Reversible stretching of homopolymers and random heteropolymers

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Abstract

We have analyzed the equilibrium response of chain molecules to stretching. For a homogeneous sequence of monomers, the induced transition from compact globule to extended coil below the $\theta$-temperature is predicted to be sharp. For random sequences, however, the transition may be smoothed by a prevalence of necklace-like structures, in which globular regions and coil regions coexist in a single chain. As we show in the context of a random copolymer, preferential solvation of one monomer type lends stability to such structures. The range of stretching forces over which necklaces are stable is sensitive to chain length as well as sequence statistics.
Experiments probing the mechanical response of individual protein molecules have demonstrated that certain domains can withstand significant stretching forces before unfolding [1–3]. At strain rates much greater than unperturbed rates of unfolding, measured restoring forces imply that the native structures remain largely intact up to a threshold force, at which they unfold to an extensible state. Threshold forces for these domains are typically in the range $\sim 10$-$100 \ T/a$, where $T$ is temperature (in units such that Boltzmann’s constant is unity) and $a$ is an average distance between neighboring, connected monomers in the chain. To be sure, these experimental systems are out of equilibrium, as highlighted by wide hysteresis upon releasing strain and broad distributions of threshold forces. Nevertheless, it is reasonable to expect that such proteins undergo sharp unfolding transitions under adiabatic stretching conditions as well.

The microscopic origin of this mechanical strength is not well understood. As Wolynes and coworkers have pointed out, small forces applied to the end-to-end distance of a protein may couple very weakly to the reaction coordinate for folding [4]. Atomically detailed simulations of rapid stretching instead suggest an important role for certain backbone topologies that are stabilized by groups of hydrogen bonds [5]. In this Letter we explore the equilibrium force-induced transition at a coarse-grained level, using simple estimates of the relevant free energetics. Specifically, we distinguish between aspects of the transition that are homopolymeric in nature, and those that arise from the quenched disorder characterizing random heteropolymers. We show that necklace-like structures, as depicted in Fig. 1 (c), occur with low probability in long homopolymers, but may be stabilized over a finite range of force and temperature by sequence heterogeneity. We describe the features of sequence statistics that affect this stability, and thereby determine mechanical strength. Our results may thus shed light on evolutionary design principles for proteins whose functions are mechanical in nature.

We consider first a chain of $N$ identical monomers in solution. In the absence of stretching force and below the $\theta$-temperature ($\theta$), the chain adopts a compact, globular conformation (Fig 1 (a)). Because this state exhibits only small fluctuations in monomer density, its free
energy is well approximated using mean field theory [8]:

\[ F_g \approx BN + \gamma N^{2/3} - TS_0. \]  

(1)

Here, \( B \) is the excess free energy per particle of a fluid of unconnected monomers at the same temperature and density as the globule. The second term in Eq. 1 includes surface energetics of the globule-solvent interface, as well as the conformational entropy \( S_{\text{sph}} \) lost upon confining the chain to a spherical volume with radius \( R \approx N^{1/3} \). \( S_0 \) is the entropy of an ideal, unconfined chain.

We imagine that the principal effect of a small stretching force, \( f \), on the ends of a homopolymeric globule is to deform its spherical geometry (Fig. 1 (b)). The favorable energy of this deformation, \( -f(R_\parallel - R) \), is offset by surface energetics as well as a loss of entropy, \( S_d \). We estimate this entropy loss through the statistics of a Gaussian chain confined to a deformed volume. In the long-chain limit, the free energy per monomer of such a chain is isomorphic with the ground state energy of a quantal particle confined to the same volume \([9]\). Treating the asymmetric boundary condition as a perturbation \([10]\), we sum an infinite class of terms in the ground-state expansion \([11]\), obtaining

\[ S_d = S_{\text{sph}} \left( \frac{2R_\parallel}{3R} + \frac{R^2}{3R_\parallel^2} - 1 \right). \]  

(2)

Because \( S_{\text{sph}} \sim N^{2/3} \), the energy gained by a reasonable deformation of the globule \( (R_\parallel \sim N^{1/3}) \), is comparable to \( S_d \) only for forces of magnitude \( N^{1/3} \) or larger. For long chains, the deformation achieved by stretching forces \( \sim T/a \) is therefore negligible. We subsequently consider the globule to be undeformed, and the globular free energy to be unaffected by stretching.

