Supporting Information

Video list:

All the videos are captured using a TIRF microscope with a 60x objective. Videos F3-F6 are cropped from the original view field, to highlight the dynamics of single DNA molecules. All the videos are sped up or slowed down, but the real acquisition time is shown in the videos. The video's bit resolution are highly compressed from the originals.

Video F1: T4 DNA are trapped under 2D pulse field electrophoresis, at 0.5 Hz. The applied field strength E_2 = 142.9 V/cm, $E_1 = \sqrt{2}\ E_2$. At the beginning of the video, the DNA molecules are at rest without electric field, showing coiled conformation. At ~20 s after the pulsed fields are applied, some molecules are immobilized in the separation sieves. The view field is 123 μm ×123 μm.

Video F2: With reverse spikes, T4 DNA are not trapped under pulsed field. The field strength and duration time of forward pulses E_1 and E_2 are the same as those in video F1. The parameters of the reverse spikes are shown in Table 1. The view field is 123 μm ×123 μm.

Video F3: The trapping of a single DNA at 2 Hz, with field strength E_2 = 142.9 V/cm, $E_1 = \sqrt{2}\ E_2$. At the beginning of the video, the observed DNA molecule is in a free state.

Video F4: The trapping of a single DNA at 0.5 Hz, with field strength E_2 = 142.9 V/cm, $E_1 = \sqrt{2}\ E_2$. At the beginning of the video, the observed DNA molecule is in a free state.

Video F5: At E_1 = 202.0 V/cm, a J-shape DNA that cannot be unhooked.

Video F6: The effect of the reverse spike field modulation. Two J-shape DNA are observed at E_1 = 202.0 V/cm. One is a freshly hooked DNA, the other one has already been pinned at the apex. The reverse spike relaxes both of the molecules and dislodges them from the apex. Afterwards, the originally pinned DNA follows the ratchet model, completing the reorientation normally.

Possibility of hooking：

DNA molecules collide with silica particles frequently while driven by electrophoretic force. The ratio of "hook" to "roll-off" is determined by the Deborah number (De) in Equation 1 [1]:

$$De = 4\mu E\tau / R_{obs} \quad (1)$$

Where μ=8.857×10^{-9} m^{2}V^{-1}s^{-1} is the DNA mobility in confined structure as mentioned in paper, R_{obs} = 345 nm is radius of silica particle and τ is the relaxation time calculated from Odijk's theory

$$\tau = \frac{\eta L^2}{k_B T}\frac{D^2}{P\ln\left(\frac{4D}{\pi w}\right)} = 46 \text{ s}$$

given viscosity $\eta = 1.16\ cP$, contour length L = 55.8 μm, persistence length P = 50 nm, pore diameter D=105 nm and width of DNA w = 2.4 nm. In our experiment, De numbers are 4.77×10^4, 7.64×10^4 and 9.55×10^4 for E_1 = 101.0, 161.6 and 202.0 V/cm, respectively. As a result, the hooking probability is nearly 100% after central collision.[1] Therefore, a

hook is one of the major DNA configurations in nanoporous structures. The two arms of the U/J hook shapes are subsequently stretched by high electric field.

Effective charge density ρ_{eff}:

In the analysis of DNA mobility, we assume the frictional force compensates the electric field force, giving:

$$\xi\mu E = Eq_{eff} \tag{2}$$

where ξ is frictional coefficient, q_{eff} is the total effective charge of T4 DNA. The effective charge density ρ_{eff} is defined as charge per base pair:

$$\rho_{eff} = q_{eff} / N = \mu_0 \xi_0 / N \tag{3}$$

In free solution, the frictional coefficient is:

$$\xi_0 = 6\pi\eta g_r \tag{4}$$

Substituting Equation 4 into Equation 3, we get the effective charge density of DNA in 4x TBE buffer:

$$\rho_{eff} = 6\pi\mu_0\eta g_r / N = 0.034\,\mathrm{e^-/bp}$$

given free solution mobility $\mu_0 = 3.175 \times 10^{-8}\, m^2/(v \cdot s)$, viscosity $\eta = 1.16\ cP$, radius of gyration of T4 DNA $g_r = 1.31$ μm and number of base pair $N = 166 \times 10^3$. The result is consistent with the value obtained by Smith et al, that $\rho_{eff} \approx 0.05\ e/base\ pair$ in 1/2 TBE buffer[2].

Debye length λ_D:

We calculate the thickness of Debye layer at the silica particle surface:

$$\lambda_D = \sqrt{\frac{\varepsilon_0 \varepsilon_b k_B T}{2e^2 I N_A}} = 0.743nm$$

given relative permittivity of 4x TBE buffer $\varepsilon_b = 77.232$, ionic strength I = 0.165 mol/L[3].

