New Directions in Single Polymer Dynamics

Amanda B. Marciel,1 Charles M. Schroeder1,2,3

1Department of Biophysics and Computational Biology, University of Illinois at Urbana-Champaign, Illinois 61801
2Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign, Illinois 61801
3Department of Materials Science and Engineering, University of Illinois at Urbana-Champaign, Illinois 61801
Correspondence to: C. M. Schroeder (E-mail: cms@illinois.edu)

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ABSTRACT: Single polymer techniques are a powerful set of molecular-level tools that enable the direct observation of polymer chain dynamics under highly non-equilibrium conditions. In this way, single polymer methods have been used to uncover fundamentally new information regarding the static and dynamic properties of polymeric materials. However, to achieve the full potential of these new methods, single polymer techniques must be further advanced to enable the study of polymers with complex architectures, heterogeneous chemistries, flexible backbones, and intermolecular interactions in entangled solutions, which reaches far beyond the current state-of-the-art. In this article, we explore recent developments in the area of single polymer physics, including single molecule force spectroscopy and fluorescence microscopy, and we further highlight exciting new directions in the field.

INTRODUCTION Long chain macromolecules play an indispensable role in modern society. Synthetic polymers are used in a wide array of industrial applications, whereas natural polymers facilitate fundamental life processes. The molecular properties of polymer chains ultimately determine the emergent bulk properties of polymeric materials. In particular, the intrinsic chemical and physical properties of polymers, in conjunction with processing conditions, give rise to polymeric materials with desired function. For many years, bulk characterization techniques such as rheometry and light scattering have been used to infer polymer orientation, conformation, and microstructure at equilibrium and during non-equilibrium processing. Recently, single molecule techniques have enabled the direct observation of polymer chains in far-from-equilibrium conditions, thereby providing a window into the molecular structure and behavior of single polymers. In this way, single polymer studies have the potential to reveal fundamentally new information regarding the processing properties and static and dynamic morphology of polymeric materials. However, to achieve the full potential and promise of single molecule techniques, these new methods must be brought to bear on polymers with complex architectures and heterogeneous chemistries, reaching far beyond the current state-of-the-art. In this article, we explore recent advances in single polymer methods and highlight promising new directions in the field.

How can single molecule studies contribute to the field of polymer science? Ultimately, single polymer techniques can elucidate the relationship between molecular scale properties and bulk-level macroscopic material response. Improved understanding of the link between the molecular scale and macroscopic properties will allow for the design and development of advanced polymeric materials with desired function. In the realm of synthetic polymers, advances in synthetic organic polymerization have led to fine scale control over chain length and monomer arrangement, thereby enabling the creation of well-defined architectures with tunable self-assembly behavior.1–4 Development of new synthesis techniques has spurred the use of synthetic organic polymers in a vast array of applications, most notably in the fields of microelectronics,5–7 energy7,8 and biotechnology.9–11 However, expensive and rapid production of synthetic organic polymers with determinate bulk properties remains a significant challenge for several reasons. First, the structural heterogeneity of the latent polymer microstructure can give rise to variable properties. In addition, industrial processing conditions can affect polymer morphology, which makes bulk materials properties difficult to predict from primary sequence alone. From this perspective, single molecule techniques can provide an improved understanding of dynamic polymer conformation and microstructure, which will provide insight into bulk materials properties and influence the design and implementation of new processes for polymer engineering.

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During processing, polymer chains often exhibit complex behavior when exposed to fluid flows and large deformation strains. Fluid flows including extensional, shear, and linear mixed flows can stretch and orient polymer chains, thereby resulting in non-Newtonian macroscopic behavior such as flow-dependent viscosity and turbulent-drag reduction.\textsuperscript{12} Classically, birefringence,\textsuperscript{13,14} and light scattering\textsuperscript{15,16} techniques were employed to infer information on chain orientation and stretching behavior in strong flows. However, results from these bulk-level studies provide an indirect view of polymer microstructure based on ensemble averages of polydisperse systems. In recent years, single molecule techniques (e.g., fluorescence microscopy, optical tweezers, magnetic tweezers) have enabled the direct observation and manipulation of polymer microstructure.\textsuperscript{17,18} A true renaissance of polymer physics began when single molecules of DNA were stained with fluorescent dye, thereby enabling observation of real-time chain dynamics directly via fluorescent videomicroscopy.\textsuperscript{17,19}