For sufficiently large stretching forces, the dominant state of a homopolymer is an expanded coil (Fig. 1 (d)). In contrast to the globule, this state is characterized by extensive density fluctuations and is strongly susceptible to deformation. Considering short-ranged attractions between monomers to be unimportant in this case, we model the extended coil as a freely jointed chain. The free energy of such a chain \([8]\), \( F_c(N) = \)
\(-NT\ln\left[\sinh\left(\frac{fa}{T}\right)\right] - TS_0\), is sensitive to the magnitude of \(f\). At a given temperature \(T < \theta\), a phase transition occurs at a force sufficient to lower the coil free energy below that of the globule. (See inset of Fig. 2.) For \(N^{2/3} \gg 1\), the phase boundary is given by

\[B + \gamma N^{-1/3} = -T\ln\left[\sinh\left(\frac{fa}{T}\right)\right].\]  

(3)

The resulting phase diagram in the force-temperature plane is shown in Fig. 2 for various \(N\). Here, we have taken \(B = T - \theta\) (accurate near \(T = \theta\)), which is unrealistic at low temperatures. It is reasonable, however, that as temperature decreases, stretching energy density of the strongly fluctuating coil grows more quickly than attractive energy density of the relatively placid globule. As a result, a reentrant coil phase appears at low temperature. The “coil” in this case is a nearly straight chain with small fluctuations in extension. Computer simulations of strained lattice heteropolymers \([12]\) appear to support our prediction of reentrance.

Although the globule-coil transition is second-order in the absence of stretching \([8]\), the force-induced transition is here predicted to be first-order, since the average chain extension is discontinuous at the phase boundary. This result is certainly correct for \(T < \theta\), where globule and coil phases are distinct. Near \(T = \theta\) and \(f = 0\), however, our caricatures of these states are oversimplified: Density fluctuations are not negligible in the globule, and attractions are not negligible in the coil. In this region of the phase diagram, extension increases smoothly with force, and the stretching transition is second-order.

In constructing a phase diagram for homopolymer stretching, we did not consider chain structures that are necklace-like (i.e., coexisting globule and coil regions, as in Fig. 1(c)). Neglecting surface effects, the free energy per monomer of a necklace lies between that of a globule and that of a coil. The entropy of a necklace is augmented by the freedom of globular regions to reside anywhere along the chain (provided they do not overlap). For a single globular region, however, this additional “translational” entropy scales as \(\ln N\), and is insufficient to overcome the \(O(N)\) deficit in free energy to either globule or coil, even for
chains of modest length. In the case of many globular regions, the gained entropy, \( \sim N \ln 2 \), is considerable, but does not compensate for the cost of presenting an extensive surface to the solvent.

The stability and importance of necklace structures may be qualitatively different for heteropolymers. In particular, fluctuations in local sequence composition yield a preference for globule or coil that varies along the chain. We assess the strength of this effect and its influence on stretching behavior, for a two-letter random copolymer. In this model, each monomer has two possible identities, \( \sigma_i = \pm 1 \), perhaps denoting hydrophobic and hydrophilic moieties. For a given sequence \( \{\sigma_i\} \), the energy of a chain conformation defined by monomer positions \( \mathbf{r}_i \) is

\[
\mathcal{H} = \mathcal{H}_0 + \Gamma \sum_{i \text{ exposed}} \sigma_i - \mathbf{f} \cdot (\mathbf{r}_N - \mathbf{r}_1),
\]

\[
\mathcal{H}_0 = \sum_{i,j=1}^N \delta(\mathbf{r}_i - \mathbf{r}_j)(B_0 + \chi \sigma_i \sigma_j),
\]

where \( B_0 \) is a mean attractive energy density stabilizing the globule at low temperatures. We consider \( \chi < 0 \), so that attractions are strongest between monomers of the same type. The second sum in Eq. 4 includes only monomers that are exposed to solvent. For \( \Gamma > 0 \), monomers of type \( \sigma_i = -1 \) are favorably solvated, while exposure of \( \sigma_i = 1 \) monomers is unfavorable. In Ref. [13], the phase diagram was determined for the model defined by \( \mathcal{H}_0 \) at the mean field level, using the replica trick to average over random sequences of monomer types [14,15]. The one-step replica symmetry breaking demonstrated in that work is consistent with a suitably chosen random energy model. In other words, the average thermodynamics are reproduced by drawing \((a^3/v)^N\) energy levels at random from a distribution \( P(E) = (\pi N \Delta^2)^{-1/2} \exp \left[-(E - \bar{E})^2/2N\Delta^2\right] \), with variance \( \Delta = |\chi| \mu^2 \rho \) and mean \( \bar{E} = B_0 N \rho \). Here, \( v \) is the volume occupied by a monomer, \( \rho \sim v^{-3} \) is the mean density in the globule core, and \( \mu \) is the variance of the monomer distribution. Here we describe the effect of solvation and stretching energetics on the effective distribution of energies [11].

