DNA-driven assembly of micron-sized colloids
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3 A finite-cluster phase in $\lambda$-DNA coated colloids

Inspired by theoretical work indicating that crystal structures can be build with a system of DNA-coated colloids, we studied the aggregation of 1 $\mu$m colloids bridged by long flexible DNA with 32 $\mu$m contour length. Hereto two species of colloids with grafted double-stranded $\lambda$-DNA displaying short, complementary single-stranded “overhangs” as free binding-ends were mixed. Confocal microscopy showed the formation of stable, size limited clusters in which the two species of colloids were at touching contact. The inter-colloidal distance and the size of the DNA used suggests that the DNA is expelled out of the cluster by excluded volume effects. Simulations indicate that the observed close contact and the limitation to grow both result from entropic exclusion of the bridging DNA from the space between nearby colloidal surfaces.

3.1 Introduction

The highly specific nature of complementary-strand binding in double-stranded DNA (dsDNA) offers many possibilities for the use of this biomolecule in micro- and nano-technology. The specificity of interaction between two complementary DNA strands makes it possible to “tune” the interactions between colloids with different DNA coatings.

Since Mirkin and coworkers [3] showed that the formation of nanoparticles into larger assemblies is possible by the specific recognition of two complementary ssDNA strands, many researchers have focused their attention on building new types of nano-materials based on these assemblies. For example, colloidal particles coated with distinct species of short single-stranded DNA (ssDNA) can bind specifically with each other via suitable DNA “linkers” [4, 6, 7, 123]. Such DNA-coated colloids can be used as sensors for nucleotide polymorphism [4, 93] and may offer a route to novel colloidal crystals [7, 123]. While most research has directed its effort on short ssDNA oligos grafted to gold colloids or nanoparticles, we choose to work with longer, more flexible DNA on micron-sized polystyrene colloids. The bigger colloids have a practical reason. With this size, direct imaging is possible with confocal microscopy. The choice for longer DNA is two fold. First, experiments that use DNA-colloid mixtures with short ss- and dsDNA cannot exploit the molecule’s polymeric potential. Second, the long DNA can function as a spacer as well as a mean of specific linking. Using very long DNA tethers will change the “sticky”-colloid phase diagram qualitatively, as the long tethers allow for rearrangement.
within the clusters. In particular, theory suggests that the reversible binding between ssDNA overhangs attached to long dsDNA linkers may yield novel crystalline phases [9] forbidden to systems that use only short DNA.

In this chapter we report the first experimental investigation of the aggregation of colloids bridged by long lengths of grafted dsDNA. Here we mixed two species of colloids with grafted λ-DNA displaying short, complementary ssDNA “overhangs” as free-ends. Direct imaging and computer simulations suggest that both the polymeric nature of the DNA and the possibility it offers for specific molecular recognition are important in this system.

3.2 Results and Discussion

3.2.1 λ-DNA driven assembly leads to finite cluster sizes.

To examine the aggregation behavior of λ-DNA coated colloids A and B colloids were mixed in equal ratios, a 20 µl aliquot of which was displayed in an imaging chamber. This mixture was observed with time by means of confocal microscopy. Some aggregates, mostly of AB doublets, can be seen 30 minutes after mixing (Figure 3.1a). Besides the few small aggregates the sample contains many colloids that remained as singlets. The system is in the induction phase. As the DNA used is long and flexible, the ssDNA ends need time to find each other and hybridize. After several hours, large clusters consisting of ∼ 10 colloids from each population have formed (Figure 3.1b), but a significant number of smaller clusters remained. Although cluster growth started, the presence of small

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1The main experiments described in this chapter are performed by Tatiana Schmatko and Nienke Geerts. The work on YOYO-died clusters is done by Tatiana Schmatko in Edinburgh. Nienke Geerts suggested the control experiment with DNA tethers, where the backbone was not ligated. The Monte Carlo simulations were performed by Behnaz Bozorgui.
3.2 Results and Discussion

Figure 3.2: Confocal image of the control experiment. Omitting the ligation step in the preparation of DNA-coated colloids and subsequent heating, leads to cos-coated colloids. The colloids have a different aggregation behavior to $\lambda$-DNA coated colloids. As they assemble on every contact they form percolating clusters. Scale bar is 10 $\mu$m.

clusters, indicates that the aggregation process is not completed during this time. Few of the latter were seen after ca one day. At this time the sample shows large clusters that have sedimented (Figure 3.1c). Inverting the sample, however, appeared to separate the sediment into independent clusters. Little change in the samples was observed after ca. one month.

