Experimental Investigation of Selective Colloidal Interactions Controlled by Shape, Surface Roughness, and Steric Layers

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Photolithography can be used to form monodisperse colloids of well-defined, nonspherical shape in a negative photoresist, SU-8. In aqueous suspension, in the presence of dextran as a depletant, we showed previously that the aggregation of these particles was highly selective for the end-to-end configuration: cylinders assembled into linear aggregates that could extend to lengths of tens of units without significant lateral aggregation. This article presents an in-depth study of the mechanisms by which these particles aggregate. In particular, we focus on the roles of global shape, roughness, and adsorbed layers of surfactants in mediating depletion, van der Waals (vdW), and electrostatic interactions between these particles. We describe in detail the fabrication and characterization of the particles. To allow for the interpretations of the experiments, we present predictions for the interactions between mathematically ideal cylinders with smooth surfaces, and a statistical thermodynamic model for the linear assemblies. We present experimental observations of the state of aggregation as a function of concentration of dextran and ionic strength for typical particles that present roughness of larger amplitude on their rounded side walls than on their flat ends. We compare this behavior to that of particles that lack this contrast in roughness; this comparison indicates that roughness can serve to attenuate strongly the attractive depletion interactions. To achieve a more quantitative measure of this effect, we analyze size distributions of linear aggregates to calculate the energies of the end-to-end “bonds” on the basis of our statistical model. We find that both the depletion and vdW interactions are attenuated ∼20 fold relative to predictions for smooth surfaces. We conclude with an assessment of outstanding questions and opportunities to exploit shape and roughness to direct the self-assembly of colloids.

Introduction

Materials with characteristic structural features on the colloidal scale (10 nm to 10 μm) are potentially important for applications in electronics, 1 optics, 2 high density memory, 3 microelectromechanical machines, 4 and tissue engineering. 5, 6 Parallel methods for the combination of thermodynamically controlled (reversible) and kinetically controlled (irreversible) steps. Despite significant progress in the pursuit of strategies of self-assembly of colloids, 2, 7–13 we still lack a sufficient diversity of the tools and depth of understanding to allow for rational design of desired materials from colloidal building blocks.

In this article, we focus on the challenge of forming selective colloidal “bonds”. For this purpose, we have exploited a new class of colloidal particles formed by photolithography in a polymeric photoresist (SU-8; Figure 1). 1, 14–16 These monodisperse “elements” present well-defined, nonspherical shape (Figure 1B). Our specific interest in these model particles stems from our previous observations of their self-assembly: 14 when stabilized with a nonionic surfactant (Figure 1D), they assemble end-to-end into linear aggregates; this assembly occurred for a specific range of concentrations of a soluble nonionic polymer (dextran) and ionic strength. Strikingly, these linear aggregates grew to lengths up to tens of units without significant lateral aggregation; the end-to-end interaction was highly selective relative to the other interparticle orientations. This degree of selectivity is unusual for colloidal particles of uniform composition, and, if understood, new avenues of controlled self-assembly may open, allowing for organization exceeding the periodicity of traditional top-down methods.
could be the basis of powerful methods to direct assembly. On the basis of our models, 17–20 we had expected that the complementarity of flat ends would lead to the most favorable interactions by depletion, as the extended contact achievable between these surfaces would expel a maximum of depleted solvent per area. Yet, the degree of selectivity was not explainable on the basis of the global geometry alone. Structural characterization of the particles by scanning electron microscopy (SEM) indicated that the particles presented heterogeneous roughness on their surfaces: the flat ends were noticeably less rough than the rounded side walls (Figure 1C). Studies by a number of investigators point to the potential importance of roughness in defining the strength of colloidal interactions, 21–25 and this work suggested that the contrast in roughness on our particles might be partly responsible for the observed selectivity. Finally, a number of investigators have illustrated the importance of adsorbed layers of surfactants in defining the interparticle interactions. 26–30

In this article, we present an investigation of the roles of shape, roughness, and adsorbed layers in defining the electrostatic, van der Waals (vdW), and depletion interactions between these lithographically defined particles. In the “Methods and Materials” section, we present the fabrication of the particles and their characterization by SEM, electrophoresis, and atomic force microscopy (AFM). In the “Theory” section we develop predictions for the interactions between mathematically ideal cylinders, with smooth surfaces; these predictions form a basis for interpreting our observations with the actual particles. In this same section, we develop a statistical thermodynamic model of the linear assemblies; this model allows us to extract free energies, energies, and entropies of the interparticle bonds within these aggregates. In the “Results and Discussion” section, we present the structures observed by optical microscopy in dispersions of these particles as a function of ionic strength and concentration of depletant, and with two different nonionic surfactants; we interpret these observations both qualitatively and quantitatively on the basis of our models.

Methods and Materials

Materials. Omnicote, SU-8 2002, SU-8 developer, and Remover-PG were purchased from Microchem Corp. (www.microchem.com). Deionized water (DI water) was obtained from a Millipore DirectQ5 system (www.millipore.com). Tris-HCl buffer was made from Tris base (J.T. Baker; Philllipsburg, NJ), DI water, and hydrochloric acid (37% (www.emdchemicals.com). Tergitol NP40 and sodium chloride (NaCl) were purchased from Sigma-Aldrich (www.sigmaaldrich.com). Tergitol NP70 was obtained from Dow Chemical (www.dow.com). Isopropyl alcohol (IPA, submicron filtered) was purchased from Fisher Scientific (www.fishersci.com). Dextran (Mr = 172 000 g/mol) was purchased from Polysciences (www.polysciences.com). Polished silicon wafers (100 mm-diameter) were purchased from Wafer Works (www.waferworks.com). Borosilicate capillaries with square cross-section (100 µm in cross-section) were purchased from Vitrocom, Inc. (www.vitrocom.com; part #ST5810). Capillary wax was purchased from Hampton Research Corp. (www.hamptonresearch.com).

Fabrication of Cylindrical Colloids. We used a photolithographic procedure to form the particles from a negative photoresist, SU-8. This process has been described in detail previously. 14 Briefly, a sacrificial layer of Omnicote was spun onto a silicon wafer (100 mm diameter) and baked at 200 °C on a hotplate for 1 min. Subsequently, SU-8 2002 was spun on this first layer at 6000 rpm (2000 rpm/s) for 40 s; this second layer was baked at 65 °C for 2 min and 95 °C for 3 min on a hot plate. A photomask (chromium-on-glass) was formed with a square array of 2000 × 2000 circular windows (diameter, 5 µm; pitch, 10 µm) with an optical pattern generator (GCA/MANN 3600F). This mask was used to expose circular regions (diameter ~ 1 µm) on the layer of SU-8 with a 5X wafer stepper (GCA Autostep 200; wavelength = 365 nm; exposure dose = 175 mJ/cm²); the pattern was repeated 425 times over the surface of the wafer. After exposure, the wafer was placed on a hotplate at 65 °C for 1 min and 95 °C for 2 min. The wafer was allowed to cool to ambient temperature prior to development of the SU-8 layer in SU-8 developer (~100 mL) with gentle manual agitation. The duration of this development step could be varied to control the roughness of the side walls of the particles: for most experiments reported in this work, a development time of 1 min was used at 65 °C.

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this paper, a development time of 1 min was used, leading to “rough” side walls (Table 1); for the experiment presented in Figure 9 with “smooth” particles (Table 1), the wafer was only dipped briefly (<5 s) in the SU-8 developer solution. The wafer was then carefully rinsed with IPA, and dried with nitrogen. To release the particles, the sacrificial layer of Omnicoat was dissolved by placing the developed wafer in a clean crystallization dish containing ~30 mL of Remover-PG and sonicating for ~30 s in an ultrasonic cleaner (Fisher Scientific model FS60H). Approximately 1.7 × 10^9 particles were generated per 100 mm wafer.

**Formulation of the Dispersions.** After release from the wafer, the particles were washed via centrifugation sequentially in clean Remover-PG, IPA, and DI water; three wash steps were performed in each solvent. After the last step in DI water, the particles were washed twice and suspended in a buffered aqueous solution (buffer = 1 mM Tris-HCl, pH = 8.3 at 23 °C) of Tergitol NP40 (75 µM) or Tergitol NP70 (60 µM). NP40 and NP70 are nonionic surfactants with a C12 alkyl chain attached, via a phenyl group, to a poly(ethylene oxide) (PEO) chain of 40 and 70 repeat units, respectively; these surfactants adsorb physically to the surface of the particles. To induce depletion interactions, we mixed the colloidal suspension with a solution of sodium chloride.

