Supplementary Materials

Improving Macromolecule Crowding Configurations in Nanopores for Protein Sensing

Fei Zheng[1,2,3,4], HongLuan Li[1,2], Jun Yang[1,2], Haiyan Wang[1,2], Guangle Qin[1,5], Dapeng Chen[1,5], Jingjie Sha[1,2*]

1. Jiangsu Key Laboratory for Design and Manufacture of Micro-nano Biomedical Instruments, Southeast University, Nanjing 211189, China

2. School of Mechanical Engineering, Southeast University, Nanjing 211189, China

3. Cavendish Laboratory, University of Cambridge, Cambridge CB3 0HE, United Kingdom

4. School of Nanoscience and Nanotechnology, University of Chinese Academy of Sciences, Beijing 101408, China

5. Jiangsu Automation Research Institute, Lianyungang 222000, China

*Correspondence to: Jingjie Sha, E-mail: major212@seu.edu.cn;

Table of Contents

Supplementary Note 1: Materials and Methods

Nanopore fabrication

Quartz glass capillaries (inner diameter: 0.7 mm; outer diameter: 1.0 mm) were sourced from Sutter Instruments (Catalog number QF100-70-7.5, California, USA). These capillaries were reduced to nanoscale dimensions using a laser-heated pipette puller (P-2000, Sutter Instrument, California, USA), with most nanopores formed to a diameter of 25 ± 3 nm and a cone semi-angle of 0.03 ± 0.01 radians. The parameters set for this process were HEAT=760, FIL=4, VEL=29, DEL=140, and PULL=168, as outlined in the P2000 manual. Following the pulling process, the capillaries were trimmed to the desired length and integrated into a custom PMMA microfluidic device. The glass pipette was positioned through a gasket separating two reservoirs, which were then securely clamped to maintain insulation. PMMA (polymethyl methacrylate) used in the setup was acquired from Sigma-Aldrich (Missouri, USA). The device underwent a 5-minute plasma treatment to make the nanopore surface hydrophilic, after which the nanopores were flushed with 1× Tris-HCl buffer solution (pH 8.0).

Nanopore characterization

The pulled glass nanopores were imaged using scanning electron microscopy (SEM) (Helios NanoLab™ 600i, FEI, USA). Before imaging, the nanopipettes were sputter-coated with a thin gold layer a few nanometers thick. They were then positioned on a sample holder at an angle of 60 degrees or greater. Imaging was performed at 3 kV with a working distance of 4.2 mm.

I-V (Current-Voltage) characterization

Nanopore measurements were carried out using a patch-clamp amplifier (Axopatch 700B, Molecular Devices, CA, USA) in voltage-clamp mode, with current signals digitized at a 250 kHz sampling rate (Axon Digidata 1550A, Molecular Devices, CA, USA) and filtered at 10 kHz. Data acquisition was managed with pClamp 10 software (Molecular Devices, CA, USA). Two Ag/AgCl electrodes, prepared by oxidizing silver wires in a 10% NaClO solution, were positioned in the *cis* and *trans* reservoirs to establish an electric circuit across the nanopore. Current-voltage characteristics were initially scanned from -800 mV to 800 mV to estimate the nanopore size before sample measurements. The recorded current data were analyzed using Clampfit software, and further processed with Python scripts. I-V characteristics were obtained using pure KCl solution, both before the addition of PEG and after flushing the system twice with PEG.

Materials

The experiments used the following commercial reagents: Bovine Serum Albumin (Sigma-Aldrich, Catalog numbers 9048-46-8), 100 × Tris-HCl buffer solution (Solarbio, Catalog number T1150-100), potassium chloride of ≥99% purity for molecular biology (Sinopharm, Catalog numbers 7447-40-7), and Polyethylene Glycol 4000 (Sigma-Aldrich, Catalog numbers 81240). Solutions and buffers for nanopore measurements, prepared with these reagents, were filtered twice using 0.22 μm Millipore syringe filters (MF-Millipore™, Sigma-Aldrich, Catalog number GSWP02500).

Supplementary Note 2: SEM Characterization of the Nanopore

The pulled glass nanopores were characterized using scanning electron microscopy (SEM) to determine their size and structural features. Figure S1 shows the SEM image of a typical nanopore with a well-defined circular opening. Prior to imaging, the nanopipettes were coated with a thin layer of gold, a few nanometers thick, using a sputtering technique to enhance image contrast. The samples were mounted on a holder at an angle of approximately 60 degrees to optimize the visualization of the nanopore structure. Imaging was performed at an accelerating voltage of 3 kV with a working distance of 4.2 mm to obtain high-resolution images. The nanopore diameter was measured to be approximately 25 nm, as indicated by the red dashed outline in the image.

