**Directing assembly of DNA-coated colloids with magnetic fields to generate
rigid, semiflexible, and flexible chains**

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Abstract

We report the formation of colloidal macromolecules consisting of chains of micron-sized paramagnetic particles assembled using a magnetic field and linked with DNA. The interparticle spacing and chain flexibility was controlled by varying the magnetic field strength and the linker spring constant. Variations in the DNA lengths allowed for the generation of chains with an improved range of flexibility, as compared to previous studies. These chains adopted the rigid-rod, semi-flexible, and flexible conformations that are characteristic of linear polymer systems. These assembly techniques were investigated to determine the effects of the nanoscale DNA linker properties on the properties of the microscale colloidal chains. With stiff DNA linkers (564 base pairs) the chains were only stable at moderate to high field strengths and produced rigid chains. For flexible DNA linkers (8000 base pairs), high magnetic field strengths caused the linkers to be excluded from the gap between the particles, leading to a transition from very flexible chains at low field strengths to semi-flexible chains at high field strengths. In the intermediate range of linker sizes, the chains exhibited predictable behavior, demonstrating increased flexibility with longer DNA linker length or smaller linking field strengths. This study provides insight into the process of directed assembly using magnetic fields and DNA by precisely tuning the components to generate colloidal analogues of linear macromolecular chains.

1 Introduction

Colloids have been used as promising atomic analogue systems to investigate multiple phenomena, such as crystallization and melting transitions.¹⁻³ As synthesis techniques have increased in sophistication, colloids have also been used as mimics for more complicated molecular systems.⁴⁻⁶ Recently, the development of colloidal polymers (long chains of linked nano or micron-sized particles that are analogous to linear polymer molecules) has generated interest.⁷⁻¹⁰ Colloidal polymer systems have multiple advantages, including being small enough to be influenced by Brownian forces yet large enough to enable studies with ‘single molecules’ using ordinary light microscopy. Various methods have been used to assemble colloidal polymer chains, including methods that produce anisotropic patches that act as binding sites¹¹⁻¹⁴ and the use of anisotropic dipolar interactions in a directed assembly of colloids using either magnetic¹⁵⁻¹⁹ or electric fields.²⁰⁻²² These structures can be considered a concrete representation of the theoretical ‘bead-spring’ system introduced by Rouse and Zimm in the 1950s to describe linear polymer chains.²³⁻²⁴

In addition to the forces used to assemble the particles, the linking moieties (the ‘chemical bonds’ between the monomers) also contribute to the stability of the structures after the external field has been removed. These linkers can also be used to control the overall flexibility of the colloidal chains, a characteristic that can greatly affect the physical properties of the assembled structure. Generally, linear polymer chains can be classified into one of three regimes depending on the relationship between the contour length (L_c) and the persistence length (L_p), which is a measure of the length scale on which bending occurs. For rigid-rod chains, L_p is much greater than L_c . For semi-flexible chains, the two parameters are of similar order. For flexible chains, L_p is much smaller than L_c . Many types of molecules have been used as linkers, including bifunctional molecules^{17, 25-26}, polymers adsorbed to the surface of the particle that then become entangled when the particles are brought together,²⁷⁻²⁸ and short single-stranded DNA linker molecules.²⁹⁻³⁰

Micron-sized colloids linked with small molecules, such as glutaraldehyde^{17, 25} and low molecular weight poly(ethylene glycol),²⁶ produce chains that are very stiff, where the persistence lengths (8-800 nm) exceed the contour lengths. These linking chemistries can only produce chains in the rigid-rod regime and are severely limited with respect to the ability to create flexible polymer analogs. Using short DNA linkers (between 30 and 150 base pairs), the persistence lengths of colloidal chains were demonstrated to range from 50nm to 1 μ m.³⁰ This method is not sufficiently versatile to yield flexible chains, and the chains that are produced are not able to robustly withstand either shear or torque, suggesting that the applications of these chains may be limited. In a recent study using an electric field, Vutukuri et al.²⁸ produced rigid chains with persistence lengths of 40nm using unmodified particles and flexible chains with persistence lengths of 14 μ m using particles grafted with polyvinylpyrrolidone. The mechanics of their system differed slightly from the approach investigated in this study; in this work, linkers are used to bridge the particles via specific binding-site interactions rather than the polymer entanglement process used in the previous study. The behavior of the chains in the intermediate regimes and the transition mechanisms between the regimes are also investigated in the present study.

