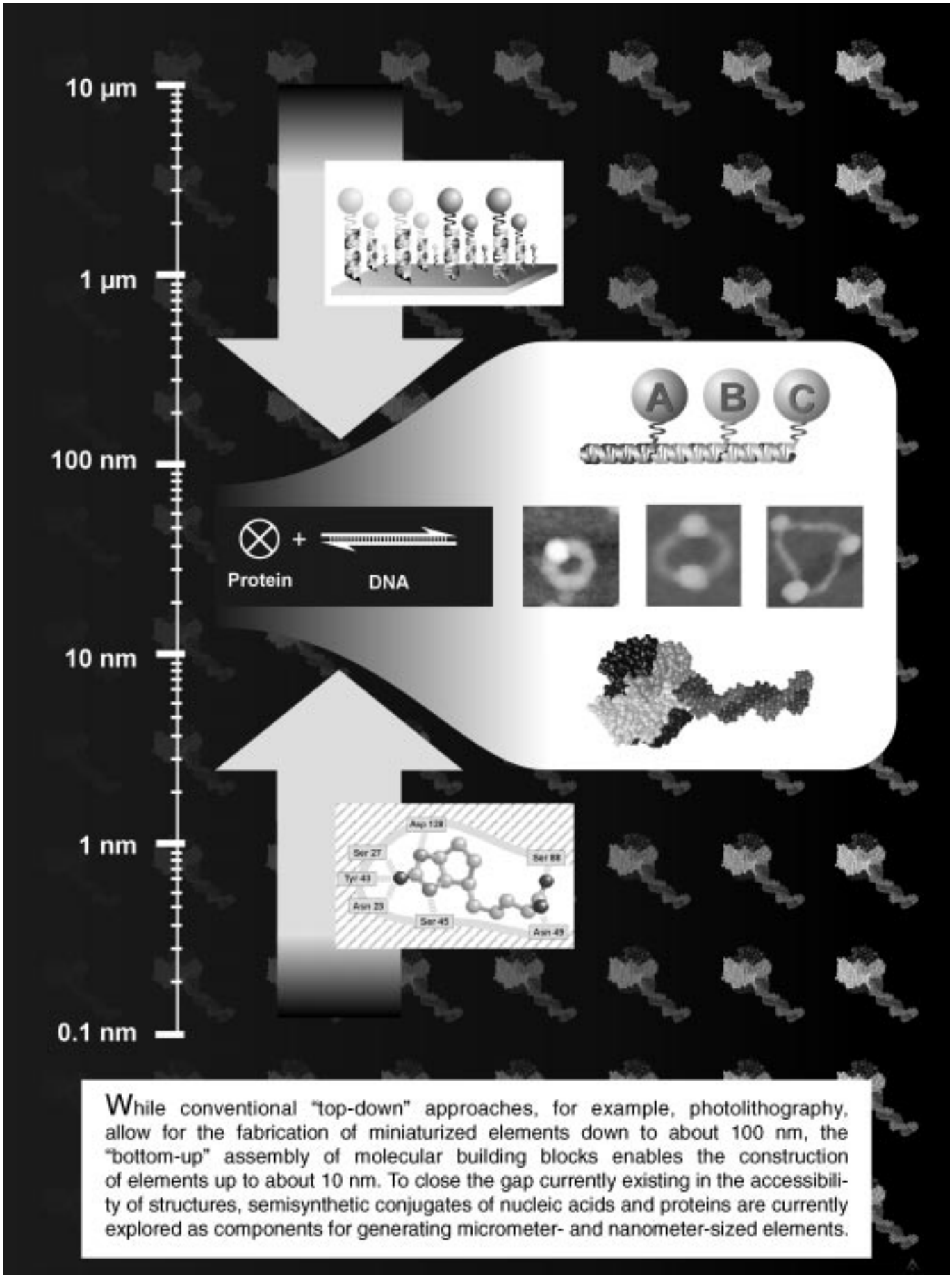

While conventional "top-down" approaches, for example, photolithography, allow for the fabrication of miniaturized elements down to about 100 nm, the "bottom-up" assembly of molecular building blocks enables the construction of elements up to about 10 nm. To close the gap currently existing in the accessibility of structures, semisynthetic conjugates of nucleic acids and proteins are currently explored as components for generating micrometer- and nanometer-sized elements.

