DNA-driven assembly of micron-sized colloids

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5 Colloidal Flying Carpets

DNA plays a special role in polymer science not just because of the highly selective recognition of complementary single DNA strands but also because natural DNA chains can be made very long yet perfectly monodisperse. Solutions of such long DNA chains are widely used as model systems in polymer science. Here, we report the unusual self-assembly that takes place in systems of colloids coated with very long double-stranded DNA. We find that colloids coated with such long DNA can assemble into unique “floating” crystalline monolayers that are suspended at a distance of several colloidal diameters above weakly adsorbing substrate. The formation of these monolayers does not depend on DNA hybridization. Floating colloidal structures have potentially interesting applications as such ordered structures can be assembled in one location and then deposited somewhere else. This would open the way to the assembly of multi-component, layered colloidal crystals.

5.1 Introduction

The preceding experimental chapters focused on the DNA-driven assembly of different types of complementary DNA-coated colloids in solution. In the present chapter we take a different approach. Instead of the assembly of complementary colloids in solution, we now consider colloids that cannot bind to each other as they are all coated with identical DNA strands. This DNA may either be terminated with sticky ssDNA ends that can bind to complementary ssDNA that is grafted to the bottom of the sample cell (Figure 5.1a), or it may only interact weakly (and non-specifically) with the substrate. But even in that case, DNA-mediated colloid-colloid binding is not possible as all colloids display the same DNA sequence (Figure 5.1b).

Immobilization of colloids coated with short DNA fragments on a surface coated with complementary DNA was extensively studied by Niemeyer and coworkers [94, 95]. In these experiments, the immobilization of the colloids on a DNA micro-array indicated that the surface adsorption proceeds with complete site selectivity, as few immobilized colloids were observed when non-complementary DNA was used. By printing small areas with ssDNA, a pattern consisting of areas with or without colloids could thus be obtained. The colloids within these areas exhibited no two-dimensional ordering; rather they formed an amorphous layer because the colloids tended to be immobilized on the spot where they landed. Niemeyer et al. also explored if two-dimensional ordering of colloids could be improved by grafting two different sequences on the colloids and
introducing additional “linker” DNA [101]. The first sequence on the colloids was again used to bind (hybridize) to the surface. The second type of colloid-bound oligomers were used to establish cross-links to neighboring colloids by means of the “linker” DNA. While the length of the linker DNA controlled the inter-particle spacings, no additional lateral order was observed in this system. Other authors [96, 97] also observed that DNA-coated colloids bound to a substrate tended to exhibit no lateral ordering. In fact, ordered DNA layers were only observed in cases where the surface had been prepared with an ordered pattern of surface grafted ssDNA strands [96, 97]. In those cases, the order was induced by the “template” rather than by the colloid-colloid interactions.

Seeman and coworkers used a DNA scaffold to align DNA-coated colloids in rows with precisely defined inter-row spacings. The scaffold was prepared before addition of the colloids and contained predetermined hybridization sites. After addition of DNA-coated colloids, rows of aligned colloids appeared. Of course, there exists an extensive literature on colloidal particles that form two-dimensional crystals upon adsorption to a flat and unstructured surface (see e.g. ref. [153, 154]). In these systems, the attraction between colloids and wall leads to a high concentration of mobile adsorbed colloids. At sufficiently high densities, these colloids undergo a (two-dimensional) freezing transition. However, when the attraction between colloids and surface is reduced, these crystals melt again. Density-driven freezing of DNA-coated colloids was observed by Luo et al. [104]. These authors were able to make hexagonally stacked crystal sheets by drying a drop of DNA-coated colloid solution confined in the pores of a super-lattice sheet. By evaporation of the excess solution a free-standing colloidal film appeared. As, in this case, the ordering was density driven, the colloids used in this study did not need complementary ssDNA ends.

In this chapter we show that it is possible to obtain two-dimensional colloidal structures that are floating well above a properly prepared but unstructured surface. While previous studies mainly used short oligonucleotides, we choose to work with longer DNA. As we shall show below, DNA-mediated colloid-surface interactions are not responsible
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5.2.1 Grafting surfaces with DNA

As a sample chamber we used a 96-well plate (Sensoplate; Greiner bio-one), allowing us to run many experiments under identical conditions. Onto the bottom glass surfaces of these wells we grafted a polymer monolayer holding ssDNA “sticky ends” (Figure 5.2c). To obtain such a layer we first coat the glass surfaces with a polylsine-poly(ethylene glycol)-biotin polymer (PLL-PEG-biotin; SurfaceSolutions). Subsequently we added a layer of streptavidin. In the final step biotinylated ssDNA could be easily attached (Figure 5.2c). Once the DNA-coated surface was formed, we tested the surface for DNA coverage, by exposing it to a suspension of colloids coated with the 12 complementary bases. The high number of colloids bound indicates proper surface coating (Figure 5.2a). Besides providing a suitable method of linking DNA to a glass surface, the polymer layer also prevents non-specific binding of colloids without DNA to the surface. Control experiments in which we disperse colloids without a DNA coating show that far fewer colloids are bound (by physisorption) to the surface (Figure 5.2b).
Figure 5.3: Aggregation of λ-DNA coated colloids above a “sticky” surface leads to 2D-flying carpets. A: Image of a flying carpet imaged with a confocal microscope. As the structure is slightly bent in the xy-plane a z-projection is shown. Scale bar is 10 µm. B: Pair correlation function of figure A. The first neighbor is at contact, the function also shows peaks for the second, third and fourth neighbor. Black triangles: Guide to the eye; positions of expected peaks for a perfect hexagonal crystal.

