

A simple method of creating a nanopore-terminated probe for single-molecule enantiomer discrimination

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Supplementary Materials

Figure S1. Size distribution of 10-nm gold nanoparticle (Au-NP) in low salt concentration.

Figure S2. Dependence of amplified DNA by PCR on the copy number of template.

Figure S3. Discriminating single molecular binding of camphanic acid enantiomers with β CD trapped in 1 nS nanopore.

Figure S4. Discriminating single molecular binding of adamantines with β CD trapped in 1 nS nanopore.

Expression to determine nanocavity profile and pore size–conductance correlation

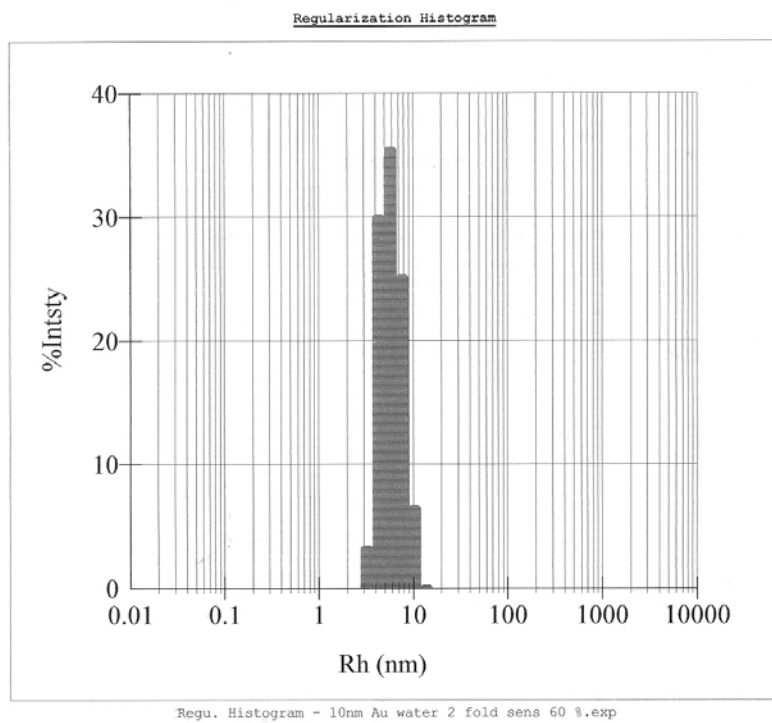
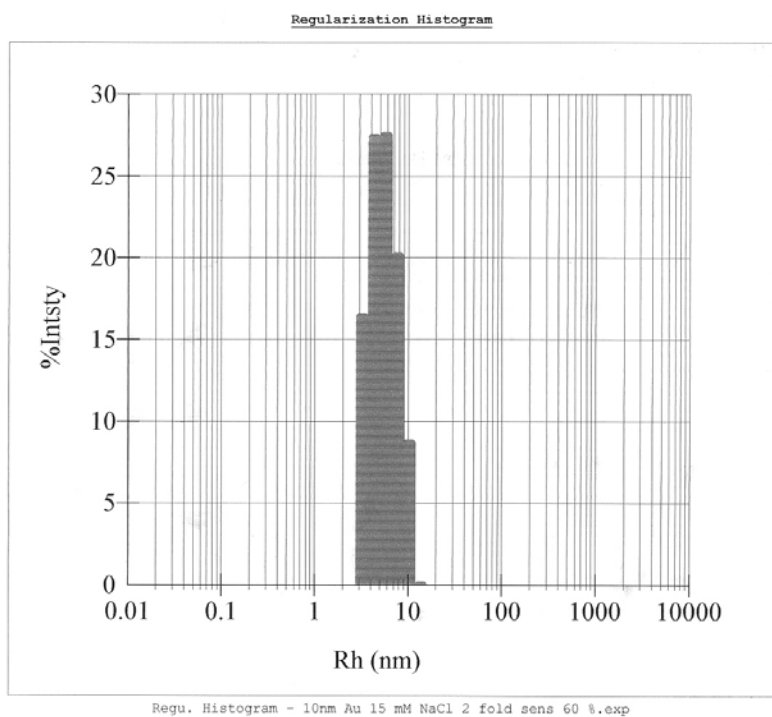
a**b**

