Topological repulsion between polymer globules

Jeffrey Chuang, Alexander Yu. Grosberg, and Toyoichi Tanaka
Department of Physics and Center for Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

(Received 16 August 1999; accepted 14 January 2000)

The work is motivated by the experimental observation of B. Chu *et al.* [Macromolecules **28**, 180 (1995)] which suggests that, while polymer globules in dilute solution in poor solvent are supposed to be very sticky, in actuality they collide many hundreds of times before merging and before aggregation starts. We argue that this slow-down is caused by an ''entanglement force'' which is operational on the prereptational time scale. This force arises from the fact that two touching globules cannot enjoy the mixing entropy gain expected in equilibrium until after they explore all conformations, including entangled ones. We report a molecular dynamics simulation in which we were able to measure the entanglement force as a function of distance. The important conclusion we can formulate so far is qualitative; the entanglement force exists and is sufficient to explain the observed slow-down of aggregation. © *2000 American Institute of Physics.* [S0021-9606(00)50214-1]

I. INTRODUCTION

One of the longstanding puzzles in polymer physics is the aggregation of globules, the process in which polymer chains in poor solvent clump together to form a macroscopic aggregate. Despite the seemingly trivial nature of the process, its current scientific understanding is quite limited.

A. The problem: Homopolymer globules are not as sticky as one might naively think

To be specific, imagine the following experiment. Let us take a dilute solution of polymer chains equilibrated in a good solvent, and then abruptly subject it to a temperature quench, during which the solvent quality becomes very poor. If the concentration of chains is low enough, every chain collapses well before different chains have a chance to meet and stick to each other, and the aggregating particles will be compact globules. Polymer chains in poor solvent attract each other very strongly. Therefore their aggregation is expected to follow the standard diffusion-limited aggregation scenario, which posits that two globules merge every time they meet each other in the course of Brownian motion. This would lead to the expectation that aggregation should develop in about the Smoluchowski[1] time

$$\tau_S \simeq \frac{1}{4\pi DRc} \simeq \frac{3}{2c}\frac{\eta}{k_B T}, \tag{1}$$

where D and R are the diffusion coefficient and radius, respectively, of a single polymer (globule), c is the concentration (the number of polymers per unit volume), η is the solvent viscosity, and T is the temperature. Meanwhile, in the most accurate experiment,[2] no significant aggregation was seen until after a much longer time. Specifically, under the conditions of the experiment,[2] $\tau_S \approx 0.1$ s, while aggregation actually started only after about 10 min. Thus the discrepancy between the naive theory and observation is several orders of magnitude. In other words, globules in poor solvent appear to bounce against each other several thousands of times before ''successful'' merging takes place.

A simple argument shows why the aggregation kinetics may indeed be nontrivial. This argument involves comparison of the chain states before and after aggregation. Initially, in the dilute solution, every collapsed globule has a size of the order of $aN^{1/3}$, with a being some microscopic size associated with monomer volume, and N the number of monomers per chain. However, at the end of the process, when the macroscopic aggregate is formed and exists as essentially a polymer melt, each chain should obey the Flory theorem,[3] i.e., have a Gaussian size of the order of $aN^{1/2}$. Thus, somewhat counterintuitively, chain relaxation in a poor solvent involves *swelling* from a compact to Gaussian conformation at the late stages. How this occurs is not known. One may speculate that some kind of reptation mechanism must be involved, but the details remain to be understood.

As suggested by the anonymous referee, the problem at hand should be also compared with that of kinetics of intermixing between two initially segregated polymer blends. Indeed, when two globules approach and touch each other, the interaction between their fringes is reminiscent of that between two flat surfaces of two separate polymer melts which are brought in a close contact with one another. This latter problem has been extensively examined by P.-G. de Gennes and his followers in the 1980s.[4–6] Two regimes were identified, depending on the value of the product χN_e, χ being the Flory parameter and N_e being the entanglement length; interdiffusion of polymers follows the Rouse mechanism when $\chi N_e \geqslant 1$, while reptation dominates the mixing process when $\chi N_e \ll 1$. Consideration of these two regimes highlights the important difference between intermixing of two blends and that of two globules. Indeed, both Rouse diffusion and reptation occur for the melt chains, which are Gaussian both at the beginning and at the end of the process. By contrast, our chains are rather far from Gaussian throughout the process; each chain starts as a globule and finishes confined in a

doubled volume which is still much smaller than the Gaussian size. Nevertheless, we shall exploit later the useful insights generated by the comparison between our system and that of the works.[4–6]

B. Why is it important?

The problem just described, which is the clumping kinetics of sticky globules in poor solvent, even apart from its significance as a fundamental issue in polymer physics, has bearing on a number of biophysical phenomena. We mention here but a few.

Protein folding is the most obvious major problem having to do with polymer chain collapse. On the one hand, most *in vitro* folding experiments, starting with that of Anfinsen,[7] have been and are still performed with very dilute protein solutions. This low concentration obviously contrasts with the overcrowded environment in which folding occurs *in vivo*. On the other hand, *in vitro* folding depends on protein concentration in a very nontrivial way.[8] If the conditions (such as temperature, *p*H, salinity, etc.) are ''right,'' folding develops correctly for the majority of chains in a fairly broad range of concentrations. But if conditions are ''wrong,'' aggregation cannot practically be prevented by any dilution which leaves the amount of dissolved proteins observable. Thus, it is a pressing necessity to understand the interaction between different protein molecules in the course of their folding.

To take the issue simply, we can imagine an egg. Its content is a concentrated solution of proteins. A raw egg remains liquid for many days, that is, proteins do not aggregate during this long time. However, if the temperature is elevated, protein globules denature, and then aggregation occurs very quickly. This is why the content of a hard boiled egg is a solid, not a liquid. Note that this process is completely irreversible, in seeming contradiction to the stability of the original liquid state.

Of course, proteins are heteropolymers, and that is of central importance. In particular, what happens to an egg is typically explained by saying that each protein globule has a hydrophobic core and a hydrophilic fringe, so when two protein globules meet, they only contact with their hydrophilic peripheral parts, which are obviously not sticky. This argument notwithstanding, the slow aggregation of homopolymer globules may shed now a new light on protein aggregation kinetics. Indeed, it may actually happen that the hydrophilic shell of each protein globule need not be so ideally insulating.

