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Nanoslit Confined DNA at Low Ionic Strengths

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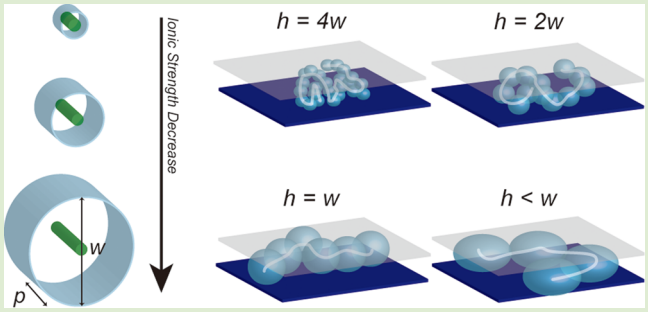
Supporting Information

ABSTRACT: We present nanoslit confined DNA conformations at very low ionic strengths and a theory to explain most measurements for single DNA molecule size under strong nanoslit confinement. Very low ionic strength conditions not only increase the DNA persistence length dramatically, but also cause DNA molecules to swell to the extent that the effective diameter of DNA becomes larger than the nanoslit height. By accounting for these effects, our results and theory provide a reasonable clue for a current controversy regarding the dependence of the DNA conformation on slit height (h), persistence length (p), and effective diameter (w).



Direct visualization of individual DNA molecules confined within a nano/microfluidic geometry has provided a new route for the study of polymer physics.[1,2] Molecular confinement has been utilized to evaluate theoretical predictions developed over several decades.[3−5] Particularly, nanoslit confined DNA molecules are an interesting topic to show the conformational transition from 3D to 2D depending on nanoslit heights.[6] Although several experiments have recently reported the dependence of DNA conformation on nanoslit confinement,[7−12] a controversy still remains because the results have not been consistent with one another nor with theoretical predictions.[13−15]

DNA properties in nanoslit have been intensively investigated with theoretical modeling.[16−19] In 1977, Daoud and de Gennes derived a scaling relationship to describe a real polymer chain trapped in a small slit ($h < R_g$) as $R_{\parallel} \cong aN^{3/4}(a/h)^{1/4}$, where $R_{\parallel}$ is the radius of gyration projected to the plane and h is the slit height.[20] For DNA confined within a nanoslit, this can be rewritten as[19]

$$R_{\parallel} \cong L^{3/4}(pw/h)^{1/4} \tag{1}$$

where L is the DNA contour length, p is the persistence length, and w is the effective diameter. Recently, several experiments investigated the height dependence in eq 1, but limitations due to the relatively short accessible range between $R_{\parallel}$ and p has plagued accurate measurement of the scaling exponent.[16] On the other hand, under strong confinement where h is comparable to w, DNA becomes effectively 2D with the size, which is given by[6]

$$R_{\parallel} \cong L^{3/4}p^{1/4} \tag{2}$$

The change of persistence length (p) due to the ionic strength (I) is also critically important in understanding DNA conformations in nanoslits.[8,12] Odijk,[21] Skolnick, and Fixman[22] theory (OSF) characterizes how electrostatic interaction affects the rigidity of a polyelectrolyte in terms of the Debye length ($1/\kappa$), which is given by

$$p_{\mathrm{OSF}} = p_0 + \frac{1}{4\kappa^2 l_{\mathrm{B}}} = p_0 + \frac{0.0324}{I}\ \mathrm{nm} \tag{3}$$

However, Dobrynin pointed out the limitation of the OSF theory[23] and proposed an empirical formula given by[24]

$$p_{\mathrm{Dob}} = 46.1 + \frac{1.1915}{\sqrt{I}}\ \mathrm{nm} \tag{4}$$

He also claimed that the validity of either theory is dependent on given conditions.[25] DNA in free solution was reported to follow eq 4 at very low ionic strength down to 1 μM.[8,26] However, experiments in nanoslits have not been definitive for two competing theories probably due to the limited range of the ionic strength examined.[8,12]

