

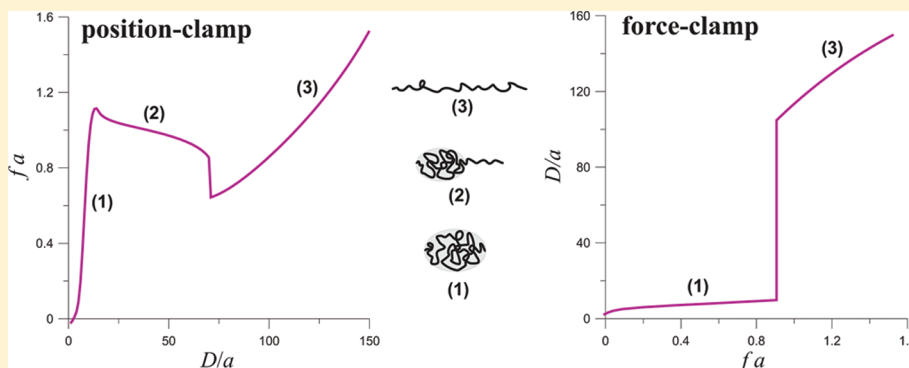
Theory of Mechanical Unfolding of Homopolymer Globule: All-or-None Transition in Force-Clamp Mode vs Phase Coexistence in Position-Clamp Mode

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ABSTRACT:



Equilibrium mechanical unfolding of a globule formed by long flexible homopolymer chain collapsed in a poor solvent and subjected to an extensional force f (force-clamp mode) or extensional deformation D (position-clamp mode) is studied theoretically. Our analysis, like all previous analysis of this problem, shows that the globule behaves essentially differently in two modes of extension. In the force-clamp mode, mechanical unfolding of the globule with increasing applied force occurs without intramolecular microphase segregation, and at certain threshold value of the pulling force the globule unfolds as a whole (“all-or-none” transition). The value of the threshold force and the corresponding jump in the distance between the chain ends increase with a deterioration of the solvent quality and/or with an increase in the degree of polymerization. In the position-clamp mode, the globule unfolding occurs via intramolecular microphase coexistence of globular and extended microphases followed by an abrupt unraveling transition. Reaction force in the microphase segregation regime demonstrates an “anomalous” decrease with increasing extension. Comparison of deformation curves in force and position-clamp modes demonstrates that at weak and strong extensions the curves for two modes coincide, differences are observed in the intermediate extension range. Another unfolding scenario is typical for short globules: in both modes of extension they unfold continuously, without jumps or intramolecular microphase coexistence, by passing a sequence of uniformly elongated configurations. The values of the critical chain length, N_{cr} , separating long and short chain behavior are slightly different for two extension modes: $N_{cr,f} < N_{cr,D}$.

INTRODUCTION

In the past decade, a substantial progress has been achieved in development of experimental single molecular manipulation techniques. The use of such instruments as atomic force microscope (AFM),^{1–3} optical tweezers^{4,5} and magnetic traps⁶ made it possible to study the mechanical properties and related conformational changes in individual (bio)macromolecules and their complexes or the dynamics and mechanisms of molecular motors. The above-mentioned methods not only became “standard” tools of biophysics, polymer physics, and molecular biology but also led to appearance of a new interdisciplinary field of molecular nanomechanics.

The choice of the proper experimental approach depends on the system and the problem under study. Each apparatus has its

effective range of applied (and measured) forces and extensions and, as a rule, more than one operating modes. For example, in AFM experiment the ends of the macromolecule under study are bound to AFM cantilever tip and to a flat surface that are moving apart. The basic operating mode of AFM is the *velocity-clamp mode* when the surface and the AFM tip holder are moved relative to one other at constant velocity. On the other hand, using a feedback loop it is possible to control the force applied to investigated object and carry on measurements either in the *force-clamp mode*, where applied force is hold constant or in the

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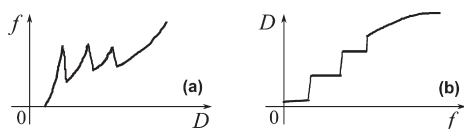


Figure 1. Schematic pictures of deformation curves for globular protein mechanically unfolded in (a) position-clamp and (b) force-clamp modes.

force ramp mode where the force increases linearly with time.^{7–9} AFM has a wide effective range of measured and applied forces: 10 pN to 100 nN. The basic operating mode of optical tweezers is the *position-clamp mode* where extension is fixed with the aid of one or two optical traps. However the use of a feedback loop allows to operate in the *force-clamp mode* too. The effective force range of optical tweezers is typically 0.1–150 pN. A magnetic trap consists of a set of magnets that provide a strong magnetic field gradient, thus exerting a force on magnetic beads tethered by a macromolecule of interest. For this setup it is most natural and convenient to study macromolecules subjected to a constant applied force (*force-clamp mode*). At the same time magnetic traps has a high resolution that allows to measure the response of macromolecule to relatively weak forces about or less than 1 pN. On the other hand, the maximum force can be increased (up to 160 pN) by using bigger beads.⁶ It should be also mentioned that magnetic traps and optical tweezers allow studying rotational degrees of freedom of single macromolecules by applying (or measuring) a torque on them.^{5,6} This feature is especially useful and informative in studying DNA and DNA–protein complexes.

Hence, different modes of mechanical action on single macromolecules are available in experiment. These modes fall far short of being equivalent with respect to the results obtained. Results of single-molecular nanomechanical experiments in the position (D) and force (f) clamp modes are expressed in the form of equilibrium deformation curves $f = f(D)$ in the former case and $D = D(f)$ in the latter case. Equilibrium deformation curves $f = f(D)$ and $D = D(f)$ can also be obtained from the velocity-clamp and force ramp experiments, respectively, if the corresponding governing parameter is changed very slowly.

From the statistical-mechanical point of view, position-clamp and force-clamp modes can be interpreted as two conjugate thermodynamic ensembles: fixed extension ensemble, or D -ensemble, and fixed force ensemble, or f -ensemble, respectively. Correspondingly, in the following text both the words “mode” and “ensemble” will be utilized as “equal in rights”. According to classical statistical mechanics¹⁰ conjugate D - and f -ensembles are equivalent, i.e. corresponding deformation curves $f = f(D)$ and $D = D(f)$ should coincide, it is enough to exchange axes on one of the dependences, for instance $D(f) \rightarrow f(D)$. However, statistical mechanics deals with macroscopic systems. For individual macromolecules this limit (which is called “thermodynamic limit”) is achieved when the chain length N tends to infinity $N \rightarrow \infty$. Macromolecules with finite chain length represent small systems (or nanosystems); therefore, their behavior in different thermodynamic ensembles should be individually studied.¹¹

Experimental force–extension dependences $f = f(D)$ obtained for stretching of macromolecules in the position-clamp mode (or in the velocity clamp mode at very low velocity) have a rather complex structure. For example, for DNA double helix, the reaction force f grows with an increase in the extension D at small and at large extensions. Two increasing branches are separated by a

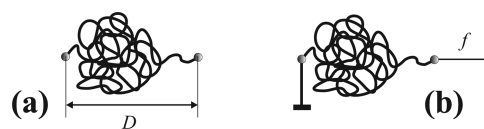


Figure 2. Homopolymer globule deformed in the position-clamp (a) and force-clamp (b) mode.

plateau at moderate extensions. Constant force on the plateau is an indication of progressive extension-induced melting of the native double helix structure of DNA.⁴ Mechanical action on different parts of protein globules (or computer simulation of such an action) leads to appearance of a single or multiple maxima on the force–extension curve followed by decrease(s) in reaction force with increasing extension,^{1–3} Figure 1 a. Each peak of this “sawtooth” curve corresponds to the unfolding of an individual domain of the protein.

In the force-clamp mode, deformation curves of globular proteins acquire appreciably different “staircase” form demonstrating a sequence of jump-wise extensions when applied force is increased,^{8,9} Figure 1 b. Each step of this “staircase” can also be associated with individual domains’ unfolding.

A globule formed by a homopolymer chain collapsed in a poor solvent is similar to a liquid droplet and has, therefore, a simpler structure than that of the protein globule which is, according to Schrödinger,¹² an “aperiodic crystal”. Nevertheless, as it follows from the theory,^{13–18} the shape of force–extension curves in the position-clamp mode for homopolymer globules were found to be not so simple as it might be expected.

The goal of the present work is to develop a theory of mechanical unfolding in the force-clamp mode of a globule formed by a homopolymer chain collapsed in a poor solvent, Figure 2. Comparison with available data on homopolymer globule unfolding in the position-clamp mode should allow revealing main regularities that determine differences in globule behavior in the f - and the D -ensembles.

The problem of the homopolymer globule unfolding attracts the attention of theorists starting from the pioneering work of Halperin and Zhulina¹³ in 1991. The following studies included the development of analytical theories and computer simulations, a review of these works can be found, for example, in¹¹ and^{17,18}. Recently we have performed an extensive self-consistent field (SCF) modeling of the globule unfolding in the position-clamp-mode¹⁷ and proposed a quantitative mean-field theory based on simple model¹⁸ which shown a very good agreement with the results obtained by SCF modeling. By now a clear theoretical picture of equilibrium unfolding of the polymer globule by extensional deformation is well developed.