Another problem which may benefit from better understanding of aggregation is that of kinetics of a single polymer collapse. This problem has attracted a great deal of attention in recent decades, starting from Ref. 9 (see Ref. 10, and references therein). Although there are several different theoretical ways to model this process, all authors agree that collapse occurs first in small parts of the chain, and then subglobules (variably called ''raisins,'' ''domains,'' ''plums,'' ''beads,'' etc.) merge at the later stages. In some fundamental sense, merging of these subglobules is similar to aggregation of different globules.

C. Entanglements as a possible mechanism slowing aggregation down

The major subject of the present paper is to examine what we see as the most plausible explanation for the slow aggregation rate of globules in poor solvent. Our idea stems from the above mentioned argument that every chain in an equilibrium aggregate must be much less compact than it is in a single globule state. For instance, consider two globules situated next to each other. Equilibrium theory[11] suggests, in accordance with naive qualitative expectations, that the free energy of such a system is significantly lower than that of two separate globules. This gives rise to the significant and negative (osmotic) second virial coefficient of the solution of globules. However, gaining this lucrative extra free energy from merging requires that both chains strongly penetrate each other, such that each of them is spread over the double volume. This cannot occur very quickly, since most likely it can be achieved only through some kind of reptation. On the (long) time scale before reptation can take over, the two globules, therefore, behave as if inter-reptation was totally suppressed, that is, as two ring polymers. In that case, conformational entropy cannot be gained, and so merging globules may be much less favorable in terms of ''free energy'' than one would expect in equilibrium. We speak here about ''free energy,'' not free energy, to emphasize that this consideration is only valid on a restricted time scale shorter than that of reptation.

Let us now return to the central point of the argument presented above and look in some further detail as to why merging of chains is entropically suppressed in the absence of reptations. To understand this, let us consider two strongly overlapping rings. Forget for a second about excluded volume and all other constraints, and suppose that the conformations of both rings are chosen at random. For this situation, it was shown a long time ago[12] (see also Ref. 13) that the two rings will be topologically linked together with probability of almost 100%. It is even more true if both rings are taken to have compact globular shapes. That means when we take two rings which are not linked together and force them to come close, they will face topological prohibition of the majority of their conformations. Thus, the approaching of two unlinked rings is entropically unfavorable. Furthermore, if we try to force two rings together, we expect to experience an entropic counterforce. It is this entropic counterforce effect that we will demonstrate and examine in this paper using the molecular dynamics technique.

D. Our computer experiment

Specifically, we report in this work the results of the following computer experiment. We take a pair of globules and apply an external force that presses the globules against one another. Since reptation does not occur on the time scale available to our current computational possibilities, globules are guaranteed not to merge. This is manifested by the fact that the globule centers approach each other after the force is applied, but do not become completely coincident, instead coming to some steady, or quasiequilibrium, state. At this state, the external force is balanced by the counterforce of

topological, entanglement, origin, which is what we wish to study. Thus, by measuring the quasiequilibrium separation of globules against the applied external force, we will obtain $f(s)$, the topological force developed between the globules as a function of the distance between them.

II. MODEL AND METHOD

We use a dimensionless Rouse molecular dynamics model[14,15] in the spirit of such works as Refs. 16 and 17. Solvent entrainment effects are not included, as we wish to understand the properties of the system determined solely by the polymer. We assume a Lennard-Jones force to operate as the pair interaction between monomers. This is the only excluded volume force present in our system.

A. Basic equations

In the Rouse model, the inertial term in the equation of motion is neglected, leading to the first order differential equation:

$$\xi\frac{d\mathbf{x}_i}{dt}=\mathbf{F}_{\text{chain}}(i,i+1)+\mathbf{F}_{\text{chain}}(i,i-1)+\mathbf{F}_{\text{therm}}(t)+\frac{4\epsilon^*}{\sigma^2}\sum_{i\neq j}\mathbf{x}_{ij}\left[12\left(\frac{\sigma}{x_{ij}}\right)^{14}-6\left(\frac{\sigma}{x_{ij}}\right)^{8}\right]. \tag{2}$$

Here ξ is the friction coefficient, $\mathbf{x}_i$ is the position of the ith monomer in a chain, $\mathbf{F}_{\text{therm}}(t)$ is the random thermal force, and ϵ^* and σ are the energy and the length scales, respectively, associated with the Lennard-Jones force. The thermal force has delta-function time correlation and variance $2\xi k_BT$ in each cartesian direction.[18] $\mathbf{x}_{ij}$ is the vector $\mathbf{x}_j-\mathbf{x}_i$. The $\mathbf{F}_{\text{chain}}$ terms are a modified springlike attraction between connected monomers along the chain. Implicitly included in this chain force is the bond length a.

These equations can be rewritten in dimensionless form:

$$\frac{d\mathbf{y}_i}{d\tau}=\mathbf{G}_{\text{chain}}(i,i+1)+\mathbf{G}_{\text{chain}}(i,i-1)+\mathbf{F}_{\text{gauss}}(\tau)+4\epsilon\sum_{i\neq j}\mathbf{y}_{ij}\left[\frac{12}{y_{ij}^{14}}-\frac{6}{y_{ij}^{8}}\right]. \tag{3}$$

Our dimensionless units are given by the relations,

$$y_i=\frac{x_i}{\sigma},\quad \tau=\frac{k_BT}{\sigma^2\xi}t,\quad \epsilon=\frac{\epsilon^*}{k_BT},\qquad \mathbf{G}_{\text{chain}}=\frac{\sigma}{k_BT}\mathbf{F}_{\text{chain}},\quad \mathbf{F}_{\text{gauss}}(\tau)=\frac{\sigma}{k_BT}\mathbf{F}_{\text{therm}}(t). \tag{4}$$

For a free particle in a viscous solvent, $\sigma^2\xi/k_BT$ is the time it takes for a particle to diffuse one Lennard-Jones length. Thus, τ is the time measured in Lennard-Jones length diffusion times. Note that if we consider Eq. (3) without the first and third lines on the right-hand side, then we are left with the diffusion equation, reduced to parameterless form.