**Characterization of the Particles.** In this subsection, we describe our methods for the characterization of the global shape, surface roughness, and zeta-potential of the lithographically defined particles, and for the determination of the thickness of the adsorbed layers of surfactants. We report the measured values of these parameters in Table 1.

### Global Shape
Before placing the particles in the buffered solutions of surfactants, a sample of particles in DI water was redeposited on a silicon wafer, and imaged by SEM (Zeiss Supra 55VP or Zeiss Ultra 55). The average and standard deviation of the heights and diameters of the particles were measured on images such as the one presented in Figure 2A. These values are reported in Table 1. We note in Figure 2A that the two ends of the cylinders were not identical: one end had sharp edges that were globally flat (although not smooth); the other end had more rounded edges (arrow). The flat end was the one that was in contact with the Omnicoat sacrificial layer.

### Surface Roughness
As is visible by SEM in Figure 2A, the surface of the particles, particularly the side walls, presented topographical roughness. For both “rough” and “smooth” particles, we quantified the roughness using AFM (Digital Instruments Dimension 3100) in contact mode. For this procedure, particles that had been redeposited on a wafer from DI water were transferred to double-sided copper tape and adhered to a wafer; the adhesion to this tape was necessary to inhibit sliding of the particles on the substrate during imaging. Figure 2B,C shows a typical AFM scan of the particles side and end surfaces, respectively. Scans were performed on a limited number of particles (11 sides, 3 ends) due to the difficulty of finding them in appropriate orientation. The root-mean-square roughness, $\Delta_{\text{rms}}$, was calculated from topographical profiles along line scans of these images (software: WSxM, Nanotec Electronics S.L., andOriginPro, OriginLab Corp.). Values are reported in Table 1.

### Hamaker Constant
To enable predictions of the vdW interactions between particles formed in SU-8 2002, we proceeded as follows: (1) We performed a spectrally resolved (400 to 1300 nm) measurement of the index of refraction on a uniform film of the resist (prebaked, exposed, postbaked, and dipped into SU-8 developer and Remover-PG as a wafer with particles would normally be processed); this measurement was performed by ellipsometry (Woollam VASE). (2) We used these values to predict (according to a procedure described in references 33 and 34) the dielectric spectrum of SU-8, and used the reported dielectric spectrum for water to estimate, in the frame of the Lifshitz theory, the vdW interaction energy between

### Table 1. Characteristics of the Two Types of Particles Studied

<table>
<thead>
<tr>
<th>particle type</th>
<th>$R_e$ [nm]</th>
<th>$H$ [nm]</th>
<th>$\Delta_{\text{rms}}$ [nm]</th>
<th>$\Delta_{\text{side}}$ [nm]</th>
<th>$\psi$ [mV]</th>
<th>$\psi_{\text{NP40}}$ [mV]</th>
<th>$\psi_{\text{NP70}}$ [mV]</th>
</tr>
</thead>
<tbody>
<tr>
<td>“rough”</td>
<td>520 ± 20</td>
<td>875 ± 33</td>
<td>12 ± 5</td>
<td>3 ± 1</td>
<td>-50 ± 10</td>
<td>-27 ± 8</td>
<td>-21 ± 6</td>
</tr>
<tr>
<td>“smooth”</td>
<td>600 ± 20</td>
<td>1420 ± 20</td>
<td>4 ± 2</td>
<td>4 ± 2</td>
<td>-50 ± 10</td>
<td>-27 ± 6</td>
<td>-21 ± 6</td>
</tr>
</tbody>
</table>

*“Rough” particles were formed with a long SU-8 development time (60 s), and “smooth” particles were formed with a short development time (5 s). Reported are the radius $R_e$ and height $H$ of the particles, as measured by SEM. The root-mean-square amplitude of the roughness on the side walls, $\Delta_{\text{side}}$, and top and bottom walls, $\Delta_{\text{top}}$, as measured by AFM; three and seven images were used for the ends and sides, respectively, of the “rough” particles; seven images were used for the sides of the smooth particles. The zeta potentials $\psi$, $\psi_{\text{NP40}}$, and $\psi_{\text{NP70}}$ are for the particles without surfactant, with Tergitol NP40, and with Tergitol NP70 adsorbed on their surface, as measured by laser doppler electrophoresis.*

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two flat plates of SU-8 in water.\textsuperscript{33,34} We did not include retardation and electrolyte screening. (3) We then fit the Lifshitz prediction with the expression derived from the Hamaker theory for flat plates, using the Hamaker constant $H$ as an adjustable parameter. The best fit ($R^2 = 0.99997$) was achieved for $H = 7.3 \times 10^{-21}$ J. This value of $H$ is in the range of that established for other polymers in water, such as poly(methylmethacrylate) ($1.05 \times 10^{-20}$ J), poly(styrene) ($9.5 \times 10^{-21}$ J), and poly(isoprene) ($7.43 \times 10^{-21}$ J).\textsuperscript{34}

**Surface Potential.** To enable predictions of the electrostatic interactions between particles, we estimated their surface potential, $\psi_0$ [mV] on the basis of electrophoretic mobility as measured by laser doppler electrophoresis (Malvern Zetasizer Nano-ZS). For these measurements, we used dilute suspensions of particles ($\Phi = 0.005$ vol %) in the presence of 1 mM Tris-HCl buffer ($pH = 8.3$ at 23 $^\circ$C), without surfactant. Our estimates were based on the following assumptions: (1) The surface potential was equal to the zeta potential (surface potential at shear plane) measured in these conditions.\textsuperscript{27,35,36} (2) The surface potential was uniform over the entire surface of the particles. (3) The electrophoretic mobility, measured experimentally, followed the Henry–Smoluchowski prediction for the mobility of a sphere as a function of surface potential; this assumption is reasonable for a Debye screening length that is small compared to the size of the particles, as was the case for our experiments.\textsuperscript{34} Both with and without adsorbed surfactant, we found the surface potential to be negative. Although other studies also report negative potentials for SU-8 surfaces immersed in water,\textsuperscript{37} the chemical origin of this net negative charge remains unclear. We measured $\psi_0 = -50.5 \pm 10.5$ mV (Table 1).

**Thickness of the Layers of Adsorbed Surfactant.** To estimate the thickness of the layers of adsorbed surfactants on the surfaces of the particles, we related the zeta-potential of the particles with adsorbed surfactants, $\psi_z (a = N\text{P}40$ or NP70) [mV], to the surface potential of the bare particle, $\psi_0$, and the thickness of the layer, $\delta_a$ [m].\textsuperscript{27,34,36}

$$\tan \left( \frac{z \psi_z}{4k_B T} \right) = \tan \left( \frac{z \psi_0}{4k_B T} \right) \exp \left( - \kappa(\delta_a - \delta_S) \right)$$

(1)

where $k_B$ [J/K] is Boltzmann’s constant, $T$ [K] is the temperature of the dispersion, $z$ is the charge number of the ion in solution ($z = 1$ in our experiments), $\kappa$ [C] is the fundamental charge, $\kappa$ [m$^{-1}$] is the Debye parameter, and $\delta_S$ [m] is the thickness of the Stern layer; the Stern layer defines the limit between the strongly adsorbed ions moving with the particle and the mobile ions in the diffuse layer. We further considered the potential at the Stern layer to be equal to the effective surface potential of the particles, $\psi_0$; we took $\delta_S = 0.4$ nm.\textsuperscript{27} Equation 1 depends on two assumptions: (1) The shear plane under electrophoresis is shifted to the edge of the adsorbed layer (several studies have shown that this assumption is accurate for layers of nonionic polymers on the surface of particles, at sufficiently low ionic strength ($\kappa^{-1} > \delta$)).\textsuperscript{27,35,36,38} (2) The adsorbed layer did not significantly alter the surface potential, $\psi_0$, or the distribution of free ions relative to those associated with the bare surface.\textsuperscript{26,27} Figure 3 presents a schematic representation of the physical situation that corresponds to these assumptions.

