DNA-driven assembly of micron-sized colloids

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DNA-coated colloids constitute a completely new class of materials. Due to extraordinary control over the interactions between these colloids, in theory, completely new thermodynamically stable phases can be obtained, that cannot be realized with normal colloidal spheres that have a less specific interaction. In this chapter I describe the route taken towards the first experimental realizations of one-, two- and three-dimensional crystals and clusters. I also briefly discuss potential applications of these new materials.

2.1 Introduction

DNA is the basic building block of life. As soon as the double-helix structure of DNA was discovered, the specific pairing of bases was suggested to be at the root of a possible mechanism to copy genetic information [10]. Indeed, hereditary information is encoded in the chemical language of DNA and reproduced in all cells of living organisms. The double helical structure is built up by two linear chains of four bases (adenine, cytosine, guanine and thymine) that are held together by a backbone of sugar-phosphate molecules. One sugar-phosphate group together with one base is called a nucleotide. Specific pairing of the Watson-Crick bases stack the nucleotides into a helical structure. The pairing of two single strands of DNA (ssDNA) into a double strand (dsDNA) via hydrogen bonds is called hybridization.

The simplicity of the ssDNA combined with the endless possible strand compositions, inspired the synthesis of artificial DNA strands. The ability to synthesize virtually any DNA sequence by automated methods [11] was realized in the mid eighties. As soon as DNA became readily available, its utility for the preparation of new biomaterials and nano-fabrication methods was soon recognized. The power of DNA as a molecular tool was enhanced even more when the ability to amplify any DNA sequence from nanomolar concentrations to macroscopic quantities by polymerase chain reaction (PCR) was discovered [12].

In the early nineties the first reports on using DNA as a building block, to create new materials emerged. Among the pioneers in this field of research were Seeman and coworkers [13–16]. They started by using branched DNA molecules to form specific motifs (small building units with specific sticky ends) that can assemble further [14]. Later more elaborate structures were realized, like cubes [15] or octahedra [16]. As DNA hybridization is a reversible process, solutions were found to lock structures once they were
Figure 2.1: DNA-Driven assembly of colloids. Two types of ssDNA coated colloids with non-complementary ssDNA A or B are mixed together with the complementary A’B’-DNA linker. Assemblies arise below the dissociation or melting temperature ($T_{m}$) of the DNA. As only hydrogen bonds are responsible for the binding, the process can be reversed. Above $T_{m}$, the colloids redisperse to their monomeric state.

synthesized [17]. At that time the theory of producing DNA structures was well ahead of experimental confirmation. It was much easier to design a structure on the computer screen, than it was to synthesize it. Fortunately, through the years experiments caught up with theory, with the experimental realization of more and more exotic structures [18–25]. To go into detail for all structures realized (ranging from geometrical objects to smileys) goes beyond the scope of this thesis. Instead we would like to focus on research in another direction, directly inspired by the realization of synthesizing basic shapes utilizing DNA hybridization.

Even before the time that the use of DNA as a building material emerged, the study of nanoparticle-based systems had evolved into a large (and growing) field of research [26]. These particles exhibit properties that are between those of a macroscopic solid or bulk material and those of a single molecule. The development of high-resolution imaging tools (e.g. transmission electron microscopy (TEM) and atomic force microscopy (AFM)) paved the way to the design of new states of matter from nano-scaled particle assemblies. To realize this, nanoparticles need to be organized in one, two or three dimensions, so that the electronic and optical coupling between the particles can be studied.

Initially, assemblies were mainly formed through covalent linkage of linear dithiols [27]. The disadvantages of this method are that the assemblies are formed irreversibly and the binding-process is difficult to control. Other attempts to assemble metal nanoparticles in well-defined structures include Langmuir-Blodgett techniques [28], molecular self-assembly by ligands [29] and electrostatic coupling to the deposition at pre-structured surfaces [30]. However, these methods suffer from a lack of binding specificity.

In contrast, biomolecules such as proteins and nucleic acids have almost perfect binding properties and biochemical functionality that have been optimized over billions of years of evolution. Self-assembly was already realized with systems of pure DNA.
Figure 2.2: DNA-driven assembly can either be initiated by the addition of a linker DNA (3-strand system) or colloids can be coated with fully or partially complementary ssDNA directly (2-strand system).

In 1996 two important articles addressed the strength of DNA as specific glue between nanocolloids. In one of them it was first shown by Mirkin and coworkers that DNA oligonucleotides could be attached to nanoparticles to direct the formation of larger assemblies [3]. For this, the authors made use of a three strand system in which particles coated with ssDNA could only bind after introduction of a linker ssDNA complementary to the two particles types in solution (Figure 2.1). As hydrogen bonds between the complementary base pairs are responsible for the aggregation, increasing the temperature can reverse the process. The work of Mirkin and coworkers focused on the use of ssDNA coated colloids to detect specific linker sequences [3]. However, if the objective is to make specific colloidal assemblies, the use of a linker is not essential. By designing sequences on both species of colloids, that are partially or fully complementary a DNA linker is no longer needed, making it a two-strand system. Figure 2.2 shows both options. The prospect of designing three-dimensional (3D) structures that would self-assemble in a controlled and reversible manner marked DNA as a potential tool in designed self-assembly. Since then, the double-stranded helical structure of the DNA is key to its use in self-assembly applications, through exploiting its storage capacity to program self-assembly on a molecular scale.