$\mathbf{G}_{\text{chain}}$ is the normalized chain force. Using our dimensionless units, the typical separation of adjacent monomers will be $b\equiv a/\sigma$. We have used the particular interpolation for the chain force,

$$\mathbf{G}_{\text{chain}}(\mathbf{r})=\begin{cases}24\epsilon\mathbf{r}/(2b^2-r^2) & \text{if } 2b^2-r^2>0.2\\ 5*24\epsilon\mathbf{r} & \text{otherwise,}\end{cases} \tag{5}$$

which is similar to that used in Ref. 17. In the simulations using this force law, the mean-square bond length was found to vary between $0.95b$ and $1.1b$. The large r regime of the corresponding potential is relevant only in rare situations when the energy of a single bond fluctuates to greater than several k_BT. This analytic continuation has been chosen simply to avoid the divergent behavior of the small r regime at $r=\sqrt{2}b$.

In the computer simulation, Eq. (3) is integrated as a finite difference equation,

$$\Delta\mathbf{y}_i=\mathbf{G}_{\text{chain}}(i,i+1)\Delta\tau+\mathbf{G}_{\text{chain}}(i,i-1)\Delta\tau+\sqrt{2\ \Delta\tau}\mathbf{R}_{\text{gauss}}+4\epsilon\sum_{i\neq j}\mathbf{y}_{ij}\left[\frac{12}{y_{ij}^{14}}-\frac{6}{y_{ij}^{8}}\right]\Delta\tau. \tag{6}$$

Here $\mathbf{R}_{\text{gauss}}$ is a vector in which each cartesian component is generated by a random number generator with a Gaussian probability distribution of standard deviation 1.

B. Chain uncrossability

1. Crossing is suppressed when beads are large and bonds are short

As mentioned above, we have chosen parameters similar to those used in a polymer melt simulation by Kremer.[17] The importance of the specific values for the parameters b and ϵ is discussed further in other sources,[19] but for the purposes of the present paper their ultimate relevance is in whether they preserve system topology. This question has two aspects. On the one hand, chain uncrossability must be preserved by the continuous equation of motion (3). On the other hand, the time step in the finite difference version (6) must be small enough to prevent direct hopping of one part of the chain through the other.

As regards the former aspect, it is described in Ref. 17, that the form (5) for the chain connectivity potential does an excellent job of preventing crossing of chains. To explain this point, we first note that if the monomers were hard spheres, of radius σ each, and if the chain bonds were all of the fixed length $a=b\sigma$, then crossing of chains would be geometrically possible for $b>\sqrt{2}$ and forbidden for $b<\sqrt{2}$. This is illustrated in Fig. 1. We have used the value $b=1$ throughout these simulations. Of course, the $b/\sqrt{2}$ criteria uniquely determines crossability for this hard model only. Since we use potentials that are somewhat soft, crossing is not strictly forbidden, but is a matter of barrier height. Thus, we have performed special tests to examine if crossing occurs with an appreciable probability.

The chain uncrossability test is as follows. We set up a system of two long chains linked together at a sliplink, using the same force parameters as in the rest of this work. The ends of the chains were fixed to opposite walls of a box in a manner such that the chains were stretched to nearly their full extension. (Imagine two copies of the letter ''U'' linked together, with the straight parts far extended and fixed in place.) We allowed this physical system to evolve for 10

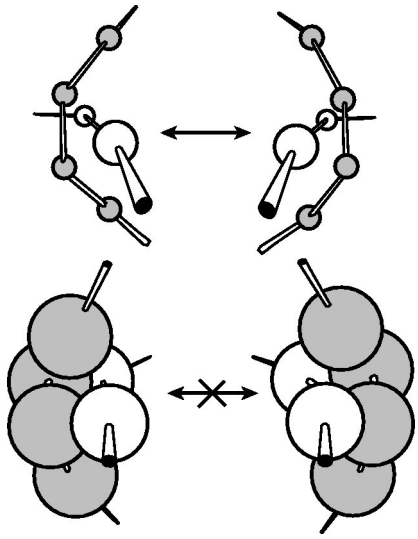

FIG. 1. In our work, as in most computer simulations of polymer chains, the monomers are hard spheres, but the bonds themselves are phantom, as checking for bond-crossing is very time consuming. (Top) Chains will freely pass through each other if the ratio of a bondlength to a hard-sphere diameter is larger than $\sqrt{2}$. (Bottom) When this ratio is less than $\sqrt{2}$, hard sphere repulsion inherently prevents chains from crossing, keeping the topology of the system fixed. In our simulation, we set the Lennard-Jones diameter of a monomer equal to a bondlength to preclude chain crossing.

times longer than the times required for any of the experimental simulations. In no cases did the link ever break. From this we conclude that chain crossing does not occur in our experimental systems.

2. Comment on integration timestep used

The test described above, as well as all our simulations, requires that the integration time step be short enough to prevent crossing during this ''dead time.'' On the other hand, as in all molecular dynamics simulations, we would like to integrate our equations of motion using the largest timestep possible. If the time step is too large, then monomers may ''hop'' over each other or out of potential wells during a single integration step. Such easy hopping would not lead to well-defined equilibrium.

Hops should be smaller than the width of the Lennard-Jones well to ensure that freezing is well-defined at low temperatures.

The Lennard-Jones force is a short-range force, since it falls away faster than r^{-d} in $(d=3)$-dimensional space. This allows us to define a finite size for the Lennard-Jones well. Particles within the well are trapped. Those not in the well are free.

As a measure of the width of the potential well, we calculated the distance between the minimum of the potential (occurring at $r_{\min}=2^{1/6}\sigma$) and the inflection point [occurring at $r_{\text{inflect}}=(156/42)^{1/6}\sigma$]. This gives a width $r_{\text{inflect}}-r_{\min}\approx 0.1\sigma$. Note that at r_{inflect}, the Lennard-Jones force has strength $0.60\epsilon/\sigma$, while at $r=1.5\sigma$, it has halved in strength to $=0.29\epsilon/\sigma$. At $r=2\sigma$, it has decayed by a factor of more than 10, to $0.045\epsilon/\sigma$.