Double stranded DNA (DNA) is ideally suited for single polymer studies. Monodisperse samples of short and long-chain DNA sequences are routinely prepared via polymerase chain reaction or extracted from genomic sources. Furthermore, DNA is readily soluble in aqueous solutions, and natural DNA is amenable to non-natural chemical modifications. In addition, the molecular properties of semi-flexible DNA facilitate single molecule visualization,\textsuperscript{20} and the monodispersity of genomic or phage DNA highly simplifies analysis.\textsuperscript{21} For many years, bacteriophage lambda DNA (\(\lambda\)-DNA) has served as the most widely used DNA sample for single molecule studies due to its commercial availability and long contour length (16.3 \(\mu\)m) that is readily visualized using optical microscopy.\textsuperscript{22} Pioneering single polymer experiments relied on \(\lambda\)-DNA tethered to microspheres manipulated via optical tweezers. These experiments investigated entanglement dynamics,\textsuperscript{23} relaxation,\textsuperscript{24} elasticity,\textsuperscript{25} and scaling of diffusion coefficients.\textsuperscript{26} Flow-based experiments have explored non-equilibrium chain dynamics in planar extensional flow and shear flow.\textsuperscript{27} Fluid flows can stretch flexible polymers into highly deformed states far from equilibrium, which has allowed for direct observation of the coil-stretch transition\textsuperscript{28,29} conformational hysteresis,\textsuperscript{30} and shear thinning properties.\textsuperscript{31} These early experiments were instrumental in establishing single molecule studies as the premier approach to test long standing theories and to elucidate new phenomena in polymer physics.

For over 20 years, DNA has retained its status as the model polymer for single molecule studies. However, DNA is a semi-flexible polymer with a relatively large persistence length \(l_p \approx 53\) nm, approximately two orders of magnitude larger than most synthetic polymers (e.g., polyethylene \(l_p \approx 0.57\) nm).\textsuperscript{21} In addition, natural DNA is a linear polymer, whereas most industrially relevant polymers have exceedingly complex architectures, including side-chain branches or comb shapes. Therefore, new model polymer systems that are truly flexible and can accommodate complex branching or heterogeneous chemistries are critically required for bulk and molecular-level studies. In this way, researchers will be able to fully capitalize on single molecule methods to explore the dynamics of industrially relevant polymers.

How pervasive are single polymer studies of chain dynamics in the field today? Strikingly, research in single polymer dynamics has not extended far beyond linear chains of DNA in dilute solution (Fig. 1). There is a dearth of single molecule studies of synthetic polymers, and exceedingly few on polymer chains with complex architectures. Recently, our group extended the field of single polymer dynamics to truly flexible polymers. We developed a platform to synthesize designer sequences of flexible single stranded DNA (ssDNA) using rolling circle replication (RCR).\textsuperscript{32} Our synthesis platform enables facile incorporation of modified nucleotides for covalent attachment of dyes for single molecule visualization via fluorescence videomicroscopy.\textsuperscript{32} We anticipate that our new model system will enable access to non-Newtonian flow properties that were previously obscured by the semi-flexible nature of DNA.
In this article, we highlight current trends in single molecule polymer studies with a particular focus on the molecular properties (semi-flexible versus flexible) and the measured physical properties (static vs. dynamic). Overall, the main goal of single molecule studies is to elucidate fundamental polymer behavior at the microscale to improve our understanding of polymer behavior at the macroscale.

RECENT WORK ON SEMI-FLEXIBLE DNA: NANOCONFINEMENT

Over the last two decades, the static and dynamic properties of semi-flexible polymers (e.g., double stranded DNA) in dilute solutions have been extensively investigated. Early experiments focused on tethered and free chain dynamics in fluid flow. More recently, single polymer experiments have focused on polymer dynamics in confined geometries or upon contact with microscale or nanoscale obstacles. Advances in microfabrication have enabled production of devices with nanometer-sized channels and microscale to nanoscale post diameters. As we will see, these nanoscale geometries result in intriguing dynamic behavior of semi-flexible polymers.

