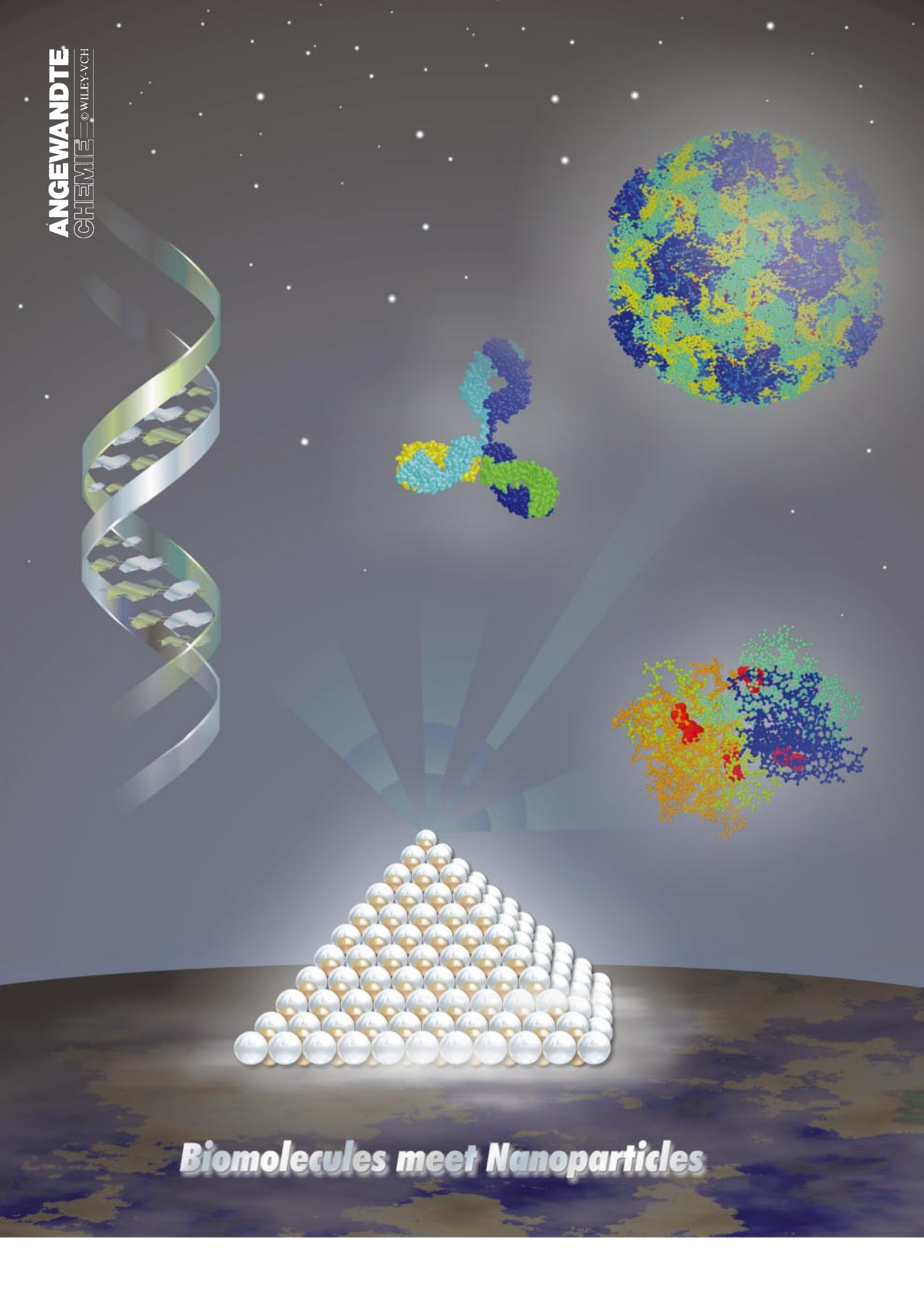




# ***Biomolecules meet Nanoparticles***

# Nanoparticles, Proteins, and Nucleic Acids: Biotechnology Meets Materials Science

Christof M. Niemeyer\*

Based on fundamental chemistry, biotechnology and materials science have developed over the past three decades into today's powerful disciplines which allow the engineering of advanced technical devices and the industrial production of active substances for pharmaceutical and biomedical applications. This review is focused on current approaches emerging at the intersection of materials research, nanosciences, and molecular biotech-

nology. This novel and highly interdisciplinary field of chemistry is closely associated with both the physical and chemical properties of organic and inorganic nanoparticles, as well as to the various aspects of molecular cloning, recombinant DNA and protein technology, and immunology. Evolutionary optimized biomolecules such as nucleic acids, proteins, and supramolecular complexes of these components, are utilized in the production of

nanoscaled and mesoscopic architectures from organic and inorganic materials. The highly developed instruments and techniques of today's materials research are used for basic and applied studies of fundamental biological processes.

**Keywords:** analytical methods • biotechnology • materials science • nanostructures • supramolecular chemistry

## 1. Introduction

"The essence of chemical science finds its full expression in the words of that epitome of the artist-scientist Leonardo da Vinci: *'Where Nature finishes producing its own species, man begins, using natural things and with the help of this nature, to create an infinity of species.'*" Nobel laureate Jean-Marie Lehn used these words to give an outlook on the future and perspectives of supramolecular chemistry.<sup>[1]</sup> In a highly interdisciplinary effort, the pioneering work of Pedersen, Cram, and Lehn on supramolecular aggregates held together by weak noncovalent interactions has developed over the past 30 years into a well-established discipline. Supramolecular chemistry concerns the investigation of nature's principles to produce fascinating complex and functional molecular assemblies, as well as the utilization of these principles to generate novel devices and materials, potentially useful for sensing, catalysis, transport, and other applications in medicinal or engineering science.<sup>[1-3]</sup> Another example of modern technical achievements concerns the development of advanced, functional, and even "smart" materials for applications in highly integrated mechanical, optical, and electronic devices, sen-

sors, or catalysts. The enormous advance attained in this area so far is illustrated impressively by comparing materials used in last centuries electrical devices such as millimeter-sized copper wires to today's (sub)micrometer-sized optical and electronical parts, comprised of modern conducting and electroluminescent organic polymers.<sup>[4]</sup>

Similar advances have been made in the biological sciences. Natural evolution has led to highly functional assemblies of proteins, nucleic acids, and other (macro)molecules which perform complicated tasks that are still daunting for us to try to emulate. As an example, the 20 nm ribosome particle is an effective supramolecular machine which spontaneously self-assembles from more than 50 individual protein and nucleic acid building blocks, thereby impressively demonstrating the power of biologically programmed molecular recognition.<sup>[5]</sup> Starting with the discovery of the double helix structure of DNA, biology has grown from a purely descriptive and phenomenological discipline to a molecular science. Recombinant DNA technology brought insights into the basic principles of many biochemical processes, and it has also opened the door to modern biotechnology. Today we are able to genetically engineer relatively simple bacterial cells, and we are on our way towards tailoring complex organisms. In view of such revolutionary developments, it seems particularly challenging to fuse biotechnology with materials science (Figure 1). Merging these disciplines will allow us to take advantage of the improved evolutionary biological components to generate new smart materials and, conversely, to

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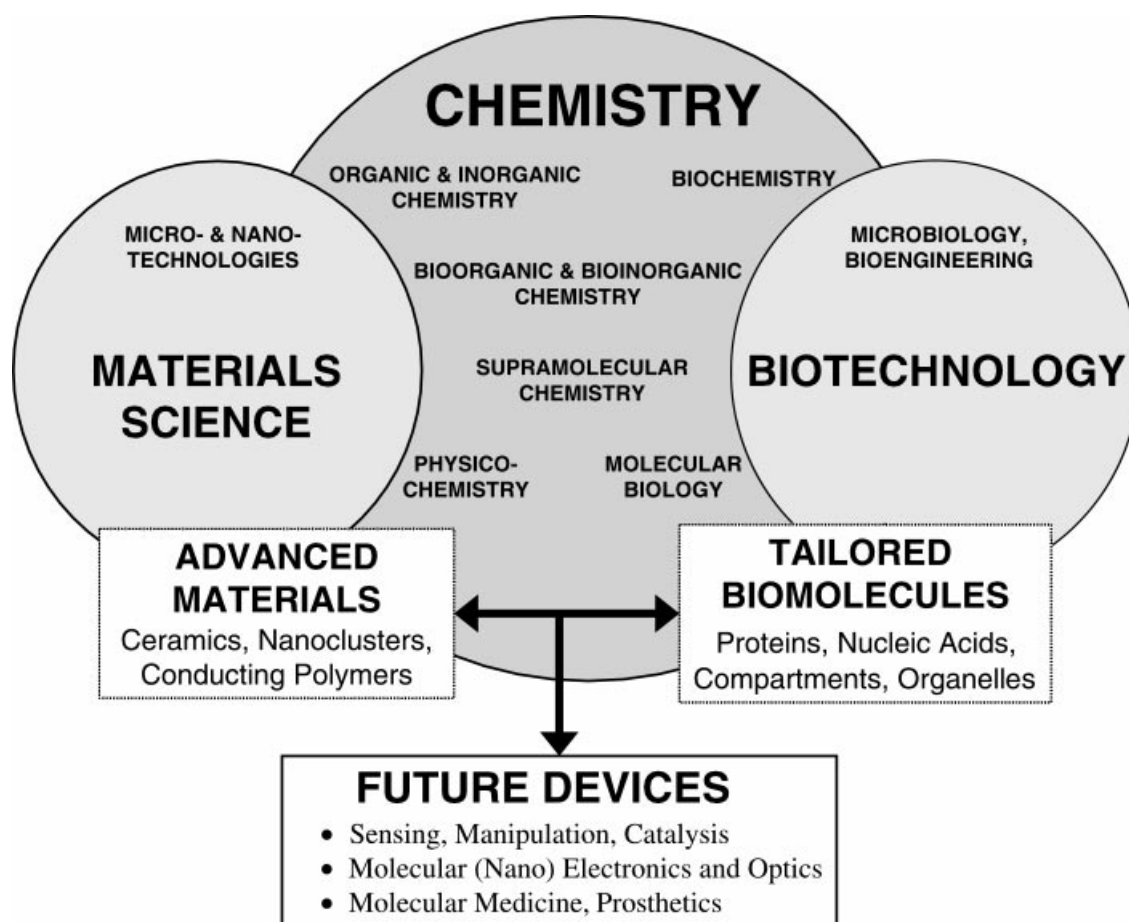


Figure 1. Chemistry is the central science for the development of applied disciplines such as materials research and biotechnology. Materials science, which is based on classic chemical research fields and engineering technologies, has led to enormous advances in tailoring advanced modern materials.

apply today's advanced materials and physicochemical techniques to solve biological problems.

Both biotechnology and materials science meet at the same length scale (Figure 2). On the one hand, biomolecular components have typical size dimensions in the range of about 5 to 200 nm. To exploit and to utilize the concepts administered in natural nanometer-scale systems, the development of nanochemistry is crucial.<sup>[6]</sup> On the other hand, commercial requirements to produce increasingly miniaturized microelectronic devices strongly motivate the elaboration of nanoscale systems.

The structural dimensions of computer microprocessors are currently in the range of about 200 nm. They are only just available by conventional top-down processes (miniaturization processes) such as photolithography, but for the foreseeable future, such technologies hardly allow the large-scale production of parts that are significantly smaller than 100 nanometers. "There is plenty of room at the bottom," as Nobel physicist Richard Feynman pointed out more than 40 years ago,<sup>[7]</sup> and thus today's nanotechnology research puts a great emphasis on the



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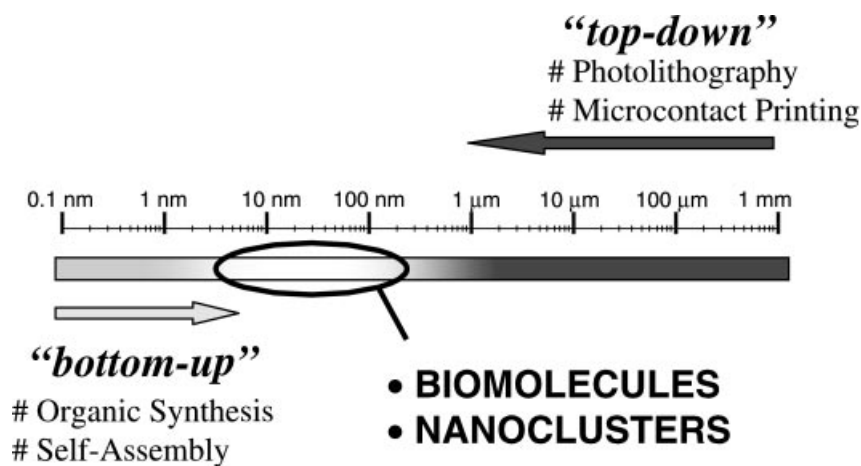


Figure 2. A gap currently exists in the engineering of small-scale devices. Whereas conventional top-down processes hardly allow the production of structures smaller than about 200–100 nm, the limits of regular bottom-up processes are in the range of about 2–5 nm. As a result of their own dimensions, two different types of compounds appear to be suited for addressing that gap: 1) biomolecular components, such as proteins and nucleic acids, and 2) colloidal nanoparticles comprised of metal and semiconductor materials.

development of bottom-up strategies (enlargement strategies), which concern the self-assembly of (macro)molecular and colloidal building blocks to create larger, functional devices.<sup>[8]</sup> The individual components to be employed in self-assembling processes should fulfill some basic requirements. First, distinct intrinsic functionality, such as steric, optical, electronic, or catalytic properties are desired to allow the particular applications envisaged. Second, the modules need to be programmable through their specific constitution, configuration, conformation, and dynamic properties to enable specific recognition for self-assembly. Third, the building blocks should have an appropriate size to bridge the gap between the submicrometer dimensions that are reachable by classical top-down engineering and the dimensions that are addressable by classical bottom-up approaches, such as chemical synthesis and supramolecular self-assembly (Figure 2).

Inorganic nanoparticles are particularly attractive building blocks for the generation of larger superstructures.<sup>[9–16]</sup> Such nanoparticles can be prepared readily in large quantities from various materials by relatively simple methods. The dimensions of the nanoparticles can be controlled from one to about several hundred nanometers, with a fairly narrow size distribution. Most often, the particles are comprised of metals, metal oxides, and semiconductor materials, such as Ag<sub>2</sub>S, CdS, CdSe, and TiO<sub>2</sub>. The nanoparticles have highly interesting optical, electronic, and catalytic properties, which are very different from those of the corresponding bulk materials and which often depend strongly on the particle's size in a highly predictable way. Examples include the wavelength of the light emitted from semiconductor nanocrystals which can be utilized for biolabelling<sup>[17, 18]</sup> or in lasers,<sup>[19]</sup> as well as the external magnetic field required to switch a magnetized particle, which is highly relevant in magnetotactic bacteria<sup>[20]</sup> and in hard disk drives.<sup>[21]</sup> Moreover, certain types of nanoparticles can be considered as “artificial atoms”<sup>[22]</sup> since they are obtainable as highly perfect

nanocrystals, which can be used as building blocks for the assembly of larger two- and three-dimensional structures.<sup>[23]</sup>

A number of outstanding monographs and reviews already deal with the rapidly increasing area of fundamental and applied nanoparticle research.<sup>[9–15]</sup> This article is intended to summarize approaches which go beyond the questions of pure materials science. These approaches currently emerge at the intersection of materials science and molecular biotechnology.<sup>[24–26]</sup> This novel field of research is closely associated with the surface chemistry and the physical properties of inorganic nanoparticles, the topics of bioorganic and bioinorganic chemistry, and the various aspects of molecular biology, recombinant DNA technology, recombinant protein expression, and immunology. In particular, this article describes the utilization of nucleic acids and proteins as programmable recog-

nition units to modify and improve the nanoparticle's assembly capabilities, and thus, to allow for nanostructured and mesoscopic supramolecular hybrid architecture. Moreover, recent efforts to utilize nanoparticles as tools for fundamental and applied studies on biomolecular interaction are summarized.

## 2. Coupling of Nanoparticles and Biomolecules

Bioorganic and bioinorganic chemistry are the interdisciplinary fields which provide the basis for joining biotechnology with materials science. For instance, bioorganic model systems have long been elaborated to provide tools for probing the mechanisms of biological principles, as well as to develop chemical means for the handling and manipulating biological components.<sup>[27, 28]</sup> Analogously to the interactions that are abundant in many reaction centers of enzymes between the amino acid side chains and the metal centers, the interaction between organic ligands and the surface of an inorganic nanoparticle paves the way for the coupling of biomolecular recognition systems to generate novel materials. Although inorganic nanoparticles can be prepared from various materials by several methods,<sup>[9, 10, 13–15]</sup> the coupling and functionalization with biological components has only been carried out with a limited number of chemical methods. Some examples are summarized in Table 1.

Typically, the wet-chemical preparation of the nanoparticles is carried out in the presence of stabilizing agents (often citrate, phosphanes, or thiols) which bind to the atoms exposed at the surface of the nanoparticles. This capping leads to a stabilization and prevents uncontrolled growth and aggregation of the nanoparticles. In the case of a labile capping layer such as citrate, biomolecules can be linked directly with the metal particle by exchange reactions with stronger binding ligands (Figure 3). This method is usually applied in the coating of colloidal gold with thiol-containing

Table 1. Coupling of inorganic nanoparticles and biomolecules.

| Particle                                 | Linker                                                                   | FG                                                  | Biomolecule                     | Reference     |
|------------------------------------------|--------------------------------------------------------------------------|-----------------------------------------------------|---------------------------------|---------------|
| Au                                       | –                                                                        | HS-Cys                                              | immunoglobulins, serum albumins | [34, 152]     |
| Au                                       | citrate                                                                  | H <sub>2</sub> N-Lys <sup>[a]</sup>                 | proteins                        | [156, 217]    |
| Au                                       | streptavidin                                                             | biotin-(CH <sub>2</sub> ) <sub>6</sub> -            | immunoglobulins, serum albumins | [218]         |
| Au                                       | <b>3</b>                                                                 | –                                                   | immunoglobulin, streptavidin    | [42]          |
| Au                                       | streptavidin                                                             | biotin-(CH <sub>2</sub> ) <sub>6</sub> -            | DNA                             | [148e, 219]   |
| Au                                       | –                                                                        | HS-(CH <sub>2</sub> ) <sub>6</sub> -                | DNA                             | [77, 82]      |
| Au                                       | –                                                                        | (HS-PO <sub>3</sub> R <sub>2</sub> ) <sub>5</sub> - | DNA                             | [30, 31]      |
| Au                                       | <b>2</b>                                                                 | –                                                   | DNA                             | [33]          |
| Au                                       | <b>4</b>                                                                 | HOOC-Glu                                            | proteins                        | [43]          |
| Au                                       | <b>5</b>                                                                 | HS-Cys                                              | proteins                        | [158]         |
| Ag                                       | citrate                                                                  | H <sub>2</sub> N-Lys <sup>[a]</sup>                 | heme proteins, immunoglobulins  | [35, 36, 166] |
| ZnS                                      | –                                                                        | HS-Cys                                              | glutathione                     | [47]          |
| CdS                                      | –                                                                        | HS-Cys                                              | peptides                        | [46]          |
| CdS                                      | Cd <sup>2+</sup> , HS-(CH <sub>2</sub> ) <sub>2</sub> -OH <sup>[a]</sup> | –                                                   | DNA                             | [39, 41]      |
| CdSe/ZnS <sup>[b]</sup>                  | –                                                                        | HS-(CH <sub>2</sub> ) <sub>6</sub> -                | DNA                             | [79]          |
| CdSe/CdS/SiO <sub>2</sub> <sup>[b]</sup> | <b>6</b>                                                                 | NHS-biotin                                          | streptavidin                    | [17]          |
| CdSe/ZnS <sup>[b]</sup>                  | HS-(CH <sub>2</sub> )-COOH                                               | H <sub>2</sub> N-Lys                                | immunoglobulin, transferrin     | [18]          |
| CdSe/ZnS <sup>[b]</sup>                  | <b>1</b>                                                                 | H <sub>2</sub> N-Lys <sup>[a]</sup>                 | leucine zipper fusion proteins  | [32]          |
| SnO <sub>2</sub> , TiO <sub>2</sub>      | HOOC-(CH <sub>2</sub> ) <sub>n</sub> -NH <sub>2</sub>                    | HOOC-Glu                                            | proteins <sup>[c]</sup>         |               |
| GaAs, InP                                | phosphoramidate ε-NH <sub>2</sub>                                        | HOOC-Glu                                            | proteins <sup>[c]</sup>         | [220]         |

Examples of chemical interactions used for the coupling of nanoparticles with biological macromolecules (FG = functional coupling group, see Figure 3). [a] Adsorption through Coulombic interactions. [b] Core/shell nanoparticles. [c] Proposed from related studies of nanoparticle binding to analogously modified solid substrates.

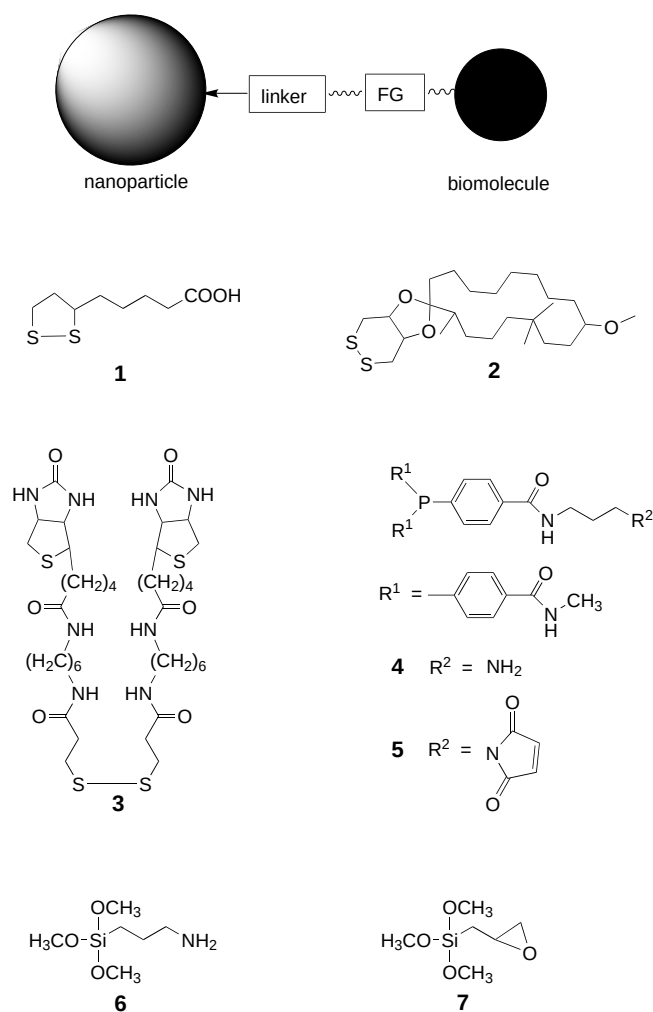


Figure 3. General schematic representation of methods to couple inorganic nanoparticles and biomolecules (FG = functional coupling group). Representative structures of typical linkers are listed beneath (see also Table 1).

proteins, for example, immunoglobulins (IgG) and serum albumins, which have cysteine residues that are accessible for the heterogeneous interphase coupling. If no such residues are available in the native proteins, thiol groups can be incorporated by chemical means (e.g. with Traut's reagent (2-iminothiolane))<sup>[29]</sup> or by genetic engineering. Similarly, DNA molecules can be synthesized with alkylthiol groups at the 3'- or 5'-ends by using appropriate phosphoramidite precursors in a solid-phase synthesis. The *n*-alkylthiolated DNA has been used extensively in the preparation of DNA-functionalized gold and semiconductor nanoparticles (Table 1). As an interesting alternative, DNA oligonucleotides that contain several adenosyl phosphothioate residues at their ends have been used to interact directly with the metal surface of nanoparticles.<sup>[30, 31]</sup> The use of cyclic disulfide linkers such as **1**<sup>[32]</sup> and **2**<sup>[33]</sup> (Figure 3) leads to nanoparticle cappings, which are more stable towards ligand exchange than the corresponding conjugates prepared with the conventional reagents that contain a single thiol group or acyclic disulfide units. The greater stability is likely a result of the anchoring of the ligands to the nanoparticles through two sulfur atoms.<sup>[33]</sup>

In the case of nanoparticles that are stabilized by anionic ligands such as citrate or lipoic acid (**1**), the biomolecules are often coupled through noncovalent electrostatic interactions. For instance, gold and silver nanoparticles, which are produced by the citrate method, have been functionalized with IgG molecules at high pH values that are slightly above the isoelectric point of the citrate ligand.<sup>[34]</sup> This allows an effective binding between the positively charged amino acid side chains of the protein and the negatively charged citrate groups of the colloids. The optimal coupling ratio can be determined by means of a flocculation assay. The addition of electrolytes to gold and silver nanoparticles causes flocculation as a result of the shielding of the repulsive double-layer charges which normally stabilize them. The aggregation can be conveniently monitored photometrically by the decrease

and/or red shift of the plasmon absorption band at about 520 nm.<sup>[35]</sup> Protein adsorption stabilizes the metal particle and the steric repulsion of the particles prevents flocculation. The amount of stabilizer needed to prevent flocculation is determined by increasing the concentration of electrolyte in various nanoparticle preparations, previously coated with distinct amounts of proteins. The amount often corresponds to a single monolayer of proteins attached to the surface of the nanoparticle. Other examples of protein coating through electrostatic interactions include the directed adsorption of heme-containing redox enzymes at citrate-stabilized silver nanoparticles,<sup>[36–38]</sup> and the binding of basic leucine zipper proteins to semiconductor particles that are stabilized with lipoic acid (**1**).<sup>[32]</sup> Similarly, the adsorption of DNA molecules to positive or neutral CdS nanoparticles has been studied in detail.<sup>[39–41]</sup> A different approach, which takes advantage of a combination of various noncovalent interactions, employs specific receptor–ligand binding for the coupling of nanoparticles and biomolecules. For instance, chemisorption of disulfide **3** (Figure 3) to colloidal gold particles provides ligands that are specifically recognized and bound by the complementary receptors (strept)avidin and antibodies that are directed against the biotin moiety.<sup>[42]</sup>