In this work, we demonstrate a robust and easily tunable method for creating colloidal particle chains using commercially available micron-sized magnetic colloids linked by double-stranded DNA. Several research groups have created higher-ordered assemblies by linking nanoparticles with DNA.³¹⁻³⁴ On the other hand, the use of micron-sized particles can facilitate visualization of the dynamics of the colloidal polymers. The DNA linkers can be easily synthesized and their sizes precisely controlled using standard molecular biology techniques. The mechanisms by which the properties of the linker molecules affect the properties of the colloidal chains are also a focus of this work. Keeping with the bead-spring analogy, two possible methods for adjusting the persistence length were investigated. First, by altering the length of the DNA, the spring constant of the linker could be tuned. Second, by changing the field strength used to link the particles, the interparticle spacing could be adjusted to either increase or decrease the area available for the linkers to form bridges,²⁹ thus varying the number of springs between the beads. Both of these variables are of vital importance to the assembly of the particle chains. As the linker

increases in length, the chains increase in flexibility. For the longest DNA used, however, the field induced exclusion of the linker between the beads, creating chains with increased stiffness. At low field strengths the chains linked with short DNA linkers became unstable, as the field strength was insufficient to bring the particles close enough to allow the DNA to bridge the gap.

Our model bead-spring system consists of micron-sized paramagnetic colloids connected with DNA linker lengths ranging from 564 base pairs (bp) to 8000bp under magnetic field strengths ranging from 19G to 275G. In this range, colloidal polymers were produced with persistence lengths spanning over three orders of magnitude—from 83mm to 19μm. With the typical chain contour lengths ranging from tens to hundreds of microns, chains were designed and constructed to exhibit properties that fall into all three flexibility regimes: rigid, semi-flexible, and flexible. These materials may advance the development of complex self-organizing materials.

2 Theory

A schematic characterizing the behavior of the DNA linkers and the streptavidin/biotin binding of our system is shown in Figure 1. The DNA linkers have reactive end-groups that bind to groups on the surfaces of the particles, either forming a bridge to another particle or looping back to form another attachment on the same particle. There are two distinct states to consider in our system: the field on and field off states. During the linking process, while the field is on, the magnetic attractive interaction is the dominant factor driving assembly. In the presence of an external field, paramagnetic colloids interact via their dipole moments to form particle chains. The strength of the dipole moment is given by

$$\vec{m} = \frac{4}{3}\pi a^3 \chi \vec{H} \quad (1)$$

where a is the particle radius, χ is the magnetic susceptibility of the particle, and $\vec{H}$ is the external field.

The interaction energy between two particles, i and j , with dipole moments m_i and m_j , respectively, is

$$U_{i,j}(r, \alpha) = \frac{m_i m_j}{4\pi\mu_0 r^3} (1 - 3\cos^2\alpha) \quad (2)$$

where μ_0 is the permeability of free space, r is the magnitude of the vector connecting the two particles' centers, and α is the angle between this vector and the direction of the external field. This equation shows that it is energetically favorable for dipolar particles to align with their dipoles in a head-to-tail fashion in the direction of the field. The magnetic field therefore serves as a means to direct the assembly of the particles into chains rather than disordered clusters, as the timescale for directed magnetic assembly is on the order of seconds—much faster than the hours or days needed for particles to assemble via random collisions.