Electrokinetic Flows

Preliminary studies on the dynamic behavior of individual semi-flexible polymers focused on the electrophoretic movement of λ-DNA through thin agarose gels. Electrophoretic migration in agarose gels was shown to induce a U-shaped conformation in λ-DNA via a unique hooking and unhooking mechanism. Volkmut and Austin employed a microfluidic platform comprised of microfabricated post arrays to demonstrate that migration was inversely proportional to polymer length. Polymer migration behavior in gels was attributed to the disentanglement time of hooked DNA chains from microscopic obstacles. To gain an improved understanding of the precise dynamics of polymer collisions, synthetic mimics of gel matrices in the form of single posts and post arrays were fabricated to precisely control polymer separation based on molecular weights.

Obstacle Arrays

In 2002, Doyle et al. formulated a readily tunable self-assembling magnetic post array microfluidic platform capable of separating restriction digested and concatemer λ-DNA driven by an electric field. The hooking behavior of λ-DNA was directly visualized using single molecule videomicroscopy. Following a collision event, chains were observed to continually stretch for a fixed time, which retarded the mobility of larger chains. The mechanism of collision-induced mobility changes is the main driving force for size-based separation of DNA fragments. In subsequent studies, Minc et al. investigated the length dependence of mobility and dispersion by manipulating the size and density of the magnetic post arrays and inferred that mobility scales linearly and dispersion scales inversely with electric field strength. More recently, Park et al. fabricated a nanofence array with a large spanning gap; however, this design facilitated efficient separation but produced poor dispersion. Olson and Dorfman explored the effect of array order on mobility and dispersion and concluded that dispersion decreased with array disorder; whereas mobility remained unaffected over a wide range of electric fields. To advance the use of post arrays as efficient electrophoretic separation devices, a quantitative understanding of the mechanism of polymer-obstacle
collision behavior is imperative. Therefore, many groups have studied polymer collisions using single obstacles.

**Single Obstacles**

When a single polymer chain impinges on an obstacle of finite size it can undergo a hooking and unhooking mechanism, or it can continue migrating in a roll-off type mechanism.\(^{43}\) The probability of a hooking event \(P_{\text{hook}}\) is depend-ent on three geometric parameters: the obstacle radius \(R_{\text{obs}}\) the polymer radius of gyration \(R_g\) and the offset distance \(b\) between the centers of mass of the polymer and obstacle. Randall and Doyle investigated single collisions of \(\lambda\)-DNA with an insulating poly(dimethylsiloxane) post and determined that \(P_{\text{hook}}\) could be classified on the basis of the dimensionless ratios of geometric parameters. Specifically, when \(R_{\text{obs}}/R_g > 1\), the hooking probability \(P_{\text{hook}}\) decreased with increasing field strength, and the roll-off mechanism was observed to be the dominant mechanism for polymer disengagement. However, when \(R_{\text{obs}}/R_g \sim 1\), \(P_{\text{hook}}\) increased with increasing electric field strength and increased with decreasing \(b/R_g\). In addition to geometric parameters, Randall and Doyle demonstrated that electric field gradients induced by obstacles cause polymer deformation analogous to hydrodynamic field gradients. Therefore, the hooking probability \(P_{\text{hook}}\) is also a strong function of the Deborah number \(D_e = 2\mu E / R_{\text{obs}}\). The Deborah number is a dimensionless group that describes the ratio of the polymer relaxation time \(\tau\) to the flow stretching time \(G^{-1}\), where \(G = 2\mu E / R_{\text{obs}}\) is the fluid strain rate, \(\mu\) is the electrophoretic mobility, and \(E\) is electric field strength.\(^{43,44}\) Based on direct observation of single DNA dynamics, \(P_{\text{hook}}\) was found to increase with increasing \(D_e\). Finally, if \(R_{\text{obs}}/R_g < 1\), then hooking dynamics can be described by four commonly observed polymer chain conformations: U, J, W, X.\(^{45}\) Interestingly, Randall and Doyle described a conformational dependence on unhooking time, which is an important parameter for molecular separations. More recently, Doyle and Dorfman formulated new models describing polymer-obstacle collisions based on single molecule data.\(^{46–50}\)