Another approach is based on thiols or disulfides, and phosphane ligands (**1**, **4**, and **5**; Figure 3), which contain terminal carboxy, amino, or maleimide groups. These can be used for the coupling of biological components by means of carbodiimide-mediated esterification and amidation, or reaction with thiol groups. To prepare gold conjugates as probes for histological applications, this strategy is routinely used in the coupling of proteins with well-defined 0.8-nm undecagold nanoclusters, stabilized with arylphosphanes and ligand **5** (Figure 4).<sup>[43]</sup> Furthermore, the monomaleimido gold clusters have been coupled with thiolated DNA oligomers to synthesize probes for homogeneous nucleic acid analyses<sup>[44]</sup> and to prepare nanocrystal molecules (Section 3.2.2).<sup>[45]</sup>

Various syntheses of semiconductor colloids in the presence of thiol ligands,<sup>[9–16]</sup> and in special cases the biosynthesis of cysteine- or glutathione-protected CdS and ZnS colloids,<sup>[46, 47]</sup> or the efficient gram-quantity colloid production of water-soluble ZnS nanocrystal powders<sup>[48]</sup> have been reported. Mercaptoacetic<sup>[\*]</sup> acid stabilized CdTe nanoparticles have been coupled with the  $\epsilon$ -amino groups of the biotin-binding protein streptavidin through covalent amide bonding.<sup>[49]</sup> In the case of silica-coated Cd/chalcogen nanoparticles, the hydroxy groups at the surface can be used for the covalent coupling of biomolecules by using a variety of commercially available silanes such as **6** and **7** (Figure 3). This method is

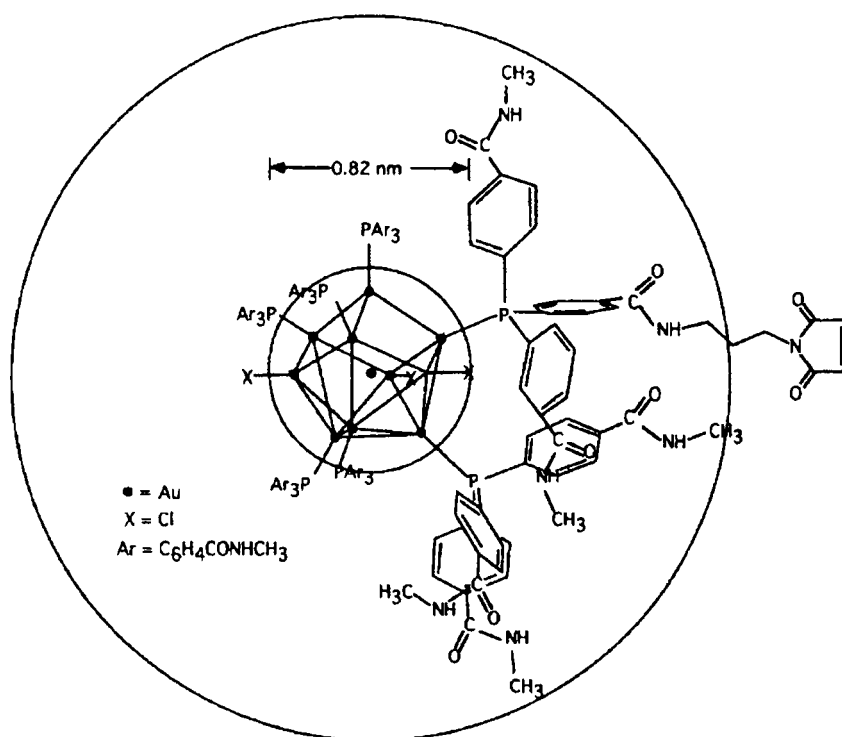


Figure 4. Molecular structure of an undecagold cluster, which is stabilized with phosphane ligands. A single maleimido group is attached per gold cluster to allow chemical coupling of thiolated components. Reproduced from <http://www.nanoprobes.com>.

commonly applied in the biofunctionalization of larger, SiO<sub>2</sub>-based microspheres and has been used to couple SiO<sub>2</sub>-coated core (CdSe)/shell (CdS) particles to biomolecules.<sup>[17]</sup> When the inorganic nanoparticles are covered with a bioorganic matrix, such as the polypeptide core in remineralized ferritin (Section 4.3), the entire spectrum of protein-modification chemistry can be applied for further functionalization and coupling steps.

The above examples demonstrate that some chemical means have already been explored for the physical linkage of inorganic and biological materials. However, there is still a great demand for alternative methods to allow for compensation of typical problems that arise in the biofunctionalization of inorganic nanoparticles. In particular, harsh reaction conditions often lead to the degradation and inactivation of sensitive biological compounds, and ligand-exchange reactions that occur at the colloid surface often hinder the formation of stable bioconjugates. Moreover, the synthesis of stoichiometrically defined nanoparticle–biomolecule complexes is a great challenge, and is particularly important from a molecular engineering point of view to generate well-defined nanoarchitectures.

### 3. Biomolecule-Directed Nanoparticle Organization

Many approaches have been described for the formation of two- and three-dimensional arrays of metal and semiconductor nanoparticles,<sup>[11–13, 15]</sup> for example, random inclusion of the particles into gel and glassy matrices, deposition of the

[\*] In this review, the commonly used prefix “mercapto-” is used rather than the IUPAC-recommended “sulfanyl-”.

particles on structured surfaces, or chemical coupling in solution by means of bivalent crosslinker molecules. An example of the latter is the alkyldithiol-directed aggregation of gold colloids,<sup>[50]</sup> which leads to extended networks of cross-linked nanoparticles. Similarly, biomolecules can also be used as cross-linkers (Figure 5). Initially, the two sets of nano-

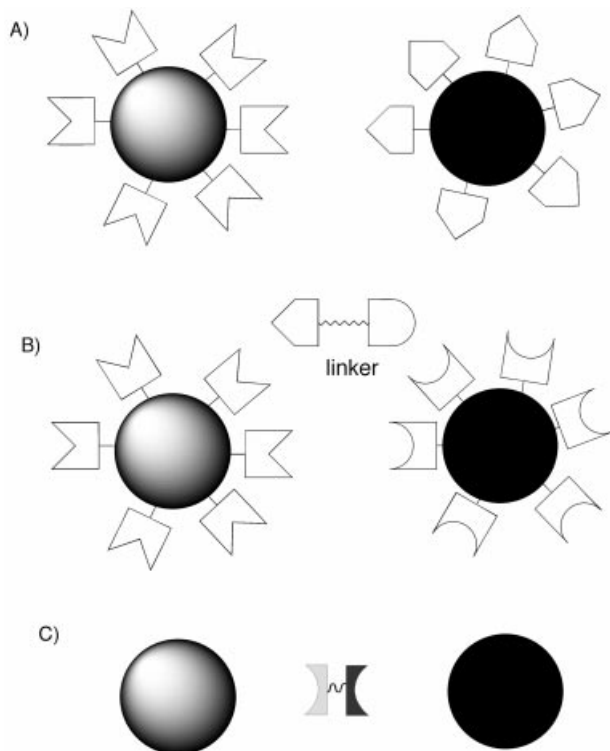


Figure 5. Solution-phase chemical coupling with biomolecular recognition elements. A) Two sets of nanoparticles are functionalized with individual recognition groups that are complementary to each other. B) The particle-bound recognition groups are not complementary to each other, but can be bridged through a bispecific linker molecule. C) A bivalent linker that directly recognizes the surfaces of the nanoparticles is used for aggregation.

particles are functionalized with individual recognition groups that are either directly complementary to each other, or else are complementary to a molecular linker (Figures 5 A and B, respectively). Driven by the biospecific interaction, the two particle batches assemble to form extended nanoparticle networks, which in some cases can grow to the size of macroscopic materials. Rather than just exploring novel, unconventional coupling systems, the major motivation for this approach is based on the unique properties of biomolecular linkers, which promise new applications such as tunable and/or switchable materials. In particular, the tremendous recognition capabilities of biomolecules, and their potential to be addressed and manipulated through biochemical procedures that use designed tools such as enzymes, should provide the basis for entirely novel routes to construct advanced materials rationally.

### 3.1. Protein-Based Recognition Systems

The coupling approaches depicted in Figure 5 have been experimentally realized by using protein-based recognition

systems. One of the first examples took advantage of the specific interaction between antibodies and low molecular weight organic compounds, the so-called hapten groups.<sup>[34]</sup> The latter can be used to cross-link nanoparticles, which are previously coated with antibody molecules that specifically recognize the hapten group (Figure 6). Shenton et al. functionalized gold and silver nanoparticles with immunoglobulins of class G and E (IgG and IgE, respectively), which had a specificity directed against either the D-biotin or the dinitrophenyl (DNP) group.<sup>[34]</sup> To allow the cross-linking of the IgG-functionalized nanoparticles, bivalent linkers that contained two terminal hapten groups were synthesized. The monospecific linker **8**, which is comprised of two DNP moieties, and the bispecific linker **9** were used (Figure 6) to induce the directed assembly of homo-oligomeric or heterodimeric aggregates, respectively. The precipitate formed was characterized by transmission electron microscopy (TEM) and large disordered 3D networks of discrete nanoparticles were found. In the case of the bimetallic Ag–Au networks, however, the degree of integration was not as high as expected for an oligomerization process that is regulated by highly specific biomolecular recognition. This suggests that further progress will require a more sophisticated engineering of the antibody–antigen interface.<sup>[34]</sup>

Other approaches to using protein-based recognition systems to organize inorganic nanoparticles made use of the unique interaction between the biotin-binding protein streptavidin (STV) and its low molecular weight ligand D-biotin.<sup>[42, 51]</sup> The remarkable biomolecular recognition of the water-soluble biotin (vitamin H) by the homotetrameric protein STV is characterized by the extraordinary affinity constant of the STV–biotin interaction of about  $10^{14} \text{ dm}^3 \text{ mol}^{-1}$ , which makes it the strongest ligand–receptor interaction currently known.<sup>[52]</sup> Another great advantage of STV is its extreme chemical and thermal stability. Biotinylated materials are often commercially available or can be prepared by using a variety of mild biotinylation procedures, and thus biotin–STV conjugates form the basis of many diagnostic and analytical tests.<sup>[53]</sup>

Connolly and Fitzmaurice have used the STV–biotin interaction to organize gold colloids that were functionalized by chemisorptive coupling to the disulfide biotin analogue **3**.<sup>[42]</sup> According to the generalized principle depicted in Figure 5 B, the subsequent cross-linking was achieved by the addition of STV as a linker. The immediate change of the sol from red to blue was indicative of the formation of oligomeric networks. This process was also monitored by dynamic light scattering (DLS), which showed that the average hydrodynamic radius of all particles in solution rapidly increased upon STV-directed assembly (Figure 7 A). TEM images revealed networks with an average of 20 interconnected particles that were separated by about 5 nm, which correlates with the diameter of an STV molecule. Since the deposition of the colloid aggregates on the graphite substrate used for TEM characterization might affect aggregation or alteration of existing structures, small angle X-ray scattering (SAXS) was employed to probe the solution structure of the networks (Figure 7 B). The angular dependence of the intensity of the scattered X-ray radiation is quantitatively related to the size



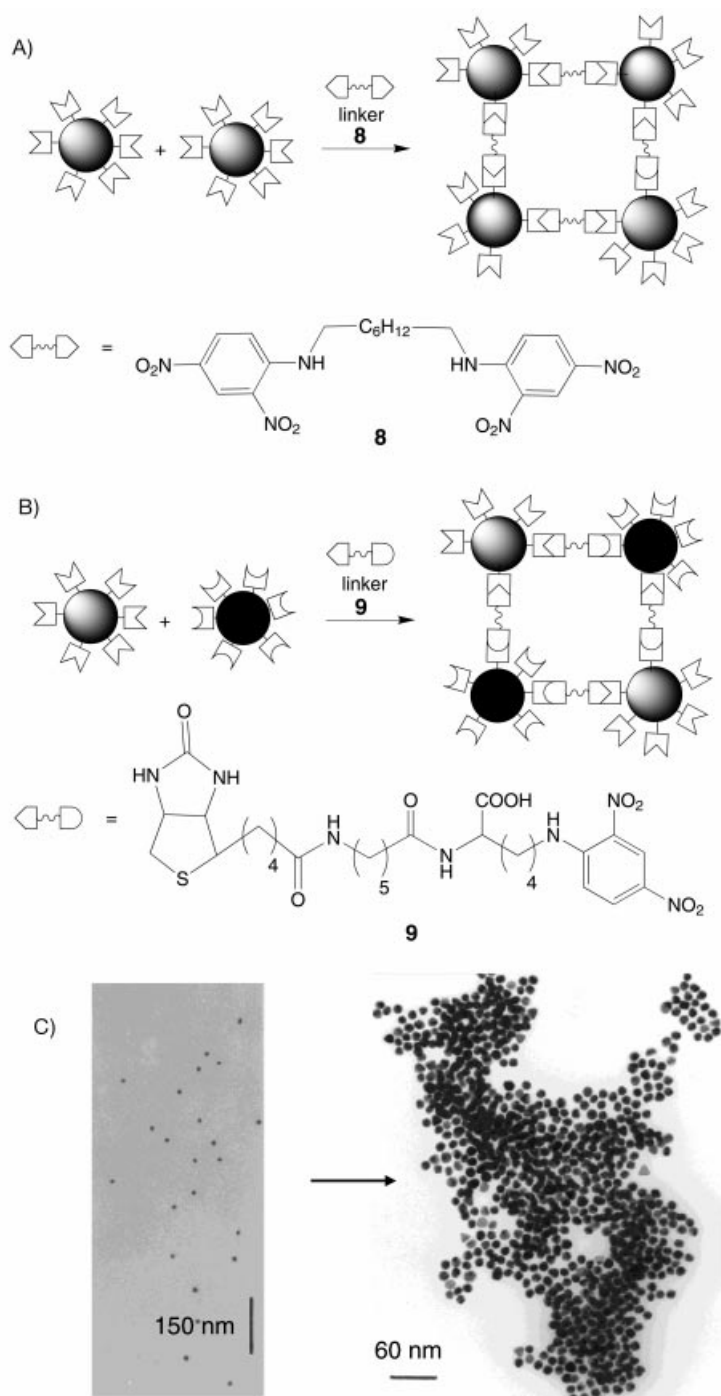


Figure 6. Cross-linking of Ag and Au nanoparticles functionalized with IgG molecules. Bivalent linkers with two terminal hapten groups, either monospecific **8** or bispecific **9**, allow the directed assembly of homo-oligomeric (A) or heterodimeric (B) aggregates, respectively. The TEM images (C) were obtained from colloidal Au/antibody aggregates before (left) and after (right) the addition of linker **8**. (Reproduced, with permission, from ref. [34].)

and shape of the assemblies measured. Thus the pair-distance distribution function (PDDF), which describes the number of pairs of scattering centers as a function of their separation, can be calculated. SAXS of nonaggregated samples showed a single maximum in the PDDF which is indicative of dispersed particles. In the case of STV-coupled nanoparticles, however, SAXS indicated the presence of

trimers of 16-nm-diameter hard spheres (the gold colloids), separated by a minimum distance of 4 nm.<sup>[42]</sup>

In agreement with earlier studies,<sup>[51]</sup> the above findings suggest that the STV–biotin system is versatile for developing novel strategies for assembling nanoparticles in solution or on a substrate. The applicability of the STV–biotin system for generating supramolecular aggregates is enhanced by the availability of various biotin analogues<sup>[54, 55]</sup> and recombinant STV mutants.<sup>[56–61]</sup> A wide range of rate and equilibrium constants can be used to allow the fine-tuning of the dynamic and structural properties of colloidal hybrid materials. It was suggested that extensive use of protein-based assembly could lead to a “factory of the future”, in which the nano-production of spatially defined aggregates takes place by means of the bottom-up assembly of nanoparticles, directed by multiple highly specific biomolecular recognition elements.<sup>[62]</sup> Such a scenario would dramatically benefit from the advances in modern biotechnology, providing us with the technical skills for tailoring novel protein components by means of directed evolution strategies. Recombinant DNA technology has already led to the development of high-affinity artificial linker systems, such as antibodies that are specific against digoxigenin and fluorescein haptens,<sup>[63, 64]</sup> or short peptides, such as the 9-mer FLAG-sequence.<sup>[65, 66]</sup> These coupling systems have proven their applicability in various diagnostic assays, and it seems likely that they are also convenient for generating inorganic nanoparticle networks.

The power of today’s biotechnological methods was recently applied to generate de novo protein linker units that directly recognize distinct surfaces of semiconductor materials, thereby avoiding the necessity of previous chemical functionalization of the particles (Figure 5C). Belcher and co-workers used phage-display techniques to select 12-mer peptides with a binding specificity for distinct semiconductor surfaces.<sup>[67]</sup> Based on a combinatorial library of about  $10^9$  random 12-mer peptides, phage clones were selected for their specific binding capabilities to one of five different single-crystal semiconductors: GaAs(100), GaAs(111)A, GaAs(111)B, InP(100), and Si(100). These substrates were chosen to allow the systematic evaluation of peptide–substrate interactions. Specific peptide binding was found that is selective for the crystal composition (for example, binding to GaAs, but not to Si) and crystal face (for example, binding to GaAs(100), but not to GaAs(111)B). As shown in Figure 8, the clone G12-3 specifically binds to a GaAs pattern, but not to the SiO<sub>2</sub> regions on the same substrate. This clone was even capable of differentiating between two zinc-blend crystal structures, GaAs and AlAs, which have almost identical lattice constants of 5.66 Å and 5.65 Å, respectively. Binding occurred specifically to the GaAs substrate.

The basis for this selectivity, whether it is chemical, structural, or electronic, is still under investigation. The



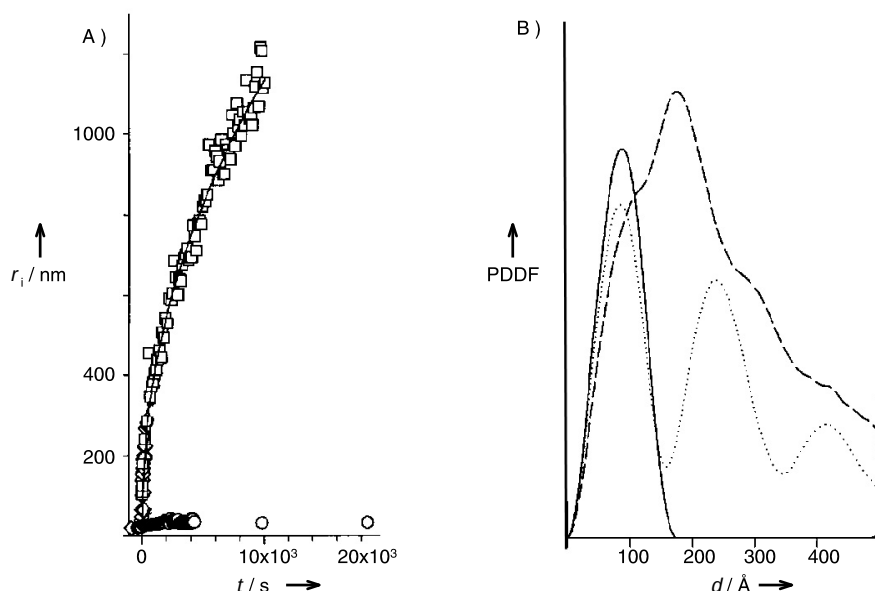


Figure 7. Characterization of streptavidin-mediated cross-linking of Au nanoparticles functionalized with disulfide **3**. A) DLS measurement to determine the average hydrodynamic radius ( $r_h$ ) of all particles in solution. The rapid increase upon the addition of STV is indicative of the aggregation of particles modified with **3** (squares). The control reaction (circles) with unmodified nanoparticles confirms that the increase in  $r_h$  is a result of the proposed specific biotin–STV interactions. B) Small-angle X-ray scattering (SAXS) allows the solution structure of the networks to be probed. Shown are the pair-distance distribution functions (PDDF) under three different conditions. A single maximum is obtained for dispersed Au colloids (continuous line). For STV-induced aggregation (dotted line), the maxima correspond to 16-nm gold spheres separated by 4 nm (consistent with the diameter of STV), whereas for salt aggregation (dashed line) the maxima correspond to touching spheres (data reproduced, with permission, from ref. [42]).

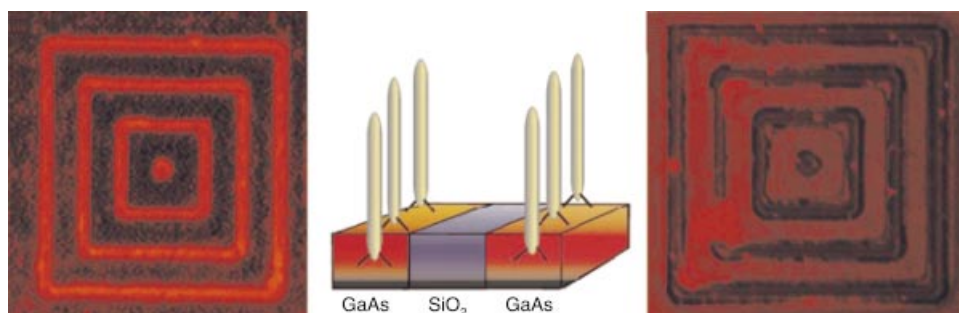


Figure 8. Recognition of an inorganic semiconductor surface by a bacteriophage, selected by phage display of a combinatorial pool of polypeptides. The diagram in the middle depicts the specific binding of the phage to the 1- $\mu\text{m}$  lines of GaAs separated by 4- $\mu\text{m}$   $\text{SiO}_2$  spacers. The phage binding was visualized with a fluorophore-labeled antibody, leading to fluorescent illumination of the GaAs lines (left). In the control experiment, no phage was present, but staining reactions were carried out (right) (adapted from ref. [67], with permission).

various substrates offer individual chemical reactivities and thus the phage selectivity is not particularly surprising. Even in the case of different GaAs surfaces, the composition of surface oxide layers is expected to be different, which in turn might also influence the peptide-surface interaction. However, the sequence analysis of various clones has revealed the presence of an average of about 40% polar functional groups and about 50% Lewis base functional groups. The statistical abundance of the latter is only 34%, which suggests that the interactions between peptide Lewis bases and Lewis acid sites on the substrates are important for specific recognition.<sup>[68]</sup> The evolved peptides may be applied to the generation of bispecific linker molecules with the ability to bind to two

different semiconductor or metal surfaces (Figure 5C), thereby paving the way for novel bottom-up approaches for producing complex nanoparticle heterostructures.<sup>[67]</sup> Furthermore, this work also has implications for the understanding of the fundamental processes of biomineralization, in which protein components control the formation of materials by specifically interacting with growing crystallites (Section 5.4.2).

Mechanical hybrid elements comprised of nanostructured inorganic components and biomolecular devices were constructed from DNA<sup>[69]</sup> and recently from motor proteins.<sup>[70]</sup> The latter was produced from a recombinant  $F_1$ -ATPase motor protein, immobilized on 80-nm diameter Ni columns and coupled with a nanopropeller of approximately 1000 nm length. The ATPase molecule, which is capable of producing about 100 pN nm of rotary torque by the hydrolysis of ATP,<sup>[71]</sup> rotates the propeller with a velocity of about 5 rps.

### 3.2. DNA-Based Nanoparticle Aggregates

The use of biomolecules for developing nanotechnology devices was already envisioned by early researchers, who suggested the use of biological macromolecules as components of nanostructured systems.<sup>[72–74]</sup> DNA is particularly suitable to serve as a construction material in nanosciences.<sup>[75, 76]</sup> Despite its simplicity, the enormous specificity of the adenine–thymine (A–T) and guanine–cytosine (G–

C) Watson–Crick hydrogen bonding allows the convenient programming of artificial DNA receptors. The power of DNA as a molecular tool is enhanced by our ability to synthesize virtually any DNA sequence by automated methods, and to amplify any DNA sequence from microscopic to macroscopic quantities by means of polymerase chain reaction (PCR). Another very attractive feature of DNA is the great mechanical rigidity of short double helices, so that they behave effectively as a rigid rod spacer between two tethered functional molecular components on both ends. Moreover, DNA displays a relatively high physicochemical stability. Finally, nature provides a complete toolbox of highly specific biomolecular reagents, such as endonucleases, ligases, and

other DNA-modifying enzymes, which allow for the processing of DNA material with atomic precision and accuracy on the Ångström level. No other (polymeric) material offers these advantages, which are ideal for molecular constructions in the range of about 5 nm up to a few micrometers. The following section (3.2.1) will focus on the use of DNA as a construction material for the production of nanoparticle assemblies.

