Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Exploring Single Molecule Interactions: Heparin and FGF-1 Proteins Through Solid-State Nanopores

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1. CURRENT-VOLTAGE (I-V) CHARACTERISTIC OF THE NANOPORES

Figures S1.A, and S1.B represent the current-voltage characteristic curve for the two CT-CDB pores used in our experiments. The conductance (*G*) of each pore was calculated by measuring the gradient of the curves. The diameter of an assumed cylindrical nanopore can be calculated using equation S1.1. The pore conductance was measured using IV plots where the conductivity was measured with Orion Star pH/conductivity multiparameter. The membrane thickness of all the pores used are 12 ± 2 nm.

$$d = \frac{G}{2s}\left(1 + \sqrt{1 + \frac{16sL}{\pi G}}\right) \qquad \text{S1.1}$$

where,

d : nanopore diameter

G : pore conductance

s : solution conductivity

L : membrane thickness

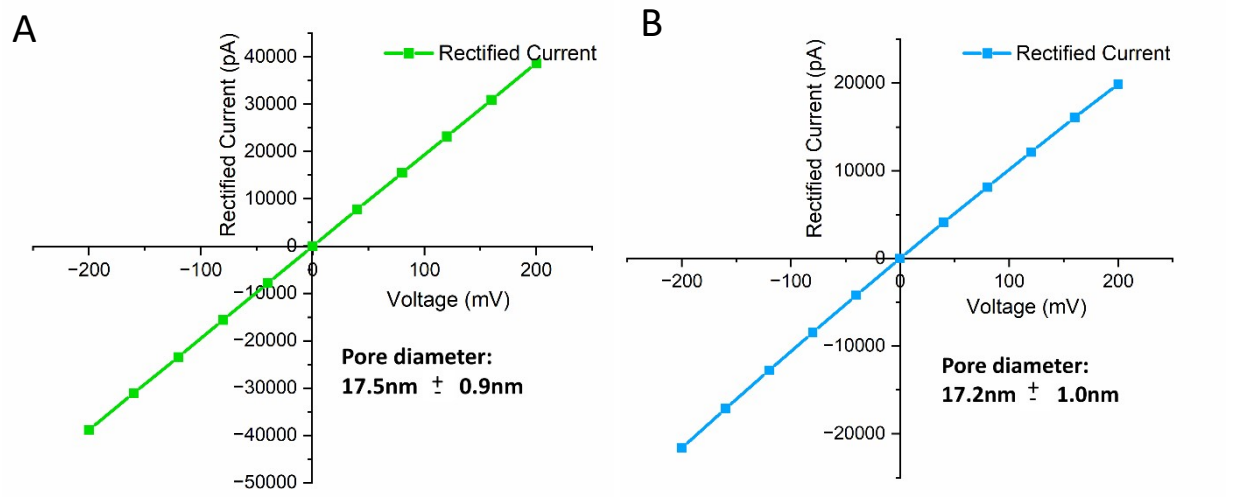

Fig. S1: I-V characteristic relationship of a CT-CDB fabricated pore: (A) A pore in 2M KCl, 10 mM TRIS, pH 10.0 electrolyte with a conductivity of 20.83 S/m (B) A pore in 1M KCl, 10 mM TRIS, pH 10.0 electrolyte with a conductivity of 11.38 S/m.

