

# Reversible self-assembly and directed assembly of DNA-linked micrometer-sized colloids

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**We present a technique for the directed assembly and self-assembly of micrometer-scale structures based on the control of specific DNA linkages between colloidal particles. The use of DNA links combined with polymer brushes provides an effective way to regulate the range and magnitude of addressable forces between pairs (and further combinations) of different particles. We demonstrate that the autoassembly of alternate microbeads as well as their directed assembly, by using laser tweezers, is reversible. The key to reversibility is preventing the particles from falling into their van der Waals well at close distances. This goal is achieved by the use of adsorbed polymers that limit the number of DNA bridges to one to three between adjacent particles.**

DNA links | reversible aggregation

Studies of reversible and specific adhesion between colloids are an important step toward understanding various phenomena involving molecular recognition. For instance, they are relevant in the study of cell adhesion (1), cell migration (2), or cell sorting during embryonic development (3). The knowledge and the control of the interplay between nonspecific repulsion and molecular recognition is also fundamental for biotechnological device improvements; e.g., a strategy to improve latex agglutination tests is to reduce aggregation due to nonspecific interactions (4). The present work on DNA links is also a contribution to these more general studies.

Controlling and tuning interactions between particles has always been a relevant challenge both experimentally (5, 6) and theoretically (7–11). For example, Tkachenko (11) predicted diverse and unusual crystal morphologies assuming a reversible contact between particles in a binary system of colloids, in which identical particles experience repulsive interactions and differing particles experience attractive ones. Particularly, he predicted a self-assembled diamond lattice structure that would be especially relevant for photonic crystal building. His work was inspired by the work by Mirkin *et al.* (12), who first used DNA chains as linkers between nanoparticles to build a reversible DNA-mediated assembly of gold nanoparticles. The specificity and magnitude of the attraction is determined by the molecular recognition of complementary DNA strands and the sensitivity of hybridization to solution conditions and temperature. Experimental work involving DNA as a linker between particles has up to now mainly focused on nanosized particles (13–18). To our knowledge, only two studies (19, 20) have been reported in the literature with microsized particles, but in both of them the assembly process was not reversible, with the DNA acting as a molecular bridge between the entities of a binary mixture. In this study, we focus on the reversibility of the aggregation process between microsized particles. The specificity and reversibility are proof that the interactions between the colloids are controlled by DNA and thus can be tuned.

## Materials and Methods

**Sample Preparation.** DNA-functionalized polystyrene microbead binary mixtures were prepared by conjugating two types (green and red) of fluorescent NeutrAvidin-coated particles (FluoSpheres

NeutrAvidin, Molecular Probes; 1- $\mu\text{m}$  diameter) with biotin-modified DNA (Integrated DNA Technologies, Coralville, IA). A first sequence of DNA, which we called “G-type,” was attached on the green particles, and another sequence, which we called “R-type,” was attached on the red particles. Both G- and R-type sequences are 61-base oligonucleotides (Fig. 1A): 50 bases common to both sequences are hybridized to form a rigid spacer. The 11 end bases of G-type are complementary to the 11 end bases of R-type and act, therefore, as “sticky ends.” Before binding the DNA to the beads, the 50-base spacers were hybridized with their complementary strand. DNA strands were displayed in a 50 mM PBS buffer with 50 mM NaCl (hereafter called PBS) at a final concentration of  $10^{-7}$  M. This solution was first brought to 90°C and then slowly cooled to room temperature. Then, 0.15 nmol of hybridized DNA was added to 10  $\mu\text{l}$  of 1% solid solution of beads ( $\approx 1.5 \times 10^8$  particles). The total volume of the solution then was adjusted to 100  $\mu\text{l}$  with PBS. The NeutrAvidin/biotin reaction was allowed to proceed for 1 h at room temperature. Samples then were washed to remove the excess reactants. A washing cycle consisted of centrifuging the solution to settle all of the beads ( $2,000 \times g$  for 20 min), removing the supernatant, and redispersing the beads in fresh PBS solution. After three washing cycles, the final bead concentration was adjusted to 2 nM in the appropriate stabilizing solution. We estimated that 20% of the beads were lost during the washing steps. DNA-grafted beads were then added to the stabilizing solution in a typical concentration of 0.1% (wt), and 10  $\mu\text{l}$  of the solution was placed between two microscope slides. The microchamber was sealed with optical glue to avoid evaporation upon heating. To avoid a rapid sticking of beads onto the surfaces of the microchamber, the cells were previously hydrophobized by silanization with hexamethyldisilazane by using the following procedure: microscopic slides were first exposed to UV-ozone (21) for 15 min and then placed in a closed Petri box for 1 h in the presence of a few drops of hexamethyldisilazane.