We now choose our time step such that the thermal force, spring force, and Lennard-Jones force all yield hops of size much less than 0.1σ.

This condition for the thermal force is

$$\sqrt{\frac{2}{d\tau}}F_{\text{Gauss}}d\tau \ll 0.1. \tag{7}$$

For a Gaussian force of order unity, we obtain an inequality for the maximum allowable time step of the system,

$$d\tau \ll 0.005. \tag{8}$$

Now we consider the spring force. Fortunately, the spring potential does not change by more than k_BT for any hop in coordinate space of 0.1σ. So if we adhere to condition (8) from the thermal force above, we should not have problems with the spring force, either. This fortunate property of the spring potential energy was constructed intentionally. This is the reason for the two regimes of the spring potential energy. If we had used the asymptotically diverging potential for all values of r, single hops could jump the particle out of the physically defined region.

The Lennard-Jones force must be handled with care because particles in close proximity can exert strong forces on each other. To avoid excessively large jumps, we implemented a variable time step integration mechanism. The mechanism limits the maximum jump at any time step to 0.1σ. At a given r, the Lennard-Jones force is calculated. Multiplying by the time step gives the test hop size. If this test hop size is greater than 0.1σ, the force is replaced by the averaged Lennard-Jones force the particle would feel while moving between the initial position and the new calculated one. The constraint on force size is then checked again recursively. This averaging process can always reduce the hop to the width of the well because the Lennard-Jones force is short ranged.

Inequality (8) places the strictest constraint on the time step. Beginning our empirical time step tests with this condition, we found that a slightly smaller time step of 0.0001 was able to satisfy all physical constraints. We have used timestep 0.0001 in all simulations described below.

C. Two chain interaction

To study the interaction of globules, we ran a molecular dynamics simulation on two interacting homopolymer chains. Apart from trivially modifying Eq. (3) to include Lennard-Jones interactions between monomers on different chains, we also applied an external force, f, pressing the centers of mass of the two globules towards each other. Without this external force, two globules, even brought initially into a close contact, do not merge and in fact diffuse away from one another. This molecular dynamics observation is consistent with the data of experiments, as we discussed above.[2] The external force holds the globules together and makes them squeeze into one another. Upon some ''equilibration'' globules achieve a steady separation distance between their respective centers of mass. This fact is naturally interpreted by saying that the topological repulsion force is operational between the globules on the prereptational time scale, and at

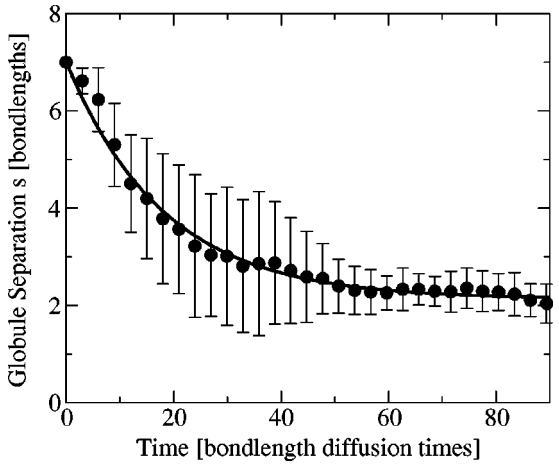

FIG. 2. Typical separation distance decay curve for two polymer globules forced towards each other. Each monomer is pushed along the direction from the center of its globule to the center of the other globule. The globules coalesce on a short time scale, then reach a steady state separation due to ''equilibration'' with the topological repulsive force. The smooth curve is a fit of the data to an exponential decay [see Eq. (11)]. In this example the pushing force on each globule is 0.64 in units of k_BT/bondlengths.

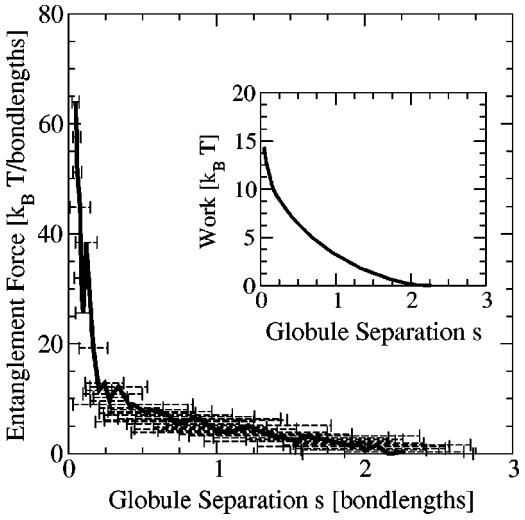

FIG. 3. (Main box) Entanglement force between two 64-mer globules vs distance s between their centers of mass. The force is repulsive due to the topological constraint. (Inset) Work vs s. The typical approach due to thermal fluctuations is $s_{\text{thermal}}=1.6$, where $V(s_{\text{thermal}})=k_BT$.

the achieved degree of interpenetration this force is just equal in magnitude to the applied external force. As before, we emphasize the fact that this ''equilibration'' is an intermediate time scale stabilization, before topological equilibration takes place. Thus, reading off the applied force will give us the topological force as a function of globule separation distance.

The equations of motion we have integrated for the two chain system are

$$\frac{d\mathbf{y}_i^\alpha}{d\tau}=\mathbf{G}^\alpha_{\text{chain}}(i,i+1)+\mathbf{G}^\alpha_{\text{chain}}(i,i-1)+\sqrt{\frac{2}{d\tau}}\mathbf{F}_{\text{Gauss}}(\tau)$$

$$+4\epsilon\sum_{(\alpha,i)\neq(\beta,j)}\mathbf{y}_{ij}^{\alpha\beta}\left[\frac{12}{y_{ij}^{\alpha\beta 14}}-\frac{6}{y_{ij}^{\alpha\beta 8}}\right]+\frac{f}{N}\,\mathbf{s}_{\beta\alpha}/|\mathbf{s}_{\beta\alpha}|. \qquad (9)$$

Here α and β are chain labels, taking on the values 1 and 2 in our two chain simulation. The last force term is the force of magnitude f/N applied to each monomer along the unit vector connecting the two chains' centers of mass. The vector $\mathbf{s}_{\beta\alpha}$ is defined as

$$\mathbf{s}_{\beta\alpha}\equiv\frac{\Sigma_i[\mathbf{y}_i^\beta-\mathbf{y}_i^\alpha]}{N}. \qquad (10)$$

Since each monomer ''feels'' applied force f/N, the total applied force on each globule is f.