2. OPEN PORE BASE CURRENTS BEFORE HEPARIN EXPERIMENTS

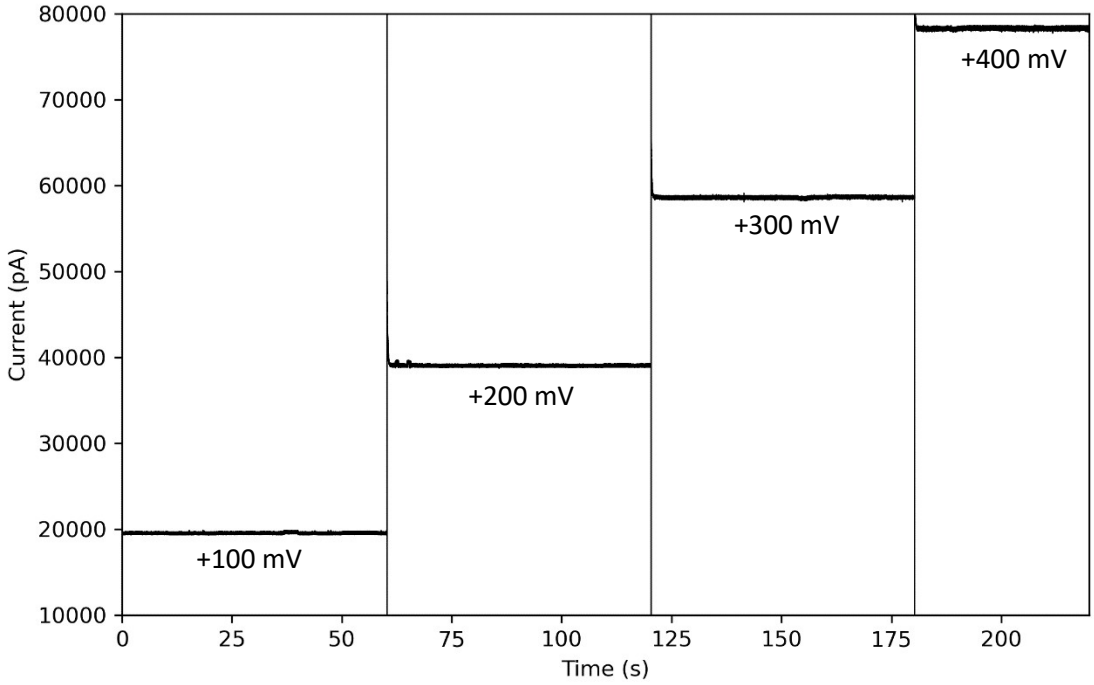

Fig. S2: Open pore current traces were recorded prior to the introduction of heparin to the *cis* with 2M KCl 10 mM TRIS, pH 7.6 as the electrolyte, and a 10 kHz low-pass bezel filter. The voltage was systematically varied in increments of 100 mV, ranging from +100 mV to +400 mV. Concurrently, the open current was meticulously measured and recorded at each voltage level over a duration of 60 seconds.

Prior to the introduction of analytes, open pore baseline currents were recorded and observed. These baseline currents were very stable in nature with minimum noise (Figure S2), indicating the stability of the pore and the absence of any impurity within it. Such characteristics are anticipated in advance of experimental procedures.

3. HEPARIN TRANSLOCATIONS WITH DIFFERENT SALT CONCENTRATION - 1M, 2M, and 3M KCl

Figures S3.A, S3.B, and S3.C illustrate the mean current drops of heparin in the presence of three electrolyte molarities: 1M KCl, 2M KCl, and 3M KCl respectively, while the event capture rates for the aforementioned electroltye molarities are presented in Figure S3.D. Compared to 1M, and 2M KCl, 3M KCl marked intensified event capture rates throughout the experimented voltage bias range.

