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Long-Range Depletion Forces Induced by Associating Small Molecules

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This is the first report of experimental observations of depletion interactions in solutions of a (hydrogen-bonded) reversible supramolecular polymer. Depletion forces were measured directly by colloidal probe atomic force microscopy. The range of the depletion force is consistent with existing independent experimental data. The interaction can be tuned by adding monofunctional chain stoppers to the solution, a possibility which is unique to supramolecular polymers. The depletion force is shown to be strong enough to induce phase separation in a colloidal suspension.

The presence of an interface generally perturbs the structure of liquids [1]. It may affect the density or the orientation of the molecules near the interface and, in the case of solutions, also the local concentration. The perturbation extends into the liquid over a characteristic distance, which is usually determined by the size of the molecules. Free energy is stored in the perturbed zone, and, when two interfaces approach each other to such a distance that their perturbation zones overlap, this gives rise to a force between the interfaces. These forces are known as "structural forces" [2]. An example is the solvation (hydration) force between smooth surfaces in a pure liquid, which occurs as a result of the layering of molecules. Solvation forces are often oscillatory with a period given by the molecular diameter, and they decay over a few periods. In solutions, structural forces may also occur due to the solute. A well-known example is the depletion force in nonadsorbing polymer solutions [3,4]. Depletion forces are of practical importance in many systems such as paints and food emulsions. They also play an important role in biological systems, where they have been studied in the context of, for example, bacterial aggregation and DNA compaction. The range of the depletion interaction does not usually exceed the size of the polymer chains. In this Letter, we focus on depletion forces in solutions of a special class of (small) molecules, namely, those capable of associating to *reversible supramolecular polymers*. In contrast with the cases mentioned above, the forces in these solutions extend over many times the molecular size.

Reversible supramolecular polymers are chains of small molecules, held together by noncovalent interactions such as hydrogen bonding or metal-ligand complexation [5]. The reversibility of the bonds gives these polymers exceptional properties compared to their covalent counterparts. For example, the chain length distribution is not fixed but depends on monomer concentration, temperature, and other parameters and can thus easily be adjusted. Furthermore, reversible supramolecular polymers have faster dynamics than covalent ones, owing to the continuous breaking and reformation of bonds [6–9].

A variety of applications in which the unique properties of supramolecular polymers are used are currently being developed [10]. Supramolecular polymers hold promise as "intelligent" materials which respond to environmental conditions such as temperature, light, or other stimuli [11]. In some respects, supramolecular polymers are similar to wormlike micelles [7], which are also long, noncovalent aggregates of small molecules. Applications of supramolecular polymers can, therefore, be expected in the same areas where wormlike micelles are used, such as the oil industry and cosmetics [12]. However, the bonds between the monomers in supramolecular polymers are much more specific than those in wormlike micelles, and this offers additional possibilities to control their properties. Interesting examples are the use of specific "chain stoppers" [8,9,13] (*vide infra*) and the regulation of colloidal stability by supramolecular brushes [14].

To form linear chains, the monomers of a supramolecular polymer must be bifunctional: a monomer contains two binding groups. If we assume that there is a single association constant K (independent of the length of the chain to which a monomer binds) which determines the equilibrium between breaking and formation of bonds, the number average degree of polymerization $\langle N \rangle$ at an overall concentration c of bifunctional monomers is $\langle N \rangle \approx 2\sqrt{Kc}$. This equation holds if the chains are long, that is, if $Kc \gg 1$ [8]. The presence of more than two binding groups per monomer leads to the formation of branched chains and networks. Monomers with only one binding group can bind to a chain, but the chain cannot grow any further. These monofunctional monomers are known as chain stoppers, and they decrease the average chain length. If a fraction x of chain stoppers is present [defined as $x = k/(c + k)$, where k is the concentration of chain stoppers], $\langle N \rangle$ is given by [8]:

$$\langle N(x) \rangle \simeq \frac{\langle N(0) \rangle}{(1 - x) + x\langle N(0) \rangle}, \qquad (1)$$

where $\langle N(0) \rangle$ is the average degree of polymerization

without chain stoppers. At sufficiently high x and $\langle N(0) \rangle$, $\langle N(x) \rangle$ is proportional to $1/x$ and independent of the overall monomer concentration.

Arrays of multiple hydrogen bonds display high association constants in apolar solvents and are useful building blocks for supramolecular polymerization [15,16]. Bisurea 2,4-*bis*(2-ethylhexylureido)toluene (EHUT) forms long semiflexible supramolecular chains in apolar solvents by the cooperative formation of four hydrogen bonds between two consecutive monomers [17]. Monofunctional monomer 2,4-*bis*(dibutylureido)toluene (DBUT) can be used as a chain stopper for EHUT [8,9,13].