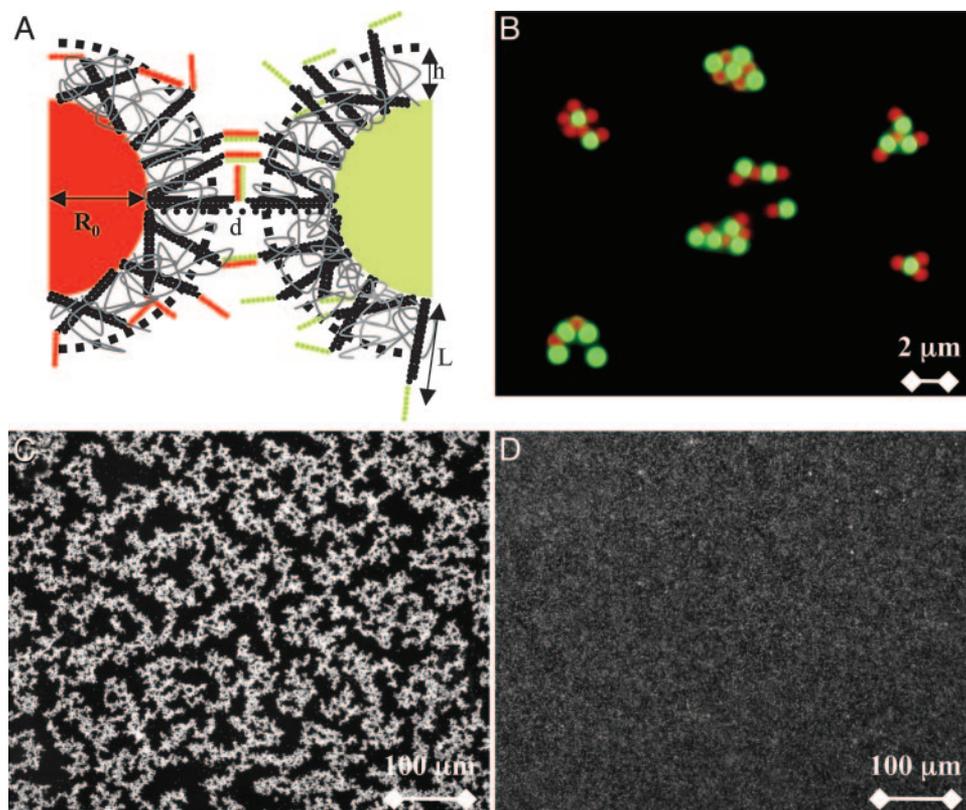
**Stabilizer.** BlockAid solution (Molecular Probes) is a protein (BSA)-based blocking solution designed to be used with NeutrAvidin microspheres. We will describe in *Results* that this BlockAid solution was not efficient enough to fulfill our experimental criteria of reversibility. Two series of copolymers were selected for the stabilization of the colloids. The first type is a neutral triblock copolymer, sold under the trade name Pluronic, which is known to adsorb on latex particles (22). Although these products exist in a variety of block lengths, we selected the longest poly(ethylene oxide) (PEO) chain F108 [129 poly(ethylene oxide) units]. Indeed, shorter ones were not able to stabilize our DNA-colloid assembly. The second type of copolymers were charged amphiphilic diblock copolymers (from Rhodia, Complex Fluids Laboratory, Cranbury, NJ). The hydrophobic part, poly(diethyleneglycol ethylether acrylate)

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Abbreviations: PAA, poly(acrylic acid); PDEGA, poly(diethyleneglycol ethylether acrylate).