Once two complementary ss-overhangs on two neighboring A and B colloids meet and hybridize, the two colloids are bridged by a continuous piece of dsDNA with 32 $\mu$m contour length. The radius of gyration of the unbound dsDNA with this contour length can be estimated to be $2^{0.58} \approx 1.5$ times that of a single $\lambda$-DNA, i.e. $\sim 1.2$ $\mu$m, so that the ratio of the radius of the colloid to that of the bridging chain is 0.5 : 1.2 $\approx$ 0.42. We denote this ratio by the symbol $\alpha$. Figure 3.1 shows that these bridging chains bring colloids very close together (neighboring A and B colloids are practically touching) to form clusters consisting of multiple colloids of each kind. The observed close approach of bridged colloids is, at first sight, surprising, given the size of the bridging DNA. Another surprise is the apparently self-limiting nature of the cluster growth.

3.2.2 $\lambda$-DNA is responsible for the formation of finite clusters.

Control experiments confirmed that the observed aggregation was caused by the grafted $\lambda$-DNA. We repeated our sample preparation protocol without ligation of the DNA backbone after the cos1-biotin and cos2-biotin have been hybridized to the linearised $\lambda$-DNA, (Figure 3.6). After incubating the unligated biotinylated $\lambda$-DNA with the neutravidin-coated colloids overnight, each batch of colloids was heated to 65 °C for 30 minutes. Hereafter the colloids were washed to remove free DNA. Mixing the resulting colloids and following the aggregation in time, showed a huge difference. Fast aggregation was observed to commence immediately and was completed within 1 hour, resulting in large
3.2.3 Expulsion of DNA from the gap between two bridged colloids can explain the finite sizes.

During the experiments two unexpected results appeared constantly. The distance between the colloids within a cluster is close to zero and all aggregates are finite in size. To begin to understand these observations, we first inquired the behavior of two colloids with large bridging polymers. Bhatia and Russel [142] made self-consistent field theoretic calculations for a system of two spherical colloids and linear polymers with strongly adhering chain ends. These show that ideal chains of such “telechelic associative polymers” induce an attraction between the colloids. For \( \alpha \), they predict an attraction with a minimum practically at touching (figure 10 of ref. [142]), with a well-depth of \( 2k_B T \) at \( \alpha \approx 0.4 \) (figure 11 of ref. [142]). These results include chain configurations in which both adsorbing ends of a telechelic chain are adsorbed onto the same colloid. Such loops contribute a repulsive component to the inter-particle interaction. We certainly do not have such “loops” in our experiments. Their removal from the calculation should deepen the attraction and move the minimum further towards touching. The results of Bhatia and Russel therefore give us reason to expect that a bridging polymer at \( \alpha \approx 0.4 \) should...
3.2 Results and Discussion

Figure 3.4: DNA gets expelled from the gap between two neighboring colloids. A: Non fluorescent colloids grafted with YOYO stained λ-DNA. Scale bar is 10 µm. B: Cartoon of λ-DNA coated colloids. DNA is long enough to stretch over neighboring colloids. No free ends have to be present for dimers or bigger assemblies.

effectively be “expelled” by the two colloids (for entropic reasons), thereby causing them to approach to practically touching.

Experimentally, such expulsion of the bridging DNA can be inferred from the close approach of AB colloids, (Figure 3.1b,c). Confocal microscopy allows us to obtain colloid coordinates [2] although finite pixel size and colloid movement between frames limit the accuracy. With these we can reconstruct 3D images of our finite sized clusters (Figure 3.3a) and construct a pair-correlation function of A-B colloids, which confirms the close contact between colloids (Figure 3.3b). The small shift from r = 1 µm of the first peak, is related to experimental errors, rather than a fixed distance between our colloids.

It can also be explicitly demonstrated by adding the fluorescent dye YOYO, which labels the DNA. Now we can see, (Figure 3.4a), that the DNA forms a “halo” surrounding the colloids in a cluster. Note that Tkachenko’s theory of long-DNA bridged colloids predicts an attractive minimum significantly away from touching [9]. His work, however, relied on the Derjaguin approximation to move from flat plates to curved surfaces; in Bhatia and Russel’s model, this procedure only gives acceptable results in the limit of α ≫ 1 [142]. The expulsion of the bridging DNA from the gap between two bridged colloids also suggests an explanation for the observation of size-limited clusters. After a certain degree of aggregation, the cluster is surrounded by DNA expelled from the space between closely-approaching colloids, (Figure 3.4a). The long length of the DNA not only forms this “cloud” of DNA that hinders further aggregation, it also leads to a situation where there are no free “sticky ends” available anymore. The DNA linkers are long enough to find a partner within the cluster (Figure 3.4b).
3.2.4 Monte Carlo simulations agree well with experimental results.