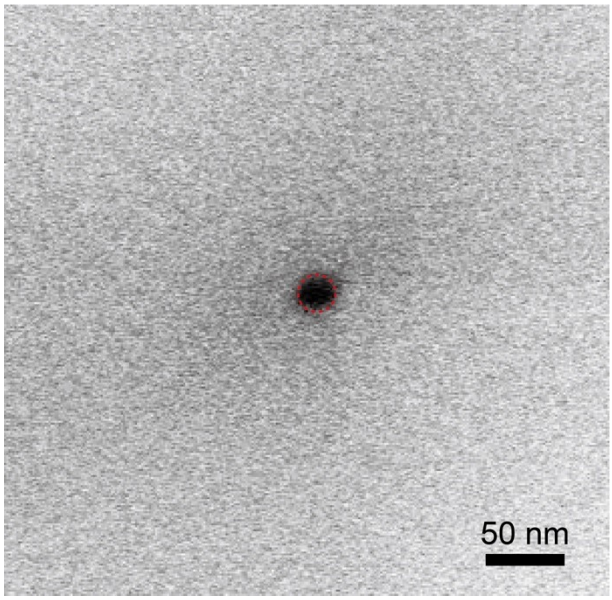

Figure S1. SEM image of a nanopore with a diameter of approximately 25 nm (highlighted by the red dashed outline).

Supplementary Note 3: I-V Characteristic Measurements

The current-voltage (I-V) characteristics of the nanopore were measured to assess its electrical properties and to estimate the nanopore diameter. Figure S2 shows the I-V curve recorded using a patch-clamp amplifier in voltage-clamp mode. Measurements were performed by varying the applied voltage from -800 mV to +800 mV, with current signals digitized and low-pass filtered at 10 kHz. The I-V curve exhibits a nonlinear trend, indicating the asymmetric nature of the nanopore structure.

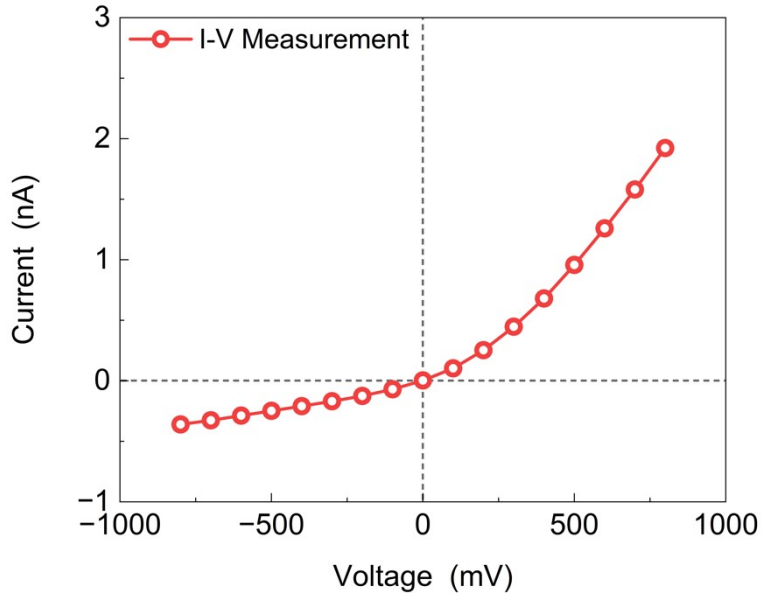

Figure S2. I-V curve of the nanopore.

Supplementary Note 4: Long-Time Current Trace Recording

A long-time current trace was recorded at +800 mV to confirm that PEG molecules do not translocate through the nanopore under experimental conditions. Figure S3 shows the current-time trace over a 300-second period, displaying a stable baseline current of around 0.5 nA with occasional minor fluctuations but no significant translocation events. This stability indicates that PEG remains on its designated side of the nanopore, validating that PEG does not interfere with the nanopore by causing false-positive signals, ensuring the reliability of subsequent biomolecule detection experiments.