Consider a flexible polymer chain comprising N monomer units, each of size a , immersed in a poor solvent. The solvent strength is characterized by the Flory–Huggins parameter $\chi > 0.5$. A typical equilibrium force–extension curve for (not too small) globule unfolded by extensional deformation (in the position-clamp mode) is presented in Figure 3a. Here and below $k_B T$ is taken as energetic unit (hence, fa is dimensionless). At small deformations the globule extends as a whole and has an elongated shape - this corresponds to the first ascending part of the curve (1). Further increase in the end-to-end distance leads to appearance of a new intramolecular microphase, i.e., a (strongly) stretched chain part. Microphase segregation in the extended globule has an analogy with the Rayleigh instability in a

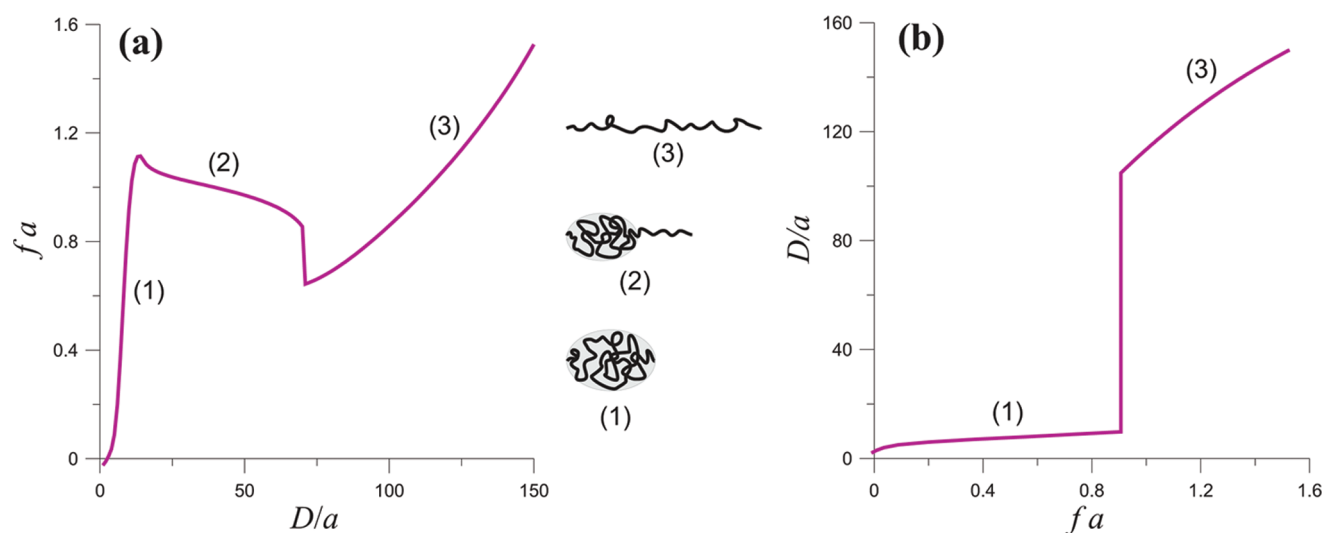


Figure 3. Homopolymer globule deformation curves calculated at $N = 200$, $\chi = 1.4$ from SCF modeling in the position-clamp (a) and force-clamp (b) modes.

liquid droplet;¹⁹ it was predicted in the first work on globule unfolding by Halperin and Zhulina.¹³ In a wide range of extensions, a microphase segregation takes place, and the globule acquires a “tadpole” conformation with a prolate globular “head” and a stretched “tail”. On the force–extension curve this regime corresponds to the weakly decreasing quasi-plateau (2). The deformation of the tadpole is accompanied by progressive unfolding of the globular core, at strong deformations the tadpole conformation becomes unstable, and at certain extension the globule completely unfolds to a uniformly extended chain. This transition is accompanied by a sharp decrease in the reaction force, the number of monomer units in the vanishing globular phase is rather large: $n \sim N^{3/4}$. This “unraveling” transition was discovered by Cooke and Williams¹⁵ in 2003. The second ascending part of the force–extension curve (3) characterizes the following extension of the unfolded chain.

Three factors have an impact on such a complex picture of globule unfolding. First, unfolding of the globule occurs as an intramolecular phase transition. Second, the globule is a small nanosized system (nanosystem). In contrast to (infinitely) large macroscopic systems, the fraction of elements (i.e., monomer units in a macromolecule) at the interface is not negligible and grows with the chain extension. Finally, the macromolecule has a “linear memory” (I. M. Lifshitz²⁰): all elements are joined into a single chain at any extension D that cannot exceed the contour length Na .

The present paper aims at the developing a mean-field theory of equilibrium globule unfolding in the force-clamp mode (which means that the kinetic aspects of globule lie out of scope of the present study). We will show that the dependence $D = D(f)$ differs fundamentally from $f = f(D)$. As it can be seen from Figure 3 b, $D = D(f)$ curve is free from peculiarities of the $f = f(D)$ curve in Figure 3 a and has a simple shape characterized by a sharp transition from the globular to the completely unfolded “open chain” state.

Note that equilibrium deformation curves shown in Figure 3 represent the dependences of the *average* reaction force (end-to-end distance) on imposed deformation (applied force), hence, strictly speaking, one should write: $\langle f \rangle = \langle f(D) \rangle$ ($\langle D \rangle = \langle D(f) \rangle$). In the following text, angular brackets at observables will be omitted.

This should not confuse the reader because the name of the argument (as well as the name of the function) in the dependence definitely points on the corresponding mode of deformation (thermodynamic ensemble).

It is important to emphasize that in the present work, the problem is solved in the framework of the *mean-field* approach. It is well-known that the mean-field approach neglects fluctuations around the ground state for the considered system, see for example.²¹ This approximation is justified for large systems, except for the vicinity of the phase transition point where mean field theory predicts a jumpwise transition. Neglecting fluctuations in the case of the globule unfolding does not affect, however, the essential physics: as it will be demonstrated below, the main “source” of the differences in globule unfolding in position- and force-clamp modes is the surface energy of the globule.

The rest of the paper is organized as follows. In the section “Self-Consistent Field Modeling of Globule Extension in Position- and Force-Clamp Modes” we show how to use the results of SCF modeling obtained for the position-clamp mode (D -ensemble) in order to derive deformation curves in the force-clamp mode (f -ensemble) without doing additional extensive calculation and compare the deformation curves in two modes. In the section “Model and Analytical Theory of Globule Unfolding in the f -Ensemble” analytical theory of the globule unfolding in the f -ensemble based on a simple model is developed and the results of theory, including detailed study of the force-induced unfolding transition and comparison between unfolding transition in two ensembles are presented, and this is followed by Discussion and Conclusions.

■ SELF-CONSISTENT FIELD MODELING OF GLOBULE EXTENSION IN POSITION- AND FORCE-CLAMP MODES

Relation between D - and f -Ensembles and “Translation” of Results. As it was already mentioned in the Introduction, position-clamp and force-clamp modes of globule extension correspond to two conjugate thermodynamic D - and f -ensembles, respectively. It is well-known that each thermodynamic ensemble has its “own” independent variables and this determines a choice of the proper thermodynamic potential that

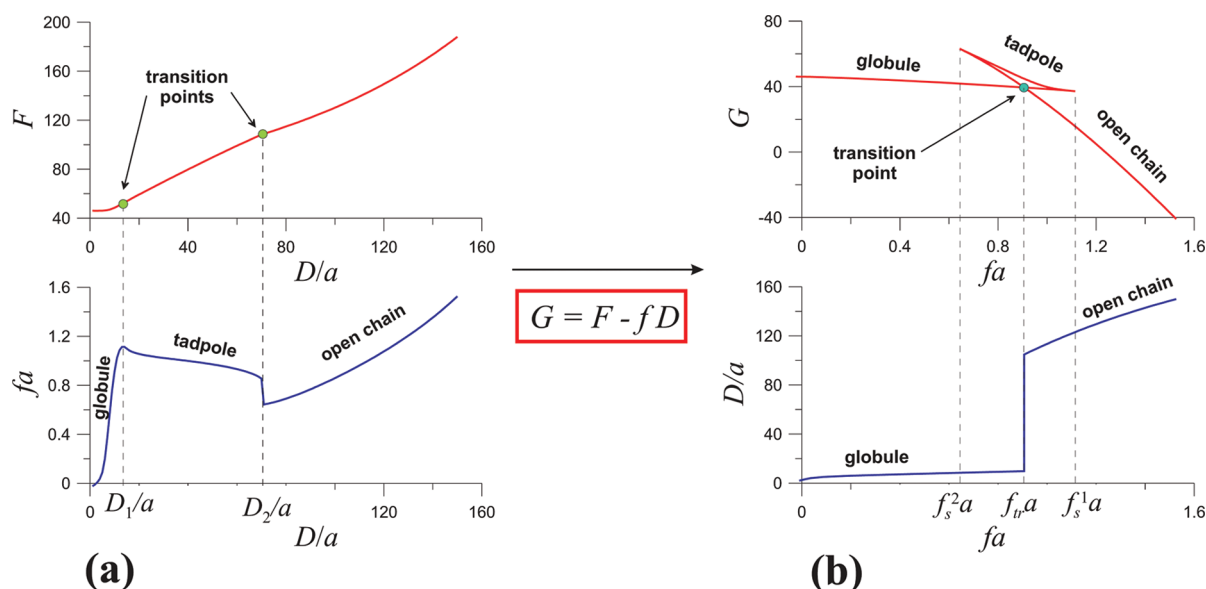


Figure 4. “Translation” of the results obtained for globule unfolding in the D -ensemble using SCF modeling into the f -ensemble: using the Legendre transform (see explanation in the text). $N = 200$, $\chi = 1.4$.

describes the thermodynamics of the system in the given ensemble. For the fixed extension (D -) ensemble the proper thermodynamic potential is the Helmholtz free energy $F(D)$, while for the fixed force (f -) ensemble one should work with the Gibbs free energy $G(f)$. These thermodynamic potentials are related via Laplace transform^{22–24}

$$G(f) = -k_B T \log \left[\int dD e^{-(F(D) - fD)/k_B T} \right] \quad (1)$$

where the integral is taken over all possible extensions (end-to-end distances) D , therefore, fluctuations of the chain end positions are taken into account. In the self-consistent field approach one neglects fluctuations and considers only one state that maximizes the exponent in eq 1; i.e., the integral is dominated by the saddle point value,²¹ and therefore, the thermodynamic potentials are related by Legendre transform:

$$G = F - fD \quad (2)$$

This relation (eq 2) suggests an easy and elegant way to transform the D -dependences of the Helmholtz free energy and the reaction force in the D -ensemble obtained earlier in¹⁷ using the Scheutjens–Fleer self-consistent field (SF-SCF) numerical approach, into f -dependences of the Gibbs free energy and extension in the f -ensemble. In other words, without doing independent extensive SCF calculations for the globule unfolding in the force-clamp mode, the results for this ensemble can be obtained by using the already existing results for the conjugate ensemble and the Legendre transform, eq 2. The scheme and the result of this “translation” for $N = 200$ and $\chi = 1.4$ are presented in Figure 4 (only the equilibrium $F(D)$ and $f(D)$ dependences are used for such the “translation”).