5.2.2 DNA-coated colloid assembly above a (complementary) surface

The main experiments were performed with λ-coated colloids. This type of DNA is monodisperse and has a 16 µm contour length (485000 bps; radius of gyration ∼ 800 nm [53]). The resulting colloids carry long dsDNA spacers with ssDNA “sticky ends” that can bind (hybridize) to the complementary ssDNA on the surface. Hybridization between colloids is not possible as they all display the same ssDNA sequence (5’-AGGTCGCCGCCC-3’). As the radius of gyration (R_g) of λ-DNA is similar to the colloid-size used, the colloids are coated with no more than 8 to 10 strands [53]. Sedimentation is minimized by density matching the DNA-coated polystyrene colloids with a sucrose-TRIS buffer. In all experiments we work at a low colloidal volume fraction of ∼ 4.10^{-4}.

Two hours after injecting a solution of colloids coated with λ-DNA into the sample cell, we observe the appearance of single layers of close-packed colloids. If left at room temperature for longer times, these structures can grow into large colloidal monolayers that span the entire field of view of the microscope (Figure 5.3a). The structures are clearly crystalline, be it that some crystals contain point defects or even grain boundaries. Indeed, the pair-correlation function of this two dimensional crystal displays distinct peaks corresponding to those of a 2D hexagonal crystal (see black arrowheads, Figure 5.3b). Interestingly, these colloidal sheets float above the bottom of the cell, with colloids surrounding the carpet on either side (Figure 5.4a), at heights ranging from the R_g of λ-DNA up to 5 µm (Figure 5.4b) with an average around 2.5 µm. Because the colloidal monolayers appear several particle diameters above the substrate, we refer to these two-
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Figure 5.4: The average flying height of colloidal carpets above the surface. A: Reconstructed 3D-image of a carpet surrounded by colloids on either side. B: The 2D colloidal crystals sit not directly on the surface. Instead they are suspended above the surface at heights ranging from $\lambda$-DNA $R_g \sim 0.8$ up to 5 $\mu$m. The bars indicate the height a carpet had in respect to the surface from three different experiments (wells). In black a Gaussian fit is drawn to obtain an estimate of the average height: 2.5 $\mu$m.

dimensional crystals as “colloidal flying carpets”. We observe this carpet-formation only above the bottom surface of the cell, but not in the bulk of the solution. Besides the two dimensional ordered crystals, also a small number of less ordered shapes is present.

$\lambda$-DNA coated colloids contain 8 to 10 strands per colloid. If all strands of DNA would anchor to the surface the carpets would be unable to move. By imaging a single carpet at different moments in time, slow movements can be detected. Figure 5.5a shows a carpet with an unusual shape at a given time. After leaving the sample for two hours we imaged the same carpet again. This time there was no sign of growth, but the carpet did rotate (Figure 5.5b). The mobility of the carpet indicates that either not all strands of DNA were hybridized or at the very least, that the hybridization at room temperature is sufficiently weak to make rearrangements possible. As room temperature is well below the melting temperature of our 12 base pair bonds, the first probability is the more likely. Another conclusion that can be drawn from Figure 5.5 is that the crystallites have some time to anneal during growth, as the 2D crystals can even show clear facets.

5.2.3 Depletion forces are not responsible for the assembly

As our colloids are designed to have no DNA-mediated interaction with each other, the appearance of close packed colloids must be due to other (non-specific) interactions. The mechanism of self-assembly depends on the effective interaction between colloids mediated by the environment such as solvent, substrate or external fields. The most common non-specific interactions between colloids are: Coulomb interactions, dispersion forces and depletion forces. In the present case, (screened) Coulomb interactions between the colloids are certainly present but, as all colloids are like-charged, the Coulomb interaction...
is unlikely to lead to condensation. Depletion forces are entropic, attractive interactions between colloids that can be caused by non-adsorbing polymers \cite{156}. In our system, there is no free DNA and hence one might be tempted to dismiss depletion forces as a possible source of attraction. However, if the colloids move in a semi-dilute mesh of long DNA strands, short-ranged depletion interactions cannot be ruled out a priori - even if the DNA is grafted to the colloids.

To test if depletion forces are responsible for the 2D-aggregation behavior in our system, we repeated the experiment with colloids without DNA coating, but with free \( \lambda \)-DNA in solution. Whereas grafted polymers led to ordered 2D-aggregates (Figure 5.6a), the same concentration of DNA free in solution only yields small 3D aggregates of \( \sim 15 \) colloids at most (Figure 5.6b). Even if we increase the \( \lambda \)-DNA concentration a hundred times we still see a clear difference in aggregation behavior. Now the aggregates comprise more colloids, but they consist of 3D branched structures (Figure 5.6c) as opposed to flat 2D crystals (Figure 5.6a). Kim and coworkers \cite{123}, who compared depletion forces of colloids of different materials, showed a similar effect for neutravidin-coated colloids.

