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Introduction

The combination of biological elements with electronics is of great interest for many research areas. Inspired by biological signal processes, scientists and engineers are exploring ways of manipulating, assembling, and applying biomolecules and cells on integrated circuits, joining biology with electronic devices. The overall goal is to create bioelectronic devices for biosensing, drug discovery, and curing diseases, but also to build new electronic systems based on biologically inspired concepts. This research area called bioelectronics requires a broad interdisciplinary and transdisciplinary approach to biology and material science. Even though at the frontier of life science and material science, bioelectronics has achieved in the last years many objectives of scientific and industrial relevance, including aspects of electronics and biotechnology. Although the first steps in this field combined biological and electronic units for sensor applications (e.g., glucose oxidase on an oxygen electrode), we see now many applications in the fields of genomics, proteomics, and celomics as well as electronics. This approach challenges both the researcher and the student to learn and think outside of their zones of comfort and training.

Today, one can fabricate electrically active structures that are commensurate in size with biomolecules. The advancement of nanotechnology has influenced bioelectronics to a large extent. New inspection tools, such as scanning probe microscopy, developed in the last two decades have become ubiquitous systems to image nanoscale structure and estimate certain structural, mechanical, and functional characteristics of biological entities, ranging from proteins and DNA to cells and tissues. Various modes of imaging and SPM-based spectroscopy have been developed to correlate structure, properties, and chemomechanical interactions between biological units in different environments. This has induced rapid improvement in control, localization, handling, assembling, and subsequent modification of these biological entities. New understanding of properties of interfaces and binding mechanisms has been achieved. In particular, the detailed investigation of self-assembling processes at the base of protein and DNA formation and ligand–receptor interactions has opened new routes to the design and engineering of hybrid systems, comprising inorganic nanostructures and biological "smart" matter. In parallel different technologies have been developed to produce structures below 100 nm with nanometer control: first, electron beam lithography, which is most often employed, but also ion beam lithography, X-ray lithography, scanning probe

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lithography, and alternative techniques such as soft lithography. The latter in particular has been demonstrated to be compatible with the handling and modification of organic and biological materials, and there exist already in literature various examples of protein patterning realized by means of this technique. Finally, it is worth mentioning the progress made by chemistry in the production of colloidal nano-objects such as spherical particles, rods, tetrapods and combinations, characterized by wide tunability in sizes and emission wavelengths, along with the development of the biochemical ability to join them to biological entities.

Having tools similar in size to biomolecules enables us to manipulate, measure, and (in the future) control them with electronics, ultimately connecting their unique functions. The combination of inorganic nano-objects with biological molecules leads to hybrid systems with special properties that provide fascinating scientific and technological opportunities. A bioelectronic interface joins structured, functional surfaces, and circuits to nucleic acids (e.g., DNA), proteins at the single molecule level. The need of development of new strategies for the functional integration of biological units and electronic systems or nanostructured materials were also facilitated by the parallel progress in biochemistry and molecular biology, namely, advances in protein engineering, with the ability to make "designer" proteins and peptides with specific functions or combinations of functions; and the establishment of surface display technologies, with the ability to generate and screen large repertoires of peptides and nucleic acids for high-affinity binding to potentially any structure (organic or inorganic). New nanostructured sensors, electronic nanocircuitries based on biomolecules, and biomolecular templates are a few examples in which biology meets nanoelectronics.

Moreover, the similar dimensions of biomolecules and electronic nanostructures have opened the way for fabrication of bioelectronic hybrid systems of novel functions. In the last years, considerable research was focused on understanding transport phenomena between biological materials and electronic systems. Recent advances in the field have demonstrated electrical contacting of redox proteins with electrodes—the use of DNA or proteins as templates to assemble nanoparticles and nanowires. This combination of biomolecules with nano-objects will find applications in various disciplines. In turn, recent studies have opened the way to the use of nanoelectrodes, nano-objects, and nanotools in living cells and tissue, for both fundamental biophysical studies and cellular signaling detection. Another research direction is based on the functional connection of neuronal signal processing elements and electronics in order to build brain–machine interfaces and future information systems.

The different aspects of bioelectronics reviewed in this book emphasize the immense developments in the field of bioelectronics and nanobioelectronics. These technological and scientific advancements show that bioelectronics is a ripe discipline based on solid ground. The range of themes addressed emphasizes key aspects and future perspectives of nanobioelectronics. The book discusses

the electronic coupling of DNA and proteins with electronic devices to build new information systems and apply the systems as biosensors. The exploitation of networks of neurons connected with electronic devices in future information processing systems and the use of nano-objectes to assess cellular function is also discussed in detail.