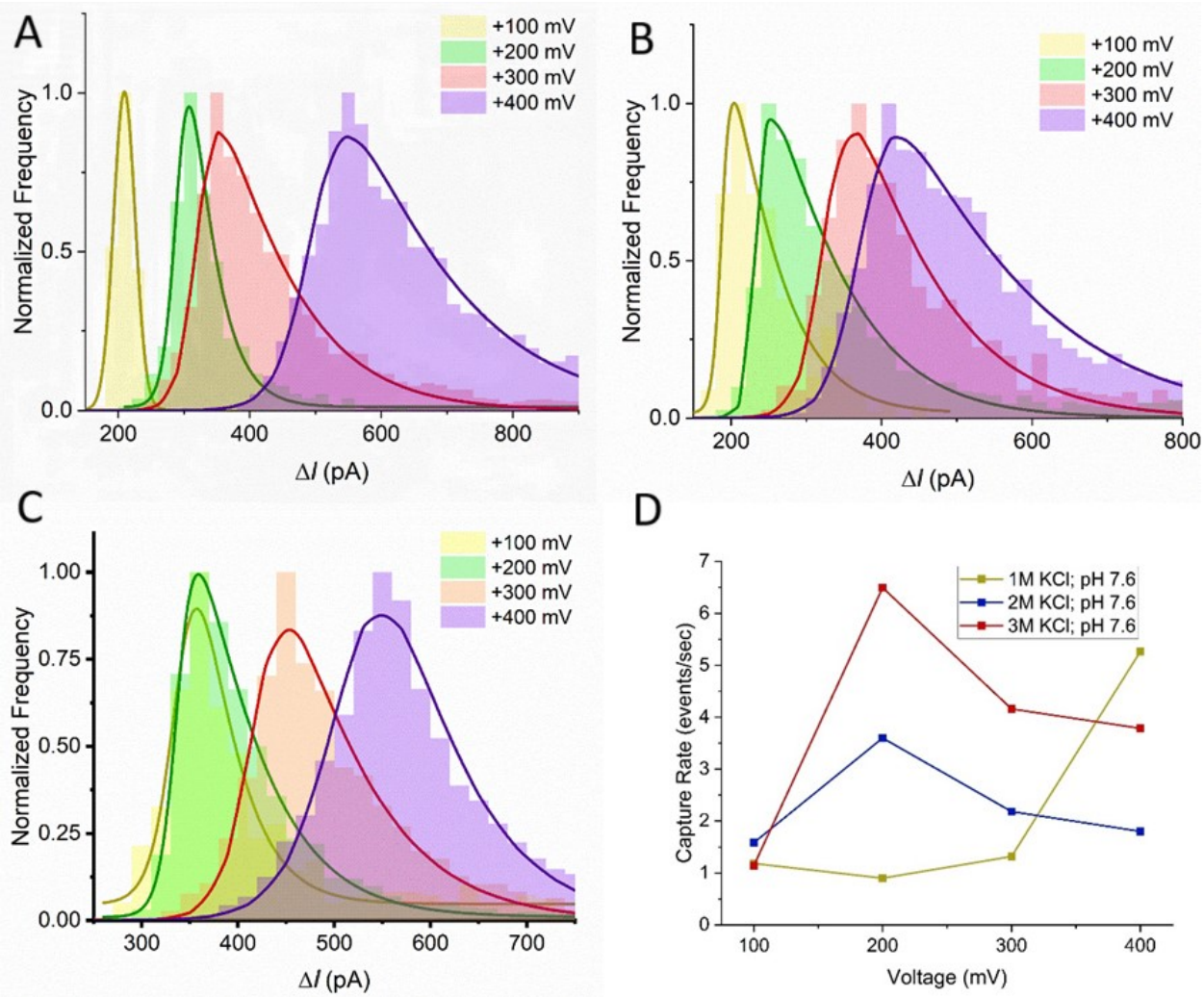

Fig. S3: (A) Translocation dynamics of heparin in 1M KCl, 10 mM TRIS, pH 7.6, (B) Translocation dynamics of heparin in 2M KCl, 10 mM TRIS, pH 7.6, (C) Translocation dynamics of heparin in 3M KCl, 10 mM TRIS, pH 7.6, and (D) Heparin capture rates through a CT-CDB pore of 17.5nm ± 0.9 nm.

In the context of our experimental conditions with a higher pore radius of ~17nm, the influence of the Debye layer can be deemed negligible. Consequently, the nanopore can be assumed to possess a well-defined geometry, particularly under low-voltage conditions. Hence, a constant pore resistance can be postulated against the ionic flow through the pore. As the applied voltage increases, the resultant current drop indicative of a translocation is expected to rise for a given molecule. This phenomenon elucidates the observed escalation in mean current blockade at each electrolyte molarity with the augmentation of applied voltage.