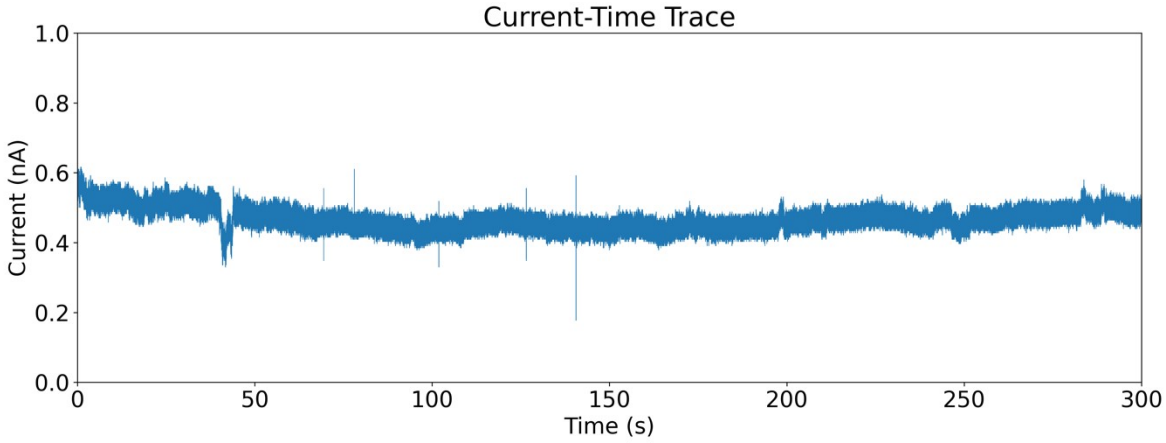

Figure S3. Long-time current-time trace recorded over 300 seconds, showing a baseline current with no detectable translocation events, confirming that PEG molecules do not translocate through the nanopore.

Supplementary Note 5: Scatter Plots of Current Blockade Maxima versus Dwell Time for Experiments without PEG and with PEG

To investigate the influence of PEG on biomolecule translocation dynamics through the nanopore, scatter plots of current blockade maxima versus dwell time were generated for experiments without PEG (Figure S4a) and with PEG (Figure S4b). The results indicate a substantial increase in the number of translocation events when PEG is present, as seen in the higher density of data points in Figure S4b compared to Figure S4a. The histograms alongside each plot illustrate the frequency distribution of these events.

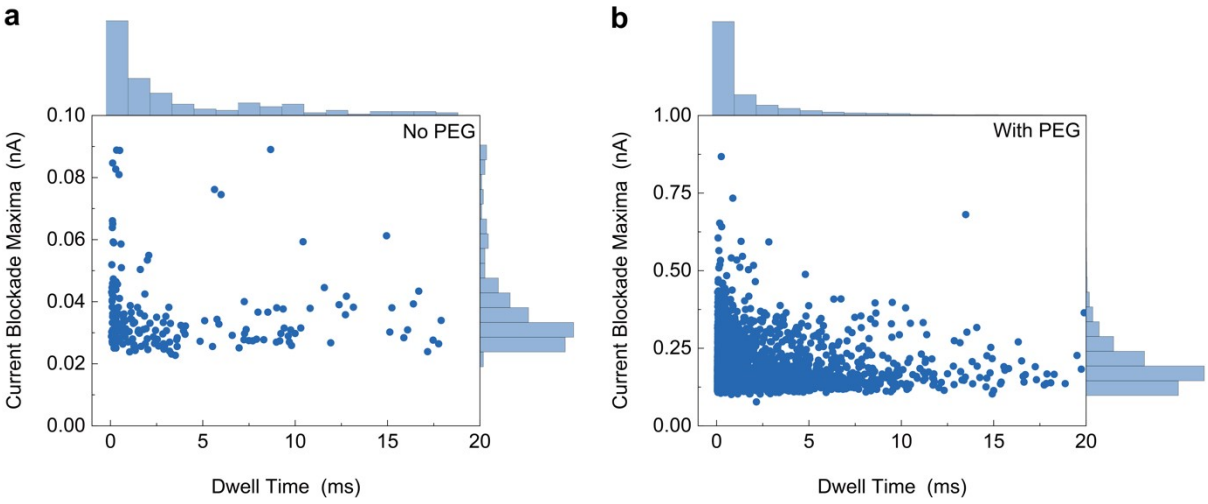

Figure S4. Scatter plots of current blockade maxima versus dwell time. (a) Without PEG and (b) with PEG.

Supplementary Note 6: Theory of Osmotic Flow Enhanced Capture Rate of Biomolecules

The capture rate of biomolecules in nanopore systems can be significantly influenced by osmotic flow induced by macromolecules, such as PEG. Here we outline the theoretical derivation of the osmotic flow enhanced capture rate.

