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# Stretching tethered DNA chains in shear flow

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**Abstract.** – We discuss the stretching of tethered chains subject to a shear flow. Fluorescence microscopy measurements of the extension of sheared tethered DNA, "model polymers", of various lengths are presented. The tethered chains approach full extension as the flow rate is increased, but very slowly. We show that a general theory to understand the large deformation of any sheared tethered chain must include both the correct nonlinear force-extension relation and chain fluctuations transverse to the flow direction, which themselves depend on the nonlinear elasticity of the chain. Direct comparison of derived scaling laws to simulations and experiments support our theory.

*Introduction.* – Polymer molecules can be deformed and stretched when subject to a hydrodynamic flow. Recently, many groups have used double-stranded DNA molecules as a model system to directly observe polymer dynamics in flow. These single-molecule studies provide detailed information about chain configurations and have revealed a host of interesting phenomena including "molecular individualism" [1], shear-enhanced extension fluctuations [2] and chain tumbling [3]. The deformation of complex molecules tethered to surfaces is of particular interest because they occur in many practical applications ranging from colloidal stabilization [4] and lubrication [5], to biological systems where molecules can protrude from lipid bilayer membranes [6]. The deformation of a single tethered chain has been modeled by Brochard and coworkers [7] using blob models. At large shear rates the model considers that a molecule adopts a conformation containing a straight portion terminated by a blob, termed the Stem and Flower model. The nonlinear elasticity of the molecule is not explicitly accounted for in these calculations. Recent DNA experiments [2] show a much slower approach to full chain extension than predicted by the Stem and Flower model, and complex molecular dynamics.

The dynamics of sheared polymers is nontrivial due to both a rotational and an extensional component to the flow. For free chains this leads to a tumbling motion [3]. Tethering the chain to a solid surface frustrates end-over-end tumbling and instead gives rise to cyclic dynamics [2]. Computer simulations suggest that the cyclic dynamics are driven by the tethering constraint and segment fluctuations perpendicular to the tethering surface which allow the chain to explore the linearly varying flow [2, 8]. These qualitatively different dynamics lead to very different steady-state mean extensions of the chain in the flow direction. These differences are most pronounced at large shear rates where a free chain approaches a mean extension

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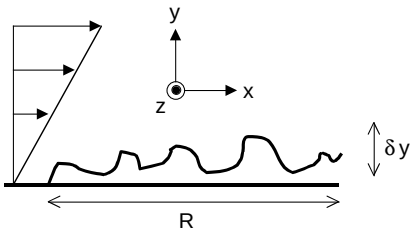


Fig. 1

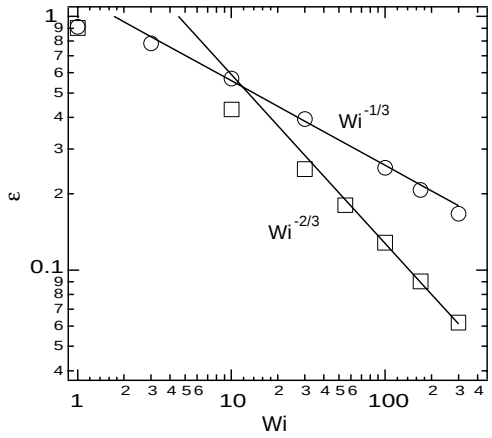


Fig. 2

Fig. 1 – Representation of a tethered chain.

Fig. 2 – Comparison of predicted scaling laws to Brownian dynamics simulations of a WLC (circles) and FJC (squares). The simulations are for a chains with 357 Kuhn steps.

of approximately 50% its contour length [3], while a tethered chain approaches complete extension [2], though very slowly.

Two recent works have shed more light on the dynamics of highly stretched chains. Hatfield and Quake [9] have shown that tension dominates the fundamental relaxation time, $\tau$, of a highly extended polymer. Furthermore, there is a symmetry breaking of $\tau$ for large extensions which leads to different transverse and longitudinal $\tau$. Brochard *et al.* [10] have arrived at similar conclusions for taut DNA chains.