Another article on DNA and nanoparticles appeared in the same issue of Nature. There, Alivisatos et al. [31] showed that the distance between particles can be manipulated by the sequence of DNA used. This is due to the long persistence length ($\sim 50$ nm) of DNA, that allows the molecule to behave as a rigid rod at short distances. As nature provided us with a wide variety of specific enzymes that allow the processing of the DNA material with atomic precision and accuracy, an endless variety of strands can be produced that specifically recognize their complementary counterparts. No other (polymeric) material offers these advantages, which are ideal for molecular construction in the range from about 5 nm up to the micrometer scale [19]. Hence, DNA holds the promise of allowing a controlled bottom-up self-assembly of complex nano-devices. Because DNA is widely available at low costs, this molecule promises to be the material of choice in future nano-fabrication.

Since theoretical work indicated that crystal structure formation is possible by the use of a binary mixture of DNA-coated colloids [9], several research groups set their
goal towards obtaining these structures experimentally. Unfortunately, most (if not all) pioneering research of DNA-driven assembly of nanoparticles, mainly resulted in random aggregation, with modest control over the placement of the particles within the assembled material and the distance between them. That is, much of the materials obtained were best classified as amorphous clusters. One disadvantage of working with nanoparticles is that detailed structural characterization of all these systems is limited to TEM of dried samples. This is a significant issue, as drying effects may be responsible for the observed particle consolidation or aggregation [32, 33]. To circumvent this, a few groups shifted their focus to assemblies of micron-sized colloids, which can be characterized through optical and confocal microscopy [5, 6]. Micron-sized colloids were also used more and more, as lattice-spacings comparable to the wavelength of light are required to achieve photonic crystals in the visible range. Even though the possibility of forming crystal structures was theoretically predicted in the beginning of this century, it took some time to realize the first experimental crystal structures. In 2005 Crocker and coworkers [8] were able to obtain the first micron-sized (hexagonal) crystal structures. While the formed structures were crystalline, it only proved possible to grow relatively small crystallites. In the beginning of 2008 the first experimental nano-crystals with body-centered cubic (bcc) and face-centered cubic (fcc) symmetry were made [34, 35]. However, many of the other structures still remain illusive [9]. Apparently, DNA driven assembly is dependent on and controlled by many factors. To obtain a crystal, optimal conditions have to be realized.

Here we give an overview of the journey taken from the early work of Mirkin and coworkers on DNA-coated colloids as “detectors” of specific DNA sequences towards the first realizations of three dimensional crystal structures. A popular saying reminds us of the fact that sometimes the journey is more important than the (preset) goal. This could not have been truer in the case of DNA-coated colloids. As we take you along our journey, assemblies with various dimensionalities will be discussed, as will be the parameters that affect DNA assembly of colloids, to convince you of the enormous potential of this topic.

2.2 DNA-coated colloids

In the available literature, numerous ways to functionalize colloids with nucleic acids are described. In general, the connection between the colloid and the probe DNA consists of a binding group, a spacer and a piece of single-stranded probe-DNA. The spacer is needed to give the probe some freedom to explore its surroundings. Usually the spacer is just an additional stretch of DNA, either single or double stranded. The binding group depends on the type of colloid used. In principle any molecule can be used to bind the DNA to the colloid as long as the strength of this bond is much larger than the subsequent probe target binding. Below we will describe some of the most commonly used techniques to graft nucleic acids to colloids.
2.2 DNA-coated colloids

2.2.1 Synthesis of DNA functionalized nanoparticles

The solid-phase synthesis of DNA oligomers is nowadays routine technology, and DNA sequences up to 140 nucleotides in length, modified with a wide variety of chemical labels (such as amino or thiol groups) attached to the 5’- or the 3’-end, are readily (and commercially) available. These relatively short synthesized DNA strands are typically used in combination with nanoparticles, ranging in size from 3 to 30 nm (diameter). Most protocols nowadays use the same method as described in the early work by Mirkin and coworkers [3]. Briefly, metallic (mainly gold, but silver is also used [36]) nanoparticles are mixed with oligonucleotides functionalized with an alkylthiol group on one end. During incubation the thiol group will bind to the gold surface (Au–S binding). Unbound DNA is removed by centrifugation and subsequent removal of the supernatant. The resulting DNA-coated particles are water soluble and stable for months. With the use of magnetic microparticles (for magnetic separation) as templates, gold-Janus nanoparticles can also be obtained with two different lengths of DNA [37] with the longest length mainly present on one side of the particle (the longer strand is present (after ligation) on the side that was docked to the template microparticle with a linker molecule).

Although this preparation method can be used every time, some factors have to be taken into account. The ssDNA probe has some affinity to the metallic (mainly gold) surface of the nanoparticles. Besides binding specifically by means of the thiol binding group also non-specific binding is possible. Both experimental and theoretical researchers [38–40] attribute this type of binding to the amines in the purine or pyrimidine rings of the nucleotides. As non-specific adsorption complicates the control and interpretation of experimental studies (fraction of active probe unknown), this type of binding needs to be prevented as much as possible. One way to reduce the amount of non-specific bound ssDNA is to treat the samples with mercaptohexanol after the adsorption step. Herne and Tarlov [41] came to this conclusion after comparing the adsorption of thiol functionalized ssDNA versus untreated ssDNA by X-ray photo-electroscopy. Choosing the sequence of the ssDNA spacer wisely also reduces the chance of high amounts of non-specific bound ssDNA strands. The Mirkin research group [42] tested the binding
affinity of the four deoxynucleotides (dA, dT, dC and dG) to gold surfaces. Using a colorimetric assay they found that deoxynucleotide dT has by far the lowest non-specific binding affinity. As the free amines of the nucleotides are held responsible for the non-specific attraction, this finding can easily be understood as dT contains only one free amine, whereas the other nucleotides contain more. Thus, DNA spacers high in dT minimize non-specific adsorption. Another simple technique to reduce the fraction of non-specifically bound ssDNA is to increase the grafting density (Figure 2.3), as non-specifically anchored chains occupy a larger surface area. Adsorption of an excess of thiol-functionalized ssDNA at high salt concentrations facilitates an increase in grafting density [43], as the salt screens the negative charge along the DNA backbone, allowing them to come closer to each other.