In order to use eq 1 to calculate $\delta_{\text{NP}40}$ and $\delta_{\text{NP}70}$, we measured $\psi_{\text{NP}40}$ and $\psi_{\text{NP}70}$ via laser doppler electrophoresis in dispersions containing 75 µM of Tergitol NP40 and 60 µM of Tergitol NP70, respectively, both buffered with 1 mM of Tris-HCl. We performed these experiments only for the “rough” particles and found $\psi_{\text{NP}40} = -26.5 \pm 8.0$ mV, and $\psi_{\text{NP}70} = -21.0 \pm 6.2$ mV. Using eq 1, we obtained $\delta_{\text{NP}40} = 6.1 \pm 3.3$ nm and $\delta_{\text{NP}70} = 8.2 \pm 3.3$ nm. Despite the large uncertainty on these values, they indicate that the NP70 layer was thicker than the NP40 layer, as expected. In the Results and Discussion section, we consider potential consequences of these uncertainties for our interpretations of the observed interparticle interactions.

**Observation of Dispersions.** We observed the evolution of the cylinders in suspension by enclosing the dispersions in wax-sealed borosilicate capillaries with 100 µm × 100 µm cross-section. The capillaries were coated with polymers to avoid adhesion of the particles to the walls. The coating procedure is described in the Supporting Information of our previous publication on this system.\textsuperscript{14} Once loaded into the capillaries, the dispersions were exposed to germicidal UV light (Spectrolite X-15G, $\lambda = 254$ nm) for 1 min at 10 cm from the lamp to prevent the growth of microorganisms. Immediately after UV treatment, the capillaries were placed on a rotating stage (3 rpm),\textsuperscript{14} with their long axis oriented horizontally; the continuous rotation maintained the particles in a suspended state within the cross-section of the capillaries, despite the mismatch in their density relative to water ($\delta_{\text{water}} = 1.2$ g/cm$^3$).\textsuperscript{39} To evaluate the state of aggregation within a dispersion, the capillary was removed from the rotating stage and placed on an inverted optical microscope (Leica DM IRB, transmission mode) with a 100× oil immersion lens. Still frames (for state diagrams, Figure 8) or videos (for statistics of aggregation number, Figures 10 and 11) were captured with a digital camera (Basler A202k) immediately after removal from the rotating stage; during video capture, the focal position of the objective was moved through the entire depth of the capillary. Still images and videos were captured at three random locations in the capillary to improve statistics. For constructing histograms of
aggregation number (Figures 10 and 11), each sequence was then analyzed by manually counting, frame by frame, the number of aggregates in multiple frames was accounted for to avoid double counting.

Theoretical Concepts

Pair Interactions between Ideal Cylinders. To provide a basis for the interpretation of our observations of aggregation in dispersions of our cylinders, we present approximate forms of the electrostatic, vdw, and depletion interactions between two mathematically perfect cylinders (smooth surfaces and sharp edges) in the three principal, relative orientations shown in Figure 4: “end-to-end” (Figure 4A), “end-to-side” (Figure 4B), and “side-to-side” (Figure 4C). We discuss the impact of the stabilization layer and roughness in the following subsections.

Electrostatic Interactions. We evaluate the electrostatic interactions using a semianalytical approximation for constant surface charge; this form has been shown to be valid out to high surface potentials over the whole range of separation distances, \( x \) [m].\(^{46} \) The change in energy upon bringing two flat, circular plates of radius \( R_p \) [m] from infinite separation to a separation \( x \) [m] is

\[
\Delta U_{\text{elec}}^{e-s}(x) = 2\pi R_p^2 N_A \kappa^{-1} k_B T A \left\{ \sinh\left( \frac{B}{\sinh(\kappa x C)} \right) - \sinh(B) \right\} \tag{2}
\]

where

\[
A = \sinh(1.854|\Psi| - 0.585|\Psi|^2 + 0.1127|\Psi|^3 - 0.00815|\Psi|^4)
\]

\[
B = 0.571|\Psi| \exp(-0.095|\Psi|^{1.857})
\]

\[
C = 0.5 - 0.00424|\Psi|
\]

\[
\Psi = \frac{ze\nu_0}{k_B T}, \quad \kappa^{-1} = \sqrt{\frac{e_0 e \nu_0 k_B T}{2IN_A^2}} \tag{3}
\]

and \( I \) [mM] is the ionic strength, \( N_A \) is the Avogadro number \((6.02 \times 10^{23} \text{ mol}^{-1})\), \( z \) is the valence of the ions (symmetric electrolyte) in solution, \( \kappa^{-1} [\text{m}] \) is the Debye length, \( e_0 \) is the permittivity of vacuum \((8.854 \times 10^{-12} \text{ F/m})\) and \( e_0 (80 \text{ for water})\) is the dielectric constant of the solvent. The other parameters are the same as those defined in the previous section.

For the two other configurations involving curved surfaces, we use the Derjaguin approximation.\(^{33,41} \) This approximation is valid when the radius of the particles is large compared to the range of the interaction (thin double layer, \( R_p \kappa \gg 1 \)). The change in energy upon bringing together cylinders oriented end-to-side is

\[
\Delta U_{\text{elec}}^{e-s}(x) = \left\{ \begin{align*}
&HR_p^2 \int_{-\pi/2}^{\pi/2} \Delta U_{\text{elec}}^{e-s}(x + R_p \frac{\theta^3}{2}) d\theta, & H \leq 2R_p \\
&2R_p^2 \int_{-\pi/2}^{\pi/2} \Delta U_{\text{elec}}^{e-s}(x + \frac{R_p \theta^3}{2}) d\theta, & H > 2R_p 
\end{align*} \right. \tag{4}
\]

and oriented side-to-side is

\[
\Delta U_{\text{elec}}^{e-s}(x) = HR_p^2 \int_{-\pi/2}^{\pi/2} \Delta U_{\text{elec}}^{e-s}(x + R_p \theta^3) d\theta \tag{5}
\]

where \( H \) [m] is the height of the particles, and \( \theta \) is the azimuthal angle of the cylinder (Figure 4D). The integrals in eqs 4 and 5 were evaluated numerically with Maple (Maplesoft).

van der Waals Interactions. We treat vdw interactions using the Hamaker theory within the Derjaguin approximation, a detailed description of which can be found in the literature.\(^{33,34,41} \) The interaction energies for the end-to-end, end-to-side, and side-to-side configurations are presented in eqs 6–8:

\[
\Delta U_{\text{vdw}}^{e-s}(x) = -\frac{\pi R_p H a}{12\pi x^2} \tag{6}
\]

\[
\Delta U_{\text{vdw}}^{e-s}(x) = \left\{ \begin{align*}
&\frac{-H a R_p^2}{12\sqrt{2} x^{3/2}}, & H \leq 2R_p \\
&\frac{-H a R_p^3}{6\sqrt{2} x^{3/2}}, & H > 2R_p 
\end{align*} \right. \tag{7}
\]

\[
\Delta U_{\text{vdw}}^{s-s}(x) = \frac{-H a R_p^2}{12\sqrt{2} x^{3/2}} \tag{8}
\]

Depletion Interactions. Following the common description of the interaction between surfaces in the presence of a nonadsorbing solute,\(^{42–44} \) we evaluate the depletion energy as

\[
\Delta U_{\text{dep}}(x) = -\Pi_0 \Delta V(x) = -n_d k_B T \Delta V(x) \tag{9}
\]

