Supplementary Material

A patterned anisotropic nanofluidic sieving structure for continuous-flow separation of DNA and proteins

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This supplementary material includes

Supplementary Text

Supplementary Figures (Fig. S1 and Fig. S2)

Supplementary Video Captions

Supplementary Text

In the Ogston sieving regime, the nanofilter jump passage rate P_x for short DNA of a bp number N can be calculated based on the equilibrium partitioning theory and the Kramer's rate theory[S1]. In the limit of low field, the passage rate P_x is proportional to $E_x^2 K/N$, where K is the DNA equilibrium partitioning coefficient that is calculated as the ratio of accessible microscopic configuration state integrals within shallow and deep regions across the nanofilter[S1,S2]. Therefore, the relative mobility μ_x^* along the x-axis across the nanofilters can be calculated as[S1]

$$\mu_x^* = (1 + \frac{\alpha N}{E_x K})^{-1} \tag{1}$$

where α is a constant with a unit of V/(m·bp). By definition, μ_x^* is the ratio between the mobility μ_x along the x-axis and the maximum sieving free mobility $\mu_{x,max}$ across a nanofilter[S1]. Thus, the tangent of the stream deflection angle θ can be approximately written as

$$\tan\theta = \frac{V_x}{V_y} = \frac{\mu_{x,\max}}{\mu_0} \cdot \frac{E_x}{E_y} \cdot \mu_x^* = \frac{\mu_{x,\max}}{\mu_0} \cdot \frac{E_x}{E_y} \cdot (1 + \frac{\alpha N}{E_x K})^{-1} \tag{2}$$

where V_x and V_y are the migration velocities along the positive x- and negative y-axis, respectively, and μ_0 is the free solution mobility. In equation (2), we have implicitly assumed that DNA fragments preserve their free draining property in the ANA deep regions along the y-axis[S3]. $\mu_{x,max}/\mu_0$ depends solely on the structural parameters of the ANA[S4], and $\mu_{x,max}/\mu_0 = 4d_s d_d/(d_s+d_d)^2 = 0.52$ for the ANA tested in the experiments. The equilibrium partitioning coefficient K can be calculated as in Ref. (S1). In the limit of short DNA, equation (2) becomes $\tan\theta = 0.52E_x/E_y$, which indicates a sieving free case in the ANA. The experimental data of $\tan\theta$ for the PCR maker sample in **Supplemental Figure 1** roughly agree with the theoretical curves calculated from equation (2). The best fitting constant α was found to be fairly constant for the different DNA fragments. The slight deviation of the theoretical curves from the deflection angle data in the low E_x regime might be attributed to the non-uniformity of E_x and E_y in the ANA.

References

S1. Fu, J., Yoo, J. & Han, J. Molecular sieving in periodic free-energy landscapes created by patterned nanofilter arrays. *Phys. Rev. Lett.* **97**, art. no. 018103 (2006).

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S3. Stellwagen, N. C., Gelfi, C. & Righetti, P. G. The free solution mobility of DNA. *Biopolymers* **42**, 687–703 (1997).

S4. Fu, J., Mao, P. & Han, J. Nanofilter array chip for fast gel-free biomolecule separation. *Appl. Phys. Lett.* **87**, art. no. 263902 (2005).

Supplementary Figure 1

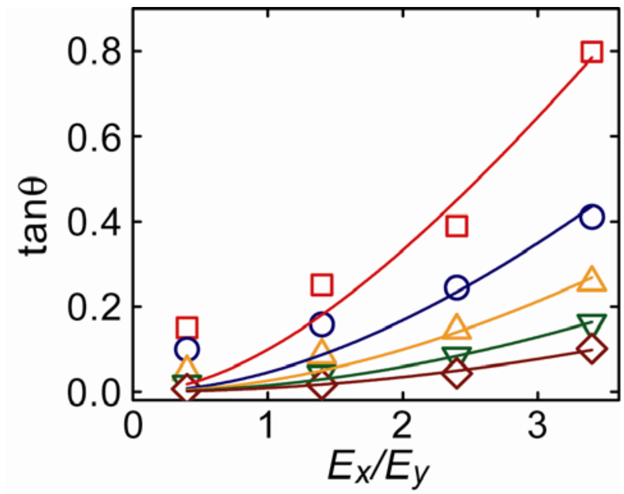

Figure S1. tanθ of different streams as a function of E_x/E_y at fixed E_y= 25 V/cm (50-bp (□), 150-bp (○), 300-bp (△), 500-bp (▽), 766-bp (◇)). The ±s.d. of θ derived from the stream half-width are all less than 1°, so statistical error bars for tanθ are not plotted. The colored solid lines are theoretical curves calculated from equation (2) in the **supplemental text**. The best fitting constant α has a mean about 177.5 and a ±s.d. about 12%.