For many applications it is essential to be able to quantify the coverage of the DNA on the particles surface. To achieve this, a fluorescence based assay [43] or a PCR method [44] can be used. DNA coated nanoparticles with low surface density can even be purified by gel electrophoresis, separating particles with one to six DNA strands from each other [45–47]. The ability to isolate nanoparticles, with low and well-defined grafting densities is important, because in the low grafting-density regime the number of DNA strands per particle, significantly affects how the particles interact. Instead of binding in every direction, as particles with a high grafting density, at low grafting densities only specific angles are open for binding. The effective “chemical valence” of a DNA-coated colloid (i.e. the number of ssDNA’s available for linking to other colloids) is very important, as the valence controls the width of the gas-liquid coexistence region [48–51].

Niemeyer and coworkers reported the synthesis of “oligofunctional” DNA particles [52]. These particles had a number of different strands ranging from two up to seven. With these particles the specificity of DNA can be optimally used, as multiple binding possibilities are present.

### 2.2.2 Synthesis of DNA functionalized micron-sized colloids

To obtain DNA-functionalized micron-sized colloids with short pieces of DNA, the same commercial strands can be used as described above. To obtain colloids grafted to longer DNA strands, plasmid DNA (obtained from bacteria) provides a good alternative [53, 54]. In general three different grafting methods are used to attach the DNA to the colloids: avidin-biotin linking, water-soluble carbodiimide linking or a polymer swelling/deswelling method.

#### Avidin-biotin chemistry

The biotin-avidin system is the strongest non-covalent biological interaction known, having a dissociation constant, $K_d$, in the order of $4 \times 10^{-14} \text{M}$ [55]. The bond forms very rapidly and is stable over a wide range of pHs and temperatures [56, 57]. The strength and the specificity of this interaction led it to be a favorite choice to graft DNA to colloidal particles especially since polystyrene colloids, coated with neutravidin (a special form of avidin) are commercially available. The neutravidin protein differs from avidin and streptavidin in that it has been specially processed to remove carbohydrates
and to lower the isoelectric point, resulting in a near-neutral protein that has significantly lower nonspecific binding than conventional avidin. The grafting method is very similar to the one used for coating nanoparticles. Neutravidin coated colloids are mixed with a solution of biotin-labeled DNA. During incubation, biotin will bind to the avidin of the colloids. Unbound DNA is removed by centrifugation and subsequent removal of the supernatant. Additionally, the DNA-colloid solution is heated to 50 °C to remove non-specifically bound biotin.

Water-soluble carbodiimide chemistry

As stable as the biotin-avidin link is, it remains a non-covalent bond. At high temperatures in non-ionic aqueous solutions, the biotin-avidin interaction can be reversibly broken [58]. Although experiments with DNA-coated colloids are never performed in nonionic solutions, some researchers nevertheless prefer a covalent link to a non-covalent one.

A convenient way to covalently link DNA to colloids is by use of water-soluble carbodiimide chemistry. This involves a two-step mechanism. First, carboxyl-functionalized colloids are activated by adding 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). The EDC facilitates the formation of amide linkage between the carboxylate group and the amine group without leaving a spacer molecule, by transforming the carboxyl-group to a nucleophile reactive intermediate [7]. Second, amino-labeled DNA is added to the reaction medium. This molecule attaches to the spheres by an amide bond. As an amide linkage has a much smaller molecular footprint than bulky proteins like neutravidin, a higher surface density of DNA can be achieved.

Despite the apparent simplicity of this method, covalent linkage is technically more difficult and time-consuming than protein binding. More reaction steps are required and often colloids aggregate into small clusters before DNA strands are added.

Swelling/deswelling-based chemistry with PEG modification.

As the covalent coupling method described above can be pre-empted by colloid instability in commonly used buffers and as the use of protein-free systems can be advantageous, a third method of obtaining DNA-grafted colloids was introduced [59]. This method was directly inspired by polymer-protein modifications to improve protein stability [60]. To obtain colloids grafted with DNA, but stable over a wide variety of conditions, triblock-polymer are used to attach the DNA to the colloids. Briefly, triblock copolymers are adsorbed onto the surface of the colloids. Then a small amount of organic solvent is added to swell the colloids. By “liquefying” the colloid, allowing the hydrophobic block of the polymer to penetrate the surface [61], anchoring is possible after deswelling (remove organic solvent by heating). The exposed hydrophilic blocks of the triblock copolymer can readily be labeled with DNA.

The main advantage of this preparation method is that subsequent stabilization by surfactants or polymers is not needed. An assembly of DNA-grafted micron-sized colloids cannot as simply be redispersed by thermal denaturation as an equivalent system of nanoparticles. Obviously, size is a parameter that affects the interactions between
colloids. In particular, strong Van der Waals interactions can lead to irreversible, non-specific binding. Crocker and coworkers [6] gave an overview of interactions that are important in micron-sized systems. Between DNA coated colloids there is an attractive part, $U_{dna}$, due to inter-particle DNA hybridization, a steric and/or electrostatic repulsive part, $U_{rep}$, and a strong Van der Waals interaction, $U_{vdw}$, at short distances. Control over aggregation and temperature reversibility requires a subtle balance between $U_{dna}$ and $U_{rep}$. For micron-sized colloids, two strategies may be adopted to obtain aggregation reversibility: decreasing $U_{dna}$ by decreasing the surface density of probe-DNA or increasing $U_{rep}$. The latter approach can be achieved by adsorbing polymer stabilizers onto the colloids.