Moreover, with increased molarity of electrolytes, the flow through the nanopore is expected to increase. This suggests that the current drop in a translocation should also increase with increasing molarity. The above is clearly observed when the three molarities are compared in Figure S3.

4. TYPICAL CURRENT TRACES OF TRANSLOCATING HEPARIN IN 3M KCl

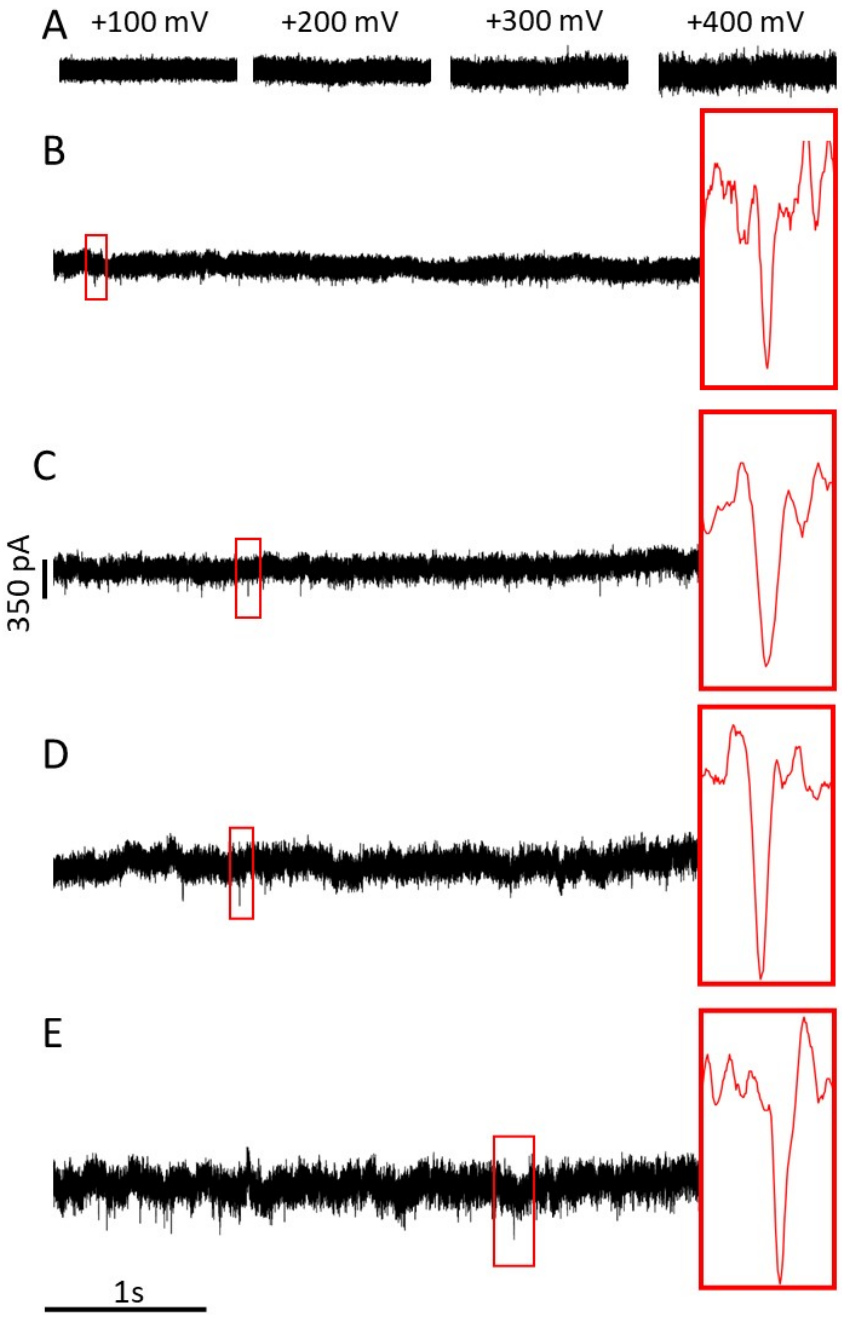

Fig. S4: (A) Baseline current traces at different voltage biases: +100mV, +200mV, +300mV, and +400mV prior to the introduction of heparin. Four-second current traces of heparin translocations are presented along with their individual translocation events (presented in red) at (B) +100mV, (C) +200mV, (D) +300 mV, and (E) +400 mV voltage biases.