In this letter we focus on highly stretched tethered chains in shear flow. First, scaling laws are derived for two different polymer models —the worm-like chain and freely jointed chain. Next, using the experimental apparatus developed in [2], we perform further fluorescent microscopy experiments for DNA chains comprising three different contour lengths. A comparison of our experimental data to the derived scalings and existing theories is performed.

*Scaling laws.* – Here we derive scalings for the approach to full extension of a sheared tethered polymer. We consider two polymer models, namely the worm-like chain (WLC) and freely jointed chain (FJC). Single-molecule experiments have shown that the force-extension curve of a double stranded DNA can be described by the WLC [11], while flexible polymers such as poly(methylacrylic acid) have been described by the FJC [12].

Consider a sheared tethered chain as shown in fig. 1. The hydrodynamic force on a section of the chain increases linearly with distance from the tethering surface, $y$. We have assumed that a strongly stretched chain is free-draining. The segment density of the chain in the $y$ direction will thus determine the hydrodynamic force exerted on the chain in the $x$ direction. For a stretched chain the breadth in the $y$ direction is determined by the transverse fluctuations $\delta y$. The solid wall confines the chain to positive $y$ and acts as a reflecting boundary.

In this section we derive a relation between the transverse fluctuations of a stretched chain and extension, use this to compute the hydrodynamic force exerted on the chain for a given extension and balance this with the elastic restoring force (spring force) of the chain to derive a scaling for the approach to full extension of a sheared tethered chain. These scalings are based on a simple monoblock representation of the chain and ignore variable tension along the chain backbone.

We first consider the worm-like chain model. By simple geometric arguments Hatfield and Quake [9] have shown that the transverse spring constant, $k_\perp$, of a stretched chain is

$$k_\perp = F(R)/R\,, \tag{1}$$

where $F(R)$ is the force required to hold the ends of a chain at distance $R$. The transverse fluctuations of the chain are driven by thermal motion:

$$\frac{1}{2}k_\perp \delta y^2 = k_\mathrm{B}T\,, \tag{2}$$

where $k_\mathrm{B}$ is the Boltzmann constant and $T$ is the temperature. Combining eqs. (1) and (2) leads to a relation between extension and fluctuations:

$$\delta y = (2k_\mathrm{B}T\,R/F)^{1/2}\,. \tag{3}$$

Equation (3) agrees with the calculations of Marko and Siggia [11] for a continuous worm-like chain in the limit of large $F$. The extension of the chain in the flow direction is determined by a balance of the spring force and the hydrodynamic force:

$$F = \zeta\dot{\gamma}\,\delta y\,, \tag{4}$$

where $\zeta$ is the drag coefficient. The neglect of hydrodynamic interactions and assumption of a constant drag coefficient are discussed in detail in the *Results and discussion* section. Marko and Siggia [11] numerically solved for the exact spring force of a worm-like chain and derived the following analytic interpolation formula:

$$Fb/k_\mathrm{B}T = 2\left[\frac{1}{4}(1-R/L)^{-2} - \frac{1}{4} + R/L\right]\,, \tag{5}$$

where $b$ is the Kuhn length. A higher-order interpolation formula exists [13] which leads to a small correction to eq. (5), but is not necessary to include at this level of model development and is negligible at large extensions. As we are interested in highly stretched chains we next define $\epsilon = 1 - R/L$. Taking the limit as $\epsilon \to 0$ the spring force is to leading order in $\epsilon$

$$F \sim \epsilon^{-2} \tag{6}$$

and the transverse fluctuations scale as

$$\delta y \sim \epsilon. \tag{7}$$

Combining eqs. (4), (6) and (7) leads to a scaling for the extension of a WLC:

$$\epsilon \sim \dot{\gamma}^{-1/3}. \tag{8}$$

Now we consider the freely jointed chain. To leading order in $\epsilon$ the force law is

$$F \sim \epsilon^{-1}. \tag{9}$$

Following the same derivation as for the WLC leads to transverse fluctuations of an extended FJC chain scaling as

$$\delta y \sim \epsilon^{1/2}. \tag{10}$$

Applying the force balance, eq. (4), to the chain and keeping the leading order term in $\epsilon$ gives

$$\epsilon \sim \dot{\gamma}^{-2/3}. \tag{11}$$

Note that the FJC is more easily extended than a WLC due to both the weaker divergence of the spring force law and also due to the larger transverse fluctuations for a given extension.

