Highly Stretched Single Polymers: Atomic-Force-Microscope Experiments Versus *Ab-Initio* Theory

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Experimental single-molecule stretching curves for three backbone architectures (single-stranded DNA, various types of peptides, polyvinylamine) are quantitatively compared with corresponding quantum-chemical (zero-temperature) *ab-initio* calculations in the high-force range of up to two nanonewtons. For high forces, quantitative agreement is obtained with the contour length of the polymers as the only fitting parameter. For smaller forces, the effects of chain fluctuations are accounted for by using recent theoretical results for the stretching response of a freely-rotating-chain model.

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Single-molecule force spectroscopy is an established tool for the study of material properties and structures [1–4]. When applied to single polymers, their conformational entropy is probed in the low force range and has been compared with the freely-jointed-chain (FJC) or worm-like-chain (WLC) model [5], while energetic contributions due to stretching of the polymer backbone have been described by *ab-initio* calculations at higher forces up to 300 pN [6]. A comparison of experimental force-extension curves with theoretical predictions over the full force range including the entropic regime (in the range of a few piconewtons) and the energetic regime (above roughly one nanonewton) is still a challenge. A popular model is the WLC model augmented by a linear stretching term, which contains the polymer length, the persistence length, and the Hookean spring constant as fit parameters [5,7,8]. Unfortunately, even with these three parameters, it is not possible to describe the force-extension traces in the full range of forces; i.e., the fit parameters depend considerably on the range of forces used for the fit [9,10]. This suggests that the spring constant determined in such studies is not a material constant but rather functions as a heuristic parameter and makes up for imperfections of the fitting model employed. This is backed up by recent theoretical investigations showing that a freely-rotating-chain (FRC) model (which is quite realistic for many synthetic and single-stranded biological polymers) exhibits a crossover from WLC behavior at small stretching forces to a regime dominated by the discrete nature of the chain at large forces [11,12]. For polymers with a bond length of $b = 0.15$ nm, this crossover occurs at a force of roughly 40 pN and thus the WLC chain behavior is preempted for most atomic-force microscope (AFM) experiments.

In this Letter we concentrate on the large-force regime above 500 pN, where conformational polymer fluctuations constitute only small corrections to the stretching response. We experimentally investigate three different polymer architectures, namely, single-stranded DNA (ss-DNA), polyvinylamine, and peptide molecules (comparing polylysine, polyGVGVP, and titin) at stretching forces up to 2 nN, made possible by very stable attachments between polymers and cantilever tips and substrate surfaces. As an illustration, we show in Fig. 1 the force-extension traces of three different single-stranded DNA molecules with widely varying contour lengths (the custom-built AFM is described in Ref. [13]). The DNA was adsorbed on a gold-coated surface and picked up with an untreated Veeco Metrology Si_3N_4 AFM tip in Tris-buffer (150 mM NaCl, 10 mM Tris, 1 mM EDTA, pH 8), leading to maximal forces of 1200 pN. For accurate data, the polymer spring constant should not greatly exceed the probe spring constant (determined via the amplitude of its thermal fluctuations). The specific force traces shown were selected on the basis of (i) maximal force before detachment and (ii) maximal polymer contour length. In the following analysis we will show that (i) these data can be collapsed by rescaling the chain extension and (ii) the resulting master curve compares well with *ab-initio* (zero-temperature) calculations in the high stretching regime with the polymer contour length as the only fitting parameter. The entropic force

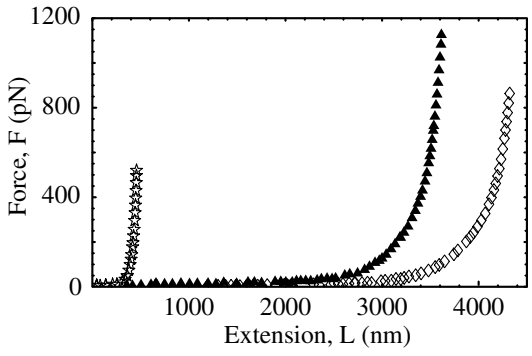

FIG. 1. AFM force-extension traces of three different single-stranded DNA molecules with approximate contour lengths varying from 400 nm to 4000 nm.

contribution due to chain conformational fluctuations can for large forces be accounted for without additional fitting parameters.