where \( \Delta V(x) \) [m\(^3\)] is the overlap of excluded volume for a separation \( x \), and we have assumed that the solution of dextran is ideal such that the osmotic pressure \( \Pi_0 = n_d k_B T \), where \( n_d \) [1/m\(^3\)] is the number concentration of polymer coils (Figure 1C). The assumption of ideality is reasonable, given that all reported experiments were performed below the estimated concentration for overlap of the dextran molecules (\( \phi_d < \phi_d^\ast = 4.1 \text{ vol} \% \)).\(^{45} \) As the volume fraction of colloids was low in all of our experiments, we evaluate the concentration of dextran with respect to the total volume of the solution, without accounting for the volume occupied by the particles.\(^{46} \) We note that, for a neutral depletant such as dextran, we can ignore its electrostatic
Predictions of Pair Interactions for Ideal Cylinders with No Steric Layer. In Figure 5A, B, we present the evaluation of the depletion and Derjaguin–Landau–Verwey–Overbeek (DLVO) \((\Delta U_{\text{vdW}} + \Delta U_{\text{elec}})\) interactions between ideal cylinders in the three principal orientations based on eqs 2–12; values of parameters were representative of those in our experimental system: \(H = 1\ \mu m, R_p = 0.5\ \mu m, R_d = 12\ \mu m, I = 20\ \text{mM}, \phi_d = 0.5\%, \text{Ha} = 7.3 \times 10^{-21}\ \text{J}, T = 293.15\ \text{K}, M_w = 1.72 \times 10^5\ \text{g/mol}, \rho_d = 1.05\ \text{g/cm}^3, \text{and } \psi_0 = -50.5\ \text{mV}; \) no account was made for the adsorbed layer of surfactant. These plots serve as a comparison to the characteristics of the interactions of our actual particles, as extracted from our experiments (see “Results and Discussion” section). The plots illustrate three important points: (1) For all three orientations, the magnitude of the predicted minimum energies for depletion (Figure 5A) and DLVO (Figure 5B) are very large relative to the thermal energy, \(k_B T\). On the basis of these predictions, ideal particles should undergo irreversible aggregation with little orientational specificity. This predicted behavior is in sharp contrast to what we observed experimentally at these conditions; this discrepancy suggests that the adsorbed layer of surfactants, nonideal shape, and roughness modified the interactions among the actual particles. (2) For both depletion and DLVO, the magnitudes of interactions in the three orientations are predicted to be different, with the end-to-end configuration having the deepest minimum. For depletion interactions, this situation arises from the larger volume of depleted solvent \((AV)\) that is released upon joining two flat surfaces relative to curved surfaces. For DLVO, the closer and more extended contact of two flat surfaces leads to a stronger vdW attraction, which is the dominant interaction at long range under the cited conditions (and, more generally, for \(I > 3\ \text{mM}\)). This observation suggests that it should be possible to obtain orientational selectivity for the end-to-end orientation if, with entropic contributions, the free energy of the end-to-side and side-to-side interactions can be weak (less than approximately \(-3kT\)) while maintaining strong interaction between the ends. (3) As seen in the plot of the depletion and DLVO components in Figure 5C, the large electrostatic barrier is positioned \((x \sim 12\ \text{nm})\) such that the attractive depletion interaction is largely canceled by the electrostatic repulsion. This prediction suggests that a layer of neutral surfactant could play an important role in mediating depletion interactions by moving the steric boundary of the particle beyond the electrostatic double layer, and thus beyond the strong electrostatic repulsion.

**Influence of a Steric Layer Adsorbed on the Surface of the Particles.** Nonionic polymers are often used to improve the stability of colloidal particles when the electrostatic repulsion is insufficient to counterbalance the vdW attraction.\(^{48}\) Layers of PEO have been shown to generate strongly repulsive steric forces when they overlap.\(^{29,48}\) The size of the polymer is thereby a determining element in the efficiency of the stabilization against


vdW aggregation. In this study, the use of PEO-based surfactants of two different molecular weights (NP40 and NP70) allowed us to investigate the role of the thickness of the steric layer in defining stable suspensions and selective colloidal interactions.

When the adsorbed polymer chains are in a good solvent, as is the case for the PEO tails of NP40 and NP70, previous studies have shown that neither the surface charge density of the particles nor the mobility of ions within the electrostatic double layer are significantly affected by the presence of the polymers at the surface. In contrast, adsorbed layers of copolymers of PEO and poly(propylene oxide) (PPO) have been shown to affect vdW interactions between coated surfaces in water: by total internal reflection microscopy, Bevan and Prieve measured an increased vdW attraction between poly(styrene) surfaces coated with PEO–PPO–PEO (thickness ~15 nm) relative to bare surfaces in water: at the contact of the layers, they measured an increase of ~2 k_BT in the depth of the vdW potential between a sphere of 6 nm sphere and a flat surface. In the present study, given our lack of specific information about the density of the adsorbed layer on our particles and the smaller thickness of our adsorbed layers (~7 nm), we choose to neglect this potential contribution to the attraction; we will comment on the potential consequences of this simplification. In sum, we adopt a simple model for the effect of the Tergitol layers in which the presence of a steric layer does not bring a significant energetic contribution to either vdW or electrostatic interactions.

To motivate a model of the effect of the adsorbed layer on the depletion interaction, we consider the interactions of dextran and poly(ethylene glycol) (PEG) in solution: although both are soluble in water, they have been shown to be strongly incompatible in solution, as evidenced by the phase separation they experience at relatively small volume fractions. Steric interactions between the two species are thought to be the main reason of this incompatibility, suggesting that the soluble polymer coils of dextran should not penetrate into the adsorbed layer. We have previously established that dextran does not adsorb to the Tergitol-coated particles. We also neglect any compression of the adsorbed layer by the dextran coils. In sum, we treat the adsorbed layer as a hard wall with regard to overlap with dextran and with the layers on other particles. On the basis of these considerations, we adopt a model of the depletion interaction between Tergitol-coated particles that is identical to the conventional model presented earlier (“Depletion Interactions” section), except that the steric boundary from which the depleted zone is measured is shifted by the thickness of the adsorbed layer, δα, into the solvent.

For end-to-end assembly, the modified form of eq 10 becomes

$$
\Delta U^e_{xg}(x) = -\frac{\pi N_A \delta_\alpha}{M_w} k_B T \phi_0 R_p^2 (2R_d + 2\delta_\alpha - x)
$$

(13)

where δα stands for δNP40 or δNP70. Figure 6 summarizes schematically our hypothesis for the form of the depletion, vdW, and electrostatic interactions in the presence of an adsorbed polymer layer: the potential energy profiles of the vdW and electrostatic interactions originate from the edge of the layer of adsorbed polymers (x = 0), whereas the effective potential profile of the depletion interaction originates from the edge of the layer of adsorbed polymers (x = δα).


**Influence of Surface Roughness.** We have considered so far a model system of particles presenting perfectly smooth surfaces. However, SEM and AFM experiments (Figure 2) clearly revealed that our particles exhibit some surface roughness, like in many real colloidal systems. It is thus necessary to consider the role of this roughness on the different interactions. Numerous studies have tried to elucidate the effect of model or random surface roughness on both components of the DLVO interaction. The recent paper of Dagastine et al. summarizes the situation for vdW interactions and presents an interesting method to estimate the influence of roughness based on AFM data and the dielectric properties of the different constituents of the system. One of their main conclusions, supported by experimental evidence, is that roughness can lead to a significant decrease in the strength of the interaction. The electrostatic interactions between rough surfaces have received less attention. A theoretical treatment indicates that roughness can slightly increase the electrostatic repulsion within the screening layer.

We now turn to the effect of surface roughness on the depletion interaction. Given the geometrical interpretation of the depletion interaction in terms of the volume of depleted solvent that is released upon joining two surfaces (eq 9), it is reasonable to propose that random and noncomplementary roughness on the approaching surface could diminish this interaction by inhibiting the overlap of the depleted zones. Figure 7 illustrates this effect schematically for three limits: (1) The depth of the roughness is small compared to the size of the depletant (Δm ~ R_d, Figure 7A); in this limit, despite the roughness, the depletant is expelled from the gap upon assembling the surfaces, and, therefore, the roughness has no significant effect on the strength of the interaction predicted for smooth surfaces. (2) The surface features are commensurate with the size of the depletant (Δm ~ R_d); in this situation, a few zones of excluded volume cannot overlap; these zones lead to a reduction of the strength of the depletion interaction. (3) The amplitude of the roughness is large compared to the size of the depletant (Δm >> R_d, Figure 7C); in this limit, the excluded volume layers cannot fully overlap, the volume of expelled solvent is smaller than for smooth surfaces, and the strength of the interaction is significantly reduced. We note that this picture depends on the assumption that the lateral size of the roughness (the characteristic wavelength) is large enough to
Figure 7. Schematic representation of the possible role of random, noncomplementary roughness on depletion interactions induced by nonadsorbing polymer coils of radius of gyration $R_d$. (A) When $R_d$ is much larger than the rms roughness, $\Delta_{R_{\text{rms}}}$, the excluded volume layers can fully overlap. As a consequence, the interaction is not expected to be significantly different from the one existing between particles presenting smooth surfaces. (B) As $R_d$ becomes commensurate with the size of the surface features, some zones of excluded volume do not overlap; these zones lead to a reduction of the depletion strength. (C) In the thin excluded volume layer limit, the weak overlap of excluded volume layers is expected to significantly decrease the strength of the interaction.

accommodate a polymer coil of radius $R_b$, as seen in Figure 2B,C, this assumption is reasonable for the roughness found on our particles. This perspective has been outlined and validated experimentally by Zhao and Mason.\(^{25}\)

**Thermodynamics of Assembly.** At intermediate values of the depletion volume fraction and ionic strength, we observed that the particles assembled end-to-end into linear assemblies.