The topics of these hybrid nanobioelectronic systems are both interesting for fundamental research and to enhance industrial competitiveness through research, education, and transfer of technology. Applications of these technologies include:

- *Nanoelectronics for the future*. The fascinating world of the bio–self-assembly provides new opportunities and directions for future electronics, opening the way to a new generation of computational systems based on biomolecules and biostructures at the nanoscale.
- *Life sciences*. Rapid pharmaceutical discovery and toxicity screening using arrays of receptors on an integrated circuit, with the potential to develop targeted "smart drugs."
- *Medical diagnostics*. Rapid, inexpensive, and broad-spectrum point-of-use human and animal screening for antibodies specific to infections
- *Environmental quality*. Distinguishing dioxin isomers for cleaning up polluted sites, improving production efficiency of naturally derived polysaccharides such as pectin and cellulose, and measuring indoor air quality for "sick" buildings.
- *Food safety*. Array sensors for quality control and for sensing bacterial toxins.
- *Crop protection*. High-throughput screening of pesticide and herbicide candidates.
- *Military and civilian defense*. Ultrasensitive, broad-spectrum detection of biological warfare agents and chemical detection of antipersonnel land mines, screening passengers and baggage at airports, and providing early warning for toxins from virulent bacterial strains.

Therefore, the different topics addressed in this book will be of interest to the interdisciplinary research community. We hope that this collection of chapters will provide physics, chemists, biologists, material scientists, and engineers with a comprehensive perspective of the field. Furthermore, the book is aimed to attract young researchers and introduce them to the field, while providing newcomers with an enormous collection of literature references.

The book is organized into three sections: The first is on nanobioelectronics and DNA, the second is on nanobioelectronics and proteins, and the third is on nanobioelectronics and cells. In each section there is a preface describing the key properties of the basic bio-units on which the sections have been focused. The sections are in turn divided in two parts: The first presents the biological element as a part of a (possible) nanoelectronic device, and the second highlights how the recent and fast progress (development) of nanothechnologies can meet the life science world to explore, understand, and possibly control mechanisms that have not been explored up to now. We hope that from the conjunction of the two ways, bio-to-nano and nano-to-bio, a new broad discipline could come up, aimed to increase the scientific progress of the whole scientific community and everyone's wellness in the near future.

Part A
DNA-Based Nanobioelectronics

Deoxyribonucleic acid (DNA) is a nucleic acid that contains the genetic instructions for the development and function of living organisms. The main role of DNA in the cell is the long-term storage of information. It is often compared to a blueprint, since it contains the instructions to construct other components of the cell, such as proteins and RNA molecules. The DNA segments that carry genetic information are called genes, but other DNA sequences have structural purposes or are involved in regulating the expression of genetic information.

DNA is a long polymer made from repeating units called nucleotides. The DNA chain is 22 to 24 Å wide and one nucleotide unit is 3.3 Å long. Although these repeating units are very small, DNA polymers can be enormous molecules containing millions of nucleotides. For instance, the largest human chromosome is 220 million base pairs long.

In living organisms, DNA does not usually exist as a single molecule, but instead as a tightly associated pair of molecules. These two long strands entwine like vines in the shape of a double helix. The nucleotide repeats contain both the backbone of the molecule, which holds the chain together, and a base, which interacts with the other DNA strand in the helix. In general, a base linked to a sugar is called a nucleoside and a base linked to a sugar and one or more phosphate groups is called a nucleotide. If multiple nucleotides are linked together, as in DNA, this polymer is referred to as a polynucleotide.

The backbone of the DNA strand is made from alternating phosphate and sugar residues. The sugar in DNA is the pentose (five-carbon) sugar 2-deoxyribose. The sugars are joined together by phosphate groups that form phosphodiester bonds between the third and fifth carbon atoms in the sugar rings. These asymmetric bonds mean a strand of DNA has a direction. In a double helix the direction of

the nucleotides in one strand is opposite to their direction in the other strand. This arrangement of DNA strands is called antiparallel. The asymmetric ends of a strand of DNA bases are referred to as the 5′ (*five prime*) and 3′ (*three prime*) ends. One of the major differences between DNA and RNA is the sugar, with 2-deoxyribose being replaced by the alternative pentose sugar ribose in RNA.

The DNA double helix is held together by hydrogen bonds between the bases attached to the two strands. The four bases found in DNA are adenine (abbreviated A), cytosine (C), guanine (G), and thymine (T). These four bases are attached to the sugar/phosphate to form the complete nucleotide.

These bases are classified into two types, adenine and guanine, which are fused five- and six-membered heterocyclic compounds called purines, whereas cytosine and thymine are six-membered rings called pyrimidines. A fifth pyrimidine base, called uracil (U), replaces thymine in RNA and differs from thymine by lacking a methyl group on its ring. Uracil is normally only found in DNA as a breakdown product of cytosine, but a very rare exception to this rule is a bacterial virus called PBS1 that contains uracil in its DNA.