5. OBSERVATION OF FGF-1 EVENTS AT POSITIVE VOLTAGE BIAS

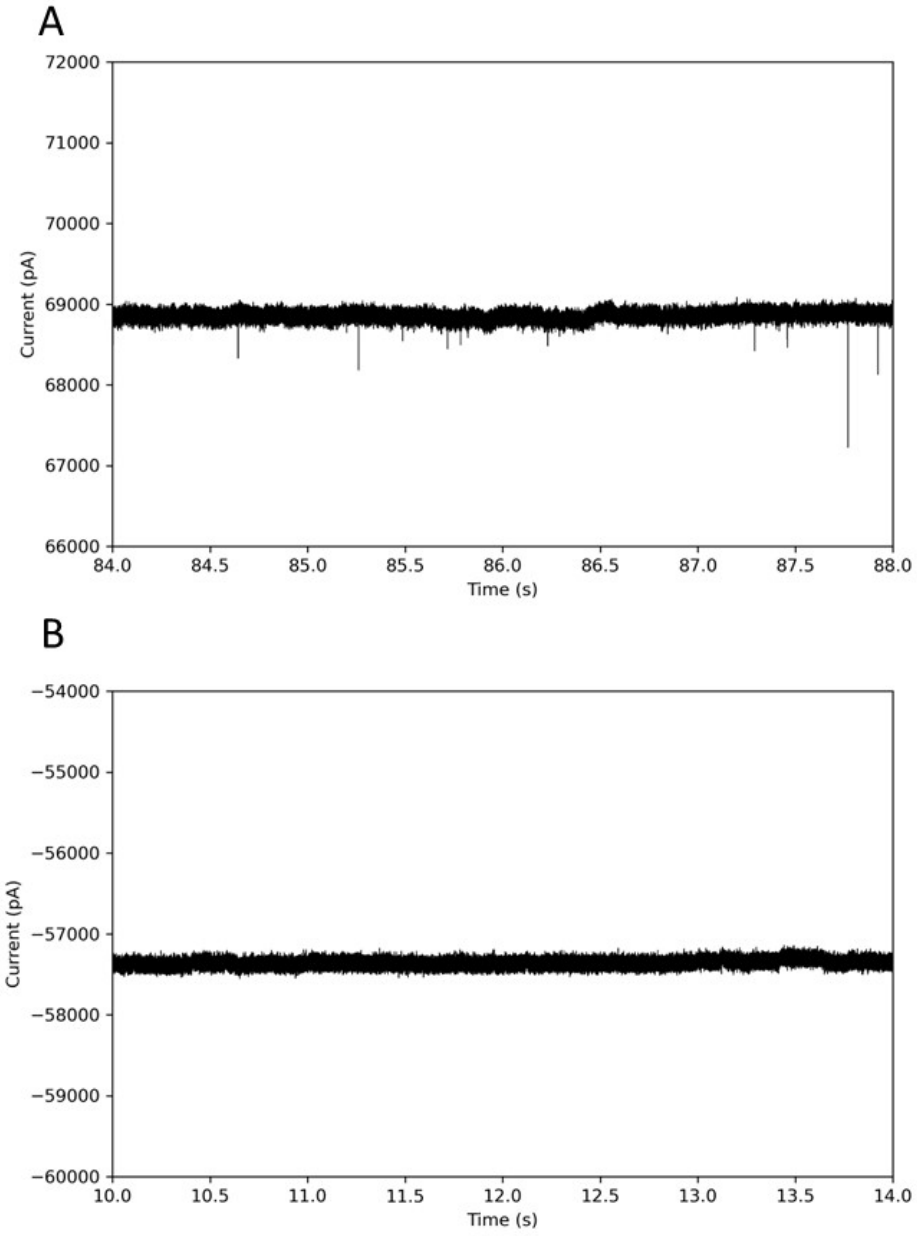

Fig. S5: FGF-1 was introduced to the *cis* side, and translocations were investigated from a pore of ~17 nm in diameter in the presence of 3M KCl, 10 mM TRIS, pH 7.6 at (A) +200 mV and (B) -200 mV voltage biases on the *trans* side. Frequent events at +200mV and no events at -200 mV suggest the overall charge of FGF-1 at pH 7.6 to be negative.