To test the intuitive picture described above, Bozorgui et al. [53] performed Monte Carlo simulations of our system using a simple model. In her model, polymer coils, modeled as soft spheres [143] are permanently tethered onto purely repulsive spheres by harmonic springs. Two soft spheres on neighboring colloids can form a single reversible bond, again modeled as a harmonic spring. Typical configurations after $10^6$ Monte Carlo cycles for 10 “polymers” per colloid are shown in Figure 3.5a. These bear striking resemblance to the experimental observations: size-limited clusters displaying many close-to-touching A-B contacts (Figure 3.1c and Figure 3.3a). Also the simulations clearly show that the polymers (represented as the small black dots) surround the clusters.

The calculated A-B pair correlation function is shown in Figure 3.5b (light gray line). The peak at contact illustrates that touching can indeed be induced by the purely repulsive interactions in this system. To compare this pair correlation function with the one from experiments, the latter is displayed in the same figure (black triangles). The simulations agree especially well with the experimental data when they are rebinned to simulate limited distance resolution (1 pixel $\approx$ 400 nm; dark gray line).

Note that in the work by Bhatia and Russel quoted earlier, the polymer bridge between two colloids is irreversible. The simulations show that touching interaction remains with a degree of reversibility in the bridges, which we believe can also be present in our experiments. Finally, in the simulations, polymers link some clusters. We do not have direct evidence for such DNA links from our experiments.
3.2.5 Increasing temperature has no effect on clusters stability
The clusters obtained experimentally are surprisingly stable. Heating to 80 °C (and leaving the sample for 6 to 12 hours at that temperature) did not redisperse the colloids. Since we linearise cyclic λ-DNA at 65 °C, we may expect the cos1-cos2 binding holding each piece of bridging DNA together at its mid-point to have dissociated by 80 °C, thereby destroying the clusters. We believe that the observed irreversibility is due to the close-to-touching inter-particle contact engendered by the initial bridging mechanism—an effect predicted both by Bhatia and Russel’s calculations[142] and Bozorgui’s simulations [53]. Close inter-particle contact will bring strong Van der Waals forces into play, causing irreversible binding. Such effects were observed by Valignat et al. when they used neutravidin-grafted short (61 bases) ssDNA with complementary free ends (11 bases) to aggregate colloids [6]. These authors found that aggregation was irreversible unless the colloids were additionally (sterically) stabilized by adsorbed block copolymers.

3.3 Conclusion
We have demonstrated by experiments and simulations a novel route for the growth of finite colloidal clusters in a system with only repulsive interactions using long bridging polymer molecules with weak reversibility. Much of the literature on colloidal self assembly to date focuses on generating structures that are potentially unlimited in spatial extent, especially photonic crystals. In contrast, generating nano-devices requires the assembly of building blocks into finite size units. The protocol we have reported represents a novel route towards such assembly.

The mechanism we investigated is an example of micro-phase separation. In our case, a mixture of non-adsorbing λ-DNA and similar-sized colloids will macro-phase separate due to depletion [144]. But the tethering of DNA to colloids leads to the arrest of this macro-phase separation, giving rise to finite-size clusters. These clusters are similar to those observed in mixtures of charged colloids and partially-charged block copolymers [145]. The formation of micelles, with tethered hydrophilic head groups and hydrophobic tails, and the finite-size clusters formed by charged attractive colloids [146] are other instances of such frustration-driven self assembly. Understanding such routes to finite-size self assembly is a key step towards implementing functional nano-devices.

3.4 Materials and Methods
3.4.1 Preparation of biotin-DNA solutions
A schematic summary of our colloid-preparation protocol is given in (Figure 3.6). We used λ-phage DNA (New England Biolabs), which has a contour length of 16 µm (48 500 base pairs (bps)). Special care was taken to handle long λ-phage DNA (New England Biolabs) to avoid any damage due to shear. This DNA was pipetted with tips that were blunted and sterilized before use. The λ-phage DNA, predominately circular at room temperature, was linearised by heating in a 25 µg/ml solution to 65 °C.
Each linear dsDNA is terminated by two complementary 12 base single strands (ssDNA; “sticky ends”), which we call cos1 and cos2 (cos1 = 5’-GGGCGGCGACCT-3’; cos2 = 5’-AGGTCGCCGCCC-3’).