**Nanoconfinement**

Polymer chains in confined geometries have been observed to adopt similar conformations to polymer chains in strong extensional flows.\(^{51}\) Polymer confinement is typically described using three variables: channel dimension \(d\), polymer persistence length \(\xi\), and polymer radius of gyration \(R_g\). A polymer chain can be in weak \((d > R_g)\), moderate \((p \ll d < R_g)\), or strong \((d < p \ll d < R_g)\) confinement. In weak confinement, the polymer chain is free to adopt a random coil configuration. In moderate or de Gennes confinement, the polymer chain adopts a coil-size on the order of \(d\). Polymer extension in this regime is due to excluded volume effects and scales as \(d^{-2/3}\).\(^{51}\) Finally, in strong or Odijk confinement, the polymer chain is unable to assume a random coil configuration, and the polymer contour length deflects the channel boundaries on an average length scale \(\lambda \simeq (d^3\xi)^{1/3}\).\(^{52}\) The first experimental evidence of the dynamic behavior in confined geometries demonstrated that polymer extension following a collision event scaled inversely with \(d\) for molecules hooked on posts in U-type configurations.\(^{53}\) Bakajin et al.\(^{53}\) attributed this behavior to an increase in hydrodynamic drag force due to the confinement that induces free draining behavior. This investigation spurred several key studies on semi-flexible polymers in confinement to validate scaling laws previously derived by de Gennes and Odijk. Several excellent reviews have been recently published on confinement-induced dynamics of polymer chains, and we refer the reader to these for more detailed discussions.\(^{54,55}\) In the following section, we highlight a few major advancements in the field of polymer dynamics under strong confinement.

**Nanochannels**

Tube-like confinement of polymers in nanochannels has enabled dynamic studies of the diffusivity, relaxation, and mobility in two dimensions. These studies have made a substantial impact on applications in molecular detection and manipulation for biological and chemical analysis.\(^{56}\) Guo and Cheng employed nanoprinting technology to formulate shallow channels with heights as small as 120 nm and widths as small as 75 nm. In these studies, it was observed that DNA extension increased upon decreasing the channel diameter.\(^{56}\) Tegenfeldt et al.\(^{57}\) corroborated de Gennes scaling laws by observing the dynamics of \(\lambda\)-DNA in a 100 nm diameter channel. In a follow up study, Reisner et al. employed a wide range of nanochannel diameters (between 400 nm to 30 nm) and reported deviation from power-law scaling below a channel diameter of 35 nm. These authors attributed the deviation from power-law scaling to a transition into the Odijk regime, which occurred at a nanochannel diameter approximately equal to twice the polymer persistence length.\(^{58}\) For channel diameters greater than 35 nm, de Gennes scaling did not hold, and polymer extension was observed to scale as \(d^{-0.85}\).\(^{58}\) More recently, Tree et al. used Monte Carlo simulations to demonstrate that semi-flexible polymers exhibit a large plateau transition from the de Gennes to Odijk regime, which was attributed to the anisotropy of DNA monomers.\(^{59}\) In light of this work, it became clear that the vast majority of previous experiments aimed at validating scaling laws were mistakenly probing the transitional state (or extended de Gennes regime), which likely accounts for discrepancies between scaling parameters.\(^{59}\)

**Nanoslits**

Nanoslit devices are defined as having a single channel dimension \((d)\) in the nanometer range. These devices are the most widely used nanodevices to investigate polymer confinement due to their ease of fabrication. Balducci et al. studied the effects of molecular weight and channel height on DNA diffusion in slitlike nanochannels with depths ranging from 75 to 545 nm and a constant width of 150 nm.\(^{60}\) For small channel heights \((d < R_g)\), polymer diffusion coefficients were observed to scale inversely with molecular weight, which provided the first experimental evidence that hydrodynamic interactions are screened in channel heights only slightly smaller than the radius of gyration.\(^{60}\) These authors also observed that the transition from de Gennes to Odijk was smooth in slitlike confinement, which is in contrast to the transition in relaxation in tube-like nanochannels.\(^{59,60}\)
Balducci et al. and Hsieh et al. further explored polymer relaxation in nanoslits and reported the existence of two distinct relaxation regimes: in the first regime, relaxation time scaled with channel depth as $d^{-1/2}$ which signifies an increase in the size of the tension blobs along the chain. However, in the second regime, relaxation time showed a stronger dependence on channel depth, scaling as $d^{-0.92}$, which can be attributed to the relaxation of a two-dimensional self-avoiding chain (Fig. 2).