We choose globules with $N=64$ for computational tractability and because proteins are of this approximate degree of polymerization.

The globule initial conditions were generated by individually starting each polymer in a random walk configuration, then annealing at low temperature ($\epsilon=2.0$) for 60 time units, which was more than long enough for the polymer to become a globule. The chains were then placed at $s=7.0$, a distance such that their surfaces were separated by approximately one bondlength. The simulation was then run at $\epsilon=1.0$ (Fig 2). For comparison, the value of epsilon at the theta temperature is on the order of $0.6\div 0.8$. The globules were molten, i.e., the temperature was not low enough to cause rigid freezing.

D. Method of computing numerical results

The data in Fig. 3 are the result of 403 computer experiments, in each of which a pair of globules was allowed to interact at one applied force. At each applied force f we determine the ''equilibrium'' center-of-mass separation s_{final}. We then invert these data to derive the function $f(s_{\text{final}})$ for the topological force, which is equal in magnitude to the applied force.

Roughly the same number of trials (≈ 10) were conducted for each of the different force data points, with slightly more tests for force values smaller than 12.8. The f values ranged from 0.00 to 64.00.

In most cases, the separation $s(t)$ quickly decayed after the applied force was switched on. In a few cases, however, because of random diffusion, the globules briefly drifted before coming into attractive range. A smaller number of these drifting globule pairs failed to come back in contact. Since our goal is to examine collisions between chains, those trials in which the pairs did not come in contact were excluded from our data. However, for all other trials, s stabilized to an ''equilibrium'' value, s_{final}. As we are only concerned with forces in our system after this ''equilibration,'' the quantity labeled s in Figs. 3, 4, and 5 refers to s_{final}.

Figure 2 is a sample plot of ''squashing'' dynamics under the external force equal to $f_{app}=0.64$. Once attraction began, the $s(t)$ curves fit well to an exponential decay law

$$s(t)=s_{\text{final}}+(s(0)-s_{\text{final}})\exp(-t/t_0). \qquad (11)$$

In each trial, we allowed the globules to interact for ≈ 100 time units. This was significantly longer than the decay time, which was found to be of the order of 20 time units in all trials, but much shorter than the time required for entanglement to occur (see Discussion).

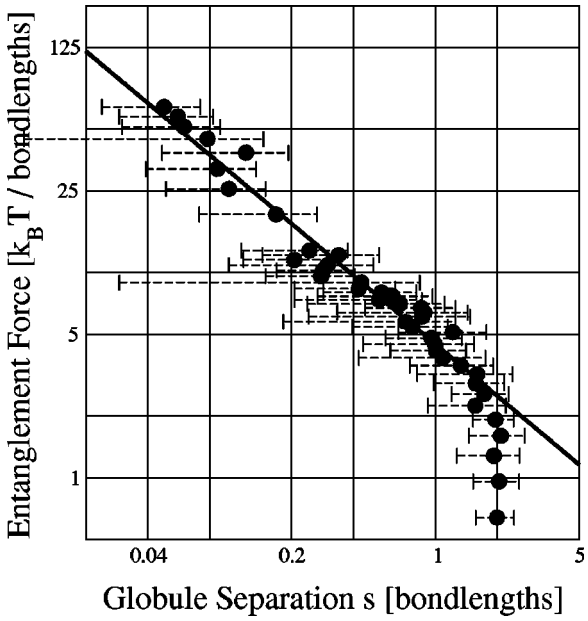

FIG. 4. Log–log plot of entanglement force versus distance between two 64-mer globule centers. There is an unexpected, but clearly defined, power law for all s within the cutoff range of the force. The line is the fit (13).

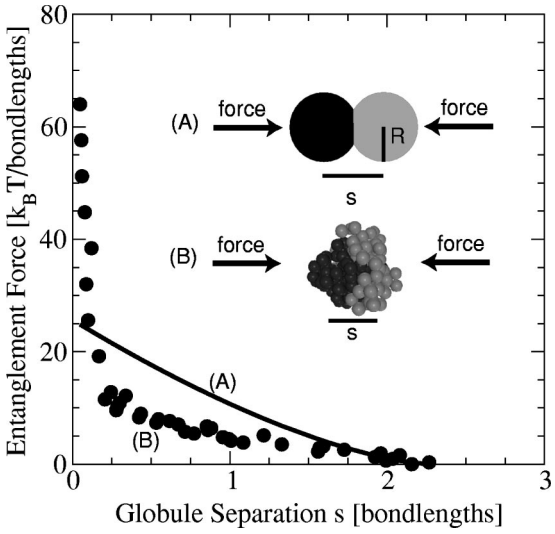

FIG. 5. Globules do not behave like rigid elastic bodies, as manifested by their poor fit to the Hertz law, $f_{\mathrm{Hertz}}(s)=\alpha(R-s/2)^{3/2}$, with α and R being our fit parameters. The force we consider here is the repulsive force that resists compression of the two bodies into one another. (A) Hertz force law for two rigid elastic spheres as a function of separation distance s. (B) Force data for two polymer globules. Globules are molten and highly deformable, making the data fit terrible for even the optimized curve shown here.

To calculate s_{final} and the error, we averaged the value of s over the last 15 time units of the runs at each f, i.e., our results are determined by only the flat tail of the decay curve, where the errorbars are much smaller.

The main result of the work, the topological force as a function of distance, is shown in Fig. 3. In the remainder of the work, we shall discuss this plot in some detail.