For a compact, spherical globule with surface area \( A \), the solvation contribution in Eq. 4
broadens the energy distribution, increasing $\Delta$ by $(\Gamma^2 \rho/4|\chi|)A/N$. This result is obtained following the analysis of Ref. [13], with the additional assumption that the spatial pattern of solvent-exposed monomers is independent of compact chain conformation. As in the case of the homopolymer, we neglect the energetic contribution of globule deformation. The resulting distribution of energies is dominated by states in the interval $E - N^{1/2}\Delta < E < E + N^{1/2}\Delta$. At energies just below a critical value, $E^* = E - N\Delta(\ln a^3/v)^{1/2}$, the number of states is $O(1)$, while just above $E^*$ the number is exponentially large. The ground states of particular random sequences are distributed narrowly about $E^* [16]$. Introducing solvation energetics thus lowers the average ground state energy by an amount $(\Gamma^2 \rho/4|\chi|)(\ln a^3/v)^{1/2}A$. If $\chi$ and $\Gamma$ are comparable in magnitude, this shift is a significant fraction of the energy gained by exposing only monomers with $\sigma_i = -1$ to the solvent. The density of states around $E^*$ in this case is sufficiently large that a ground state with favorable solvation energetics may always be selected.

In an extended coil, nearly all monomers are exposed to solvent. For sequences with fixed total composition, $\sum_{i=1}^{N} \sigma_i = 0$, thermodynamics of the coil state are unaffected by solvation energetics. We consider the heteropolymer coil to have essentially the same physics as the homopolymer coil described above.

For necklace structures of a random heteropolymer, free energy depends on the positions of globular regions along the chain. In effect, these globules move in a random potential generated by sequence disorder. (See Fig. 3.) The scale and correlation length of this potential are determined by the size of the globule, and by statistics of the sequence. Although the total composition of the chain is fixed, a globular region with $n < N$ monomers, situated at a given point on the chain, has in general an excess of one monomer type:

$$q \equiv \frac{1}{n} \sum_{i \in \text{globule}} \sigma_i \neq 0.$$  \hspace{1cm} (6)

Because the local composition $q$ is a sum of many independent random variables, its distribution is Gaussian, with variance $\mu n^{-1/2}$. This distribution of sequence compositions leads to localization of globules along the chain.
At a particular location, a globular region has an apparent distribution of monomer types that is modified according to the local value of $q$, $p(\sigma_i; q) \propto \exp[-(\sigma_i - q)^2/2\mu^2(1 - q^2)]$. The ground state energy of the globule at this location, determined from the random energy model described above, is

$$E^* = (B_0 - |q|^2/\mu n - \Gamma q/\mu n^{2/3} - \left(\ln \left(\frac{a^3}{\sigma_i}\right)\right)^{1/2} \left[2|q|^2(1 - q^2)/\mu n + \frac{\Gamma^2 \mu}{4|q|} n^{2/3}\right]). \tag{7}$$

Since local composition is not fixed for a heteropolymer, $E^*$ is a random variable for different globule locations. But its fluctuations are weak: $\langle(\delta E^*)^2\rangle^{1/2} = \Gamma/\mu n^{1/6} + O(n^0)$, where angled brackets denote an average over the distribution of $q$.

For a single globular region incorporating $n$ monomers, the remaining $N - n$ monomers of the necklace belong to coil regions. When the globule resides at a given chain location with composition $q$, the complementary composition in coil regions is $-q$. Consequently, the solvation energy of these fully exposed regions, $-\Gamma nq$, is also a random variable, with fluctuations of magnitude $\Gamma/\mu n^{1/2}$. These relatively strong fluctuations in coil solvation energy establish the scale of the random potential for globule motion along the chain. Below a critical temperature $T_c$, the globule will become localized in the deepest minimum of this potential. Following Derrida’s analysis of randomly distributed energies \cite{17}, we find that $T_c = \Gamma/\mu n^{1/2}/\sqrt{2s}$, and that the free energy due to globule motion is

$$F_{\text{rand}} = \begin{cases} -sT[1 + \left(\frac{T_c}{T}\right)^2], & T > T_c \\ -2sT_c, & T \leq T_c. \end{cases} \tag{8}$$

In Eq. $8$, $s$ is the logarithm of the number of possible globule locations. The number of statistically independent locations is approximately a factor of $n$ smaller, so that $s$ is appropriately renormalized, $s \simeq \ln (N/n) - 1$. For $T \gg T_c$, randomness of the potential is irrelevant, and the homopolymer result, $\sim T \ln N$ is recovered.