The dependence of the Gibbs free energy obtained by “translation” of the results from D - to f -ensemble has a loop, Figure 4b. Each part of this looplike dependence is unambiguously identified with the corresponding conformation of the globule (ellipsoidal globule, tadpole, stretched chain), by analogy with the force–extension curve for the D -ensemble. We see that the lowest free

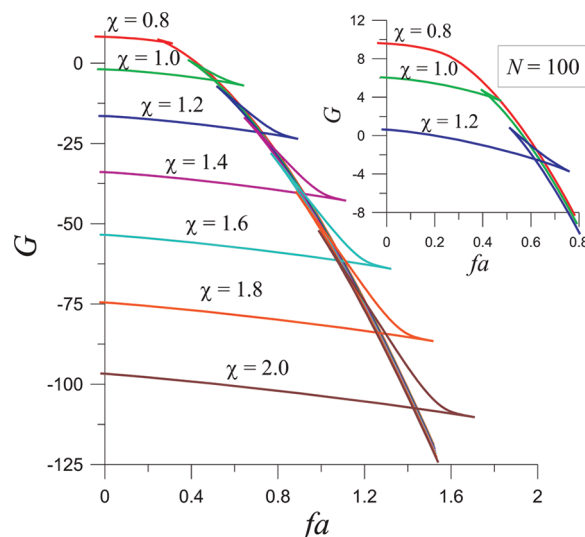


Figure 5. Gibbs free energy of the globule extended by applied force obtained from SCF modeling at $N = 200$ and 100 (inset) and different χ values.

energy always corresponds to one of two pure states: weakly extended globule at small forces and stretched “open” chain at large applied forces. Two free energy branches corresponding to pure states intersect at certain value of $f = f_{tr}$ which is the unfolding transition point. The transition point does not coincide with none of the two characteristic points in the D -ensemble, forces f_s^1 and f_s^2 corresponding to extensions D_1 and D_2 in the D -ensemble but lies between them (note also that D_1 and D_2 are the points bounding the range of the tadpole (two-phase) state stability, Figure 4a). Figure 4 b demonstrates that the free energy of the microphase segregated tadpole state is larger than that of pure states at any value of applied force f . This points to disadvantage of the microphase segregation in the f -ensemble. Extension-force curves in the force-clamp mode, Figure 4 b

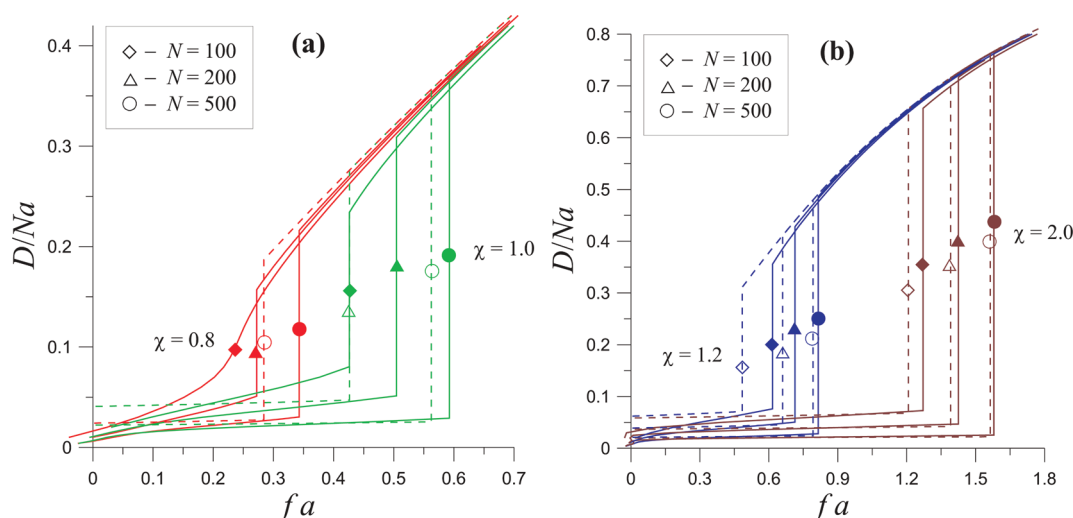


Figure 6. Equilibrium degree of extension (relative extension) vs applied force curves for the globules with different N and χ calculated from SCF modeling data (solid lines) and using analytical theory (dashed lines).

reproduce the “all-or-none” phase transition from weakly extended globule to the open chain state.

Free Energy and Deformation Curves. The above-described D -to- f -ensemble “translation” was made for the SCF data obtained for different chain lengths $N = 100, 200, 500$ and a series of Flory interaction parameters $\chi = 0.8, 1.0, \dots, 2.0$. The SCF results for globule unfolding in the D -ensemble $N = 200$ and 500 were presented in ref 17; the results for $N = 100$ was calculated in the present work.

In Figure 5, the Gibbs free energy as a function of applied force is shown for $N = 100$ and 200 at different values of χ . Most of the dependences have the looplike shape, the branches corresponding to open chain state collapse into a single curve. Extended globule branches demonstrate close to linear dependence. With an increase in χ the globule branch shifts down so that the point of its intersection with the open chain branch (i.e., the transition point) moves toward larger values of applied force f . Unstable parts of loops at $N = 200, \chi \geq 1$ and $N = 100, \chi \geq 1.2$ in Figure 5 correspond to the two-phase tadpole conformation which is the stable state of the globule unfolded in the D -ensemble. On the other hand, at $N = 200, \chi = 0.8$ and $N = 100, \chi = 1.0$ globule unfolding in the D -ensemble is not accompanied by microphase segregation and formation of the tadpole state¹⁷ since the system is below the critical point, i.e. the chain length N is lower than the critical value $N_c(\chi)$, the minimal chain length at which the formation of the tadpole structure is possible.¹⁸ Under precritical conditions ($N < N_c$), intermediate state in the D -ensemble is that of the “sparse” globule: upon stretching the globule becomes more asymmetric and the density of its core decreases. However, the “anomalous” decrease of the reaction force f with an increase in D retains in this regime. Unstable part of the loop in Figure 5 at precritical values of parameters correspond to “sparse” globule conformation. At $N = 100, \chi = 0.8$ when even the unperturbed globule is on the edge of stability, the transition becomes continuous.

Deformation (extension-force) curves obtained for different values of N and χ from SCF modeling data according to the above-described procedure (see Figure 4) are presented in Figure 6 by solid curves. Note that for the sake of comparison, it is more convenient to consider the relative extension, or degree

of stretching, $D/(Na)$ rather than its absolute value D . It can be seen that in all presented examples, with the exception of the case $N = 100, \chi = 0.8$, we obtain that globule unfolding in the f -ensemble occurs according to the “all-or-none” principle, or as a jumpwise first order phase transition. At small applied force there is a weakly extended globular phase, its relative linear size $D/(Na)$, weakly increasing with growing f , decreases with an increase in N and χ . At certain value of $f = f_{tr}$ corresponding to the transition point the globule disintegrates and the macromolecule acquires a strongly stretched “open” conformation with the end-to-end distance D proportional to N but independent of χ : relative extension vs force dependence above the transition point ($f > f_{tr}$) is described by a unique curve independent of both N and χ . The position of the transition point f_{tr} where the end-to-end distance abruptly changes depends primarily on χ and in a less extent on N : f_{tr} increases with increasing N and χ ; correspondingly, the value of the jump in the relative end-to-end distance in the transition point increases too.

As opposed to the above considered jumpwise transition, for the smallest N and χ values presented in Figure 6, $N = 100, \chi = 0.8$, globule unfolding in the f -ensemble occurs continuously.

Comparison of Deformation Curves in Force- and Position-Clamp Modes. One of the most important goals of the present study is to compare the deformation curves in force- and position-clamp modes of globule deformation. Making such a comparison is easy: the deformation curves in two modes should be plotted together in the D – f coordinates (therefore, we exchange the axes for deformation curves in the f -ensemble).