In the section discussing the statistical analysis of columns, we exploit this simple state of aggregation to extract an estimate of the energy of the end-to-end bonds, $\Delta U_{\text{bond}}$, via comparison of the observed distribution of linear aggregates to a prediction from statistical thermodynamics. We follow the formulation of Sciortino et al.\(^{57}\) to arrive at a predicted distribution. Their analysis is based on the Wertheim perturbation theory (WPT).\(^{28}\) In the limit of low total volume fraction of particles, $\Phi_p \approx 1$, for which the reference state of a dispersion of hard-core monomeric particles would be uniform, the distribution of $N$-mers of bonding particles is approximately

$$
\Phi_N = N \Phi_1^N \left[ \frac{2V_{\text{bond}}}{V_p} \exp \left( -\frac{\Delta U_{\text{bond}}^{\text{e-e}}}{k_B T} \right) - 1 \right]^{-1} \quad (14)
$$

where $\Phi_N$ and $\Phi_1$ are, respectively, the volume fraction of cylinders in columns of length $N$ and the volume fraction of monomers, $V_{\text{bond}}$ [m\(^3\)] is the free volume associated with the bound state, $V_p = \pi R_p^2 H$ [m\(^3\)] is the volume of a particle, and $\Delta U_{\text{bond}}^{\text{e-e}}$ [J] is the energy per bond. The relationship in eq 14 is constrained by the conservation of particles: $\Phi_b = \sum_i \Phi_i = \text{constant}$. Given that aggregation will only occur if $\Delta U_{\text{bond}}^{\text{e-e}}$ is large relative to $k_B T$, we approximate eq 14 as

$$
\Phi_N = N \Phi_1^N \left[ \frac{2V_{\text{bond}}}{V_p} \exp \left( -\frac{\Delta U_{\text{bond}}^{\text{e-e}}}{k_B T} \right) \right]^{-1} \quad (15)
$$

We note that eq 15 can be understood as an expression of thermal equilibrium between particles in the monomeric state

$$
K = \frac{\Phi_N}{\Phi_1} = \exp \left[ -\frac{\Delta U_{\text{bond}}^{\text{e-e}}}{k_B T} \right] \quad (16)
$$

where $\Delta U_{\text{bond}}^{\text{e-e}}$ is the change in Helmholtz free energy associated with the formation of a single end-to-end bond between particles; the formation of an $N$-mer from $N$ monomers involves the formation of $(N - 1)$ such bonds. Combining eqs 15 and 16, we have

$$
\Delta U_{\text{bond}}^{\text{e-e}} = \Delta U_{\text{bond}}^{\text{e-e}} - k_B T \ln \left( \frac{2V_{\text{bond}}}{V_p} \right) \quad (17)
$$

In eq 17, we can identify the entropic costs of forming a bond, $\Delta U_{\text{bond}}^{\text{e-e}} = k_B T \ln (2V_{\text{bond}}/V_p)$. We note that pressure-volume terms are absent from the expression for the Helmholtz free energy; this absence is consistent with the fact that $\Phi_p \ll 1$, and thus the osmotic pressures and changes of osmotic pressure associated with the particles are small (the osmotic pressures associated with the depletant are accounted for in the depletion energies included in $\Delta U_{\text{bond}}^{\text{e-e}}$).

In the section discussing the statistical analysis of columns, we use eqs 16 and 17 to analyze the experimentally observed distributions of columnar aggregates. For this purpose, we exploit the following equation:

$$
\frac{1}{N} \ln \left( \frac{\Phi_N}{\Phi_1} \right) = \ln(\Phi_p) - \left( N - 1 \right) \left[ \frac{\Delta U_{\text{bond}}^{\text{e-e}}}{k_B T} \right]
$$

$$
\ln(\Phi_p) - \left( N - 1 \right) \left[ \frac{\Delta U_{\text{bond}}^{\text{e-e}}}{k_B T} - \ln \left( \frac{2V_{\text{bond}}}{V_p} \right) \right] \quad (18)
$$

Thus, if we plot $(1/N) \ln(\Phi_N/\Phi_1)$ versus $(N - 1)/\Phi_1$, we should find a straight line with slope $= -\Delta U_{\text{bond}}^{\text{e-e}}/k_B T$. In interpreting these values, we take $\Delta U_{\text{bond}}^{\text{e-e}} = \Delta U_{\text{deg}} + \Delta U_{\text{edw}} + \Delta U_{\text{elec}}$, with the individual components based on eqs 2, 6, and 13, evaluated at an equilibrium state that we must specify. For $V_{\text{bond}}$, we use the following form:

$$
V_{\text{bond}} = \lambda \pi R_p^2 \quad (19)
$$

where $\lambda$ is the typical magnitude of thermally induced displacements of bound particles relative to one another. The value of $\lambda$ depends on the shape of the potential well about the equilibrium separation; we will provide an estimate for this value in the section “van der Waals Interactions at High Ionic Strength”.

We note that our use of the WPT as formulated by Sciortino et al. for the treatment of cylindrical particles is not strictly appropriate, as it was developed for spherical particles. In particular, the reference state for their formulation is a dispersion of hard spheres. Yet, in a dilute dispersion for which we assume a spatially uniform distribution of monomers in this reference state, the distinction between spheres and cylinders should not have any significant impact, as hard cylinders should also be uniformly and isotropically distributed at infinite dilution.

**Results and Discussion**

**General Morphology of the State Diagrams.** Figure 8 shows state diagrams for suspensions of “rough” particles (see Table 1), at $\Phi_p = 0.45$ vol %, stabilized by NP40 (Figure 8A) and NP70 (Figure 8B) in dispersions with various volume fractions of dextran, $\phi_d$, and ionic strength, $I$. States were assigned after ensuring, by optical microscopy (Figure 8C–F), that the dispersions showed no evolution over a 24 h period. Most states

stabilized within 48 h after the preparation of the suspensions. Both diagrams present the same global morphology, with four distinct states: (1) homogeneously dispersed monomers (circles; Figure 8C); 2) linear aggregates or columns of particles in the end-to-end orientation (triangles; Figure 8D); (3) aggregates of particles arranged predominately in the three principal orientations defined in Figure 4 (stars; Figure 8E); and (4) large aggregates of particles in random relative orientations (squares; Figure 8F). The general structure of these diagrams is understandable based on simple considerations: at low ionic strength, the long-range electrostatic repulsion inhibits the assembly of the particles by overwhelming the vDW and depletion-based attraction. At high values of \( \phi_d \), strong depletion attractions induce random aggregation because the interaction strength for all particle–particle orientations are large compared to \( k_B T \). Subtler features of the observed states are also qualitatively consistent with the predictions in Figure 5 for interactions between ideal cylindrical particles: the observation that the dispersion first transitions into a columnar state (circles) for rising \( \phi_d \) or \( I \) is qualitatively consistent with the prediction that the end-to-end interaction is more favorable relative to the other principal interactions.