III. DISCUSSION

A. Force and work

Since we have measured the topological repulsion force, $f(s)$, as a function of separation, s, between centers of globules, we can easily compute the work, W, necessary to smash one globule into the other by simply integrating the force $f(s)$. Specifically, to bring the globules to the separation s the work

$$W(s)=\int_s^\infty f(s')ds' \tag{12}$$

must be performed, if the reptation does not have time to occur. This work, as a function of s, is shown in the inset in Fig. 3. It is important to note, that in our dimensionless variables the work W is measured in k_BT units. In particular, using the inset of Fig. 3, we can estimate how close two globules may come to each other in a typical collision, when no external force is applied. Indeed, since the work against entanglement force must be performed by a fluctuation, the typical ''thermal'' proximity, s_{thermal}, is estimated via $W(s_{\mathrm{thermal}})\simeq k_BT$. According to the figure, in our experiment $s_{\mathrm{thermal}}\simeq 1.6$. This is, of course, significantly smaller than the sum of geometric radii of the two globules, which is about 5 or 6, but still far from being negligible. Therefore, our results do indeed show, at least qualitatively, that the entanglement force prevents globules from merging.

B. Power law dependence of entanglement force

Figure 4 presents the same data as Fig. 3, but in the double logarithmic scale. As can be seen, the force law exhibits a sharp transition at $s\approx 2$, from very sharp decay of force at large distance, when globules are essentially not touching each other, to a smoother power law dependence at smaller s. In the power law region, the curve is a close fit to $\ln f\simeq(-0.84\pm 0.02)\ln s+(1.50\pm 0.03)$, i.e., the force law is

$$f(s)\simeq 4.5s^{-0.84\pm 0.02}. \tag{13}$$

There is no particular reason for us to have expected a power law behavior, but the results are a surprisingly good fit. At the moment we have no satisfactory explanation for the origin of the power law. Since the entire phenomenon is of topological origin, the exponent may or may not be related to ''classical'' exponents such as the excluded volume or Gaussian scaling exponents.

One can also compare the entanglement force between two globules with that computed in the classical Hertz problem of the force resulting from elastic deformation of two colliding elastic balls.[20] That force can be written as $f_{\mathrm{Hertz}}(s)\sim(R-s/2)^{3/2}$, R being the ball radius.

Not surprisingly, the entanglement force between globules does not fit the Hertz formula. The character of deformation in a polymer globule is very different from that in a regular solid. Globule deformation is not elastic. Still, globules are not liquid drops either. Like rigid elastic balls, globules accumulate deformation energy over their volume, rather than only in the surface layer the way a liquid drop would. As we show in the next section, globules deform very strongly, such that there is no hope for linear elasticity, including Hertz theory, to remain valid.

Our squeezing together of two globules is also reminiscent of the system of two polymer-covered plates squeezed

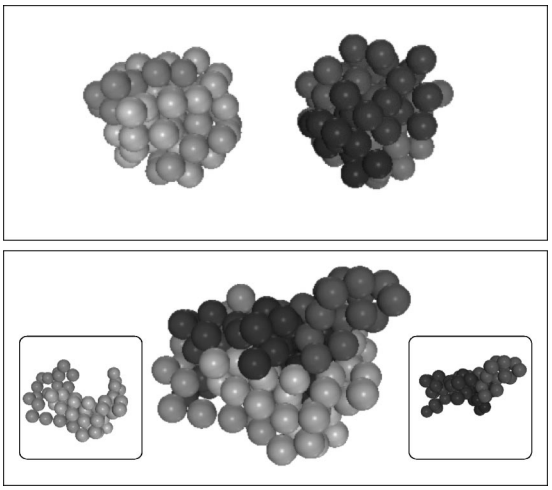

FIG. 6. Globules deform from a spherical geometry when they come in contact. (Top) Two compact globules placed close to each other at $t=0$. (Bottom) The same two globules 113 time units later under the influence of squeezing force $f=2.88$. The globules have severely deformed from their initial shapes. This means the magnitude of topological polymer repulsion can not be calculated perturbatively from rubber elasticity theory. Random fluctuations have even altered the orientation of the vector connecting the globule centers of mass. Monomers are color coded to indicate connectivity of the chains. The color smoothly changes from white to light gray on chain 1 and from black to dark gray on chain 2. For clarity, the two small boxes display each chain rendered in the absence of the other.

together at an interface in good solvent, for which much work has been done to determine the force of repulsion as a function of separation distance.[21,22] However, we stress that our force is of topological origin, while the forces in such good solvent plate systems are caused by excluded volume repulsion. Volume compression is the chief determinant of the force power law for systems in good solvent. Our system is in poor solvent, and the density of our system changes insignificantly when force is applied. Hence our force is unrelated to those measured in such polymer-covered plate experiments or other systems in good solvent.

C. Severe deformation of interacting globules

It is important to remember that throughout this mixing process, the globules remain molten. At lower temperatures, some kind of freezing or glass transition may occur, which would make further entanglement impossible. We do not consider this regime. Visual inspection of the system on a computer screen convincingly indicates that the globules are liquidlike fluctuating bodies as well.

On a more quantitative level, measuring the gyration radius of each globule (before it enters a contact with another globule) shows that it fluctuates with time in the range $1.9 \div 2.3$ (spherical radius $2.5 \div 3.0$). This is roughly consistent with theoretical predictions on the fluctuations of a homopolymer globule gyration radius[23] (see specifically Fig. 2 in that work; one can compare by noting that the chain contraction parameter, $\alpha \equiv R_g / R_g^{\text{ideal coil}}$, is about 1/2 for the globules considered in this work. This value of α corresponds to a reduced temperature at which R_g fluctuations are about 10% of the average R_g value).

When two globules start merging together, each of them undergoes deformation and shape changes which are far greater than these fluctuations. Figure 6 is an image of a typical conformation for two globules at $s=s_{\text{thermal}} \simeq 1.6$, which is the closest fluctuation for two globules in the absence of an applied force. As can be seen in the figure, entanglement of chains has not occurred, as there is little interior penetration of the two globules. On the other hand, the spheres have compressed into two pancakes. They have slightly wrapped around one another, but it is difficult for them to merge any more from this position. Clearly, the gyration radius of each globule *increases* when it ''hugs'' another globule. The darker globule has increased its radius of gyration during the course of interaction from 2.0 at time zero to 2.7. The lighter globule R_g has increased from 1.9 to 2.6.