To confirm the scalings in eqs. (8) and (11) we have performed Brownian dynamics simulations of the FJC and WLC models. Details of the simulations are presented elsewhere [2,8]. The dimensionless shear flow strength is defined by $Wi = \dot{\gamma}\tau$ which is the ratio of the characteristic flow deformation rate $\dot{\gamma}$, where $\dot{\gamma}$ is the shear rate, to the polymer's slowest fundamental relaxation rate, $1/\tau$. Comparison between the simulations and the predicted scaling laws is shown in fig. 2 for chains with 357 Kuhn steps. This corresponds to DNA of length 37.8 $\mu$m, or a $2\lambda$ molecule. Good agreement with the proposed scaling laws are found. Further simulations of various chains lengths also follow the scaling laws [8].

Recently, Brochard-Wyart and coworkers [7] have developed the Stem and Flower model for highly stretched tethered chains. This model considers a stretched chain as a rod terminated by a single blob or flower. The Stem and Flower model predicts that the extension of a sheared tethered chain scales as

$$\epsilon \sim \dot{\gamma}^{-1}. \tag{12}$$

Note that the nonlinear entropic force of the chain is not considered in this model.

*Experimental materials and methods.* – $\lambda$-phage DNA chains (Pharmacia Biotech) were hybridized and ligated with T4 DNA ligase (Biolabs) to form lengths of 48 kbp, 96 kbp and 144 kbp. We will refer to these molecules as $\lambda$, $2\lambda$ and $3\lambda$. A biotynlated oligonucleotide complimentary to the 12 base pair overhang of the $\lambda$-phage DNA (Genset) was then ligated to the end of the molecules. The DNA were fluorescently labeled with YOYO-1 (Molecular Probes) at a ratio of one dye molecule per eight base pairs. At this staining ratio the molecules have contour lengths, $L$, of 18.9 $\mu$m, 37.8 $\mu$m and 56.7 $\mu$m ([1]). A polydimethylsiloxane elastomer flow channel of 100 $\mu$m in width, 200 $\mu$m in height and 2.5 cm in length was prepared on a microscope coverslip coated with streptavidin. DNA molecules were attached by one end to the bottom of the microchannel and submitted to shear flow created by a syringe pump (Kd Scientific). The flow cell was placed in a Zeiss Axiovert 135 TV inverted microscope equipped with a $\times$100 1.4 numerical aperture oil immersion objective and fluorescence capabilities. Stained DNA were excited with the 488 nm line of an argon-ion laser (Coherent) and visualized using a cooled intensified CCD camera (LHESA, Les Ulis, France). The experiments were performed at 24 °C in *p*H 8.3 TBE buffer. 4% $\beta$-mercaptoethanol, glucose oxidase, catalase and glucose were added to the solution to minimize photocision of the molecules. The images were transferred to a computer and the extension, $R$, of the molecules was determined using custom programmed software in NIH Image. Further experimental details can be found in [2].

The DNA's fundamental relaxation time $\tau$ was obtained by stretching the chains at a shear rate of 57 $s^{-1}$, stopping the flow and measuring the relaxation of the chain back to its equilibrium coiled configuration. For each contour length ensemble averages were taken over 15 chains. After the chains have relaxed to $\frac{1}{3}L$ the relaxation is dominated by the slowest mode and the plot of $R^2(t)$ is well described by a single exponential, $R^2 = c_1 \exp[-t/\tau] + c_2$, where $c_1$, $\tau$ and $c_2$ are free parameters. A representative fit to the experimental data is shown in fig. 3a). We determined that $\tau_\lambda = 0.45$ s, $\tau_{2\lambda} = 1.45$ s and $\tau_{3\lambda} = 2.56$ s.

([1])The intercalation of the dye molecules increases the DNA contour length from the unstained value of 16.3 $\mu$m for $\lambda$-phage DNA.