Figure 7 presents equilibrium deformation curves for D - and f -ensembles calculated using SF-SCF approach for $N = 200$ (Figure 7a) and 100 (Figure 7b) (f -ensemble, solid lines; D -ensemble, dashed lines). Deformation curves for the D -ensemble were already presented in.¹⁷ At weak and strong extensions the curves for two ensembles coincide; differences are observed in the intermediate extension range. In this range of extensions, at large enough values of N and χ , microphase segregated tadpole structure forms and the force–extension dependence has a quasi-plateau shape with “anomalous negative” slope and a jump in the point where the globular phase disappears. In the cases $N = 200, \chi = 0.8$ (Figure 7a) and $N = 100, \chi = 1.0$ (Figure 7b)—a

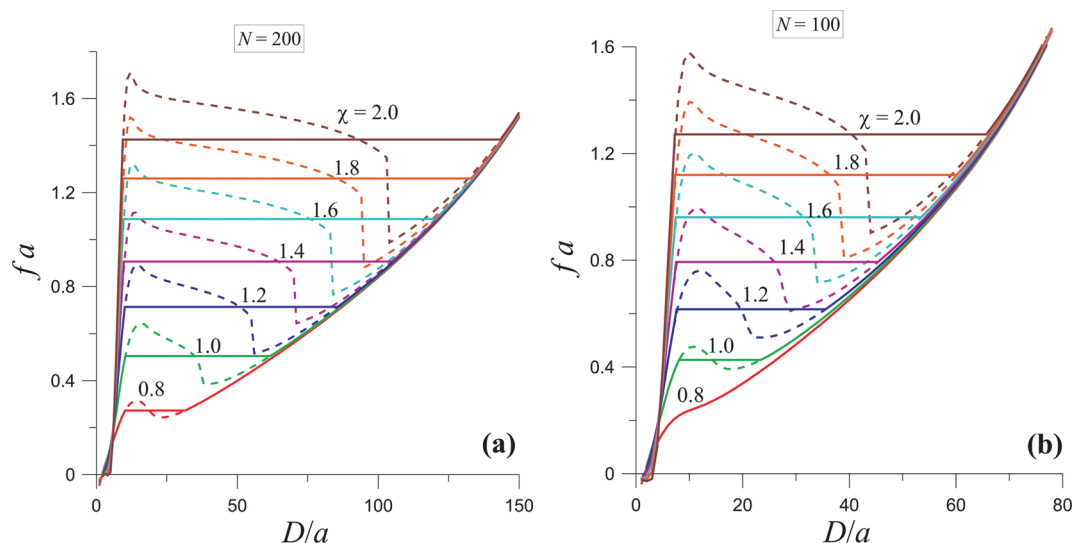


Figure 7. Comparison of deformation curves for f - (solid lines) and D -ensembles (dashed line) calculated using SCF modeling data for $N = 200$ (a), $N = 100$ (b), and different values of χ .

short macromolecule in a moderately poor solvent—intramolecular microphase segregation does not occur but the globule deforms as a whole and its density decreases (the system is below the critical point^{17,18}), at the same time the deformation curve in the D -ensemble has a part with the “anomalous” negative slope.

When the globule is deformed by applied force, equilibrium intermediate state (either the tadpole or sparse globule) does not form; i.e., the globule “hurdles” these intermediate states. Horizontal plateaus on the dependences in Figure 7 are virtual since they correspond to the jump in the end-to-end distance only.

Note that the quasi plateaus with “anomalous” decay of the reaction force with increasing D in the D -ensemble may seem to be a sign of instability. However, in the position-clamp mode they do represent stable equilibrium states. Their “anomalous” shape is caused by the small size of the (nano)system. Nevertheless, it can be shown that the value of threshold force f_{tr} that induces the phase transition in the force-clamp mode can be found from the deformation curves obtained in the position-clamp mode with the aid of the Maxwell equal area rule,²² which is commonly used to exclude unstable states. Indeed, in the transition point $G_{globule}(f_{tr}) = G_{chain}(f_{tr})$. On the other hand, using the Legendre transform, eq 2:

$$G_{chain}(f_{tr}) - G_{globule}(f_{tr}) = F_{chain}(f_{tr}) - F_{globule}(f_{tr}) - f_{tr}(D_{chain} - D_{globule}) \quad (3)$$

Since

$$F_{chain}(f_{tr}) - F_{globule}(f_{tr}) = \int_{D_{globule}}^{D_{chain}} \frac{\partial F}{\partial D} dD = \int_{D_{globule}}^{D_{chain}} f dD \quad (4)$$

then

$$G_{chain}(f_{tr}) - G_{globule}(f_{tr}) = \int_{D_{globule}}^{D_{chain}} (f - f_{tr}) dD = 0 \quad (5)$$

and this means that the area between the horizontal solid line and the part of the dashed line above it is equal to the area between the horizontal solid line and the part of the dashed line below it. Fulfillment of the Maxwell rule in this case is a direct consequence of the correctness of the Legendre transform.

The threshold force f_{tr} is found to be smaller than the reaction force at the onset of the stretched phase formation in the D -ensemble and larger than the reaction force corresponding to disappearance of the globular phase in the D -ensemble. In other words, in the f -ensemble, a passage from the growing force branch at small deformations (when the globule shape is close to that of a prolate ellipsoid) to the unfolded open chain branch takes place at smaller deformation and/or force compared to the onset of the microphase segregation in the D -ensemble. This means that in the position-clamp mode a larger maximum extension of the ellipsoidal globule can be reached compared to the force-clamp mode. In the range of large deformations, a similar effect is observed: position-clamp mode allows obtaining less extended open chain states than is possible for the globule deformed in the force-clamp mode.

In Figure 7, one can find only one situation when deformation curves totally coincide in both ensembles, this corresponds to $N = 100$, $\chi = 0.8$ in Figure 7 b. In this case, the globule is deformed as a whole, its density decreases with extension and the force monotonously increases with deformation (or, vice versa, where the deformation increases as the value of applied force grows).

■ MODEL AND ANALYTICAL THEORY OF GLOBULE UNFOLDING IN THE F -ENSEMBLE

SF-SCF analysis of the globule unfolding in the position-clamp mode made in ref 17 allowed us not only to obtain thermodynamic characteristics of deformed globule and to calculate force–extension curves but also to analyze changes in the structure of the macromolecule upon increasing deformation. Using these findings, a simple model of extended globule was introduced and analytical mean field theory of the globule unfolding in the D -ensemble based on this model was developed in ref 18 that goes beyond the limits of the SCF modeling. In the present section, we extend the analytical approach suggested in ref 18 to the globule unfolding in the f -ensemble. Our approach consists in calculating and then comparing the free energies of the possible conformational states of the deformed globule (1–3) shown in Figure 3. Preliminary results of this part of work were presented in ref 25.

Weakly Deformed Globule. Similarly to ref 18, the shape of the unperturbed globule (at $f = 0$) formed by a macromolecule consisting of N monomer units and immersed into a poor solvent can be approximated by a sphere of uniform density. The sphere has a volume V , polymer density within the globule $\varphi = N/V$ is determined by the value of the Flory parameter χ (i.e., by solvent quality). The radius of the unperturbed globule is

$$R_0 = \left(\frac{3N}{4\pi\varphi} \right)^{1/3} \quad (6)$$

An important model assumption is the restriction imposed on possible deformation of the globule. It is assumed that upon extension, the globule undergoes only the shape deformation whereas its volume $V = 4\pi R_0^3/3$ is conserved: the sphere acquires a prolate shape which we approximate by a prolate uniaxial ellipsoid (often called spheroid), the major axis of the ellipsoid is equal to the given end-to-end distance D . Then the Helmholtz free energy of the globule deformed in the D -ensemble is¹⁸

$$F_{\text{globule}} = \mu N + \gamma S = \mu N + \gamma 4\pi R_0^2 g(x) \quad (7)$$

where $g(x)$ is a universal function of the extension parameter $x \equiv D/(2R_0)$

$$g(x) = \frac{S}{4\pi R_0^2} = \frac{1}{2x} + \frac{1}{2} \frac{x^2}{\sqrt{x^3 - 1}} \arcsin \sqrt{\frac{x^3 - 1}{x^3}} \quad (8)$$

In eq 7, the first term describes the preference for the monomer units to be in the globule, $\mu < 0$ is the monomer chemical potential in the globular phase. μ amounts to the monomer free energy change when it is transferred from the pure solvent (dilute phase, which is taken as a reference state for calculation of the chemical potential) to the globular phase.

The second term accounts for energetic penalties at the globule surface which are proportional to the surface area S , $\gamma > 0$ is the interfacial tension coefficient. In order to pass from the D - to the f -ensemble, the Legendre transform, eq 2, should be used. This gives for the Gibbs free energy:

$$G_{\text{globule}} = F_{\text{globule}} - fD = \mu N + \gamma 4\pi R_0^2 g(x) - fD \quad (9)$$

Equilibrium end-to-end distance (ellipsoid major axis) D at given applied force is obtained by minimizing G_{globule} , eq 9 with respect to D : $\partial G_{\text{globule}}/\partial D = 0$. This leads to equation that contains $D = D(f)$ in implicit form:

$$2\pi R_0 \gamma g'(x) = f \quad (10)$$

Here $g'(x)$ is the $g(x)$ derivative with respect to x :

$$g'(x) = \frac{1}{2} \left[-\frac{1}{x^2} + \frac{3x}{2(x^3 - 1)} + \frac{x(x^3 - 4)}{2(x^3 - 1)^{3/2}} \arcsin \sqrt{\frac{x^3 - 1}{x^3}} \right] \quad (11)$$

Note that in the D -ensemble the expression for the reaction force $f = f(D)$ was obtained in ref 18 according to $f = \partial F_{\text{globule}}/\partial D$ from eq 7. This expression coincides with eq 10.