From the obtained pictures it is clear that depletion forces are not responsible for the formation of the “colloidal flying carpets”. These results indicate that the crystallization mechanism is different from previous observations where 2D crystallization from a very dilute colloid/polymer mixture was observed \cite{157}.

### 5.2.4 Colloidal flying carpets are temperature stable

On the basis of the above discussion, it seems most likely that short-range dispersion forces play a key role in the formation of the dense colloidal carpets. As the primary minimum of the dispersion interaction between two colloids is extremely deep, we should expect that colloidal carpets, once formed are very stable. To test this hypothesis, we examined the behavior of the carpets at elevated temperatures. We first prepared
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Figure 5.6: Comparison between aggregation of DNA-coated colloids and colloids surrounded by DNA free in solution. A: λ-DNA coated colloids aggregate into a flying colloidal carpet. B: Colloids surrounded by a solution of λ-DNA (λ-DNA is present at the same concentration as used in figure A) hardly aggregate; only small 3D clusters appear. C: If the concentration of λ-DNA is increased 100x, more aggregation is visible. In contrast to figure A this leads to 3D branches rather than 2D carpets. All scale bars are 10 µm.

crystalline colloidal carpets at room temperature (Figure 5.7a). Once these had formed, the sample was heated to 70 °C for several hours (Figure 5.7b). The two-dimensional crystal structures were found to be remarkably stable against heating: even after more than 20 hours at 70 °C only minor changes can be seen (Figure 5.7c). Only at the edges of the carpet some colloids disappeared (compare Figure 5.7a, b and c white arrow heads).

These results strongly suggest that the colloidal carpets are held together by Van der Waals forces. In previous experiments (chapters 3, 4) the 12-base overhang grafted to colloids (with or without spacer) proved difficult to dissociate with elevated temperatures and it seems likely that in that case the DNA linkers “catalyzed” the formation of aggregates but that, once formed, the DNA links were irrelevant for the thermal stability of the aggregates - instead, the colloids stuck together due to dispersion forces. Of course, this short-ranged dispersion attraction can only function if the colloids can get close enough. Hence, if colloids are coated with a dense brush of short ssDNA, we should not expect them to stick to each other, nor to the substrates (provided the temperature is high enough to dissociate the DNA links). We tested whether cos-oligonucleotide functionalized colloids could dissociate from a surface with complementary ssDNA coating upon heating to 70 °C (Figure 5.8a). As this system only showed few colloids bound to the surface at a low volume fraction (∼ 4.10⁻⁴) a significantly higher volume fraction was used (∼ 4.10⁻³). First the surface coverage is imaged at room temperature (Figure 5.8b). Then the sample was heated to 70 °C. As can be seen from the figure, after removal of unbound colloids only few colloids remain bound to the surface (Figure 5.8c). This indicates that at 70 °C the 12-base DNA duplex is dissociated. As we showed before, the carpets that form when the colloids are coated with the longer DNA remain near the surface. In section 2.6 we shall argue that this fact is most likely due to gravity rather than to any specific DNA-mediated attraction.
Figure 5.7: Flying colloidal carpets are temperature stable. A: Carpet formation at room temperature. B: After heating at 70 °C for two hours the same carpet is still intact. C: Even after more than 20 hours at 70 °C, the carpet did not melt. Marginal changes appeared in the border of the carpet (white arrowheads, compare to A and B) All scale bars measure 10 µm.

5.2.5 Examining different control parameters of the system

As the colloids only crystallize near a surface and not in the bulk, other factors than dispersion forces also play a role in the membrane formation. To get more insight into the mechanism by which colloidal carpets form, we performed a series of experiments, where some of the system-parameters that could affect the aggregation process were varied. These are: the method used to attach the ssDNA to the bottom of the sample cell, additives like sucrose present in the buffer and the length of the DNA-coating on the colloids.

Method of surface coverage does not control carpet formation

To test the importance of a polymer-based method to graft DNA to the surface, a different approach was chosen to provide a DNA-coated surface. Instead of using a polylsine-PEG coating, we coat the glass surface with a protein-based multilayer. Bovine serum albumin (BSA; Sigma-Aldrich) adsorbs to glass if charge neutral. By using a biotinylated version of BSA and multiple layers of the protein in combination with a streptavidin linker, a DNA-coated surface can easily be built up (Figure 5.9a). Although the surface coverage is lower than before (Figure 5.9b), carpets do form above this surface as well (Figure 5.9c), be it that they are smaller in size and occur less frequently.

Carpet formation is not dependent on choice of buffer

In order to test if the sucrose that we used for density matching is responsible for carpet formation, we repeated the experiment in a Tris-HCl buffer in combination with heavy water (D₂O; 50-50; 100 mM final concentration) to density match the DNA-coated colloids. The appearance of similar two-dimensional crystalline structures (Figure 5.10a), demonstrates that sucrose is not essential for the aggregation process.
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Figure 5.8: Cos-oligonucleotide grafted DNA-coated colloids can be melted off a flat surface functionalized with the complementary ssDNA. A: Schematic representation of cos-oligonucleotide grafted DNA-coated colloids on a surface. B: At RT an amorphous colloidal layer in direct contact with the polymer layer forms. C: When the sample is heated to 70 °C for several hours most colloids dissociate from the surface. Both scale bars indicate 10 µm.