Despite the considerable interest in supramolecular polymers in recent years [5], few reports address the behavior of supramolecular polymers at interfaces. This is particularly true for depletion forces, which have been treated only theoretically [18,19] (and references therein). Depletion forces can be observed experimentally by studying depletion interaction-induced phase separation of colloidal suspensions, but they can also be measured directly [3]. There are reports of the experimental observation of depletion forces induced by covalent polymers but also by spherical [20] and wormlike [21] micelles. In this Letter, we report the first experimental observations of depletion forces in reversible supramolecular polymer solutions, both by direct measurement by means of colloidal probe atomic force microscopy (CP-AFM) and by depletion interaction-induced phase separation.

Depletion forces were analyzed quantitatively for the first time by Asakura and Oosawa [22]. They argued that the polymer concentration near a nonadsorbing wall is lower than in the bulk solution, due to the loss of entropy a polymer suffers when it is close to the surface. The layer with a reduced polymer concentration is called the depletion layer. When two particles approach each other to such a distance that their depletion layers overlap, there is an osmotic pressure difference between the bulk solution and the gap separating the surfaces, which pushes the surfaces together. Let us assume that the polymer concentration is zero within a distance δ (the depletion layer thickness) from the surface and equal to the bulk value at distances $\geq \delta$. In that case, the osmotic pressure difference $\Delta\Pi$ between the depletion layer and the bulk solution simply equals the bulk osmotic pressure (Π_{bulk}). If there is a gap of width h between the surfaces, the depletion layers overlap if $h < 2\delta$. The overlap volume ΔV depends on h and is determined by the geometry of the surfaces. The depletion free energy G_{dep} is given by $G_{dep} = -\Delta V \Delta\Pi$. Differentiating G_{dep} with respect to h gives the depletion force F_{dep}. For a spherical particle with radius R and a planar surface at separations $h \ll R$, the typical geometry in CP-AFM experiments, a good approximation of the depletion force at small separations ($h \lesssim 1.5\delta$) is given by [21]:

$$F_{dep}(h) = \begin{cases} -2\pi R(2\delta - h)\Pi_{bulk} & \text{if } 0 < h \leq 2\delta, \\ 0 & \text{if } h > 2\delta. \end{cases} \quad (2)$$

Using the Derjaguin approximation [2], the force can be converted into the interaction free energy per unit area between two planar surfaces of area A: $F_{dep}/2\pi R = G_{dep}/A$. In dilute solutions, the depletion layer thickness is approximately equal to the radius of gyration R_g of the polymer, and the osmotic pressure is given by Van't Hoff's law. Note that for supramolecular polymers (without chain stoppers), the average degree of polymerization increases with the overall monomer concentration. This implies that the concentration of chains is not proportional to the overall monomer concentration, as is the case for covalent polymers, but to $c^{0.5}$. In semidilute solutions (above the overlap concentration), the polymers form a transient network with an average mesh size ξ, the correlation length. This length depends only on the overall concentration and not on the length of the individual chains. Hence, when considering supramolecular polymers, ξ is not affected by the concentration dependence of $\langle N \rangle$. In this regime, $\delta \approx \xi$, and also the osmotic pressure is determined by ξ: $\Pi_{bulk} \sim \xi^{-3}$ [19,21].

In CP-AFM experiments, a colloidal sphere is firmly attached to an AFM cantilever [23]. The cantilever with the probe is placed above a planar surface (substrate), and the distance between the probe particle and the substrate is varied. By measuring the deflection of the cantilever, the interaction force between the colloidal probe and the substrate as a function of the separation distance can be calculated. In our experiments, silica surfaces were used, coated with stearyl alcohol (SiO_2-C_{18}) [24,25], with cyclohexane as the solvent. The van der Waals interaction in this system is negligibly small due to the matched refractive indices of the materials. Therefore, only hard-wall interactions are present between the surfaces in the pure solvent. The experimental procedure was previously described in detail by Wijting *et al.* [25]. The spring constant of the cantilever was determined by the thermal noise method [26] to be 0.075 ± 0.05 N/m. Force curves were measured using small scan ranges (50–150 nm) and low scan velocities (10–50 nm/s). Note that for all separations used in our experiments, $h \ll R$, and we may therefore use Eq. (2) to fit our experimental data at separations $\lesssim 1.5\delta$.

