

Interactions between Colloids Induced by a Soft Cross-Linked Polymer Substrate

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Using videomicroscopy imaging, we demonstrate the existence of a short-ranged equilibrium attraction between heavy silica colloids diffusing on soft surfaces of cross-linked polymer gels. The intercolloid potential can be tuned by changing the gel stiffness or by coating the colloids with a polymer layer. On sufficiently soft substrates, the interaction induced by the polymer matrix leads to large-scale colloidal aggregation. We correlate the in-plane interaction with a colloid-surface attraction.

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Interactions between colloids confined to surfaces [1–3] or interfaces [4] are key to the fundamental understanding of many physical phenomena. For instance, self-assembly of ordered phases [5] can be induced, which finds applications in the engineering of photonic crystals [5–7]. Recent studies have shown that aspects of cellular morphology [8,9], mechanical properties [9,10], mobility [9,11], and differentiation [9,12] are sensitive to the elastic response of the environment. Experiments in this field often employ cross-linked polymer-gel surfaces [8,10,11] as a model for biological tissues. We demonstrate that soft polymer substrates may themselves actively induce non-negligible interactions between inanimate, micrometer-sized, objects. It is vital to recognize that the same phenomena may affect living systems of similar dimensions and therefore must be taken into account when studies such as those above are carried out. In this Letter, we explore the nature of such substrate-induced intercolloidal forces and show how they are modified by changing the physical properties of the soft environment. After a brief introduction of our system, we report qualitative observations on the collective behavior of sedimented colloids. Then, we describe quantitative measurements of in-plane colloid-colloid interactions using two complementary techniques. Finally, we report colloid-surface potential energy measurements and micro-rheology results on the viscoelastic properties of the substrate, which help us understand the observed in-plane interactions.

We used plain silica colloids with diameter $\sigma = 1.16 \pm 0.05 \mu\text{m}$ (Microparticles GmbH, Berlin) and a nominal density of $1.5\text{--}2 \text{ g cm}^{-3}$ suspended in 100 mM tris(hydroxymethyl)aminomethane buffer at $p\text{H}$ 8. Under these conditions, the silica colloids have a net negative charge with an average ζ potential of $-42 \pm 1 \text{ mV}$. As soft surfaces, we used $150 \mu\text{m}$ thick polyacrylamide (PAA) cross-linked gel films deposited on a microscope coverslip. The polymerization of PAA was triggered by adding

tetramethylethylenediamine and ammonium persulfate to a solution containing acrylamide monomers and the cross-linker bis-acrylamide (bis-AA), all in phosphate buffered saline buffer ($p\text{H}$ 7.4). For control experiments, we coated the same silica colloids with positively charged

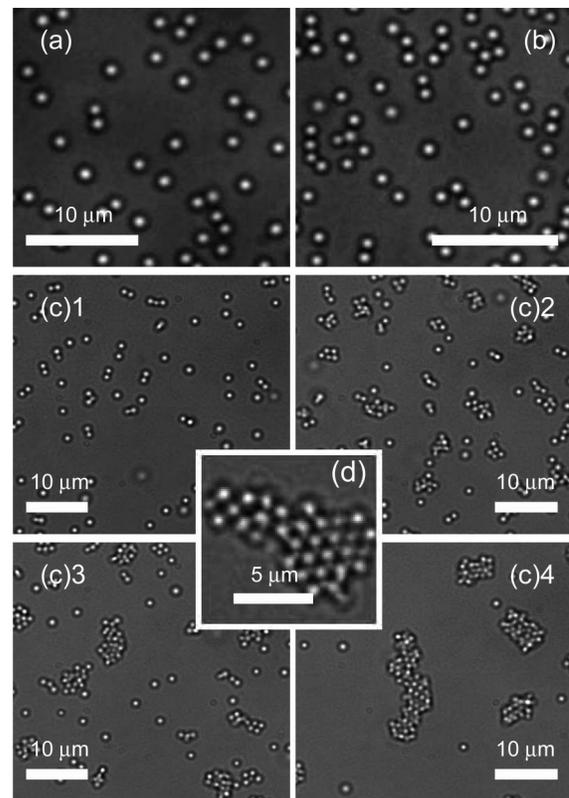


FIG. 1. (a),(b): Microscope images of plain silica colloids sedimented on a PAA soft gel with different surface coverage. (c) 1–4: Aggregation process of plain silica colloids sedimented on an ultrasoft PAA gel surface at $t = 0$ (1), $t \approx 15$ (2), $t \approx 30$ (3), and $t \approx 60$ min (4). (d): Highlight of an ordered aggregate on the ultrasoft gel.