But is density matching a necessary requirement for the 2D crystallization? Repeating the experiment in a pure TRIS buffer (no density matching) indicated that carpets form even without density matching of the DNA-coated colloids (Figure 5.10b), although they appear smaller and closer to the surface. In time, all colloids eventually settle down, so the carpets get buried underneath sedimented colloidal particles (Figure 5.10c).

Reducing the DNA length inhibits carpet formation

The carpet formation that we described above was observed in systems of colloids coated with very long (lambda) DNA. In order to test the role of the length of the grafted DNA, we repeated the experiments with shorter DNA strands grafted to the colloids. To keep all other interactions similar, DNA with the same 12 base ssDNA overhangs were used: pBelo-DNA (7500 bp; Rg ~ 200nm (100 mM Tris-HCl pH 8)) and cos-oligonucleotides (12 bases ssDNA; no spacer). The length of the DNA plays an important role in carpet formation. Whereas λ-DNA (Figure 5.11a) and pBelo-DNA coated colloids (Figure 5.11b) form colloidal carpets, the colloids coated with the cos-oligonucleotides do not (Figure 5.11c). Rather, we observe an amorphous colloidal layer in direct contact with the polymer layer that coats the bottom surface of the sample well. While we did observe the formation of floating 2D crystals with pBelo-colloids, the maximum sizes of the colloidal sheets seemed, on average, smaller than for the λ-DNA coated colloids. Furthermore, the carpets appeared closer to the surface (average ~ 1 µm; data not shown), suggesting that the DNA separates the colloids from the surface: the length of the DNA determines the “flying height” of the floating 2D crystal structures.
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5.2.6 Specific binding through ssDNA overhangs is redundant to form flying colloidal carpets

As increasing the temperature can dissociate the 12-base duplex as shown in figure 5.8c, DNA hybridization cannot be the only factor responsible for keeping the carpets near the surface. Non-specific binding of the dsDNA spacer to the surface may, however, play a role. Whether or not this is the case can be tested by repeating the carpet formation experiments under conditions where DNA-surface hybridization is impossible. Indeed, ref. [158] reports a study on the non-specific DNA interaction with pLL-PEG films. As shown in ref. [158], DNA can adsorb to the polymer layer (Figure 5.12a). The strength of this adsorption depends on the ionic strength of the solution. To test whether non-specific interactions are sufficient to obtain carpets, we prepared polylysine-PEG monolayers on the well-surfaces and grafted colloids with blunt pBelo-DNA. The results (Figure 5.12b) show that DNA hybridization is indeed not necessary to obtain the crystal structures. Note that this is only true for a surface coated with pLL-PEG (or at least a surface coating with a positive charge). The other coating tested, a BSA protein layer (Figure 5.9), is negatively charged [159] at the pH used in the experiments. In this case it seems plausible that ssDNA is necessary to impose an attractive interaction between the surface and the colloids, but we did not test this.

The behavior observed in Figure 5.12a suggested an additional experiment, namely one where the electrostatic attraction between the long (negatively charged) DNA and the (positively charged) polylysine is strongly screened. Indeed, we found that no carpets form when the experiment is performed in the usual buffer but with 100 mM of added salt (data not shown). It would be interesting to test the behavior of the carpets at intermediate ionic strengths. Is there a threshold for carpet formation or does the average size of the carpets decrease continuously to zero?
Figure 5.10: Carpet formation is not dependent on choice of buffer. A: \(\lambda\)-DNA-coated colloids also aggregate into flying colloidal carpets when \((\text{D}_2\text{O})\) is used to match the density of the colloids instead of sucrose. B: Carpets form even without minimizing sedimentation. C: Without any type of density matching carpets get buried under colloids that settle down on the surface. As these carpets are harder to see, a white curve is drawn around one of the buried carpets. Scale bar indicates 10 \(\mu\text{m}\) for A and 5 \(\mu\text{m}\) in B and C.

### 5.2.7 Two factors are essential for the formation of crystalline colloidal membranes

The colloids coated with cos-oligonucleotides (no dsDNA spacer; 12 bases ssDNA attached directly to the colloids) were unable to form crystalline sheets. Instead, an amorphous colloidal layer in direct contact with the polymer layer coating the surface of the sample well is formed. This randomness as well as the fact that these colloids can be “melted off” above the hybridization temperature of the 12 bp-bonds (\(T_m \sim 42^\circ\text{C}\); Figure 5.8c) suggests that two factors are essential for the formation of crystalline colloidal membranes: a. weak binding to the surface that allows colloids to diffuse and b. weak steric stabilization of the colloids against the formation of direct contacts due to

Figure 5.11: The formation of flying colloidal carpets depends on the dsDNA spacer length. A: \(\lambda\)-DNA coated colloids (48500 bps). B: pBelo DNA-coated colloids (7500 bps). C: Colloids coated with 12 bp ssDNA (no spacer). All scale bars measure 10 \(\mu\text{m}\).
strongly attractive dispersion forces. If the colloids are too strongly bound to the surface of the cell, as is the case for the colloids coated with many short ssDNA strands, surface diffusion of the colloids is inhibited and hence amorphous layers can form, since annealing is not possible. In addition, the short DNAs provide steric stabilization against the formation of direct colloid-colloid contacts. Hence, neither condition for the formation of crystalline colloidal carpets is satisfied in the case of colloids coated with short ssDNA.