Function $g'(x)$ is a nonmonotonic bounded function: it increases from zero at $x = 0$, passes through a maximum at $x = x^* \approx 2.1942$ corresponding to $g'(x^*) \equiv g^* \approx 0.2214$, and then decays with extension asymptotically tending to zero. Hence, in the framework of given model one can adequately describe globule extensions up to $D/(2R_0) \approx 2.2$, which corresponds to

Table 1. Values of Monomer Chemical Potential (μ), Polymer Density (φ), and Interfacial Tension Coefficient (γ) Calculated for Different χ Using SCF Approach¹⁸

	$\chi = 0.8$	$\chi = 1.0$	$\chi = 1.2$	$\chi = 1.4$	$\chi = 1.6$	$\chi = 1.8$	$\chi = 2.0$
μ	-0.10	-0.23	-0.37	-0.54	-0.71	-0.89	-1.08
φa^3	0.54	0.70	0.80	0.87	0.92	0.94	0.96
γa^2	0.088	0.18	0.27	0.38	0.48	0.58	0.67

applied forces that do not exceed a certain value

$$f \leq f^* = 2\pi R_0 \gamma g^* \quad (12)$$

Let us also give approximate expressions in the limit of small extensions that will be used in the following analysis:

$$\begin{aligned} g(x) &\approx 1 + \frac{2}{5}(x-1)^2 \\ g'(x) &\approx \frac{4}{5}(x-1) \end{aligned}, \quad x-1 \ll 1 \quad (13)$$

By substituting eq 10 and eq 13 into eq 9, approximate expression for the Gibbs free energy of the globule as a function of applied force in the limit of small forces is obtained:

$$G_{\text{globule}} \approx \mu N + 4\pi \gamma R_0^2 - 2R_0 f - \frac{5f^2}{8\pi \gamma} \quad (14)$$

Correspondingly, approximate expression for D :

$$D_{\text{globule}} \approx 2R_0 + \frac{5f}{4\pi \gamma} \quad (15)$$

The model described above depends on the (partial) parameters φ (polymer density within the globule), μ (monomer chemical potential within the globule), and γ (interfacial tension coefficient) characterizing the globular state. In our model it is assumed that they are virtually N -independent and can be found as functions of the solvent quality only. In ref 18, it was demonstrated that the dependence of φ and μ on χ can be easily found in the framework of the Flory lattice model of polymer solution:

$$\chi = -\frac{\log(1 - \varphi a^3)}{(\varphi a^3)^2} - \frac{1}{\varphi a^3} \quad (16)$$

$$\mu = 2 + \frac{2 - \varphi a^3}{\varphi a^3} \log(1 - \varphi a^3) \quad (17)$$

For the interfacial tension coefficient γ a closed form analytical solution was obtained for moderately poor solvent (close to the coil-globule transition point):

$$\gamma a^2 = \frac{3}{16} (1 - 2\chi)^2 \quad (18)$$

Alternatively, the values of φ , μ , and γ were calculated in¹⁸ from SCF modeling of free globules; they are presented in Table 1. The numerical results for φ and μ agree very good with the Flory theory in the whole range of χ , whereas for γ an agreement with analytical result is observed, as expected, only at small χ . In the following numerical calculations we will use the values of φ , μ , and γ given in Table 1. Hence, the proposed theory of globule unfolding is a quantitative one.

Dependences of G/N for deformed globule, eq 9, as functions of f at $0 \leq f \leq f^*$ for various N and χ are shown below in Figure 9.

Extreme right points correspond to the values of f^* increasing with an increase in χ .

Stretched Open Chain. When the applied force is large, the globule is completely unfolded and strongly stretched, all the monomer units are exposed to solvent. In refs 17 and 18, the Helmholtz free energy F_{chain} in the D -ensemble was calculated on the basis of the Gibbs free energy G_{chain} in the f -ensemble subjected to the Legendre transform, eq 2. Expression for the Gibbs free energy of a freely jointed chain consisting of N monomer units and subjected to the force f has the following form:

$$G_{chain} = -N \log \left\{ 1 + \frac{1}{2k} [\cosh(fa) - 1] \right\} \quad (19)$$

where k is the rigidity parameter of the chain. Equation 19 was obtained in ref 18 for the lattice model, and k is associated with the statistics of corresponding lattice walks.

The average end-to-end distance D at given force f is obtained by differentiation of the Gibbs free energy, eq 19, with respect to the force:

$$D = -\frac{\partial G_{chain}}{\partial f} = Na \cdot \frac{\sinh(fa)}{2k + \cosh(fa) - 1} \quad (20)$$

Equation 19 and eq 20 contain k as the only one parameter. In refs 17 and 18, the value of $k = 3/4$ was accepted. Below in Figure 9 a universal dependence of $D/(Na)$ on f is shown.

Let us also write down the approximate expressions for G_{chain} and D obtained for small forces via Taylor expansion:

$$G_{chain} \approx -N \frac{f^2 a^2}{4k} \quad (21)$$

$$D_{chain} \approx Na^2 \frac{f}{2k} \quad (22)$$

Approximate expressions are in good agreement with the exact ones only in a narrow force range: $0 \leq fa \lesssim 0.5$, the value of average extension turns out to be overestimated.

Microphase-Segregated "Tadpole" State. Consider now the third of the possible "candidates", which is the mixed microphase-segregated tadpole state where globular and extended part coexist in equilibrium. Let us assume that the ellipsoidal head comprises n monomer units and the tail, therefore, consists of $N - n$ units. Both the head and the tail are subjected to the force f . Then the Gibbs free energy of the microphase-segregated structure can be written using eq 8, eq 9, and eq 19

$$G_{tadpole} = G_{globule}(n, f) + G_{chain}(N - n, f) \quad (23)$$

(in expressions for $G_{globule}$, eq 9, and G_{chain} , eq 19, instead of N as argument, n and $N - n$, respectively, are substituted). The size of the head, n , assures phase equilibrium in the tadpole conformation, hence, the free energy $G_{tadpole}$ should be minimized with respect to n .

It was shown above, see Figure 5, that according to the SCF results the free energy of the two-phase state, $G_{tadpole}$, is larger than the free energies of the one-phase states, $G_{globule}$ and G_{chain} . Analytical theory provides us with opportunity of deeper analysis of this issue. Figure 8 presents as an example a series of dependences of the tadpole free energy on the distribution of monomer units between two phases. It can be seen that all $G_{tadpole}(n)$ curves are convex up, that is, $G_{tadpole}(n)$ has only

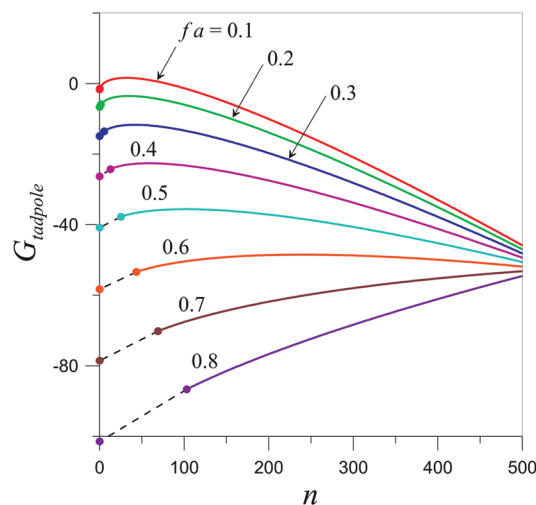


Figure 8. Gibbs free energy of the tadpole conformation as a function of the number of monomers in the globular head calculated for $N = 500$, $\chi = 1$, and various values of applied force f .

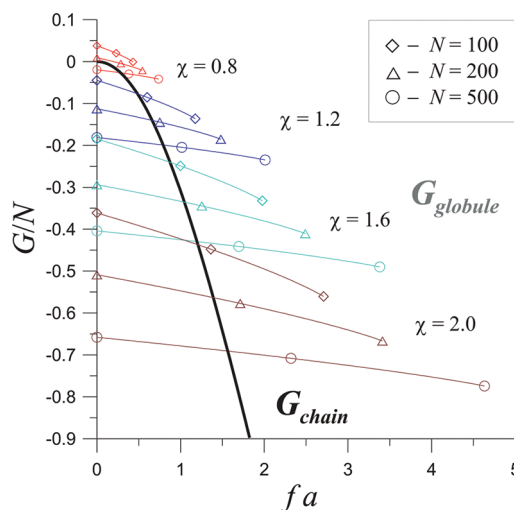


Figure 9. Gibbs free energy per monomer unit of compact globular and stretched states as a function of applied force for various values of N and χ calculated using analytical theory.

boundary minima corresponding to pure states: globule at $n = N$ or open chain at $n = 0$. Hence, in the equilibrium the microphase-segregated tadpole state with $0 < n < N$ is unstable and is not implemented. The system will be in one of two pure states: weakly extended compact globule at small forces or stretched open chain at large f . For the particular choice of parameters in Figure 8 ($N = 500$, $\chi = 1$), at $f \leq 0.5$ the globule state with $n = N$ is stable while at $f \geq 0.6$ the free energy minimum corresponds to the open chain state with $n = 0$. Therefore, the transition between two states occurs in the force range $0.5 < f < 0.6$. Note also that the curves in Figure 8 have a gap at small n between $n > 0$ and $n = 0$. This is because according to the condition (eq 12) small globular head becomes unstable if the applied force exceeds the threshold value $f = f^*$ which depends on the number of monomer units in the globular phase.

Gibbs Free Energy as a Function of Applied Force. In the preceding subsection, it was shown that a microphase-segregated

tadpole state is not formed in the globule subjected to a pulling force, the system can be found in one of two pure states: weakly extended ellipsoidal globule or stretched open chain. Therefore, for the analysis it suffices to consider the free energies of these two states. Note that in the framework of the approach that we use, only one state corresponding to the free energy minimum (or, equivalently, the ground state) is physically implemented in the system at the given value of the governing parameter (i.e., of the force).

Figure 9 shows dependences of the reduced Gibbs free energy G/N of two pure state of stretched macromolecules on applied force calculated using analytical theory for a series of N and χ values. As follows from eq 19, the monomer free energy in the open chain state, G_{chain}/N , is a universal function of the force independent of the chain length and the solvent quality whereas the globule free energy per monomer, $G_{globule}/N$, depends on both N and the solvent quality. $G_{globule}/N$ dependences are plotted in the range $0 \leq f \leq f^*$, where f^* is the limiting maximum value of the force determined by eq 12.