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In addition, low ionic strength also increases effective diameter (w), resulting in a more swollen DNA molecule. Stigter derived the dependence of w on I, given by[27]

$$w = \kappa^{-1}\left[0.7704 + \ln\left(\frac{\pi}{2l_B\kappa}\left(\frac{y_0}{\gamma K_0(x_0)}\right)^2\right)\right] \quad (5)$$

where $\kappa^{-1} = 0.304\ \text{nm}/\sqrt{I}$, $x_0 = \kappa a$ (a = 1.2 nm, DNA radius), y_0 is the dimensionless surface potential, K_0 is the zeroth order modified Bessel functions of the second kind, and γ is the correction factor defined in Stigter's paper.[28] A previous study showed that w could become even larger than 1 μm at 1 μM ionic strength.[8] It could thus be expected that large effective diameter at low ionic strength strongly affect DNA conformation in a nanoslit.

First, we investigated nanoslit confined DNA conformations under various ionic strengths (I) from 0.18 mM to 1.73 mM (Figure 1). According to eq 1, DNA sizes in a nanoslit can be described by $R_\parallel \sim (pw)^{1/4}$ from the dependence of the ionic strength (I). Yet, it is not straightforward to obtain the appropriate p and w from given ionic strength condition. First, w is determined using eq 5 with parameters obtained by numerically solving the Poisson–Boltzmann equation for a charged rod (see SI).[27,29] It is noteworthy that the largest w (135 nm: 0.183 mM) is even larger than the smallest nanoslit height of 130 nm (see SI, Table 1). However, the interaction between DNA segments and the wall also depend on the wall surface charge density, and the effective DNA width for the wall may be smaller. In order to determine the persistence length (p), we analyze log-scale graphs for the DNA size ($R_\parallel$) with p_{OSF} from eq 3 (Figure 1-i) and p_{Dob} from eq 4 (Figure 1-ii). This comparison shows $p_{Dob}w$ better fitted than $p_{OSF}w$, though not perfect. Thus, we used p_{Dob} for the remaining calculations in this work. However, corresponding graphs using p_{OSF} are also included in SI.

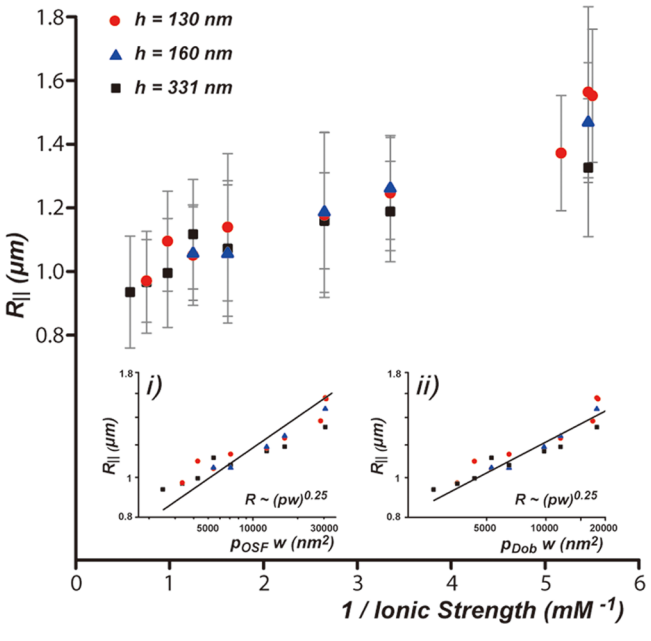

Figure 1. Nanoslit confined DNA ($R_\parallel$) as a function of the ionic stregnth (I). The insets depict a log–log plot $R_\parallel$ as a function of p and w (*i*: OSF, *ii*: Dobrynin). Each data points represent a measurement from 100 to 300 molecules; error bars show standard deviations.