Colloids grafted with either λ-DNA, or pBelo-DNA with the same ssDNA overhangs do not aggregate in the bulk under the conditions used in the present experiments (chapter 4). This implies that in the bulk of the solution, where the colloidal concentration is low, the DNA cloud that surrounds the colloids is sufficient to prevent aggregation due to non-specific Van der Waals interactions. However, near the bottom cell surface, this situation changes: the charged DNA strands on the colloids are attracted weakly and non-specifically to the (oppositely charged) polylysine layer on the surface. As a consequence of the DNA-surface attraction, the colloidal concentration is significantly enhanced on the surface and colloids come into contact sufficiently frequently to overcome the weak entropic barrier provided by the long dsDNA. Indeed, in the 2D crystals the colloids are effectively touching each other and, as we argued above, the thermal stability in the colloidal membranes is therefore most likely due to the action of short-range dispersion forces. At the same time, the fact that highly ordered 2D crystals form indicates that crystal growth is slow: aggregation is definitely not diffusion limited.
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Figure 5.13: Carpet formation precedes (binary) cluster formation for long DNA. A: Schematic representation of the experiment. In the binary colloid mixture, colloid-colloid binding is possible by complementary base pairing. B: Cos-oligonucleotide and pBelo-DNA coated colloids aggregate into 3D-assemblies (cos-cluster depicted) and carpet formation is not observed. C: λ-DNA coated colloids form carpets of single-color. Carpets of green-fluorescent colloids coexist with carpets of red-fluorescent colloids (insert). Note that in the bulk solution red and green colloids are well mixed. Both scale bars are 10 \( \mu m \).

5.2.8 Carpet formation precedes (binary) cluster formation for long DNA

Thus far, we have studied carpet formation in colloids that cannot bind to each other via DNA hybridization. We now consider what happens if such interactions can occur. By allowing colloid-colloid binding we introduce a competition between colloid-colloid binding and colloid-to-surface interactions. We limit ourselves to the case where the colloids cannot hybridize to the surface (Figure 5.13a). All three DNA-coated colloid systems were examined. Both the colloids coated with the cos-oligonucleotides and the colloids grafted to pBelo-DNA formed three-dimensional aggregates comprising both green- and red-fluorescent colloids (Figure 5.13b). The structures that formed were similar to those observed in our previous experiments in bulk (chapter 4). For both systems, colloid-colloid binding is preferred to the interaction with the surface.

In contrast, the samples containing both types of λ-DNA colloids aggregate into two dimensional crystalline structures (flying carpets). Apparently, here the competition turned out in favor of the surface interactions. Surprisingly, all carpets consist of either pure red, or pure green colloids (Figure 5.13c) even though the red and green colloids are well mixed in solution. By comparing the relative aggregation speeds of the different types of DNA-coated colloids, we can arrive at a qualitative explanation for the observed competition between bulk aggregation and surface adsorption. The colloids coated with cos-oligonucleotides bind on contact by DNA hybridization. Aggregation takes place in minutes, i.e. in a time that is much shorter than the time needed to adsorb onto the substrate. Colloids coated with pBelo-DNA can in principle form carpets but they can also aggregate in the bulk. Which of the two scenarios dominates depends on the relative rate of the two processes. In the present experiments, only three dimensional
structures were found. This is not surprising because, under the conditions of these experiments, the formation of carpets with colloids coated with pBelo-DNA takes 5-7 hours. However, as was shown in chapter 4, appreciable bulk clustering of pBelo-coated colloids can take place on this time scale. In fact, the results of chapter 4 suggest that in 5-7 hours pBelo-clusters can form that contain up to \( \sim 90 \) colloids (Figure 5.14). The clustering via DNA hybridization is therefore faster than carpet formation. Aggregation of \( \lambda \)-DNA coated colloids is comparable in speed to pBelo-DNA coated colloids, but in contrast to pBelo-DNA coated colloids, carpet formation is faster (\( \sim 1-2 \) hours). On this timescale, \( \lambda \)-DNA clusters are barely present (Figure 5.14). This explains why we see carpet formation in the \( \lambda \)-DNA coated colloids and not with the other two types of DNA coated colloids.

Explaining why the \( \lambda \)-DNA carpets form, does not explain the puzzling observation that individual carpets are made of one type of colloid. One possible explanation could be that the different fluorescent dyes give slightly different physical properties to the red and green colloids. This might affect the range of the DNA “corona” of the colloids or possibly even their density. If this were to be so, the carpets of different colors could appear at different heights above the surface. However, our data shows no difference in the flying height of the red or green carpets (data not shown). There is, however, clear evidence that the dyes do affect the surface charge of the differently labeled colloids. This follows from measurements of the zeta-potential (measured with a Malvern “Zetasizer 2000” - averaged over 5 measurements). For non-fluorescently labeled \( \lambda \)-colloids, the zeta-potential is \(-18.3 \pm 1.6 \) mV. In contrast, the red fluorescent \( \lambda \)-colloids have a zeta-potential of \(-13.1 \pm 1.6 \) mV and the green fluorescent \( \lambda \)-colloids \(-23.0 \pm 5.6 \) mV. These differences in the zeta-potential affect the behavior of the colloids and their DNA corona - clearly, such differences may result in different kinetics of carpet formation. However, at this stage, it is unclear how the differences in electrostatic properties could result in red-green phase separation and further experiments would be needed to sort this out.