First, it was checked that EHUT does not adsorb to the SiO_2-C_{18} surfaces. There was no measurable decrease in EHUT concentration (measured by UV absorption) upon the addition of SiO_2-C_{18} particles to an EHUT solution, which indicates that EHUT does indeed not adsorb to the particles. Also, previous experiments showed no indication of any adsorption [27]. A typical force curve in pure cyclohexane is shown in Fig. 1 (inset). Even though the van der Waals forces between SiO_2-C_{18} surfaces in cyclohexane are very small, perfect hard-wall interactions between a colloidal probe and a planar substrate are usually not found in CP-AFM experiments. This is generally explained by roughness of the surfaces. For (stearylated) silica, this roughness is typically a few nanometers. The force curve for an EHUT-DBUT solution (100 g/l EHUT,
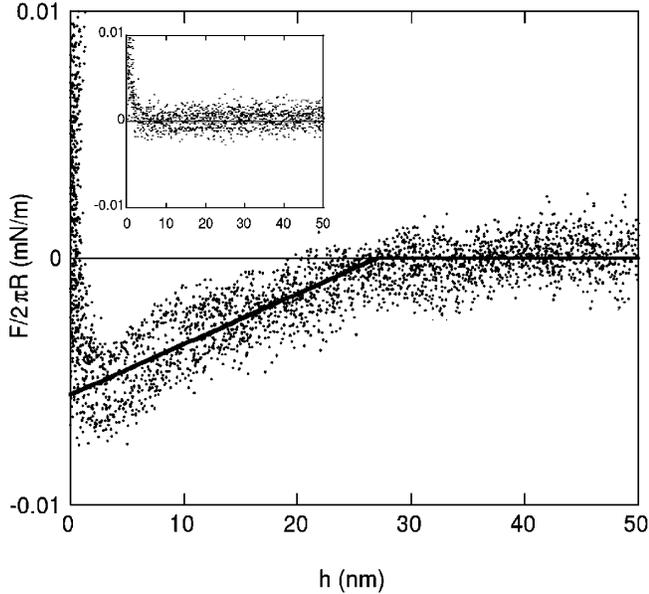
FIG. 1.   Normalized interaction force between SiO_2-C_{18} surfaces in a 100 g/l EHUT solution containing DBUT ($x = 0.10$) in cyclohexane. Eight force curves are plotted. The line is a fit to Eq. (2). Inset: Interaction in pure cyclohexane.

FIG. 2.   Depletion layer thickness δ as a function of the inverse stopper fraction $1/x$. The inverse overlap fraction $1/x^*$ is denoted in the figure. Inset: Viscosity of the same solutions.

$x = 0.10$) is also shown in Fig. 1. The chain stopper was added to decrease the viscosity of the solution to such an extent that it could be injected into the AFM liquid cell. At the EHUT concentration and stopper fraction used in this experiment, the solution is still highly viscous (5 Pa s, over 5000 times the solvent viscosity). This indicates that the solution is far above the overlap concentration. Hence, the depletion layer thickness and the osmotic pressure are determined by the mesh size of the transient polymer network. An attractive interaction is clearly visible in the force curve. A fit to Eq. (2) using the data at separations between 3 and 20 nm is shown in Fig. 1 as the solid line. From the slope of the fitted line and the intersection with the abscissa, values of Π_{bulk} and δ were obtained.

The attraction starts at a separation of 26 ± 4 nm, indicating that the depletion layer thickness is 13 ± 2 nm. Previous light scattering experiments showed that the correlation length in the semidilute regime follows the scaling law $\xi = 120c^{-0.5}$ [8]. If we apply this relation and assume that $\delta = \xi$, the depletion layer thickness in the present experiment ([EHUT] = 100 g/l) should be 12 nm. We conclude that the measured depletion layer thickness is in good agreement with the light scattering results. The fitted line in Fig. 1 has a slope of 230 ± 70 N/m^2. Unfortunately, we have no independent measure of the osmotic pressure to confirm this value.