Fig. 1 Schematic representation of linker behavior. The biotinylated DNA linkers can bind to the streptavidin-coated particles in either a bridge or loop configuration. a) Top: Behavior at surface-to-surface distances, d , larger than the size of the linker molecule, $N^{1/2}\ell$. Bottom: A chain linked with 8000bp in the $d/N^{1/2}\ell > 1$ regime. b) Top: Behavior for surface-to-surface distances smaller than the size of the linker. At this point, the DNA linker molecules are entropically excluded from between the particles. Bottom: A chain linked with 8000bp DNA in the $d/N^{1/2}\ell < 1$ regime. Scale bar: 5 μm .

The strength of the magnetic field can be used to control the interparticle spacing along the chain, as the dipolar attraction balances the steric and electrostatic repulsion of the DNA in the gap²⁹. The distance between the particles determines the total surface area on a given particle that is accessible by DNA on a neighboring particle to form bridges. The ability to control the total number of bridges between the particles along the chain can be used to tune the flexibility of the chain.

After the chains have been linked and the field is removed, the intermolecular interactions caused by the linker molecules dominate. Our DNA linkers induce an attractive bridging interaction between the particles. It has been shown that bridging with ideal chains, the energy minimum at separations equal to the size of the chains and the attractive bridging force varies linearly with the particle separation.³⁵ With DNA chains, there are additional electrostatic and steric repulsions between the particles²⁹. When the dipolar interactions are removed, these repulsive forces balance the attractive bridging interactions. Van der Waals interactions are not significant, since the particle surfaces do not come in close contact due to repulsive interactions. The last important factor is the configurational entropy of the linkers. For small enough interparticle spacing, it becomes entropically favorable for the linkers to be depleted from the gap

In our system, there are two relevant size ratios. The first is the ratio between the size of the gap between the particles and the polymer size: $d/N^{1/2}\ell$, where d is the surface-to-surface distance between particles, ℓ is the polymer segment length (this value is twice the persistence length, or approximately 100nm for double-stranded DNA), and N is the number of segments. The second is the ratio between the particle and the polymer sizes: $a/N^{1/2}\ell$, where a is the particle radius. Bhatia and Russel³⁶ theoretically modeled a system of particles bridged by associative polymers. They observed that when $d/N^{1/2}\ell < 1$, the interaction potential between particles becomes attractive, with the position of the attractive minimum occurring at a point at which the particles are almost touching. As shown in Figure 1b, the DNA linkers will be entropically excluded from the space between particles. Additionally, the potential well depth increases with decreasing $a/N^{1/2}\ell$ (as the linker molecules become larger). When $a/N^{1/2}\ell = 1$ the well

depth is approximately equal to kT . The values of the linker sizes and the particle/linker size ratios used in our experiments are shown in Table 1. For most cases, the ratio $a/N^{1/2}\ell$ is greater than one and the well depth of the interaction potential is less than kT . This is not the case for linkers of 8000bp, where $a/N^{1/2}\ell = 1$ and the entropic exclusion of the linkers will begin to affect the overall properties of the colloidal particle chains.

DNA Length (bp)	$N^{1/2}\ell$ (nm)	$a/N^{1/2}\ell$
564	138	3.9
1250	206	2.6
2000	261	2.1
4000	369	1.5
8000	522	1.0

Table 1. Relevant length scales and sizes ratios for different DNA linker lengths (in base pairs) used in this study. $N^{1/2}\ell$ represents the size of the linker molecule. $a/N^{1/2}\ell$ is the ratio between the size of the particle (radius 535nm) and the linker.