### 3.2.1. DNA-Directed Oligomerization of Nanoparticles

Mirkin and co-workers have used DNA hybridization to generate repetitive nanocluster materials (Figure 9). Following the generalized scheme in Figure 5B, two noncomplementary oligonucleotides were coupled in separate reactions

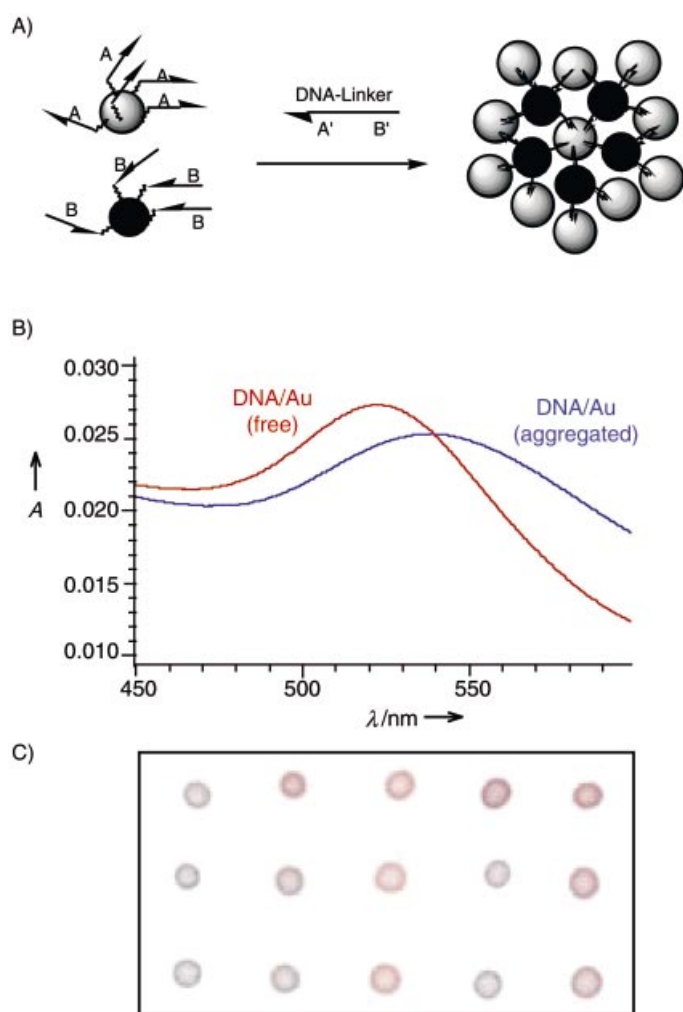


Figure 9. Assembly of gold colloids by using DNA hybridization. A) Two batches of gold particles are derivatized with noncomplementary oligonucleotides, either through 5'- or 3'-thiol groups. The nanoparticles are oligomerized by using a single-stranded nucleic acid linker molecule. Network formation leads to a characteristic change in the plasmon absorption (B). The color change can be used to macroscopically detect DNA hybridization by using an assay in which DNA/Au conjugates and a sample with potential target DNA is spotted on a hydrophobic membrane (C). Red spots indicate the absence of fully matched DNA, whereas blue spots are indicative of complementary target DNA. (Data in C are reproduced, with permission, from ref. [89]. Copyright 1999 American Chemical Society.)

with 13-nm gold particles by means of thiol adsorption.<sup>[77]</sup> A DNA duplex molecule that contains a double stranded region and two cohesive single-stranded ends, which are complementary to the particle-bound DNA, was used as a linker. The addition of the linker duplex to a mixture of the two oligonucleotide-modified colloids led to the aggregation and slow precipitation of a macroscopic DNA-colloid material. The reversibility of this process was demonstrated by the temperature-dependent changes of the UV/VIS spectroscopic properties. Since the colloids contained multiple DNA molecules, the oligomerized aggregates were well-ordered and three-dimensionally linked, as deduced from the TEM analysis. Images of two-dimensional, single-layer aggregates revealed close-packed assemblies of the colloids with uniform particle separations of about 6 nm, which corresponds to the length of the DNA linker duplex.<sup>[77]</sup>

More recent work by the Mirkin group concerns the generation of binary networks. For example, two types of gold clusters of either 40- or 5-nm diameter were modified with individual 12-mer oligonucleotides, and were assembled by using a complementary 24-mer oligonucleotide linker.<sup>[78]</sup> As a result of the specificity of Watson–Crick base-pairing, only heterodimeric A–B composites with alternating particle sizes are formed (Figure 9). In the case of an excess of one particle, satellite-like aggregate structures were generated and characterized by means of TEM. In a similar approach, oligonucleotide-functionalized CdSe/ZnS quantum dots and gold nanoparticles were incorporated into binary nanoparticle networks.<sup>[79]</sup> TEM analysis revealed that the hybrid metal/semiconductor assemblies adopted the expected A–B-structure, and fluorescence and electronic absorption spectroscopy indicated cooperative optical and electronic phenomena within the network materials. The oligomerization of DNA-coated gold nanoparticles depicted in Figure 9 can also be mediated by conjugates comprised of oligonucleotides and the STV protein. Interestingly, the thermodynamically most stable oligomeric networks are only formed upon heating the kinetically controlled adducts produced initially.<sup>[80]</sup>

Further studies of DNA-linked gold nanoparticle assemblies concerned the influence of the DNA spacer length on the optical<sup>[81]</sup> and electrical<sup>[82]</sup> properties of the networks. The experiments provided evidence that the linker length kinetically controls the size of the aggregates, and that the optical properties of the nanoparticle assemblies are governed by the size of the aggregate.<sup>[81]</sup> In contrast, it was found that the electrical properties of dried DNA–nanoparticle aggregates are not influenced by the length of the linker. Although SAXS clearly indicated that distances between particles in solution are related to the length of the linker, the networks collapse upon drying, thereby forming bulk materials comprised of nanoparticles covered with an insulating film of DNA. These materials show semiconductor properties that are not influenced by the linker lengths.<sup>[82]</sup>

Despite these advances, very little is known about the manipulation and tailoring of such nanoparticle networks, for instance, on ways to influence the structure and topography of the DNA hybrid materials subsequent to their formation by self-assembly. Within the context of fundamental studies on DNA-linked nanoparticle networks, the oligomeric aggre-

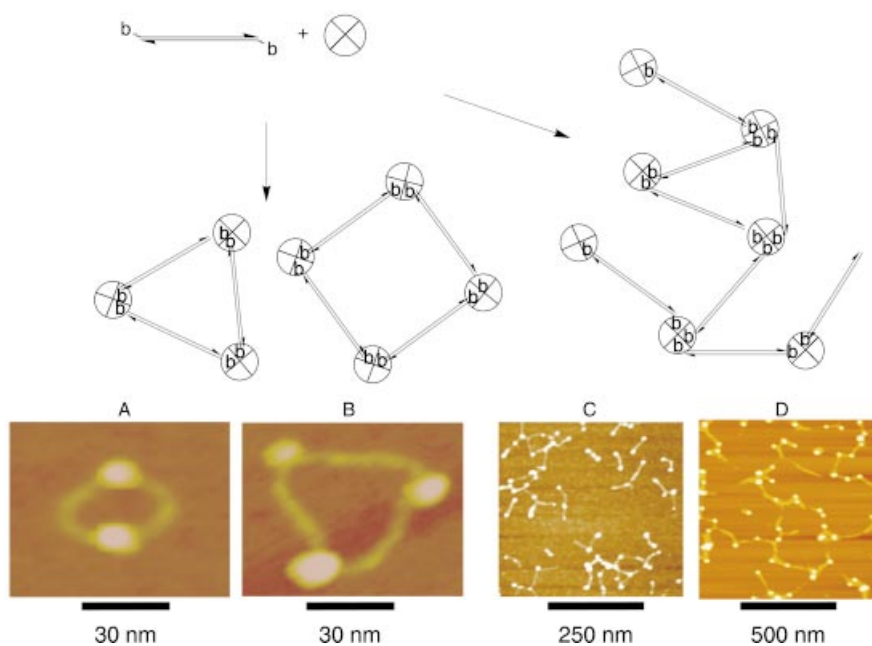


Figure 10. Synthesis of nanoparticle networks by using dsDNA as spacer groups. The dsDNA fragments contain two binding sites (b) attached to the two 5'-ends of the dsDNA. The binding sites are either biotinyl groups that allow cross-linking of the biotin-binding protein streptavidin as a model nanoparticle (A–C), or thiol groups that allow the connection of 5-nm gold colloids (D). Adapted in part from reference [86], with permission.

gates generated from bioorganic streptavidin (STV) particles and bisbiotinylated dsDNA are suitable model systems to gain insight into the properties of complex particle networks (Figure 10). The STV functions as a 5-nm model particle that can undergo a limited number of interconnections to other particles within the network. Either one, two, three, or four biotinylated DNA fragments can be conjugated with STV by means of the high-affinity STV–biotin interaction. This simplifies the complexity of the supramolecular particle networks, and thus allows the convenient analysis of effects that occur from variations, for example, in the immobilization parameters. Furthermore, the size of the dsDNA linker fragments, which are typically about 30 to 170 nm in length, allow convenient direct observation by means of scanning force microscopy (SFM; Figure 10).<sup>[83–86]</sup> These findings are convenient for both disciplines involved: the use of SFM allows insight into biomolecular structure and assembly dynamics; in turn, the well-defined and clearly distinguishable DNA–STV nanostructures can be used as calibration standards for soft materials in the detailed study of tip-convolution effects and of the influence of measuring parameters in SFM analyses.<sup>[87, 88]</sup>

### 3.2.2. Individual Nanoscaled Particle Assemblies

The work of Mirkin and co-workers described above leads to novel hybrid materials with promising electronic and optical properties, potentially useful for applications in materials science,<sup>[24, 89, 90]</sup> and the results also have a tremendous impact on immediate diagnostic applications, in particular, DNA microarray-based nucleic acid analyses (Section 5.2.2). As a result of the enormous potential of these

novel compounds, it is particularly important to overcome limitations which may result from the lack of stoichiometric control during the assembly process. To control the architecture of nanomaterials, spatially defined arrangements of molecular devices are required. For example, to organize metal and semiconductor nanocrystals into ultra-small electronic devices one may consider a linear aggregate of several individual components (Figure 11 A). Similarly, as initially demonstrated for proteins,<sup>[91]</sup> gold nanoclusters that contain a single nucleic acid moiety,<sup>[45, 92]</sup> have been rationally assembled into stoichiometrically defined nanoscale aggregates (Figure 11 B).

To control the stoichiometry and architecture of nanomaterials, Alivisatos and co-workers synthesized well-defined monoadducts from commercially available 1.4-nm gold clusters that contain a single reactive maleimido group and thiolated 18-mer oligonucleotides.<sup>[45, 92]</sup> Subsequent to purification, these conjugates allowed the rational construction

of well-defined nanocrystalline molecules by means of DNA-directed assembly with a single-stranded template that contains the complementary sequence stretches (Figure 11 B).

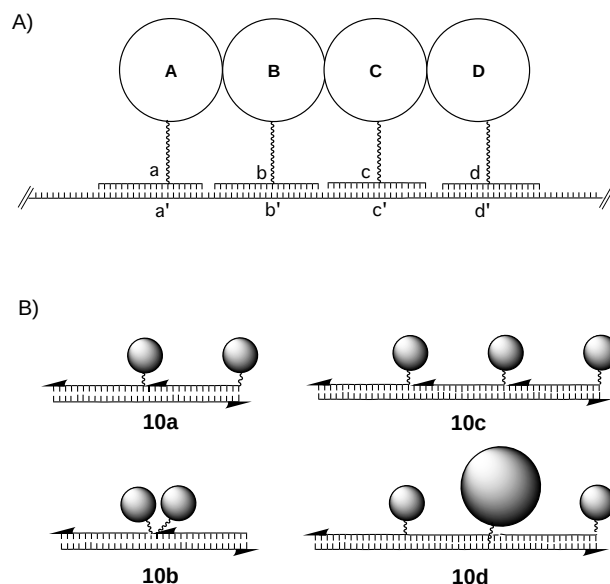


Figure 11. A) DNA-directed assembly of four different nanoscaled building blocks to form a stoichiometrically and spatially defined supramolecular aggregate. B) Self-assembly of nanocrystal molecules by means of DNA hybridization.<sup>[45]</sup> Conjugates from gold particles (shaded spheres) and 3'- or 5'-thiolated oligonucleotides allowed the formation of head-to-head (10a) or head-to-tail (10b) homodimers. A template that contains the complementary sequence in triplicate effects the formation of the trimer 10c. The concept described by A) has also been used in the DNA-directed assembly of proteins,<sup>[91]</sup>  $\beta$ -galactosyl carbohydrates,<sup>[215]</sup> and organometallic compounds.<sup>[216]</sup>

Depending on the template, the DNA-nanocluster conjugates were assembled to generate the head-to-head (**10a**) and head-to-tail (**10b**) homodimeric target molecules in approximately 70 % purity, as determined by TEM analysis. Consistent with model calculations, the center-to-center distances observed in the two isomers were about 3–10 nm and 2–6 nm in **10a** and **10b**, respectively. TEM images also confirmed the structure of trimeric molecules **10c**. More recently, multiple gold nanocrystal aggregates were generated by DNA-directed assembly.<sup>[92]</sup> The preparations, which contain up to three nanoclusters of different sizes organized in several ways, were then purified by electrophoresis. TEM characterization indicated that the nanocrystal molecules have a high flexibility when the DNA backbone is nicked (e.g. **10c**), whereas an unnicked double helix (e.g. **10d**) significantly lowers the flexibility. UV/VIS absorbance measurements indicated changes in the spectral properties of the nanoparticles as a consequence of the supramolecular organization.<sup>[92]</sup>

The concept of using DNA as a framework for the precise spatial arrangement of molecular components was carried out originally with the covalent conjugates of single-stranded DNA oligomers and STV protein.<sup>[91]</sup> The STV–DNA conjugates **11** were used as model systems for a variety of important fundamental studies on the DNA-directed assembly of macromolecules.<sup>[85]</sup> In addition to their model character, the covalent DNA–STV conjugates are also convenient as versatile molecular adapters in the nanoproduction of supramolecular assemblies. The covalently attached oligonucleotide moiety supplements the four native biotin-binding sites of STV with a specific recognition domain for a complementary nucleic acid sequence. This bispecificity allows the use of the DNA–STV conjugates as adapters for the assembly of basically any biotinylated compound along a nucleic acid template.<sup>[85]</sup> As an example, the strong biotin–STV interaction and the specific hybridization capabilities of the DNA–STV conjugates **11** were used to organize gold nanoclusters (Figure 12).<sup>[51]</sup> In this work, 1.4-nm gold clusters that contain a single amino substituent were derivatized with a biotin group, and the biotin moiety was used subsequently to organize the nanoclusters into the tetrahedral superstructure, defined by the geometry of the biotin-binding sites of the STV. Subsequently, the nanocluster-loaded proteins self-assemble in the presence of a complementary single-stranded nucleic acid carrier molecule, thereby generating novel biometallic nanostructures such as **12** (Figure 12). Since the DNA–STV conjugates can be used as a molecular construction kit, this approach even allows the combined assembly of inorganic and biological components to produce functional biometallic aggregates such as **13**, which contain an immunoglobulin molecule (Figure 12). Aggregate **13** was produced from STV conjugates **11a–11e** as well as from conjugates of **11f** and biotinylated antibodies, which were previously coupled in separate reactions. The functionality of the antibody in this aggregate allows the targeting of the biometallic nanostructures to specific tissues, substrates, or other surfaces. This approach impressively demonstrates the applicability of protein–ligand interaction and DNA hybridization for the nanoconstruction of novel inorganic/bioorganic hybrid systems.<sup>[51]</sup>

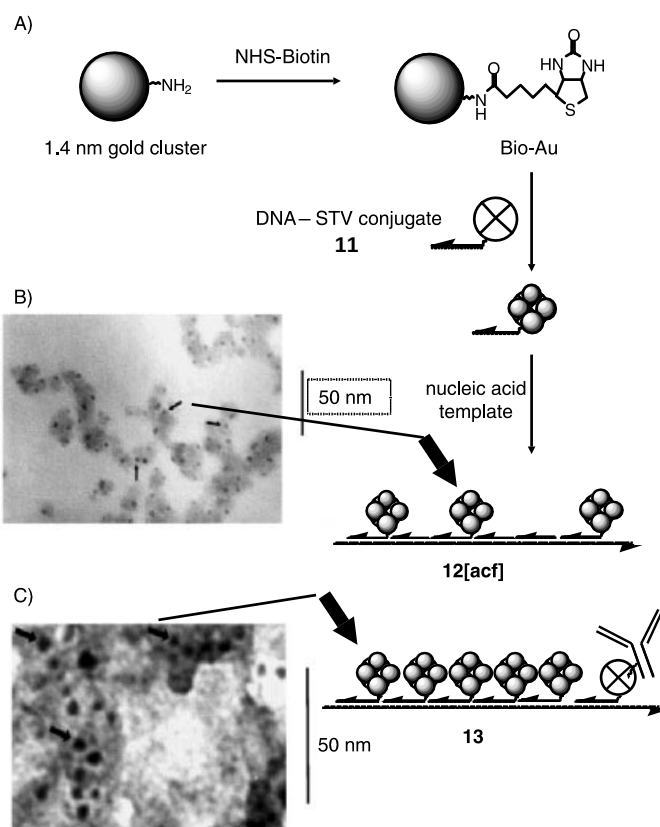


Figure 12. A) DNA-directed assembly of biotinylated gold (Bio–Au) nanoclusters with DNA–STV conjugates **11** as molecular adapters. Specific nucleic acid hybridization leads to biometallic aggregates **12**. TEM images of B) the biometallic aggregate **12[acf]** (the letters in brackets indicate the protein components bound to the carrier) and C) an antibody-containing biometallic construct **13**. Data from reference [51], with permission.

### 3.3. Biomimetic Systems

Analogously to the biomolecule-based oligomerization of nanoparticles (Figure 5), reversible abiotic recognition systems have been applied to the supramolecular organization of nanoparticles. For example, Fitzmaurice and co-workers have reported the heterosupramolecular generation of novel inorganic materials.<sup>[93]</sup> Semiconducting titanium dioxide particles (22 nm) were stabilized with a modified pyridine substituent, which can specifically recognize a uracil derivative that is covalently linked with the electron-acceptor viologen. Hydrogen bonding of the uracil–viologen conjugate to the nanoparticles led to the formation of a heterosupramolecular inorganic–organic hybrid. Band–gap excitation of the self-assembled donor–acceptor system induces electron transfer from the donor (TiO<sub>2</sub> nanocrystallite) to the acceptor (viologen).<sup>[93]</sup> More recently, gold<sup>[94]</sup> and silver<sup>[95]</sup> nanoparticles, stabilized with a monolayer comprised of  $\omega$ -undecylthiol and an alkane thiol **14** that contains a dibenzo-18[crown]-8 moiety, were aggregated by pseudorotaxane assembly. Bis(dibenzylammonium) cations **15** were used as linker units (Figure 13). As indicated from NMR spectroscopic and DLS measurements, the extent of nanocrystal aggregation can be controlled by the relative amount of linker

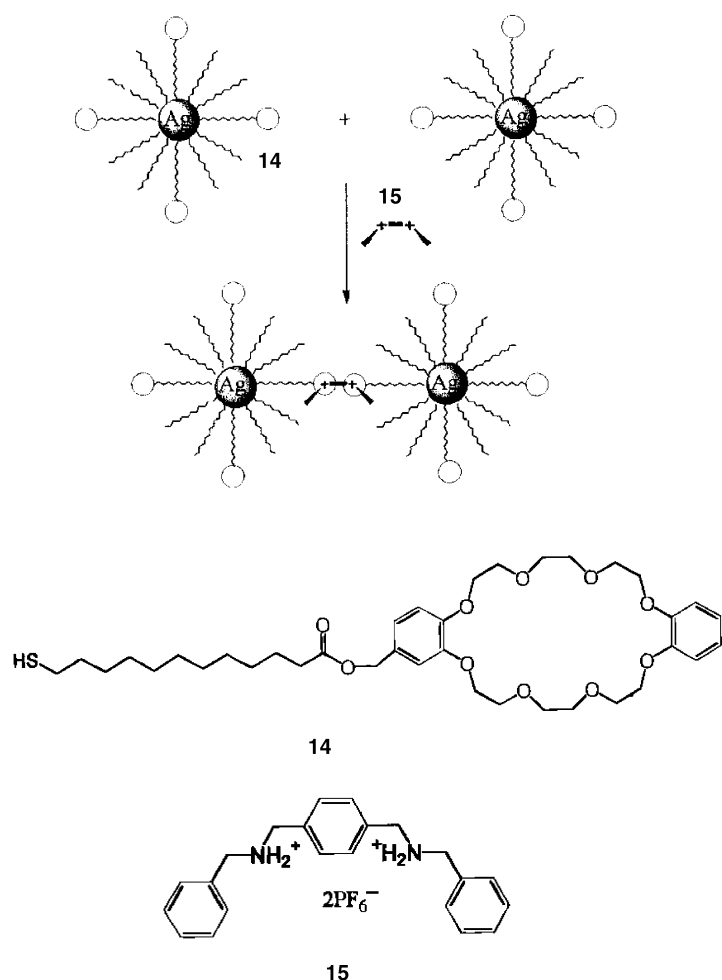


Figure 13. Silver nanocrystals stabilized with a monolayer of an alkyl thiol and **14**, aggregated through pseudorotaxane formation in the presence of bis-dibenzylammonium cations **15** as linker units. Reproduced in part from ref. [95], with permission. Copyright 2000 American Chemical Society.

**15**, or by the addition of inhibitors such as free dibenzo-18[crown]-8.<sup>[95]</sup>

In general, the above self-assembled monolayer (SAM) functionalized gold colloids (SAM-colloids)<sup>[96]</sup> provide a versatile platform for the production of multivalent receptors. In these systems, the number of functional ligands can be controlled during particle formation or displacement reactions,<sup>[96b]</sup> and the mobility of thiol ligands on the surface of the gold colloids can be utilized to create environmentally responsive systems.<sup>[96d]</sup> To this end, gold nanoparticles were coated with a mercaptooctanol SAM that contained two receptor groups: diacyldiaminopyridine **16** and pyrene **17** (Figure 14). The exposure of flavin **18** to the doubly functionalized colloids induced a guest-templated surface rearrangement of the two receptor groups, thereby forming a self-optimized, bivalent binding site for the flavin. Although the imprinted formation of binding sites on two-dimensional planar gold surfaces has previously been reported,<sup>[97]</sup> the nanoparticle-based receptors open novel ways to generate diffusible probes for the recognition of biomolecular protein and cell surfaces.<sup>[96d]</sup> Moreover, this approach should be expandable to catalytically and optically active chemical

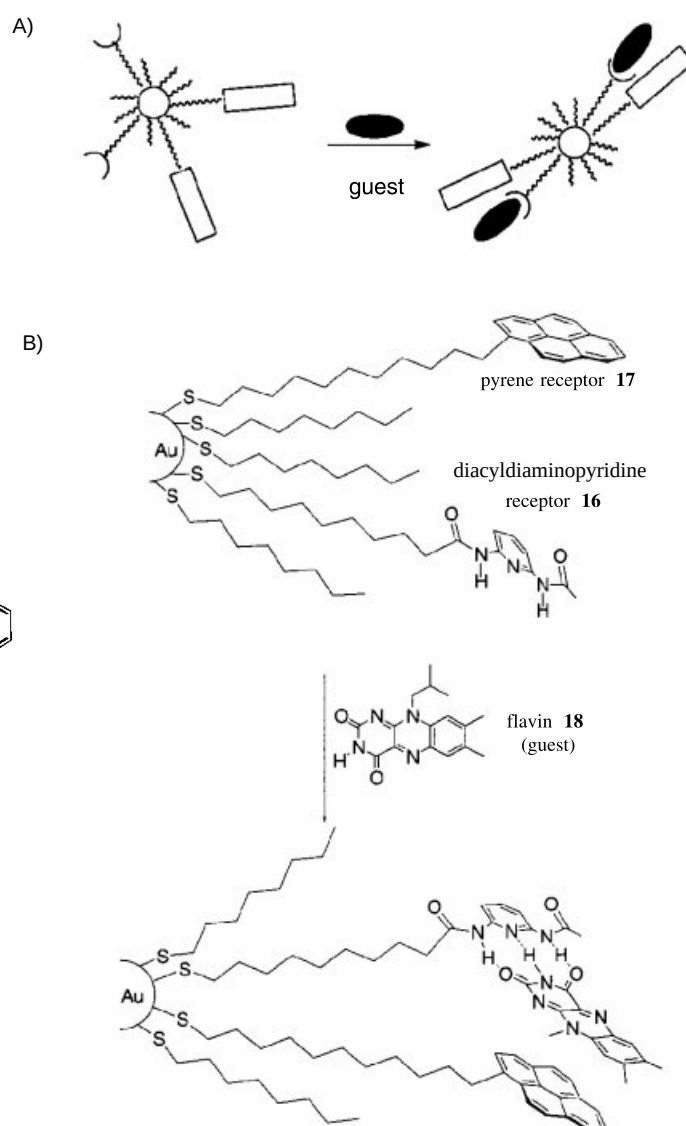


Figure 14. Supramolecular template formation of a polyvalent binding site on SAM-stabilized gold colloids that contain the receptor groups **16** and **17**. Adapted from ref. [96d], with permission. Copyright 2000 American Chemical Society.

groups,<sup>[98]</sup> as well as to SAM-bound biomolecules, such as enzymes, receptors, and antibodies,<sup>[99]</sup> thereby providing novel tools for immunoassays, biosensors, and model systems for the study of membrane diffusion processes.

#### 4. Biotemplating

The electrostatic and topographic properties of biological macromolecules and their derived supramolecular complexes can be used for the synthesis and assembly of organic and inorganic components. In this biological templating approach, regular two-dimensional lattices of bacterial cell surface proteins, nano- and micrometer-sized nucleic acid components, as well as hollow biomolecular compartments such as virus particles, have already been exploited for the generation of nanoparticles and supramolecular architecture.



#### 4.1. Bacterial Surface Layers

Crystalline cell surface layers, S-layers, are the most abundant structures in procaryotic organisms. S-layers consist of multiple copies of a single polypeptide, which spontaneously forms highly regular nanoporous superlattices of varying symmetry (Figure 15). As a result of their extraordinary

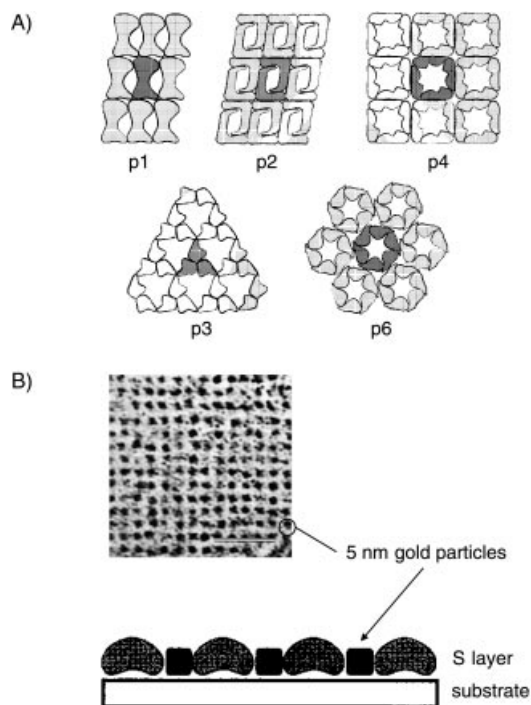


Figure 15. Bacterial surface layers (S layers). A) Different types of S layers with a diagonal (p1, p2), square (p4), or hexagonal (p3, p6) symmetry. B) TEM image of a gold superlattice prepared by using a square S layer lattice mounted on a substrate. Data from ref. [100], with permission.

regularity, S-layers are unique systems to study structure, genetics, and dynamic self-assembly processes of biomolecular components. Furthermore, isolated native and chemically or genetically engineered S-layers offer a broad range of applications ranging from biotechnology, diagnostics, and vaccines to molecular nanotechnology.<sup>[100]</sup> The regular pores within the two-dimensional protein crystals of S-layers have been used as templates for the generation of nanoparticle arrays. Shenton et al. described the synthesis of CdS superlattices by using recrystallized S-layers from *Bacillus stearothermophilus* on electron microscope slides.<sup>[101]</sup> The immobilized S-layer was mineralized by incubation in a CdCl<sub>2</sub> solution, and subsequent treatment with H<sub>2</sub>S. TEM analyses showed that the monodisperse 5-nm CdS nanocrystals grown were organized in a regular array, located within the nanopores of the protein matrix.