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**Fig. 1.** Specific and reversible aggregation. (A) Microbeads surfaces grafted with oligonucleotides, G-type (sequence, ATCGCTACCCCTTCGCACAGTCAATCCAGAGAGCCCTTCATTACGACCAAGTTATGA) and R-type (sequence, ATCGCTACCCCTTCGCACAGTCAATCCAGAGAGCCCTTCATTACGATCATAACTGG). A polymer brush imparts a steric repulsion between the particles and reduces the number of links that may form between them. Part of the DNA sticky ends is hidden in this layer of thickness  $h$ . (B) Specific aggregation. Green, G-type beads are specifically linked to red, R-type beads as shown by fluorescence microscopy. The 1- $\mu\text{m}$ -diameter particles are stabilized by 3- to 12-kDa copolymer. (C)  $T = 23^\circ\text{C}$ , reversible aggregation by means of bright field microscopy of particles stabilized in F108 solution observed 8 h after mixing G- and R-type beads. (D) The temperature has been increased to  $50^\circ\text{C}$ , and the beads are completely redispersed.

(PDEGA), is an ethoxylated acrylate with chemical properties close to those of Pluronics. The hydrophilic part, poly(acrylic acid) (PAA), is a weak acid that is fully charged in our working conditions. We have used three types of PDEGA-*b*-PAA, referred to as PDEGA-*b*-PAA 1–4 kDa (6 diethyleneglycol ethylether acrylate units; 55 acrylic acid units), 3–12 kDa (18 diethyleneglycol ethylether acrylate units; 165 acrylic acid units), and 6–24 kDa (36 diethyleneglycol ethylether acrylate units; 330 acrylic acid units). The three PDEGA-PAA samples have been synthesized by controlled radical polymerization (Rhodia, U.S. Patent no. R03117) with an index of polydispersity of  $\approx 1.5$ . The stabilizing solutions of F108 and PDEGA-PAA were prepared at concentrations of, respectively, 10 and 4 g/liter PBS stock solution (adjusting the pH to 7.6 to fully dissociate the PAA).

**Imaging of Suspensions.** All suspensions were imaged by means of direct or inverted optical microscopy (HC or DM IRB/E, Leica, Deerfield, IL) by using a  $\times 10$  or  $\times 100$  objective and a camera. Direct imaging was used for fluorescence measurements with a type A filter (Leica) allowing us to image simultaneously red and green particles. Inverted imaging was used for the laser tweezers experiments.

**Temperature Measurements.** To study the equilibrium behavior of DNA/microbeads, we displayed 20  $\mu\text{l}$  of a DNA/beads solution between two microscope slides 5 cm in length. Two Peltier elements were placed on each edge of the sample and laid on a copper block cooled with a water flow. Imposing different temperatures to each Peltier element induced a typical temperature gradient in the

sample of  $2^\circ\text{C}/\text{cm}$ . Once the temperature of each Peltier had been set, the temperature along the sample was measured with a thermocouple. The system was covered with a polystyrene block to minimize heat exchange with the surrounding atmosphere and was left standing for 8 h. We then removed the microscope cell from the gradient temperature stage and quickly recorded images across the sample with an inverted microscope. The recorded images vs. temperature are complex and, therefore, too difficult to analyze by means of computerized image analysis. To get quantitative measurements out of our images, we simply counted the number of unbound beads vs. the total number of beads.