3 Experimental Methods

3.1 Synthesis of DNA fragments

DNA fragments (sizes: 564bp, 1250bp, 2000bp, 4000bp, and 8000bp) were created using standard polymerase chain reaction (PCR) techniques with an original template of λ -DNA (New England Biolabs, Ipswich, MA). The DNA polymerase Vent_R (New England Biolabs) was used to create fragments from 564bp to 4000bp. To synthesize the 8000-bp fragment, the Phusion polymerase (New England Biolabs) was used, as this polymerase displayed a fourfold increase in the extension rate and the ability to handle large amplicon sizes. In both cases, the manufacturer's suggested PCR protocols were followed. The primers (Integrated DNA Technologies, Coralville, IA) used in all experiments were biotinylated on the 5' ends to allow the final DNA product to bind to the streptavidin coating on the particles. The same reverse primer (5'-Biotin-TTC CTG ACG GAA TGT TAA TTC TCG -3') was used

for all fragments. The forward primers used had the following sequences: 564bp, 5'-Biotin-TTA GAG CGA TTT ATC TTC TGA A-3'; 1250bp, 5'-Biotin-ACG TAA GGA ATT ATT ACT ATG TAA ACA CCA GGC -3'; 2000bp, 5'-Biotin-ACT TAC GGC CAA TGC TTC GTT TCG-3'; 4000bp, 5'-Biotin-GCA TGG TGT GCT CCT TAT TTA TAC-3'; and 8000bp, 5'-Biotin-CTC GCA CAT TGC AGA ATG GG-3'. After the sizes were verified using gel electrophoresis, each sample was purified using a PCR DNA fragment extraction kit (IBI Scientific, Peosta, IA).

3.2 Linking experiments and image capture

Linking was performed in a chamber constructed with double-sided tape sandwiched between two glass coverslips and sealed using epoxy glue, generating a final volume of approximately 35-40 μ L. Immediately prior to use, the coverslips were cleaned using oxygen plasma followed by soaking in a concentrated potassium hydroxide bath for 30 minutes. The KOH bath was used to distribute a negative charge on the surface of the glass, preventing the particles from sticking to the surface of the chamber. The particles used in the experiments were Dynabeads MyOne Streptavidin C1 beads (Life Technologies, Carlsbad, CA), with a diameter of 1.07 μ m and a hydrophilic surface coated with the protein streptavidin. The particles contained 26% iron content (ferrites) dispersed throughout a cross-linked polystyrene matrix, as specified by the manufacturer. The density of the particles (1.8 g/cm³) was much greater than that of the solution, facilitating the almost immediate settling of the beads on the surface of the chamber and ensuring that the chains existed in a quasi-2D environment. Prior to use, the particles were rinsed thoroughly with DI water to eliminate the preservatives present in the stock solution. Each experimental solution consisted of 0.01wt% of Dynabeads and 5nM of the biotinylated DNA fragment in a 10mM phosphate buffer (pH ~7.4).

The sealed chambers were placed on a hot plate at 50°C. A magnetic field was applied for 1.5 hours to allow the magnetic particles to assemble into chains and the DNA fragments to form bridges between the particles. The field was created using two ferrite bar magnets with dimensions of 2in. x 1in. x 0.5in. (McMaster Carr, Atlanta, GA). The field strengths were varied between 19G and 275G, as

measured via a magnetometer (AlphaLabs, Inc., Salt Lake City, UT), by changing the magnet spacing. Experiments were performed to determine the linking time needed to ensure that the properties of the resulting colloidal chains were independent of time and suggested that at times exceeding 1.5 hours, the persistence length of the chains was stable, with homogeneity along the length of the chain. The linking process was performed at an elevated temperature to enhance diffusion of the sticky DNA ends to the surface of the beads. We expect that the DNA coats the beads before the field is applied. To test this, we performed experiments varying the time between when the DNA is added to the bead solution and when the field is applied. There was no observable difference if chains were linked one minute after mixing or thirty minutes after mixing. The DNA concentration was chosen to be 5nM, as this concentration ensured that the persistence length was uniform along the length of the chain. Below this concentration, the chains were heterogeneous, with some joints between the beads exhibiting increased flexibility.