2.3 Phase behavior of DNA-coated colloids

The properties of materials formed by the self-assembly of DNA-coated colloids are affected by a number of parameters. The ones judged most important are: the number of base-pair matches, which tune the strength of the attraction between colloids; the grafting density of the DNA strands to tune the strength of $U_{dna}$; the ionic strength of the surrounding medium, which shields the repulsion between negatively charged ssDNA strands and the length of possible spacers connecting the “reactive” single-stranded DNA (ssDNA) to the colloids. Below we give a brief overview on the effects these parameters have on the phase behavior of DNA-coated colloids.
2.3.1 The number of base-pair matches and spacer length

The strength of the attraction between colloids can be tuned by the number and type of base-pair matches between colloids coated with complementary ssDNA strands. The Watson-Crick base pairs are not of equal strength. The three hydrogen bonds between a G-C base pair impart greater stability than the two of an A-T base pair. Thus, increasing the G-C content raises the melting temperature at which complementary strands dissociate. The strength of the bond between a pair of nucleotides is also influenced by the chemical nature of the neighboring base pairs. For instance an A-T surrounded by G-C pairs has an enhanced stability. Predicting the DNA duplex stability from the base sequence is possible [63].

Increasing the number of base matches also raises the melting temperature. Crocker et al. [5] found in 2003 that lowering the number of matching base pairs leads to a decrease in melting temperature (Figure 2.4b). Later, other researchers came to the same conclusion [62, 64]. Thorough research on the effect of the number of base pairs on the melting temperature, showed two interesting results. First, linker sequences (3-strand system) with an uneven number of base-pair matches have a slightly lower melting temperature than a linker with one base less would have. DNA free in solution does not display this behavior [64]. It is believed that in DNA-linked nanoparticle assemblies, duplexes with different lengths introduce binding energy disorder and, therefore, slightly lower the stability of the system.

Second, the length of spacer in between the colloid and the probe also influences the melting temperature. Increasing the spacing between adjacent colloids (by increasing the spacer length), leads to higher melting temperatures of the assemblies [65].

Above, one difference between DNA free in solution or grafted to colloids is mentioned. There is another, very important one: the melting curve associated with aggregation of colloids is narrower than the melting curves of DNA without nanoparticles (Figure 2.4a) [65, 66]. While all research groups report comparable melting behavior, different physical mechanisms are held responsible for it. Mirkin and coworkers [4] claim that inter-particle bridges influence each other. The breaking of bridges lowers the local salt concentration, which leads to destabilization of neighboring bridges. This cooperativity is translated into a narrowing of the melting curves. Lukatsky and Frenkel [67] consider the influence of local salt concentrations as less important. Instead their theory stresses the importance of entropic effects on the sharpness of the melting curve in DNA-coated colloid systems with multiple bonds.

2.3.2 Probe-DNA density on the colloidal surface

Specific interactions are obtained through DNA hybridization. With most types of binding, the strength of binding increases with the number of bonds made. The same is true for DNA-coated colloids. With an optical tweezers set-up the force of interaction between two DNA coated colloids can be measured directly [68]. Different DNA densities give different strengths of interaction. So by playing with the DNA density a preferred interaction can be found. As a high DNA surface density on the colloid is advantageous in terms of particle stabilization, especially for (gold) nanoparticles at elevated salt con-
centrations [3]), colloids are usually coated with a combination of binding (probe/linker) and non-binding DNA strands (neutral; Figure 2.5). Biancaniello et al. [69] examined the aggregation behavior of micron-sized colloids for different percentages of hybridizing strands. They found that the affinity between complementary DNA strands in a single duplex is too weak to promote adhesion between colloids of this size. Increasing the probe-DNA concentration 10-fold led to an onset of aggregation, but in their system at least 33% of all DNA on a colloid must be probe-DNA to obtain a system in which all colloids are hybridized. Besides controlling the sizes of obtained aggregates by varying the percentage of probe-DNA [70], also the melting temperature is directionally proportional to the probe-DNA surface density (for short DNA tethers) when the colloid and target concentrations are kept constant. Lowering the density leads to a decrease in melting temperature and a slight broadening of the melting transition [4, 62]. Valignat et al. [6] used a different approach to reduce the number of hybridizing events between two neighboring colloids. They coated their colloids with DNA and an inert polymer. By increasing the length of the stabilizing polymers, colloids were held further apart, thereby reducing the number of possible links. Besides polymers, also dsDNA can be used as stabilizing polymers. With the help of restriction enzymes, appropriate lengths can be created [71]. Again the same relation was found: the existence of fewer links reduces the melting temperature significantly. Only recently it was found that strands neighboring the hybridized bridge in between two colloids, can additionally stabilize the aggregate by non-perfect base pair matching, known as “slipping” [72]. As this type of binding is difficult to interpret, one may choose to prevent them from happening. Diluting strands can help to prevent this type of binding.