6. FGF-1 CURRENT TRACES IN 3M KCl

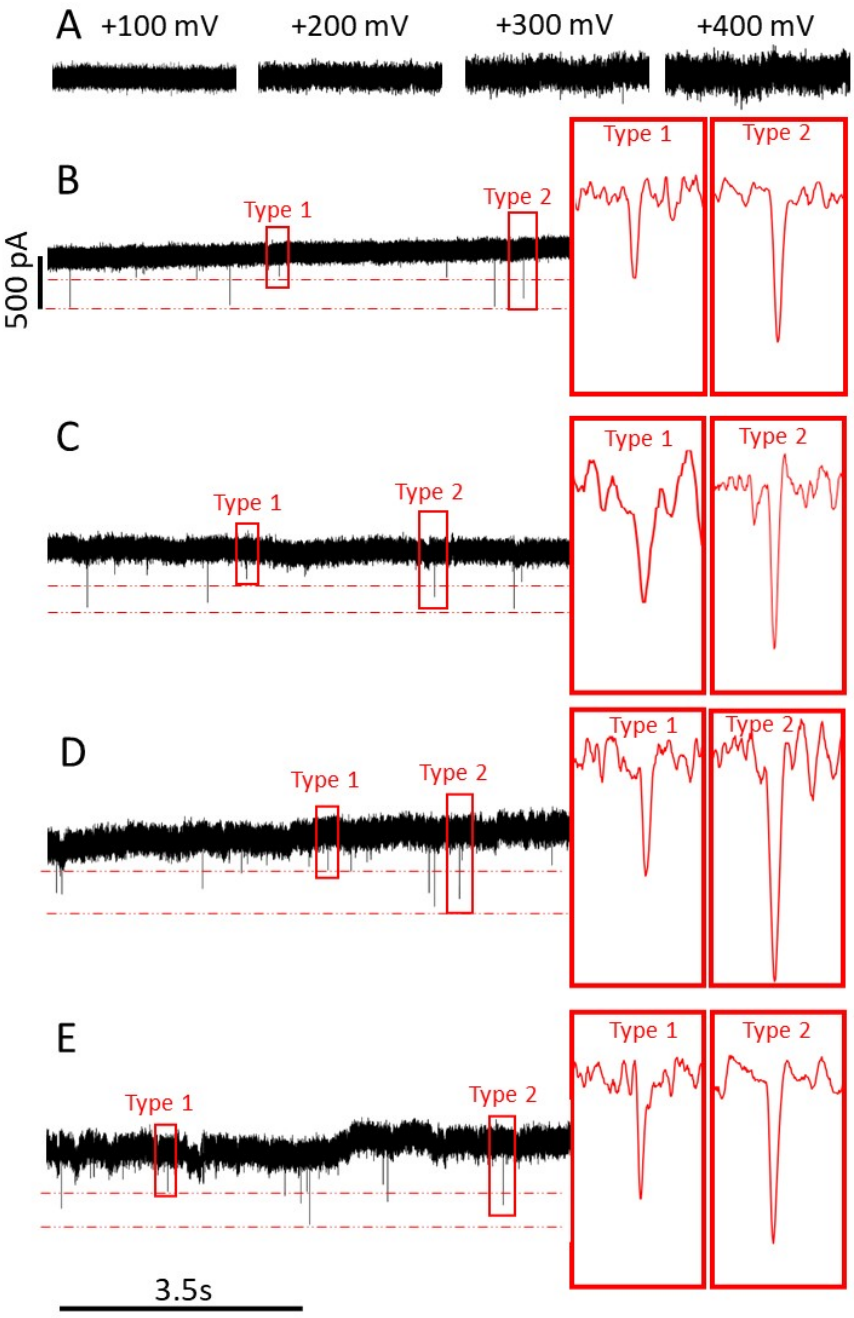

Fig. S6: (A) Baseline current traces at different voltage biases: +100mV, +200mV, +300mV, and +400mV prior to the introduction of FGF-1. Seven-second current traces of FGF-1 translocations are presented along with their individual translocation events (presented in red) at (B) +100mV, (C) +200mV, (D) +300 mV, and (E) +400 mV voltage biases. Two types of current blockades are reported (Type 1 and Type 2). These two types of events can be discriminated as Type 1 being the smooth translocation