The double helix is a right-handed spiral. As the DNA strands wind around each other, they leave gaps between each set of phosphate backbones, revealing the sides of the bases inside. There are two of these grooves twisting around the surface of the double helix: one groove is 22 Å wide and the other 12 Å wide. The larger groove is called the major groove, while the smaller, narrower groove is called the minor groove. The narrowness of the minor groove means that the edges of the bases are more accessible in the major groove. As a result, proteins like that can bind to specific sequences in double-stranded DNA usually read the sequence by making contacts to the sides of the bases exposed in the major groove.

Each type of base on one strand forms a bond with just one type of base on the other strand. This is called complementary base pairing. Here, purines form hydrogen bonds to pyrimidines, with A bonding only to T, and C bonding only to G. This arrangement of two nucleotides joined together across the double helix is called a base pair. In a double helix, the two strands are also held together by forces generated by the hydrophobic effect and pi stacking, but these forces are not affected by the sequence of the DNA. As hydrogen bonds are not covalent, they can be broken and rejoined relatively easily. The two strands of DNA in a double helix can therefore be pulled apart like a zipper, either by a mechanical force or high temperature. As a result of this complementarity, all the information in the double-stranded sequence of a DNA helix is duplicated on each strand, which is vital in DNA replication. Indeed, this reversible and specific interaction between complementary base pairs is critical for all the functions of DNA in living organisms.

The two types of base pairs form different numbers of hydrogen bonds, AT forming two hydrogen bonds, and GC forming three hydrogen bonds. The GC base pair is therefore stronger than the AT base pair. As a result, it is both the percentage of GC base

pairs and the overall length of a DNA double helix that determine the strength of the association between the two strands of DNA. Long DNA helices with a high GC content have strongly interacting strands, whereas short helices with high AT content have weakly interacting strands. The strength of this interaction can be measured by finding the temperature required to break the hydrogen bonds, their melting temperature (also called T_m value). When all the base pairs in a DNA double helix melt, the strands separate and exist in solution as two entirely independent molecules.

Based on these properties DNA is of great interest for applications in bioelectronics. This is in the focus of the first part which is divided into two sections: The first focuses on the use of DNA for future nanoelectronic devices, whereas the second relates to recent developments in the fields of biodiagnostics and genomincs.

"DNA-Mediated Assembly of Metal Nanoparticles: Fabrication, Structural Features, and Electrical Properties" is the title of the first chapter of the first section. It is a great challenge to organize nanoparticles in one to three dimensions in order to study the electronic and optical coupling between the particles, and to even use these coupling effects for the set-up of novel nanoelectronic, diagnostic or nanomechanical devices. Here the authors describe the principles of DNA-based assembly of metal nanoparticles in one, two, and three dimensions together with structural features, and summarize different methods of liquid-phase synthesis of metal nanoparticles as well as their functionalization with DNA. Concepts, which have been developed up to now for the assembly are explained, whereas selected examples illustrate the electrical properties of these assemblies as well as potential applications.

The second chapter, "DNA-Based Nanoelectronics" reports about the exploration of DNA to implement nanoelectronics based on molecules. The unique properties in self-assembling and recognition in combination with well established biotechnological methods makes DNA very attractive for concepts of auto-organizing nanocircuits. Nevertheless, the conductivity of DNA is still under debate. Here the author briefly reviews the state-of-the-art knowledge on this topic.

The first chapter of the second section, entitled "DNA Detection with Metallic Nanoparticles" draws attention to the development of detection schemes with high specificity and selectivity needed for the detection of biomolecules. Here, the authors describe the use of metal nanoparticles as markers to overcome some of the obstacles of the classical DNA labeling techniques. The unique properties of nanoparticles can be used for a variety of detection methods such as optical, electrochemical, electromechanical, or electrical detection methods. In this chapter the authors give an overview of the use of metal nanoparticles as labels for DNA detection in solution and in surface-bound assays.

Finally, the last chapter of this part of the book, "Label-Free, Fully Electronic Detection of DNA with a Field-Effect Transistor Array," gives an introduction into label-free detection of DNA with an electronic device. Electronic biosensors based on field-effect transistors (FET), offer an alternative approach for the direct

and time-resolved detection of biomolecular binding events, without the need to label the target molecules. These semiconductor devices are sensitive to electrical charge variations that occur at the surface/electrolyte interface and on changes of the interface impedance. Using the highly specific hybridization reaction of DNA molecules, which carry an intrinsic charge in liquid environments, unknown—so-called target—DNA sequences can be identified.