poly-*L*-lysine-poly(ethylene glycol) (PLL-PEG, Surface Solutions, Switzerland) that is easily adsorbed onto silica and provides steric stabilization. PLL-PEG coated beads were almost neutral with a ζ potential of -2.2 ± 0.2 mV. Nonadhesive hard surfaces were obtained by coating the glass bottom of 8 mm diameter incubation wells (Sensoplate, Greiner bio-one) with PLL-PEG. Using a PAA concentration of 5%, we tuned the stiffness of the material by changing the bis-AA fraction. The observations of weak attractive interactions between colloids were qualitatively and quantitatively similar for gels with elastic shear modulus G' [13] between 55 and 522 Pa; thus, we focused on a substrate prepared with 4×10^{-4} vol/vol bis-AA resulting in a $G' = 240 \pm 5$ Pa—we refer to this gel as the “soft” substrate. In contrast, colloids on “ultrasoft” gels produced with a bis-AA concentration of 1×10^{-4} vol/vol and with $G' = 12 \pm 3$ Pa exhibited stronger interactions.

On soft gels, sedimented colloids did not undergo large-scale aggregation. Instead, we observed the formation of dimers, triplets, and small chains and clusters, as shown in Figs. 1(a) and 1(b) for two different surface densities. On ultrasoft gels, sedimented beads initially formed small clusters that were still mobile and merged into larger ones. The aggregation stopped after about 1 h as the reduced mobility of big clusters kinetically hindered the process. A sequence of images of the aggregation is shown in Fig. 1(c). The larger aggregates could be either amorphous or ordered in a hexagonal close-packed lattice [see Fig. 1(d)]. The mobility of colloids within the aggregates was small, and we only observed rearrangement of the beads located at the outer perimeter. By using PLL-PEG coated colloids instead of plain ones, the aggregation still took place on the same time scale, but the clusters showed liquidlike behavior with a continuous rearrangement of single colloids in the bulk of the aggregates.

We measured the effective pair potential of supported beads with two complementary techniques. For the first one, referred to as “blinking optical tweezers” (BT) [14,15], we positioned two isolated colloids at separations between 1.5 and 10 μm by using optical tweezers [16]. After releasing the colloids, movies were taken at 30 frames per second, and the separation r between beads was obtained by tracking their positions with conventional algorithms [17]. After discretizing r with a mesh size of 25 nm, we sampled the transition matrix elements P_{ij} that express the probability for r to evolve from bin i to bin j in the time between two consecutive frames. The probability distribution of r is $\rho_i^s = P_{ij}\rho_j^s$, and the effective pair potential is given by $V(r)/(k_B T) = -\log(\rho^s)$. The second method consisted of extracting $V(r)$ from the equilibrium radial distribution function $g(r)$, which was estimated for every sample by taking a few hundred snapshots from an area of about 1.5×10^{-2} mm² with a surface coverage of 0.5%–1%. At these densities, we could neglect many-body

effects and extract the pair potential as $V(r)/(k_B T) \approx -\log[g(r)]$. In both techniques, superposition of colloid diffraction images can bias the measurement of the intercolloid distance in microscopy images, leading to systematic errors in the estimate of $V(r)$ [2,18]. We corrected our data by following the method proposed by Polin, Grier, and Han [2]. The potential extracted by using the BT method arises from forces that may not have an underlying equilibrium potential, including hydrodynamic coupling [3]. The evaluation of $V(r)$ from $g(r)$ is generally preferable, being unaffected by nonequilibrium effects. However, due to rapid aggregation, $g(r)$ measurements on the ultrasoft gels in the low-density limit were unfeasible. The BT technique was applicable to all of our surfaces and was therefore adopted as a reference technique. $g(r)$ measurements were used to spot nonequilibrium artifacts.

For the case of plain silica colloids supported by soft PAA surfaces, the apparent $V(r)$ measured with BT evidences a sharp minimum for $r \approx \sigma$, as shown in Fig. 2, curve A. The same measurement done by using PLL-PEG coated colloids on the same soft gels is displayed in Fig. 2, curve B, and demonstrates a weakened short-range attraction. In Fig. 2, curve C, we show $V(r)$ measured with BT for PLL-PEG coated colloids on ultrasoft gels [19]. In this case, the short-range attraction is stronger than for soft gels. In Fig. 2, curves A, B, and C show a maximum in the potential at $r \approx 1.2\sigma$. This cannot be ascribed to screened Coulomb repulsion as the Debye screening length is estimated to be only a few nanometers [20]. As a control, we