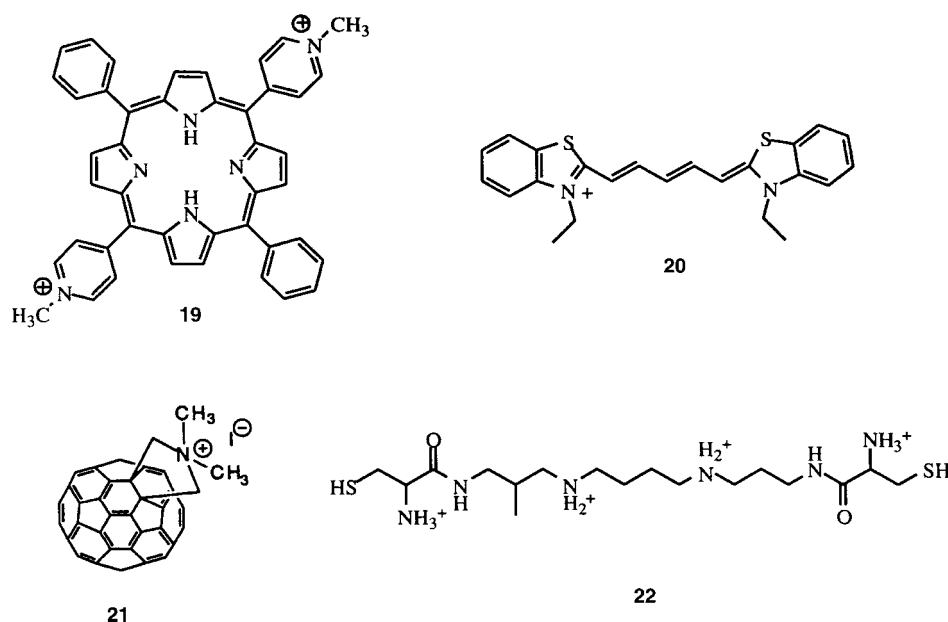
Dieluweit et al. produced a square superlattice of uniform 4–5 nm gold particles with a repeat distance of about 13 nm, by using an S-layer lattice of *Bacillus sphaericus* that was chemically modified to contain thiol groups within the individual protein building blocks.<sup>[102]</sup> For this purpose, a solution of tetrachloroauric(III) acid was used to generate a

thin gold coating of the S-layer. Electron irradiation during TEM investigation of the samples led to the formation of the 5-nm gold particles (Figure 15). Mertig et al. prepared a highly oriented 2-nm platinum nanocluster array by means of biomolecular templating with an S-layer of *Sporosarcina ureae*.<sup>[103]</sup> In contrast to the above approaches, the cluster array was prepared in homogenous solution by treating a suspension of the S-layer with a solution of K<sub>2</sub>PtCl<sub>4</sub> and subsequent reduction using NaN<sub>3</sub>. As indicated by TEM analysis, the Pt clusters have clear metallic character, and the lateral p4-symmetric cluster arrangement is defined by the underlying protein crystal.<sup>[103]</sup>

Recently, Hall et al. reported the precipitation of pre-formed gold colloids by using a hexagonally packed intermediate (HPI) S-layer of *D. radiourans*.<sup>[104]</sup> The HPI layer has a periodic structure of hexameric units and each forms a hollow cone-shaped protrusion with a positively charged cavity with a width of about 2 nm. Exposure of surface-mounted HPI layers to an aqueous solution of citrate-stabilized 5- and 10-nm gold colloids led to the formation of micrometer-sized arrays of regularly spaced nanoparticles, hexagonally organized with center-to-center spacings of about 18 nm, which have the same pattern as the underlying S-layer lattice. In competition experiments with a 1:1 mixture of the 5- and 10-nm particles, the HPI layer exhibited exclusive binding of the 5-nm colloids. The binding of the 10-nm particles led to a preferential adsorption of particles with an average diameter of  $8.0 \pm 1.3$  nm, which is significantly smaller than the mean particle diameter of  $9.7 \pm 2.2$  nm. No long-range periodicity was observed for the adsorption of 20-nm particles or of positively charged 5-nm particles. These results demonstrate that the shape and electrostatic properties of a biotemplate can be used to control the spatial arrangement of micrometer-sized arrays of inorganic nanoparticles. Future developments of this approach are particularly promising since the engineering of only a single gene is required for tailoring the topographical and chemical properties of the S-layer.<sup>[105]</sup>

#### 4.2. DNA-Templated Assembly

The electrostatic and topographic properties of the DNA molecule can also be used for the templated synthesis of supramolecular aggregates of inorganic and organic building blocks. An early example of this approach was reported by Gibbs et al.,<sup>[106]</sup> who demonstrated that porphyrin derivatives that contain cationic side chains (e.g. **19**), form long-range structures on a DNA template. These supramolecular porphyrin assemblies have the same helical sense as the nucleic acid template. Further studies gave more insight into the kinetic and thermodynamic properties of the DNA–porphyrin assemblies.<sup>[107, 108]</sup> The use of DNA as a nanotemplate for the spontaneous assembly of cationic cyanine dye **20** to form supramolecular aggregates was reported by Armitage and co-workers.<sup>[109, 110]</sup> Dye **20** dimerizes in the presence of dsDNA that contains alternating A/T residues. Strikingly, the dimer binding is highly cooperative. The binding of the first dimer greatly facilitates the binding of a second dimer, leading



to the formation of an extended helical cyanine dye aggregate in the case of longer DNA templates. Thus, the DNA structure precisely controls the spatial dimensions of the supramolecular aggregate.

Tour and co-workers have used DNA to congregate macromolecular C<sub>60</sub> fullerene molecules **21**, which were modified with an *N,N*-dimethylpyrrolidinium iodide substituent.<sup>[111]</sup> The electrostatic binding of **21** to the phosphate backbone of DNA could be directly imaged by TEM without the need for heavy metal shadowing or other staining techniques. TEM analysis of the DNA fullerene hybrid materials revealed that the complexation had significantly altered the structure of the DNA. In the case of circular plasmid DNA templates, it was found that the diameter of the hybrid complexes were condensed about fivefold, whereas the thickness of the double helix ranged between 15 and 30 nm, instead of 2 nm for native DNA. This condensation (shortening and thickening) of the dsDNA is probably a consequence of extensive hydrophobic interactions between the fullerene moieties attached to the DNA. Similar condensation effects are well known from polyamines, such as spermine and spermidine, which induce a significant packing of DNA structures. The dynamics of such DNA condensates at the solid–liquid interface have been studied recently by SFM.<sup>[112]</sup> The spermine-induced packing of DNA to generate calibrated bioorganic particles of nanometer-sized dimensions was reported by Blessing et al.<sup>[113]</sup> They used the polymerizable cation **22**, which can bind to the minor groove of B-DNA. The air-induced thiol/disulfide oligomerization of **22** in the presence of a plasmid DNA led to the physical collapse of the DNA, together with the formation of 50 ± 15 nm particles. Such nanometric monomolecular DNA particles are potentially useful for gene delivery.<sup>[114]</sup>

Pioneering work on the DNA-templated generation of metal and semiconductor nanoparticle arrays was carried out by Coffey and coworkers.<sup>[115–118]</sup> They used the negatively charged phosphate backbone of the DNA double helix to

accumulate Cd<sup>2+</sup> ions, which were subsequently treated with Na<sub>2</sub>S to form CdS nanoparticles. For this, solutions of calf thymus DNA and Cd<sup>2+</sup> ions were mixed, and subsequently, molar amounts of Na<sub>2</sub>S initiated the formation of CdS nanoparticles. TEM analysis indicated that the particles were fairly monodisperse with an average diameter of about 5.6 nm.<sup>[115]</sup> Since the actual role of the DNA was vague from this study, later experiments investigated the potential influence of the sequence of the DNA template on the particle binding.<sup>[116]</sup> The adenine content of the DNA particularly affects the size of the nanoparticles, thereby providing indirect evidence for the suggested template mechanism.

Subsequent studies concerned the synthesis of surface-bound mesoscopic nanoparticle aggregates.<sup>[117, 118]</sup> For this purpose, the 3455 base pair circular plasmid DNA pUCLeu4 was used as a template, forming a circle with a diameter of about 375 nm. The DNA was mixed with Cd<sup>2+</sup> ions in solution, and subsequently, the Cd<sup>2+</sup>-loaded DNA was adsorbed on an amino-modified glass substrate. CdS nanostructures were formed by treatment with H<sub>2</sub>S.<sup>[117]</sup> TEM analysis revealed that CdS particles with a diameter of about 5 nm had developed, which were assembled close to the circular DNA backbone. A measurement of its circumference indicated the intactness of some of the DNA molecules. However, various other aggregates with irregular shapes were also observed.

In addition to its use as a nanotemplate, DNA can also be employed to produce micrometer-scaled elements, which are potentially useful in microelectronics. Braun et al. described an example in which a 16-μm λ-DNA molecule, which contained two cohesive ends, was used to bridge the ≈12–16 μm distance between two gold microelectrodes, prepared by standard photolithography (Figure 16).<sup>[119]</sup> Subsequent to the functionalization of the electrodes with individual capture oligonucleotides, the DNA fragment was allowed to hybridize. Successful interconnection of the electrodes was confirmed by optical microscopy of the fluorescently labeled λ-DNA. Next, the sodium ions bound to the phosphate backbone of the λ-DNA were exchanged with Ag<sup>+</sup> ions, and the latter were chemically reduced by hydroquinone. The small silver aggregates formed along the DNA backbone were then used as catalysts for further reductive deposition of silver, eventually leading to the formation of a silver nanowire. This micrometer-sized element with a typical width of 100 nm had a granular morphology, as determined by SFM (Figure 16). Two terminal electrical measurements of the Ag nanowire revealed non-linear, history-dependent *I*–*V* curves, possibly a result of polarization or corrosion of the individual 30–50 nm Ag grains that comprised the wires.<sup>[119]</sup>



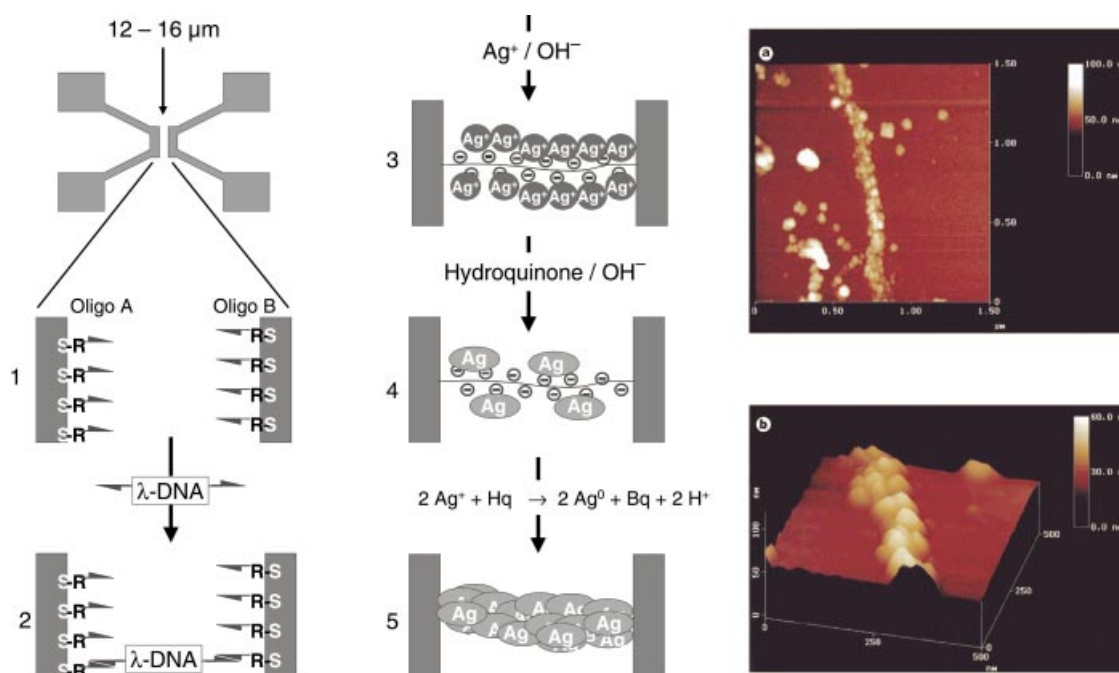


Figure 16. DNA-templated synthesis of a conducting silver wire. SFM images revealed the granular structure of the wire. Reproduced from ref. [119], with permission.

Richter et al. reported the use of  $\lambda$ -DNA for the templated growth of Pd clusters.<sup>[120]</sup> This was achieved by activation of the DNA with palladium acetate and subsequent reduction of the DNA-bound Pd ions with a sodium citrate/lactic acid/dimethylamine borane solution. This led to the formation of DNA-associated one- and two-dimensional arrays of well-separated 3–5 nm Pd clusters.<sup>[120]</sup> Subsequent work has focused on the preparation of highly conductive nanowires by using DNA templates<sup>[121]</sup> as well as on the mechanism of the initial nucleation of the metal clusters.<sup>[122]</sup> The DNA-templated production of mesoscale structures from pre-formed, positively charged 3-nm CdS particles with a thiocholine-modified surface was reported by Torimoto et al.<sup>[123]</sup> As a result of the electrostatic interaction between positively charged nanoparticle surfaces and the phosphate groups of DNA, the particles were found to be assembled into chains, as determined by TEM analysis. The CdS nanoparticles were arranged in a quasi-one-dimensional dense packing, which revealed interparticle distances of about 3.5 nm, correlating with the height of one helical turn of the DNA double strand.<sup>[123]</sup> The electrostatic assembly of lysine-stabilized gold particles on DNA was also reported.<sup>[124]</sup>

The examples described above give initial impressions of how DNA can be utilized as a template in the synthesis of nanometric and mesoscopic aggregates. However, the studies emphasize the importance of fundamental research on how the interaction between DNA and the various binders, such as metal and organic cations, influences the structure and topology of the nucleic acid components involved. Potential applications include the use of DNA strands<sup>[119]</sup> and networks<sup>[125]</sup> to prepare microelectronic devices, or the use of DNA circles<sup>[84, 126–130]</sup> to produce nanoscaled Aharonov–Bohm rings.<sup>[131]</sup>

### 4.3. Bio-Nanoreactors

Nature has developed a variety of protein components that function as carriers or storage devices for metal components. Of these systems, the iron-storage protein ferritin is probably the most intensively studied and best understood.<sup>[132]</sup> Ferritin consists of a central core of hydrated iron(III)oxide encapsulated with a multisubunit protein shell. During formation, the protein shell self-assembles in the first step to form a hollow cage of about 8 nm in diameter. As a consequence, subsequent inorganic mineralization leads to spatially confined nanoparticles of an antiferromagnetic iron oxide similar to the mineral ferrihydrite.<sup>[133]</sup> Because of the pores within the polypeptide shell, the mineral can be removed by reductive dissolution, leading to the formation of a demetallated protein, termed apoferritin.<sup>[134]</sup> It has long been known that apoferritin can be remineralized by chemical procedures,<sup>[135]</sup> and the reconstitution of the ferrihydrite core in ferritin has served to investigate the catalytic properties of the apoferritin with respect to iron(II) oxidation and nucleation of ferrihydrite within the protein compartment.<sup>[136]</sup>

In the early 1990's, Mann and co-workers began to explore the use of ferritin as a nanometer-sized bioreactor for producing metal particles other than the natural ferrihydrite (Figure 17). Horse spleen apoferritin was used as a nanometer-sized reaction vessel to generate manganese oxide and uranyl oxyhydroxide crystals.<sup>[137]</sup> As an example, nanoparticles were synthesized by basic hydrolytic polymerization of uranyl acetate, leading to the formation of uniform 6-nm uranyl oxyhydroxide particles within the spatially confined supramolecular protein cages. Later work concerned the production of magnetic proteins by reconstituting apoferritin with the ferrimagnetic minerals magnetite ( $\text{Fe}_3\text{O}_4$ )<sup>[138]</sup> and

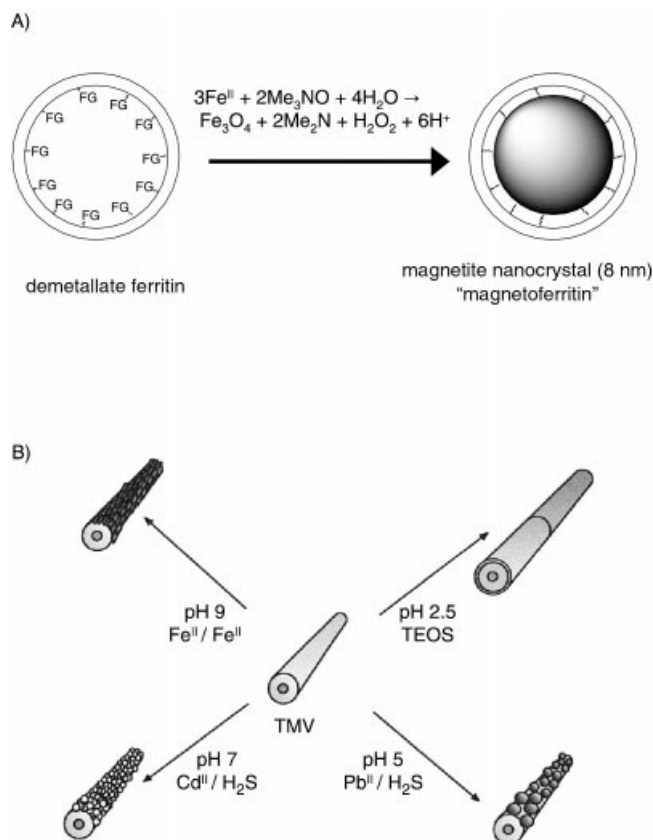


Figure 17. A) Hollow protein cages used as spatially confined bioreactors for the growth of inorganic nanoparticles (FG = functional group, which acts as a nucleation site for biomineralization). B) Synthesis of nanotube composites by using TMV templates. (TEOS = tetraethoxysilane). Reproduced from ref. [144], with permission.

magnetite/maghemite ( $\text{Fe}_3\text{O}_4/\gamma\text{-Fe}_2\text{O}_3$ ).<sup>[139]</sup> As a result of the inner diameter of the nanoreactors, about 6–7 nm diameter crystalline inorganic particles were formed, as determined by TEM. The magnetite/maghemite particles were generated by oxidation of apoferritin with trimethylamino-*N*-oxide, which was loaded with various amounts of iron(II) ions. The magnetic properties were studied by using SQUID magnetometry, and an approximately linear dependence of the superparamagnetic blocking temperature on iron loading was found.<sup>[138]</sup> The monodisperse magnetic proteins might be utilized in biomedical imaging, cell labeling, and separation procedures (Section 5.3). Moreover, the coupling of biotin recognition groups to the ferritin allowed the assembly of the bioinorganic particles with streptavidin connectors (see Section 3.1) to form oligomerized superstructures.<sup>[140]</sup>

In a recent contribution, 15-nm lumazine synthase capsid particles, comprised 60 polypeptide subunits in the form of an  $\approx 8$ -nm hollow cage, were used as a nanobioreactor to produce iron oxide nanoparticles.<sup>[141]</sup> Virus particles are another type of biological structure that can be applied as a template for biomineralization. Virus particles typically consist of several hundred to thousands of protein molecules that assemble in the form of a hollow compartment, which holds the viral nucleic acid. The self-assembly of the tobacco mosaic virus (TMV) is the most comprehensively understood system of biological self-assembly.<sup>[142, 143]</sup> TMV is a stable virion and

can withstand temperatures of up to 60 °C and pH values between 2 and 10. It is made up of 2130 identical protein building blocks, arranged in a helical motif around the viral RNA to form a hollow tube of  $300 \times 18$  nm in size with a central channel with a diameter of 4 nm. The internal and external surfaces of the supramolecular protein assembly consists of a repeated pattern of charged amino acid residues such as glutamate, aspartate, arginine, and lysine, which should offer a variety of nucleation sites for surface-controlled biomineralization. This assumption led Shenton et al. to use TMV particles as a template to generate inorganic–organic nanotube composites by means of template mineralization.<sup>[144]</sup> As indicated in Figure 17B, several routes were explored for the synthesis of nanotube composites that use TMV templates. The sol–gel condensation of TEOS led to the formation of electron-absorbing  $\text{SiO}_2$  rods, which were identified as TMV particles covered with a uniform 3-nm silica shell. Coprecipitation of TMV and  $\text{CdCl}_2$  or  $\text{Pb}(\text{NO}_3)_2$  and  $\text{H}_2\text{S}$  led to the production of hybrid materials in which disordered aggregates of CdS (5 nm) or PbS (30 nm) nanoparticles, respectively, were adsorbed on the external surface of the TMV template. Similar CdS nanoparticles had previously been synthesized by using a ferritin nanoreactor.<sup>[145]</sup> The TMV particles were also subjected to iron oxide mineralization by adding NaOH to an acidic dispersion of iron(II)/iron(III) that contained the virions. This led to a poorly crystalline ferrihydrite deposition on the external surface of the protein template. In contrast, slow aerial oxidation of TMV in anaerobic iron(II) solution yielded virions covered with an even 2 nm thick iron oxide film.<sup>[144]</sup>

Spherical virus protein cages were used for the host–guest encapsulation of inorganic and organic materials. Douglas and Young used well-defined cowpea chlorotic mottle virus (CCMV) particles, obtained from in vitro assembly of purified viral-coat protein subunits (Figure 18).<sup>[146, 147]</sup> Each protein

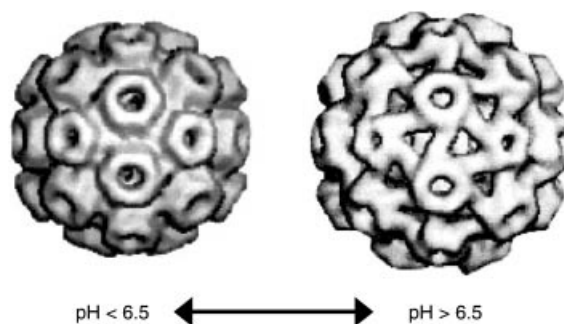


Figure 18. Cryoelectron microscopy and image reconstitution of the CCMV virus. The structure in an unswollen condition at low pH values (left) and in a swollen condition at high pH values (right). Swelling results in the formation of 60 pores with a diameter of 2 nm. Reproduced from ref. [146], with permission.

building block has nine basic amino acid residues (arginine or lysine), which point into the interior of the  $\approx 18$ -nm cavity. Thus, the positively charged inner compartment of the  $\approx 28$ -nm diameter CCMV particles provides a suitable interface for inorganic crystal nucleation, such as the growth of polyoxometalate species, paratungstate and decavanadate.

For this purpose, a pH-dependent gating mechanism of the CCMV virions was used. At pH values greater than 6.5, CCMV particles swell up by about 10 %. This leads to the formation of 60 separate 2-nm pores, which allow free molecule exchange between the cavity and the bulk medium. In contrast, no exchange of large molecules occurs at pH values lower than 6.5. Therefore, empty virions were incubated with inorganic molecular tungstate ( $\text{WO}_4^{2+}$ ) at pH 6.5, concentrated and subsequently washed at pH 5, at which point the pores in the protein shell close and the tungstate ions oligomerize. The latter process is a crystallization that yields uniform 6.7-nm diameter nanocrystals. Besides the polyoxometalate species, organic polymer nanobeads have also been produced within the spatially constrained virions. The pH-dependent encapsulation of an anionic polymer (polyanetholsulfonic acid) was carried out at pH 7.5, and the subsequent lowering of the pH to 4.5 resulted in the formation of polymer-loaded virus particles of uniform size distribution, as determined by TEM analysis.<sup>[146]</sup>

The above examples demonstrate that biomolecular compartments are suitable in applications for the formation of nanophase materials as well as for supramolecular encapsulation strategies. It is safe to assume that future developments will focus on the generation of altered protein building blocks for the self-assembly of novel improved biological reaction compartments using today's powerful techniques of molecular biology. Based on the native proteins, rational or random mutagenesis will allow us to design recombinant proteins that have optimized recognition capabilities for the assembly of distinctively shaped superstructures and possess appropriate amino acid residues for the effective nucleation of organic and inorganic nanomaterials.

## 5. Applications

From a biotechnological point of view, the term application is strictly associated with commercial products that (at least potentially) address significant markets. With respect to this stringent definition, real applications of the bioinorganic hybrid materials described above are rare at the present time. However, the fusion of biotechnology with materials science has great potential for generating advanced materials, and in turn, today's advanced materials and devices, as well as modern physicochemical techniques can be applied to solve biological problems. Thus, bioanalytical applications are currently the most practical outcome of the interdisciplinary work. Biological sciences greatly benefit from the refinement of analytical techniques, originally developed for material and physicochemical sciences.

### 5.1. Physicochemical Techniques

The most prominent physical characterization method for biological systems is scanning force microscopy (SFM). SFM has become a tremendously useful tool to map biological surfaces with high spatial resolution. In particular, the application of functionalized tips enables biospecific chemical

force microscopy.<sup>[148]</sup> Moreover, SFM allows biomolecules to be stretched, and thus brings novel insight into the exploration of nucleic acid conformation and protein folding.<sup>[149]</sup>

Surface plasmon resonance (SPR) spectroscopy is another advancing technique which contributes to the comprehensive understanding of biomolecular structure and dynamics.<sup>[150]</sup> The surface-sensitive optical technique of SPR imaging is used to characterize ultrathin organic and biopolymer films at metal interfaces in a spatially resolved manner, for example, at the boundary between a gold layer and a liquid phase. Because of its high surface sensitivity and its ability to measure in real time the interaction of unlabeled biological molecules with arrays of surface-bound species, SPR imaging has been successfully implemented in the characterization and monitoring of biomolecular interactions, such as heterogeneous antibody–antigen binding at surfaces, and the study of solid-phase DNA hybridization.