We then examine the controversy of the height dependence of the DNA size in nanoslits. An experimental study in 2008 first reported observing DNA conformation transition from moderate to strong nanoslit confinement.[7] They found that $R_\parallel \sim h^{1/4}$ in nanoslits of height from 100 to 400 nm and that $R_\parallel$ does not change significantly below 100 nm.[7] It was claimed that DNA's Kuhn length of 100 nm ($2p$) separates the moderate confinement de Gennes regime (eq 1) and strong confinement Odijk regime. More recent experimental results,[9−12] however, reported values of the scaling exponents within the −0.18 to −0.22 range for slit heights from 20 to 780 nm with no clear transition from de Gennes to Odijk regime. In this context, we aim to investigate these current controversies.

Figure 2a presents an overview of the DNA conformation dependence on 20 different slit heights ranging from 130 nm to 1900 nm at four different ionic strengths. The largest DNA size ($R_\parallel$) observed is 1.56 μm in the 130 nm nanoslit at the lowest ionic strength of 0.18 mM. In contrast, the smallest DNA size ($R_\parallel$) is 0.73 μm for h = 1.13 μm. These observations allow us to evaluate the scaling exponent for $R_\parallel \sim h^\delta$, as shown in Figure 2b. For I = 0.18 mM, the best fit over the 100−1000 nm range finds the scaling exponent $\delta = -0.18$. This agrees well with that reported by Strychalski et al.[13] It is also observed that the

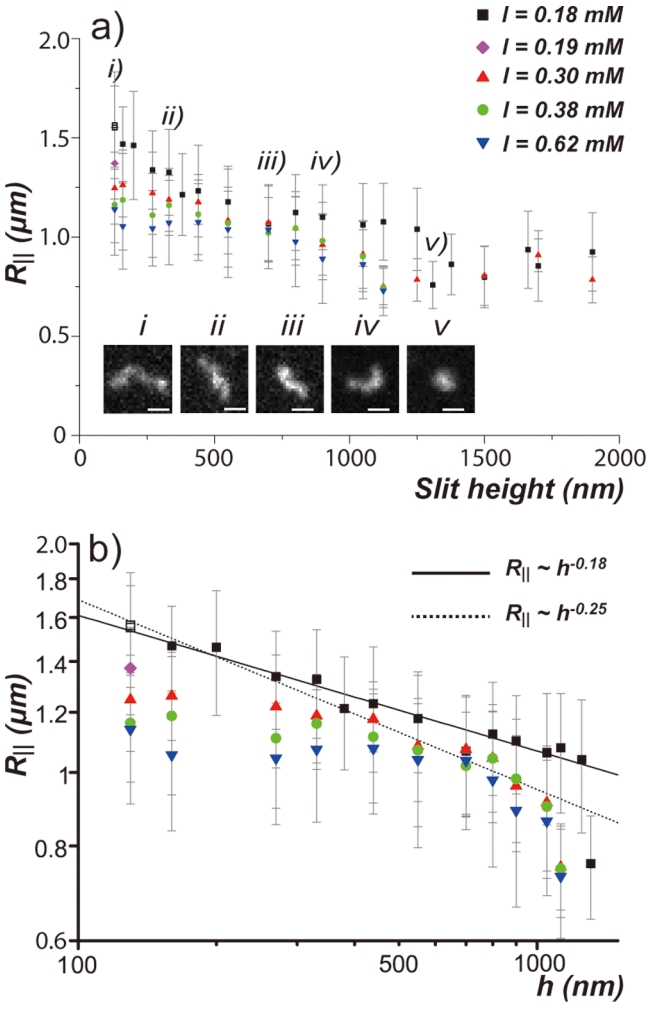

Figure 2. (a) $R_\parallel$ as a function of slit heights. Fluorescence micrographs show representative DNA molecules (scale bars, 2 μm.). (b) Scaling analysis for $R_\parallel$ vs h.

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measured $R_{\parallel}$ follow $R_{\parallel} \sim h^{-0.25}$ for other I and more limited range of h.