In a follow up experiment, Balducci et al. probed DNA extension in nanoslits, where they showed that polymer stretching was enhanced under confinement compared to bulk solution. In particular, smaller strain rates were required to achieve the same degree of polymer stretching under confinement. Moreover, the authors found that the time scale governing the stretching process is the (confinement-independent) longest relaxation time.

Recently, Dai et al. used Monte Carlo simulations to investigate chain extension in nanoconfinement and observed four distinct regimes: the de Gennes regime, an extended de Gennes regime, a self-crossing regime, and a non-self-crossing regime (deflection theory). Interestingly, these authors determined that the transition to the Odijk regime is quite broad and depends on the chain width, which is a function of ionic strength.

**Entropic Trapping**

A combination of channels with nanoslit and microslit dimensions has been used to study entropic recoil. In entropic recoil, a polymer is transported based on size due to a change in entropy and the concomitant drive to overcome the resulting free energy increase. A recent review by Dorfman highlights the underlying physics of entropic trapping. Here, we aim to only provide a brief qualitative overview of this process. The first observation of entropic trapping by Han and Craighead revealed that a device with alternating deep regions $d > R_g$ ($d = 1 \mu m$) and shallow regions $d < R_g$ ($d = 90 \text{ nm}$) preferentially increased the mobility of high molecular weight DNA in electric field gradients 20–60 V/cm. Turner et al. later explored the behavior of DNA in two regions with differing entropies, including regions with a dense array of pillars and no pillars. DNA was positioned at the boundary of the pillar array, and a series of electric field pulses was used to drive the DNA into the entropically unfavorable pillar region. Polymer recoil into the pillar-free zone was monitored. During this process, it was observed that polymer extension showed a 35% extension in length for a device with a 60-nm channel depth containing 35-nm diameter pillars. The recoil phenomenon was then used to separate long DNA molecules using three steps: gather, drive, and recoil.

In 2006, Mannion et al. took a more sophisticated approach to understand the conformational dependence on polymer escape from nanoslits. They directly observed stretching, relaxing, and recoiling for linear and folded strands. In a related study, Levy et al. inserted previously folded DNA and observed larger polymer extensions as a result of excluded volume effects. Recently, it was shown that a plug of oil butted against a device wall may act as an entropic trap. Based on this study, the mechanism was envisioned as a three-step process: pre-stretching, pseudo-tethering by the trap, and rapid chain extension by an intensified field within the film.

**RECENT WORK ON SINGLE FLEXIBLE POLYMERS**

In the previous sections, we explored recent single molecule studies on the dynamics of DNA under nanoconfinement. These studies highlight the amenability of DNA to single polymer studies, which is largely due to the intrinsic molecular properties of DNA (e.g., large persistence length, long contour lengths on the order of microns, and relaxation times in the range of seconds). However, DNA is a semi-flexible polymer with vastly different local molecular properties...
compared to synthetic organic polymers, most of which are truly flexible chains.\textsuperscript{21} The static properties of flexible polymers such as entropic elasticity and force-induced conformational transitions have been measured using single molecule force spectroscopy (SM-force spectroscopy).\textsuperscript{72-74} Atomic force microscopy (AFM) and magnetic tweezers are the most commonly used force spectroscopy techniques for investigating the static properties of flexible polymers. These methods typically involve tethering a single polymer molecule to a surface at one terminus and either specifically or nonspecifically tethering to a bead or cantilever at the opposite terminus. Manipulation of one or both beads results in polymer deformation and stretching; in this way, a force-extension curve can be generated.\textsuperscript{72}