**Role of the Steric Layers.** Other features of these state diagrams shed light on the role of the adsorbed layer of surfactant in the definition of particle–particle interactions. We note that for higher values of \( I \) (\( I > 20 \text{ mM} \) for NP70 and \( I > 50 \text{ mM} \) for NP40), the transitions between states occur for constant \( \phi_d \); the state of the system appears to be independent of the electrostatic component of the interaction. This observation is consistent with the hypothesis presented in Figure 6 and eq13 that the layer of adsorbed surfactant shifts the origin of the depletion potential away from the surface of the particle while leaving the electrostatic potential unchanged: the electrostatic barrier recedes into the adsorbed layer, such that, with respect to depletion, the particles behave as if they had only a hard-core repulsion. The observation that the transition into this “steric” regime occurs at higher ionic strength for NP40 than for NP70 agrees qualitatively with our estimate that the layer of NP40 is thinner than the layer of NP70: for the thinner steric layer, the ionic strength must be raised further to ensure that the electrostatic barrier is buried. We also point out that, in the state diagram of particles stabilized by NP40, we observed the formation of short columns (less than four cylinders long) at high ionic strength (\( I \geq 50 \text{ mM} \)) with no depletant (\( \phi_d = 0 \)). This aggregation is not observed for NP70, even at ionic strengths as high as 200 mM. The observed aggregation in absence of depletant was likely the result of the attractive vDW interactions. The difference in behavior between particles with NP40 and NP70 supports the hypothesis (Figure 6) that the adsorbed layers, if sufficiently thick, can provide a steric barrier that holds the particles outside the range of the vDW attraction. These observations regarding the roles of the adsorbed layer of nonionic polymers are consistent with other experimental studies.29

**Influence of Surface Roughness.** Relative to the predictions in Figure 5 for ideal cylinders, the experimentally observed state clearly illustrates that the interactions among the actual particles are much weaker than calculated: in the absence of depletant, the particles are stable up to high ionic strength (50 mM for NP40 and up to over 200 mM for NP70; data not shown); for volume fractions of depletant larger than \( \phi_d = 0.1 \sim 0.2\% \), the aggregates exhibit orientational preferences (Figure 8D,E) rather than the random orientation that would be expected for the strong, predicted interactions in Figure 5A. The fact that we did not see this aggregation cannot be explained by the presence of the adsorbed layers based on our estimates of their thicknesses, as

Figure 8. State of aggregation of “rough” particles. (A,B) Diagrams representing the different states observed in suspensions of cylindrical particles stabilized by the nonionic surfactants Tergitol NP40 (A) and NP70 (B), at various ionic strengths \( I \) [mM] and volume fractions of dextran \( \phi_d \) [%]. Four distinct states are observed: dispersed (blue circle), columnar (pink triangle), parallel and orthogonal aggregation (gray star), and isotropic aggregation (yellow square). (C–F) Representative optical micrographs of states identified in panels A and B. Characteristics of dispersions: Particles in C–F were all stabilized with NP40: (C) \( I = 5 \text{ mM} \), \( \phi_d = 2\% \); (D) \( I = 15 \text{ mM} \), \( \phi_d = 0.5\% \); (E) \( I = 30 \text{ mM} \), \( \phi_d = 0.5\% \); (F) \( I = 40 \text{ mM} \), \( \phi_d = 0.75\% \). All dispersions evolved on a rotating stage. Scale bars = 10 \( \mu \text{ m} \).

the predicted minimum would be outside of the steric barrier. This discrepancy suggests that the roughness of the surfaces of the particles may significantly influence the DLVO and depletion interactions. We explore this possibility further in the next subsections.

Another inconsistency between the observed states of aggregation for “rough” particles and the predictions for ideal cylinders is that the experimentally observed selectivity for end-to-end aggregation was greater than can be explained by the calculations. On the basis of the predicted differences in energies for end-to-end versus side-to-side interactions (Figure 5A), columns should start to aggregate laterally once they reach a length of \( \sim 5\sim 6 \). Yet, as seen in Figure 8D (see also movie in Supporting Information for a dramatic example: \( I = 10 \text{ mM} \), \( \phi_d = 0.6\% \), and NP70) the columns had little tendency to aggregate laterally even for columns of length in excess of 10 particles. To test whether this selectivity arose in part due to the contrast in surface roughness on the sides versus the tops of the “rough” particles (\( \Delta d_{\text{side}} = 13 \text{ nm} \); \( \Delta d_{\text{top}} = 3 \text{ nm} \)), we prepared dispersions with “smooth” particles that presented reduced roughness on the side walls (\( \Delta d_{\text{side}} = 4 \text{ nm} \)). Figure 9 shows a typical optical micrograph of the aggregates observed in a dispersion with NP40,
larger crystals with such an organization previously. The contrast in selectivity of aggregation for the different "rough" particles may have played an important role in defining the "smooth" particles, the assemblies were aggregates of columns (Figure 8A, triangles; Figure 8D). Characteristics of dispersion: "Smooth" particles with NP40, $I = 15$ mM and $\phi_d = 0.3\%$. Scale bar = 10 $\mu$m.

Figure 10. Steady state distribution column sizes observed in a single dispersion after evolution on a rotating stage (dispersed, open squares), after settling on a stationary, horizontal surface (settled, open circles), and after evolution again on a rotating stage (dispersed, open triangles). Distributions were counted in two videos at each state ($\geq 300$ particles in each video). The reported values are the average from these two videos; the error bars show the spread of the two values. Characteristics of dispersion: "rough" particles stabilized by NP70. $I = 10$ mM and $\phi_d = 0.45\%$.

$\phi_d = 0.3\%$ and $I = 15$ mM; for the "rough" particles, these conditions would fall in the columnar regime (Figure 8D); for the "smooth" particles, the assemblies were aggregates of columns of moderate length. Qualitatively, these structures correspond more closely to the predicted level of selectivity for end-to-end versus side-to-side interactions for smooth particles (Figure 5). This observation suggests that the contrast in roughness on the "rough" particles may have played an important role in defining the contrast in selectivity of aggregation for the different orientations. We treat the potential of roughness more quantitatively in the subsection titled "Analysis of Energy of End-to-End Bonds".

We also note that the clusters seen in Figure 9 are crystalline: the cylinders of each column are aligned laterally with the cylinders of the neighboring columns. We reported the observation of larger crystals with such an organization previously. The origin of this intercolumnar alignment remains unclear, although it could be related to ordering in liquid crystalline systems.

Figure 11. Typical plot of $(1/N) \ln(\Phi_N/N)$ as a function of $(N − 1)/N$, where $\Phi_N$ is the volume fraction of particles in columns of length $N$. The line is the best fit to the experimental data (squares). Over 300 particles were analyzed to form the distribution. From eq 18, the slope gives $-\Delta F_{\text{end}}/k_B T$, where $\Delta F_{\text{end}}[I]$ is the free energy of an end-to-end bond. Characteristics of the dispersion: "rough" particles stabilized by NP40. $I = 10$ mM and $\phi_d = 0.7\%$.

Demonstration of Reversibility of Assembly. To proceed with a more quantitative analysis of the energetics of assembly, we would like to use the statistical thermodynamic predictions developed in the section “Thermodynamics of Assembly” (eq 18) to analyze the aggregates formed in the columnar state. For this purpose, we must establish that these states were near thermodynamic equilibrium. We use the condition that a thermodynamic state forms reversibly to demonstrate this point. A first, qualitative indication of the reversibility of the aggregated state is the observation of the Brownian nature of the cylinders inside the columns: In the movie provided as Supporting Information (see Supplemental Movie1), we note the Brownian fluctuations of individual particles within the columnar aggregates. We also note the occurrence of scission and recombination of some columns due to thermal agitation. We tested reversibility more quantitatively by measuring the steady state distribution of column sizes (NP70, $\Phi_d = 0.06\%$, $\phi_d = 0.45\%$, and $I = 10$ mM) in a single capillary after the initial evolution of the dispersion on the rotating stage, after evolution with the capillary lying stationary on a flat bench, and after evolution back on the rotating stage. Figure 10 presents the distributions observed for these three cases. Upon settling in the stationary capillary (circles in Figure 10), we see that the distribution evolved toward higher degrees of aggregation relative to the original dispersed state (squares in Figure 10), as would be expected due to the higher concentration of particles in the settled state and a potential templating effect provided by the flat boundary. Upon redispersion on the rotating stage (triangles in Figure 10), the distribution returned very nearly to that of the original dispersed state. This observation implies that the steady state distributions of columns observed in dispersions that have evolved on the rotating stage form in a reversible, path-independent manner. We will thus proceed to analyze distributions formed in the dispersed state with our thermodynamic model.