The extensive studies of interfacial processes between biomolecules and metal surfaces have also led to the refinement of distinguished spectroscopic methods, in particular, surface-enhanced Raman scattering (SERS). In SERS, molecules adsorbed on roughened gold or silver surfaces exhibit vibrational spectral intensities that are enhanced up to 100 000-fold.<sup>[151]</sup> Since the SERS method only detects principally the first monolayer adsorbed on the enhancing substrate, this technique allows the elucidation of specific interactions that occur between solid substrates and adsorbed biomolecules. For example, citrate-reduced silver colloids demonstrate excellent SERS enhancements and good biocompatibility. Thus, this substrate has been used for detailed studies of adsorbed proteins, which indicated that several enzymes such as chlorocatechol dioxygenase,<sup>[36]</sup> cytochrome c (Cc),<sup>[37]</sup> and several cytochromes P450,<sup>[38]</sup> retain their activity while adsorbed on this type of metal surface. However, since colloidal Ag preparations are often obtained as a heterogeneous population of different particle sizes and shapes, this leads to poorly reproducible optical properties and protein aggregation characteristics. On the other hand, although methods for preparing monodisperse protein-coated gold nanoparticles are well developed,<sup>[152]</sup> Au colloids unfortunately reveal significantly lower SERS enhancement factors with visible excitation, and thus SERS intensities of biomolecule/Au colloid conjugates are relatively weak.

Natan and co-workers reported a solution to this problem: the small heme-containing redox enzyme Cc can be used as a model system which adsorbs to citrate-stabilized gold surfaces in an oriented fashion through its lysine-rich heme pocket.<sup>[35]</sup> Au nanoparticles with a diameter of 12 nm were coated with Cc, and the resulting conjugates were used to deliver the protein to colloidal Ag for SERS measurements (Figure 19). SERS spectra of the Ag/Cc/Au sandwich aggregates indicated that the protein retains its native conformation. The advantage of this approach is that the Ag/Cc/Au sandwich can be made at the well-defined concentration of the Au carrier particles, and the SERS measurement can be made at another concentration of the Ag colloids (which may be less defined). Moreover, this approach allows one to overcome the normal dependence of SERS intensity on increasing distance from the Ag substrate by positioning the analyte between two metal

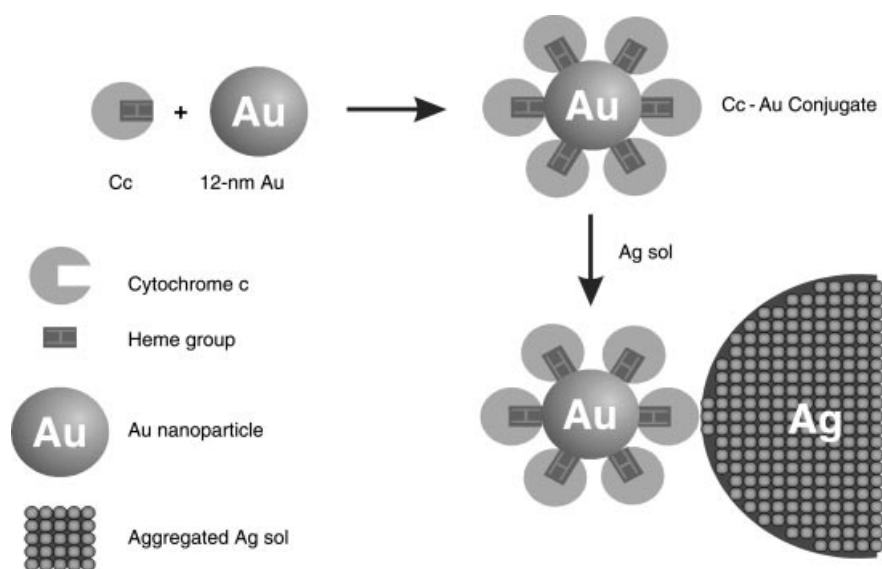


Figure 19. Formation of a metal–Cytochrome c (Cc) metal sandwich for SERS. The heme-containing redox enzyme Cc adsorbs to citrate-stabilized gold particles in an oriented fashion through its lysine-rich heme pocket. The resulting conjugates are used to deliver the protein to colloidal Ag for SERS measurement. Adapted from ref. [35].

surfaces.<sup>[35]</sup> It was observed that the metal/protein/metal sandwich conjugates revealed significantly higher electromagnetic fields between the particles.<sup>[153]</sup> The strong interparticle electromagnetic coupling was deduced from the wavelength-dependent SERS enhancement ratios, and the presence of wavelength-shifted single-particle surface-plasmon bands. Thus, such studies are important for experimental verification of theoretically predicted electromagnetic effects between closely spaced metal nanoparticles.<sup>[153]</sup> In addition to basic studies, the SERS technique also has the potential for the development of analytical methods, for example, as a structure-specific detection method for capillary electrophoresis,<sup>[154]</sup> or for quantifying dsDNA in bioanalytical assays.<sup>[155]</sup>

## 5.2. Nanoparticles as Biolabels

### 5.2.1. Protein-Coated Particles

Gold nanoparticles that are functionalized with proteins have long been used tools in the biosciences.<sup>[156]</sup> For instance, antibody molecules adsorbed on colloidal gold (10–40 nm) are routinely used in histology, thus allowing the biospecific labeling of distinguished regions of tissue samples and subsequent TEM analysis. More advanced small gold clusters with a diameter of 0.8 or 1.4 nm can be used for the site-specific labeling of biological macromolecules (Figure 4).<sup>[43, 157, 158]</sup> These labels have a number of advantages over colloidal probes, including better resolution, stability, and uniformity. Moreover, their small size improves the penetration and more quantitative labeling of antigenic sites. The 1.4-nm particles are the smallest gold clusters that can be seen directly under a conventional electron microscope, allowing a spatial resolution of about 7 nm when covalently attached to antibody fragments. The visibility of the clusters

can be improved by a wet-chemical silver enhancement step for use in electron or light microscopy for histological purposes, or to detect picogram amounts of antigens in immunoblots.<sup>[159]</sup>

The silver enhancement can also be applied for the electrical sensing of biological binding events, for instance, through the short circuiting of microelectrodes (Figure 20). Gold nanoparticles are immobilized in the gaps of microelectrodes through biospecific interactions such as immunosorption of antibodies.<sup>[160]</sup> The colloids serve as catalytic cores for the reductive deposition of a conducting layer of silver, which short circuits the two electrodes. The resulting decrease in ohmic resistance is used as a positive signal for the sensing of the biospecific interaction to be detected. Other approaches used electrode-immobilized layers of gold colloids for the adsorption of the redox

enzyme horse-radish peroxidase to prepare biosensors for the electrocatalytic detection of hydrogen peroxide.<sup>[161–163]</sup> Gold nanoparticles can also be applied to enhance detection limits in SPR-based real-time biospecific interaction analysis (Figure 21).<sup>[164, 165]</sup> The dramatic enhancement of SPR biosensing with colloidal Au was initially observed in a sandwich immunoassay in which Au nanoparticles were coupled to a secondary antibody. The latter was used to detect a primary antibody bound through specific immunosorption to the antigen immobilized on the gold sensor surface. The immunosorptive binding of the colloidal gold to the sensor surface led to a large shift in plasmon angle, a broadened plasmon resonance, and an increase in minimum reflectance, thereby allowing picomolar detection of the antigen.<sup>[164]</sup> Similarly, an improvement in sensitivity by about 1000-fold was obtained in nucleic acid hybridization analysis, when a colloidal gold/oligonucleotide conjugate was used as a probe (Figure 21).<sup>[165]</sup> These results suggest that the detection limit of SPR<sup>[150]</sup> now begins to approach that of traditional fluorescence-based DNA hybridization detection.

The use of colloidal silver plasmon-resonant particles (PRPs) as optical reporters in biological assays has recently been described.<sup>[166]</sup> PRPs can readily be observed individually with a microscope configured for dark-field microscopy, with standard white-light illumination. The use of PRPs, whose surfaces are coated with bioactive ligands, was illustrated in a model sandwich immunoassay for goat anti-biotin antibody. Because PRP labels are nonbleaching and bright enough to be identified and counted rapidly, they might soon be applicable in ultrasensitive assay formats based on single-target molecule detection.<sup>[166]</sup>

Semiconductor nanoparticles are also powerful fluorescent probes, which can be used for the labeling of biological components.<sup>[17, 18, 32, 167]</sup> The quantum dots have several advantages over conventional fluorescent dyes. Their fluorescence

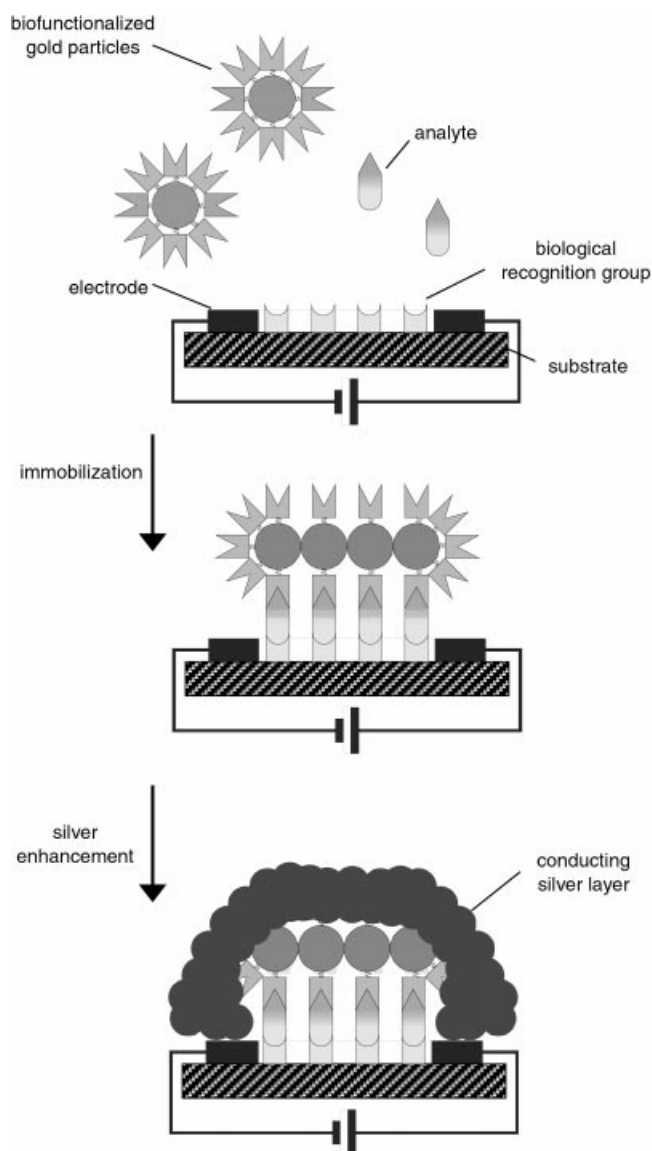


Figure 20. Electrical detection of biorecognition processes. Receptor groups such as antibodies or DNA oligomers are immobilized in the gap between two microelectrodes and used as a capture agent to bind complementary target molecules specifically. In a sandwich-type assay, the captured analyte is tagged with colloidal gold by a second biological recognition unit. Subsequent reductive deposition of silver leads to the formation of a conducting metal layer which short-circuits the two electrodes.

absorption and emission are conveniently tunable by their size and material composition, and the emission peaks have a narrow spectral linewidth (Figure 22). Typically, emission widths are 20–30 nm (full-width at half-maximum, FWHM), which is only one third of the emission linewidth of a conventional organic dye. The high quantum yields often range from 35–50% for core (CdSe)–shell (ZnS) nanoparticles. Moreover, quantum dots are about 100 times as stable against photobleaching as organic dyes, and they often reveal a long fluorescence lifetime of several hundred nanoseconds. This allows time-delayed fluorescence measurements, which can be used to suppress the autofluorescence of biological matrices.<sup>[168]</sup>

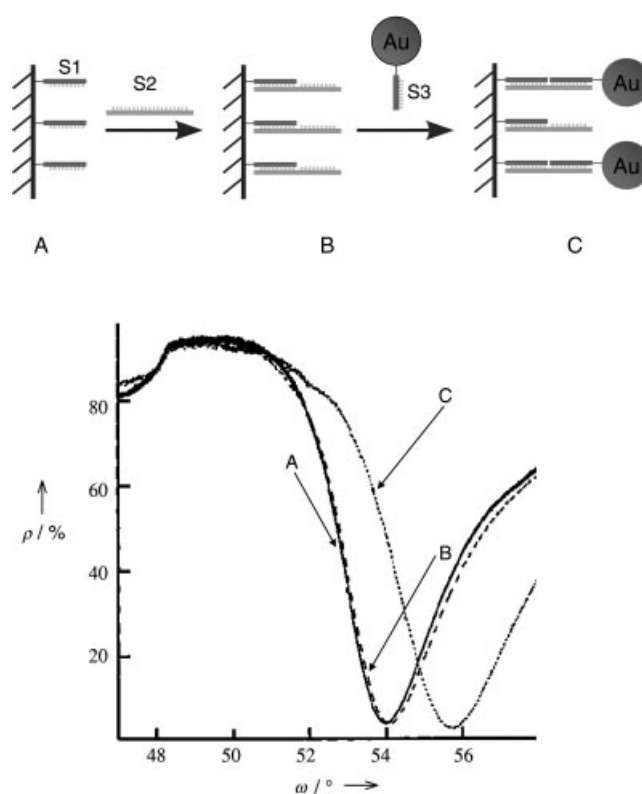


Figure 21. Sensing of DNA hybridization by means of SPR. A gold sensorchip functionalized with capture oligonucleotides S1 (A), is used to bind complementary target DNA S2 specifically (B). Introduction of a colloidal gold–oligonucleotide conjugate S3 allows the detection of the surface-immobilized complementary target (C). The corresponding SPR curves (reflectance  $\rho$  as a function of the SPR angle  $\omega$ ) show the large increase in  $\omega$  in the transition from B to C, compared to that from A to B. Data from ref. [165], with permission. Copyright 2000 American Chemical Society.

Bruchez, Jr. et al. used core (CdSe)–shell (CdS) nanoparticles that were coated with an additional layer of silica for biolabeling.<sup>[17]</sup> The silica layer makes the nanoparticles water soluble and also allows covalent coupling of functional groups by means of organosilane chemistry. To demonstrate this principle, mouse fibroblast cells were labeled with two different sizes of nanocrystals. Green, 2-nm particles that were coated with methoxysilylpropyl urea and acetate groups were found to bind specifically to the cell nucleus by electrostatic interactions. Red, 4-nm biotinylated nanocrystals were used to label F-actin filaments, owing to the biotin–STV interaction. The surface of the nanocrystal was covalently modified with biotin groups, and the biotinylated nanoparticles were used for the specific staining of the actin filaments, previously labeled with phalloidin–biotin conjugate and STV. The dual labeled fibroblast were imaged with a conventional wide-field confocal fluorescence microscope, using a mercury lamp as a light source with a fluorescein isothiocyanate excitation filter and a single long-pass detection filter. Despite this simple setup, both colors were clearly visible simultaneously, and the green and red labels could be distinguished with the naked eye (Figure 22).<sup>[17]</sup>

Chan et al. used 4.2-nm ZnS-protected CdSe quantum dots, stabilized with a layer of mercaptoacetic acid.<sup>[18]</sup> The latter



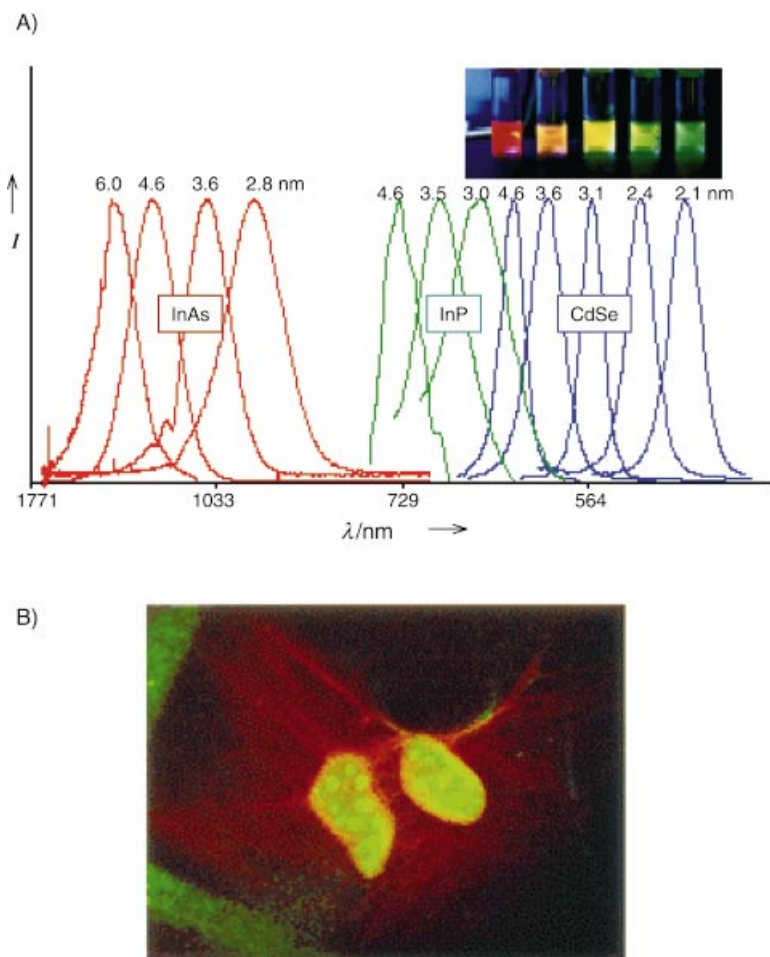


Figure 22. A) Fluorescence emission spectra of semiconductor quantum dots; B) cross-section through dual-labeled mouse fibroblasts. The actin fibers are stained red. The nonspecific labeling of the nuclear membrane by both the red and the green probes results in a yellow color. Reproduced from ref. [17], with permission. Copyright 1998 American Association for the Advancement of Science.

improved the water solubility of the nanoparticles, and the carboxy groups also allowed the covalent coupling to various biomolecules by cross-linking to reactive amino groups. The quantum dots were coupled with transferrin, a protein that induces receptor-mediated endocytosis.<sup>[169]</sup> A HeLa cell culture was incubated with the resulting nanoparticle–protein conjugates, and confocal microscopy revealed that the conjugates were transported into the cell. (HeLa cells are epithelial cells; they were isolated from a cervix carcinoma of a patient, *Henrietta Lacks*, and were the first human cells from which a permanent cell culture was established.) In another example, the suitability of the nanocrystals for sensitive immunoassays was investigated. Conjugates with IgG molecules were prepared and subjected to immunoprecipitation by using a complementary antibody with a binding specificity for the particle-bound proteins. The extensive aggregation observed in this simple experiment clearly indicated that the attachment of nanocrystals does not harm the functionality of the biomolecule.<sup>[18]</sup> In a recent continuation of this work, micrometer-sized polymer particles were tagged with various quantum dots to achieve an optical “barcode” for biomolecules.<sup>[170]</sup>

Mattoussi et al. recently described a novel approach to the coupling of biomolecules with semiconductor quantum dots.<sup>[32]</sup> They prepared a recombinant protein linker that contained a basic leucine zipper motif, which induces a net positive charge, thereby allowing electrostatic binding to negatively charged semiconductor quantum dots (Figure 23). Functional proteins, such as bioactive enzymes or binding proteins can be attached to the leucine zipper motif through the flexible linker chain by means of genetic engineering. The use of a model fusion protein which consists of the maltose binding protein (MBP) allowed the preparation of biofunctionalized 4-nm CdSe/ZnS quantum dots that containing about 19 protein molecules per nanoparticle. The conjugates had the full biological functionality of the MBP as well as the undisturbed photoluminescent properties of the quantum dots. Moreover, it was observed that the binding of the leucine zipper is beneficial to the stability of the nanoparticle stability in aqueous solutions.<sup>[32]</sup> Stable, water-soluble oligonucleotide conjugates were recently prepared from hydroxy-capped quantum dots.<sup>[171]</sup> These nanoparticles can be used as labels in FISH (fluorescence in situ hybridization).

Besides problems of solubility, physicochemical stability, and quantum efficiency of the semiconductor nanocrystals, which still remain to be solved, the routine application of such nanoparticles as biolabels is still controversial, particularly because of general environmental concerns regarding the use of highly toxic cadmium compounds in biomedical diagnostics. As a result of

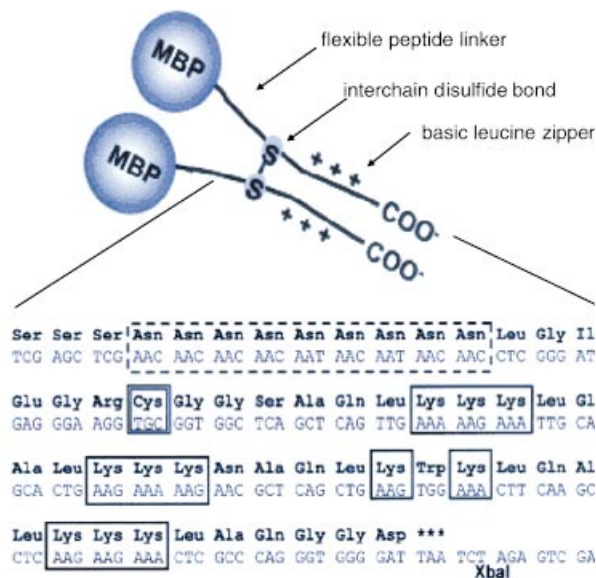


Figure 23. Recombinant protein linker which contains a functional protein moiety, MBP, and a basic leucine zipper motif. The primary amino acid sequence indicates the flexible peptide linker (boxed with dashed lines), the cysteine residue for covalent interchain linking (double boxed), and the lysine residues, which contribute to the net positive charge of the leucine zipper (single boxed). Reproduced from ref. [32], with permission. Copyright 2000 American Chemical Society.



their lower toxicity, colloids made up of other materials such as the chemically stable, highly luminescent, and less toxic  $\text{LaPO}_4\text{:Ce,Tb}$  nanocrystals<sup>[172]</sup> might provide a solution to this problem.

### 5.2.2. Nucleic Acid Coated Particles

Whereas protein-coated gold colloids have long been used in bioanalytical techniques, applications of DNA-functionalized Au particles were only introduced five years ago by Mirkin and co-workers.<sup>[77, 89]</sup> The DNA-directed nanoparticle aggregation depicted in Figure 9 can be used for simple and economically viable sensors in biomedical diagnostics, for example, for the detection of nucleic acids from pathogenic organisms. Although detailed studies of the optical phenomena associated with the supramolecular colloid assembly are still in progress,<sup>[81]</sup> several analytical applications that allow the detection of nucleic acids in homogenous solutions have already been reported.<sup>[44, 89, 173]</sup> Furthermore, the use of DNA-functionalized nanoparticles in the heterogeneous nucleic acid hybridization with capture oligonucleotides attached to solid supports have recently attracted much attention. Mirkin and co-workers<sup>[174]</sup> as well as other groups<sup>[175–180]</sup> have reported the DNA-directed immobilization of gold nanoparticles to form supramolecular surface architecture. 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 analyses.<sup>[181]</sup> The latter approach is based on the reduction of silver ions in the presence of gold particles (Figure 24) and allows a 100-fold increase in sensitivity over conventional fluorescent DNA detection.<sup>[182]</sup> The size-selective light scattering of Au nano-

particles with diameters of 50 and 100 nm has been used for the two-color labeling of oligonucleotide arrays.<sup>[183]</sup>

The use of colloidal gold nanoparticles also allows a signal enhancement in the DNA hybridization detection by means of a quartz crystal microbalance,<sup>[31, 184–186]</sup> angle-dependent light scattering,<sup>[187]</sup> and surface-plasmon resonance (Figure 21).<sup>[165]</sup> Willner and co-workers recently reported the use of DNA-functionalized 2.6-nm CdS nanoparticles for the photoelectrochemical detection of nucleic acid hybridization. An array of quantum dots was assembled at the surface of an electrode by using the specific hybridization of complementary DNA fragments. Upon irradiation, a photocurrent is generated which is proportional to the amount of target DNA.<sup>[188]</sup>

DNA-tagged proteins can also be readily immobilized on DNA-coated gold nanoparticles.<sup>[99]</sup> This approach has several advantages over the conventional methods to adsorb proteins on colloidal gold. Not only do the biofunctionalized nanoparticles retain the undisturbed recognition properties of the proteins immobilized, but they also reveal an extraordinary stability that even allows the regeneration of the DNA/Au particles. The bioinorganic hybrid components have been used as reagents in a sandwich immunoassay for the detection of proteins. A silver development technique promoted by gold particles was used to analyze fmol-amounts of antigens, and can also be used in the spatially-addressable detection of chip-immobilized antigens.<sup>[99]</sup>

### 5.3. Therapeutic Applications

Gold particles are well-established carriers for the delivery of dsDNA in the so-called gene-gun technology.<sup>[189]</sup> In this

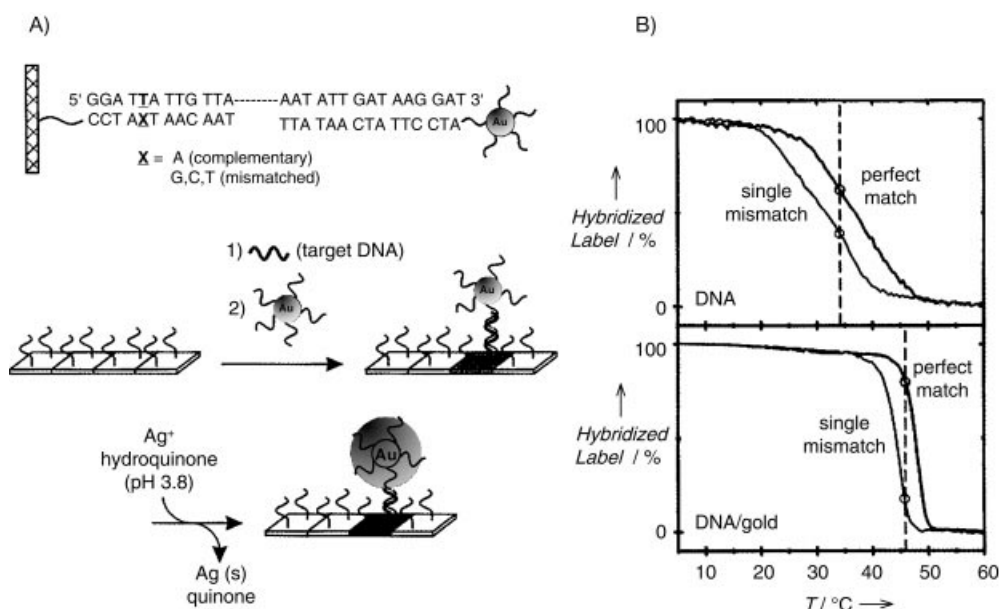


Figure 24. Scanometric detection of nucleic acids in DNA chip analyses. Capture oligonucleotides are immobilized on glass slides and used for the specific binding of target nucleic acids. Oligonucleotide-functionalized gold nanoparticles are employed as probes in solid-phase DNA hybridization detection. Subsequent to a silver enhancement step, the immobilization of the colloidal gold probe is detected by scanning the glass substrate with a conventional flat-bed scanner. The extraordinary sharp melting point of the immobilized DNA–nanoparticle networks (see melting curves on the right) allows single-mismatches to be detected by means of this method. Reproduced from ref. [181], with permission. Copyright 2000 American Association for the Advancement of Science.

method, plasmid DNA with a typical length of several 1000 base pairs is adsorbed on Au or tungsten colloids with a typical size of about 500 nm to several micrometers. The DNA- or RNA-coated particles are loaded into a gun-like device in which a low pressure helium pulse delivers the particles into virtually any target cell or tissue. An advantage of this technique is that cells do not have to be removed from the tissue to transform cells. The particles penetrate the cells and release the DNA, which is diffused into the nucleus and, for example, incorporated into the chromosomes of the organism. Since its development in the mid-1980s, the gene gun technology has led to numerous examples of the tremendous potential of this method for “biolistic” transfection of organisms<sup>[189]</sup> as well as for DNA immunization.<sup>[190]</sup> The latter technique, also known as DNA vaccination, or genetic immunization, is a vaccine technology that can be used to stimulate protective immunity against many infectious pathogens, malignancies, and autoimmune disorders in animal models. Plasmid DNA, which encodes a polypeptide antigen, is introduced into the host cell (e.g. by means of gene gun particle bombardment), where it serves as a template for the highly efficient translation of its antigen. This leads to an immune response that is mediated by the cellular and/or humoral immune system and is specific for the plasmid-encoded antigen.<sup>[190]</sup>

Paramagnetic nanoparticles are currently being investigated for biomedical applications in the treatment of cancer.<sup>[191]</sup> Biocompatible dextran- or silane- coated superparamagnetic magnetite nanoparticles can be incorporated into malignant cells by means of endocytosis. The intracellular magnetic fluids are excited with alternating magnetic fields, thus leading to an increase in the local temperature, thereby inducing hyperthermia. It has been demonstrated that magnetic fluid hyperthermia (MFH) affects mammary carcinoma cells *in vitro* and *in vivo*.<sup>[191]</sup>

## 5.4. Nanoparticles as Model Systems

Two general aspects of metal and semiconductor nanoparticles make them suitable model systems for the study of fundamental biological phenomena. On the one hand, their size matches the macromolecular components employed in living systems. Proteins, nucleic acid fragments and their supramolecular complexes such as the nucleosome, the various complexes involved in DNA replication and transcription, and the ribosome, have typical dimensions in the range of 2–200 nm. On the other hand, nanoparticles of inorganic materials are essential building blocks in biomineralization, a fundamental biological process in which nature chemically generates morphology by means of genetic instructions. It is thus not surprising that the current advances in the study of metal and semiconductor nanoparticles has led to their consideration as model systems for the study of the interactions between proteins and nucleic acids and also for the understanding of the fundamental principles of biomineralization.