2.3.3 Ionic strength in surrounding medium

As the backbone of DNA consists of negatively charged sugar-phosphate groups, ssDNA has a net negative charge. To allow close proximity necessary for duplex formation, counter-ions are needed. Adding some salt to the medium is a simple method to increase...
the affinity between complementary strands and to prevent later helix dissociation. Already in one of the first studies on micron-sized DNA-coated colloids [5] it was discovered that colloids aggregate faster if the salt concentration was increased. In principle one can speak of two distinct regimes of aggregation kinetics. At low salt concentrations aggregation is shown to be dependent on the rate of duplex formation between colloids, i.e. reaction limited. At higher salt concentrations, aggregation behaves diffusion-limited as it depends on the rate of diffusion of the colloids [73]. Biancaniello et al. [69] were the first to obtain a phase diagram for DNA-functionalized microspheres as a function of salt concentration. They found that to obtain a fully aggregated system a minimum of 100 mM NaCl was necessary. Intermediate concentrations (25-75 mM) led to a system containing small aggregates and monomers, while a homogeneous fluid phase, consisting of dispersed microspheres, was seen for salt concentrations below 20 mM. The absence of or incomplete DNA-mediated colloidal aggregation at low salt concentrations is attributed to dominant electrostatic repulsions. The appearance of larger aggregates at higher salt concentrations was also reported by Jin et al. [4], who studied (gold) nanoparticles.

Besides controlling the speed and size of aggregation, the melting temperature is also affected by an increase in salt. As the double helix is more stable in the presence of counter ions, the melting temperature increases with increasing salt concentration [4].

### 2.4 Assemblies of DNA-coated colloids

DNA-driven assembly of colloids can be classified into three main categories: One-dimensional (1D), two-dimensional (2D) and three-dimensional (3D) assembly. 1D assemblies include long chains, which lead to the formation of nanowires. With a future as nano-electronics, wires are most useful when made from metal or magnetic material. That is why this type of assembly is only performed with nanoparticles. 2D assembly of DNA coated colloids leads to sheets of particles above a surface. Last, 3D assembly leads to a range of structures, from finite to percolating and from ordered to amorphous. The latter categories are used for both nano- as micron-sized colloids.

#### 2.4.1 One-dimensional assemblies

In the same issue in which Mirkin introduced the concept of DNA assembly, Alivisatos and coworkers [31] showed that a discrete number of nanoparticles can be assembled along the backbone of DNA. They were able to align the particles with distances of 2 or 6 nm apart, depending on the direction of assembly (head-to-head or head-to-tail). These nanoparticle-DNA conjugates have a high flexibility when the backbone is nicked, while an un-nicked (backbone without a discontinuity) in the double helix significantly lowers the flexibility [74]. Though an elegant approach, it has thus far proven to be a difficult task to assemble the particles in direct contact to each other over extended domains with an inter-particle spacing that is identical and small enough to allow direct dipolar coupling or even electronic transport along the array. Noyong et al. [75] have reported the synthesis of a string-of-beads alignment of 4 nm gold nanoparticles along DNA strands. To achieve identical inter-particle spacings they make use of cisplatin, a
platinum complex with high affinity for neighboring guanine-guanine nucleotides. Cysteamine stabilized nanoparticles can then selectively bind to the platinum center by exchange of the NH$_3$-ligands with NH$_2$-groups of the ligand shell. While small particles have a greater potential to serve as building blocks in future nano-electronic devices due to their enhanced charging energy, Hutchinson and co-workers have elaborated a method to organize 1.5 nm gold nanoparticles into linear chains with precisely controlled inter-particle spacings [76]. In this configuration, the spacing between two neighboring nanoparticles is determined by the thickness of the ligand-shell and can thus be varied.

Another method to obtain one dimensional assemblies is the direct metallization of DNA. This method (the metallization by metal-ion reduction) has successfully led to the formation of highly conductive nanowires, which could be applied as metallic interconnections. The use of DNA as a template for the generation of silver nanowires [77] is depicted in Figure 2.6. The 12-16 $\mu$m gap between two microelectrodes was bridged by a nucleic acid attached with its two ends to complementary thiolated nucleic acids associated with the microelectrodes. Ion exchange of the phosphate counter ions, followed by the hydroquinone induced reduction of the ions resulted in silver nanoclusters associated with the DNA wire template. The subsequent catalytic enlargement of the Ag$^0$ nanoclusters using an acidic Ag$^+$ hydroquinone solution resulted in the formation of continuous Ag$^+$ nanowires that were ca. 100 nm wide. Related methods were applied to synthesize other metal nanowires such as platinum [78], palladium [79] or copper [80]. However, all nanoparticles formed during such processes suffer from an extraordinarily
2.4 Assemblies of DNA-coated colloids

Figure 2.7: DNA mismatch detection with DNA coated colloids compared to detection with conventional fluorophores. A: Cartoon both of the fluorophore-labeled and the colloid-labeled ssDNA binding to the complementary strands on a flat support (e.g. DNA-array). B: Adsorption intensity of dsDNA at 260 nm as function of temperature. At a given temperature the difference between the number of complementary strands hybridized to perfect ssDNA and that to one with a single mismatch is far bigger for a system with DNA coated colloids.

broad size distribution. Thus the metal structures along the DNA wires are highly disordered and none of the size-specific electronic transport properties, which are based on single-electron tunneling, could be observed or even utilized in nanodevices so far.

2.4.2 Two-dimensional assemblies

Besides arranging nanoparticles in a 1D-fashion on a DNA strand, attaching oligonucleotides to a solid support to obtain two dimensional assemblies also attracted much attention. Surface-bound ssDNA was initially used to detect specific DNA sequences or to detect single-nucleotide mutations within a DNA sequence [81, 82]. Later, DNA-coated colloids were also attached to a surface for a more material-oriented goal.