After the linking process, the sample was transferred from the hot plate to the microscope stage. The chains were imaged using an Olympus IX71 inverted microscope (Olympus, Tokyo) with an attached ORCA-HR digital camera (Hamamatsu Corp., Hamamatsu City, Japan) using a 100X oil immersion objective with a numerical aperture of 1.40 (Olympus, Tokyo). For each video, a single chain was located that was sufficiently isolated from its neighbors to avoid hydrodynamic coupling effects. Images were captured every 0.5 seconds with an exposure time of 5 milliseconds for a duration of 75-80 minutes, generating a total of approximately 9,000-10,000 frames. Each experiment was performed at least twice and during each experiment, roughly 3-6 chains were recorded.

3.3 Image processing and Fourier mode analysis

The process of using a filament's Brownian motion to determine persistence lengths has been outlined in detail in the literature.^{30, 37-38} Briefly, the core requirement for use of this method is a reliable way to track the particle locations as a function of time using experimentally captured images. The centroid position of each particle can be determined by fitting the pixel intensity to a Gaussian

distribution after performing both a boxcar averaging and a Gaussian convolution on the images to remove noise.³⁹

Knowing the centroid positions (x_i, y_i) for each particle, the bending angles can be determined at each point along the chain, allowing for a determination of the curvature, $\theta(s)$, of the chain in each video frame. This curvature can then be decomposed into a series of cosine modes, as shown in Eq. 3:

$$\theta(s) = \sum_{n=0}^{\infty} \theta_n(s) = \sqrt{\frac{2}{L}} \sum_{n=0}^{\infty} a_n \cos\left(\frac{n\pi s}{L}\right) \quad (3)$$

where L is the length of the chain, n is the mode number, a_n is the amplitude of mode n , and s is the arclength along the chain.

Information on the flexibility of the chain is contained in the variance of the mode amplitudes. This factor can be found from the bending energy of the chain and the equipartition theorem. The bending energy is represented by the following equation:

$$U_{Bend} = \frac{L_p k_B T}{2} \int_0^L \left(\frac{d\theta}{ds} - \frac{d\theta_0}{ds} \right)^2 ds \quad (4)$$

By differentiating Eq. 3 with respect to s and substituting into Eq. 4, an approximation can be obtained, as represented by the following equation:

$$U_{bend} = \frac{L_p k_B T}{2} \sum_{n=1}^{\infty} \left(\frac{n\pi}{L} \right)^2 (a_n - a_n^0)^2 \quad (5)$$

In this equation, a_n^0 is the amplitude of the n^{th} mode in the absence of thermal forces (zero for an intrinsically straight rod). The equipartition theorem suggests that at equilibrium, each of the terms in the energy equation contributes $k_B T/2$ to the total energy, relating the variance of the cosine mode amplitudes to the persistence length via the following relation:

$$\text{var}(a_n) = \langle (a_n - a_n^0)^2 \rangle = \frac{1}{L_p} \left(\frac{L}{n\pi} \right)^2 \quad (6)$$

The behavior of each mode can provide an independent estimation of the persistence length of the chain.

This method has many advantages, including both simplicity and non-invasiveness; however, as with any system that relies on digital imaging, image noise can become a problem. Uncertainty in determining the particle positions can lead to increased variance estimates with artificially low persistence lengths. This effect is more pronounced for high mode numbers, with smaller relevant length scales. The noise floor can be found using the following expression.³⁷⁻³⁸

$$\text{var}(a_n)^{\text{noise}} = \frac{4}{L} \langle \varepsilon_{xy}^2 \rangle \left[1 + (N - 1) \sin^2 \left(\frac{n\pi}{2N} \right) \right] \quad (7)$$

where $\langle \varepsilon_{xy}^2 \rangle$ is the standard error of the centroid position.