Figure S1. Histograms for size distributions of Au-NP (10 nm in diameter) in water (a) and in 15 mM NaCl (b), analyzed with a DynaPro99 Molecular Size Instrument. Both

histograms are copies from the original test reports, where the x-axis [Rh (nm)] represents the radius of the nanoparticle, and the y-axis (%Intensity) represents the percentage of particles at each radius. The bottom comment describes the test condition. The peak value was given by the instrument as the particle radius. Based on multiple tests (n=3), the radii of the particle species were 5.12 ± 0.17 nm in water (a) and 5.09 ± 0.21 nm in 15 mM NaCl (b). The corresponding particle diameters were 10.24 nm and 10.18 nm. The identical diameter demonstrates that there is no particle aggregation in the 15 mM low salt concentration.

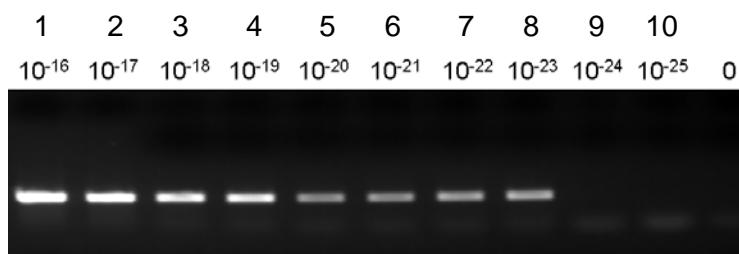


Figure S2. Amplification of DNA by PCR at various copy numbers of template. The 10 nM template stock was first diluted 100 folds to 100 pM. 1 μ l of the 100 pM solution was taken out to mix with 10 μ l dd-H₂O as Sample#1. Then 1 μ l of Sample#1 was taken out to mix with 10 μ l dd-H₂O as Sample#2. This 10-fold dilution process was repeated 10 times. The final copy numbers of template in these samples were: #1, $\sim 10^{-16}$ mol (6×10^7); #2, $\sim 10^{-17}$ mol (6×10^6); #3, $\sim 10^{-18}$ mol (6×10^5); #4, $\sim 10^{-19}$ mol (6×10^4); #5, $\sim 10^{-20}$ mol (6×10^3); #6, $\sim 10^{-21}$ mol (6×10^2); #7, $\sim 10^{-22}$ mol (6×10^1); #8, $\sim 10^{-23}$ mol (6×10^0); #9, $\sim 10^{-24}$ mol (6×10^{-1}); #10, $\sim 10^{-25}$ mol (6×10^{-2}). Each sample was used to participate in a 50 μ l PCR. The amplified DNAs were shown in the gel. The PCR result revealed an uneven change in DNA amount as the template amount was decreased from thousands (Lane-5) to several copies (Lane-8), suggesting that PCR is able to identify, but is difficult to quantify the template at very low copy number.