Many groups have reported the DNA-directed immobilization of gold nanoparticles to form supramolecular surface architectures. This way of assembly led to the first application of DNA coated colloids. The specific nucleic-acid mediated immobilization of gold nanoparticles can be utilized for the topographic labeling of surface bound DNA targets
This readily allows the highly sensitive scanometric detection of nucleic acids in DNA-chip analysis. Oligonucleotide array technology depends on the quantitative detection of target DNA hybridized to complementary array elements. Mirkin and coworkers [82] reported a “scanometric” method for detecting DNA targets via hybridization of 13 nm gold nanoparticles to an array functionalized with various oligonucleotides (Figure 2.7a,b). Because of the unusually sharp temperature-induced “dissociation” or melting profiles of the nanoparticles from the surface of the array, the selectivity of the scanometric DNA detection system is intrinsically higher (by a factor of 4) than that of a conventional array system based upon fluorophore probes (Figure 2.7a,b). In addition, enlarging the array bound nanoparticles by gold-promoted reduction of silver permitted the arrays to be imaged, in black and white by a flatbed optical scanner, with 100 times the sensitivity typically observed by confocal fluorescent imaging. Later, the same group found that different sizes of nanoparticles can be used to identify two target sequences in solution, due to their size-selective light scattering [84]. Besides using silver, the use of colloidal gold nanoparticles also allows a signal enhancement in the DNA hybridization detection by means of a quartz crystal microbalance [85, 86], dendritic amplification [87], angle dependent light scattering [88] and surface plasmon resonance [89]. A very sensitive method to detect target DNA is the combination of localizing nanoparticles in an electrode gap and silver deposition. This leads to readily measurable conductivity changes and is able to measure concentrations as low as 500 femtomolar with a point mutation selectivity factor of \( \sim 100,000:1 \) [90, 91]. Recently, a detection limit of 1 femtomolar was claimed by using thiolated detection-probe-conjugated nanoparticles that exhibit a high level of unblocked active sites. These sites are easily accessed by p-nitrophenol and NaBH\(_4\). Electroactive p-aminophenol is generated at these sites and is then electro-oxidized to p-quinoneimine at the electrode. The p-aminophenol redox cycling by NaBH\(_4\) offers large signal amplification [92]. DNA mismatch detection depends on (mismatched) DNA strands to dissociate at a lower temperature than the perfectly complementary strand [93].

Immobilizing nanoparticles onto a surface is used for a more material-oriented goal as well. As the “bottom-up” approach in which small molecular building blocks self-assemble to form larger devices, gave irregular shapes in three-dimensional assemblies, the two-dimensional approaches gained more attention [94, 95]. By imprinting surfaces with specific areas (complementary DNA strands) and non-specific areas, site-selective immobilization of gold nanoparticles functionalized with DNA-oligomers was realized [94–97]. Instead of these surfaces, also a DNA grid can be used to arrange nanoparticles into square periodic lattices, resulting in a uniform layer of distributed nanoparticles organized with precise control on inter-particle distance [98, 99]. By coating nanoparticles with multiple DNA strands [52], a next step towards 2D assembly for novel materials can be made. Now one of the strands can be used to immobilize particles onto a surface, while the other type of DNA strand is still available. This one can be used to establish cross links between the particles [100]. By changing the length of the DNA used, the inter-particle distance should be adjustable. Indeed, bifunctional DNA-modified gold nanoparticles at solid substrates generates particle layers with programmable interparticle spacing [101].
While working with DNA oligonucleotides grafted to a surface it is important to graft them well. Non-specific binding of neutravidin proteins to the surface in combination with biotinylated DNA strands can lead to problems as forces above 2 pN per bond can rip the neutravidin from the substrate [102]. The way of surface coating can also lead to surprising effects. A recent study on immobilizing micron-sized colloids onto a DNA-functionalized surface reported the existence of “floating” crystalline 2D membranes above the surface. In this study it seems the polymer alone was sufficient to obtain these structures [103]. Two-dimensional sheets were also formed with nanoparticles dried within a grid [104]. Also in this case, Watson-Crick base paring is not responsible for the assembly. But whereas the micron-sized colloids were closely packed, the nanoparticles are further apart, with distances depending on the DNA length used.

2.4.3 Three-dimensional assemblies

Following the initial approach of Mirkin’s group, DNA hybridization has been used to generate repetitive, three dimensionally linked nanoparticle assemblies (Figure 2.1). As there are numerous reports on nanoparticles as well as micron-sized colloids, we split the two subjects to avoid confusion.

Three-dimensional assemblies of nanosized particles

The assembly scheme of Mirkin and co-workers [3] can also be used for the generation of binary networks comprised of different particle sizes. Due to the specificity of Watson-Crick base-pairing, only heterodimeric “A-B” composites of alternating particles are formed [105]. By playing with the ratio between the two sizes used (8 and 31 nm) different aggregates can be attained, ranging from random to satellite structures (one big colloid coated with smaller ones). Ten years after realizing satellite structures, bigger particles can now also be coated on one side only, creating so called “Janus particles” [106]. While experimental efforts do not go beyond binary networks, simulations already studied the aggregation behavior of four different DNA coated particles [107]. In that case a hexagonal crystal can be formed with two alternating bands containing two of the four possible particles.