Extraction of Free Energy of Bonds from Statistical Analysis of the Distribution of Column Sizes. For quantitative analysis, we chose columnar states with low degrees of aggregation ($N \leq 7$); these states were amendable to direct counting of the number and size of columns and contained no noncolumnar aggregates (e.g., branching structures). Figure 11 presents a typical plot of a distribution of column sizes as $(1/N) \ln(\Phi_N/N)$ versus $(N − 1)/N$. Equation 18 predicts that such a plot should be a straight line with slope $(-\Delta F_{\text{end}}/k_B T)$, the negative of the free energy of an end-to-end bond in units of the thermal energy, $k_B T$. In this form, the data is indeed linear; this observation

$\Phi_N/(\Phi_N / N)$

N

$1/N \ln(\Phi_N / N)$

$\Phi_N / N$

$\Phi_N / N$

$\Phi_N / N$

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I by considering the data at each point, we extracted above the transition from the dispersed to the columnar state. At fraction interaction between “rough” particles as a function of the depletant volume dependence of the bond volume, in Equation 20, we neglect the electrostatic interaction and ∆ by Tergitol NP40 (A) and Tergitol NP70 (B). The values of ∆ values are 18–25 fold smaller than the geometric area, the actual depletion interaction was thus 18–25 fold smaller than predicted.

Potential factors that could have led to this significant attenuation of the depletion interaction include: (1) The roughness of the end surfaces as measured by AFM; this roughness could act in the manner indicated in Figure 7. Yet, given that ΔRMS = 3 nm is 4 times smaller than the radius of gyration of the nonadsorbing polymer, Rg = 11.8 nm, we would expect to be in the intermediate regime between Figures 7A and 7B for which the interaction would be only moderately reduced. If roughness caused this attenuation, it is more plausible that a few large asperities held the surfaces at a distance of separation that was large compared to the average amplitude of the roughness; this mechanism would be consistent with the experiments of Zhao and Mason. (2) The global curvature of the ends of the cylinders could inhibit overlap of the deplet ed zones over the entire geometric area; as pointed out in Figure 2A, such curvature was perceptible on one end of each particle. We note though, that given the observed asymmetry between the curvature of the tops and bottoms, if this global curvature had a large impact, we would expect to have seen a preponderance of dimers in distributions of column sizes at low φd (Figure 10): the flat ends of two particles would bind strongly and leave the curved ends exposed. We did not see this effect in the dispersions that evolved on the rotating stage (dimers did appear in excess in settled dispersions (circles in Figure 10) but this may have been a kinetic state). (3) Weak adsorption of the dextran to the surfaces of the particles could weaken the depletion interaction in two ways: first, this adsorption could reduce the difference in osmotic pressure between the deplet ed zone and the bulk solvent (Πd in eq 9). Second, a low density of dextran coils adsorbed on the particles could induce strong repulsive forces that could hold the particles at a distance of separation large enough that the remaining depleted zone could not be excluded; a strong repulsion due to such coils at a separation of ~2 Rg could lead to an attenuation of the depletion interaction of the magnitude observed. We note further that any high molecular weight polymer adsorbed at low density could potentially have the same impact.61 Our previous note that the predicted linear scaling with φd holds; this observation supports the use of the osmotic picture of the depletion interaction, as stated in eq 9. Of course, this prediction was developed assuming that the ends of the particles were perfectly smooth, whereas the actual particles presented roughness of amplitude, ΔRMS = 3 nm. To investigate the impact of this roughness, we use the slope (m = (δΔFbond/δφd)/kBT) of the experimental data in Figure 12 to estimate the effective area of the rough ends of the particles, Areaeff dep = πR2eff. The ratio of this effective area to the actual geometric area, Areaeff (measured by SEM), is a measure of the factor by which the depletion interaction between the experimental particles was lower than the predicted interaction between ideal cylinders with idealized behavior of the depletant (eq 13). On the basis of eq 20, we have

\[
\text{Area}_{\text{eff dep}} = - \frac{M_w}{2\pi R_d N_A \rho_d} \frac{m}{m_{\text{obs}}}
\]  

We obtain Areaeff dep = (4.7 ± 0.8) × 10^{-2} μm² for particles stabilized by NP40 and Areaeff dep = 3.4 × 10^{-2} μm² for those stabilized by NP70. These values are 18–25 fold smaller than the geometric area, the actual depletion interaction was thus 18–25 fold smaller than predicted.

Figure 12. (A,B) Plots of the interaction energy ∆Fbond/kBT for end-to-end interactions between “rough” particles as a function of the depletant volume fraction φd [vol %], at different ionic strengths I. Particles were stabilized by Tergitol NP40 (A) and Tergitol NP70 (B). The values of ∆Fbond/kBT are those obtained from linear regression to distributions of column sizes represented as in Figure 11. The error bars are the standard deviation of those obtained from linear regression to distributions of column sizes at low φd (Figure 12) for end-to-end bonds, as illustrated in Figure 11. Figure 12 presents these free energies for particles stabilized by NP40 (Figure 12A) and NP70 (Figure 12B).

Depletion Interactions at High Ionic Strength. We begin by considering the data at I = 60 mM (circles in Figure 12) for which we assume that the electrostatic interaction was negligible and the particles were held apart by the adsorbed layer of Tergitol at a distance x = 2Δ, . We further assume that this separation distance remains constant as the depletant volume fraction φd is raised on the basis of our assumption of a hard wall steric potential. In this limit, we predict the following simplified expression for ∆Fbond, on the basis of eqs 13 and 17

\[
\frac{\Delta F_{\text{end}}} {k_BT} = \frac{1}{k_BT} \left[ \Delta U_{\text{dep}}(x = 2\Delta, ) + \Delta U_{\text{vdW}}(x = 2\Delta, ) \right] - \ln \left( \frac{2V_{\text{bond}}}{V_p} \right)
\]

In Equation 20, we neglect the electrostatic interaction and dependence of the bond volume, Vbond, on φd. Qualitatively, we
assumption of the binding of dextran to SU-8\textsuperscript{14} (see section “Formulation of the Dispersions”) cannot exclude these effects, as the level of sensitivity was on the order of a monolayer. If this third mechanism was responsible for the attenuation of the depletion at all surfaces of the particles, then the contrast in roughness between the sides and the ends could still explain the observed selectivity for end-to-end interactions (Figures 8 and 9) via the mechanism presented in Figure 7A–C. A repulsion could arise due to colloidal forces, such as hydration forces,\textsuperscript{62} that we do not account for explicitly in this work.

van der Waals Interactions at High Ionic Strength. We continue to consider the data for high ionic strength ($I = 60$ mM; circles in Figure 12) to investigate the vdW component of the end-to-end interaction. As for the depletion interaction, we define an effective area for the vdW interaction between rough surfaces, $\text{Area}_{vdW}$. On the basis of eqs 20 and 6, we have the following relationship between this effective area and the intercept ($\Delta F_{bond}^{vdW}(\phi_d = 0)/k_BT$) from the plots in Figure 12:

$$\text{Area}_{vdW} = -\frac{12\pi(x_0 - 2\delta_d)^2 k_BT}{\ln \left(\frac{2V_{bond}}{V_p}\right)}$$

To proceed with the evaluation of eq 22, we must estimate $V_{bond}$: In eq 19, we use an approximate form for the thermally induced displacement,

$$\lambda = k_BT \left( \frac{\partial (\Delta U_{vdW}(x))}{\partial x} \right)^{-1}$$

with $U_{vdW}(x)$ from eq 6 (i.e., for smooth surfaces), and the separation distance at equilibrium between bound particles, $x_0 = 2\delta_d$. For NP40 ($\delta_{NP40} = 6$ nm), we find ln($2V_{bond}/V_p$) = -9.5; for NP70 ($\delta_{NP70} = 8$ nm), we find ln($2V_{bond}/V_p$) = -8.6. With these estimates and experimental values for the intercepts ($\Delta F_{bond}^{vdW}(\phi_d = 0)/k_BT = -4.5$ for NP40 and $\Delta F_{bond}^{vdW}(\phi_d = 0)/k_BT = -2.1$ for NP70), eq 22 predicts $\text{Area}_{vdW}$ = $4.3 \times 10^{-2}$ $\mu m^2$ and $6.0 \times 10^{-2}$ $\mu m^2$ for NP40 and NP70, respectively; the geometrical area, as measured by SEM, was $\text{Area}_{exp}$ = 0.85 $\mu m^2$. We conclude that the magnitude of the vdW interaction is $\sim 15$–$20$-fold lower than that predicted for perfectly flat surfaces.