High field gradient region:

The size of the high field gradient region that could cause a significant external force (Red in Figure 7) is estimated from Figure S4. Because trapping doesn't occur at E_1 = 101.0 V/cm where the maximum $\nabla|E|^2$ is $0.8 \times 10^{17} mkg^2/(s^6A^2)$, the region with $\nabla|E|^2 > 1 \times 10^{17} mkg^2/(s^6A^2)$ was taken as the high field gradient regime. For E_1 = 202.0

V/cm, the high $\nabla|E|^2$ regime corresponds to the arc length (0, 0.024) μm in Figure S4, which gives a central angle α = 0.437. Therefore, the length of the yellow segment across which a strong field gradient occurs, shown in Figure S4 inset, is 34.9 nm. This distance is 47 times the Debye length, indicating the size of high field gradient region is much larger than the double layer. Figure S4 also shows the size of the high field gradient regime is increased with the field strength.

Rationale for using 105 nm pore size:

The most common separation media for the pulsed field electrophoresis is gel. Experimental evaluation of the pore sizes of gels are 150-350 nm and 5-100 nm for agarose and polyacrylamide, respectively.[4] Trapping is observed in these materials across this pore size range. Thus, with a 105 nm pore size in the colloidal crystal arising from the choice of 690 nm particle diameter, a comparison of models for trapping in gels and the particle beds is appropriate based on pore size.

The critical field strength of DNA trapping varies with pore size. According to the definition of De number (Eq. 1), the pore size affects the probability of DNA colliding with particles and the formation of the U/J shape. Larger pores will have less collision events, relative to smaller pores. However, with 55.8 μm contour length and 1.31 μm radius of gyration, T4 DNA molecules are strongly confined in 5-350 nm pores. Thus, collision and hooking are frequent across this pore size range, resulting in trapping of large size DNA during electrophoresis. The Odijk model of friction coefficient and relaxation time is appropriate for the specific 105 nm pore size used in this study.

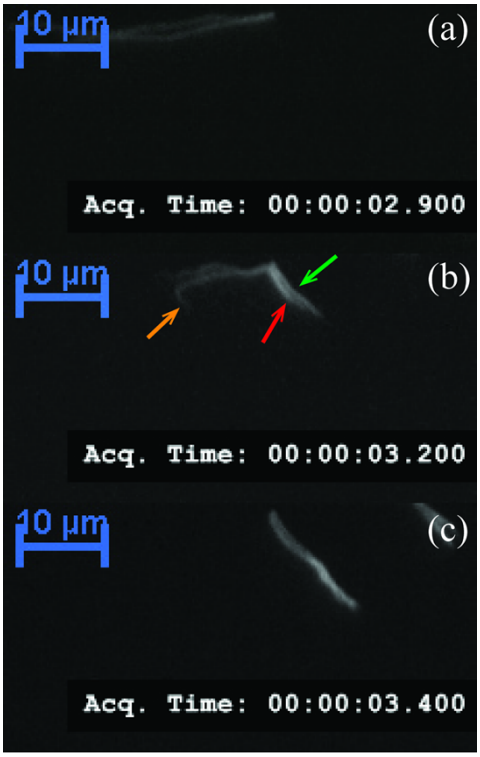

Figure S1 Time-lapse images of video F4. DNA is trapped at 0.5 Hz. In (b), both near-apex hernia (red arrow) and distant hernias (orange arrow) form. The green arrow points at the apex segment. The distant hernia disappears with time, while the near-apex hernia is substantially stretched. As an overall result, the apex is immobilized.

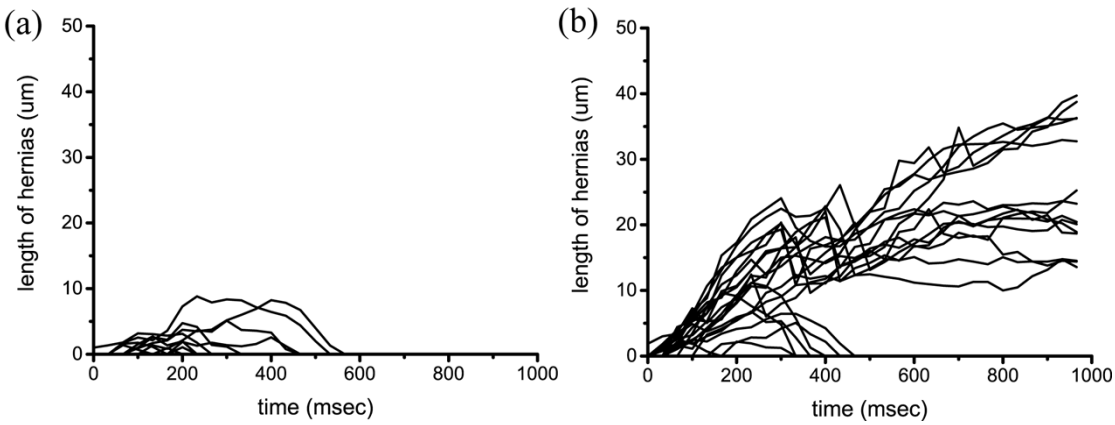

Figure S2 The total lengths of hernias are shown as a function of time. 30 hernias forming at E_2 = 142.9 V/cm are studied and identified as (a) distant hernia or (b) near-apex hernia. The near-apex hernia is within a range of 2.8 μm (5% of contour length) of the U/J apex.