DNA for Electronics

1

DNA-Mediated Assembly of Metal Nanoparticles: Fabrication, Structural Features, and Electrical Properties

Monika Fischler, Melanie Homberger, and Ulrich Simon

1 INTRODUCTION

Many different synthetic routes have been developed in order to obtain metal nanoparticles of different sizes and shapes. The evolution of high-resolution physical measurements together with the elaboration of theoretical methods applicable to mesoscopic systems inspired many scientists to create fascinating ideas about how these nanoparticles can provide new technological breakthroughs; for example, in nanoelectronic, diagnostic, or sensing devices (de Jongh 1994; Schön and Simon 1995; Simon 1998; Feldheim and Foss 2002; Schmid 2004; Willner and Katz 2004; Rosi and Mirkin 2005). Nanoparticles with a diameter between one and several tens of nanometres possess an electronic structure that is an intermediate of the discrete electronic levels of an atom or molecule and the band structure of a bulk material. The resulting size-dependent change of physical properties is called the *quantum size effect* (QSE) or *size quantization effect* (Halperin 1986).

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This behavior raises fundamental questions about the design of "artificial molecules" or "artificial solids" built up from nanoscale subunits which finally lead to a new state of matter. Therefore, ordered assemblies of uniform nanoparticles in one, two, or three dimensions are required. Such arrays of nanoparticles exhibit delocalized electron states that depend on the strength of the electronic coupling between the neighboring nanoparticles, whereas the electronic coupling depends mainly on the particle size, the particle spacing, the packing symmetry, and the nature and covering density of the stabilizing organic ligands (Remacle and Levine 2001).

Thus, it is a great challenge to organize nanoparticles in one to three dimensions in order to study the electronic and optical coupling between the particles, and to even utilize these coupling effects for the set-up of novel nanoelectronic, diagnostic, or nanomechanical devices (Willner and Katz 2004).

This chapter focuses on how DNA can be used as a construction material for the controlled assembly of metal nanoparticles. The enormous specificity of Watson-Crick base-pairing together with the chemists ability to synthesize virtually any DNA sequence by automated methods allow the convenient programming of artificial DNA architectures. Furthermore, short DNA fragments (up to approximately 100 nm) possess great mechanical rigidity. Thus, upon using short DNA fragments the DNA effectively behaves like rigid rod spacers between two tethered functional molecular components (e.g., nanoparticles). Moreover, DNA displays a relatively high physicochemical stability. Hence, DNA holds the promise of allowing the bottom-up self-assembly of complex nanodevices, where, for example, in the course of further miniaturization, conductive DNA-based structures could reduce time and costs in future nanofabrication (Stoltenberg and Woolley 2004).

We aim to acquaint the reader with the principles of DNA-based assembly of metal nanoparticles. Starting with a brief introduction into the different methods of liquid-phase synthesis of metal nanoparticles and their functionalization with DNA, we give an overview on the assembly of nanoparticles in one, two, and three dimensions. The structural features and electrical properties will be exemplarily described together with emerging applications.

2 MATERIALS SYNTHESIS

2.1 LIQUID PHASE SYNTHESIS OF METAL NANOPARTICLES

The common way for the synthesis of metal nanoparticles is the reduction of soluble metal salts in the presence of stabilizing ligand molecules (typically in excess) in solution (Fig. 1.1).

The reduction is achieved either by suitable reducing agents (e.g., hydrogen, boron hydride, methanol, citric acid, and others) or electrochemically. In order to stabilize the formed nanoparticles it is necessary to perform the reduction in the presence of molecules that are able to bind to the nanoparticles surface. These are all molecules with electron donor functionalities (e.g., carboxylates, amines, phosphines, thiols). The stabilization effect refers to sterical and electrostatic effects. Sterical stabilization means that the protecting molecules surround the nanoparticles comparable to a protective shield due to the required space of the molecules. Electrostatic stabilization refers to coulombic repulsion between the particles caused by the charge introduced by the ligand.

The protected metal nanoparticles synthesized this way can be further modified by ligand exchange reactions. This allows varying the nanoparticle properties (e.g., solubility) or the chemical functionality of the nanoparticle system.

The great variety of different reducing agents together with the great variety of different types of stabilizing molecules has led to a huge diversity of metal nanoparticles with different sizes, shapes, and ligand molecules. In the following, the preparation of selected metal nanoparticles is exemplarily described. For detailed overviews on the synthetic routes and surface modification methods one could refer to Schmid, Daniel and Astruc, and Richards and Boennemann (Daniel and Astruc 2004; Schmid 2004; Richards and Boennemann 2005).

2.1.1 REDUCTION OF SOLUBLE METAL SALTS WITH REDUCING AGENTS

For a long time the most popular route for synthesizing metal nanoparticles in the liquid phase was the reduction of $HAuCl_4$ with sodium citrate in aqueous solution, a route that was first reported in 1951 (Turkevitch et al. 1951). This route allowed the preparation of gold nanoparticles with sizes ranging from 14.5 ± 1.4 to 24 ± 2.9 nm. Thereby, the sizes of the formed nanoparticles could be controlled by the ratio of the gold precursor and the citrate. This method is still often used due to the fact that the citrate ligand can easily be exchanged and, thus, further modifications of the nanoparticle surface are enabled (see Chapter 2.2).