To verify that the colloidal chains exhibit the predicted behavior, the variance and noise floor for each chain can be plotted as a function of the mode number. On a log-log plot, the variance would be expected to be linear with respect to the mode number, exhibiting a slope of minus two (based on Eq. 6). The effect of noise on the shorter modes results in deviations from the expected slopes. To calculate the persistence length of a chain only those values not affected by image noise were taken from the modes. Typically, anywhere from 4 to 12 modes were used to calculate the average persistence length. Among the chain types studied, rigid chains were more strongly affected by noise, as the variances in amplitudes

were small even for long modes. Representative variance vs. mode plots for chains in the rigid, semi-flexible, and flexible regimes can be found in Figure 2.

Fig. 2. Representative plots of the variance of the mode amplitude as a function of the mode number for four chains across the persistence length spectrum. a) A rigid chain linked with 564-bp DNA at 140G, $L_p=84\text{mm}$. b) A semi-flexible chain linked with 1250-bp DNA at 100G, $L_p=2\text{mm}$. c) A semi-flexible chain linked with 2000-bp DNA at 19G, $L_p=230\mu\text{m}$. d) A flexible chain linked with 4000-bp DNA at 46G, $L_p=89\mu\text{m}$. The solid black lines represent the noise floor.

To calculate the surface-to-surface distance between beads, we subtract the particle diameter from the inter-centroid distance. Previously, Li et al.⁴⁰ demonstrated that by averaging the interparticle spacing of all the bead pairs along a chain, sub-nanometer resolution can be achieved and we use this technique for our analysis. For each DNA linker size and field strength, 6-10 chains of greater than 30 beads each were analyzed. The spacing was averaged over all the bead pairs of a chain, and then the results were

averaged for all chains. Additionally, by averaging over all bead pairs, we minimize potential end effects caused by the mutual induced dipoles of the particles, which as shown by Zhang and Widom⁴¹ can increase the magnetic attractive force by up to 20%. However, it has also been shown that the many-body effect becomes negligible once you are sufficiently far away from the edge of a cluster or chain.⁴² Thus, we analyze only chains of greater than 30 beads.

4 Results and Discussion

Using a system consisting of micron-sized colloids linked with DNA, this study investigates strings of particles that vary in rigidity over several orders of magnitude. Our study characterizes the chain properties as a function of the particle/DNA linker size ratio by varying the length of the DNA and the strength of the magnetic field to alter the ratio between the surface-to-surface distance and the linker size. Representative images of the chains at various points along the flexibility spectrum are shown in Figure 3. The lengths of the analyzed chains typically ranged from 30-100 particles (30-100 μm). Videos of chains from all three flexibility regimes undergoing Brownian motion are presented in the Supplementary Information. The persistence lengths for all experimental conditions are plotted in Figure 4. The surface-to-surface distances between the particles during the linking process under various magnetic fields are shown in Figure 5. In the remainder of this section, the effects of the properties of the nanoscale DNA linkers on the overall properties of the microscale particle chains are reviewed and discussed in three parts: short linkers (564bp), intermediate linkers (1250-4000bp), and long linkers (8000bp).

Fig. 3 Chains in the a) rigid-rod regime (564bp, 140G), b) semi-flexible regime (2000bp, 140G), c) flexible regime (4000bp, 19G), and d) a semi-flexible chain that results from the depletion of the long 8000bp DNA linkers at higher field strengths (8000bp, 140G).

Fig. 4 Average persistence lengths as a function of field strength applied during assembly for linkers of size 564bp (gold circles), 1250bp (red stars), 2000bp (blue diamonds), 4000bp (black squares), and 8000bp (purple triangles). Error bars represent the standard deviation of persistence length over all chains measured for each point.

Fig. 5 Surface-to-surface distances between the particles as a function of field strength for linkers with the following sizes: 564bp (gold circles); 1250bp (red stars); 2000bp (blue diamonds); 4000bp (black squares); and 8000bp (purple triangles). Error bars represent the standard deviation of interparticle spacing over all chains measured for each point.