5.2.9 DNA is not evenly distributed around the colloidal flying carpets

We have now established that grafting long DNA strands is essential for carpet formation. However, we have not yet shown directly where the \( \lambda \)-DNA resides once the carpets have formed. As the colloids within the carpets are closely packed there is little room for the \( \lambda \)-DNA inside the colloidal membrane. To determine the location of the DNA we use YOYO-staining. Because this dye is fluorescent, we use it in combination with non-fluorescent colloids.

The non-fluorescent colloids cannot be imaged with bright field light in the black sample chambers, used in all previous carpet-experiments. Therefore a clear 96-well plate was used instead. These plates are only available with a bottom surface made of plastic rather than of glass. The surface coverage of the polymer-ssDNA is tested in these sample-chambers as well. Figure 5.15a shows that although for some areas the specific binding is similar for both types of surface used (compare to insert), the
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Figure 5.14: Histogram of cluster sizes of λ-DNA and pBelo-DNA coated colloids measured after the typical time the systems need to grow carpets above the surface. Clustering started long before carpet formation for pBelo-DNA coated colloids could take place. λ-DNA coated colloids are only present in small clusters during this time.

Surface coating on the plastic plates also shows more and larger “holes” on the surface. Presumably, in these places there is insufficient polymer coating present. Comparing the number of colloids that stick non-specifically to the surfaces (Figure 5.15b) shows the effect of these so-called “holes”. Whereas the number of colloids on the bottom plate made of glass (Figure 5.15b, insert) is very low, this number increases significantly in the case of a plastic bottom plate. The figure indicates that colloids gather in the holes and stick to the unprotected surface.

Samples were loaded and imaged the next day after ∼15 hours. First the sample was checked with bright field microscopy. Although two-dimensional shapes were found, they contained less order than the colloidal flying carpets depicted earlier in this chapter, but similar to the less ordered shapes mentioned before. After localizing the “carpets”, YOYO is added to the solution. After 20 minutes incubation (in the dark), carpets are imaged (Figure 5.16). The DNA is not evenly distributed. At the surface (Figure 5.16b) non-specifically bound DNA-coated colloids are found. A faint glow is visible from the carpet floating above the surface. Moving up in the sample (∼3 µm) the edges of the carpet become visible as the carpet is slightly bent ((Figure 5.16c and a,b-insert). Moving further up, we reach the midsection of the carpet, where individual colloids can be seen, due to the DNA staining (Figure 5.16d; note the lack of order). Here the DNA remains mainly around the edges except for a small piece in the middle. Even higher, just above the colloidal carpet (Figure 5.16e) most of the DNA is found, mainly in the midsection. To show the uneven distribution of DNA even clearer a z-projection is made of the entire stack (Figure 5.16f). As there is little DNA below the carpet, the uneven coloring of the DNA on top of the carpet is most likely due to imperfect DNA staining, rather than DNA depleted areas.
The coverage is tested as depicted in Figure 5.2c: A: Specific binding; the surface coverage is lower than for the samples with glass bottom plates (insert), some areas seem to be not covered by polymer (holes). B: Non-specific binding; the difference between the two surfaces is most apparent here (compare picture to insert obtained for a glass surface). The holes on the plastic surface now get filled with colloids that stick non-specifically to the plastic bottom plate. Both scale bars indicate 10 µm.

The λ-DNA coated non-fluorescent colloids assemble into less ordered carpets. We point out that the same non-ordered carpets were also found with fluorescent colloids in the 96-well plates with a glass bottom (Figure 5.17b). These structures appeared within the same sample as the ordered ones (Figure 5.17a). For now we do not know what influences the system to create both ordered and non-ordered carpets within one sample.

The results of the DNA staining applies to the disordered carpets. Although it is likely that similar behavior would be observed in the case of ordered carpets, this conclusion is not obvious. Hence, the present experiments provide an ambiguous answer to the question where most of the DNA resides.

5.2.10 Testing a different sample holder

It would be interesting to test if we can first assemble a carpet and then transport it elsewhere. One option for this type of transport would be “dragging” a carpet with an optical tweezer, provided that the carpet retains its integrity during such manipulation.

With the 96-well plate such optical-tweezer experiments cannot be done as the bottom plate (175 +/− 15 µm) is thicker than the focusing distance of the laser. To perform the proposed experiments in the future, new sample holders are needed. Here, we describe a preliminary test of one such sample holder.