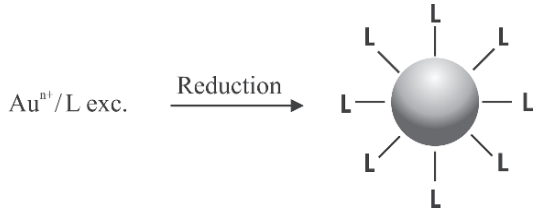

FIG. 1.1. General reaction scheme for the preparation of metal nanoparticles via reduction of a metal salt in the presence of stabilizing ligand molecules (L).

In 1995 Möller and co-workers introduced an approach utilizing amphiphilic block copolymers as templates for the preparation of small gold nanoparticles of a diameter of 2.5, 4, and 6 nm (Spatz et al. 1995; Spatz, et al. 1996). Amphiphilic block copolymers tend to form micelles in solvents that dissolve only one block of the co-polymer well. The shape and stability of the micelles depend on the solvent (polar or non-polar), the relative composition of the block co-polymer, and the concentration. In their approach Möller and co-workers used symmetrical polystyrene-b-polyethylene oxide (PS-b-PSO). Under the conditions employed, this block co-polymer assembled to spherical micelles in toluene. Upon addition of the metal salt precursor $LiAuCl_4$, the Li^+ ions formed a complex with the polyethylene oxide block, whereas the tetrachloroaurate ions were bound as counter-ions within the core of the micelles. After reduction of the metal ions either by adding hydrazine or initiating the electron beam of the TEM, nanoparticles were formed inside the micellar core. The size of the formed nanoparticles depended on the size of the micelle and the loading ratio $LiAuCl_4$/PS-b-PSO. This approach provides a good tool for the formation of polymer films containing gold nanoparticles of defined size.

The most prominent example for the synthesis of gold nanoparticles with a narrow size-distribution or even uniformity is the preparation of the so-called Schmid-cluster: $Au_{55}(PPh_3)_{12}Cl_6$ (Schmid et al. 1981). The prominence rises from the quantum size behavior of the cluster, a fact that makes these clusters promising particles for future nanoelectronic applications (Schmid 2004). The cluster was prepared by the reduction of the metal salt $Au(PPh_3)Cl$ with in situ formed B_2H_6 and could be isolated as black microcrystalline solid, and characterized by TEM and small-angle X-ray diffraction (Schmid et al. 1999). This cluster is an example of a so-called full-shell cluster. Full-shell clusters are considered to be constructed by shells, each having $10n^2 + 2$ atoms (n = number of shells) (Schmid et al. 1990; Schmid 2004). Further examples for full-shell clusters are $[Pt_{309}phen^*_{36}O_{30}]$ and $[Pd_{561}phen_{36}O_{200}]$ (phen* = bathophenantroline and phen = 1,10-phenantroline) (Vargaftik et al. 1985; Schmid et al. 1989; Moiseev et al. 1996). The Pt_{309} cluster is synthesized by the reduction of Pt(II)acetate with hydrogen in the presence of phenantroline and following oxidation with O_2. The Pd_{561} cluster is one product of the analogous reduction of Pd(II) acetate with hydrogen in the presence of phenantroline or bathophenantroline, respectively.

2.1.2 ELECTROCHEMICAL REDUCTION OF METAL SALTS

An electrochemical route for the synthesis of nanoparticles from Pd, Ni, or Co was described by Reetz and Helbig (1994). This route allowed controlling the particle size by adjustment of the current density. The electrochemical

setup was a two-electrode one, in which the anode consisted of the bulk metal and the supporting electrolyte contained tetraalkylammonium salts, which served as ligand molecules. The process itself can be described as follows: The bulk metal is oxidized at the anode, the metal cations migrate to the cathode, and a consecutive reduction takes place, resulting in the formation of the tetraalkylammonium-stabilized nanoparticles. Using this technique, particle sizes in the range of 1.4 to 4.8 nm with a narrow size distribution could be obtained. One advantage seems to be the broad variation range of the corresponding ligand shell. Since the tetraalkylamonium ions are added to the reaction mixture, the thickness of the ligand shell can be varied by changing the length of the alkyl chain.

2.2 PREPARATION OF DNA-FUNCTIONALIZED METAL NANOPARTICLES

For the construction of nanoparticle assemblies in one, two, and three dimensions DNA oligomers as ligand molecules have become an important tool. The design and synthesis of the DNA oligomers as well as of other native and non-natural nucleic acid derivatives is a routine technology today. DNA sequences up to 120 nucleotides in length, modified with a large variety of chemical substituents, such as amino- and thiol groups attached to the 3′ or 5′-terminus, are readily available by a multitude of commercial suppliers. This chapter outlines a selection of methods to functionalize nanoparticles with DNA oligomers. An overview on general procedures is given in Fig. 1.2.