4.1 Short DNA Linkers

Stable chains could not be formed at field strengths below 100G for a linker length of 564bp. At low field strengths, the distance between the particle surfaces ranged from 307 ± 7 nm to 218 ± 5 nm, 15-60% larger than the contour length of the DNA linker (approximately 192nm), suggesting that bridging would be unlikely. The chains that did form were very short (between 5-20 beads), with many constructs breaking apart over the course of the experiment. For chains that were linked above 100G, the surface-to-surface distances were still very close to the DNA's contour length (from 190 ± 10 nm to 176 ± 9 nm), as shown in Figure 5. These observations indicate that the DNA was acting as a rigid bridge between the particles, which led to the large persistence lengths shown in Figure 3 for the 564-bp chains.

4.2 Intermediate DNA Linkers

The chains produced using DNA linkers of 1250bp – 4000bp follow a clear trend. With increasing DNA length, the colloidal chains increase in flexibility. With increasing field strength, the chains increase in stiffness, with the persistence length saturating at 140G. The persistence lengths of these chains ranged from $5 \pm 2 \mu\text{m}$ to $40 \pm 10 \mu\text{m}$, falling in the rigid-rod, semi-flexible, and flexible regimes. These characteristics can be explained with the bead-spring analogy. DNA is often modeled as a finite-extensible nonlinear elastic (FENE) spring, with a size-dependent spring constant. Longer DNA lengths resulted in decreased spring constants, reducing the elastic energy penalties that must be paid by chains to bend at short length scales. Higher field strengths lowered the spacing between particles during linking, creating an increased surface area available to form bridges between particles. More linkages can be considered to increase the number of springs in parallel between the beads, increasing the effective spring constant and creating stiffer chains.

In Figure 5, the surface-to-surface distances between particles begin to plateau at high field strengths which is especially prominent for DNA of size 2000bp and longer. The results of Bhatia and Russel,³⁶ as presented in Section 2, suggest that as the gap spacing becomes smaller than the size of the linker molecules, the system will fall into the attractive portion of the interaction potential. At this point, the linkers then become entropically excluded from the gap between the particles, and increasing the magnetic field strength will have little effect on the interparticle spacing. In Figure 6, we plot the normalized spacing $d/N^{1/2}\ell$ for chains linked with 2000bp, 4000bp, and 8000bp DNA. This scaling shows that the 2000bp chains will deplete around 140G, the 4000bp around 100G and the 8000bp chains around 46G. The chains linked with shorter DNA do not enter the depleted region for any field strengths used in this study.

Fig. 6. Surface-to-surface distance normalized to linker size vs. field strength for chains linked with 2000bp (blue diamonds), 4000bp (black squares), and 8000bp (purple triangles). The shaded region represents $d/N^{1/2}\ell < 1$, the region in which the linkers will be depleted.

As shown in Table 1 the particle-to-linker size ratios, $a/N^{1/2}\ell$, are less than one for DNA lengths below 8000bp, meaning that the depth of the interaction potential is less than $k_B T$.³⁶ Consequently, with the removal of the added attraction provided by the magnetic field, the chains relax and revert from the excluded linker state denoted in Figure 1b back to the bead-spring state of Figure 1a. Even though at higher field strengths the particle spacing has a diminished dependence on the DNA length, the persistence lengths of the resulting colloidal particle chains still exhibits significant dependence on the linker size. This would not be expected if the linker molecules were still in the depleted state. Consequently, for intermediate-length linkers the spring constant of the nanoscale linker molecule directly affects the flexibility of the microscale chain, which can be further varied by using the magnetic field strength to control the number of bridges between particles.

4.3 Long DNA Linkers

The last region to be considered corresponds to chains produced with 8000bp DNA linkers. At low field strengths, increasing the DNA length from 4000bp to 8000bp increases the flexibility of the chains, but only slightly. This result is not unexpected, as the chains are beginning to approach the theoretical minimum persistence length of $1\mu\text{m}$ —the size of one bead. Bending cannot take place on length scales any smaller than this theoretical minimum value, as the bead or ‘monomer’ represents the smallest indivisible unit of the colloidal particle chain.