### 5.4.1. DNA – Protein Interaction

Besides using semiconductor and metal nanoparticles for tagging antibody and oligonucleotide probes, the nanoparticles themselves can also be used as probes for studying biological processes. They have an appropriate size similar to that of proteins and their ligand sphere can be adjusted readily with a variety of chemical groups. Thus, they can be considered as inorganic model proteins to investigate non-specific DNA – protein interactions.<sup>[39–41]</sup> The protein-induced bending and wrapping of DNA by proteins is particularly important on the scale of a few base pairs up to about 150 base pairs, which corresponds to lengths of about 1–50 nm.<sup>[192]</sup> The most prominent example of sequence-independent binding and bending of DNA by proteins is the wrapping of about 150 base pairs of DNA around the nucleosome particle (diameter  $\approx 11$  nm), which consists of eight basic histone proteins (Figure 25).<sup>[193]</sup> Recent studies suggest that the histones, which

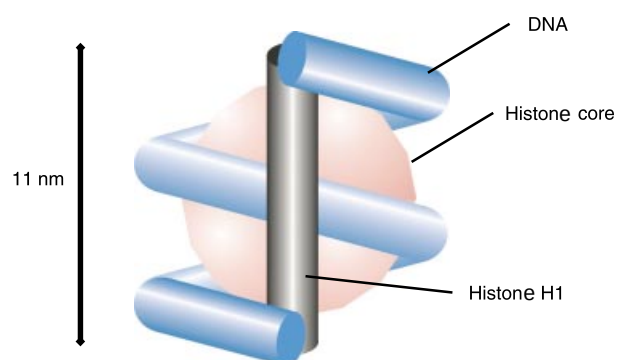


Figure 25. Model of the nucleosome particle: eight basic proteins make up the histone core, which serves as a carrier to bind 165 base pairs of dsDNA, which is wrapped around the protein core in 1.8 turns of a left-handed DNA superhelix. An additional histone molecule H1 locks the nucleosome, thus leading to a particle with a diameter of about 11 nm.

for a long time have been considered as passive structural substrates for DNA binding, might indeed influence gene expression by competing with transcription factors for DNA binding.<sup>[194]</sup> Thus, a detailed understanding of the nonspecific DNA – protein interactions and protein-induced DNA bending is important for the elucidation of the fundamental biological processes of DNA storage and processing.

Murphy and co-workers developed an unconventional way to study the adsorption of DNA onto protein-sized quantum dots.<sup>[39–41]</sup> The nanoparticle mimics a protein-like surface in terms of size and surface groups with the additional benefit of a spectroscopic probe to monitor DNA – nanoparticle interactions. As an example, if a particular sequence of DNA leads to an unusual shape or flexibility of the molecule, it is likely that these properties affect its binding to the protein-sized nanoparticle. The latter process can be conveniently monitored by photoluminescence quenching titration of the semiconductor nanoparticles. This strategy allows the adsorption of a kinked DNA molecule to 4 nm CdS nanocrystals to be compared with that of a straight DNA molecule (Figure 26). In particular, the self-complementary DNA sequence 5'-GGTCATGGCCATGACC-3', which has a crystallographic kink of  $23^\circ$  at the central GGCC in the presence of magnesium

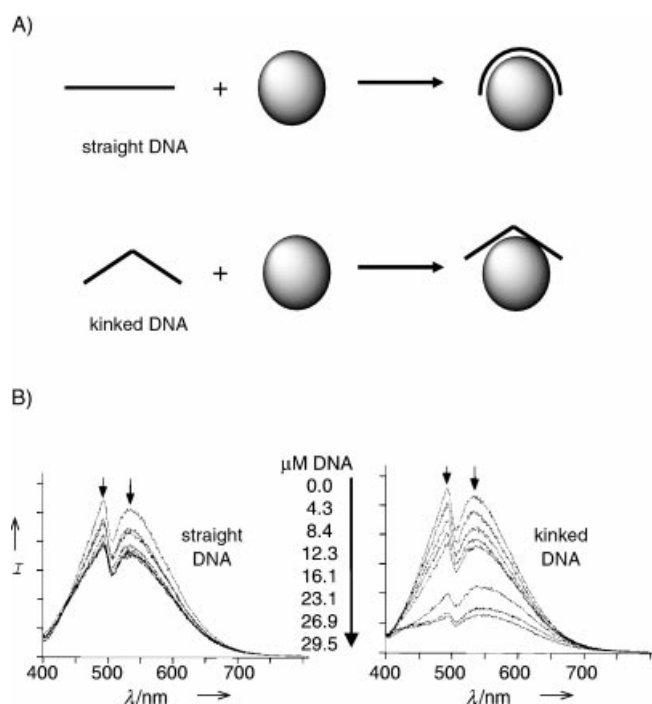


Figure 26. Semiconductor nanoparticles can mimic a protein-like surface. A) Unusual shapes of DNA molecules can affect its binding to protein-sized particles. B) The interaction between a straight and a kinked DNA molecule and a nanoparticle was monitored by photoluminescence quenching titration of 4-nm CdS nanoparticles capped with mercaptoethanol. The concentration-dependent quenching is more efficient for the kinked DNA molecule. Data from ref. [40], with permission. Copyright 1996 American Chemical Society.

ions,<sup>[195, 196]</sup> is adsorbed on the CdS quantum dots much more readily than the straight 5'-GGGTCCTCAGCTTGCC-3' and the bent 5'-GGTCCAAAAATTGCC-3' oligomer.<sup>[39]</sup> Studies with neutralized mercaptoethanol-capped CdS particles indicated that magnesium is not essential for the preferred binding of the kinked DNA, whereas the other DNA molecules do not adsorb without a magnesium-enriched surface (Figure 26B).<sup>[40]</sup>

Recent studies on the temperature- and salt-dependent binding of long calf thymus DNA to 4.5-nm CdS quantum dots by photoluminescence spectroscopy suggest that the adsorption of the DNA is driven by entropy, whereas the enthalpy contribution to the DNA–surface binding is slightly unfavorable. In agreement with the mechanism of nonspecific DNA–protein interactions, the increase in entropy is likely to be a result of the release of solvent and counterions from the interface.<sup>[41]</sup> These studies demonstrate that semiconductor nanoparticles can be utilized in biological model studies owing to their size, adjustable chemical functionality of the ligand sphere, and their intrinsic fluorescence. Instead of using quantum-dot-based fluorescence-quenching analysis, gold or silver particles may also serve for similar spectroscopic studies with SERS.<sup>[197]</sup>

#### 5.4.2. Biomineralization

The formation of the biological minerals that make up bones, teeth, or shells, is one of the most intriguing examples

of Nature's unique capabilities to program the genesis of macroscopic forms genetically.<sup>[198]</sup> The power of the process by which nanometer-sized building blocks of mineral crystals are deposited in an organized fashion in the cellular or extracellular matrix of living organisms to build a macroscopic structure is impressively illustrated by the various genera of diatoms, each of which has an individual phenotype (Figure 27).<sup>[199]</sup> Recent studies suggest that the group-specific morphology of the nanostructured silica diatom cell walls is related to species-specific sets of polycationic peptides, termed silaffins, which have been isolated from diatom cell walls. Silaffins contain covalently modified lysines that bear polyamines consisting of *N*-methylpropylamine units, and  $\epsilon$ -*N,N*-dimethyllysine groups.<sup>[200]</sup> The chemical structure of long-chain polyamines, which represent the main organic constituent of the biosilicates, were found to induce silica precipitation rapidly in vitro. Different morphologies of precipitating silica were generated from polyamines of different chain lengths.<sup>[201]</sup>

Although fundamental research into biomineralization over the past 25 years has brought insight into the mechanisms that control these processes, many questions still persist, in particular, in terms of vertebrate mineralization. For example, the biomineralization of the dental enamel matrix with a carbonated hydroxyapatite mineral is a well-studied system.<sup>[198c]</sup> Nevertheless, the chemical nature of the first mineral crystals formed in bone and dentin, the factors that lead to the initial deposition of these crystals, and the functions of macromolecules associated with these crystals are not understood. Recent studies on the molecular biology of the ameloblast gene products revealed the primary structures of some principal proteins involved in this extracellular mineralizing system. However, details of the secondary, tertiary, and quaternary structures of amelogenins, tuftelins, ameloblastins, enamelines, and proteinases involved in dental enamel matrix mineralization, their interactions with other matrix and cell surface proteins, and their distinct functions are still largely unknown. However, our current knowledge of these molecules, the probable molecular structure of the enamel matrix, and the functional role of the extracellular matrix proteins is constantly increasing,<sup>[198c]</sup> which suggests that further research might provide opportunities for the therapeutic manipulation of diseases in which biomineralization is impaired.

Biological antifreezes provide another impressive example of macromolecules with specific recognition capabilities for inorganic structures. The antifreeze proteins (AFPs) and the antifreeze glycoproteins (AFGPs) are a diverse class of proteins which have the ability to inhibit the growth of ice. Antifreezes are consequently found in organisms that live in environments in which sub-zero temperatures are routinely encountered, for instance, in Arctic fish, amphibians, insects, and plants.<sup>[202]</sup> Although still under active investigation, the molecular basis of the ice growth inhibition mechanism is in principle understood. AFPs have the ability to specifically adsorb to ice, thereby inhibiting its growth. The ice-binding mechanism of the linear alpha-helical type I AFPs has been attributed to their regularly spaced polar residues, which match the ice lattice along a pyramidal plane. As an example, the AFP of winter flounder binds to a specific set of pyramidal

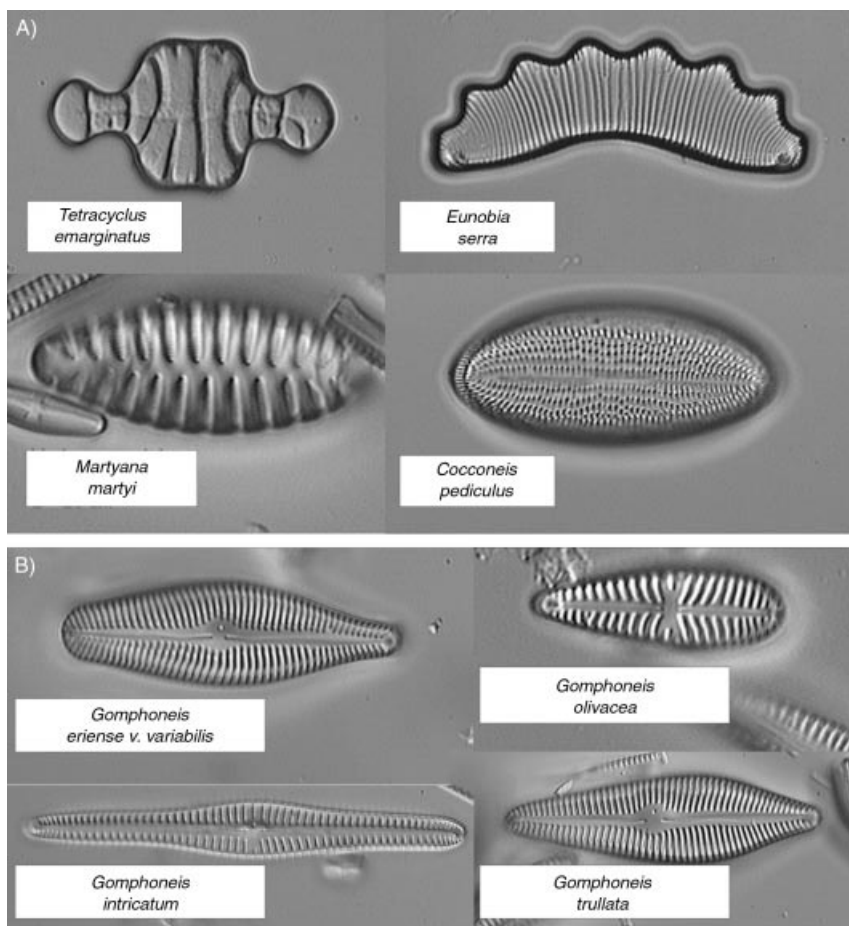


Figure 27. Genera (A) and species (B) of diatoms. Reproduced from: <http://www.calacademy.org/research/diatoms/>.

surface planes. Four groups of Asp, Asn, and Thr residues in the AFP can interact with the ice surface simultaneously through both hydrogen bonding and van der Waals interactions.<sup>[202e]</sup> Globular antifreeze proteins such as type III AFP, which lack repeating ice-binding residues, bind to ice with a remarkably flat amphipathic ice-binding site where hydrogen-bonding atoms match two ranks of oxygen atoms on the distinct planes of the ice prism plane.<sup>[202f]</sup> While some insight into the molecular basis of AFP-mediated ice growth inhibition has already been gained, more studies are required for a comprehensive understanding of the antifreeze action. One of goals is the rational construction of novel antifreezes, which have great potential for commercial applications as food additives to prevent cellular damage and to improve the texture and taste of frozen foods.<sup>[202a]</sup>

Biomineralization is also important in bacteria. Magnetotactic bacteria, which orient themselves and migrate along geomagnetic field lines, produce intracellular magnetic nanoparticles, the magnetosomes.<sup>[203]</sup> These comprise nanometer-sized monodisperse crystals of magnetic iron mineral, most often magnetite  $\text{Fe}_3\text{O}_4$ , coated with a magnetosome membrane, which prevents agglomeration (Figure 28). Depending on the bacterial species, the size of the magnetosomes range from 35 to 120 nm, and thus the particles represent a single magnetic domain. The formation of magnetosomes is achieved by means of a biological mechanism that controls

the accumulation of iron and the biomineralization of the magnetic crystals with a characteristic size and morphology within membrane vesicles, which consist of protein-containing lipid bilayers. The molecular biology of magnetosome formation is under active investigation, and more than ten different proteins have already been identified to be part of the magnetosome membrane. Bacterial magnetosomes have significant biotechnological potential since they possess advantages over conventional synthetic magnetic particles, in particular their structural perfection and uniformity. Possible applications include the coupling of bioactive compounds, such as nucleic acids, enzymes, and antibodies, which would allow the magnetic manipulation of biocomponents. Moreover, purified and receptor-functionalized magnetosomes have already been used as contrasting agents in the detection of tumors by magnetoresonance tomography.<sup>[203]</sup>

Early biotechnological applications of bacterial biomineralization processes were described by Dameron et al., who discovered that the yeast *Candida glabrata* produces CdS nanoparticles when cultured in the presence of cadmium salts, which results in the sequestration and detoxification of intracellular cadmium ions.<sup>[46, 204, 205]</sup> The crystallites are

peptide-coated, and the coating peptide varies with the nutrient conditions of the growth medium. When cultured in rich nutrient broth, the yeast forms intracellular CdS particles

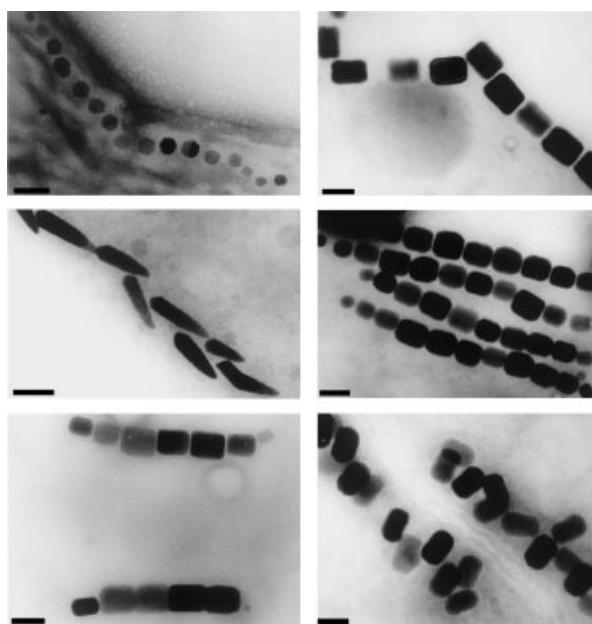


Figure 28. TEM images of magnetosomes obtained from various magnetotactic bacteria. The scale bar corresponds to 100 nm. Reproduced from ref. [203a], with permission.

that are coated with a mixture of glutathione and a  $\gamma$ -glutamylcysteine dipeptide. Cultures in synthetic minimal medium yield particles coated with polymerized peptides of the general structure  $(\gamma\text{-Glu-Cys})_n\text{-Gly}$ . The biologically produced peptide-coated particles exhibited properties analogous to those of the semiconductor quantum dots, in particular, size-dependent optical properties, photoluminescence, and photoreduction. More recently, a related approach allowed the biological production of ceramic–metal composites, cermets.<sup>[206]</sup> The silver-accumulating bacterial strain *Pseudomonas stutzeri*, which was originally isolated from a silver mine, was used to produce silver–carbon composite materials, which have the potential to produce optical thin-film coatings.

Besides immediate biotechnological applications, fundamental studies of bacterial nanoparticle formation and the interactions that occur between the organism and the inorganic surfaces<sup>[207]</sup> are also relevant for a comprehensive understanding of the fundamental material properties of artificial nanoparticles. For example, natural iron oxyhydroxide biomineralization products have recently provided strong evidence for aggregation-based crystal growth and microstructure development.<sup>[208]</sup> Since it is convenient to prepare perfect nanocrystals in large amounts, the question of whether small nanocrystals can be assembled into pure extended solid structures is of tremendous importance for the development of new optical and electronic materials.<sup>[23]</sup> For a long time, it seemed that aggregation of preformed nanocrystals assemble to form disordered solids. Recent findings by Banfield et al. suggest that some natural minerals grow by means of an oriented attachment mechanism, which means that a nanoparticle docks to the next in such a way that the two crystals are perfectly aligned.<sup>[208]</sup> Although there is a strong thermodynamic driving force for aligned attachment since the interface elimination leads to a reduced surface energy, it is still surprising that two crystals fuse together perfectly without getting stuck in any of the almost innumerable misoriented arrangements.<sup>[23]</sup>

In view of the intriguing power of biological mineralization processes, researchers have started to apply modern advances in molecular biology and biotechnology towards the development of artificial biomineralization approaches. The protein-based recognition of organic and inorganic surfaces has been studied by Addadi and co-workers.<sup>[209]</sup> They have isolated IgG molecules from animals immunized with crystals of monosodium urate monohydrate, or magnesium urate octahydrate. The antibodies revealed a high specificity and could distinguish between nuclei of crystals with similar molecular and structural characteristics, which suggests that the antibodies bear an imprint of the crystal surface structure in their binding sites. Such antibodies can act as nucleating templates that accelerate crystal formation in vitro.<sup>[209a]</sup> The generality of this approach was proven by the production of monoclonal antibodies against crystals of 1,4-dinitrobenzene (DNB). The reactivity of the antibodies was specifically directed against the DNB crystals, and they did not interact with molecular DNB conjugated to carrier proteins. Thus, these antibodies are not specific to the DNB molecule but rather to a repetitive motif of molecular moieties exposed at the crystal

surfaces.<sup>[209b]</sup> Similarly, several antibodies were generated which specifically interact with distinct faces of cholesterol monohydrate crystals.<sup>[209c]</sup> These antibodies, which were raised against the three-dimensional supramolecular antigens, also bind specifically to the two-dimensional geometrically reduced forms of the antigen, such as fluid membranes of cholesterol.<sup>[209d,e]</sup>

The above results indicate that the binding sites of the antibody recognize a number of molecular moieties that are exposed at the surface in a specific structural organization. Besides serving as a model for the study of antibody recognition and related applications regarding the use of antibodies for targeting defined supramolecular structures in biological systems, the antibody–crystal surface interactions may also pave the way for the development of tailored (semi)synthetic binding proteins for the biomineralization of novel materials. In this context, recombinant DNA technology is a powerful tool for the biological production of novel protein compounds, and the phage-display technique has already allowed the in vitro evolution of protein linkers that directly recognize the surfaces of semiconductor materials (Figure 8).<sup>[67]</sup> Further research might lead to the development of procedures that enable the genetically coded biological growth of artificial inorganic materials.

## 6. Conclusions and Perspectives

This article summarizes a young and rapidly increasing research field located at the crossroads of materials research, nanosciences, and molecular biotechnology. An important point concerns the functionalization of inorganic nanoparticles by means of evolutionary optimized biological components. Since the nanoparticles and biomolecules typically meet at the same nanometer length scale, this interdisciplinary approach will contribute to the establishment of a novel field, descriptively termed biomolecular nanotechnology or nanobiotechnology.<sup>[76]</sup>

Although biomolecules and inorganic materials can be chemically coupled by means of various methods (Section 2), there is still a great demand for mild and selective coupling techniques that allow the preparation of thermodynamically stable, kinetically inert, and stoichiometrically well-defined bioconjugate hybrid nanoparticles. Examples in Nature suggest that enzymatic procedures combined with modern methods of bioorganic syntheses<sup>[210]</sup> will open novel routes for the generation of well-defined inorganic/biomolecular components, which are useful as building blocks for the bottom-up assembly of sophisticated nanostructured architecture. The first steps in this direction have already been taken by using protein- or nucleic acid based recognition elements (Section 3). In both systems, a large number of complementary binding pairs with a wide variety of free energies of association is available. It therefore remains to be seen whether protein-based conjugation might offer advantages over nucleic acid based assembly.<sup>[62]</sup> One should expect that nucleic acids are superior for the functionalization of nanoparticles, since the physicochemical properties of a single 20-mer oligonucleotide represents  $4^{20}$  ( $=10^{12}$ ) different recog-

nition elements. The binding strength can be conveniently adjusted by varying the lengths and GC contents of native DNA, or else by using non-natural DNA analogues with altered backbone properties and/or base-pairing specificity. Furthermore, directed evolution, already applied to tailor protein linkers,<sup>[67]</sup> should also be applicable in the generation of novel nucleic acid based linkers. It seems safe to predict that the tremendous advances in the development of artificial nucleic acid receptors will soon lead to the use of aptamers, which specifically recognize any target structure,<sup>[211]</sup> in the de novo generation of nucleic acid receptors that are capable of recognizing inorganic surfaces. Consequently, combinations of the two fundamental biological systems, nucleic acids and proteins, are very promising to synergistically cooperate and thus allow novel functions and applications.<sup>[85]</sup>

Another crossover of biotechnology and materials science concerns the utilization of the chemical and topographical properties of biological components for templating the spatial assembly of organic and inorganic components to form nanometer- and micrometer-sized elements (Section 4). There is still a tremendous need for systematic fundamental research into the interaction between biomolecules and the abiotic components; future perspectives should address these and should also concern the tailoring of the shape and physico-chemical properties of the biocomponent by means of genetic engineering and bioconjugate chemistry. Although commercial applications are currently rare, the interdisciplinary work has a great potential for generating advanced materials, which might lead to novel devices for sensing, signal transduction, catalysis, as well as for new biocompatible materials and interfaces, currently being developed for biomedical sciences and tissue engineering.<sup>[212]</sup> In addition to long term perspectives, today's advanced materials as well as the modern physicochemical techniques originally developed for materials research and physics, are already used in bioanalytical applications or as model systems to solve biological problems (Section 5).