Osmotic Pressure and Flow Rate

The osmotic pressure difference (ΔΠ) across a nanopore is a critical factor driving the transport of fluids and solutes due to concentration gradients. It can be described by the equation:

$$\Delta\Pi = RT\Delta C_{PEG} \tag{4.1}$$

where R is the gas constant, T is the absolute temperature, and ΔC_{PEG} is the concentration difference of PEG across the nanopore. The osmotic flow rate Q_{osmotic} through the nanopore is given by the Hagen–Poiseuille equation[1]:

$$Q_{\text{osmotic}} = \frac{\Delta\Pi \cdot \pi {r_p}^4}{8\eta L} \tag{4.2}$$

Where r_p is the radius of the nanopore, η is the viscosity of the solution, and L is the effective length of the nanopore. Substitute $\Delta\Pi = RT\Delta C_{PEG}$:

$$Q_{\text{osmotic}} = \frac{RT\Delta C_{PEG} \cdot \pi {r_p}^4}{8\eta L} \tag{4.3}$$

Drift Velocity with Distance Dependence

The drift velocity v at a distance r from the nanopore decays with the square of the distance. It is inversely proportional to the surface area over which the flow spreads:

$$v_{\text{drift}}(r) = \frac{Q_{\text{osmotic}}}{4\pi r^2} \tag{4.4}$$

Substitute Q_{osmotic}:

$$v_{drift}(r) = \frac{RT\Delta C_{PEG} \cdot \pi {r_p}^4}{8\eta L} \cdot \frac{1}{4\pi r^2} \tag{4.5}$$

Simplifying:

$$v_{drift}(r) = \frac{RT\Delta C_{PEG} \cdot {r_p}^4}{32\eta L r^2} \tag{4.6}$$

Balance Between Drift and Diffusion at r^*

At the capture radius r^*, the drift velocity is balanced by the diffusive velocity of the biomolecule[2,3], which is given by:

$$v_{\text{diffusion}} = \frac{D}{r^*} \tag{4.7}$$

At $r = r^*$, the drift velocity equals the diffusion velocity (boundary condition):

$$\frac{RT\Delta C_{PEG} \cdot {r_p}^4}{32\eta L(r^*)^2} = \frac{D}{r^*} \tag{4.8}$$

Now, solve for r^*:

$$r^* = \frac{RT\Delta C_{PEG} \cdot {r_p}^4}{32\eta LD} \tag{4.9}$$

This gives the capture radius r^*, which is directly proportional to ΔC_{PEG}.

The capture radius is directly proportional to the concentration difference of PEG (ΔC_{PEG}). This indicates that as the concentration gradient of PEG increases, the effective region for capturing biomolecules expands. Thus, higher osmotic pressure enhances the ability to attract and capture biomolecules.

Capture Rate

The capture rate R_c is proportional to the flux of molecules arriving at the nanopore. The flux depends on the concentration of biomolecules and the surface area of the capture radius. The capture rate is given by:

$$R_c = J_{\text{diff}} \cdot A_{\text{sphere}} = D \cdot \frac{c_0}{r^*} \cdot 4\pi(r^*)^2 \tag{4.10}$$

Where J_{diff} is the diffusion flux, D is the diffusion coefficient of the target biomolecule, and c_0 is the bulk concentration of the biomolecule. Substituting r^*:

$$R_c = 4\pi D c_0 r^* \tag{4.11}$$

Now, substitute the expression for r^*:

$$R_c = 4\pi D c_0 \cdot \left(\frac{RT\Delta C_{PEG} \cdot {r_p}^4}{32\eta LD}\right) \tag{4.12}$$

Simplifying:

$$R_c = \frac{\pi c_0 \cdot RT\Delta C_{PEG} \cdot {r_p}^4}{8\eta L} \tag{4.13}$$

Where:

- D is the diffusion coefficient of the biomolecule.
- c_0 is the concentration of biomolecules near the nanopore.
- η is the viscosity of the fluid.
- L is the length of the nanopore.

- r_p is the nanopore radius.
- ΔC_{PEG} is the PEG concentration difference across the nanopore.
- R is the gas constant.
- T is the absolute temperature.

Similar to the capture radius, the capture rate is also directly proportional to the PEG concentration difference. This emphasizes the role of osmotic pressure in enhancing the capture efficiency, as more substantial concentration gradients drive more substantial osmotic flow, facilitating biomolecule translocation through the nanopore.

Notably, the capture rate itself also does not directly depend on the diffusion coefficient (D) of the biomolecule. This independence indicates that while diffusion influences how fast a biomolecule can move towards the pore, the osmotic flow and the overall configuration of the system predominantly dictate the capture rate. This is similar to the electric-field-dominated capture rate where it is also independent of biomolecule diffusion.