Gearheart and co-workers described the functionalization of citrate stabilized gold nanoparticles with unmodified DNA oligomers by ligand exchange (Fig. 1.2A). Here, the oligomer with the negatively charged phosphate backbone binds electrostatically to the gold nanoparticles and thereby replaces citrate ligands on the nanoparticles' surface (Gearheart et al. 2001). Thus, the binding ability of the DNA depends on the nanoparticle size and curvature as well as on the kinked, bent, or straight DNA morphology.

Numerous other protocols for the synthesis of DNA-modified gold nanoparticles are related to the initial description of these materials by Mirkin and coworkers who used the chemisorption of thiol- or amino-functionalized oligomers on the gold nanoparticle (Mirkin et al. 1996). This method is displayed in Fig. 1.2B. Briefly, citrate-stabilized gold nanoparticles are mixed with DNA oligomers derivatized with alkylthiolgroups at the 3′- or 5′-terminus, incubated for prolonged times up to several days, and purified by repeated centrifugation to remove unbound oligomers in the supernatant. The resulting DNA-functionalized gold nanoparticles are water soluble and stable for months.

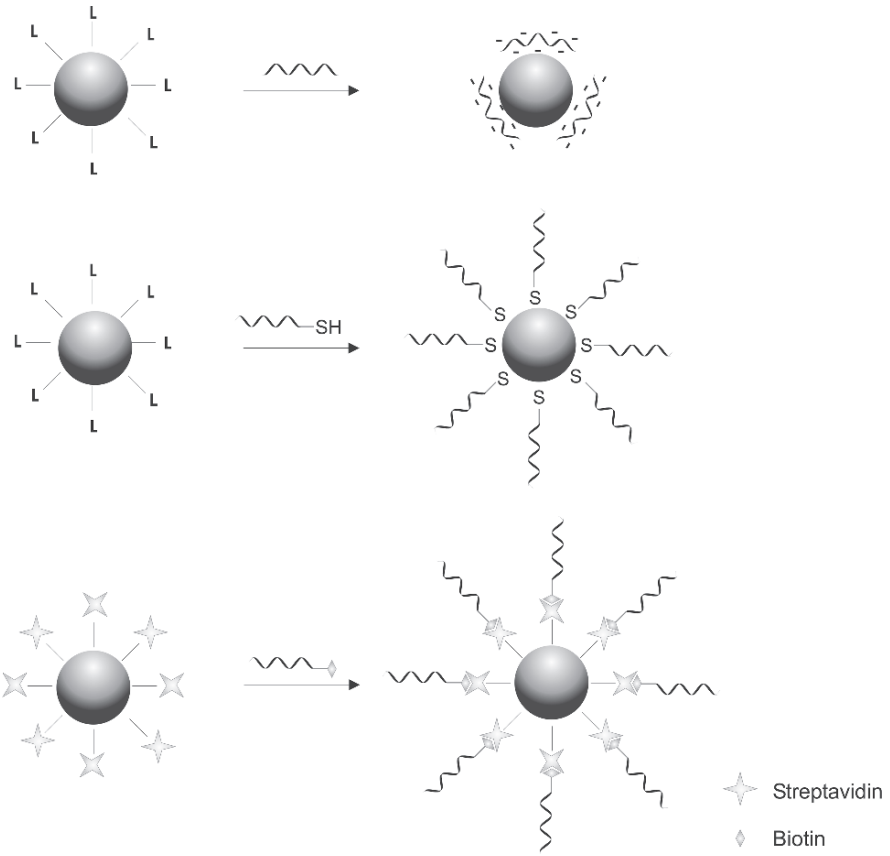

FIG. 1.2. Schematic presentation of three different principles for the preparation of DNA-oligomer functionalized nanoparticles. **A.** Via ligand exchange: Oligomers are attached electrostatically to the nanoparticle surface. **B.** Via ligand exchange: Thiol-terminated oligomers are attached covalently to the nanoparticle surface. **C.** Via streptavidin/biotin system: Biotinylated oligomers bind specifically to streptavidin-modified particles.