We analyzed the same data sets with the ratio of the height to the effective diameter (h/w) for the swelling and the persistence length (h/p) for the stiffness to categorize regimes as illustrated in Figure 3. For instance, if the effective diameter is larger than the slit height $(h < w)$, it is very difficult to put a swollen DNA molecule into a nanoslit. As expected, we usually had difficulty in loading DNA molecules into a thin nanoslit under very low ionic strength conditions. This can be considered as electrostatic trapping to confine molecules to geometry smaller than the physical boundaries. Therefore, we consider the $h/w < 1$ region as the strongest confinement regime in which a DNA molecule exists in a nanoslit without 2D crossing: that is, a nanoslit-confined quasi-2D regime.[16] This regime can be extended to the second strongest confinement regime $(h/w < 2)$, because a nanoslit is not high enough to allow two DNA segments to cross each other. Previously, Dai et al. reported simulation results that $h/w = 2.5$ would be a boundary condition for separating Odijk regime and de Gennes regime.[18]

The strong confinement regime is originated from Odijk's theory to predict DNA stretch $(L_{\parallel})$ within a small tube.[30] Thus,

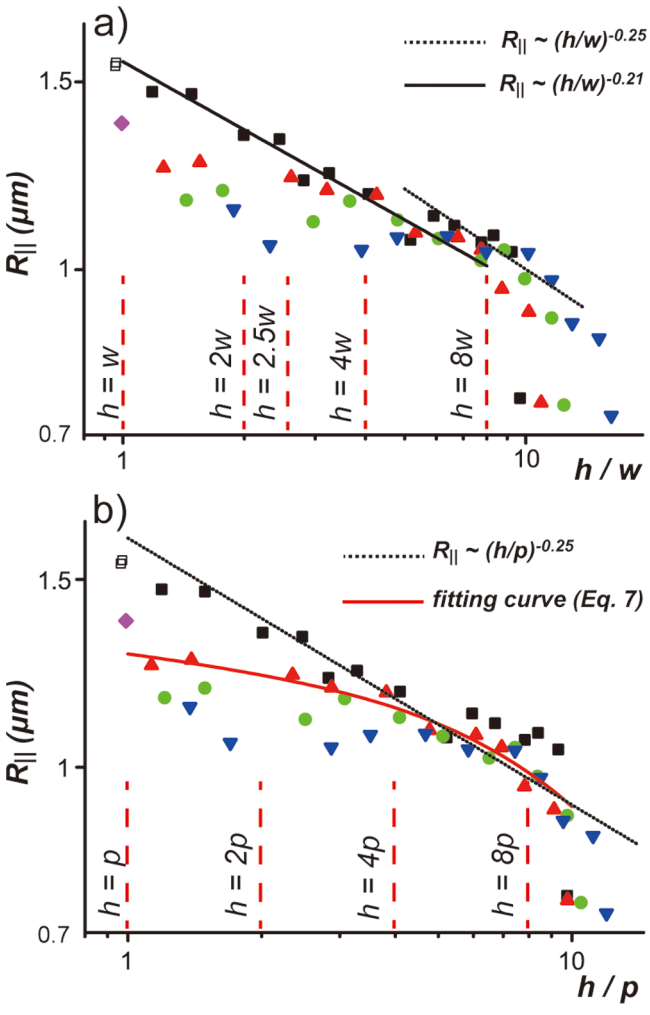

Figure 3. (a) $R_{\parallel}$ as a function of ratio of h/w. (b) $R_{\parallel}$ as a function of ratio of h/p. For I = 0.18 mM (■), 0.19 (♦), 0.30 (▲), 0.38 (●), and 0.62 (▼).