Potential factors that could have led to this significant attenuation of the vdW interaction include the following: (1) Roughness may have held the surfaces apart such that the global vdW interactions were lower than for flat surfaces. To test this hypothesis, we follow the suggestion of Bevan and Prieve\textsuperscript{23} to estimate a lower bound on the magnitude of the vdW attraction between rough surfaces: in eq 22, we take the equilibrium separation $x_0 = (2\delta_d + 4\Delta R_{\text{SMS}})$ for the vdW terms; this estimate neglects the interactions due to the asperities. For $\Delta R_{\text{SMS}} = 3$ nm, as observed by AFM, this estimate still leads to predicted interactions that are 5–$6$-fold stronger than those observed; for $\Delta R_{\text{SMS}} = 12$ nm, this estimate would agree with the observed strength of the vdW interaction. Thus, if the observed attenuation is due to roughness, there must have been a few large asperities ($\sim 24$ nm tall) per surface; the existence of such features is plausible, although they were not evident in our AFM images. (2) As with the depletion, the curvature of the ends of the particles could have limited the vdW attraction. Again, at high ionic strengths for which aggregation did occur by purely vdW interaction (NP40 case), we did not observe an excess of dimers, as would be expected if this mechanism were important. (3) A number of aspects of our proposed model of the vdW interaction could have led to an overprediction of its strength: our neglect of retardation and screening by the electrolyte could have led to an overestimate, although we would expect this correction to be less than a factor of 2 for surfaces near contact.\textsuperscript{23,33} An underestimate of the thicknesses of the adsorbed layers could account for some of the discrepancy, as could polydispersity in the length of adsorbed chains of Tergitol.\textsuperscript{28} (4) The presence of a low density of high molecular weight polymer chains adsorbed on the particle surfaces (see section “Depletion Interactions at High Ionic Strength”). A repulsion could arise as a result of colloidal forces, such as hydration forces,\textsuperscript{62} that we do not account for explicitly in this work. We note that our neglect of the dielectric contrast of the PEO layer in water should tend to make our predictions of the vdW attraction weaker rather than stronger.\textsuperscript{23}

We also note that our use of the vdW potential for smooth surfaces in eq 23 likely leads to an underestimate of $V_{bond}$, because the observed vdW potential was much weaker than this prediction. Thus, we have likely overestimated the magnitude of the negative, entropic term in eq 22, and our experimental estimate of the vdW attraction is larger than it would be with a more accurate estimate of the entropic component.

We further note that the experimental values of the intercepts, $\Delta F_{bond}^{vdW}(\phi_d = 0)/k_BT = -4.5$ for NP40 and $-2.1$ for NP70 are consistent with the fact that NP40-stabilized particles form short columns, while NP70-stabilized particles did not, since the aggregation point between colloidal particles is generally accepted to be approximately $-3k_BT$.\textsuperscript{63}

Interactions at Low Ionic Strength. We now turn to the data for lower ionic strengths: $I = 10$ mM and $I = 30$ mM for NP40 in Figure 12A. Under these conditions, we expect two added complications relative to high ionic strength ($I = 60$ mM): (1) the electrostatic repulsion should have played a non-negligible role in the particle–particle interaction, and (2) the distance of separation at equilibrium, $x_0$, should have been a function of both ionic strength, $I$, and volume fraction of depletant, $\phi_d$. We first note that the observation of the calculated $\Delta F_{bond}^{vdW}$ is linear in $\phi_d$ over the limited range studied (Figure 12A), as was observed for $I = 60$ mM. A naïve interpretation of this observation is that $x_0$ was a function of $I$ only, such that the depletion interaction behaved as predicted in eq 13 for a hard repulsive wall. If we pursue this hypothesis, the Helmholtz free energy is, approximately,

$$\Delta F_{bond}^{vdW} = \frac{\pi R^2}{6 M_w} N_A \phi_d [2R_d + 2\delta_d - x_0(I)] + \frac{\Delta U_{DLVO}(\phi_d(I))}{k_BT}$$

where we have ignored variations of the entropic term ($\ln(2V_{bond}/V_p)$) with $I$ and $\phi_d$. Thus, by interpolating the experimental data to $\phi_d = 0$, we can estimate the variation of the DLVO potential at equilibrium with the ionic strength. As seen in Figure 12A, this potential becomes more attractive (more negative) for increasing ionic strength; this observation is qualitatively consistent with a stronger screening of the electrostatic interaction. Additionally, the magnitude of the slope of the trend lines increases with increasing ionic strength; this observation is again consistent with the stronger screening of the electrostatic interaction, and, more particularly, with the reduction of the range of the interaction with increasing ionic strength (note the dependence of the depletion interaction on $x_0$). Unfortunately,
we can not justify the hypothesis that \( x_0 \) is independent of \( \phi_0 \). In particular, this hypothesis would require a steep repulsive barrier in the DLVO potential relative to the depletion induced attraction; this situation may have held, but, our data (Figure 12A and \( \delta_{\text{NP40}}, \delta_{\text{NP70}}, \psi_0, \text{Ha} \)) and approximate predictions (eqs 2, 6, and 13) do not support it. Therefore, in absence of any further information or a more complex model, we cannot provide a more definitive analysis of the data obtained in the electrostatically stabilized regime.

**Conclusion**

With this study, we have demonstrated that lithographically defined colloids provide a rich platform for the investigation of the roles of specific characteristics of particles—global shape, surface roughness, adsorbed surface layers—on self-assembly and particle–particle interactions. Our experimental observations showed that these particles could be induced to assemble preferentially into linear assemblies in which their flatter, smoother ends were bound and their rounded, rougher sides remained unbound. Comparison to assemblies of particles of similar global shape that lacked the contrast in surface roughness suggested that this strong selectivity arose from the combination of global shape and roughness. This observation points to the use of shape and surface roughness to define the preferred “bonding” between colloidal particles. By working with two different neutral surfactants, we found that small changes in the thickness of the stabilization layer could have significant influence on the assembled structures. Finally, we exploited the simplicity and near-equilibrium nature of the linear assemblies of these particles to analyze the distribution of sizes quantitatively with predictions of WPT. This analysis allowed us to extract the free energies, entropies, and energies associated with the particle–particle bonds as a function of depletant concentration and ionic strength. Not surprisingly, we found that the interactions energies between the particles with finite roughness were weaker than those predicted for ideal, smooth cylinders. Yet, the magnitude of the discrepancy between the predicted and observed energies (\( \sim \)20 fold for both depletion and vdW) was surprising, as the roughness observed by AFM was not large enough to explain this degree of attenuation of the interactions.

Future experiments with this system could help clarify the observed behavior. In particular, to aid in the interpretation of the depletion interactions, the following studies would be valuable: varying the size of the depletant in order to further elucidate the role of roughness in defining depletion interactions in this system, and measuring the degree of reversible binding of dextran to the surfaces more precisely to evaluate whether this adsorption played a significant role in reducing, relative to the predictions, the dependence of the observed interactions on the concentration of polymer. Further analysis of the roughness of the particles by AFM might reveal the existence of sparse asperities of large height; the presence of these features could aid in the interpretation of both the depletion and the vdW interactions. Finally, we have not excluded the possibility that the large orientational selectivity observed at moderate ionic strength (Figure 8D) could have arisen, in part, as a result of heterogeneity in the surface potential on the “rough” particles, i.e., if the sides presented higher surface potential than the ends. This study could be performed by solution-phase AFM or, potentially, by total internal reflection microscopy.

This study points to future developments in both fundamental colloid science and applications. The ability to form well-defined colloidal particles by direct fabrication opens the possibility of incorporating functional materials (e.g., silicon and III–V compound semiconductors) and shapes that are not accessible via synthetic routes into building blocks for self-assembly. The identification of selectivity based on shape and surface roughness provides a general rule for the design of particles that could assemble into nonclose packed structures. The apparent importance of roughness in our experimental system suggests that tuning this surface feature might allow one to define the selective interactions in the absence of complementarity of global shape: e.g., smooth patches on an otherwise rough sphere could act as directionally selective binding sites. Finally, the development of well-defined dispersions of nonspherical colloids provides interesting new targets for simulations and theories of self-assembly.

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**Supporting Information Available:** Movie depicting the Brownian fluctuations of individual particles within columnar aggregates. This material is available free of charge via the Internet at http://pubs.acs.org. LA801718J