Using quantum-chemical *ab-initio* methods, the elastic properties of polymeric bulk substances [14,15] and the stretching behavior of single poly-ethylene-glycol molecules [6,16] have been successfully predicted and compared with experiments. Here one obtains the ground-state energy of a certain molecular configuration from the Schrödinger equation within various numerical approximation schemes. As an illustration, we present in Fig. 2(a) the total binding energy (relative to the ground state) as a function of the unit-cell-length a for a propane molecule [structure shown in Fig. 2(f)], which forms the smallest geometric subunit of an alkane chain. In the calculations we only fix the distance of the two outer carbon atoms a, while the positions of all other nuclei are optimized such as to minimize the overall energy. We compare results for three different levels of quantum-chemical calculations [17], namely, Hartree-Fock (HF) with a rather small basis set consisting of six Gaussian Slater-type orbitals (STO-6G, diamonds), HF with the more refined Triple-Zeta-Valence basis set (TZV, stars), and Moller-Plesset-2 which takes electron correlations into account (TZV-MP2, squares). The latter, most advanced calculation is subsequently compared with experiments. The difference between the various schemes illustrates the systematic errors involved in such *ab-initio* calculations. The lines in Fig. 2(a) denote polynomial fits according to

$$E = E_0 + a_0 \sum_{n=2}^{\infty} \gamma_{n-1}(a/a_0 - 1)^n/n, \qquad (1)$$

where a_0 is the equilibrium distance ($a_0/2 = 0.1280$ nm, 0.1275 nm, and 0.1280 nm for the STO-6G, TZV, and TZV-MP2 calculations, respectively) and E_0 the ground-state energy. Our fitting results for the elastic coefficients γ_n are given in Table I. We used a sequential fitting procedure, where the leading polynomial terms are fitted first using restricted data subsets centered around the ground state. What is really measurable experimentally is the force, which follows from Eq. (1) by a derivative

$$F = \partial E/\partial a = \sum_{n=1}^{\infty} \gamma_n(a/a_0 - 1)^n. \qquad (2)$$

The first coefficient in this series, γ_1, is the polymer stretching modulus. The complete force functions are plotted in Fig. 2(b) and demonstrate that higher-order, nonlinear terms are important in the force range considered in AFM experiments. Figure 2(c) compares binding energies for alkane chains consisting of 3, 7, and 11 carbon atoms (diamonds, triangles, squares, respectively), all performed with the restrictive basis set STO-6G, showing that finite-size effects are less important than the quality of basis sets. We have also checked for the influence of side chains (in specific amine, hydroxyl, and carboxyl groups) or solvent properties (by modeling water as a uniform polarizable continuum medium with relative dielectric constant $\varepsilon = 78$) on the elastic properties and found no effects at forces larger than about 50 pN. Figure 2(d) shows the propane bond angle γ (solid line) and the bond length b [broken line; both are defined in Fig. 2(f)] as a function of the applied force: the elastic response of a chain involves simultaneous changes of both bond angle and bond length. Figure 2(e) presents the main theoretical result, the force versus relative elongation L/L_0 for the different polymer structures considered experimentally, namely, alkane,