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Bioorganic Applications of Semisynthetic DNA – Protein Conjugates

Christof M. Niemeyer[a]

Abstract: Semisynthetic DNA – protein conjugates are versatile molecular tools useful, for instance, in the self-assembly of high-affinity reagents for immunological detection assays, the fabrication of highly functionalized laterally microstructured biochips, and the biomimetic "bottom-up" synthesis of nanostructured supramolecular devices. This concept paper summarizes the current state-of-the-art concerning the synthesis, characterization, and applications of such hybrid molecules, and also draws perspectives on future developments.

Keywords: analytical methods · microtechnology · nanostructures · nucleic acids · proteins

Introduction

An important goal of synthetic supramolecular chemistry is to understand and to mimic nature's fascinating principles, in particular, to form complex and highly functional supramolecular aggregates from small molecular building blocks by means of self-assembly processes.[1] Basic studies on such biomimetic "bottom-up" approaches have strong implications on the development of artificial nanometer-scaled elements, currently elaborated due to commercial requirements of producing microelectronics and micromechanical devices of increasingly minimized dimensions. Current technologies hardly allow the generation of technical elements smaller than 100 nm, but, to refer to the famous lecture of the Nobel physicist Richard Feynman, *"there is plenty of room at the bottom"*.[2] It is apparent that a further reduction of the available microsystems through photolithographic "top-down" methods is becoming increasingly uneconomical, and, thus, the biomimetic bottom-up assembly is considered a highly attractive alternative. In this context, the ribosome has to be mentioned as a brilliant natural example of this approach; it demonstrates the power of biologically programmed molecular recognition. This cellular nanomachine is capable of synthesizing polypeptide chains by using an RNA molecule as the informational template. The ribosome spontaneously self-assembles from its more than 50 individual building blocks, driven by an assortment of low-specific, noncovalent contacts between discrete amino acids of the protein components interacting with distinct nucleotide bases and the phosphate backbone of the ribosomal RNAs.[3] Motivated by such fascinating models, early researchers have suggested to fabricate synthetic nanometer-sized elements from biomolecular building blocks.[4] Under these, DNA is a particularly promising construction material for the fabrication of nanometer-sized elements owing to its unique and simply predictable recognition capabilities, high physicochemical stability, mechanical rigidity, and high precision-processing ability.[5]

[a] Priv. Doz. Dr. C. M. Niemeyer
Universität Bremen, FB2-UFT
Biotechnologie und Molekulare Genetik
Leobener Str., 28359 Bremen (Germany)
Fax: (+49) 421-218-7578
E-mail: cmn@biotec.uni-bremen.de

Self-Assembled Oligomeric Networks That Consist of DNA and Streptavidin

Despite the enormous biological importance, only little has been reported on the synthesis of artificial nucleic-acid – protein conjugates.[6, 7] A convenient approach to readily produce semisynthetic DNA – protein conjugates is based on the remarkable biomolecular recognition of the water-soluble molecule biotin (vitamin H) by the homotetrameric protein streptavidin (STV). The affinity constant of the STV – biotin interaction of about $10^{14}\,dm^3\,mol^{-1}$ indicates the strongest ligand – receptor interaction currently known.[8] Another great advantage of STV is its extreme chemical and thermal stability. Since biotinylated materials are often commercially available or can be prepared with a variety of mild biotinylation procedures, biotin – STV conjugates form the basis of many diagnostic and analytical tests.[9] For example, biotinylated oligodesoxyribonucleotides, prepared on a routine basis by today's automated nucleic acids synthesis, are versatile tools in the various applications of molecular biology and nucleic acid analyses. Although broadly applied, the formation and structure of conjugates of STV and biotinylated nucleic acids are not yet fully exploited.