Figure 2 shows δ for a number of stopper fractions, ranging from 0.10 to 0.40. By increasing the amount of chain stoppers, the average degree of polymerization $\langle N \rangle$ is decreased, as is the solution viscosity. As long as the solution remains semidilute, this will not affect ξ, and therefore also the range of the depletion interaction is
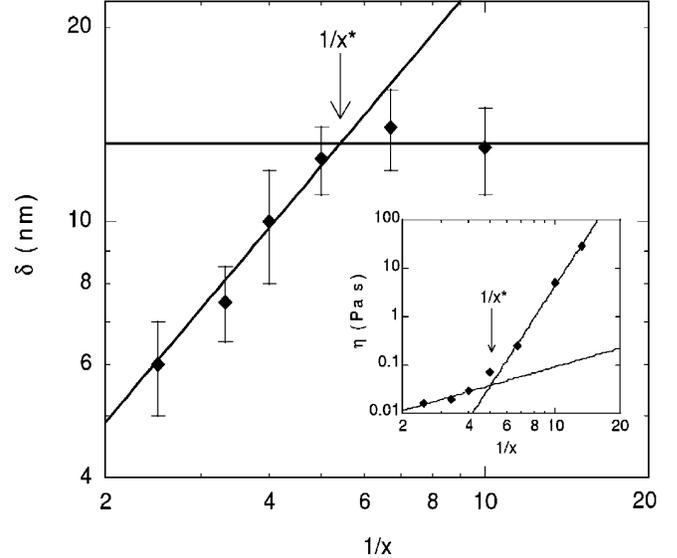
expected to be constant. On the other hand, if x is sufficiently high, the chains become so short that they do not overlap anymore and the solution passes from the semidilute to the dilute regime. In that case, δ is determined by the (average) size of the chains, which is a function of x [Eq. (1)]. The "overlap stopper fraction" where this crossover occurs is denoted by x^*. The depletion interaction can thus be tuned by adding monofunctional monomers to the solution. This is possible only owing to the reversibility and specificity of the interactions between the monomers, and this effect can therefore never occur in covalent polymers nor in wormlike micellar systems. Figure 2 shows that δ can indeed be changed by the addition of chain stoppers. Data are plotted as a function of $1/x$, since $\langle N \rangle$ is predicted to be proportional to this quantity at sufficiently high x [Eq. (1)]. At stopper fractions lower than approximately 0.20 ($1/x > 5$), δ is constant within the experimental error, as denoted by the horizontal line at $\delta = 13$ nm. When x is increased further ($1/x \leq 5$), a clear decrease in δ is observed, indicating a crossover to the dilute regime. The line corresponds to $\delta = 2.4/x$. EHUT chains are semiflexible, with a persistence length ≥ 200 nm [8]. On the length scale of δ, the chains can therefore be regarded as rigid rods, for which $\delta \approx R_g \sim \langle N \rangle$ (in the dilute regime). The proportionality of δ with $1/x$ indicates that $\langle N \rangle \sim 1/x$, which is indeed expected for high stopper fractions [Eq. (1)]. From the intersection of the lines, the overlap stopper fraction can be found: $x^* \approx 0.18$.

Besides the transition of δ at x^*, the solution viscosity is also expected to show a crossover from a relatively weak (power law) dependence on x in dilute solutions to a much stronger dependence in the semidilute regime. Therefore, the viscosity of the solutions was also measured, as shown in Fig. 2 (inset). The viscosity indeed starts to increase
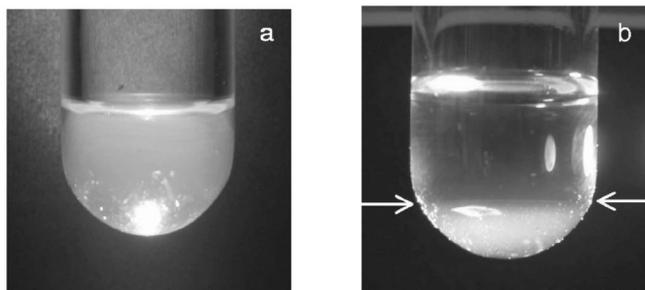
FIG. 3. Tubes containing suspensions of SiO_2-C_{18} spheres ($R = 14$ nm) in (a) pure cyclohexane and (b) 100 g/l EHUT, $x = 0.10$. Arrows indicate the position of the interface between the two phases.

steeply at $x \approx 0.20$, whereas the dependence at higher stopper fractions is much weaker. This observation also leads to an estimate of x^* of approximately 0.20, in agreement with the value obtained from the measured depletion layer thickness.

It is interesting to investigate if the depletion forces caused by EHUT are strong enough to induce phase separation in a colloidal suspension. To study this, equal volumes of an EHUT-DBUT solution and a suspension of SiO_2-C_{18} particles ($R = 14$ nm, 200 g/l) in cyclohexane were mixed in glass tubes. After homogenization, the suspensions were allowed to equilibrate. A suspension of particles in pure cyclohexane is shown in Fig. 3(a). It consists of a single phase and scatters homogeneously. Figure 3(b) shows a suspension with the same particle concentration in a solution of 100 g/l EHUT with added DBUT ($x = 0.10$). The suspension has separated into a particle-rich bottom phase (which has the highest turbidity) and a polymer-rich top phase. The phases are separated by a mirrorlike interface (not visible on the photograph). This result demonstrates that the depletion forces are strong enough to induce phase separation.

In summary, depletion interactions in solutions of a hydrogen-bonded reversible supramolecular polymer (bifunctional monomer EHUT and monofunctional chain stopper DBUT) were studied experimentally for the first time. This was done by directly measuring the depletion force by means of CP-AFM. The thickness of the depletion layer was analyzed, and the results are consistent with previous light scattering measurements of the correlation length. It is demonstrated that the depletion interaction can be tuned by the addition of chain stoppers. This effect is unique for reversible supramolecular polymers. It is also shown that the depletion forces induced by supramolecular polymers can give rise to phase separation in a colloidal suspension.

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