DNA-driven assembly of micron-sized colloids
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Summary

In the natural world, DNA provides a kind of blueprint that directs a complex molecular dance which culminates in the creation of a much larger, more complex object be it a bacterium or an elephant. In the material world however, this molecule proofs very useful in constructing larger assemblies. This assembly method relies on the attractive forces between complementary bases of two single DNA strands.

Designing new materials
A monodisperse colloidal system with non-specific interactions will typically crystallize into a closed-packed structure or body-centered cubic (bcc) lattice. Attractive specific forces, on the other hand, could potentially drive colloidal crystallization at much lower volume fractions through recognition-mediated assembly. In addition, specific forces can potentially enable us to construct unique colloidal structures that are otherwise entropically unfavorable. An example of a molecule that can provide a colloidal system with specific attractive forces is DNA. Indeed, theoretical work by Tkachenko predicted numerous crystal structures for a system of equal sized colloids coated with DNA [9]. The predicted structures range from a simple cubic lattice to the more complex diamond structure. The prediction of a self-assembled diamond lattice is especially exciting, because of its potential as a photonic crystal. That is why this system shows great promise in obtaining new materials.

The DNA-zipper
Not only is DNA the carrier of the genetic code - it also turns out to be a beautiful building material due to its intrinsic properties. Chemically, DNA is composed of building-blocks called nucleotides consisting of a sugar molecule, a phosphate group, and one of the four bases - adenine (A), thymine (T), guanine (G), and cytosine (C). The phosphates and the sugars of adjacent nucleotides are linked together through covalent bonds, forming a long linear polymer. Together, two of such polymer chains form a twisted upright ladder due to the formation of hydrogen bonds between the bases. The rungs of the ladder can only be formed by complementary bases (A with T and C with G). This process - also called hybridization - is highly selective: a chain of bases will bind strongly to its complementary partner, but not (or scarcely) to any other DNA chain.

Hydrogen bonds are not a covalent bonds, this makes them much weaker. Therefore the formation of a bond between two single DNA strands can be made undone by increasing the temperature. At elevated temperatures the hydrogen bonds are not strong enough to keep the two chains together. The number of hydrogen bonds between two DNA strands determines at what temperature the double DNA helix dissociates into two single strands.
DNA as a building material
The ability of single DNA strands to hybridize and thus form a double-stranded DNA structure can be used for the design of complex structures. By decorating building-blocks with pieces of single-stranded DNA, one can direct building-block 1 to bind to building-block 2, but not to building-block 3. In this manner, one can link different building-blocks in a directed fashion by utilizing single DNA strands as glue.

Typical examples of building-blocks are colloidal spheres that range in size from a few nanometers up to several micrometers in diameter. By mixing two solutions containing DNA-coated colloids with complementary sequences, aggregates will form due to hybridization between the two complementary DNA strands. As, besides mixing, no further measures are needed to obtain aggregates, this process is called “self-assembly”. If one is not content with the obtained aggregate, the solution can be heated in order to redisperse the colloids. Subsequent cooling leads to a new hybridization process, which could lead to a different (better) cluster.

Applications
This research-area is still in its early stages. Eventually the goal is to obtain artificial material that can repeatedly copy itself. The ability to create new materials in this way could provide new routes to building regular structures like those used in microelectronics. Or it could produce entirely new materials, such as photonic crystals that control the movement of light through them in a way akin to how electricity flows through a semiconductor. An application that is already utilized is the use of DNA-coated colloids as bio-sensors. Several diseases that are caused by DNA mutations can be diagnosed with these sensors. This technique is very sufficient, with sensors sensitive enough to detect concentrations as low as a few molecules.

This thesis
The subject of my dissertation is DNA-driven assembly of micron-sized colloids. As mentioned above colloids are small spheres. I mostly use spheres with a diameter that is one thousands of a millimeter (micron). They are made of plastic (polystyrene). As the spheres are very small, one can not distinguish them individually by eye. Though, with the help of a microscope they can be seen. By coating these colloids with DNA, I was able to assemble monomers into more complex structures. By using colloids with a specific protein-coating (neutravidin), coating them with DNA is simple. By modifying one end of the DNA strands with a molecule (biotin) that strongly binds to the proteins on the colloids, mixing of DNA and colloids is enough. This process together with some properties of the DNA are described in chapter 1.

In chapter 2 a short overview of available literature is given. Here, nano- and micron-sized colloids are discussed. Different coating procedures are mentioned as well as important parameters to control the assembly. Finally, some applications, as the ones briefly mentioned above, are explained in more detail.

Starting from chapter 3, I discuss the experiments I performed during my PhD-research. We started with coating colloids with a very long DNA chain. Of this strand only the very last 12 bases are single strand and thus “sticky”. The reasoning behind this was to introduce a linker as well as a spacer with one molecule. As the spacer was
of similar size as the colloids, some space between adjacent colloids was expected. The results of chapter 3 proof this basic assumption wrong. Aggregates obtained with this type of colloids are finite in size and colloids are closely packed. As colloids are closely packed, the DNA is expelled to the outer sides of the cluster. Because the DNA I used is very bulky and carries a net negative charge, it acts as a repulsive barrier, preventing further growth of the aggregates.

While finite size building-blocks can be useful, we next tried to improve the size of the clusters obtained. In chapter 4 it is shown that reducing the length of the DNA spacer, while keeping the sticky-end the same, leads to percolating clusters. A good example of this is shown in figure 4.3. Both the clusters obtained with the very long DNA as the ones formed with DNA of intermediate length were unable to redisperse at temperatures well above the melting temperature of the DNA (i.e. the temperature where a 12 base double stranded DNA strand will dissociate). Reducing the spacer length drastically, leaving only the sticky overhangs on the colloid surface, resulted in a temperature reversible system. Preliminary conclusions suggested that complete covering of all neutravidin on the colloidal surface might be responsible for this.

More research indicated that this assumption was not completely true (appendix A). It turns out that micron-sized colloids coated with long and flexible DNA strand are so closely packed within aggregates that after assembly, colloids are held together by van der Waals forces instead of DNA bridges. Hence the lack of redispersal of the colloids at elevated temperatures.

In chapter 5 a different approach is chosen. Instead of a binary system of two types of colloids in solution, now only one type of colloid is allowed to assemble above a surface coated with complementary DNA. This type of assembly resulted into two-dimensional structured aggregates. As the DNA keeps these crystals from direct contact with the surface, we named them “flying carpets”.

Besides the most common DNA structure (the double helix), also more exotic structures are possible. One of these structures is a 4-way junction (also called a Holiday junction) made of four DNA strands. With this structure, I would like to link four colloids together, obtaining well defined, finite sized clusters. In chapter 6 I describe the steps already taken to obtain these aggregates. Formation of the Holiday junction is realized as well as increasing the length of the construct to make it suitable for micron-sized colloids. Even though the first trial experiments look promising, the yield of this reaction is still very low.

The work described in this thesis is part of fundamental research, increasing the knowledge of DNA as a building material. Self-assembly becomes more and more important as there is a constant demand for smaller and smaller machinery and building-parts. To be able to utilize DNA in its full extent, the molecule and all its properties need to be understood extremely well.