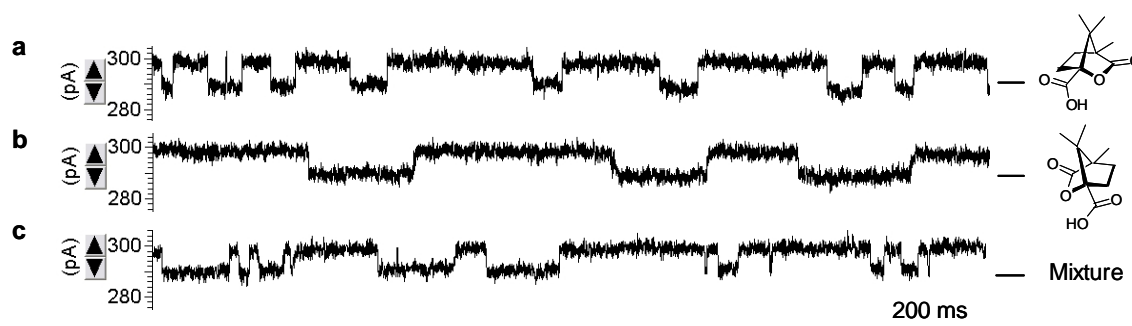


Figure S3. Discriminating single molecular binding of camphanic acid enantiomers with β CD (Reference ¹) in 1 nS nanopore. These traces were recorded at +1 V for **a**, 50 μ M (1R)-(+)-camphanic acid, with a block duration of 46 ± 7 ms; **b**, 50 μ M (1S)-(-)-camphanic acid, with a block duration of 160 ± 20 ms, and **c**, a mixture of 50 μ M (1R)-(+)-camphanic acid and 50 μ M (1S)-(-)-camphanic acid, with two block durations, 46 ms and 160 ms.

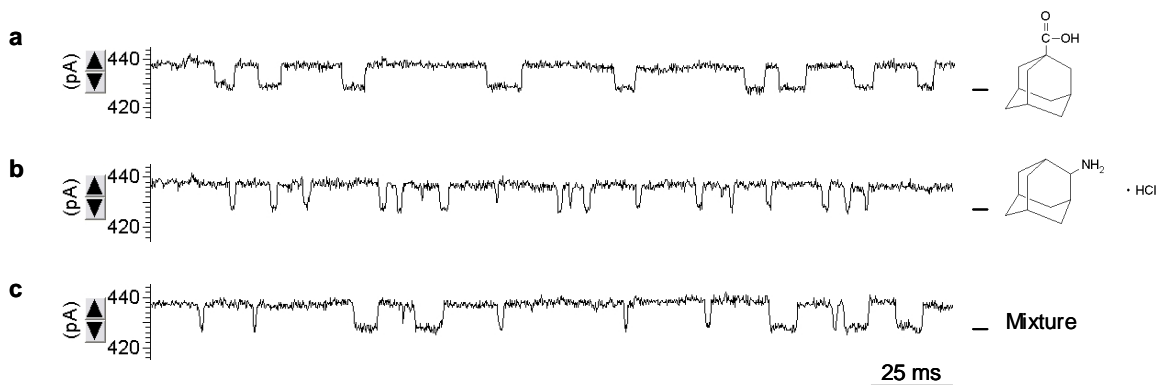


Figure S4. Discriminating single molecular binding of adamantines with β CD trapped in 1 nS nanopore. These traces were recorded at +1 V for **a**, 100 μ M 1,3-adamantanedicarboxylic acid, with a block duration of 11 ± 0.2 ms, **b**, 100 μ M 2-adamantanamine, with a block duration of 1.8 ± 0.5 ms, **c**, their mixture, with two block durations, 11 ms and 1.8 ms.

Expression to determine nanocavity profile and pore size–conductance correlation

An analytical method is proposed to determine the profile of nanocavity and the pore size-conductance correlation.

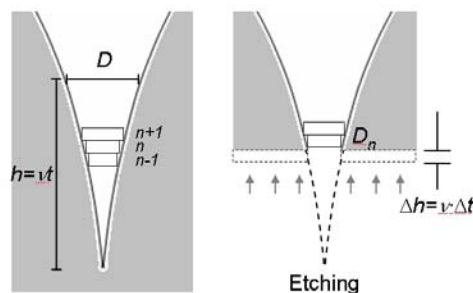


Figure S5. Cartoon showing the formation of a nanopore from the nanocavity by external etching. Left, nanocavity sealed in the pipette terminal. The profile of nanocavity is described by $D=f(h)$. The solution in nanocavity is considered as a series of conductive layers stacked one on another. Right, nanopore formation by progressive removal of n conductive layers by the bottom-up etching.

Fig.S5 shows the formation of different size nanopores from an arbitrary nanocavity. The nanocavity profile can be described by $D=f(h)$, where h is the length starting from the narrow end of the nanocavity, and D is the diameter of nanocavity at the length h . The solution in the nanocavity is equivalent to a series of conductive layers that stack one on another, with D_n , S_n and Δh represent the diameter, area and thickness of the n^{th} layer. The resistance of the n^{th} layer is

$$R_n = \frac{1}{K} \cdot \frac{\Delta h}{S_n} = \frac{4}{\pi K} \frac{\Delta h}{D_n^2} \quad (\text{S1})$$