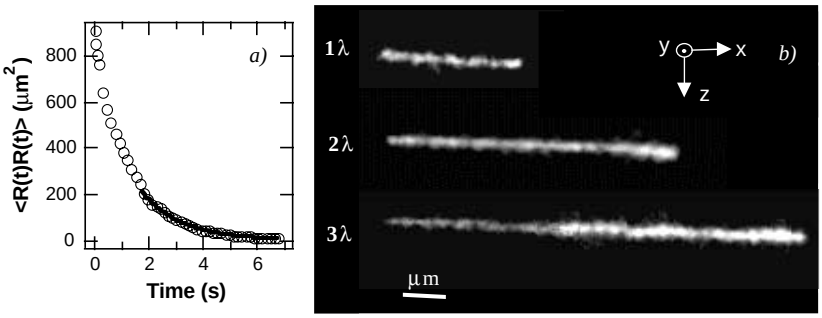


Fig. 3 – a) Relaxation of a $2\lambda$ molecule after flow is stopped. The symbols are experimental data and the line is the result from a fit to a single exponential. b) Sample DNA configurations at $Wi = 55$. The flow is in the positive $x$ direction and the images are in the $x$-$z$ (vorticity) plane.

*Results and discussion.* – In fig. 3b) we show sample chain configurations at $Wi = 55$. For all three chain lengths the deformation of the molecule is progressive with fluorescence intensity (or segment density) increasing to a maximum at the free end of the chain. The longest molecule ($3\lambda$) has approximately 535 Kuhn steps (assuming a Kuhn length of 0.106 $\mu$m) and is more representative of a highly flexible polymer. Sample chain configurations of the $3\lambda$ molecule are shown in fig. 4 for varying $Wi$.

A quantitative measure of the chain deformation is the mean fractional chain extension in the flow direction, $\langle R \rangle / L$, shown in fig. 5. To obtain each data point we took averages over 10 separate molecules and time averaged over 40 seconds for each molecule. The data points for all lengths roughly collapse onto a single curve. The collapse of the data at small $Wi$ is not surprising since linear polymer rheology predicts that a chain will begin to stretch when the flow deformation rate ($\dot{\gamma}$) is larger than the slowest response rate of the chain ($1/\tau$), resulting in the appropriate dimensionless flow strength defined by $Wi$. More significant, though, is that all the chains show a progressive and rapid increase in $R$ up to $Wi \approx 20$. At larger $Wi$ the chains continue to stretch and approach full extension, but very slowly.

In fig. 6 we have plotted $\epsilon$ *vs.* $Wi$ in a log-log plot. When the data is displayed in this manner it is clear that the average extension of the chains is approaching $L$, though very slowly. In fig. 6 we also have plotted the scaling laws for the WLC, FJC and the Stem and Flower model. The experimental data follow very closely the scaling derived for the WLC,

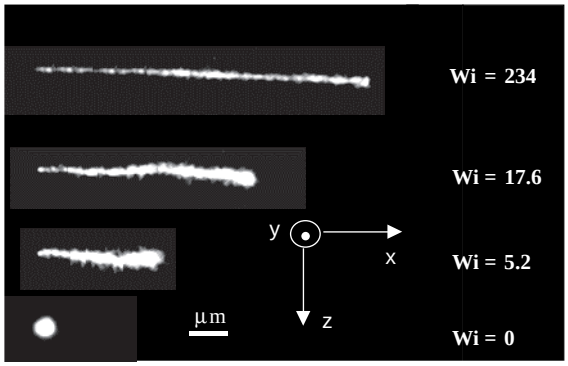


Fig. 4 – Sample chain configurations of a $3\lambda$ DNA at various $Wi$.

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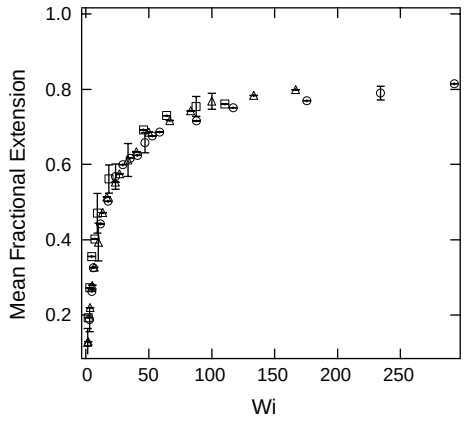