the stretch in a nanochannel is given by $L_{\parallel} = L(1 - \alpha(h/p)^{2/3} - \alpha(b/p)^{2/3})$, where b is the channel width,[3] and α is 0.09137.[31] The Odijk regime has been clearly observed for DNA in the nanochannel.[4,5,32] Odijk's theory can be simply extended to nanoslit, in which the apparent contour length can be described as $L_{\parallel} = L(1 - \alpha(h/p)^{2/3})$.[18] Therefore, for the Odijk regime, eq 2 can be rewritten as

$$R_{\parallel} \cong L_{\parallel}^{3/4} p_{\parallel}^{1/4} = L^{3/4}\{1 - \alpha(h/p)^{2/3}\}^{3/4} \times p_{\parallel}^{1/4} \quad (6)$$

where $p_{\parallel}$ is the longitudinal segmental correlation length.[18] Recent simulations have shown that $p_{\parallel}$ on the slit confinement follows the form $p_{\parallel}/p = l_{2D} - \exp[-0.8(p/h - l_c)]$ for $p/h > l_c$, where $l_{2D} = 2$ for the persistence length of a 2D ideal semiflexible chains and $l_c = 0.2$ signifies an arbitrary cutoff length beyond which confinement does not affect $p_{\parallel}$.[17] Increased intrapolymer repulsion due to the stronger confinement may be explained by considering the excluded area between polymer segments in the 2D plane as $A = (p_{\parallel} + (1/2 + \pi/4)w)^2$.[18] Considering the excluded area, the DNA size can be written as

$$R_{\parallel} \cong L_{\parallel}^{3/4} p_{\parallel}^{1/4}\left(1 + \left(\frac{1}{2} + \frac{\pi}{4}\right)\frac{w}{p_{\parallel}}\right)^{1/2} \quad (7)$$

Equation 7 is applied to fit $R_{\parallel}$ for I = 0.30 mM, as shown in Figure 3b. It is notable that the fitting curve captures a wide regime covering from $h = p$ to $h = 8p$, though only $h < 2p$ is generally accepted as the Odijk regime. In addition, the data for different ionic strengths appear to follow a scaling exponent of −0.25 for $h = 4p$ to $8p$, as has been observed in prior experiments.[9,12] As seen in Figure 3b, the exponent varies depending on the data range. Thus, the differences between these prior reports may be attributed to the limited data range. Interestingly, the transition to Odijk regime is not observed for I = 0.18 mM. At this low ionic strength, $p/w \approx 1$, and the DNA conformation more closely follow that of a flexible self-avoiding walk than that of a semiflexible polymer.

By accounting for strong electrostatic effects at low ionic strength, we aim to derive a theory to explain all measured DNA sizes confined in a nanoslit. First, we evaluated eq 1 by combining all the data into a single graph. Unfortunately, we cannot find any region to be well explained by eq 1 (see SI, Figure S2). Therefore, we rewrite eq 7 as a function of the height (h), the persistence length (p), and the effective width (w).

$$R_{\parallel} \cong f(h, w, p) = L_{\parallel}^{3/4} p_{\parallel}^{1/4}\left(1 + \left(\frac{1}{2} + \frac{\pi}{4}\right)\frac{w}{p_{\parallel}}\right)^{1/2} \quad (7)$$

$$= L^{3/4}(1 - \alpha(h_{\mathrm{eff}}/p)^{2/3})^{3/4} \times p_{\parallel}^{1/4}\left(1 + \left(\frac{1}{2} + \frac{\pi}{4}\right)\frac{w}{p_{\parallel}}\right)^{1/2} \quad (8)$$

where $p_{\parallel} = p\ (2 - \exp[-0.8(p/h_{\mathrm{eff}} - 0.2)])$ and $h_{\mathrm{eff}} = h - w$. In this analysis, we additionally considered the effective height (h_{eff}), explaining the swollen DNA at ultralow ionic strength. As had been recently reported,[15] the dependence for $R_{\parallel}$ can be collapsed by plotting with h_{eff} (see SI: For comparison, Figures 2 (S5) and 3 (S6) are shown with h_{eff} and Figure 4a (S7) with h). Figure 4a shows that eq 8 successfully captures the dependence on h and I, except for weakly confined DNA. The