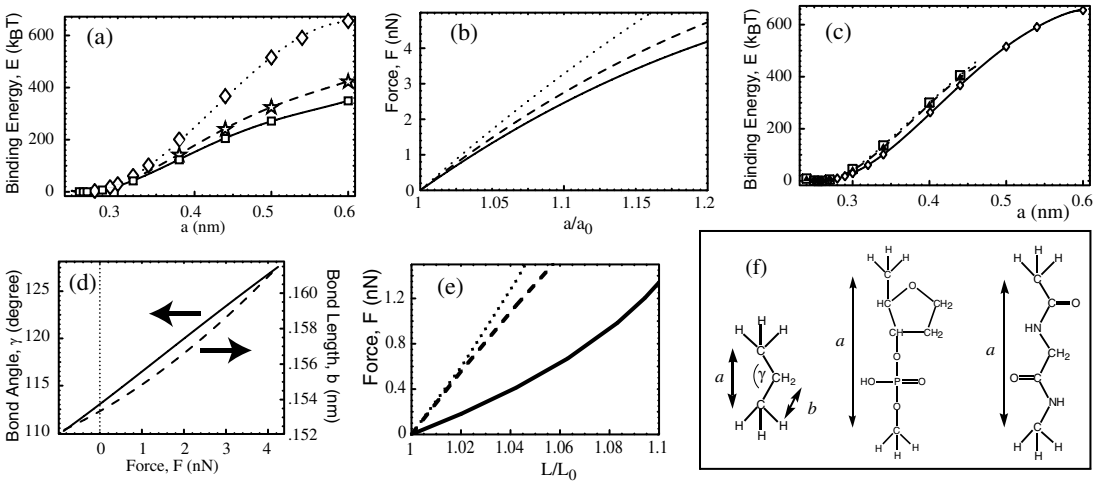

FIG. 2. (a) Total binding energy (relative to the ground state) and (b) stretching force for a propane molecule using three different quantum-chemical schemes, namely, STO-6G (diamonds), TZV (stars), and TZV-MP2 (squares), see text. The lines denote fits according to Eq. (1) and (2), respectively, with coefficients presented in Table I. (c) Binding energies per unit cell for an alkane chain consisting of 3, 7, 11 carbon atoms (diamonds, triangles, squares, respectively), showing the irrelevance of finite-size effects. (d) Bond angle (solid line) and bond length (broken line) as a function of the force for a propane molecule. (e) *Ab-initio* (TZV-MP2) force-extension relation for ss-DNA, propane, and peptide (solid line, broken line, dotted line, respectively). (f) Structural formulas used in the calculations for propane, DNA, and a di-peptide unit.

TABLE I. Elastic constants obtained from the *ab-initio* calculations and unit-cell length a_0 for an alkane, ss-DNA, and peptide backbone. The linear elastic modulus is γ_1; the other coefficients are nonlinear corrections which become important at the higher force range probed in the experiments.

	a_0 [nm]	γ_1 [nN]	γ_2 [nN]	γ_3 [nN]	γ_5 [nN]
Alkane	0.256	28.7	−42.0	16.9	···
ss-DNA	0.71	8.44	29.5	···	19 637
Peptide	0.73	27.4	109.8	···	···

ss-DNA, and peptide backbones (broken, solid, dotted line, all on the HF-TZV-MP2 level). By definition, all curves cross for zero elongation $L/L_0 = 1$. The various polynomial fit functions are defined by Eq. (2) and the elastic constants are given in Table I. Figure 2(f) shows the corresponding chemical structures and unit cells used in the calculations. In all calculations, we strip off side chains, as they have been shown not to influence the results and we consider the minimal unit cell which can be periodically repeated to build a long polymer.