of FGF-1 molecules, and Type 2 being the interaction of positively charged heparin binding domains of FGF-1 with the pore walls.

7. MIXTURE CURRENT TRACES IN 3M KCl

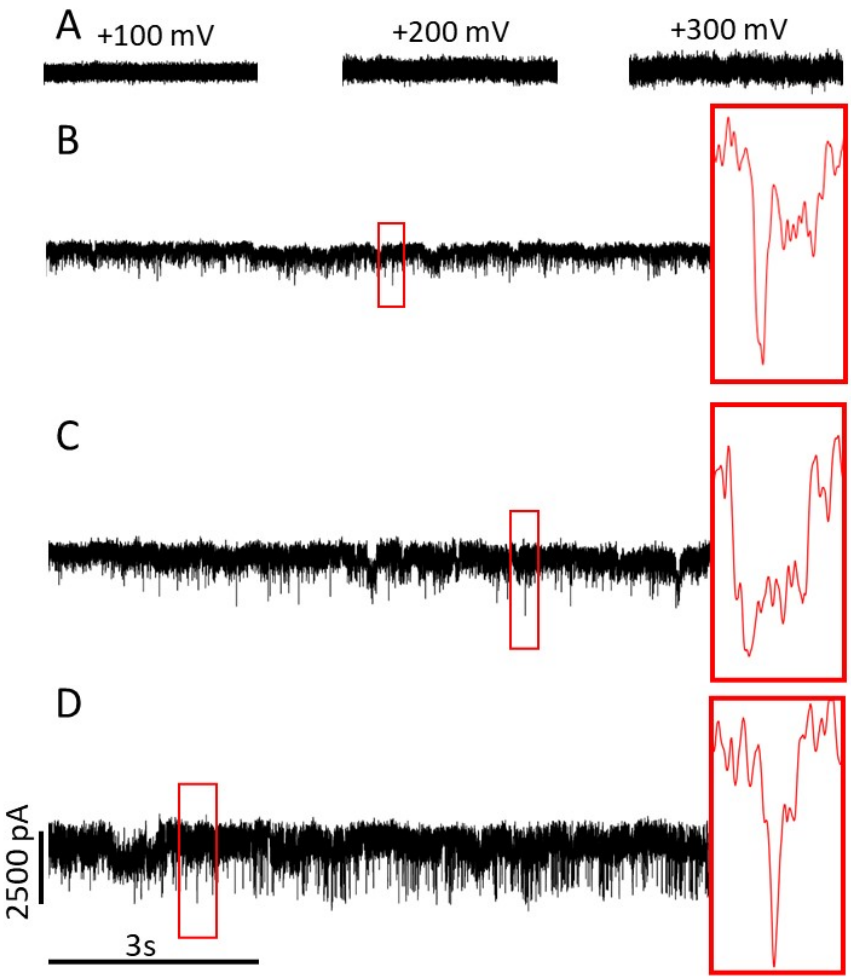

Fig. S7: (A) Baseline current traces at different voltage biases: +100mV, +200mV, and +300mV prior to the introduction of the mixture (heparin 100 nM + FGF-1 100 nM). Five-second current traces of mixture translocations are presented along with their individual translocation events (presented in red) at (B) +100mV, (C) +200mV, and (D) +300 mV voltage biases.