Fig. 5

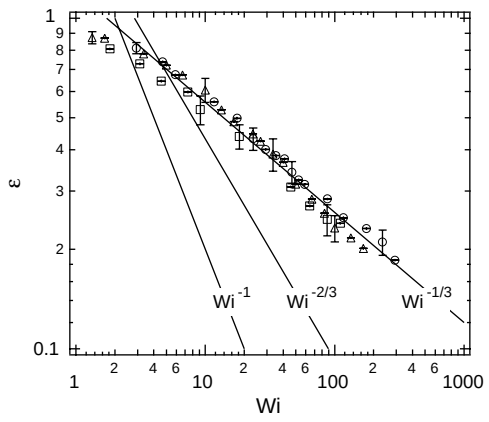


Fig. 6

Fig. 5 – Mean fractional chain extension *vs.* $Wi$: $\lambda$ (squares), $2\lambda$ (triangles), and $3\lambda$ (circles).

Fig. 6 – Comparison of experimental data to the predicted scaling laws for the WLC, FJC and Stem and Flower Model. The symbols are the same as in fig. 5.

$\epsilon \sim Wi^{-1/3}$. We see progressively better agreement with the WLC scaling as the DNA length increases. This trend is expected since polymer scaling models are approximations for large chains (*i.e.* comprising many Kuhn lengths).

Such a comparison to the proposed scalings is sensitive to the value taken for the contour length, $L$, of the DNA molecule. Chu and coworkers [1,3,14] have routinely reported a value of $L = 21$–$22$ $\mu$m for a $\lambda$-phage DNA at a staining ratio of one YOYO per four base pairs. In our analysis we have assumed that there is a linear relation between the contour length increase of the DNA molecule and the staining ratio. Furthermore, we used the average of the values reported by Chu and coworkers to compute $L$ for our staining ratios. The error bars in fig. 6 represent deviations for using values of 21 or 22 $\mu$m to determine $L$ at our staining ratios. Even accounting for the uncertainty in $L$, it is clear that the data more closely follow the WLC scaling than that for the FJC or Stem and Flower model.

In the derivation of our scalings we have neglected the effect of hydrodynamic interactions, both between segments of the chain and with the surface, and assumed a constant drag coefficient. Calculations by Larson *et al.* [15] and by Hatfield and Quake [9] have shown that for DNA in free solution (far from surfaces) the inclusion of hydrodynamic interactions between chain segments for these lengths of DNA molecules only slightly modifies the drag coefficient (by $\approx 20\%$ for $\lambda$ DNA [9]). Furthermore, the work of Hatfield and Quake [9] has demonstrated that the relaxation times of highly extended chains is dominated by the tension in the chain, *i.e.* the nonlinear force law of the molecule, and not by hydrodynamic interactions. Thus it is appropriate that we have concentrated on developing scalings which correctly handle the nonlinear elasticity of the chain, rather than concentrate on hydrodynamics at this level of modeling. Additionally, the present study is different from the previously mentioned ones in that the chains are near a solid interface. Theoretically, it is expected that the hydrodynamic interactions will be screened when a chain is placed near a solid interface [16]. The screening of hydrodynamic interactions for DNA chains in thin slits has recently been shown by Bakajin *et al.* [17].

*Concluding remarks.* – In this paper we have discussed the deformation of tethered chains in shear flow. The high shear rate deformation can be understood by a scaling model which

considers the interplay of the transverse fluctuations of an extended chain and the nonlinear elastic force law. The transverse fluctuations themselves depend on the nonlinear elasticity of the chain and are damped at large extensions. Thus as the shear rate increases, a stretched chain adopts an increasingly thinner transverse dimension and is confined to a region closer to the surface, where the flow velocity is smaller. This effect, combined with the nonlinear elasticity of the chain, is what gives rise to the slow approach to full extension for sheared tethered chains.

A scaling of $\epsilon \sim Wi^{-1/3}$ is predicted for the WLC and $\epsilon \sim Wi^{-2/3}$ for the FJC. Our experiments for 3 different contour lengths of DNA follow closely the predicted WLC scaling and support our theories.

The development of our scaling theory is not specific to DNA and can be generalized for any tethered chain. These concepts are not only relevant to understand chain deformation, but also eventual chain pull-off. Additionally, the boundary conditions could be altered to consider polymers or large proteins which are tethered to deformable fluid membranes [6].

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