Ever since it was found that the optical properties of DNA-linked nanoparticle assemblies are governed by aggregate size, regardless of the oligonucleotide linker-length [108], efforts concentrated on controlling this size. A solution was soon found: the extent of aggregation can be controlled by the addition of ssDNA oligomers to an aggregating colloid of the gold nanocrystals modified with complementary strands of DNA. The ss-DNA oligomers rapidly form duplexes with the immobilized DNA and the aggregation process can be instantly terminated. Depending on the time of addition of the ssDNA oligomers, this enables one to limit the size of the aggregates formed [73].

The elaborate research done on the phase behavior of these particles, as described before (section 2.3), led to a broader understanding of the colloids and resulted in the first crystal structures of DNA coated nanoparticles [34, 35]. Later, a phase diagram was presented, showing the relation between the ratio of linker DNA to nanoparticles and the volume fraction of particles [109]. In addition to the reasons addressed in the former
section, also complete temperature control seemed of crucial importance. In 2001 it was already found that the thermodynamically most stable assemblies are formed upon heating of the kinetically controlled adducts [110]. Both research groups that obtained these crystal structures stress this point in their article. DNA self-assembly is always accompanied by a competition between the entropic and enthalpic contributions involved in the assembly process at different temperatures. From an entropic standpoint, a close-packed structure is favored over a non-close-packed structure, because the entropy of the entire system can be maximized if the nanoparticles possess the largest possible local free volume [111, 112]. Therefore, it is vital that particles begin to assemble near the melting temperature of the DNA. Here the binding strength is very weak and the enthalpic contribution is small. The assembly process will be dominated by the entropic contribution and thus a close-packed structure forms. However, if the particles are combined several degrees below the melting temperature, the enthalpic contribution will govern the assembly process and a non-close-packed structure forms that maximizes the number of DNA hybridization events.

Besides the most common type of DNA hybridization (double helix), also more exotic DNA structures are used to obtain DNA-driven assembly. DNA can form numerous structures depending on the strands and/or condition used. Examples include G-quadruplexes, a square arrangement of guanines (a tetrad; Figure 2.8a), stabilized by hydrogen bonding; i-motifs, based on intercalated C-C\(^+\) base pairs (Figure 2.8b) and Holliday junctions (see chapter 6), a cross-structure of four DNA strands. Two different research groups used “G-quadruplexes” rather than the double helix to aggregate particles [113, 114]. This DNA-structure depends on the concentration of a cation like Na\(^+\) or (to a lesser extent) K\(^+\) in solution. The “i-motif” was used to self-assemble DNA coated colloids as well [115, 116]. With this DNA structure the pH is responsible for switching between the bound and unbound state, instead of the temperature. See-

man and co-workers [117] used a Holliday structure in combination with nanoparticles to estimate the structural integrity of a combination of 3 to 4 junctions, realizing the first quartet of particles stringed together. Recently, DNA tubules decorated with gold nanoparticles were obtained by closing up 2D DNA tiles [118].

Three-Dimensional Assemblies of micron-sized colloids

Seven years after the initial DNA-driven assembly experiment of nanoparticles, assemblies of micron-sized colloids were realized as well. Before the first crystal structure was obtained experimentally, Ratna et al. [119] already looked ahead. As spherical colloids can only make “simple” cubic structures, they asked themselves whether one can build complex super lattices by first building a mesoscale sub cell [120]. By using specific diameter ratios between the colloids used (0.23 and 0.42) they were able to construct either a tetrahedron or an octahedron [119]. Both were found, but with an estimated yield of 20% also larger aggregated assemblies were present. While Ratna et al. [119] needed different sized colloids, simulations suggests that equal-sized colloids can also aggregate into geometrical objects [121]. The second study on micron-sized DNA coated colloids [5] was not based on creating finite sized clusters. By mixing two different sizes of colloids only heterodimeric “A-B” composites of alternating colloids are formed. The
clusters obtained were strand-like aggregates that were temperature stable due to reasons discussed earlier. Two years later, the first temperature reversible systems were reported [6, 7]. As creating a system that is reversible with temperature proved more difficult for micron-sized colloids than for nanoparticles, other techniques were explored to obtain a reversible system. For example, Tison and Milam [122] used competing ssDNA strands to break up assemblies of DNA coated colloids.

Due to the difficulties to obtain appropriate temperature control, it came as a surprise that crystals of micron-sized colloids were experimentally realized before obtaining one made from nanoparticles [8, 123, 124]. Crocker and coworkers were able to grow small hexagonally stacked crystals of micron-sized DNA coated colloids. They proposed that besides temperature response also the surface of the colloids used was of vital importance [123]. The creation of these crystals by Crocker and coworkers was a tour de force as most systems of micron-sized DNA-coated colloids get stuck in a non-ordered structures [6, 7, 53, 54, 69]. In fact, most of these disordered structures are not compact but gel-like with a “fractal” structure. Even though order within a gel-like structure is possible [125], gel structures obtained with DNA coated colloids are not ordered. Finite-sized systems have also been realized [53]. It appears that if the length of the polymeric dsDNA spacer is too long (comparable to the size of the colloids), all “sticky ends” of one colloid can bind to those of a neighboring one, forming small clusters. In the experiment the finite clusters were of different size, but simulations suggests that a low density solution containing only dimers can be realized provided that all DNA-coated colloids have the same valency [126].
2.5 Applications of DNA-coated colloids

At present, most practical applications of DNA-coated colloids are related to detection of small molecules or DNA sequences. Above we mentioned the ability to detect DNA on a surface (two-dimensional assembly), but DNA detection is also possible in the bulk (three-dimensional). Methods range from detecting clusters obtained with different target DNA, taking the inter-particle distance into account [66] to nanoparticles coated with DNA end-capped by a fluorophore. If single-stranded, the DNA lies on the particle surface, quenching the fluorophore. Upon binding of a target DNA, the molecule stretches, freeing the fluorophore from the surface. This restores the fluorescence [127].

