated with Fab HED10 (0.1 nmol) before injecting into the 1 mL *cis* chamber. The final concentration of the poly(dT)₄₅ in 1 mL *cis* chamber was 0.1 μ M for the control experiment. Unless otherwise noted, all the other chemicals were of analytical grade.

The lipid bilayers were created by applying 30 mg/mL diphytanoyl-phosphatidyl-choline in decane (\geq 99%, Sigma-Aldrich St. Louis, MO. USA) to a 150 µm orifice in a 1 mL Delrin cup integrated into a lipid bilayer chamber (Warner Instruments, Hamden, CT, USA) filled with 1.2 M KCl and 10 mM Tris-HCl (pH = 7.8) buffer. A bilayer was deemed stable by monitoring its resistance and capacitance.^{1, 2}The two compartments of the bilayer cell are termed *cis* and *trans*, where the trans compartment is defined as virtual ground. The potential was applied at +100 mV from the *cis* side by an Ag/AgCl electrode. The experiments were run under voltage-clamp condition using a ChemClamp (Dagan Corporation, Minneapolis, MN, USA) instrument. Currents were filtered at 10 kHz by DigiData 1440A (Axon Instruments, Forest City, CA, USA) hardware and recorded by a PC running PClamp 10.2 (Axon Instruments, Forest City, CA, USA).

The α -HL was injected adjacent to the aperture in the *cis* chamber, and pore insertion was determined by a well-defined jump in current value.³ The electrolyte solution used throughout all bilayer measurements was 1.0 M KCl in 10 mM phosphate buffer (pH =7.8). Thus, the open pore current is 120 pA at +100 mV. Once a stable single-pore insertion was detected, the analyte was added to the *cis* chamber, proximal to the aperture. All the experiments were carried out at room temperature.

The ionic current blockages that were larger than a threshold value of 15 pA were recorded. These events, defined by any current blockages that exceeded the threshold value, were strung together in series and each event was analyzed by the home designed software. The raw current data events were analyzed by measuring both the magnitude of the ionic current blockage and the duration of the current blockage. Thus, each event consists of two data points described as blockage current magnitude and duration. Based on previous literature,⁴ the blockage of ionic flow under applied potentials is the result of large linear biopolymers transiting the pore, resulting in a restriction in ionic flow. Each event consists of two parameters, named blockage current and

blockage duration, thus a convenient method to view the data is via a 2-dimensional histogram matrix, where the bins are divided into small current and duration blocks resulting in a contour plots. In addition, the population distribution of blockage currents can be determined from a 1-dimensional histogram and curve fitting a Gaussian function that describes the population of events. In this manuscript, the characteristic double-step"partial-then-deep" events were analyzed into two levels, as shown in figure S1. The blockage current and blockage duration for each level term as $i_{\text{level II}}$, $i_{\text{level II}}$, and $t_{\text{level II}}$, respectively. The errors of the blockage currents and duration times were based on three separate experiments.



Fig.S1 Typical current trace of a poly(dT)₄₅ translocation event in pH=7.8 Tris-HCl at 100 mV. The event is divided into two level as level I and level II. The blockage current of each levels is labeled as $i_{\text{level I}}$ and $i_{\text{level II}}$, respectively. The duration time of each level is assigned as $t_{\text{level I}}$ and $t_{\text{level II}}$.

2. Nanopore sensing poly(dT)₄₅



Fig.S2 Current trace and the models show the $poly(dT)_{45}$ is captured and translocates through the α -HL pore. $poly(dT)_{45}$ enters the vestibule of α -HL causing the abruptly dropping of current from its initial open pore current (i); then it remains in the vestibule area while exploring for the narrow

entrance of the stem (ii); eventually it threading through the pore resulting in an further increase in current blockage (iii).

3. Nanopore sensing Fab HED10



Fig.S3 The current traces for the open state of α -HL in 1.2 M KCl at pH=7.8 Tris-HCl buffer by applying + 100 mV.



Fig.S4 The current traces of 0.1 μ M Fab HED10 in 1.2 M KCl at pH=7.8 Tris-HCl. The positively charged Fab HED10 causes bumping events with short blockage current.



Fig.S5 The histogram of blockage currents for Fab HED10 in 1.2 M KCl at pH=7.8 Tris-HCl. The distribution is fitted by a Gaussian function with peak current at 31.6 pA.

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Fig.S6 The duration time histogram for Fab HED10 in 1.2 M KCl at pH=7.8 Tris-HCl.

References

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