The future development of the joint venture between biotechnology and materials science will profit from the rapid current advances in chemistry as a central science. In addition, the current genome and proteome research will also be beneficial for this field, since it provides data that will allow us to produce even more suitable biocomponents. This might open the door to nanosensors, catalytic and light-harvesting devices, ultrafast molecular switches and transistors, supramolecular mediators between electrical and living systems, and other bio- and optoelectronic parts. Clearly, we are still far from the fabled autonomous nanomachines that heal wounds and perform surgery in a living organism. However, at the beginning of this new century one should remember the dramatic development that total organic synthesis has undergone from the early 1900s to the race for "molecular summits" in the late 1990s.<sup>[213, 214]</sup> A similar rate of progress in supramolecular sciences at the interface of biotechnology and materials research promises plenty of excitement from future developments.<sup>[221]</sup>

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- [1] J.-M. Lehn, *Supramolecular Chemistry. Concepts and Perspectives*, VCH, Weinheim, **1995**.
- [2] F. Vögtle, *Supramolecular Chemistry*, Wiley, Chichester, **1991**.
- [3] J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vögtle, *Comprehensive Supramolecular Chemistry*, Elsevier, Oxford, **1996**.
- [4] For reviews, see: a) H. Shirakawa, *Angew. Chem.* **2001**, *113*, 2642–2648; *Angew. Chem. Int. Ed.* **2001**, *40*, 2574–2580; b) A. G. MacDiarmid, *Angew. Chem.* **2001**, *113*, 2649–2659; *Angew. Chem. Int. Ed.* **2001**, *40*, 2581–2590; c) A. J. Heeger, *Angew. Chem.* **2001**, *113*, 2660–2682; *Angew. Chem. Int. Ed.* **2001**, *40*, 2591–2611; d) F. Würthner, *Angew. Chem.* **2001**, *113*, 1069–1071; *Angew. Chem. Int. Ed.* **2001**, *40*, 1037–1039.
- [5] The structure of the 50S ribosomal subunit has recently been resolved at a resolution of 2.4 Å: N. Ban, P. Nissen, J. Hansen, P. B. Moore, T. A. Steitz, *Science* **2000**, *289*, 905–920. The atomic structures of this subunit and its complexes with two substrate analogues revealed that the ribosome is, in fact, a ribozyme: P. Nissen, J. Hansen, N. Ban, P. B. Moore, T. A. Steitz, *Science* **2000**, *289*, 920–930. For reviews on the ribosome structure, see: E. Westhof, N. Leontis, *Angew. Chem.* **2000**, *112*, 1651–1655; *Angew. Chem. Int. Ed.* **2000**, *39*, 1587–1591; D. M. J. Lilley, *ChemBioChem* **2001**, *2*, 31–35.
- [6] D. Philp, J. F. Stoddart, *Angew. Chem.* **1996**, *108*, 1242–1286; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1154–1196.
- [7] R. P. Feynman in *Miniaturization* (Ed.: H. D. Gilbert), Reinhold, New York, **1961**, pp. 282–296.
- [8] G. M. Whitesides, J. P. Mathias, C. T. Seto, *Science* **1991**, *254*, 1312–1319.
- [9] G. Schmid, *Clusters and Colloids*, VCH, Weinheim, **1994**.
- [10] U. Kreibitz, *Optical Properties of Metal Clusters*, Springer, New York, **1995**.
- [11] H. Weller, *Angew. Chem.* **1996**, *108*, 1159–1161; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1079–1081.
- [12] M. Antonietti, C. Göltner, *Angew. Chem.* **1997**, *109*, 944–964; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1079–1081.
- [13] A. P. Alivisatos, *Science* **1996**, *271*, 933–937.
- [14] D. L. Feldheim, C. D. Keating, *Chem. Soc. Rev.* **1998**, *27*, 1–12.
- [15] A. N. Shipway, E. Katz, I. Willner, *ChemPhysChem* **2000**, *1*, 19–52.
- [16] F. Remacle, R. D. Levine, *ChemPhysChem* **2001**, *2*, 21–36.
- [17] M. Bruchez, Jr., M. Moronne, P. Gin, S. Weiss, A. P. Alivisatos, *Science* **1998**, *281*, 2013–2015.
- [18] W. C. W. Chan, S. M. Nie, *Science* **1998**, *281*, 2016–2018.
- [19] M. Grundmann, *Physica E* **1999**, *5*, 169.
- [20] R. E. Dunin-Borkowski, M. R. McCartney, R. B. Frankel, D. A. Bazylinski, M. Posfai, P. R. Buseck, *Science* **1998**, *282*, 1868–1870.
- [21] S. Sun, C. B. Murray, D. Weller, L. Folks, A. Moser, *Science* **2000**, *287*, 1989–1992.
- [22] A. Eychmüller, *J. Phys. Chem. B* **2000**, *104*, 6514–6528.
- [23] A. P. Alivisatos, *Science* **2000**, *289*, 736–737.
- [24] R. F. Service, *Science* **1997**, *277*, 1036–1037.
- [25] C. M. Niemeyer, *Angew. Chemie* **1997**, *109*, 603–606; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 585–587.
- [26] C. A. Mirkin, T. A. Taton, *Nature* **2000**, *405*, 626–627.
- [27] H. Dugas, *Bioorganic Chemistry*, Springer, New York, **1989**.
- [28] U. Diederichsen, T. K. Lindhorst, B. Westermann, L. A. Wessjohann, *Bioorganic Chemistry*, Wiley-VCH, Weinheim, **1999**.
- [29] S. S. Ghosh, P. M. Kao, A. W. McCue, H. L. Chappelle, *Bioconjugate Chem.* **1990**, *1*, 71–76.
- [30] A. Bardea, A. Dagan, I. Ben-Dov, B. Amit, I. Willner, *Chem. Commun.* **1998**, 839–840.
- [31] F. Patolsky, K. T. Ranjit, A. Lichtenstein, I. Willner, *Chem. Commun.* **2000**, 1025–1026.
- [32] H. Mattoussi, J. M. Mauro, E. R. Goldman, G. P. Anderson, V. C. Sundar, F. V. Mikulec, M. G. Bawendi, *J. Am. Chem. Soc.* **2000**, *122*, 12142–12150.



- [33] R. L. Letsinger, R. Elghanian, G. Viswanadham, C. A. Mirkin, *Bioconjugate Chem.* **2000**, *11*, 289–291.
- [34] W. Shenton, S. A. Davies, S. Mann, *Adv. Mater.* **1999**, *11*, 449–452.
- [35] C. D. Keating, K. M. Kovalski, M. J. Natan, *J. Phys. Chem. B* **1998**, *102*, 9404–9413, and references therein.
- [36] J. B. Broderick, M. J. Natan, T. V. O'Halloran, R. P. Van Duyne, *Biochemistry* **1993**, *32*, 13771–13776.
- [37] I. D. G. MacDonald, W. E. Smith, *Langmuir* **1996**, *12*, 706–713.
- [38] B. N. Rospendowski, K. Kelly, C. R. Wolf, W. E. Smith, *J. Am. Chem. Soc.* **1991**, *113*, 1217–1225.
- [39] R. Mahtab, J. P. Rogers, C. J. Murphy, *J. Am. Chem. Soc.* **1995**, *117*, 9099–9100.
- [40] R. Mahtab, J. P. Rogers, C. P. Singleton, C. J. Murphy, *J. Am. Chem. Soc.* **1996**, *118*, 7028–7032.
- [41] R. Mahtab, H. H. Harden, C. J. Murphy, *J. Am. Chem. Soc.* **2000**, *122*, 14–17.
- [42] S. Connolly, D. Fitzmaurice, *Adv. Mater.* **1999**, *11*, 1202–1205.
- [43] D. E. Safer, L. Bolinger, J. S. Leigh, *J. Inorg. Biochem.* **1986**, *26*, 77–91.
- [44] B. Dubertret, M. Calame, A. J. Libchaber, *Nat. Biotechnol.* **2001**, *19*, 365–370.
- [45] A. P. Alivisatos, K. P. Johnsson, X. Peng, T. E. Wilson, C. J. Loweth, M. P. Bruchez, Jr., P. G. Schultz, *Nature* **1996**, *382*, 609–611.
- [46] C. T. Dameron, R. N. Reese, R. K. Mehra, A. R. Kortan, P. J. Carroll, M. L. Steigerwald, L. E. Brus, D. R. Winge, *Nature* **1989**, *338*, 596–597.
- [47] W. Bae, R. Abdullah, D. Henderson, R. K. Mehra, *Biochem. Biophys. Res. Commun.* **1997**, *237*, 16–23.
- [48] R. Kho, C. L. Torres-Martinez, R. K. Mehra, *J. Colloid Interface Sci.* **2000**, *227*, 561–566.
- [49] "Lumineszierende CdTe-Nanoteilchen als Markierungssubstanzen in der klinischen Chemie": K. Hoppe, A. Eychmüller, H. Weller, A. Böttcher, A. Götz, G. Schmitz, poster P146, presented at the 99. Hauptversammlung der Deutschen Bunsengesellschaft für Physikalische Chemie (Würzburg, Germany, June 1–3, 2000).
- [50] M. Brust, D. Bethell, D. J. Schiffrin, C. Kiely, *Adv. Mater.* **1995**, *7*, 795–797.
- [51] C. M. Niemeyer, W. Bürger, J. Peplies, *Angew. Chem.* **1998**, *110*, 2391–2395; *Angew. Chem. Int. Ed.* **1998**, *37*, 2265–2268.
- [52] P. C. Weber, D. H. Ohlendorf, J. J. Wendoloski, F. R. Salemme, *Science* **1989**, *243*, 85–88.
- [53] M. Wilchek, E. A. Bayer, *Methods Enzymol.* **1990**, *184*, 51–67.
- [54] B. K. Sinha, C. F. Chignell, *Methods Enzymol.* **1979**, *62*, 295–308.
- [55] U. Piran, W. J. Riordan, *J. Immunol. Methods* **1990**, *133*, 141–143.
- [56] T. Sano, M. W. Pandori, X. M. Chen, C. L. Smith, C. R. Cantor, *J. Biol. Chem.* **1995**, *270*, 28204–28209.
- [57] T. Sano, C. R. Cantor, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 3180–3184.
- [58] G. O. Reznik, S. Vajda, C. L. Smith, C. R. Cantor, T. Sano, *Nat. Biotechnol.* **1996**, *14*, 1007–1011.
- [59] T. G. M. Schmidt, J. Koepke, R. Frank, A. Skerra, *J. Mol. Biol.* **1996**, *255*, 753–766.
- [60] T. Sano, G. O. Reznik, P. Szafranski, C. M. Niemeyer, S. Vajda, C. L. Smith, C. M. Mello, D. L. Kaplan, M. Ruszkowski, D. J. Hnatowich, C. R. Cantor, *Proceedings of the 50th Anniversary Conference of the Korean Chemical Society* (Yonsei University, Seoul, May 24–25, 1996), pp. 359–363.
- [61] T. Sano, S. Vajda, C. R. Cantor, *J. Chromatogr. B* **1998**, *715*, 85–91.
- [62] S. Mann, W. Shenton, M. Li, S. Connolly, D. Fitzmaurice, *Adv. Mater.* **2000**, *12*, 147–150.
- [63] C. Kessler, *Mol. Cell. Probes* **1991**, *5*, 161–205.
- [64] J. Chevalier, J. Yi, O. Michel, X. M. Tang, *J. Histochem. Cytochem.* **1997**, *45*, 481–491.
- [65] A. Knappik, A. Plueckthun, *BioTechniques* **1994**, *17*, 745–761.
- [66] R. G. Chubet, B. L. Brizzard, *BioTechniques* **1996**, *20*, 136–141.
- [67] S. R. Whaley, D. S. English, E. L. Hu, P. F. Barbara, A. M. Belcher, *Nature* **2000**, *405*, 665–666.
- [68] Transition metal complexes that contain several cupric ions have been designed to specifically target a complementary histidine pattern on the surface of a protein: M. A. Fazal, C. R. Bidhan, S. Sun, S. Mallik, K. R. Rodgers, *J. Am. Chem. Soc.* **2001**, *123*, 6283–6290.
- [69] For a nanomechanical device based on the ion-dependent B-to-Z conformational transition of a short DNA double helix, see: C. Mao, W. Sun, Z. Shen, N. C. Seeman, *Nature* **1999**, *397*, 144–146; for ionic switching of nanoparticles by means of DNA supercoiling, see ref. [86]; an array of cantilevers was used to translate DNA hybridization into a nanomechanical response: J. Fritz, M. K. Baller, H. P. Lang, H. Rothuizen, P. Vettiger, E. Meyer, H. Guntherodt, C. Gerber, J. K. Gimzewski, *Science* **2000**, *288*, 316–318; for a DNA molecular tweezer which can be opened and closed by the addition of single-stranded DNA, see: B. Yurke, A. J. Turberfield, A. P. Mills, Jr., F. C. Simmel, J. L. Neumann, *Nature* **2000**, *406*, 605–608.
- [70] R. K. Soong, G. D. Bachand, H. P. Neves, A. G. Olkhovets, H. G. Craighead, C. D. Montemagno, *Science* **2000**, *290*, 1555–1558.
- [71] J. K. Lanyi, A. Pohorille, *Trends Biotechnol.* **2001**, *19*, 140–144.
- [72] K. E. Drexler, *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 5275–5278.
- [73] N. C. Seeman, *J. Theor. Biol.* **1982**, *99*, 237–247.
- [74] K. E. Drexler, *Nanosystems: Molecular Machinery, Manufacturing, and Computation*, Wiley, New York, **1992**.
- [75] N. C. Seeman, *Trends Biotechnol.* **1999**, *17*, 437–443.
- [76] C. M. Niemeyer, *Curr. Opin. Chem. Biol.* **2000**, *4*, 609–618.
- [77] C. A. Mirkin, R. L. Letsinger, R. C. Mucic, J. J. Storhoff, *Nature* **1996**, *382*, 607–609.
- [78] R. C. Mucic, J. J. Storhoff, C. A. Mirkin, R. L. Letsinger, *J. Am. Chem. Soc.* **1998**, *120*, 12674–12675.
- [79] G. P. Mitchell, C. A. Mirkin, R. L. Letsinger, *J. Am. Chem. Soc.* **1999**, *121*, 8122–8123.
- [80] S.-J. Park, A. A. Lazarides, C. A. Mirkin, R. L. Letsinger, *Angew. Chem.* **2001**, *113*, 2993–2996; *Angew. Chem. Int. Ed.* **2001**, *40*, 2909–2912.
- [81] J. J. Storhoff, A. A. Lazarides, R. C. Mucic, C. A. Mirkin, R. L. Letsinger, G. C. Schatz, *J. Am. Chem. Soc.* **2000**, *122*, 4640–4650.
- [82] S.-J. Park, A. A. Lazarides, C. A. Mirkin, P. W. Brazis, C. R. Kanneur, R. L. Letsinger, *Angew. Chem.* **2000**, *112*, 4003–4006; *Angew. Chem. Int. Ed.* **2000**, *39*, 3845–3848.
- [83] C. M. Niemeyer, M. Adler, B. Pignataro, S. Lenhart, S. Gao, L. F. Chi, H. Fuchs, D. Blohm, *Nucleic Acids Res.* **1999**, *27*, 4553–4561.
- [84] C. M. Niemeyer, M. Adler, S. Gao, L. F. Chi, *Angew. Chem.* **2000**, *112*, 3183–3187; *Angew. Chem. Int. Ed.* **2000**, *39*, 3055–3059.
- [85] C. M. Niemeyer, *Chem. Eur. J.* **2001**, *7*, 3188–3195.
- [86] C. M. Niemeyer, M. Adler, S. Lenhart, S. Gao, H. Fuchs, L. F. Chi, *ChemBioChem* **2001**, *2*, 260–265.
- [87] S. Gao, L. F. Chi, S. Lenhart, B. Anczykowsky, C. M. Niemeyer, M. Adler, H. Fuchs, *ChemPhysChem* **2001**, *2*, 384–388.
- [88] B. Pignataro, L. F. Chi, S. Gao, B. Anczykowsky, C. M. Niemeyer, M. Adler, H. Fuchs, *Appl. Phys. A* **2001**, *2*, submitted.
- [89] J. J. Storhoff, C. A. Mirkin, *Chem. Rev.* **1999**, *99*, 1849–1862.
- [90] Polymers that are functionalized with DNA oligomers have been used to prepare nanoparticle aggregates (K. J. Watson, S.-J. Park, J.-H. Im, S. T. Nguyen, C. A. Mirkin, *J. Am. Chem. Soc.* **2001**, *123*, 5592–5593), and protein networks (R. B. Fong, Z. Ding, C. J. Long, A. S. Hoffman, P. S. Stayton, *Bioconjugate Chem.* **1999**, *10*, 720–725) by means of nucleic acid hybridization.
- [91] C. M. Niemeyer, T. Sano, C. L. Smith, C. R. Cantor, *Nucleic Acids Res.* **1994**, *22*, 5530–5539.
- [92] C. J. Loweth, W. B. Caldwell, X. Peng, A. P. Alivisatos, P. G. Schultz, *Angew. Chem.* **1999**, *111*, 1925–1929; *Angew. Chem. Int. Ed.* **1999**, *38*, 1808–1812.
- [93] L. Cusack, S. N. Rao, D. Fitzmaurice, *Chem. Eur. J.* **1997**, *3*, 202–207.
- [94] D. Fitzmaurice, S. N. Rao, J. A. Preece, J. F. Stoddart, S. Wenger, N. Zaccheroni, *Angew. Chem.* **1999**, *111*, 1220; *Angew. Chem. Int. Ed.* **1999**, *38*, 1147–1150.
- [95] D. Fitzmaurice, S. N. Rao, J. A. Preece, J. F. Stoddart, S. Wenger, N. Zaccheroni, *J. Am. Chem. Soc.* **2000**, *122*, 6252–6257.
- [96] a) M. Brust, M. Walker, D. Bethell, D. J. Schiffrin, R. J. Whyman, *J. Chem. Soc. Chem. Commun.* **1995**, 801; b) R. S. Ingram, M. J. Hostetler, R. W. J. Murray, *J. Am. Chem. Soc.* **1997**, *119*, 9175–9178; c) J. Liu, R. Xu, A. E. Kaifer, *Langmuir* **1998**, *14*, 7337–7339; d) A. K. Boal, V. M. Rotello, *J. Am. Chem. Soc.* **2000**, *122*, 734–735; e) A. K. Boal, F. Ilhan, J. E. DeRouchey, T. Thurn-Albrecht, T. P. Russell, V. M. Rotello, *Nature* **2000**, *404*, 746–748; see also refs. [94] and [95].

- [97] V. M. Mirsky, T. Hirsch, S. A. Piletsky, O. S. Wolfbeis, *Angew. Chem.* **1999**, *111*, 1191; *Angew. Chem. Int. Ed.* **1999**, *38*, 1108.
- [98] a) SAM colloids that contain surface-bound Ru catalysts revealed a combination of homogeneous and heterogeneous properties in ring-opening-metathesis polymerization reactions: M. Bartz, J. Küther, R. Sesharadi, W. Tremel, *Angew. Chem.* **1998**, *110*, 2646–2649; *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 2466–2468; b) the photochemical reactivity of stilbene moieties bound to SAM colloids differs from that of stilbene groups attached to planar surfaces: J. Hu, J. Zhang, F. Liu, K. Kittredge, J. K. Whitesell, M. A. Fox, *J. Am. Chem. Soc.* **2001**, *123*, 1464–1470; c) 2-nm gold particles that are functionalized with thiolated neoglycoconjugate moieties can be used as model systems for polyvalent carbohydrate interactions: J. M. de la Fuente, A. G. Barrientos, T. C. Rojas, J. Rojo, J. Cañada, A. Fernández, S. Penadés, *Angew. Chem.* **2001**, *113*, 2318–2321; *Angew. Chem. Int. Ed.* **2001**, *40*, 2258–2261; d) for cyclodextrin-modified nanoparticles, see: J. Liu, S. Mendoza, E. Roman, M. J. Lynn, R. L. Xu, A. E. Kaifer, *J. Am. Chem. Soc.* **1999**, *121*, 4304–4305.
- [99] C. M. Niemeyer, B. Ceyhan, *Angew. Chem. Int. Ed.* **2001**, *40*, 3685–3688; *Angew. Chem.* **2001**, *113*, 3798–3801.
- [100] U. B. Sleytr, P. Messner, D. Pum, M. Sara, *Angew. Chem.* **1999**, *111*, 1098–1120; *Angew. Chem. Int. Ed.* **1999**, *38*, 1034–1054.
- [101] W. Shenton, D. Pum, U. B. Sleytr, S. Mann, *Nature* **1997**, *389*, 585–587.
- [102] S. Dieluweit, D. Pum, U. B. Sleytr, *Supramol. Sci.* **1998**, *5*, 15–19.
- [103] M. Mertig, R. Kirsch, W. Pompe, H. Engelhardt, *Eur. Phys. J.* **1999**, *D9*, 45–48.
- [104] S. R. Hall, W. Shenton, H. Engelhardt, S. Mann, *ChemPhysChem* **2001**, *2*, 184–186.
- [105] A genetically engineered alpha-hemolysine membrane protein was functionalized with a DNA oligonucleotide within its 4-nm internal cavity. The resulting DNA-nanopore was used for sequence-specific detection of individual DNA targets: S. Howorka, S. Cheley, H. Bayley, *Nat. Biotechnol.* **2001**, *19*, 636–639. Similar nanopores were used to simultaneously accommodate two different cyclodextrin molecules, thereby opening the way for the production of novel multi-analyte sensors: L. Q. Gu, S. Cheley, H. Bayley, *Science* **2001**, *291*, 636–640.
- [106] E. J. Gibbs, I. Tinoco, Jr., M. F. Maestre, P. A. Ellinas, R. F. Pasternack, *Biochem. Biophys. Res. Commun.* **1988**, *157*, 350–358.
- [107] R. F. Pasternack, J. I. Goldsmith, S. Szep, E. J. Gibbs, *Biophys. J.* **1998**, *75*, 1024–1031.
- [108] R. F. Pasternack, E. J. Gibbs, P. J. Collings, J. C. dePaula, L. C. Turzo, A. Terracina, *J. Am. Chem. Soc.* **1998**, *120*, 5873–5878.
- [109] J. L. Seifert, R. E. Connor, S. A. Kushon, M. Wang, B. A. Armitage, *J. Am. Chem. Soc.* **1999**, *121*, 2987–2995.
- [110] M. Wang, G. L. Silva, B. A. Armitage, *J. Am. Chem. Soc.* **2000**, *122*, 9977–9986.
- [111] A. M. Cassel, W. A. Scrivens, J. M. Tour, *Angew. Chem.* **1998**, *110*, 1670–1672; *Angew. Chem. Int. Ed.* **1998**, *37*, 1528–1531.
- [112] M. Y. Ono, E. M. Spain, *J. Am. Chem. Soc.* **1999**, *121*, 7330–7334.
- [113] T. Blessing, J.-S. Remy, J.-P. Behr, *J. Am. Chem. Soc.* **1998**, *120*, 8519–8520.
- [114] A. D. Miller, *Angew. Chem.* **1998**, *110*, 1862–1880; *Angew. Chem. Int. Ed.* **1998**, *37*, 1768–1785.
- [115] J. L. Coffey, S. R. Bigham, R. F. Pinizzotto, H. Yang, *Nanotechnology* **1992**, *3*, 69–76.
- [116] S. R. Bigham, J. L. Coffey, *Colloids Surf. A* **1995**, *95*, 211–219.
- [117] J. L. Coffey, S. R. Bigham, X. Li, R. F. Pinizzotto, Y. G. Rho, R. M. Pirtle, I. L. Pirtle, *Appl. Phys. Lett.* **1996**, *69*, 3851–3853.
- [118] J. L. Coffey, *J. Cluster Sci.* **1997**, *8*, 159–179.
- [119] E. Braun, Y. Eichen, U. Sivan, G. Ben-Yoseph, *Nature* **1998**, *391*, 775–778.
- [120] J. Richter, R. Seidel, R. Kirsch, M. Mertig, W. Pompe, J. Plaschke, H. K. Schackert, *Adv. Mater.* **2000**, *12*, 507–510.
- [121] J. Richter, M. Mertig, W. Pompe, I. Monch, H. K. Schackert, *Appl. Phys. Lett.* **2001**, *78*, 536–538.
- [122] L. C. Ciacchi, W. Pompe, A. De Vita, *J. Am. Chem. Soc.* **2001**, *123*, 7371–7380.
- [123] T. Torimoto, M. Yamashita, S. Kuwabata, T. Sakata, H. Mori, H. Yoneyama, *J. Phys. Chem. B* **1999**, *103*, 8799–8803.
- [124] A. Kumar, M. Pattarkine, M. Bhadbhade, A. B. Mandale, K. N. Ganesh, S. S. Datar, C. V. Dharmadhikari, M. Sastry, *Adv. Mater.* **2001**, *13*, 341–344.
- [125] E. Di Mauro, C. P. Hollenberg, *Adv. Mater.* **1993**, *5*, 384–386.
- [126] W. Han, S. M. Lindsay, R. E. Dlakic, R. E. Harrington, *Nature* **1997**, *386*, 563.
- [127] J. Shi, D. E. Bergstrom, *Angew. Chem.* **1997**, *109*, 70–72; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 111–113.
- [128] H. Kuhn, V. V. Demidov, M. D. Frank-Kamenetskii, *Angew. Chem.* **1999**, *111*, 1544–1547; *Angew. Chem. Int. Ed.* **1999**, *38*, 1446–1449.
- [129] M. S. Shchepinov, K. U. Mir, J. K. Elder, M. D. Frank-Kamenetskii, E. M. Southern, *Nucleic Acids Res.* **1999**, *27*, 3035–3041.
- [130] M. Scheffler, A. Dorenbeck, S. Jordan, M. Wüstefeld, G. v. Kiedrowski, *Angew. Chem.* **1999**, *111*, 3514–3518; *Angew. Chem. Int. Ed.* **1999**, *38*, 3312–3315.
- [131] Y. Aharonov, D. Bohm, *Phys. Rev.* **1959**, *115*, 485.
- [132] N. D. Chasteen, P. M. Harrison, *J. Struct. Biol.* **1999**, *126*, 182–194.
- [133] S. Mann, J. V. Bannister, R. J. P. Williams, *J. Mol. Biol.* **1986**, *188*, 225.
- [134] F. Funk, J.-P. Lenders, R. R. Crichton, W. Schneider, *Eur. J. Biochem.* **1985**, *152*, 167–172.
- [135] I. G. Macara, T. G. Hoy, P. M. Harrison, *Biochem. J.* **1972**, *126*, 151–162.
- [136] D. M. Lawson, P. J. Artymiuk, S. J. Yewdall, J. M. A. Smith, J. C. Livingstone, A. Treffry, A. Luzzago, S. Levi, P. Arosio, G. Cesareni, C. D. Thomas, W. V. Shaw, P. M. Harrison, *Nature* **1991**, *349*, 541–544.
- [137] F. C. Meldrum, V. J. Wade, D. L. Nimmo, B. R. Heywood, S. Mann, *Nature* **1991**, *349*, 684–687.
- [138] F. C. Meldrum, B. R. Heywood, S. Mann, *Science* **1992**, *257*, 522–523.
- [139] K. K. W. Wong, T. Douglas, S. Gider, D. D. Awschalom, S. Mann, *Chem. Mater.* **1998**, *10*, 279–285.
- [140] M. Li, K. W. K. Wong, S. Mann, *Chem. Mater.* **1999**, *11*, 23–26.
- [141] W. Shenton, S. Mann, H. Cölfen, A. Bacher, M. Fischer, *Angew. Chem.* **2001**, *113*, 456–459; *Angew. Chem. Int. Ed.* **2001**, *40*, 442–444.
- [142] L. Stryer, *Biochemistry*, Freeman, New York, **1988**.
- [143] G. Stubbs in *The Viruses, Vol. 1* (Eds.: A. McPherson, F. Jurnak), Wiley, New York, **1984**, pp. 149–202.
- [144] W. Shenton, T. Douglas, M. Young, G. Stubbs, S. Mann, *Adv. Mater.* **1999**, *11*, 253–256.
- [145] K. K. W. Wong, S. Mann, *Adv. Mater.* **1996**, *8*, 928.
- [146] T. Douglas, M. Young, *Nature* **1998**, *393*, 152–155.
- [147] T. Douglas, M. Young, *Adv. Mater.* **1999**, *11*, 679–681.
- [148] For examples for the SFM characterization of biomolecular systems, see: a) review: H. G. Hansma, *Annu. Rev. Phys. Chem.* **2001**, *52*, 71–92; b) enzymatic processing of nucleic acids: S. Kasas, N. H. Thomson, B. L. Smith, H. G. Hansma, X. S. Zhu, M. Guthold, C. Bustamante, E. T. Kool, M. Kashlev, P. K. Hansma, *Biochemistry* **1997**, *36*, 461–468; c) mobility of DNA molecules on solid supports: M. Argaman, R. Golan, N. H. Thomson, H. G. Hansma, *Nucleic Acids Res.* **1997**, *25*, 4379–4384; d) crystallization of transfer RNA: J. D. Ng, Y. G. Kuznetsov, A. J. Malkin, G. Keith, R. Giege, A. McPherson, *Nucleic Acids Res.* **1997**, *25*, 2582–2588; e) characterization of DNA nanostructures: X. Yang, L. A. Wenzler, J. Qi, X. Li, N. C. Seeman, *J. Am. Chem. Soc.* **1998**, *120*, 9779–9786; E. Winfree, F. Liu, L. A. Wenzler, N. C. Seeman, *Nature* **1998**, *394*, 539–544; for the characterization of synthetic supramolecular DNA-protein conjugates, see refs. [83, 84]; f) improved resolution of the SFM can be attained by using carbon nanotubes as tips: H. Dai, J. H. Hafner, A. G. Rinzler, D. T. Colbert, R. E. Smalley, *Nature* **1996**, *384*, 147–151; C. L. Cheung, J. H. Hafner, C. M. Lieber, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 3809–3813. The nanotubes can be functionalized by covalent methods (S. S. Wong, E. Joselevich, A. T. Woolley, C. L. Cheung, C. M. Lieber, *Nature* **1998**, *394*, 52–55) to enable the specific chemical and biological discrimination during the imaging, for instance, to detect DNA sequences specifically: A. T. Woolley, C. Guillemette, C. Li Cheung, D. E. Housman, C. M. Lieber, *Nat. Biotechnol.* **2000**, *18*, 760–763; g) the functionalization of SFM tips with phospholipase A allows the nanostructured hydrolysis of supported dipalmitoylphosphatidylcholine bilayers: H. Clausen-Schaumann, M. Grandbois, H. E. Gaub, *Adv. Mater.* **1998**, *10*, 949–952.