Where K is the solution conductivity, As each conductive layer is removed through the bottom-up etching process, the nanopore resistance $\sum_{i=n}^{\infty} R_i$ (the total resistance of all the remaining layers) is reduced to $\sum_{i=n+1}^{\infty} R_i$, resulting in the increase of nanopore conductance from g_n to g_{n+1} . The progressive change in resistance can be described as follows:

$$\begin{aligned}
\sum_{i=1}^{\infty} R_i &= \frac{1}{g_1} = \frac{4}{\pi K} \left(\frac{\Delta h}{D_1^2} + \frac{\Delta h}{D_2^2} + \dots + \frac{\Delta h}{D_n^2} + \frac{\Delta h}{D_{n+1}^2} + \dots \right) \\
\sum_{i=2}^{\infty} R_i &= \frac{1}{g_2} = \frac{4}{\pi K} \left(\frac{\Delta h}{D_2^2} + \dots + \frac{\Delta h}{D_n^2} + \frac{\Delta h}{D_{n+1}^2} + \dots \right) \\
&\vdots \\
\sum_{i=n}^{\infty} R_i &= \frac{1}{g_n} = \frac{4}{\pi K} \left(\frac{\Delta h}{D_n^2} + \frac{\Delta h}{D_{n+1}^2} + \dots \right) \\
\sum_{i=n+1}^{\infty} R_i &= \frac{1}{g_{n+1}} = \frac{4}{\pi K} \left(\frac{\Delta h}{D_{n+1}^2} + \dots \right) \\
&\vdots
\end{aligned} \tag{S2}$$

Subtracting the $n+1^{\text{th}}$ line from the n^{th} line in Equation S2 results in the expression for the n^{th} layer's resistance,

$$R_n = \frac{1}{g_n} - \frac{1}{g_{n+1}} = \frac{\Delta g_n}{g_n^2} = \frac{4\Delta h}{\pi K D_n^2} \tag{S3}$$

where $g_n \cdot g_{n+1} \approx g_n^2$ when Δh is very small. For h , we have $\Delta h / \Delta t = h_n / t_n = v$, where v is the etching speed, Δt is the time for removing a single conductive layer (Δh) and t_n is the total time for removing n conductive layers with the total length of h_n . Given this relationship, we derived the equations from Equation S3,

$$D_n = \left(\frac{4vg_n^2}{\pi K} \bigg/ \frac{\Delta g_n}{\Delta t} \right)^{0.5} \quad (\text{S4})$$

$$h_n = v \cdot t_n$$

The differential equation form of Equation S4 is

$$\boxed{D = \left(\frac{4vg^2}{\pi K} \bigg/ \frac{dg}{dt} \right)^{0.5}} \quad (\text{S5})$$

$$h = v \cdot t$$

Equation S5 can be used to calculate nanocavity profile ($D-h$ curve) and pore size-conductance correlation ($D-g$ curve). The conductance g and its derivative dg/dt are obtained directly from the etching curve ($g-t$ curve), the increasing nanopore conductance with the etching time. The conductivity K is an important issue. The surface charge in nanopore can cause spatial variation of the conductivity, thus K is a function of D and h , $K(D, h)$. However, K is presumably a constant and similar to the bulk conductivity at high ion concentration (such as 1 M), which effectively shields the surface charge.

The application of Equation S5 is exemplified by a conical-shaped nanopore.

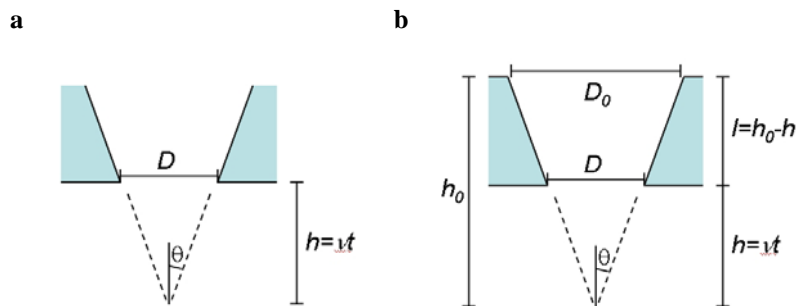


Figure S6. Formation of conical nanopore from a cone-shaped nanocavity by etching from bottom up. **a.** Conical pore with an infinitely long pore length. **b.** Conical pore with a pore length of l .