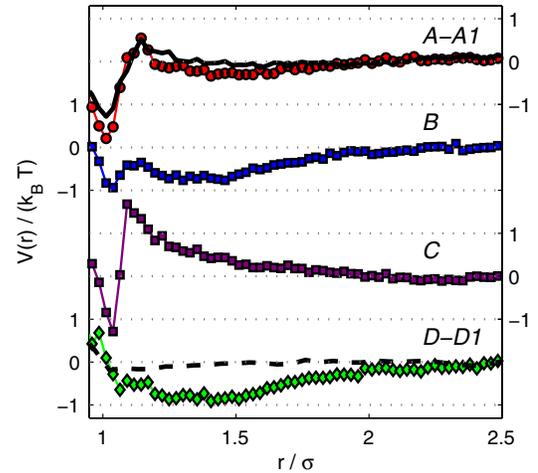


FIG. 2 (color online). Pair potentials of colloids sedimented on gels and glass surfaces measured with the BT or pair-distribution [$g(r)$] methods. Bare silica colloids sedimented on a soft PAA gel surface measured with BT (A, \circ) and with $g(r)$ (A1, solid line); PLL-PEG coated silica colloids sedimented on a soft PAA gel surface measured with BT (B); PLL-PEG coated silica colloids sedimented on an ultrasoft PAA gel surface measured with BT (C); PLL-PEG coated silica colloids sedimented on a PLL-PEG coated rigid glass surface measured with BT (D, \diamond) and with $g(r)$ (D1, dashed line). The curves have been vertically shifted for clarity.

measured $V(r)$ with BT for the case of PLL-PEG coated colloids sedimented on a PLL-PEG coated rigid glass surface. The potential, plotted in Fig. 2, curve *D*, does not show the short-range minimum observed on PAA surfaces. The broader, longer-ranged minimum observed in BT measurements of $V(r)$ for both soft gels (Fig. 2, curves *A* and *B*) and rigid glass surfaces (Fig. 2, curve *C*) can be ascribed to nonequilibrium artifacts [3]. This hypothesis is confirmed by equilibrium measurements of $V(r)$ from $g(r)$ for plain colloids settled on a soft gel surface and PLL-PEG coated colloids settled on a rigid glass surface, shown in curves *A1* and *D1* of Fig. 2. In both cases, the long-range minimum is absent, consistent with the explanation that this attraction was due to hydrodynamic coupling. As demonstrated with both BT and equilibrium measurements from $g(r)$, the short-range attraction is present only on PAA gels but not on the rigid surface, indicating that it is an equilibrium effect mediated by the polymer-gel substrate. The same argument applies to the repulsive maximum in the colloid pair potential located at $r \approx 1.2\sigma$ that is present when the colloids are on soft gels but absent when colloids are on a hard glass interface. We can deduce that the short-range attraction is responsible for the small aggregates on the soft gel substrates and for the large-scale clustering on the ultrasoft ones, where it became stronger. The weakening due to the PLL-PEG coating accounts for the liquidlike behavior of clusters on the ultrasoft gels.

The range and shape of the short-range attractive well are compatible with a description in terms of depletion [21]. The PAA gel can, in principle, release free polymers in solution that may act as depletants. However, the absence of aggregation on bare glass in the same sample cell that contains gels with associating colloids excludes this possibility. Chen and Ma [22] predicted that a combination of excluded volume effects and polymer-colloid interfacial energy can give rise to an interaction between colloids embedded in a polymer brush. A similar effect can occur in our system by assuming a partial inclusion of the colloids in a weakly cross-linked polymer layer on top of the substrates.

We tested this possibility by measuring the colloid-surface interaction energy and the effective viscoelastic properties of the gel surfaces by using microrheology. We tracked fluctuations of sedimented beads in the z direction by videomicroscopy measurements [23]. The diffraction image of a colloid changes as a function of the distance between the bead and the focal plane of the objective lens. We took a set of a few hundred pictures of an immobile colloid by moving the focus with steps of 25 nm and associating an intensity $I(z)$ to each reference image by integrating the pixel value within a circle concentric with the bead. Fitting the intensity as a function of z , we obtained a calibration curve that is monotonic within an interval of a few microns. Working with the same

illumination conditions, we took movies of single freely diffusing colloids, measured $I(t)$ for each frame, and found the corresponding $z(t)$ by using the calibration curve. We sampled the probability distribution $\rho(z)$ by taking a histogram of $z(t)$. The colloid-surface potential is given by $V(z)/(k_B T) = -\log[\rho(z)]$. As a control we measured $V(z)$ for PLL-PEG coated colloids sedimented on a PLL-PEG coated glass surface. As expected, the only potential attracting the bead towards the surface is the gravitational one, as shown in Fig. 3(a), curve *A*. Fitting the linear region of $V(z)$, we estimated the weight of the colloid $mg \approx 2.6$ fN, which is in good agreement with the nominal value ≈ 3 fN. For plain silica colloids settled on the surface of a soft PAA gel, $V(z)$ reveals an additional attraction [see Fig. 3(a), curve *B*]. We quantify this attraction with a parabolic fit that gives an effective spring constant of 4.1 nN m $^{-1}$. For the case of an ultrasoft gel, Fig. 3(a), curve *C*, shows that the colloid-surface attraction is even stronger with an effective spring constant of 7.2 nN m $^{-1}$. The attraction points to a partial inclusion of the beads into the gel surface that should be deeper for the case of ultrasoft gels.