- [149] For reviews, see: A. Engel, H. E. Gaub, D. J. Muller, *Curr. Biol.* **1999**, 9, 133–136; A. Janshoff, M. Neitzert, Y. Oberdörfer, H. Fuchs, *Angew. Chem.* **2000**, 112, 3346–3374; *Angew. Chem. Int. Ed.* **2000**, 39, 3229–3355.
- [150] For reviews on surface plasmon resonance measurement, see: a) J. M. Brockman, B. P. Nelson, R. M. Corn, *Annu. Rev. Phys. Chem.* **2000**, 51, 41–63; b) C. Williams, *Curr. Opin. Biotechnol.* **2000**, 11, 42–46; c) R. L. Rich, D. G. Myszk, *Curr. Opin. Biotechnol.* **2000**, 11, 54–61.
- [151] For reviews on SERS, see: a) H. Kneipp, I. Itzkan, R. R. Dasari, M. S. Feld, *Chem. Rev.* **1999**, 99, 2957–2975; b) A. Campion, P. Kambhampati, *Chem. Soc. Rev.* **1998**, 27, 241–250.
- [152] M. A. Hayat, *Colloidal Gold: Principles, Methods, and Applications*, Academic Press, New York, **1989**.
- [153] C. D. Keating, K. M. Kovalski, M. J. Natan, *J. Phys. Chem. B* **1998**, 102, 9414–9425.
- [154] L. He, M. J. Natan, C. D. Keating, *Anal. Chem.* **2000**, 72, 5348–5355.
- [155] X. Dou, T. Takama, Y. Yamaguchi, K. Hirai, H. Yamamoto, S. Doi, Y. Ozaki, *Appl. Opt.* **1998**, 37, 759–763.
- [156] J. Kreuter in *Microcapsules and Nanoparticles in Medicine and Pharmacy* (Ed.: M. Donbrow), CRC, Boca Raton, **1992**.
- [157] D. E. Safer, J. Hainfeld, J. S. Wall, J. E. Reardon, *Science* **1982**, 218, 290–291.
- [158] J. F. Hainfeld, F. R. Furuya, *J. Histochem. Cytochem.* **1992**, 40, 177–184.
- [159] J. F. Hainfield, *Proc. Natl. Acad. Sci. USA* **1992**, 89, 11064.
- [160] O. D. Velev, E. W. Kaler, *Langmuir* **1999**, 15, 3693–3698.
- [161] J. Zhao, R. W. Henkens, J. Stonehuerner, J. P. O'Daly, A. L. Crumbliss, *J. Electroanal. Chem.* **1992**, 327, 109–119.
- [162] Y. Xiao, H.-X. Ju, H.-Y. Chen, *Anal. Chim. Acta* **1999**, 391, 73–82.
- [163] F. Patolsky, T. Gabriel, I. Willner, *J. Electroanal. Chem.* **1999**, 479, 69–73.
- [164] L. A. Lyon, M. D. Musick, M. J. Natan, *Anal. Chem.* **1998**, 70, 5177–5183.
- [165] L. He, M. D. Musick, S. R. Nicewarner, F. G. Salinas, S. J. Benkovic, M. J. Natan, C. D. Keating, *J. Am. Chem. Soc.* **2000**, 122, 9071–9077.
- [166] S. Schultz, D. R. Smith, J. J. Mock, D. A. Schultz, *Proc. Natl. Acad. Sci. USA* **2000**, 97, 996–1001.
- [167] A. P. Alivisatos, *Pure Appl. Chem.* **2000**, 72, 3–9.
- [168] Europium is often applied as a label in time-resolved immuno-fluorometric assays. For a review, see: I. Hemmila, S. Dakubu, V. M. Mukkala, H. Siitari, T. Lovgren, *Anal. Biochem.* **1984**, 137, 335–343. The labeling is either achieved by using an Eu-chelate (example: A. Scorilas, A. Bjartell, H. Lilja, C. Moller, E. P. Diamandis, *Clin. Chem.* **2000**, 46, 1450–1455) or -cryptate reagents (G. Mathis, *Clin. Chem.* **1995**, 41, 1391–1397). Time-resolved fluorescence imaging for quantitative histochemistry has recently been described using lanthanide chelates in nanoparticles: V. Vaisanen, H. Harma, H. Lilja, A. Bjartell, *Luminescence* **2000**, 15, 389–397.
- [169] E. Smythe, G. Warren, *Eur. J. Biochem.* **1991**, 202, 689–699.
- [170] M. Han, X. Gao, J. Z. Su, S. M. Nie, *Nat. Biotechnol.* **2001**, 19, 631–635.
- [171] S. Pathak, S. K. Choi, N. Arnheim, M. E. Thompson, *J. Am. Chem. Soc.* **2001**, 123, 4103–4104.
- [172] K. Riwozki, H. Meyssamy, H. Schnablegger, A. Kornowski, M. Haase, *Angew. Chem.* **2001**, 113, 574–578; *Angew. Chem. Int. Ed.* **2001**, 40, 573–576.
- [173] The colloidal gold-based detection of nucleic acids in homogeneous solutions can be enhanced by subsequent adsorption of the aggregates on filter membranes: a) R. Elghanian, J. J. Storhoff, R. C. Mucic, R. L. Letsinger, C. A. Mirkin, *Science* **1997**, 277, 1078–1081; b) J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin, R. L. Letsinger, *J. Am. Chem. Soc.* **1998**, 120, 1959–1964; c) R. A. Reynolds, C. A. Mirkin, R. L. Letsinger, *J. Am. Chem. Soc.* **2000**, 122, 3795–3796.
- [174] T. A. Taton, R. C. Mucic, C. A. Mirkin, R. L. Letsinger, *J. Am. Chem. Soc.* **2000**, 122, 6305–6306.
- [175] J. Reichert, A. Csaki, J. M. Köhler, W. Fritzsche, *Anal. Chem.* **2000**, 72, 6025–6029.
- [176] R. Möller, A. Csaki, J. M. Köhler, W. Fritzsche, *Nucleic Acids Res.* **2000**, 28, E91.
- [177] C. M. Niemeyer, B. Ceyhan, S. Gao, L. F. Chi, S. Peschel, U. Simon, *Colloid Polym. Sci.* **2001**, 279, 68–72.
- [178] A. Csaki, R. Möller, W. Straube, J. M. Köhler, W. Fritzsche, *Nucleic Acids Res.* **2001**, 29, E81–81.
- [179] S. Han, J. Lin, F. Zhou, R. L. Vellanoweth, *Biochem. Biophys. Res. Commun.* **2000**, 279, 265–269.
- [180] Particles of different morphology have also been assembled by means of DNA hybridization: a) gold nanowires: J. K. N. Mbindyo, B. D. Reiss, B. R. Martin, C. D. Keating, M. J. Natan, T. E. Mallouk, *Adv. Mater.* **2001**, 13, 249–254; b) gold nanorods: E. Dujardin, L. B. Hsin, C. R. C. Wang, S. Mann, *Chem. Commun.* **2001**, 1264–1265.
- [181] T. A. Taton, C. A. Mirkin, R. L. Letsinger, *Science* **2000**, 289, 1757–1760.
- [182] For reviews on DNA microarray technology, see: a) D. H. Blohm, A. Guiseppi-Elie, *Curr. Opin. Biotechnol.* **2001**, 12, 41–49; b) C. M. Niemeyer, D. Blohm, *Angew. Chem.* **1999**, 111, 3039–3043; *Angew. Chem. Int. Ed.* **1999**, 38, 2865–2869; c) E. M. Southern, K. Mir, M. Shchepinov, *Nat. Genet.* **1999**, 21, 5–9; see also the series of review articles published in this supplement of *Nature Genetics* and in the January issue of *Curr. Opin. Chem. Biol.* **2001**, 5.
- [183] T. A. Taton, G. Lu, C. A. Mirkin, *J. Am. Chem. Soc.* **2001**, 123, 5164–5165.
- [184] L. Lin, H. Zhao, J. Li, J. Tang, M. Duan, L. Jiang, *Biochem. Biophys. Res. Commun.* **2000**, 274, 817–820.
- [185] S. B. Han, J. Q. Lin, M. Satjapipat, A. J. Baca, F. M. Zhou, *Chem. Commun.* **2001**, 609–610.
- [186] X. D. Su, S. F. Y. Li, S. J. O'Shea, *Chem. Commun.* **2001**, 755–756.
- [187] G. R. Souza, J. H. Miller, *J. Am. Chem. Soc.* **2001**, 123, 6734–6735.
- [188] I. Willner, F. Patolsky, J. Wasserman, *Angew. Chem.* **2001**, 113, 1913–1916; *Angew. Chem. Int. Ed.* **2001**, 40, 1861–1864.
- [189] For reviews on gene-gun technology, see: a) M. T. Lin, L. Pulkkinen, J. Uitto, K. Yoon, *Int. J. Dermatol.* **2000**, 39, 161–170; b) see ref. [156]; c) examples for biolistic transfection: A. Udvardi, I. Kufferath, H. Grutsch, K. Zatloukal, B. Volc-Platzer, *J. Mol. Med.* **1999**, 77, 744–750; S. Kuriyama, A. Mitoro, H. Tsujinoue, T. Nakatani, H. Yoshiji, T. Tsujimoto, M. Yamazaki, H. Fukui, *Gene Ther.* **2000**, 7, 1132–1136; B. Wetterauer, K. Salger, P. Demel, H. Koop, *Biochim. Biophys. Acta* **2000**, 1499, 139–143; V. B. Vassilev, L. H. Gil, R. O. Donis, *Vaccine* **2001**, 19, 2012–2019; d) silica nanoparticles for DNA transfection: C. Kneuer, M. Sameti, U. Bakowsky, T. Schiestel, H. Schirra, H. Schmidt, C. M. Lehr, *Bioconjugate Chem.* **2000**, 11, 926–932.
- [190] For reviews on DNA vaccination, see: S. Gurunathan, D. M. Klinman, R. A. Seder, *Annu. Rev. Immunol.* **2000**, 18, 927–974; D. J. Shedlock, D. B. Weiner, *J. Leukocyte Biol.* **2000**, 68, 793–806; E. F. Fynan, R. G. Webster, D. H. Fuller, J. R. Haynes, J. C. Santoro, H. L. Robinson, *Proc. Natl. Acad. Sci. USA* **1993**, 90, 11478–11482; overview on delivery systems for gene-based vaccines: T. W. Dubensky, M. A. Liu, J. B. Ulmer, *Mol. Med.* **2000**, 6, 723–732; immunization of mice with peptomers covalently coupled to aluminum oxide nanoparticles: A. Frey, N. Mantis, P. A. Kozlowski, A. J. Quayle, A. Bajardi, J. J. Perdomo, F. A. Robey, M. R. Neutra, *Vaccine* **1999**, 17, 3007–3019.
- [191] a) A. Jordan, P. Wust, R. Scholz, B. Tesche, H. Föhling, T. Mitrovics, T. Vogl, J. Cervos-Navarro, R. Felix, *Int. J. Hyperthermia* **1996**, 12, 705–722; b) A. Jordan, P. Wust, R. Scholz, T. Mitrovics, H. Föhling, J. Gellermann, T. Vogl, J. Cervos-Navarro, R. Felix, *Int. J. Hyperthermia* **1997**, 13, 587–605; c) A. Jordan, R. Scholz, P. Wust, T. Schiestel, H. Schmidt, R. Felix, *J. Magn. Magn. Mater.* **1999**, 194, 185–196; d) A. Jordan, R. Scholz, P. Wust, H. Föhling, *J. Magn. Magn. Mater.* **1999**, 201, 413–419.
- [192] Examples of sequence-specific binding and bending of DNA by proteins: a) S. Becker, B. Groner, C. W. Muller, *Nature* **1998**, 394, 145–151; b) N. C. Horton, J. J. Perona, *J. Mol. Biol.* **1998**, 277, 779–787; c) D. N. Paoletta, Y. Liu, A. Schepartz, *Biochemistry* **1997**, 36, 779–787; d) *DNA-Protein Interactions* (Ed.: D. M. J. Lilley), IRL/Oxford University Press, New York, **1995**.
- [193] K. Luger, A. W. Maeder, R. K. Richmond, D. F. Sargent, T. J. Richmond, *Nature* **1997**, 389, 251–260.
- [194] J. L. Workman, R. E. Kingston, *Ann. Rev. Biochem.* **1998**, 67, 549–579.

- [195] D. S. Goodsell, M. L. Kopka, D. Cascio, R. E. Dickerson, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 2930–2934.
- [196] I. Brukner, S. Susic, M. Dlakic, A. Savic, S. Pongor, *J. Mol. Biol.* **1994**, *236*, 26–32.
- [197] L. A. Gearheart, H. J. Ploehn, C. J. Murphy, *J. Phys. Chem. B* **2000**, submitted.
- [198] For reviews, see: a) *Biom mineralization: Chemical and Biological Perspectives* (Eds.: S. Mann, J. Webb, R. J. P. Williams), VCH, Weinheim, **1989**; b) S. Mann, *Angew. Chem.* **2000**, *112*, 3532–3548; *Angew. Chem. Int. Ed.* **2000**, *39*, 3392–3406; c) structural biology of the developing dental matrix: A. G. Fincham, J. Moradian-Oldak, J. P. Simmer, *J. Struct. Biol.* **1999**, *126*, 270–299; d) For the biomimetic production of ceramic mesostructures with self-assembled surfactants and polymers, see: D. M. Dabbs, I. A. Aksay, *Annu. Rev. Phys. Chem.* **2000**, *51*, 601–622.
- [199] F. E. Round, R. M. Crawford, D. G. Mann, *The Diatoms: Biology and Morphology of the Genera*, Cambridge University Press, Cambridge, **1990**.
- [200] N. Kroger, R. Deutzmann, M. Sumper, *Science* **1999**, *286*, 1129–1132.
- [201] N. Kroger, R. Deutzmann, C. Bergsdorf, M. Sumper, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 14133–14138.
- [202] a) For a recent review, see: R. N. Ben, *ChemBioChem* **2001**, *2*, 161–166; for examples of the molecular structure of antifreeze proteins, see: b) S. P. Graether, M. J. Kuiper, S. M. Gagne, V. K. Walker, Z. Jia, B. D. Sykes, P. L. Davies, *Nature* **2000**, *406*, 325–328; c) S. P. Graether, C. I. DeLuca, J. Baardsnes, G. A. Hill, P. L. Davies, Z. Jia, *J. Biol. Chem.* **1999**, *274*, 11842–11847; d) W. Gronwald, M. C. Loewen, B. Lix, A. J. Daugulis, F. D. Sonnichsen, P. L. Davies, B. D. Sykes, *Biochemistry* **1998**, *37*, 4712–4721; e) A. Cheng, K. M. Merz, *Biophys. J.* **1997**, *73*, 2851–2873; f) Z. Jia, C. I. DeLuca, H. Chao, P. L. Davies, *Nature* **1996**, *384*, 285–288.
- [203] For reviews, see: a) D. Schüller, R. B. Frankel, *Appl. Microbiol. Biotechnol.* **1999**, *52*, 464–473; c) M. Sarikaya, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 14183–14185; c) vertebrate magnetoreceptor: C. E. Diebel, R. Proksch, C. R. Green, P. Neilson, M. M. Walker, *Nature* **2000**, *406*, 299–302.
- [204] C. T. Dameron, B. R. Smith, D. R. Winge, *J. Biol. Chem.* **1989**, *264*, 17355–17360.
- [205] C. T. Dameron, D. R. Winge, *Trends Biotechnol.* **1990**, *8*, 3–6.
- [206] R. Joerger, T. Klaus, C. G. Granqvist, *Adv. Mater.* **2000**, *12*, 407–409.
- [207] S. K. Lower, M. F. Hochella, Jr., T. J. Beveridge, *Science* **2001**, *292*, 1360–1363.
- [208] J. F. Banfield, S. A. Welch, H. Zhang, T. Thomson Ebert, R. L. Penn, *Science* **2000**, *289*, 751–754.
- [209] a) M. Kam, D. Perl-Treves, R. Sfez, L. Addadi, *J. Mol. Recognit.* **1994**, *7*, 257–264; b) N. Kessler, D. Perl-Treves, L. Addadi, *FASEB J.* **1996**, *10*, 1435–1442; c) D. Perl-Treves, N. Kessler, D. Izhaky, L. Addadi, *Chem. Biol.* **1996**, *3*, 567–577; d) D. Izhaky, L. Addadi, *Adv. Mater.* **1998**, *10*, 1009; e) D. Izhaky, L. Addadi, *Chem. Eur. J.* **2000**, *6*, 869–874.
- [210] D. Kadereit, J. Kuhlmann, H. Waldmann, *ChemBioChem* **2000**, *1*, 145–169.
- [211] M. Famulok, G. Mayer, M. Blind, *Acc. Chem. Res.* **2000**, *33*, 591–599.
- [212] *Nat. Biotechnol.* **2000**, *18*, Supplement IT 56–58.
- [213] R. F. Service, *Science* **1999**, *285*, 184–185.
- [214] K. C. Nicolaou, D. Vourloumis, N. Winssinger, P. S. Baran, *Angew. Chem.* **2000**, *112*, 47–126; *Angew. Chem. Int. Ed.* **2000**, *39*, 44–122.
- [215] K. Matsuura, M. Hibino, Y. Yamada, K. Kobayashi, *J. Am. Chem. Soc.* **2001**, *123*, 357–358.
- [216] S. M. Waybright, C. P. Singleton, K. Wachter, C. J. Murphy, U. H. F. Bunz, *J. Am. Chem. Soc.* **2001**, *123*, 1828–1833.
- [217] J. E. Gestwicki, L. E. Strong, L. L. Kiessling, *Angew. Chem.* **2000**, *112*, 4741–4744; *Angew. Chem. Int. Ed.* **2000**, *39*, 4567–4570.
- [218] K. C. Grabar, J. E. Deutsch, M. J. Natan, *J. Polym. Prepr.* **1995**, 69–70.
- [219] W.-L. Shaiu, D. D. Larson, J. Vesenska, E. Henderson, *Nucleic Acids Res.* **1993**, *21*, 99–103.
- [220] S. K. Chopra, J. C. Martin, *Heteroat. Chem.* **1991**, *2*, 71–79.
- [220] Since the submission of this manuscript, further progress in this rapidly expanding field of research has been published: a) DNA–nanoparticle conjugates: DNA-modified superparamagnetic 3-nm iron oxide particles, which self-assemble in the presence of complementary single-stranded DNA, could be used as magnetic nanosensors: L. Josephson, J. M. Perez, R. Weissleder, *Angew. Chem.* **2001**, *113*, 3304–3306; *Angew. Chem. Int. Ed.* **2001**, *40*, 3204–3206; patterns of mercaptohexadecanoic acid produced by Dip-Pen nanolithography were chemically modified with DNA oligomers to assemble orthogonally gold nanoparticles, functionalized with complementary oligonucleotides: L. M. Demers, S.-J. Park, T. A. Taton, Z. Li, C. A. Mirkin, *Angew. Chem.* **2001**, *113*, 3161–3163; *Angew. Chem. Int. Ed.* **2001**, *40*, 3071–3073; DNA-modified core-shell Ag/Au nanoparticles: Y. W. Cao, R. Jin, C. A. Mirkin, *J. Am. Chem. Soc.* **2001**, *123*, 7961–7962; DNA transcription is inhibited by cationic mixed monolayer protected gold clusters: C. M. MacIntosh, E. A. Esposito, A. K. Boal, J. M. Simard, C. T. Martin, V. M. Rotello, *J. Am. Chem. Soc.* **2001**, *123*, 7626–7629; b) gold nanoparticles functionalized with proteins and biomimetic linker units: characterization of protease-coated colloidal gold: A. Gole, C. Dash, C. Soman, S. R. Sainkair, M. Rao, M. Sastry, *Bioconjug. Chem.* **2001**, *12*, 684–690; Lectin-induced association of gold particles modified with  $\alpha$ -Lactosyl- $\omega$ -mercaptopoly(ethylene glycol): H. Otsuka, Y. Akiyama, Y. Nagasaki, K. Kataoka, *J. Am. Chem. Soc.* **2001**, *123*, 8226–8230; gold nanoparticles functionalized with elastin biopolymers: N. Nath, A. Chilkoti, *J. Am. Chem. Soc.* **2001**, *123*, 8197–8202; resorcinarene tetrathiolates were used as biomimetic linker units to organize large gold nanoparticles: B. Kim, S. L. Tripp, A. Wei, *J. Am. Chem. Soc.* **2001**, *123*, 7955–7956; c) SFM cantilever-based nanomechanical sensor devices (ref. [69]) have been applied in the detection of cancer antigens (G. Wu, R. H. Datar, K. M. Hansen, T. Thundat, R. J. Cote, A. Majumdar, *Nat. Biotechnol.* **2001**, *19*, 856–860) and single nucleotide polymorphisms (K. M. Hansen, H. F. Ji, G. Wu, R. Datar, R. Cote, A. Majumdar, T. Thundat, *Anal. Chem.* **2001**, *73*, 1567–1571). Systematic DNA hybridization experiments revealed that the nanomechanical motion response of the cantilever is caused by changes in configurational entropy and intermolecular energetics induced by specific biomolecular interactions: G. Wu, H. Ji, K. Hansen, T. Thundat, R. Datar, R. Cote, M. F. Hagan, A. K. Chakraborty, A. Majumdar, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 1560–1564.