Fig.S6a shows a cone-shaped nanocavity with an infinitely long pore length. Perforation from the bottom up leads to the formation of a conical nanopore. h is the length from the terminal of cavity. $h=vt$, where v is the etching speed. D is the diameter of the narrow opening of the nanopore at the position h . The profile of nanocavity can be described as

$$D = 2tg\theta \cdot h = 2tg\theta \cdot vt \quad (\text{S6})$$

According to Equation S5,

$$\frac{1}{g^2} \frac{dg}{dt} = \frac{4v}{\pi\kappa D^2} = \frac{1}{\pi\kappa \cdot tg^2\theta \cdot vt^2} \quad (\text{S7})$$

The integration of Equation S7 with the boundary condition is

$$\int_g^\infty \frac{1}{g^2} dg = \int_t^\infty \frac{1}{\pi\kappa \cdot tg^2\theta \cdot vt^2} dt \quad (\text{S8})$$

The solution of Equation S8 is

$$\boxed{g = \pi\kappa \cdot tg^2\theta \cdot h = \frac{\pi\kappa \cdot tg\theta \cdot D}{2}} \quad (\text{S9})$$

Equation S9 describes the D - g curve for an infinitely-long conical nanopore.

Equation S9 can also be derived based on the Ohm's law. The pore resistance at the position h is

$$\begin{aligned}
R &= \int_h^\infty \frac{4}{\pi\kappa D^2} dh = \int_h^\infty \frac{1}{\pi\kappa \cdot tg^2\theta \cdot h^2} dh \\
&= \frac{1}{\pi\kappa \cdot tg^2\theta} \cdot \frac{1}{h} = \frac{2}{\pi\kappa \cdot tg\theta \cdot D}
\end{aligned} \tag{S10}$$

Thus, the conductance is

$$g = \frac{1}{R} = \frac{\pi\kappa \cdot tg\theta \cdot D}{2} \tag{S11}$$

Equation S11 is identical to Equation S9.

Fig.S6b shows a conical nanopore with a pore length of l . In this pore, h_0 is the pore length at $t=0$ (before etching); $h = vt$ is the length of cavity removed by the bottom-up etching process. $h_0 - h$ gives l . The diameter of the wide opening is D_0 , and the diameter of the narrow opening at the time t is D . The integration of Equation S5 is

$$\int_g^\infty \frac{1}{g^2} dg = \int_t^{h_0/v} \frac{1}{\pi\kappa \cdot tg^2\theta \cdot vt^2} dt \tag{S12}$$

The solution of Equation S12 is

$$\boxed{g = \pi\kappa \cdot tg^2\theta \cdot h_0vt = \frac{\pi\kappa \cdot tg^2\theta \cdot h_0vt}{h_0 - vt} = \frac{\pi\kappa \cdot D_0D}{4l}} \tag{S13}$$

Equation 13 is the exact form of the pore size-conductance correlation reported previously for the ion track-etched conical nanopore in polymer film^{2,3}.

In conclusion, Equation S5 can be used to determine nanocavity profile and pore size-conductance correlation.

References

1. Rekharsky, M. & Inoue, Y. Chiral recognition thermodynamics of beta-cyclodextrin: The thermodynamic origin of enantioselectivity and the enthalpy-entropy compensation effect. *Journal of the American Chemical Society* **122**, 4418-4435 (2000).
2. Apel, P. Y., Korchev, Y. E., Siwy, Z., Spohr, R., & Yoshida, M. Diode-like single-ion track membrane prepared by electro-stopping. *Nuclear Instruments & Methods in Physics Research Section B-Beam Interactions with Materials and Atoms* **184**, 337-346 (2001).
3. Siwy, Z., Gu, Y., Spohr, H. A., Baur, D., Wolf-Reber, A., Spohr, R., Apel, P., & Korchev, Y. E. Rectification and voltage gating of ion currents in a nanofabricated pore. *Europhysics Letters* **60**, 349-355 (2002).