In comparing experimental stretching curves (e.g., for ss-DNA as shown in Fig. 1) with the *ab-initio* prediction in Fig. 2(e), the polymer contour length L_0 is unknown. By plotting the experimental force-versus-distance data as a function of the rescaled extension L/L_0, the parameter L_0 is adjusted until the experimental data match the *ab-initio* curve at large forces. Figure 3(a) shows the result of this fitting procedure with the data for ss-DNA already displayed in Fig. 1. The resulting contour lengths are $L_0 =$ 438 nm, 3318 nm, 4017 nm (open stars, filled triangles, open diamonds). In Fig. 3(b) we show data for Polyvinylamine (PVA) polymers with a hydrolysis fraction of 0.1 (filled triangle) and 0.5 (diamonds, square). The molecules were covalently attached to an epoxy-silanized Si_3N_4 AFM tip and an epoxy-silanized glass slide. The measurements were performed under different electrolyte concentrations: 5 mM NaCl, 40 mM NaCl, and 20 mM NaCl (triangles, diamonds, squares) [9]. Contour lengths of $L_0 = 2697$ nm, 311.5 nm, 4932 nm (diamonds, triangles, squares) were obtained. Finally, in Fig. 3(c) we show three different peptide polymers, poly-peptide C-$(GVGVP)_{nx251}$-C (kindly provided by Dan Urry and attached to gold-coated glass slides and Olympus Biolever AFM tips in Millipore water [10]) with a fitted contour length $L_0 = 263.6$ nm (open diamonds), titin (kindly provided by Mathias Gautel and adsorbed on a gold-coated surface and picked up with an untreated Si_3N_4 AFM tip in PBS-buffer) with $L_0 = 129.5$ nm (filled triangles), and poly-L-lysine (Sigma, 300 kD, adsorbed on a gold-coated surface and picked up with an untreated Si_3N_4 AFM tip in PBS with additional 500 mM NaCl) with a contour length $L_0 = 638.6$ nm (open squares). All data in Fig. 3(a)–3(c) nicely collapse and agree with the corresponding *ab-initio* curves for larger forces. For the different peptide chains this indicates that they are described by their backbone stretching behavior only. For titin [Fig. 3(c) , filled triangles] the data in the force range below 900 pN fall significantly above the curves of the other peptides. This is probably due to partial desorption of the poly-peptide chain from either tip or substrate at about 900 pN, which leads to an increase in contour length L_0 above 900 pN [note that the data in Fig. 3(c) were rescaled by the value of L_0 for forces above 900 pN]. In conclusion, for forces above 0.5–1.0 nN (depending on the polymer type) the stretching behavior of a wide class of backbone architectures can be accurately modeled by zero-temperature quantum-chemistry calculations without any model assumptions, with the only fitting parameter being the polymer contour length.

Up to now, we do not account for polymer conformational fluctuations, which presumably cause the considerable deviations between the *ab-initio* curves and experimental data in Fig. 3 at small forces. How robust is our data analysis with respect to the presence of such thermal effects, and, specifically, how will the fitted contour length shift as a result? Recently, the stretching response of the so-called freely-rotating-chain (FRC) model (which is quite accurate for alkane chains) was considered theoretically [11]. The most important finding was that at forces larger than the threshold $F^* \sim \ell k_B T/b^2$ (where ℓ is the effective persistence length), in the *discrete regime*, the stretching behavior is that of a freely-jointed-chain model (FJC) but with an effective bond length twice the true bond length b. Since the force-induced bond length and angle increase is negligible for small forces, the polymer extension, R_z, can

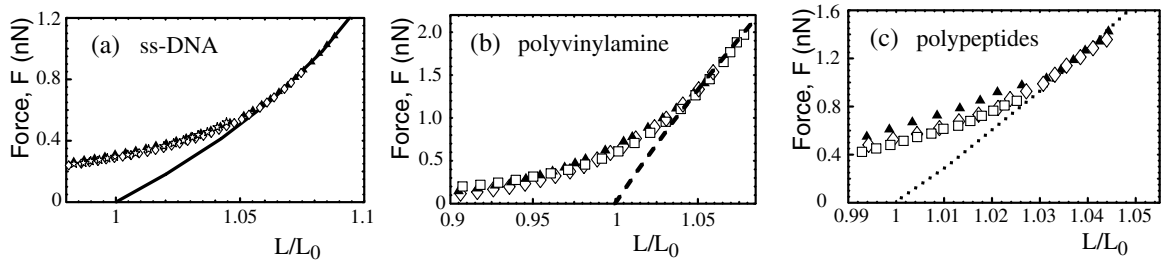

FIG. 3. Comparison of the *ab-initio* predictions from Fig. 2(e) with each three experimental curves for (a) ss-DNA, (b) polyvinylamin, and (c) peptide chains, namely, polyGVGVP (open diamonds), titin (filled triangles), poly-lysine (open squares). All experimental data were normalized by the polymer's contour length at zero force, L_0, as described in the text.