Unlike heparin, and FGF-1 events, the mixture events (100 nM heparin + 100 nM FGF-1) had considerably high capture rates and clogging events. It was observed that the amplification of clogging events increased with the voltage. At the voltage of +400mV, continuous clogging was reported and unavoidable. Hence, the data only up to +300mV are presented.

8. COMPARISON OF HEPARIN, FGF-1, AND THE MIXTURE EVENTS AT DIFFERENT VOLTAGE BIAS

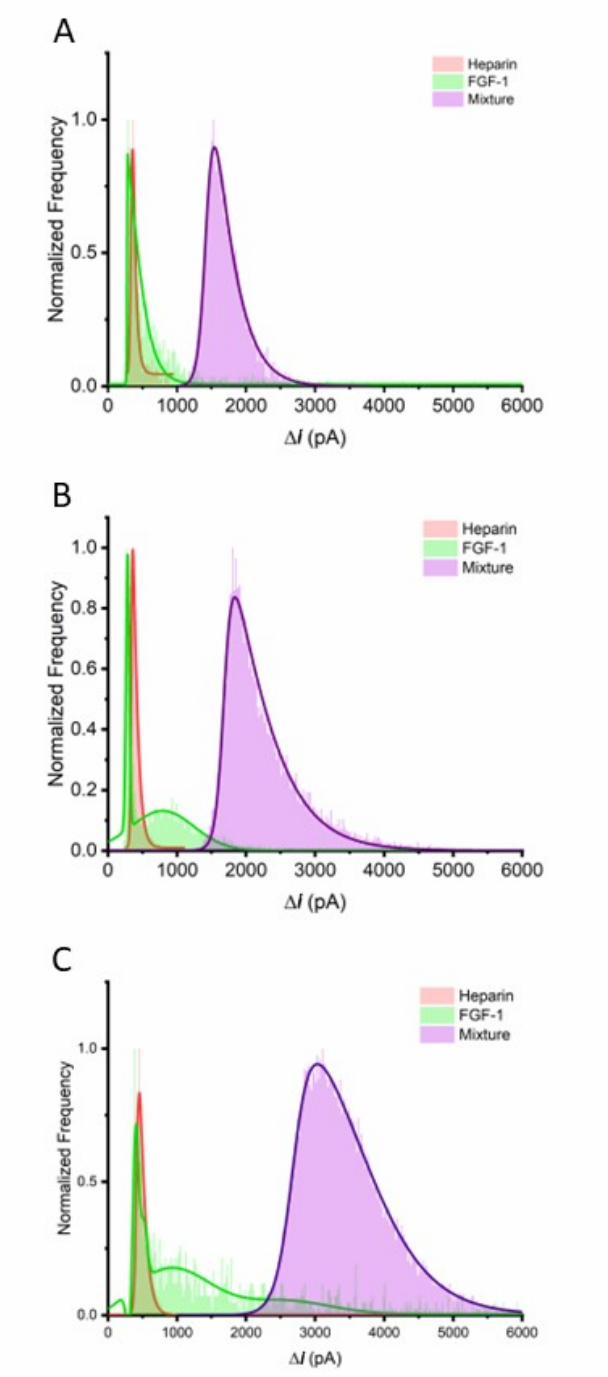

Fig. S8: Current blockade of heparin, FGF-1, and mixture (heparin: FGF-1 = 1:1) events in 3M KCl, 10 mM TRIS, pH 7.6 at (A) +100 mV, (B) +200 mV, and (C) +300 mV

Under each voltage bias, a distinct increase in the mean current drop can be observed in mixture events (light purple) when compared to individual heparin (red) and FGF-1 (green) events. This is a clear indication of the binding of protein, heparin, and FGF-1.