**Fluorimetry Measurements.** Fluorescein-labeled DNA were grafted on nonfluorescent beads. The fluorescence of the grafted beads was measured by using a fluorescence spectrophotometer [F-4500 Hitachi (Tokyo)] on solutions of 0.005% (wt) beads. A background curve taken on a solution of beads without DNA was subtracted. The result showed a peak of fluorescence intensity for comparison with a calibration curve obtained with a solution of free DNA molecules.

## Results

In a standard experiment, G- and R-type beads were mixed in equal ratio in an aliquot of which 10  $\mu\text{l}$  was displayed between two microscope slides. All suspensions were observed by optical fluorescence microscopy  $\approx 10$  min after mixing the beads. At room temperature with the four copolymers above as stabilizing agents, we observed the rapid formation of clusters, with sizes varying from several particles to tens of particles. The use of two types of



fundamentally different behavior between nanoparticles and microbeads.  $U_{\text{dna}}$  is roughly proportional to the radius of the sphere, Eq. 1, and is  $\approx 2$  orders of magnitude higher for 1- $\mu\text{m}$ -size beads than for 10-nm-size beads. For microbeads, two strategies may be adopted to obtain reversibility: decreasing  $U_{\text{dna}}$  by decreasing the surface density of DNA strands  $\Gamma$  (Eq. 1) or increasing  $U_{\text{rep}}$ . The latter approach, achieved by adsorbing special diblock or triblock copolymer stabilizers on the beads rather than the usual protein-based BlockAid, was the more successful.

The reversibility of the autoassembled structure means the association of beads can be treated as a thermodynamic equilibrium between colloids. We consider here only the equilibrium between two unbound beads and a pair. This structure contains the main physics of the aggregation process and demonstrates the specific role of DNA. The mass action law for this equilibrium leads to the expression for the unbound fraction  $f$

$$f = \frac{\sqrt{4KC_b + 1} - 1}{2KC_b}, \quad [3]$$

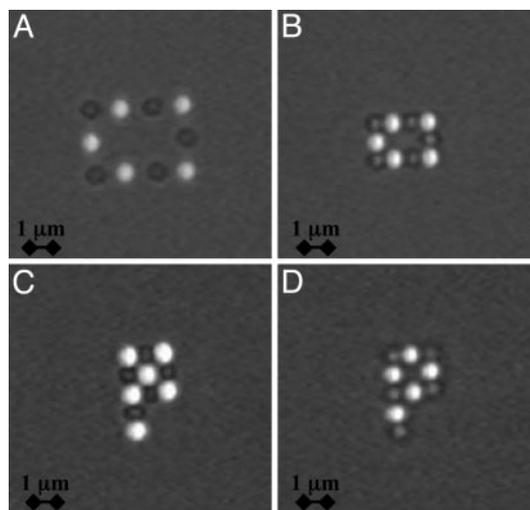
where  $K$  is the equilibrium constant and  $C_b$  is the initial volume concentration of single beads. From microscope images  $C_b \approx 10^{-11}$  mol of beads per liter. The constant  $K$  depends on temperature according to the van't Hoff relation:

$$K(T) = \exp(-\Delta G_b^0/RT), \quad [4]$$

where  $\Delta G_b^0$  is the standard Gibbs free energy of the reaction between beads. Fitting the data of Fig. 2 to Eqs. 3 and 4, we find that  $\Delta G_b^0$  takes the form  $\Delta G_b^0 = \alpha \Delta G_s^0$  with  $\alpha$  values ranging from 1.55 to 2.4 depending on the stabilizer. The physical meaning of  $\alpha$  can be understood in a simplified view of the problem: if the two beads in a pair are attached by  $N$  links, one can write  $\Delta G_b^0 = N \Delta G_s^0$ ; hence,  $\alpha$  is the effective number of links. How meaningful is this number? We have neglected the numerous combinations with which  $N$  links can be made between two beads, each bearing  $N_{\text{tot}}$  DNA strands. We also have neglected the change of rotational entropy  $\Delta S_r$  of beads upon aggregation. Its value may be estimated to be  $\Delta S_r \approx R \ln[(L/R_0)/2\pi] \approx -5R$ . Both of these effects only add a multiplication factor  $C$  in the expression for  $K = C \exp(-\Delta G_b^0/RT)$  and will not change the values of  $\alpha$  fitting the experimental melting curves. The dominant effects are the large enthalpy and entropy of the sticky ends, which put a tight limit of one to three DNA links per microbead pair for our reversible samples. These are surprisingly small numbers as compared with the total number of binding sites  $N_{\text{tot}} \approx 10^4$  (as determined by adsorption measurements using a fluorimeter).