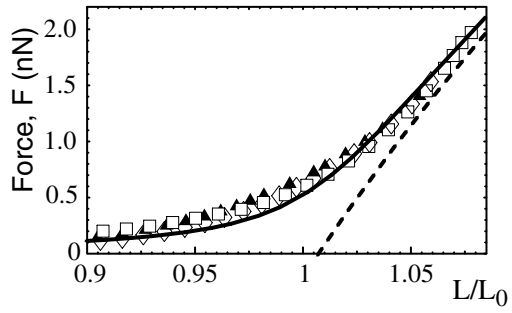

FIG. 4. PVA stretching data compared with the discrete-chain prediction Eq. (3) including the *ab-initio* contour length variation (solid line). The broken line denotes the zero-temperature limit without conformational fluctuations.

for $F > F^*$ approximately be written as

$$R_z = L[F][1 - k_B T/(2bF)], \quad (3)$$

where $L[F]$ is the force-dependent contour length. For smaller forces, WLC behavior is predicted [11]. Since for an alkane chain $F^* \approx 40$ pN, Eq. (3) is valid for all stretching data presented in this Letter. The force-dependent contour length $L = L[F]$ is obtained from Eq. (2) via inversion. In Fig. 4 we compare the PVA data [already shown in Fig. 3(b)] with the discrete-chain prediction Eq. (3) (solid line), taking into account the energetic contribution from the *ab-initio* calculation (via $L[F]$) and the entropic chain-fluctuation contribution. No additional parameter enters, since the known bond length $b = 0.154$ nm is used. The agreement of the solid line with the experimental data is remarkable, keeping in mind that no fitting parameter is used other than the chain contour length L_0. By including the effects of fluctuations, the fitted contour lengths increase by a factor of 1.007, as is visualized by the broken line which follows from Eq. (3) by setting $k_B T = 0$ and thus corresponds to the zero-temperature limit. Fitting with the *ab-initio* curves alone (without fluctuation corrections) thus gives reliable estimates of the chain lengths already, which is relevant for the more complicated peptide and ss-DNA molecules, where no effective model in terms of a simple FRC model is available.

In summary, we compare the experimental force-distance relations in the high-force range (up to 2 nN) for three different polymer types with the corresponding zero-temperature *ab-initio* calculations. With the contour length L_0 as the only parameter, the agreement is good for large forces. Fluctuation effects can be reasonably described, without introducing additional free parameters, by incorporating recent results for the asymptotic large-force behavior of a FRC model [11].

At large forces of roughly 1 nN, the chemical polymer structures change substantially. This could guide the development of mechanically controlled chemistry (e.g., certain reactions could be enhanced or inhibited under mechanical stress) or the understanding of bond breakage under stress (which is important for controlling lubrication wear and failure). Chemical single-polymer analysis is feasible by comparison of experimental force traces and *ab-initio* calculations at large forces, since different backbone architectures show quite different stretching responses. Our results for the energetic and entropic polymer compliance, expressed via Eqs. (2) and (3), and the elastic constants summarized in Table I, can be useful in a variety of different polymer applications where large tensile forces act on polymers. Examples are strongly stretched polymer networks, where tensile stress is typically concentrated on a small fraction of all available chains and therefore locally very high, or dissolved polymers in high shear situations such as sheared polymer solutions at interfaces.

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