The analysis for a single globule is readily generalized for a necklace with several globular regions. In this case, $e^s \simeq n^{-M}(N - n + 1)(N - 2n + 1)\ldots(N - Mn + 1)/M!$, where $M$ is the number of globules. For small globules, $n = O(1)$, the minimum value of $F_{\text{rand}}$ is obtained for $M = O(N)$, giving $\min F_{\text{rand}} = O(N^{1/2})$. For large globules, $n = O(N)$, $M$
is necessarily $O(1)$, and again $\min F_{\text{rand}} = O(N^{1/2})$. Globule sizes are thus expected to be distributed broadly, with a preference for a small number of large globules dictated by surface tension. Due to the $O(N^{1/2})$ stabilization provided by globule localization, a phase rich in necklace structures covers an appreciable range of force and temperature for $N = 10^2$ (Fig. 4 (a)). Indeed, simulated stretching of a short, nearly random heteropolymer involves partially extended structures [12]. For $N = 10^3$ (Fig. 4 (b)), the necklace phase covers a much smaller region of the phase diagram, due to the extensive free energy cost of mixing globule and coil structures. Although necklaces become unstable as $N \to \infty$, our results suggest that they may be prevalent for macromolecules of finite size.

From our results for uncorrelated sequence statistics, we may deduce the basic effects of introducing correlations. For “blocky” sequences, in which monomers within a correlation length $\xi$ are likely to be of the same type, fluctuations in local composition are large. Specifically, $\langle (\delta q)^2 \rangle = O(n^0)$ for $n < \xi$. If $\xi$ scales with chain length (or if sequence correlations decay algebraically with distance along the chain), the resulting free energy contribution due to localization is $O(N)$, and necklace structures persist as $N \to \infty$. By contrast, sequences that are anticorrelated on a scale $\xi$ exhibit small fluctuations in local composition. If $\langle (\delta q)^2 \rangle = O(n^{-1/2})$, fluctuations in solvation energy are $O(1)$, and the stretching behavior will be homopolymeric.

The equilibrium behavior of strained polymers described in this Letter is not directly related to the intrinsically dynamical, nonequilibrium response measured in experiments. Nonetheless, it is reasonable to associate the equilibrium stability of various structures with their kinetic accessibility. In particular, when necklaces are stable over a wide range of stretching forces, the kinetic transition from compact globule to extended coil is likely not sharp. Instead, we expect that the chain visits an ensemble of necklace structures as it passes through the transition region. The breadth we have predicted for this ensemble in the case of random heteropolymers suggests that the restoring force to applied strain should exhibit large fluctuations as the chain is stretched. Such a scenario has in fact been observed for
the protein barnase [18]. The observation that certain protein domains do undergo sharp stretching transitions is thus an indication of evolutionary design for mechanical strength, as is reflected by the roles of such proteins in cell adhesion and muscle elasticity.
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FIGURES

FIG. 1. Possible states of a strained polymer: (a) compact, spherical globule; (b) compact globule, deformed from a spherical geometry, with extension $2R_\parallel$ in the direction of stretching; (c) necklace of alternating compact and non-compact regions; and (d) fully non-compact, extensible coil.
FIG. 2. Phase diagram of a homopolymer subject to an applied force, $f$, on the end-to-end distance, as estimated by Eq. 3. The boundary between globule and coil phases is drawn for $N = 10^2$ (dot-dashed line), $N = 10^3$ (dotted line), $N = 10^4$ (dashed line), and $N \to \infty$ (solid line). These results are obtained for a surface energy density that is comparable to monomer interactions, $\gamma/\theta \approx 1$. Inset: Schematic picture of the influence of stretching on the free energy of extension for $T < \theta$. As force is increased ($f_1 < f_2 < f_3 < f_4$), the coil state first becomes metastable (represented by the local free energy minimum at large $R$) and then stable. Free energy of the globular state (represented by the local minimum at small $R$) is not sensitive to $f$. 

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FIG. 3. Motion of a globular region along a random heteropolymer. A sequence of monomer identities is represented schematically by black and white circles on the chain. Different sequence locations, $x$, of the globule result in different compositions (here, fractions of black and white circles) of the globule and of the extended coil regions. Consequently, the ground state energy of the globule and solvation energy of the coil depend on $x$. Because the monomer identities are independent random variables, the globule effectively experiences a random, one-dimensional potential $u$ for translation along the sequence.
FIG. 4. Phase diagram of the random copolymer defined by Eq. 4 for (a) $N = 10^2$ and (b) $N = 10^3$, estimated using mean-field arguments described in the text. Shading denotes regions dominated by necklace-like structures. Boundaries of these regions are taken to be chain compositions of 75% globule (lower dashed lines) and 25% globule (upper dashed lines). The solid line denotes a chain composition of 50% globule, i.e., $N/2$ monomers belong to globular regions, and $N/2$ belong to coil regions. The chain is assumed to be flexible on the scale of monomer size, $\overline{\rho}a^3 \approx 1$. 
FIG. 1. Geissler and Shakhnovich
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