One can see that at small applied forces $G_{globule} < G_{chain}$ i.e., the global free energy minimum corresponds to the globule state. With an increase in the value of f the free energy difference for two states, $G_{chain} - G_{globule}$, decreases, and at some value $f = f_{tr}$ the curves intersect and $G_{globule} = G_{chain}$. At $f > f_{tr}$ $G_{chain} < G_{globule}$ and the globule is unfolded. Therefore, at $f = f_{tr}$ transition from the compact globule to the unfolded state occurs, and this transition is a jumpwise first order phase transition. With an increase of the Flory parameter χ (at fixed chain length) the position of the transition point moves to the right: indeed, the more dense the globule is, the more effort is needed to unfold it.

With a decrease in the chain length the value of the relative free energy $G_{globule}/N$ increases which is easily explained: surface area of the globule $\sim N^{2/3}$, correspondingly, the surface contribution to the free energy per monomer unit is $\sim \gamma N^{-1/3}$; i.e., it increases with decreasing N (volume contribution to the free energy per monomer unit is N -independent and equal to μ). Since G_{chain}/N does not depend on N this means that the larger is N , the larger f is required to unfold the globule. Increasing N also leads to broadening of the stability range for the globule state $0 \leq f \leq f^*$, in accordance with eq 12.

It is interesting to note that for relatively small value of $\chi = 0.8$ for $N = 200$ and 100 formally calculated free energy of the globule lies above the chain free energy in the whole f range. This means that the macromolecule does not form a globule. This is a consequence of the model simplifications. More accurate (and assumption-free) SCF calculations show that at $\chi = 0.8$ unfolding of small globules occurs according to conventional scenario of the phase transition: there are two branches that cross at the transition point. The difference is, however, that extension of a small globule in a moderately poor solvent in the D -ensemble occurs without formation of the microphase segregated tadpole state but with a progressive decrease in the density of the globule.¹⁷

Deformation Curves (Theory and SCF Modeling). Let us compare deformation curves $D = D(f)$ obtained in the force-clamp mode by SCF modeling and by using the analytical theory. In order to be able to compare deformation curves corresponding to different N and χ , not the absolute but the relative extension of the macromolecule with respect to its contour length, $D/(Na)$, should be considered. An example of such a comparison is presented in Figure 6 which was already discussed above (deformation curves calculated in SCF modeling are

shown by solid lines, “theoretical” curves—by dashed lines). One can see that a good agreement is observed for longer chain and for stronger solvent. At these values of parameters the suggested model properly accounts for main peculiarities of the globule behavior upon deformation in the force-clamp mode. While the applied force is small enough, the globule is good described by an ellipsoid of constant density, when the force reaches its threshold value, the globule unfolds according to “all-or-none” mechanism, and the end-to-end distance abruptly increases. The value of the jump grows with increasing N and χ . The threshold value of the force obtained in the framework of the theory is somewhat lower than that obtained from the SCF modeling.

With an decrease in the chain length and/or solvent strength the simple model fails to describe adequately the behavior of the globule. Note that in Figure 6 a for $N = 100$ $\chi = 0.8$ and 1.0 and $N = 200$, $\chi = 0.8$ there are only curves obtained by SCF modeling (solid lines) whereas corresponding theoretical curves are missing. In all these cases globule deformation in the conjugated D -ensemble occurs by strong extension and depletion (decrease in the density) of the globule which cannot be taken into account in our simple model. However, at $N = 100$ and $\chi = 1.0$, as well as at $N = 200$, $\chi = 0.8$, $f(D)$ dependence in the D -ensemble contains a part with “anomalous” dependence, see Figure 7, hence, in the f -ensemble $G(f)$ dependence has a loop and globule unfolding occurs with a jump (in this case “depleted” states are “hurdled”), Figure 6 a. At $N = 100$ and $\chi = 0.8$ $f(D)$ dependence in the D -ensemble is monotonously increasing which lead in the f -ensemble to monotonous $G(f)$ and $D(f)$ dependences. Moreover $D(f)$ dependence in the f -ensemble is obtained by simply inverting the $f(D)$ dependence in the D -ensemble.

The results here presented demonstrate a good agreement between SCF modeling and analytical theory based on simple model. This correspondence is seemed to us essential in two respects.

On the one hand, data of the SCF modeling were not obtained by directly modeling (i.e., performing SCF calculations) globule unfolding in the f -ensemble but were derived from the results of SCF modeling in the D -ensemble using the principles of statistical physics. The obtained agreement gives support to the correctness of such an approach.

On the other hand, the observed correspondence demonstrates a wide applicability range of the analytical approach and allows using the results of the analytical theory for analysis of the system behavior.

Transition Point. Analytical theory allows to carry out a more detailed study of the unfolding transition point and of the changes in globule characteristics at the transition. In the transition point, the free energy minima corresponding to the globule and the open chain states have the same depth and the position of the transition can be found, therefore, from the following equation:

$$G_{globule}(N, f_{tr}) = G_{chain}(N, f_{tr}) \quad (24)$$

Figure 10 shows the dependence of the threshold force f_{tr} on the polymerization degree N at different values of the Flory parameter χ while in Figure 11 corresponding dependences of the average end-to-end distance in globule (lower branches) and open chain (upper branches) states calculated in the transition point are presented. One can see that with an increase in χ and/or N both the threshold force, f_{tr} , and the jump in the end-to-end

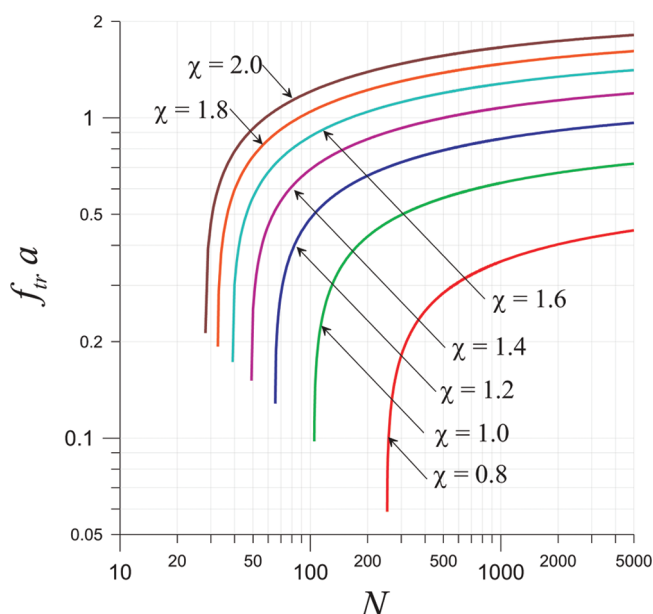


Figure 10. Threshold (transition) force in the f -ensemble as a function of polymerization degree at various values of χ .

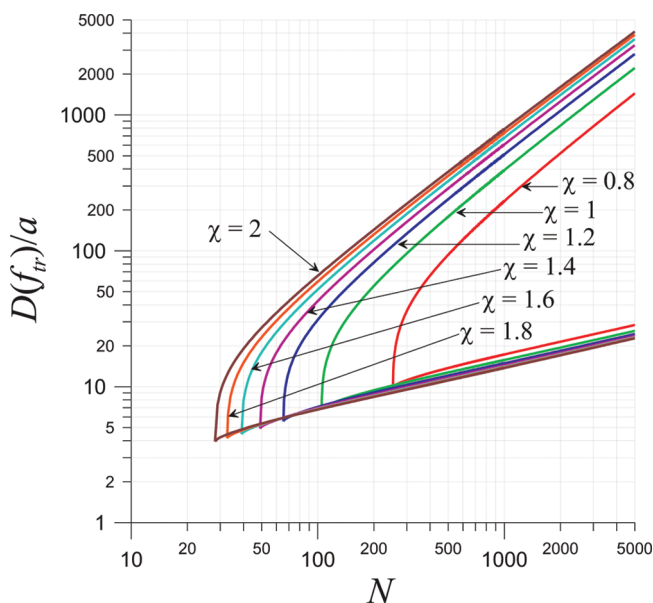


Figure 11. Globule and open chain extension at threshold (transition) force as a function of polymerization degree at various values of χ . The lower branch corresponds to the globule state and the upper branch to the open chain state.

distance, $D_{\text{chain}}(f_{\text{tr}}) - D_{\text{globule}}(f_{\text{tr}})$, increase. At small N , two branches corresponding to D_{chain} and D_{globule} meet at a certain point $N = N_{\text{cr},f}(\chi)$ ($f_{\text{tr}}(N)$ also terminates in this point). At $N < N_{\text{cr},f}$, eq 24 has no solution (and the model itself is not applicable at small N where the crucial assumption about conservation of the globule density ϕ upon extension does not work; this is also illustrated by Figure 9). It can be concluded that $N = N_{\text{cr},f}$ is a critical point for the given model.