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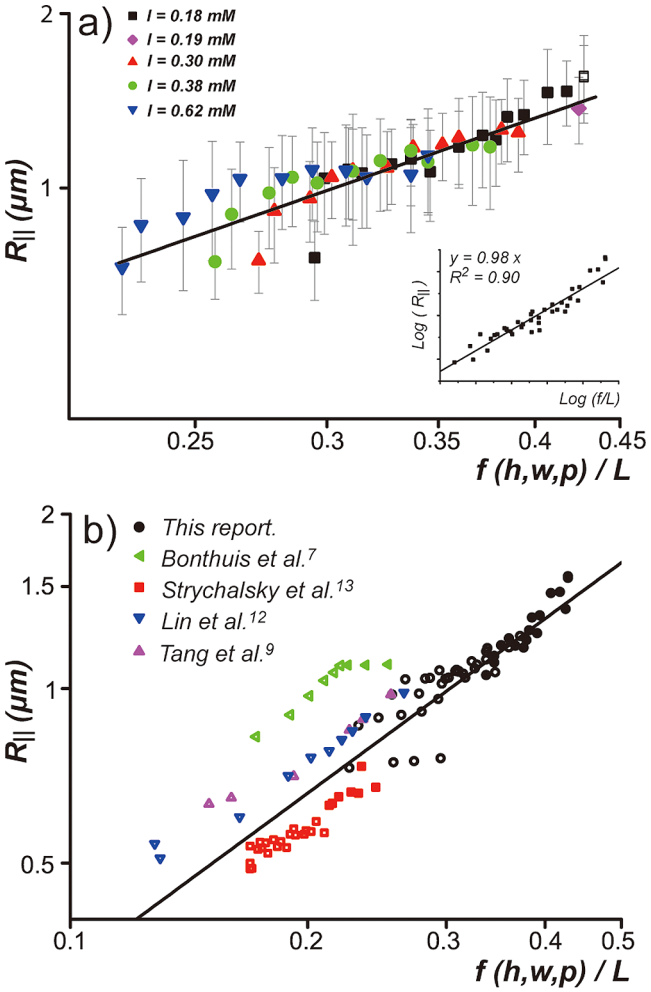

Figure 4. (a) DNA size ($R_{\parallel}$) as a function of height (h), effective width (w), and persistence length (p) in eq 8. The line represents $R_{\parallel} \cong f(h,w,p)/L$. (b) Interstudy comparison. The size variation of DNA molecules in nanoslit confinement are compared with other reports. Closed symbols represent Odijk regime ($h_{\mathrm{eff}} = h - w < 2p$). Data from other studies were manually obtained from plots as published.

linear regression analysis shows the slope of 0.98 with the *r*-square of 0.90.

The current results are also compared with previous studies, as shown in Figure 4b.[7,9,12,13] Overall, there is significant consistency from multiple data sets, although these data sets do not agree perfectly with one another. This discrepancy may be attributed by a possibility that each measurement utilizes a little bit different approach to obtain DNA sizes. On the other hand, it makes more sense if this analysis is limited to only strong confinement regime because Odijk assumed that polymers had deflection dominant conformations.[30] Although it is hard to define the onset boundary for Odijk's regime, it should be valid at least when the effective height is less than a Kuhn segment ($h_{\mathrm{eff}} < 2p$). Therefore, the data in that regime are denoted as filled symbols in Figure 4b.

In conclusion, this work provides a plausible clue long-standing controversy of the scaling dependence of DNA size confined in nanoslits. We performed experiments with various low ionic strength conditions, which increase the repulsion between DNA−DNA and DNA−nanoslit. From these measurements, we are able to show that a scaling relation (eq 8) explains the DNA conformation as a function of the slit height (h), the persistence length (p), and the effective diameter (w) of DNA.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, effective diameter calculation, corresponding graphs with p_{OSF}, and graphs with h_{eff}. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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