To enable experiments with optical tweezers we need to make carpets in a sample containing two thin coverslips as bottom and top surfaces. Not only can we then use optical tweezers to try to move our carpets, but we can also turn the sample to see
Figure 5.16: DNA is not evenly distributed around the colloidal flying carpets. A: Cartoon of image taken. Non-fluorescent λ-DNA coated colloids form a less ordered carpet above the surface. After YOYO-staining images are taken at different heights from the surface to determine where the DNA is. B: Colloids are stuck to the surface, the glow from the DNA of the carpet above can be seen faintly. Insert: side-view of the stack taken to show the slight bend of the carpet. C: More DNA appears to be present at the borders. D: At this height individual colloids can be seen. The reduced order in this system is clear. The DNA is mainly present at the borders except for a spot in the middle. E: Just above the carpet, a lot of unevenly distributed DNA is present. F: z-Projection of the entire stack showing DNA on top of the assembly and the monomers surrounding the carpet.

whether carpet formation is possible on both bottom and top surface simultaneously.

In the new sample holder (made with two thin coverslips separated with parafilm) surface coverage is tested with neutravidin colloids (Invitrogen; 1 μm; Figure 5.18a), on both pLL-PEG-biotin (specific; Figure 5.18b) and pLL-PEG (non-specific; Figure 5.18c) coated surfaces. We observe that there are approximately equal amounts of colloids present on both surfaces, indicating that the polymer layer is most likely not uniform or not dense enough. Therefore, we cannot conclude that the colloids in Figure 5.18b are bound specifically.

Despite the poor surface coverage, we tested if we could obtain carpets in these sample holders. The first experiments show that this is possible, but conditions are not yet optimized. In figure 5.19 some results are depicted. For the moment small carpets (Figure 5.19a,b) can be found as well as larger but less ordered ones (Figure
Figure 5.17: In one sample both perfectly ordered and less ordered carpets were found. A: Nicely ordered colloidal flying carpet. B: Two-dimensional shape with less order. Both scale bars indicate 10 µm.

5.19c). Two shortcomings of these new sample holders are that the number of carpets formed is far lower than in the 96-well plates (from ~ 50 to 4) and that the aggregation time increases to a few days as opposed to a couple of hours needed in the well plates. When the conditions are properly optimized, these sample holders could also be used for YOYO-staining on ordered carpets.

5.2.11 Conclusion

We have found that colloids coated with long DNA strands can spontaneously form a crystalline 2D colloidal carpets, hovering several microns above the support surface. Our control experiments suggest that the formation of these colloidal membranes is facilitated by the weak, non-specific adsorption of the DNA-coated colloids (or, more precisely, of the DNA coating of these colloids) to a weakly positively charged lower surface of the sample cell [158]. Under these conditions, the steric stabilization of the colloids by the grafted dsDNA is insufficient to prevent the slow formation of dense 2D colloidal crystals that are subsequently stabilized by short-ranged dispersion forces. Colloids that are coated with very short ssDNA strands bind strongly to the surface via specific interactions between the complementary strands and form an amorphous adsorbate rather than a crystalline floating carpet.

The ability to make floating, yet surface-bound structures, could provide an interesting route to make novel colloidal structures: in particular, by making use of suitable DNA linkers, it should be possible to induce the self-assembly of multiple layers of 2D crystals that can be attached on top of the carpets. Another option would be to use the free “sticky ends” to close the carpets, forming a tube of colloids with DNA on both sides, as possible docking sites to bind other molecules or materials.
5.3 Materials and Methods

5.3.1 Coating glass-surfaces with DNA

Polymer coating

The coating of the glass bottom plates of the 96 well-plates with a polymer is a three step procedure. Firstly, the wells are rinsed with a strong soap solution (Hellmanex; 10% solution) for at least 5 hours. After removing all the soap (rinsing with ddH₂O) a polylysine-poly(ethylene glycol)-biotin polymer solution (PLL-PEG-biotin (0.5 mg/ml; 50 µl); SurfaceSolutions) is added. Subsequently we remove the excess of polymer solution. Next, a layer of streptavidin (0.5 µg/µl; 50 µl; Invitrogen) is added. We can now easily attach ssDNA in our third step using a biotin-streptavidin bond. A short single strand of 12 bases (5’-GGGCGGCGACCT-3’) with a biotin attached to the 3’-
end is added (1 $\mu M$; 50 $\mu l$; Eurogentec). Surface coverage is tested in a Tris-HCl buffer (100 mM; pH 8) with neutravidin-coated polystyrene colloids (PS-colloids; Invitrogen, 1 $\mu m$ diameter) coated with the complementary 12 bases. DNA coated surfaces that are not immediately used are kept at room temperature in a sucrose-TRIS buffer (150 mg/ml sucrose; 100 mM TRIS).

**Protein coating**

To obtain protein coating we rinse the wells with a strong soap solution (Hellmanex; 10% solution) for at least 5 hours. After removal of all soap residues (rinsing with ddH$_2$O) stacked layers of biotin-Bovine serum albumin (biotin-BSA; Sigma-Aldrich) and streptavidin are added. First a layer of biotin-BSA (2.5 $\mu g/\mu$l; 50 $\mu l$) is attached in an acetate-buffer (20 mM acetic acid/ 80 mM sodium acetate; pH 5.2). Then a layer of streptavidin is added (1 $\mu g/\mu$l; 50 $\mu l$; Invitrogen). As the biotin-BSA contains multiple biotins/molecule ($\sim$ 3), a branched structure of biotin-BSA can be generated by repeating the two steps once more (Figure 5.9a). Now we can, easily attach ssDNA in our third step using a biotin-streptavidin bond. A short single strand of 12 bases (5'-GGGCGGCGACCT-3') with a biotin attached to the 3'-end is added (1 $\mu M$; 50 $\mu l$; Eurogentec).