Although SM-force spectroscopy can be used to measure the static properties of polymer chains, single molecule fluorescence microscopy (SM-fluorescence microscopy) can be used to determine the dynamic properties of polymers in non-equilibrium conditions. However, direct visualization of single flexible polymers has been a longstanding challenge due to difficulties in sample preparation and single polymer imaging. In this section, we highlight recent work that aims to measure the static properties of flexible polymers. In addition, we describe recent advances in our group that aim to develop a new platform for single molecule studies of flexible polymers with complex architectures. Recently, our work has enabled the direct observation of flexible polymer dynamics in non-equilibrium fluid flows for the first time using SM-fluorescence microscopy.\textsuperscript{72}

**Single Molecule Force Spectroscopy**

SM-force spectroscopy has enabled measurements of the entropic and enthalpic chain elasticity for flexible polymers, including RNA, ssDNA, carbohydrates, and synthetic organic polymers. Biological polymers such as carbohydrates exhibit interesting force-extension behavior attributed to the underlying chain architecture.\textsuperscript{74,75} In some cases, the elasticity of flexible polymer chains has been described using the extensible freely joints chain model (FJC) or worm like chain model (WLC). However, both of these models neglect monomer–monomer interactions (e.g., excluded volume interactions or H-bonding), and more recent work (described below) provides a more accurate description of the elastic properties of flexible polymers.

**Carbohydrates and Synthetic Polymers**

Early AFM experiments revealed a plateau in the force-extension curve of dextran and amylose polysaccharides.\textsuperscript{76,77} The plateau signifies a force-induced structural transition attributed to a change from the low energy chair to the high energy boat conformation of the \(\alpha\)-d-glucopyranose rings.\textsuperscript{77,78} The elasticity was modeled using the extensible FJC with a Kuhn length of 6 Å.\textsuperscript{76,77} With the success of this work, many groups began to adopt AFM as the preferred tool to study elastic properties of synthetic polymers. Li et al.\textsuperscript{78,79} observed similar plateau behavior for poly(vinyl alcohol) (PVA). The extensible FJC model fits the experimental data well at low forces (entropy driven chain re-orientation) and high forces (enthalpy driven chain straightening), but not at intermediate forces, potentially indicating formation of polymeric suprastructure. In a subsequent study, Li et al. investigated poly(acrylic acid) (PAA) and modeled the extension with the extensible FJC using a Kuhn length of 6.4 Å.\textsuperscript{80} Interestingly, unlike previous studies of different polymers, the force-extension behavior of PAA could be effectively modeled using the extensible FJC model.\textsuperscript{80}

Oesterhelt et al.\textsuperscript{81} studied the effects of solvent on poly(ethylene glycol) (PEG) force-extension behavior. In water, PEG cannot be fit to the extensible FJC model, which may be due to intramolecular hydrogen bonding affecting net polymer length. This was further corroborated by repeating the experiments in hexadecane, where extension could be modeled with the extensible FJC.\textsuperscript{81} Poly(acrylamide) derivatives enabled side group and solvent choice to be probed simultaneously. Zhang et al. investigated the dimethyl, diethyl, and isopropyl derivatives of poly(acrylamide) (PAAM). The elasticity of PAAM and the isopropyl derivative could be both modeled by extensible FJC in 8 M urea. However, in water, the elasticity of PAAM could not be fit to the extensible FJC model in the intermediate force regime, which was attributed to a suprastructure via hydrogen bonding.\textsuperscript{82,83} In particular, bulky diethyl groups were observed to increase chain stiffness compared to the dimethyl groups. For both derivatives, the chain elasticity was observed to be modified significantly in 8 M urea, thereby indicating a large enthalpic elasticity via hydrogen bonding.\textsuperscript{83}

In addition to homopolymers, block copolymer architectures were also studied using SM-force spectroscopy. Early experiments by Bemis et al.\textsuperscript{84} varied relative block length of polystyrene-b-poly-2-vinylpyridine polymers. FJC and WLC models fit comparably well with persistence lengths \(\approx 3\) Å. Poly(methyl methacrylate)-polystyrene block copolymers displayed distinct force-extension behavior for each individual block. Individual PS blocks have a plateau in water, which is attributed to hydrophobic interactions.\textsuperscript{85} Individual PMMA blocks displayed a saw tooth-shaped force-extension curve in water, which is postulated to result from the unraveling of polymeric suprastructure. Stretching the PMMA-PA block copolymer resulted in distinct force extension regions for PS and PMMA.\textsuperscript{86}