Besides detecting DNA, also some metal ions can be detected. Examples are Pb$^{2+}$ [128] and Hg$^{2+}$ [129]. The detection methods for both metal ions differ. Pb$^{2+}$ detection depends on clustering nanoparticles with DNA containing one RNA nucleotide (Figure 2.9a). The mixture is completed by a DNAzyme, whose activity depends on the presence of Pb$^{2+}$. The metal ion is thus detected by the destruction of nanoparticle clusters. Concentrations of $\mu$M could be detected [128]. In contrast, Hg$^{2+}$-ions are detected by assisting in DNA release from nanoparticles. When Hg$^{2+}$-ions interact with the thymidine units of the DNA molecules bound to the nanoparticles, the conformations of these DNA derivatives change from a linear to a hairpin structure. This causes the release of some of the DNA molecules from the surface of the nanoparticles into the bulk solution. There they can be detected with the help of OliGreen, a fluorescent probe that binds to ssDNA but not to dsDNA. The fluorescence of OliGreen-DNA complexes increased with increasing concentration of Hg$^{2+}$, and Hg$^{2+}$ could be detected at concentrations as low as 25 nM [129].

The optical monitoring of bio-recognition processes through the separation of DNA-nanoparticle clusters can also be used for the detection of cocaine [130]. One longer strand of DNA is designed not only to link nanoparticles, but also to wrap around cocaine. Due to this wrapping the link between the nanoparticles is destroyed and the assembly falls apart (Figure 2.9b). Again concentrations of $\mu$M could be detected [130].

As a last example of biosensing with DNA-coated particles, we briefly discuss the assay of telomerase. Telomerase is a ribonucleoprotein that elongates the chromosomal telomere units and transforms them into immortal cells. Thus, telomerase is a versatile marker for cancer cells [131]. In a DNA-nanoparticle based assay of telomerase, CdSe/ZnS core shell quantum dots are modified with thiolated nucleic acid 17 that is recognized by telomerase. In the presence of a nucleotide mixture (dNTPs) and the Texas-Red-labeled dUTP together with telomerase extracted from HeLa cancer cells, telomerization of the nucleic acid 17 was initiated in a similar path that proceeds on the ends of the chromosomes [132]. The telomerization results in the incorporation of the Texas-Red units into the telomeres, and the activation of the fluorescence resonance energy transfer (FRET) from the quantum dots to the dye labels (Figure 2.9c). The fluorescence generated by the dye is then used to follow the activity of the telomerase.

Another field of application of DNA-coated (nano) particles is DNA or RNA delivery into cells. It is important to note that in this case the DNA is not permanently grafted to the colloid, rather it is physisorbed so directed delivery is possible. Gold particles are
2.5 Applications of DNA-coated colloids

Figure 2.9: Applications of DNA-coated colloids. A: With the use of DNA-coated colloids and a so-called DNAzyme, lead-ions can be detected. B: Also cocaine is a substance that can be detected utilizing DNA-coated colloids. C: Besides detecting substances, DNA-coated colloids can also be used to determine the activity of the enzyme telomerase (All three examples are discussed in more detail in the text).
well-established carriers for the delivery of dsDNA in the so-called gene-gun technology [133]. Gene-gun technology uses DNA (or RNA [134]) that adheres to biologically inert gold nanoparticles. By accelerating this DNA-particle complex in a partial vacuum and placing the target tissue within the acceleration path, DNA is effectively introduced into the cell. An advantage of this technique is that cell removal from the tissue is not necessary. The particles penetrate the cells and release the DNA. Because cells are not removed from the tissue, cell sampling for gene gun bombardment should focus on the superficial cells [135]. As gold is not biodegradable, adverse side effects could arise when particles get accumulated [136]. With that fact in mind, also biodegradable alternatives, like chitosan particles, are examined [137, 138].

2.6 Outlook

When Mirkin and co-workers [3] and the group of Alivisatos reported DNA-driven assembly of nanoparticles, many researchers saw the potential of this new class of material. During the past 13 years, progress has been impressive - primarily in the area of detection of biologically relevant bio-molecules. However, there has also been progress in the “materials science” of DNA-coated colloids. Examples are the successful engineering of finite clusters of DNA-coated colloids [119] and the creation of the first crystals of such building blocks [34, 35, 123]. In the present chapter, I presented a brief review of the studies that have been reported that focused on the physics of DNA-based self-assembly. However, realizing the full potential of these new materials certainly still requires much more research. The crystals formed thus far are roughly 90% water, due to the length of spacer used [139]. This makes them too fragile for direct use. Also, as slow annealing appears vital to obtain order within the assemblies, forming DNA-linked crystals is a time consuming business. A solution to speed up the process has been found for nanoparticles [140]. Assemblies can be directed to form on a platform with an electric field. Crystals up to 20 layers can be built this way from a solution of low concentration.

Even though the quest for new materials is still on, DNA-coated colloids already proved useful in other areas as well. Examples include biosensors [81–86, 128–130] or as delivery capsules for therapeutic agents [133]. In time, combining all knowledge comprising theoretical models, simulations and experiments should lead to even more sophisticated self-assembled materials. As with the assembly of pure DNA systems before, the design is ahead of experimental realization, but as experiments with DNA are quickly catching up with more and more elaborate structures [141], it seems safe to speculate that many amazing discoveries lie in front of us.