Further insight on colloid-surface interactions was obtained by analyzing the horizontal fluctuations of a single sedimented colloid applying microrheology techniques [24]. Note that we report only effective values about the viscoelastic properties of the surfaces, as conventional

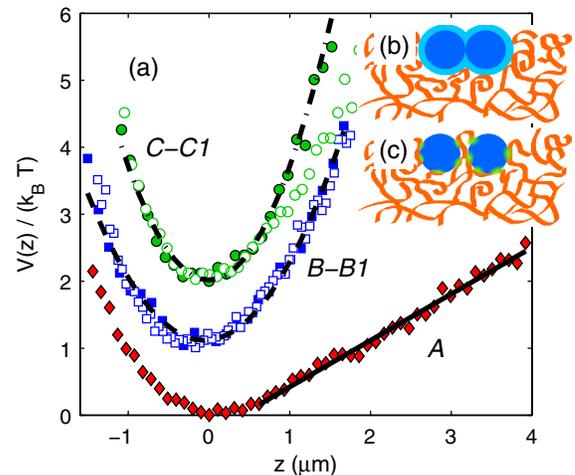


FIG. 3 (color online). (a) Experimental colloid-surface interaction potentials for PLL-PEG coated silica colloids confined by a PLL-PEG coated glass surface (*A*); plain (*B*, filled symbols) and PLL-PEG coated (*B1*, empty symbols) silica colloids confined by a soft PAA gel surface; and plain (*C*, filled symbols) and PLL-PEG coated (*C1*, empty symbols) silica colloids confined by an ultrasoft PAA gel surface. The solid line is a linear fit. Dashed and dot-dashed lines are quadratic fits. The curves are shifted vertically and the minima aligned horizontally for clarity. Insets: Not-to-scale sketch of two beads partially included in the gel surface and interacting through excluded volume effects (b) and colloid-polymer adhesion (c).

microrheology techniques are derived for probe colloids embedded in an isotropic environment [25]. The effective surface viscosity η_s , estimated from the effective loss modulus [24], was 1.7 and 5.5 mPa s for soft and ultrasoft gels, respectively. For both substrates, η_s was larger than the value expected for water at room temperature, even in the presence of hydrodynamic coupling with a boundary [26]. This indicates a coupling between the probe bead and the PAA gels, compatible with partial inclusion. Bulk measurements of the viscosity show that the soft gel has a higher viscosity ($\eta = 24 \pm 0.5$ Pa s) than the ultrasoft gel ($\eta = 1.7 \pm 0.5$ Pa s), which is expected as the soft gel has a higher cross-link density. The opposite trend between the effective surface and bulk viscosities can be explained as follows. The bead penetrates more deeply in the ultrasoft gel than the soft gel because of the former's lower cross-link density. This results in a larger contact area between the bead and gel and thus a larger dissipation energy, which, in turn, diminishes the diffusion of the particle on the ultrasoft gel accounting for its larger effective loss modulus.

We suggest two possibilities to explain the observed interparticle attraction of colloids on PAA gels. The first, partial encapsulation, accentuates a depletionlike colloid-colloid interaction [22], as sketched in Fig. 3(b). The second, direct polymer bridging mediated by the colloid-polymer surface adhesion, can be responsible for the in-plane attraction, as shown in Fig. 3(c) [27]. For the ultrasoft gels, the deeper penetration of the beads explains the stronger in-plane attraction either by increasing the excluded volume between two adjacent colloids or increasing the bead-gel contact surface. The repulsion found for $r \approx 1.2\sigma$ can be interpreted as the elastic response of polymer coils squeezed between two adjacent beads. We also measured $V(z)$ on the soft and the ultrasoft gels by using PLL-PEG coated beads, as shown in Fig. 3, curves B1 and C1. In the first case steric stabilization does not affect $V(z)$, while in the case of ultrasoft gels the potential is slightly less attractive.

In summary, we demonstrated that silica colloids sedimented onto soft cross-linked polymer-gel surfaces interact through a strong short-range equilibrium attractive potential that can be tuned by changing either the substrate stiffness or bead-surface properties and, for the case of ultrasoft substrates, produces large-scale aggregation, eventually leading to the formation of ordered phases. The in-plane interaction correlates with a colloid-gel attraction. All the experimental observations are consistent with the in-plane interactions arising from either depletion effects or direct polymer bridging. Further investigations are needed to determine which of these proposed mechanisms accounts for the aggregation of colloids on surfaces of soft polymer gels.

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