**ssDNA**

The elasticity of individual ssDNA polymers has also been studied using SM-force spectroscopy. Smith et al. used optical tweezers to obtain the first ssDNA force-extension curve using denatured \(\lambda\)-DNA. In this work, polymer extension was described using the extensible FJC model with a persistence of 7.5 Å and contour length of 27 \(\mu\)m.\textsuperscript{87} It was postulated that a transition similar to dextran and amylose polysaccharides could account for the overstretcing transition.\textsuperscript{76,77,87} More recently, homopolymer single stranded poly(dA) was observed to exhibit two plateaus in the force-extension curve, the first (at low force) due to strong base stacking interactions, and the second (at higher force) corresponding to the deoxyribofuranose rings transitioning from C3’ endo to C2’
In light of these findings, it was appreciated that enthalpic effects and monomer–monomer interactions must be accounted for in ssDNA elasticity models. The FJC and WLC models have proven successful for modeling high-force SM-force spectroscopy data for ssDNA, wherein long-range monomer interactions are screened. However, at low forces, the elasticity for both the FJC and WLC models yields an ideal linear Hookean response, such that \( f \sim \frac{x}{L} \), where \( x \) is chain extension and \( L \) is contour length. Such over simplified models cannot be expected to provide a quantitatively accurate description for the elastic force-extension behavior for flexible chains such as ssDNA. Although the original FJC and WLC models have been recently extended to account for backbone bending rigidity at large extensions, these models fail to provide an accurate description of the elasticity for real polymer chains over the full range of polymer extension; indeed, long-range monomer interactions must be accounted for in modeling the elasticity behavior of these molecules.

For real polymers with dominate excluded volume interactions, the force-extension behavior is governed by four distinct scaling regimes (Fig. 3). Unlike linear Hookean chains, real polymers exhibit a (non-linear) power law force relation in the low force regime, with force scaling as \( f \sim \left(\frac{x}{L}\right)^{3/2} \). Saleh et al. experimentally validated the power law regime for the elasticity of flexible polymers using a 10.5 kb fragment of \( \lambda \)-ssDNA using a magnetic tweezer assay referred to as single molecule elasticity at low forces (SM-ELF). For a series of salt concentrations (ranging between 20 mM to 5 M), these authors observed a crossover force marking the transition from a power law regime to a logarithmic regime in polymer elasticity. The crossover force increased with increasing salt concentration. At a relatively high monovalent salt concentration of 3 M NaCl (likely resulting in \( \Theta \)-solvent conditions), the force-extension behavior of denatured ssDNA could be well described using the WLC model with a bare, non-electrostatic persistence length of 0.62 nm. McIntosh and Saleh further employed SM-ELF to probe the effect of multivalent salts on ssDNA elasticity.
ssDNA elasticity using KCl, MgCl$_2$, and CaCl$_2$, observing ion association as a function of force.$^{35,36}$ More recently, molecular dynamics simulations and quantitative scaling arguments have been used to elucidate the origin of the logarithmic regime. Stevens et al.$^{97}$ ascribed the logarithmic regime to the straightening of short length scale structure originating from backbone interactions with the condensed ions. Toan and Thirumalai$^{98}$ quantitatively determined that polyelectrolyte effects account for the logarithmic regime. Recent single molecule experiments have been able to validate two of the four regimes in the force-extension relation for flexible polymers.$^{94}$

Recently, a computational study by Radhakrishnan and Underhill$^{99}$ worked toward formulating a quantitative model to accurately describe all four regimes.

Although SM-force spectroscopy has revealed a wealth of static property data on semi-flexible and flexible polymers, these techniques cannot probe the dynamic behavior of polymers in industrially relevant flow fields. Therefore, there is a strong need for a flexible polymer system that can enable the direct visualization of single polymer dynamics in non-equilibrium conditions such as strong fluid flow.