**Stability of the polymer coating at elevated temperatures**

A pLL-PEG-biotin surface is coated with neutravidin colloids as shown in Figure 5.18a. After imaging the surface coverage at room temperature (Figure 5.20a) the sample was heated to 70 °C. Before imaging, the sample was rinsed to remove unbound colloids. Images were taken after 2, 7, 12 or > 20 hours. Most of the time there was no evidence of colloids being removed (Figure 5.20b), but sometimes after a sample had been at 70 °C for at least 12 hrs, some colloids disappeared from the surface (Figure 5.20c). After some control experiments we realized this was only the case if an older pLL-PEG-biotin solution was used (over $\sim$ two/three months old at 4 °C).

To test if the colloid removal was due to complete removal of the pLL-PEG-biotin polymer or to a breakage in the biotin-neutravidin bond, we repeated the experiment once more. Two samples were placed at 70 °C for > 20 hrs, while being softly shaken to remove as much colloids as possible (Figure 5.20d,g). After imaging, new colloids were added to the samples. One sample received neutravidin colloids (Figure 5.20e), the other sample carboxylated colloids (Figure 5.20h; both of different color to be able to distinguish them). If the whole polymer dissociated, both types of colloids will bind as there is nothing to protect the surface from non-specific binding anymore. If only the biotin-neutravidin bond got broken, the neutravidin colloids will be able to rebind, whereas the carboxylated colloids cannot. The results show (Figure 5.20e,h) that the polymer dissociates from the surface at elevated temperatures. When an overlay is made of the colloids present after heat treatment and of the new colloids bound, we find no difference between neutravidin or carboxylated colloids and complete surface coverage again (Figure 5.20f,i).
5.3.2 Preparation of DNA-coated colloids

Colloids coated with DNA with a 12 base ssDNA overhang

In this chapter three types of colloids were used with a 12 base ssDNA overhang: λ-DNA, pBelo-DNA and the cos overhang. Colloids are prepared in the same way as described in chapters 3, 4. The only difference is in the final dilution step. Now the DNA-coated colloids were diluted in a fresh TRIS-HCl buffer (100 mM final concentration, pH = 8) to obtain a 0.5% solution and stored at 4 °C until used. For a typical experiment 15 µl of the colloidal solution was added in 200 µl sucrose-TRIS buffer (150 mg/ml sucrose; 100 mM TRIS).

Colloids coated with blunt ended DNA

To obtain blunt-ended DNA the pBelobac11 plasmid was used once more. To obtain an almost full length piece of DNA with one side blunted and the other end biotinylated, the plasmid was restricted with two enzymes: BamHI and HpaI. As these enzymes perform optimally in the same buffer conditions, the restriction can be performed simultaneously. After restriction all DNA is loaded onto a gel and the DNA piece is extracted from it. BamHI leaves a 5'-overhang of 4 bases that can be filled up nucleotide by nucleotide with Klenow exo-. (To obtain biotinylated DNA regular dTTP, dCTP, dGTP-nucleotides were used in combination with biotin-dATP; all Invitrogen). The reaction solution was then cleaned from excess nucleotides and enzyme (PCR-purification; Qiagen). The obtained blunt ended DNA was then attached to neutravidin-colloids as described before (see chapters 3, 4).

5.3.3 YOYO-staining of DNA

To visualize DNA YOYO-staining can be used. For these experiments non-fluorescent colloids were coated with λ-DNA in the same way as described before. Once aggregates form YOYO is added to the solution (5 µM YOYO, final concentration; Invitrogen). After incubating for 20 min in the dark, images can be taken. (YOYO can be excited with the 488 nm laser).

5.3.4 Confocal imaging

All suspensions were imaged by means of an inverted microscope (DMIRB, Leica) with a confocal spinning disc scan head (CSU22, Yokogawa Electric Corp.) and a 60x water immersion objective. Fluorescence of the colloids was excited at either 488 nm or 512 nm. Emission was observed at 505 nm or above 600 nm respectively. The use of fluorescent colloids and confocal microscopy allowed us to extract colloid coordinates and to reconstruct 3D images [2].
5.4 Acknowledgements

I would like to thank W. Threels for his help with measuring the zeta-potential of the λ-DNA coated colloids (Wageningen University).
Figure 5.20: Stability of the polymer coating at elevated temperatures. A: Surface coverage of neutravidin colloids above a pLL-PEG-biotin surface at room temperature. B: As long as “fresh” solutions of the polymer are used, elevated temperatures do not disrupt the surface coating. C: With older solutions of the polymer, elevated temperatures can disrupt the surface coverage. D: Image of a sample that has been at 70 °C for 20 hrs. Clearly, almost half of the colloids previously bound are now unbound. E: Addition of a fresh batch of neutravidin colloids fills up the “holes” in the sample. F: figure D and E combined to show complete surface coverage is realized once more. G: same as D, but different sample slot. H: Addition of a fresh batch of carboxylated (COOH) colloids fills up the “holes” in the sample. I: figure G and H combined to show complete surface coverage is realized once more.