To discuss the obtained dependences let us make use of approximate expressions for the Gibbs free energy of the globule,

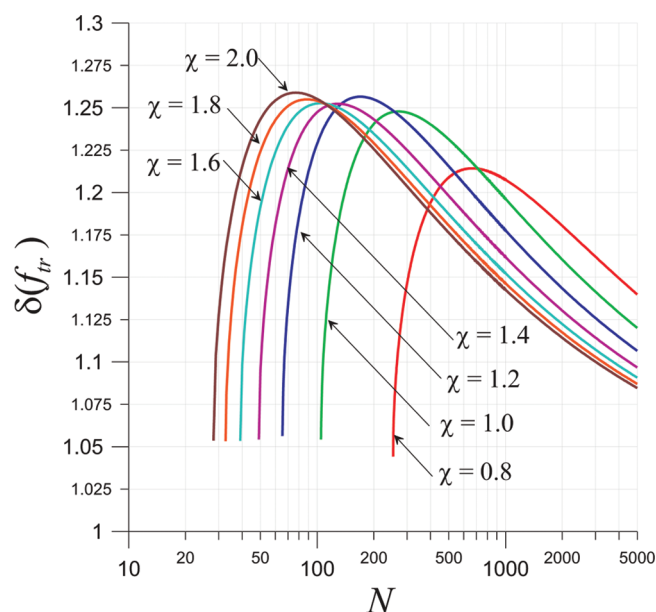


Figure 12. Asymmetry of the globule in the transition point as a function of chain length at various values of χ .

eq 14, and the open chain, eq 21, at small applied forces $fa \ll 1$, and the corresponding expressions for the average end-to-end distance, eq 15 and eq 22. By equating the free energies of two states, a quadratic equation with respect to f is obtained:

$$\mu N + 4\pi\gamma R_0^2 - 2R_0f + \frac{1}{4k}f^2a^2N - \frac{5f^2a^2}{8\pi\gamma} = 0 \quad (25)$$

By keeping only leading in N terms (of the order N and $N^{2/3}$) we obtain that

$$f_{\text{tr}} \approx \frac{2\sqrt{k|\mu|}}{a} (1 - \Delta_{\text{tr}}) \quad (26)$$

where

$$\Delta_{\text{tr}} = \left(\frac{9}{2}\right)^{1/3} \frac{\gamma}{|\mu|} \left(\frac{\pi}{\phi^2 N}\right)^{1/3} \quad (27)$$

is the first correction term of the order $1/N^{1/3}$. With an increase in N this term decreases and the threshold force increases tending to the limiting (maximum) value $f_{\text{tr}}(N \rightarrow \infty) = 2(k|\mu|)^{1/2}/a$. The value of the limiting force grows with χ due to $\mu(\chi)$ dependence. The correction term Δ_{tr} weakly decreases with an increase in χ (as follows from the data of Table 1). Using eq 26, eq 15, and eq 22 for the average end-to-end distance, asymptotic expressions for D_{globule} and D_{chain} in the transition point can be found:

$$D_{\text{tr,globule}} \approx 2R_0 + \frac{5f_{\text{tr}}}{4\pi\gamma} \approx \left(\frac{6N}{\pi\phi}\right)^{1/3} + \frac{5\sqrt{k|\mu|}}{2\pi\gamma} (1 - \Delta_{\text{tr}}) \quad (28)$$

$$D_{\text{tr,chain}} \approx Na^2 \frac{f}{2k} \approx Na \sqrt{\frac{|\mu|}{k}} (1 - \Delta_{\text{tr}}) \quad (29)$$

As follows from eq 28, $D_{\text{tr,globule}} \sim N^{1/3}$ and weakly depends on χ . The value of $D_{\text{tr,chain}}$ at not too small N and χ is proportional to

N . The difference between $D_{tr,globule}$ and $D_{tr,chain}$ demonstrates a jump in the size in the transition point that grows with an increase in N and χ .

Consider now another characteristic of the globule in the transition point—the asymmetry, or the long-to-short axis (longitudinal-to-transverse size) ratio $\delta = D/(2R_{\perp})$ presented in Figure 12. One can see that the asymmetry in the transition point is a nonmonotonous function of N : with an increase in N it grows, passes through a maximum, and then decays. Position of the maximum depends on the value of the Flory parameter: as χ increases, it shifts toward smaller N . Note that the height of the maximum is a little larger than 1.25; i.e., the shape of the globule in the transition point only slightly deviates from the spherical one (when $\delta = 1$).

The nonmonotonicity of the function $\delta(N)$ follows also from simple arguments. The asymmetry δ is related to the degree of stretching of the globule provided that the globule volume is conserved. As it was shown in,¹⁸ $\delta = \alpha^{3/2}$, where $\alpha = D/(2R_0)$ was used above in eq 8, eq 11, and eq 13. It follows from eq 28 that

$$\alpha = 1 + C_1 N^{-1/3} (1 - C_2 N^{-1/3}) \quad (30)$$

where C_1 and C_2 are N -independent functions of χ . It is easy to see that α is a nonmonotonous function of $N^{-1/3}$ with a maximum at $N^{1/3} = 2C_2$.

DISCUSSION

Deformation Curves and Characteristics of the Transition.

In Figure 7, force—extension curves in two modes of extension calculated by using SCF modeling are compared. Similar dependences were calculated using the analytical theory (not presented here). It was shown above how the complex deformation curve obtained in the position-clamp mode is transformed into connected by a jump two branches of the deformation curve in the force-clamp mode, see Figure 4 and Figure 7. From the general thermodynamic consideration it follows that the value of f_{tr} at which the abrupt unfolding transition takes place in the force-clamp mode (in the f -ensemble) can be found from the Maxwell rule applied to the deformation curve in the position-clamp mode (in the D -ensemble); see eqs 3–5 above. Therefore, if the globule is unfolded by applying a force, the states corresponding to “anomalous” part of the deformation curve in the D -ensemble characterized by the force decay with increasing extension D are “hurdled”. It is this range of extensions in the position-clamp mode where multistep globule unfolding occurs including (i) formation of extended tail, (ii) growth of the latter with extension in the phase coexistence regime, and (iii) the abrupt breakdown of the diminished globular head.

Let us compare the value of the threshold force f_{tr} in the f -ensemble with characteristic values of the reaction force in the D -ensemble: the force f_1 at the extension D_1 corresponding to the onset of extended phase formation (i.e., to formation of the tadpole) and the force f_2 at the extension D_2 where the globular phase disappears. Analytical expressions for f_1 and f_2 were derived in ref 18 in the same approximation as eqs 26–29 and have the following form:

$$f_i = \frac{2\sqrt{k|\mu|}}{a} (1 - \Delta_i) \quad (31)$$

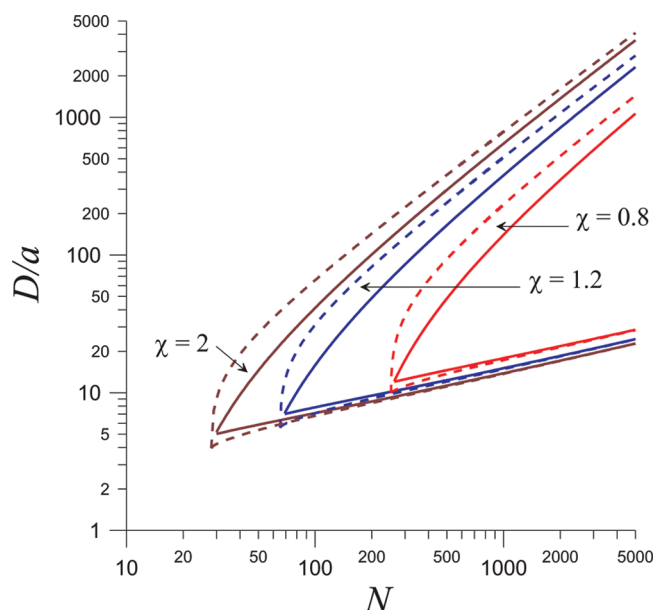


Figure 13. Globule and open chain extension at threshold force, $D_{tr}(globule)$ and $D_{tr}(chain)$, in the f -ensemble (solid lines) and phase coexistence boundaries, $D_1(globule)$ and $D_2(chain)$, in the D -ensemble (dashed lines) as functions of polymerization degree calculated at different χ values. Lower branches correspond to the globule and upper branches to the unfolded open chain state.

where $i = 1, 2$ and

$$\Delta_i = K_i \left[\frac{\gamma}{|\mu|} \left(\frac{\pi}{\phi^2 N} \right)^{1/3} \right]^{\alpha_i} < 1 \quad (32)$$

Here $\alpha_1 = 1$, $\alpha_2 = 3/4$, and $K_1 = (4/3)^{1/3}$, $K_2 = 3^{3/4} 2^{1/4}$. Comparing eq 31 and eq 26 one can see that f_{tr} , f_1 , and f_2 differ only in Δ_i . It is easy to check that $\Delta_1 < \Delta_{tr} < \Delta_2$ and, correspondingly, $f_1 > f_{tr} > f_2$. Indeed, Δ_1 and Δ_{tr} differ only in numerical coefficients, hence, the left part of inequality is obvious. For Δ_{tr} and Δ_2 one has

$$\frac{\Delta_{tr}}{\Delta_2} = \frac{1}{3^{1/3} 2^{2/3}} \Delta_2^{1/3} < 1 \quad (33)$$

The latter inequality follows from the observation that $\Delta_2 < 1$.

The end-to-end distances D_1 and D_2 in pure globular and extended phases are related to reaction forces f_1 and f_2 by expressions similar to eq 28 and eq 29 via changing f_{tr} and Δ_{tr} to f_1 , Δ_1 and f_2 , Δ_2 , respectively. From the inequality connecting f_{tr} , f_1 , and f_2 , it follows that

$$\begin{aligned} D_{tr}(globule) &< D_1(globule) \\ D_{tr}(chain) &> D_2(chain) \end{aligned} \quad (34)$$

Phase Diagrams. In Figure 13 phase diagrams of deformed globule in two modes of deformation are presented in (N, D) coordinates for various values of χ . For the f -ensemble, upper and lower branches of the diagram are determined by the values of $D_{tr}(globule)$ and $D_{tr}(chain)$ calculated in the transition point. For the D -ensemble the branches are the phase coexistence boundaries $D_1(globule)$ and $D_2(chain)$. Both diagrams have similar shape (both upper and lower D 's grow with an increase in N),

phase diagrams for the D -ensemble lie completely within corresponding diagrams for the f -ensemble. In both ensembles the area below the lower boundary corresponds to globular state whereas the area above the upper boundary - to completely unfolded “open” state. However, an essential difference between the diagrams is that the area inside the diagram in the D -ensemble corresponds to real stable microphase segregated tadpole state while in the f -ensemble these state are unattainable. The “difference” between diagrams (i.e., area between solid and dashed lines) are related to the above-discussed pure states of “strongly stretched globule” (below) and “weakly stretched open chain” (above) which can be obtained only in the position-clamp mode.