**Single Molecule Fluorescence Microscopy of Flexible Polymers**

Recently, our group developed a new synthesis platform to produce ssDNA polymers with user-defined sequences covalently labeled with fluorescent dyes for direct visualization of polymer dynamics.$^{72}$ Specifically, we employed a biochemical synthesis technique called RCR to generate ssDNA polymers with moderate to low polydispersity. RCR is a template-based synthesis method, which allows the design and synthesis of tailored ssDNA sequences to largely preclude intramolecular interactions (e.g., base pairing or base stacking). Following ssDNA synthesis, we investigated chain length using pulsed field gel electrophoresis and verified the synthesis of ssDNA with an average contour length of approximately 50 μm. During biochemical synthesis, a primary amine modified nucleotide (aminally-dUTP, aa-dUTP) is incorporated as a reactive chemical moiety to covalently attach succinimidyl ester dyes in a second step via NHS conjugation chemistry, thereby enabling direct visualization of the polymer backbone. Through a series of control experiments, we showed that incorporation of aa-dUTP does not affect the synthesized contour lengths of ssDNA polymers using several different ratios of modified to natural nucleotide (1:0, 4:1, 3:2, 2:3, 1:4, and 0:1). Dye loadings were quantified using UV and visible wavelength absorption. Detailed synthesis and characterization experiments, in combination with SM-fluorescence microscopy, clearly showed that a dye loading ratio of ~1:100 dye:base facilitates visualization of ssDNA backbones with clear, crisp single molecule images, approximately equivalent to those obtained from SM-fluorescence imaging of DNA molecules labeled with intercalating dyes at 1:4 dye:base ratio (Fig. 4). In addition, we employed poly(dimethylsiloxane)-based microfluidic devices to directly visualize and stretch fluorescently-labeled ssDNA polymers in a planar extensional flow. Using this approach, we were able to quantify stretched polymer contour lengths, and we observed that a majority of the ssDNA polymer chains were stretched to extensions greater than 20 μm. For this range of contour lengths, ssDNA chains contain ~10$^4$ Kuhn steps (assuming a persistence length of ~1–2 nm for ssDNA), whereas DNA of a similar contour length only contains ~150 Kuhn steps.

**PERSPECTIVE**

Single molecule studies have proven essential in elucidating the fundamental behavior of semi-flexible and flexible
polymers, including static and dynamic materials properties. Until now, single polymer investigations have been limited to linear polymer architectures in mainly dilute polymer solutions (Fig. 1). To better understand polymer dynamics and non-equilibrium phenomena in industrially relevant processing conditions, single molecule studies must advance further to enable studies of entangled linear flexible polymers and polymers with complex architectures in entangled solutions. Recently, the static elastic properties of single PMMA-b-P4VP block copolymer brushes in semi-dilute environments have been studied using SM-force spectroscopy measurements. Moving beyond static measurements, single molecule studies must also be extended to SM-fluorescence microscopy to enable the study of flexible polymer dynamics. Direct visualization of the dynamic behavior of single polymers in concentrated solutions will require preferential labeling of the desired polymer with fluorescent dyes. Our recent work on ssDNA has moved the field in this direction, but more work remains to be done. To achieve these broader goals, our group is currently working to extend the biochemical synthesis scheme for ssDNA to synthesize branched polymers with fluorescently labeled backbones, which will enable simultaneous monitoring of backbone and side branch fluctuations in non-equilibrium fluid flows (Fig. 5). Aside from our work, Ube et al. has employed scanning near field microscopy to directly observe conformational relaxation of perylene-labeled PMMA chains in a PMMA entangled films. In addition to studying the dynamics of linear polymers in entangled solutions, there is a clear need to study polymers with complex architectures at the single molecule level. To this end, Shi et al. applied SM-force spectroscopy to explore the static properties of dendronized poly(p-phenylenes) with hydrophobic/hydrophilic or hydrophobic/hydrophilic dendrons. SM-force spectroscopy has also been used to determine persistence lengths of highly branched cylindrical PNIPAM brushes. Studies of the diffusion of branched DNA in dilute and concentrated solutions, as well as electrophoretic mobility, have revealed remarkable differences from the mobility of linear chains. Overall, single polymer techniques represent a powerful approach to probing the non-equilibrium dynamics of long-chain macromolecules, especially in entangled polymer solutions. Combined with bulk-level analysis, single polymer methods are poised to fundamentally transform our understanding of polymer physics for "real" polymer systems in the near future.

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REFERENCES AND NOTES