D. Aggregation time: Aggregation as an activated process

If globules are to merge spontaneously, an amount of free energy equal to the work W must be provided by a fluctuation. Therefore, the probability that two globules merge after they initially touch each other is governed by the factor $\exp(-W/k_BT)$. In other words, provided that every globule in solution collides with other globules roughly once in a Smoluchowski time, τ_S, the number of attempted collisions before successful merging of two globules must be proportional to

$$\frac{t_{\text{aggreg}}}{\tau_S} \simeq \exp\left(\frac{W^*}{k_BT}\right) \simeq \exp\left(\int_{s^*}^{\infty} f(s)\,ds/k_BT\right), \tag{14}$$

where s^* is some characteristic separation at which the interpenetration of globules becomes effectively irreversible, or globules become entangled.

It is not easy to estimate s^*, except for the obvious fact that it must be somewhat smaller than twice the geometrical radius of a globule (or the sum of two radii, in the case of two different globules). One can also speculate that the critical penetration depth must be somehow related to the entanglement length N_e, parameter known in reptation theory. Although we do not have any means of a reliable estimate of s^*, we can check what happens if we replace it by 0, which corresponds to purely fluctuational merging of globules. In this case, we obtain

$$\frac{t_{\text{aggreg}}}{\tau_S} \simeq \exp(W(0)/k_BT) \approx \exp(17.7) \approx 10^8. \tag{15}$$

Thus, our force law successfully reproduces the phenomenon that aggregation is harshly suppressed by this entanglement effect. Of course, quantitatively, the result (15) is a vast overestimate (experimentally, in the conditions of the experiment,[2] the t_{aggreg}/τ_S ratio was found to be about 10^3). That means that replacing s^* by 0 is not a good approximation.

Instead, we can extract an estimate of s^* from the condition that formula (14) yields a result of about 10^3, consistent with experimental data. Comparing with the work function in Fig. 3, we obtain $s^*=0.44$, a penetration of 1.8 bond

lengths after surface contact between the globules. In general, however, a deeper understanding of entanglements is badly needed here, since we do not understand the dependence of s^* on the temperature, chain length and/or globule radius, etc. The important conclusion we can formulate so far is only qualitative; the entanglement force, operational on the prereptational time scale, is sufficient to explain the observed slow-down of aggregation.

E. Time scales: Aggregation time and validity of "unchanged topology" approximation

Our starting assumption in this paper has been that the mutual topology of two chains is fixed during some prolonged time. Therefore, we must now estimate the time τ_{entangle} it takes the system topology to change. For a polymer melt, this would be equivalent to the reptation time.

Our procedure to determine the force law above ignores the slow changes in the system topology. This can be valid, i.e., the entanglement force can be decoupled from the topology change, if and only if the simulation time satisfies $\tau_{\text{simulation}} \ll \tau_{\text{entangle}}$. As described in Sec. II D, $\tau_{\text{simulation}} \sim 100$ for our experiments to determine the force law.

To measure τ_{entangle} we did the following experiment. We first applied a strong attractive force between two globules such that they reached $s=0.123$. Then we turned off the force and waited to see how long it would take for the polymers to entangle.

The globules quickly repelled each other. That confirmed once again that the topological force was still in effect. Only after a long time ($\approx$1000 time units) did s come back below unity. The diminishing of s towards zero is the topological equilibration of the system.

In Fig. 7 we have plotted the distance s as a function of time for this system. When the attractive force is turned off, there is a marked repulsion of the two globules. s jumps from 0.123 to more than 1.5 within the first 100 time units. Because of topological frustration, the dynamics of the globule are slower than those for repulsion according to the force law. However, the data indicate that the repulsion certainly exists, meaning that our previous simulation time was short enough that topological equilibration has not occurred, i.e our force law data are valid.

As time passes, the distance s fluctuates, and eventually after about 1000 time units, the system decays back down to $s<1.0$. Further fluctuations increase s again up to more than 2, and the system decays to $s<1.0$ after another 1500 time units.

From this behavior we can estimate that τ_{entangle} must be at least of the order of 10^3 time units, if not longer. We have been able to simulate 7000 total time units so far, and strong fluctuations in s persist throughout. The system is completely entangled when s stabilizes towards 0. Since there is no clear trend of s approaching zero, it is likely that τ_{entangle} is even greater than 7000. This is much larger than the simulation time of 100 required to determine the entanglement force.

The decay time sets the lower bound on the simulation time required to determine the entanglement force. Using the exponential decay fit described in Sec. II D, we found that the decay time was of the order of 20 time units or less at all values of applied force. This is five times shorter than the simulation time, providing even more convincing evidence that system topology changes are decoupled from the topological force we have measured.

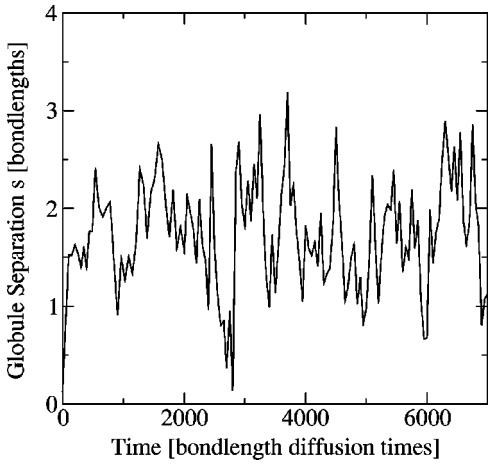

FIG. 7. The entanglement "force" is a good approximation because polymer mixing times are much longer than globule–globule coalescence times. The figure shows the evolution of two globules after they have been squeezed to $s=0.123$ by a strong attractive force. At $t=0$ the applied force is released, upon which the entanglement force induces a fast repulsion of the two globules. s jumps to 1.5 in the first 100 time units, and then fluctuates over a much longer time scale. 7000 time units later, the polymers have still not stably reptated back to the entangled high entropy state $s=0$, i.e., mixing has not occurred. In contrast,in our experiments to measure the entanglement force, the time it took for globules to reach a stable separation distance was a mere $\sim$20 units. The large disparity in time scales is strong evidence that topology can be considered fixed on the short time scale.