We prepared two populations of linear DNA. In each population, one “sticky end” was hybridized and ligated to cos1-biotin or cos2-biotin (5 µl; 20 µM; Eurogentec) using T4 DNA ligase (New England Biolabs). To remove the excess of oligonucleotides and enzyme the samples were centrifuged and washed three times on a Microcon YM100 membrane (Milipore) with Tris-HCl buffer (250 mM, pH = 8). The biotin-DNA solution was then recovered in a clean tube.

3.4.2 Preparation of DNA-coated colloids

To obtain DNA-coated colloids the two populations of DNA were then separately mixed with neutravidin-coated green-fluorescent (“A” hereafter; depicted in green or white) and red-fluorescent (“B” hereafter; depicted in red or black) polystyrene colloids (diameter 1 µm, Molecular Probes) dispersed in a Tris-HCl buffer (250 mM, pH = 8). In each case the DNA and colloids were reacted overnight during which they were continuously tumbled.

The next day the samples were pelleted and washed five times to remove excess of non-conjugated DNA. In between these washing steps, samples were heated once for 10 min. at 50 °C and washed once in a NaOH solution (0.15 µM) to remove poorly bound DNA. The supernatant was retained to quantify (by UV-spectrometry at 260 nm) the amount of DNA that had effectively bound to the colloids. For λ-DNA this leads to an estimate of some 10 DNA chains per colloid. Given the relative sizes of the DNA and colloids, we expect each colloid to be completely surrounded by grafted DNA. The DNA-coated colloids were diluted in a fresh TRIS-HCl buffer (250 mM final concentration, pH = 8) with D2O to minimize sedimentation. This yielded two batches of colloids with ds-λ-DNA grafted onto their surfaces by strong biotin-neutravidin bonds, displaying complementary single stranded cos1 and cos2 free ends respectively.
Under these conditions, we estimate (by dynamic light scattering, data not shown) the coil size of the linear λ-DNA to be $\sim 800$ nm (radius).

### 3.4.3 Preparation and coating imaging chambers

To avoid any nonspecific interactions between DNA-coated colloids and glass surfaces of the imaging chambers, coverslips were coated with polyethylene glycol (PEG). The coating of glass slides with PEG is a two-step procedure. First the glass slides were silanized with 3-mercaptoptriethoxysilane (97%, Fluka), then a 5-kDa maleimide modified PEG (Laysan Bio Inc) was tethered to the terminal thiol group of the silane.

Prior to the silanization, the glass slides were cleaned in an UV/ozone box. The cleaned slides were placed in contact with silane vapor overnight. Subsequently, the slides were rinsed with Ethanol, dried with Nitrogen and left in an oven ($100 \, ^\circ C$) for 30 minutes. Next a drop of concentrated maleimide modified PEG (250 mg/ml in TRIS-HCl buffer) is trapped between two silanized slides and left overnight at room temperature. The excess of PEG is removed by rinsing with ddH$_2$O.

A sample chamber is made from two glass coverslips of different size separated by a parafilm spacer (100 µm). The parafilm sealed the chamber on three sides. After loading the chamber with 10 µl of each population of DNA-coated colloids, reaching a final particle volume fraction of $\phi \approx 0.004$, the chamber is completely sealed with glue.

### 3.4.4 Confocal imaging

A and B colloids were mixed in equal ratios, a 20 µl aliquot of which was displayed in an imaging chamber (colloids are suspended in a TRIS-HCl buffer (250 mM final concentration, pH = 8) with D$_2$O to minimize sedimentation). The mixtures were observed at room temperature using either a Nikon TE 300 microscope and a Biorad Radiance 2100 MP confocal scan head or a Zeiss axiovert microscope with a Perkin Elmer ultraview RS spinning-disc scan head. Fluorescence of the two populations of colloids was excited at 488 nm and 512 nm, and emission observed at 505 nm and above 600 nm respectively. The use of two types of fluorescent colloids and confocal microscopy allowed us to extract colloids coordinates and to reconstruct 3D images [2].

### 3.5 Acknowledgements

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