At small N , diagrams for both deformation modes have a critical point, but the position of the latter is slightly different in two ensembles. For the globule in the D -ensemble the critical point corresponds to the smallest chain length (for a given solvent quality) which makes microphase segregation within the globule (i.e., formation of the tadpole structure) possible. In the critical point the range of extensions where the tadpole structure is stable [$D_1(\text{globule}); D_2(\text{chain})$] degenerates into a point. On the left of the critical point in the D -ensemble deformation of the globule gives rise to its gradual elongation accompanied by the decrease in density.

The critical point in the force-clamp mode where the globule phase diagram ($f_{tr}(N)$ curve, Figure 10) ends and the jump in the end-to-end distance $D_{tr}(\text{chain}) - D_{tr}(\text{globule})$ disappears is a critical point for our particular model of the globule unfolded by applied force. We recall that phase diagrams for two modes of extension presented in Figure 13 were calculated in the framework of the simple model assuming extension-independence of the globule density. A true critical point in the f -ensemble must correspond to shorter chains, as it follows from the results of SCF modeling, because $f(D)$ dependence in the D -ensemble remains nonmonotonous even if intramolecular microphase segregation does not occur and globule is deformed “as a whole”, with decreasing density, if we are not far from the critical point in the D -ensemble. Such a nonmonotonicity becomes apparent through “anomalous” $f = f(D)$ dependence in the position-clamp mode corresponding to the decrease of D with increasing f . In the force-clamp mode this range of extensions is always “hurdled”. In the true critical point for the f -ensemble $N_{cr,f}(\chi)$ this nonmonotonicity disappear and at $N < N_{cr,f}$ deformation curves for both ensembles coincide.

CONCLUSIONS

We have presented a theoretical study of homopolymer globule unfolding by a force applied to the ends of the macromolecule forming the globule, i.e., in the force-clamp mode of extension equivalent to thermodynamic f -ensemble. Two approaches were used to solve the problem: first, the results of SF-SCF modeling of globule unfolding in the conjugate D -ensemble, or, equivalent, in the position-clamp mode, were “translated” into the f -ensemble. Namely, the dependence of the Helmholtz free energy and the reaction force on the imposed end-to-end distance in the D -ensemble were transformed according to the Legendre transform, eq 2, to obtain the Gibbs free energy and equilibrium end-to-end distance as functions of applied force in the f -ensemble, the scheme of this “translation” is shown in Figure 4. On the other hand, analytical mean-field theory of the globule unfolding in the force-clamp mode was developed by

using simple model of deformed globule introduced in.¹⁸ The theory makes it possible to go beyond the limits of the SCF calculations: first of all, it allows calculations for large N , where the system size is large and numerical SCF calculations become very time and memory consuming. Moreover, in the framework of the developed theory it is easy to calculate the transition point and to find corresponding conformational characteristics in a wide range of N and χ .

One of the main goals of the study was to compare the globule unfolding in two modes of extension. Our analysis, like all previous analysis of this problem, shows that the globule behaves essentially differently in these two situations. In the position-clamp mode, the globule unfolding upon an increase in the end-to-end distance occurs via formation of the microphase-segregated tadpole conformation with globular head and stretched tail coexisting in equilibrium in a wide range of extension. As the extension increases, the tail size grows, the head diminishes and this is accompanied by “anomalous” decrease of the reaction force with extension. However, when the head contains approximately $\sim N^{3/4}$ monomer units, it loses its stability and disintegrates jumpwise. As a result the force–extension curve has a complex structure. This behavior in the position-clamp mode is caused by the small size of the system under consideration. Polymer globule is a typical example of a nanosystem where surface effects play a significant role. An additional circumstance is the linear memory—connectivity of monomer units into a chain.

In the force-clamp mode no such peculiarities are manifested. Mechanical unfolding of a globule by applied force occurs without intramolecular microphase segregation (formation of microphase-segregated tadpole state): at certain threshold value of the pulling force the globule unfolds as a whole. This transition (of “all or none type”) is accompanied by a jump in the end-to-end distance. The values of both the threshold force and of the jump in the end-to-end distance in the transition point grow with an increase in N and/or solvent strength.

In our previous work,¹⁸ statistical-mechanical analogy between globule unfolding in the D -ensemble and liquid–gas transition in the van der Waals gas in the (V,T) -ensemble below the critical temperature, $T < T_{cr}$, was discussed. It was shown that in spite of the obvious similarity between phase states (globular phase \leftrightarrow liquid, stretched (unfolded) phase \leftrightarrow gas), governing parameters (end-to-end distance $D \leftrightarrow$ volume V) and rearrangements in the systems upon increase in the governing parameter [dense phase (globular or liquid) \rightarrow phase coexistence \rightarrow low density phase (unfolded or gas)], comparison of $f = f(D)$ and $p = p(V)$ curves indicate a marked difference in the behavior of small (globule) and macroscopic (gas) systems which is observed in the phase coexistence regime. In the macrosystem $[(V,T)$ -ensemble] the pressure p is constant at any volume where two phases coexist while in the extended small globule the reaction force dependence on D is “anomalous”: an increase in D in the phase coexistence regime is accompanied by a decrease in f . In the asymptotic (thermodynamic) limit $N \rightarrow \infty$, however, the reaction force in the phase coexistence regime is constant (i.e., in eq 31 and eq 32, $\Delta_1 = \Delta_2 = 0$ and $f_1 = f_2$).

In the f -ensemble (force-clamp mode) considered in the present paper, the small system (globule) changes its phase state jumpwise with an increase in applied force, similarly to what occurs in macroscopic system in the (p,T) -ensemble. The state curves for the macroscopic system in (p,V) coordinates coincide in conjugate ensembles while for the globule, the state

(i.e., deformation) curves differ in conjugate ensembles and satisfy the Maxwell area rule (Figure 7 and eqs 3–5); the latter gives the value of the threshold force f_{tr} corresponding to the all-or-none transition in the f -ensemble.

Comparison of the deformation curves in two ensembles also shows that not only the microphase segregated tadpole state but also some of the pure states that are stable in the D -ensemble cannot be accessed in the f -ensemble. This concerns “strongly extended globules” (close to the ellipsoid-tadpole transition point in the D -ensemble) and “weakly stretched open chains” (just after the tadpole-open chain [i.e., complete unfolding] transition point in the D -ensemble). Or, in other words, the stretched phase is obtained at larger extensions in the position-clamp mode than in the force-clamp mode. Moreover, in the former case only a portion of the macromolecule is in the stretched phase whereas in the latter case the whole macromolecule is unfolded and stretched. Similarly, formation of a globule upon a decrease in the extension of a strongly stretched chain (i.e., folding of the macromolecule into a globule) in the f -ensemble occurs easier (at larger extensions) than a nucleation of the globular phase in the D -ensemble.

We have shown that the jump in the globule unfolding in the force-clamp mode is directly related to the “anomalous” part on the force–extension dependence in the position-clamp mode: the states corresponding to the force decay are unstable (unfavorable) in the f -ensemble and the system simply jumps over these states. When $f = f(D)$ curve in the D -ensemble is monotonously increasing, globule unfolds similarly in two modes, deformation curves coincide, and this case corresponds to the precritical regime $N < N_{cr,f}(\chi)$, where $N_{cr,f}$ is the critical point for the f -ensemble, or the minimal chain length necessary for the jump-wise unfolding of the globule. The value of $N_{cr,f}$ is slightly less than the critical point for the D -ensemble, $N_{cr,D}$, a minimal chain length below which the intramolecular microphase segregation in the extended globule does not occur and the globule is deformed “as a whole”, without intramolecular segregation but by progressive “dissolution” of its core.

Let us mention that the developed theory may also help in understanding the unfolding of more complex globular structures. Indeed, in the position-clamp mode, force–extension curves of both homopolymer globule and globular protein are nonmonotonous consisting of ascending and “anomalous” descending force branches. In the homopolymer case there is one “anomalous” part whereas on the protein deformation curves—they are multiple, separated by ascending parts, thus leading to appearance of the “sawtooth” pattern (Figure 1a). Drawing an analogy with the homopolymer globule unfolding, we can relate each decreasing part of the protein globule force–extension curve with unraveling of individual domains of the protein. In the force-clamp mode, deformation (extension vs force) curves of homopolymer and protein globules have a “staircase” structure. The homopolymer globule “staircase” has only one step corresponding to jumpwise complete unfolding of the globule. Deformation curve of globular protein has several steps, each one is related to unfolding of individual protein domains. Therefore, one can speak about universal features of both simple (homopolymer) and complex globule unfolding in the D - and f -ensembles.

Finally, let us recall that both the theory we developed and the SCF approach are of the mean-field type that neglect fluctuations around the ground state for the considered system. On the other hand, proper account of fluctuations and exact calculation of the partition function of a deformed macromolecule may help in

revealing fine differences in behavior of finite-length polymer chain deformed in D - and f -ensembles. There is an ongoing discussion in the literature about possible inequivalence of statistical ensembles in stretching of individual macromolecules, even in simplest “minimal” polymer models such as Gaussian^{26,27} and semiflexible²³ chains in athermal solvent. A comprehensive critical review of these and related works can be found in.²⁴ In the case of the globule deformation considered in the present paper, marked differences in deformation behavior in two ensembles are clearly seen already on the mean-field level. Taking into account fluctuations should lead to “smoothing” of the transition—instead of a jump, the transition should occur in a narrow interval of the governing parameter. This issue will be considered in detail in a forthcoming work; in particular, attention will be devoted to fluctuations in the distribution of monomer units between globular and stretched phases in the deformed globule.

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