Let us estimate the number of links between two beads from a geometric approach. We assume that the copolymer forms a brush of characteristic extension  $h$ . The effect of the stabilizer on the distribution of sticky ends in the space between the microbeads and on the number of links between beads is shown schematically in Fig. 1A. Typical thicknesses of brushes are comparable to the DNA length,  $L = 0.34 \times 61 = 20.7$  nm. The thickness of the F108 layer on polystyrene particles is known to be  $h = 13 \pm 5$  nm (24), whereas the effective thickness of polyelectrolyte brushes is more difficult to estimate. Because of the flexibility of the DNA attachment to the beads, the distribution of sticky ends vs. distance from the bead surface is expected to be linear. A fraction of them are therefore hidden in the polymer brush. Moreover, the brush imposes a minimum distance between the beads that directly affects the number of links. Assuming  $R_0 \gg L$ , the number  $N_s$  of sticky ends available for hybridization between two microbeads can finally be written as

$$N_s = \frac{2\pi R_0}{L} (L - h)^2 \Gamma, \quad [5]$$



**Fig. 4.** Directed assembly of particles. Fluorescent and nonfluorescent particles bear complementary strands of DNA. (A) Particles are first captured in discrete time-shared traps induced by laser tweezers. (B–D) Particles are moved in contact to promote hybridization between the DNA strands and form the following rigid structures: a rectangle (B), a “full” P (C), and an “empty” P (D).

$N_s$  varies from 0.5 to 72 for layer thickness ranging from 19 to 8 nm and for a total number of DNA strands per bead of  $N_{\text{tot}} = 10^4$ . This simple estimation roughly confirms the low number of links obtained by thermodynamics analysis. Geometry and statistics further reduces the number of bonds. For a number  $N_s \approx 1$ –70 sticky ends of length  $\approx 4$  nm randomly distributed over an accessible area  $\approx 10^4$  to  $10^5$  nm<sup>2</sup>, it is relatively easy to form one bond and to rotate the contact area to find a second, but a third bond is only likely for the larger values of  $N_s$ .

We have developed a system based on the DNA-mediated assembly of micrometer-size polystyrene particles. This approach should provide a basis for binary assembly in solution. It would be interesting to build complex structures starting from a mesoscale template or seed followed by self-assembly. In Fig. 4, we demonstrate fabrication of mesoscopic units. We optically manipulate the particles by using laser tweezers (25). The beads are first immobilized on an array of discrete optical traps. The gap between particles then is decreased to bring the beads in contact and allow the DNA to hybridize. As the tweezers are turned off, the structures formed are stable. We present examples of a rigid rectangle and letter “P” structures on Fig. 4. The directed assembly was achieved with all four stabilizers but the 6–24 kDa. With the 6–24 kDa layer, the number of links  $\langle N \rangle$  is the lowest and close to unity, which explains the low probability of attachment. These protostructures remain rigid but exhibit Brownian rotational and translational motion about their axes and center of mass. Upon heating above their melting temperature, they readily dissociate. The highly temperature-dependent nature of the DNA hybridization means that the structures can be annealed, cycled, and made hierarchical. With reversible links and temperature cycling we envision the fabrication of complex static and dynamic systems. Potentially PCR-like self-replication and programmable growth of designed configurations are enabled.

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