As an aside, there was a rough trend of decreasing decay time with increasing values of applied force, but there was so much variation in the times that more explicit curve-fitting was fruitless.

τ_{entangle} is at least two orders of magnitude larger than the decay time. This makes the entanglement force a good approximation.

IV. CONCLUSION

Let us now return to the most fundamental issue and discuss once again what determines the aggregation time of the solution of globules. When two globules meet each other, which happens roughly once every Smoluchowski time,[1] they spend together a time which is about the time for globule diffusion over their own diameter,

$$t_{\text{contact}} \sim R^2/D \sim R^3 \eta/k_B T \sim N(a^3 \eta/k_B T). \tag{16}$$

During that time, two globules look somewhat like a polymer–polymer interface,[4,5] except chains are Gaussian for the latter but not Gaussian for the former. In the language adopted in the works,[4,5] our major question is whether globules succeed to form connecting polymer bridges while they are touching each other. While the works[4,5] concentrated on how the number of bridges scales with time, our problem is less delicate in the sense that even one bridge would perhaps suffice to anchor globules together. Thus, aggregation is governed by the ratio $t_{\text{contact}}/t_{\text{relax glob}}$, where $t_{\text{relax glob}}$ is the relaxation time of a strongly non-Gaussian globular chain; if

$t_{\text{contact}}/t_{\text{relax glob}} > 1$, aggregation is diffusion-limited and aggregation time is the Smoluchowski time;[1] by contrast, if $t_{\text{contact}}/t_{\text{relax glob}} < 1$, many trials are needed for globules to connect, which is just the reformulation of Eq. 14. Thus, better insight into the chain dynamics and reptation in globule will be needed to achieve complete understanding of aggregation, or to compute s^*. However, our results do show that this relaxation occurs slowly and causes globules to touch each other many times before effectively making the connecting bridge.

To conclude, we have established the existence of an ''entanglement force'' acting between two approaching polymer globules in poor solvent and operational on the prereptational time scale. In terms of magnitude, this force is found to be very significant. An amount of work much greater than k_BT, on the order of tens k_BT, is needed to overcome the entanglement force. Accordingly, this force may well slow down the aggregation of globules in dilute solution in poor solvent. This may explain the puzzling observation of the experiment.[2] We have also found an interesting power law decay of the entanglement force with distance which awaits a theoretical explanation.

Our findings have a potential of significant implications in other fields of polymer physics. To mention but a few, we repeat our referral to the dynamics of coil–globule transition and to the problem of aggregation/precipitation of proteins and proteinlike heteropolymers.

To further develop our idea, a better understanding of entanglements seems desirable. Indeed, we will have to have an insight into what precisely the entanglements are in order to decide how deeply the globules have to penetrate each other before they become entangled and penetration becomes effectively irreversible. In terms of simulation, better understanding is also impeded by the fact that entanglements are poorly defined computationally.[24] All these complications notwithstanding, the very presence of the entanglement force is demonstrated beyond doubts.

ACKNOWLEDGMENTS

We wish to thank the anonymous reviewers of this article for insightful comments and for referring us to some important reference works. J.C. acknowledges support from the National Science Foundation Graduate Fellowship.

[1] M. Smoluchowski, Z. Phys. Chem. (Leipzig) **92**, 129 (1917).
[2] B. Chu, Q. Ying, and A. Yu, Macromolecules **28**, 180 (1995).
[3] P. G. DeGennes, *Scaling Concepts in Polymer Physics* (Cornell University Press, Ithaca, 1979).
[4] P. G. DeGennes, C.R. Acad. Sci. Paris B **291**, 219 (1980).
[5] P. G. DeGennes, C.R. Acad. Sci. Paris II **308**, 13 (1989).
[6] F. Brochard, J. Jouffroy, and P. Levinson, Macromolecules **16**, 1638 (1983).
[7] C. B. Anfinsen, Science **181**, 223 (1973).
[8] M. A. Speed, D. I. C. Wang, and J. King, Nat. Biotechnol. **14**, 1283 (1996).
[9] P. G. DeGennes, J. Phys. (France) Lett. **46**, 639 (1985).
[10] A. Halperin and P. Goldbart, Phys. Rev. E **61**, 565 (2000).
[11] A. Yu. Grosberg and D. V. Kuznetsov, J. Phys. II **2**, 1327 (1992).
[12] A. V. Vologodskii, A. V. Likashin, and M. D. Frank-Kamenetskii, Sov. Phys. JETP **40**, 932 (1975).
[13] T. Deguchi and K. Tsurusaki, in *Lectures at Knots 96*, edited by S. Suzuki (World Scientific, Singapore, 1997).
[14] P. E. Rouse, J. Chem. Phys. **21**, 1273 (1953).
[15] M. Doi and S.F. Edwards, *The Theory of Polymer Dynamics* (Clarendon, Oxford, 1986).
[16] K. Kremer, G. S. Grest, and I. Carmesin, Phys. Rev. Lett. **61**, 566 (1988).
[17] K. Kremer, in *Computer Simulation in Chemical Physics*, edited by M. P. Allen and D. J. Tildesley (Kluwer Academic, Dordrecht, 1993).
[18] A. Yu. Grosberg and A. R. Khokhlov, *Statistical Physics of Macromolecules* (AIP, New York, 1994).
[19] K. Binder, in *Monte Carlo and Molecular Dynamics Simulations in Polymer Science*, edited by K. Binder (Oxford University Press, New York, 1995).
[20] L. D. Landau and E. M. Lifshitz, *Theory of Elasticity*, 3rd ed. (Pergamon, Elmsford, 1986).
[21] P. F. Luckham, Adv. Colloid Interface Sci. **34**, 191 (1991).
[22] P. G. DeGennes, Adv. Colloid Interface Sci. **27**, 189 (1987).
[23] A. Yu. Grosberg and D. V. Kuznetsov, Macromolecules **25**, 1980 (1992).
[24] R. Everaers and K. Kremer, Phys. Rev. E **53**, 37 (1996).