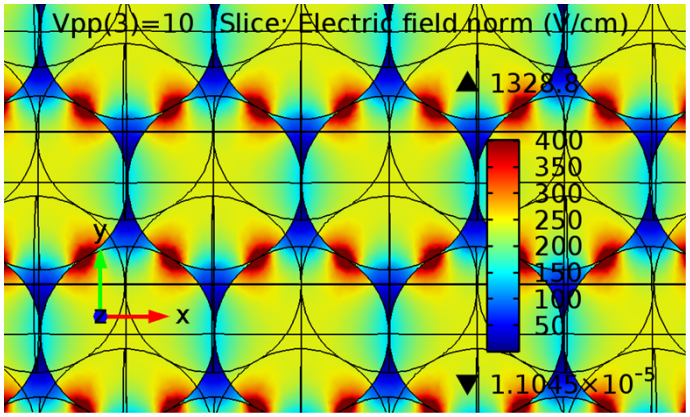

Figure S3 The simulation results, as also shown in Figure 7, here showing the electric field strength is non-uniform in the nanoporous structure. This is a two-dimensional slice taken along a plane passing through the particle's centre, parallel to the direction of the field, showing the field in the pore.

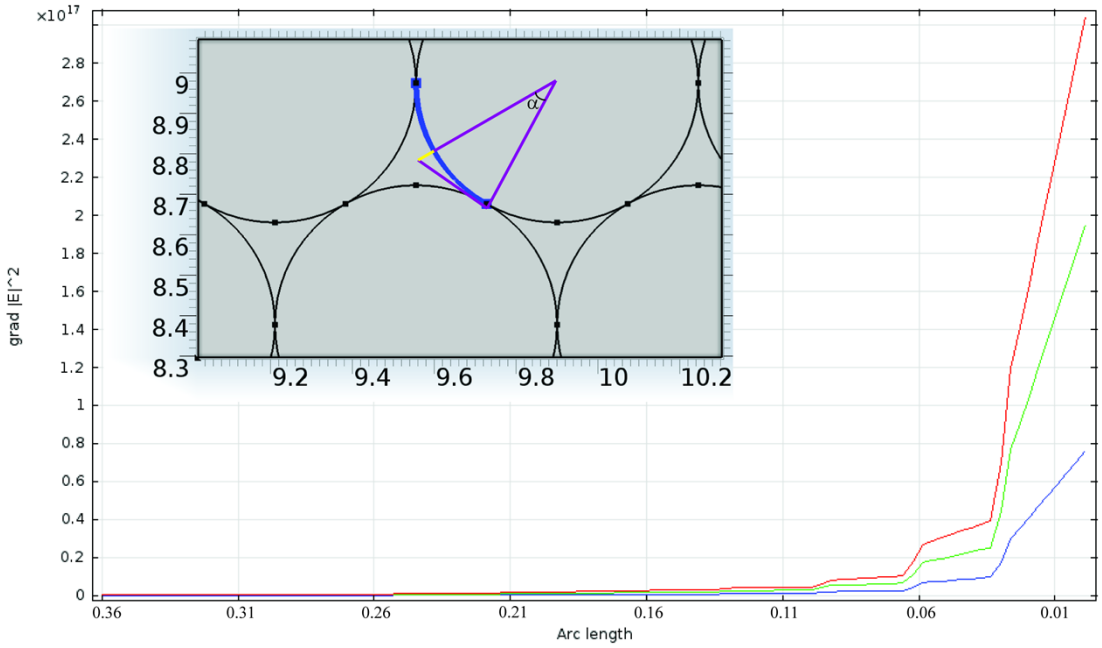

Figure S4 Field gradient $\nabla|E|^2$ (unit: $mkg^2/(s^6A^2)$) on a selected buffer-silica interface (blue arc in the inset). This result is extracted from the same simulation as those shown in Figure 7. The red, green and blue lines represent the field gradient at an average field E_1=202.0, 161.6 and 101.0 V/cm, respectively. The x-axis shows the arc length in micrometers, with x = 0 occurring when the angle α shown in the inset is zero, and arc length is a maximum of 0.361 when α = 60°.

Reference:

1. G. C. Randall and P. S. Doyle, *Phys. Rev. Lett.*, 2004, 93, 058102.
2. S. B. Smith and A. J. Bendich, *Biopolymers*, 1990, 29, 1167-1173.
3. C.-C. Hsieh, A. Balducci and P. S. Doyle, *Nano Lett.*, 2008, 8, 1683-1688.
4. J.-L. Viovy, *Rev. Mod. Phys.*, 2000, 72, 813-872.