Slight variations of this protocol have been reported again by Mirkin and co-workers and Niemeyer and co-workers (Storhoff et al. 1998; Niemeyer et al. 2003; Hazarika et al. 2004). The latter reports the synthesis of DNA-modified gold nanoparticles that contain more than one single-stranded oligo sequence (Fig. 1.3). The number of different sequences attached to the nanoparticle ranges from two (difunctional) up to seven (heptafunctional). These oligofunctional gold nanoparticles reveal almost unaltered hybridization capabilities compared with conventional monofunctional conjugates. Because of the extraordinary specificity of Watson-Crick base pairing, the various

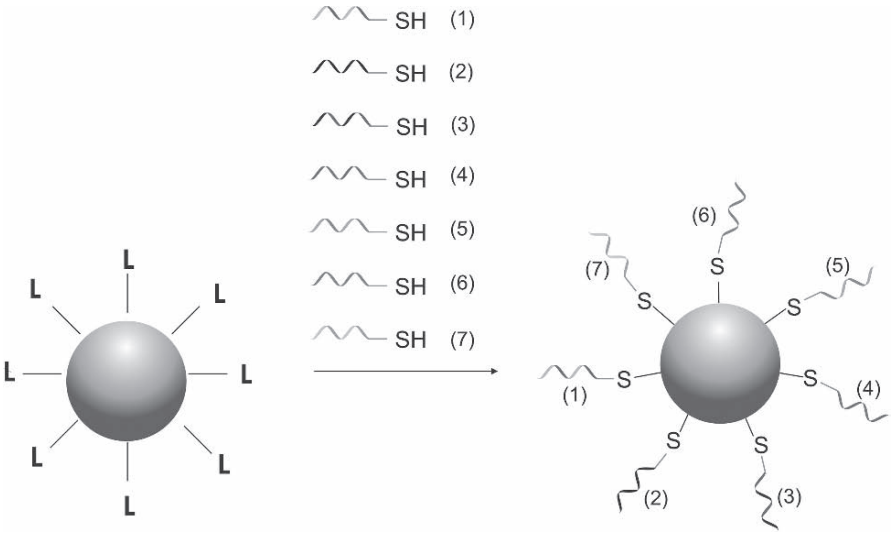

FIG. 1.3. Synthesis of oligofunctional DNA-gold nanoparticles by ligand exchange with different thiolated oligos (1–7) yielding up to heptafunctional particles.

oligonucleotide sequences can therefore be individually and selectively addressed as members of an orthogonal coupling system present at the particle's surface. Applications of such oligofunctional DNA gold nanoparticles are reported later in this chapter.

Further approaches for the preparation of DNA-functionalized gold nanoparticles including the use of polymers (Chen et al. 2004), dithiol- (Letsinger et al. 2000), and trithiol-linkers (Li et al. 2002) in between the DNA moiety and the gold particle have been reported.

Other methods for the coupling of DNA oligomers and a large variety of other biomolecules to the nanoparticle surfaces take advantage of the highly specific binding affinity of the streptavidin (STV)/biotin system (Niemeyer 2001a; Cobbe et al. 2003; Willner and Katz 2004). Streptavidin offers four native binding sites for biotin and therefore serves as an ideal linker between nanoparticles and biotinylated DNA oligomers or other biomolecules that are modified with biotin moieties (Fig. 1.2C).

For many applications it is essential to purify the DNA-gold nanoparticles and quantify the density of oligomer coverage on their surface. This can be achieved by gel electrophoresis for example, as described by Alivisatos and co-workers (Zanchet

et al. 2001; Parak et al. 2003). Another possibility to quantify the oligomer coverage density is the fluorescence-based assay of Demers and co-workers (Demers et al. 2000).

3 NANOPARTICLE ASSEMBLIES AND PROPERTIES

3.1 THREE-DIMENSIONAL ASSEMBLIES

The formation of three-dimensional assemblies of gold nanoparticles was first reported by Mirkin and co-workers. Up to now many groups have applied this assembly scheme for the preparation of various two- and three-dimensionally linked nanoparticles. Extensions of this approach use the specificity of the streptavidin/biotin system together with the advantages of the Watson-Crick base pairing scheme. In the following examples for these methods are presented. Furthermore, some properties of the resulting DNA-based networks are summarized exemplarily.

In the original work Mirkin and co-workers used 13 nm gold nanoparticles that were modified according to the method described in Chapter 2.2 with non-complementary thiol-terminated oligonucleotides. Upon the addition of a double-stranded DNA containing two single-stranded ends ("sticky ends"), complementary to the particle-bound DNA, aggregation due to DNA hybridization occurred (Fig. 1.4A) (Mirkin et al. 1996). This aggregation process became visible in the slow precipitation of the macroscopic DNA-nanoparticle network and was shown to be reversible. Because in this route the nanoparticle surface is covered with multiple DNA molecules, the aggregates are two- or three-dimensionally linked. Evidence of the assembly process can be given by TEM images (Fig. 1.4B and C). Typical images reveal close-packed assemblies of the colloids with uniform particle separations of about 6 nm, corresponding to the length of the double-stranded DNA linker.