Electric Field Contribution to Drift Velocity

In addition to osmotic pressure, the application of an electric field induces a drift velocity for charged biomolecules, given by:

$$v_{\text{electric}}(r) = uV(r) \tag{4.14}$$

where u is the electrophoretic mobility and $V(r)$ is the electric potential at distance r from the nanopore. For our purposes, we can use the potential defined as:

$$V(r) = \frac{r^2}{8Lr} \cdot \Delta V \tag{4.15}$$

Where ΔV is the applied potential across the nanopore. This expression indicates how the electric field enhances the drift of biomolecules towards the nanopore.

Total Drift Velocity

The total drift velocity at the capture radius (r^*) combines both contributions:

$$v_{\text{total}}(r^*) = v_{\text{osmotic}}(r^*) + v_{\text{electric}}(r^*) \tag{4.16}$$

Substituting the drift velocities from the previous steps gives us:

$$v_{\text{total}}(r^*) = \frac{RT\Delta C_{PEG} \cdot {r_p}^4}{32\eta L(r^*)^2} + u \cdot \frac{{r^*}^2}{8Lr^*} \cdot \Delta V \tag{4.17}$$

At r^*, the total drift velocity balances with the diffusion velocity of the biomolecule:

$$\frac{RT\Delta C_{PEG} \cdot {r_p}^4}{32\eta L(r^*)^2} + u \cdot \frac{{r^*}^2}{8Lr^*} \cdot \Delta V = \frac{D}{r^*} \tag{4.18}$$

The capture radius here is more complex and dependent on multiple parameters involving the flow field and electric field.

Supplementary Note 7: Additional Data of PEG-Enhanced Protein Sensing

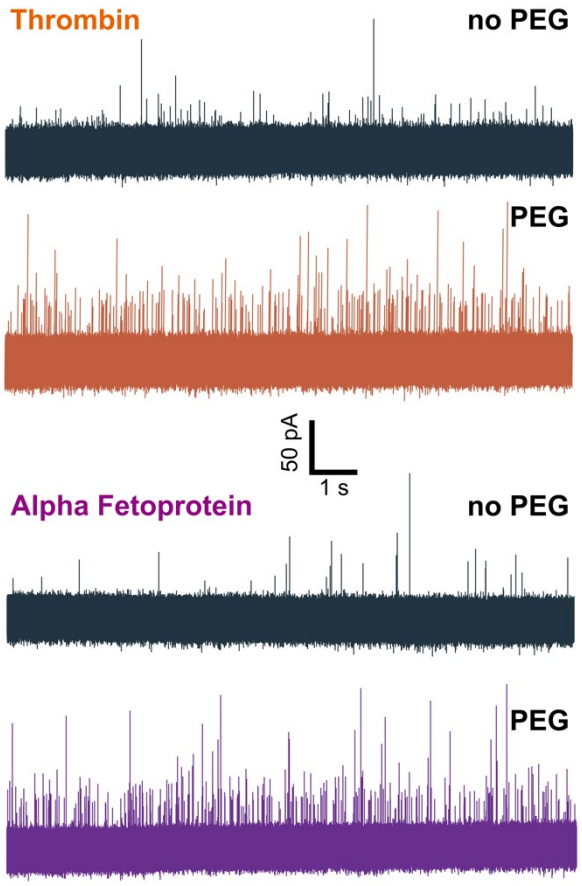

Figure S5. Current traces showing the translocation events of thrombin (top) and alpha-fetoprotein (bottom) in the absence (no PEG) and presence (PEG) of macromolecule crowding conditions. In both cases, the addition of PEG significantly increases the frequency of translocation events, indicating an enhanced capture rate.

Supplementary Note 8: Pulling Parameters of Glass Nanopores

Table S1. Pulling parameters for the glass nanopipette used in this work. To obtain nanopores with varied diameters, the Pull parameter was modified while other parameters were keeping unchanged.

Heat	Filament	Velocity	Delay	Pull
760	4	29	140	168

Reference:

1. Aluru, N. R.; Aydin, F.; Bazant, M. Z.; et al. *Chem. Rev.* **2023**, *123* (6), 2737–2831.
2. Wanunu, M.; Morrison, W.; Rabin, Y.; et al. *Nat. Nanotechnol.* **2010**, *5* (2), 160–165.
3. Qiao, L.; Ignacio, M.; Slater, G. W. *J. Chem. Phys.* **2019**, *151* (24), 244902.