At field strengths exceeding 46G, the 8000-bp linked chains become more rigid than the chains linked with the 4000bp DNA and are seen to re-enter the semi-flexible regime. The rate of change of the persistence length was also substantially higher than the rate of change for the chains linked with intermediate-length DNA. The stiffening of the chains can be explained by again turning to Figure 6. As discussed previously, the 8000bp DNA linkers will be excluded from the gap at field strengths above 46G. However, unlike for the 2000bp and 4000bp chains, with 8000bp the ratio $a/N^{1/2}\ell$ is equal to one and the well depth is now approximately equal to $k_{\text{B}}T$. As such, the entropy gained by the increased configurational freedom of the linker molecules outside the gap is sufficient to maintain the chains in the excluded linker state shown in Figure 1b, even after removal of the magnetic field. Under these conditions, the linkers cannot relax back into the gap, which further restricts the movement of the particles within the chains and contributes to the higher persistence lengths shown in Figure 4 for the 8000bp chains.

This depletion phenomenon was also reported by Schmatko et al.^{1, 43} for the aggregation of 3D colloidal clusters linked using the full sequence of λ -DNA (48,500bp). The clusters in this system did not grow past a finite size, and the particle spacing was much smaller than the size of the linker molecule suggesting that the DNA in this system is depleted and forms a cloud around the particles. We also performed preliminary experiments using the full λ -DNA, 48,500-bp, sequence and the characteristics of these chains (spacing and flexibility) were not significantly different from those of the 8000bp DNA chains. For the full λ -DNA molecule, the potential well depth should be approximately $2k_{\text{B}}T$ ³⁶ and the

spacing at which the transition to attraction takes place should be approximately $1.2\mu\text{m}$. For almost any magnetic field strength, the linker is excluded from the interparticle gap and stiff chains are formed. Therefore, at linker sizes that exceed the critical value where $a/N^{1/2}\ell = 1$, the depletion forces appear to dominate all other interactions, reducing our ability to affect the properties of the colloidal chains by tuning the linker molecules.

5 Conclusions

In this paper, DNA fragments of varying sizes were used to link magnetic particles into colloidal analogues of the bead-spring polymer model. By controlling the length of the DNA linkers (the spring constant) and the linking area available on the particles (the number of springs), chains were created that exhibited rigid, semi-flexible, and flexible characteristics with persistence lengths ranging over three orders of magnitude, from 84nm to $19\mu\text{m}$. Both the length of the DNA used to link the colloids and the field strength used in the assembly process played vital roles in determining the final properties of the chain, including both stability and flexibility characteristics.

For the shortest DNA (564bp) used in this study, rigid chains were formed with chain stability only observed for applied magnetic field strengths exceeding 46G. The surface-to-surface distances for these chains were similar to the contour length of the DNA, indicating that the DNA was behaving as a rod-like linker. For the intermediate DNA lengths from 1250bp to 4000bp, the chains were stable at all field strengths, exhibiting characteristics that encompassed all three flexibility regimes, with persistence lengths ranging from 5nm to $40\mu\text{m}$. The effects of field strength on the flexibility of the chains were similar for these intermediate DNA lengths, reaching a plateau for fields exceeding 140G. This range of linker sizes seems to be ideal for creating chains with predictable behavior. At 8000bp, the chains produced using fields exceeding 46G exhibited increased rigidity compared to the chains linked using 4000bp DNA due to the DNA being excluded from the gap between particles.

For future studies, an examination of the effects of time-dependent magnetic fields on the dynamic behavior of the chains may be instructive. The process used to link the chains was shown to induce stability for long periods of time, conferring resistance to both shear and torque. These characteristics suggest new ways for studying chain relaxation processes after contraction using a magnetic field, as well as the behaviors of these chains in various flow fields.

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Supplementary Information

Videos showing chains of various persistence lengths fluctuating under the influence of thermal forces.

This information is available free of charge via the Internet at <http://pubs.acs.org/>.

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