In a published extension of their work Mirkin and Li showed that care has to be taken if nanoparticles modified with deoxyguanosin-rich DNA strands are used for the assembly process. Within this study they showed that in the case of deoxyguanosin-rich DNA-modified nanoparticles self-assembly already occurs upon increasing the buffer salt concentration, and stable networks are formed in the presence of potassium (Li and Mirkin 2005).

The DNA hybridization scheme developed by Mirkin and co-workers was also applied for the preparation of nanoparticle networks comprised of different types of nanoparticles. For example gold nanoparticles of either 31 or 8 nm diameter

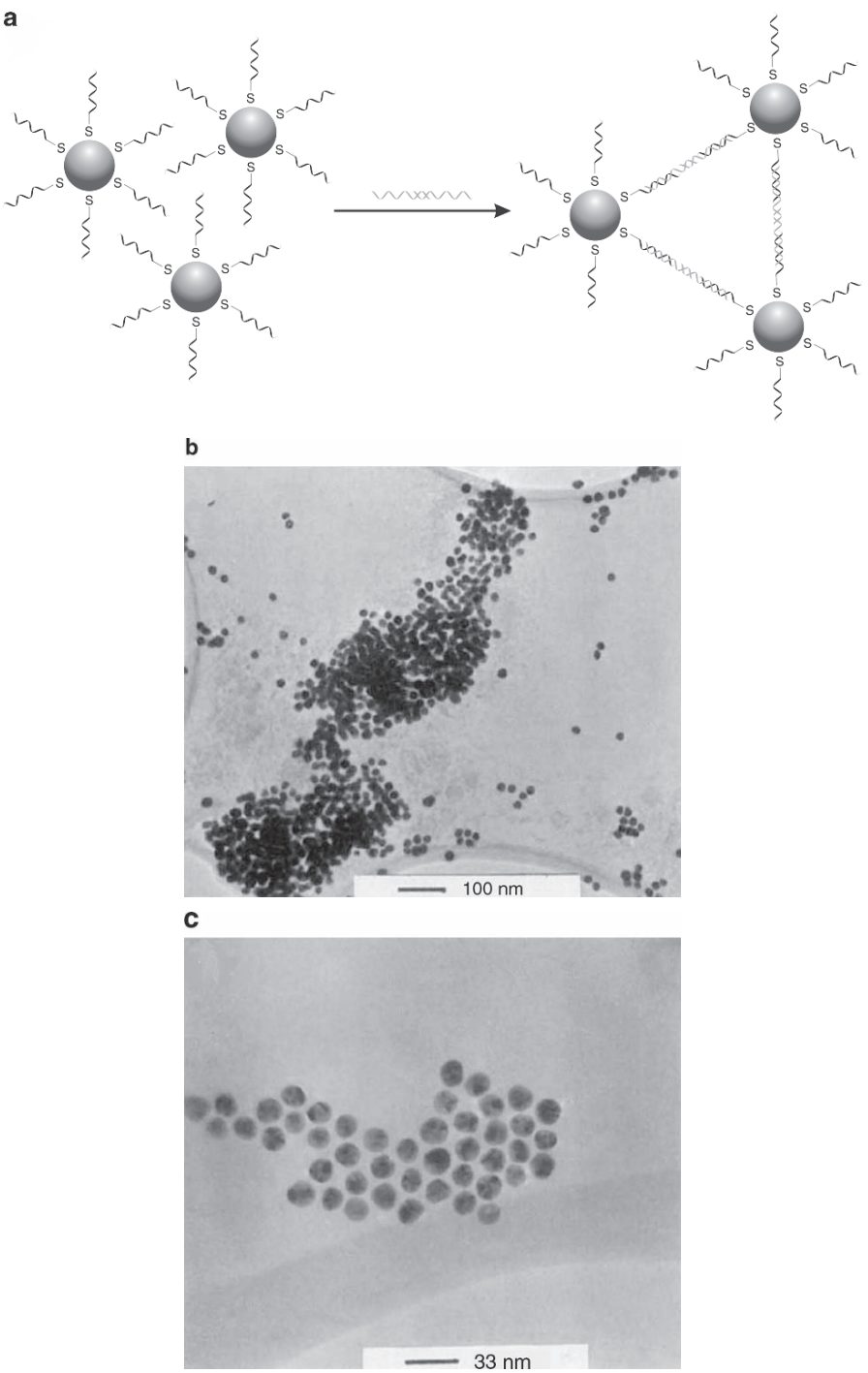

FIG. 1.4. **A.** Scheme for the formation of three-dimensional assemblies of gold nanoparticles using DNA hybridization. **B.** TEM image of an aggregated DNA/colloid hybrid material. **C.** TEM image of a two-dimensional colloid aggregate showing the ordering of the DNA linked gold nanoparticles (**B** and **C** reprinted from Mirkin et al. 1996 with permission of the Nature Publishing Group.).