Supplementary Figure 2

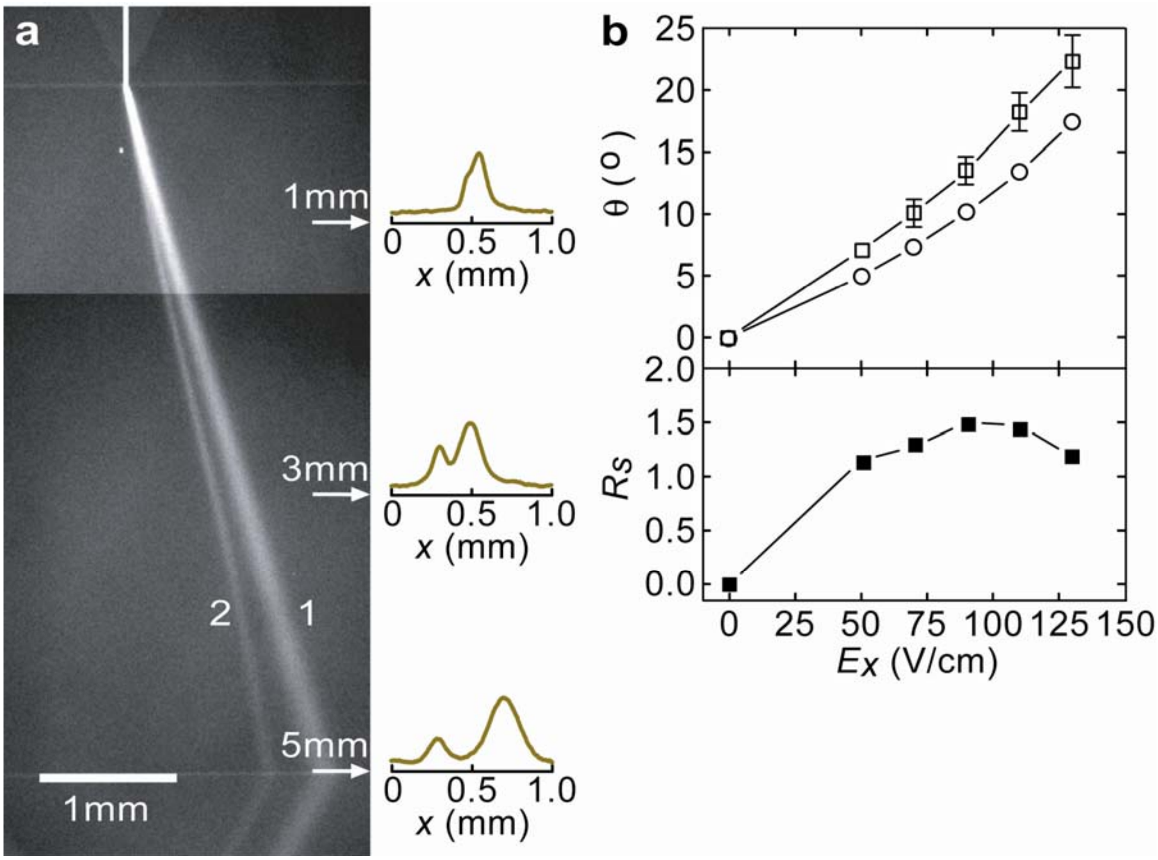

Figure S2. Continuous-flow separation of proteins under denaturing conditions through the ANA. a, Composite fluorescent photograph showing separation of Alexa Fluor 488-conjugated cholera toxin subunit B (band 1, MW~11.4-kDa) and *β*-galactosidase (band 2, MW~116.3-kDa) with E_x=75 V/cm and E_y= 50 V/cm. The protein stream widths at 1 mm, 3 mm, and 5 mm from the injection point corresponded to resolutions R_s of 0.57, 0.94 and 1.47, respectively. **b,** Measured deflection angle θ (top) of cholera toxin subunit B (□) and *β*-galactosidase (○) as a function of E_x when E_y=50 V/cm. The bottom shows the corresponding separation resolutions. The ±s.d. of θ are indicated as error bars (drawn if larger than the symbol).

Supporting Videos

Movie S1. Continuous-flow separation of the PCR marker in the ANA, with the Ogston-sieving mechanism. This video corresponds to the still images shown in **Fig. 3a–b**, with exposure time of 1300 ms/image and image size of 1300 µm × 1620 µm. The time scale in this movie has been compressed by a factor of 20. At the beginning of the movie, only E_y=25 V/cm was applied. The orthogonal field E_x=35 V/cm was applied at 3 sec in the movie, and the separation was finished at about 12 sec in the movie. Video contributed by Jianping Fu.

Movie S2. Continuous-flow separation of λ DNA – Hind III digest in the ANA, with the entropic trapping mechanism. This video corresponds to the still images shown in **Fig. 4a–b**, with exposure time of 600 ms/image and image size of 3270 µm × 4080 µm. The time scale in this movie has been compressed by a factor of 20. At the beginning of the movie, only E_y=100 V/cm was applied. The orthogonal field E_x=185 V/cm was applied at 1 sec in the movie, and the separation was finished at about 5 sec in the movie. Video contributed by Jianping Fu.

Movie S3. Continuous-flow separation of proteins under denaturing conditions in the ANA. This video corresponds to the still images shown in **Fig. S2a**, with exposure time of 1000 ms/image and image size of 3270 µm × 4080 µm. The time scale in this movie has been compressed by a factor of 20. At the beginning of the movie, only the vertical field E_y=50 V/cm was applied. The horizontal field E_x=75 V/cm was applied at 1 sec in the movie, and the separation was finished at about 6 sec in the movie. Video contributed by Jianping Fu.