We have recently reported on the self-assembly of STV **1** and 5′,5′-bisbiotinylated double-stranded DNA fragments **2** (Scheme 1).[10] The bivalent double-stranded DNA (dsDNA) molecules cross-link the tetravalent STV, thereby generating

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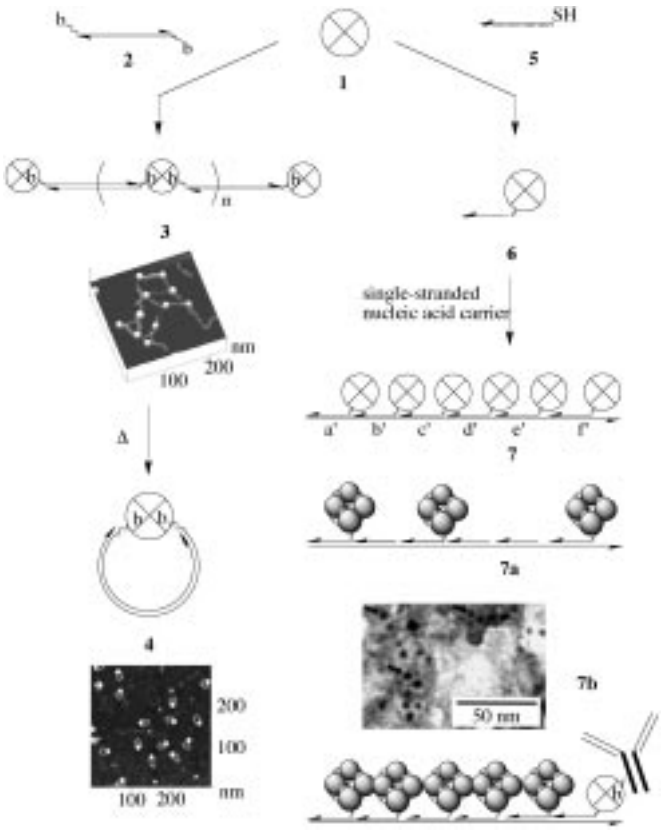

Scheme 1. Synthesis of DNA – streptavidin (STV) conjugates. Noncovalent oligomers **3** were assembled from STV **1** and 5′,5′-bis-biotinylated DNA **2**. Note that the schematic structure of **3** is simplified, since a portion of the STV molecules function as tri- and tetravalent linker molecules between adjacent DNA fragments (see the SFM image in the inset below **3**). The supramolecular networks of **3** can be disrupted by thermal treatment, leading to the formation of DNA – STV nanocircles **4** (see also the SFM image in the inset below **4**). DNA – STV conjugates **6** are obtained by covalent coupling of a 5′-thiol-modified oligonucleotide **5** and STV. Due to their bispecificity, the adducts **6** can be functionalized by the coupling of biotinylated compounds, such as proteins and gold nanoclusters. Stoichiometrically controlled, nanostructured aggregates can be generated from a set of conjugates **6** with distinct nucleotide sequences, for example **a** through **f**. The adducts self-assemble in the presence of a single-stranded nucleic acid carrier molecule, which contains complementary sequence stretches, to form supramolecular aggregates **7**. An antibody-containing biometallic construct **7b** was fabricated from gold-labeled **6a** – **6e** and a conjugate of **6f** and a biotinylated immunoglobulin, previously coupled in separate reactions. The 3′-end of the oligonucleotides is indicated by an arrowhead, the spacer-chains between DNA and protein are represented by wavy lines. The transmission electron microscopy image shown in the inset on top of **7b** was obtained subsequent to the specific immunosorption of **7b** to surface-immobilized complementary antigen.

three-dimensionally linked networks. Studies with nondenaturing gel electrophoresis and scanning force microscopy (SFM) indicated that **1** and **2** reproducibly self-assemble to form oligomeric DNA – STV conjugates **3**, which, most strikingly, predominantly contain bivalent STV molecules that bridge adjacent DNA fragments. Despite of the tetravalency of the STV protein, trivalent STV branchpoints occur with a low frequency, and the presence of tetravalent STV in the supramolecular networks is scarce (see image in Scheme 1). As a consequence of the STV's low valency, the oligomeric conjugates have a large residual biotin-binding capacity that can be utilized for further functionalization of the complexes. As an example, biotinylated antibodies can be coupled with the oligomers **3**. The resulting conjugates can be applied as reagents in immunoassays. Due to the tremendous amplification potential of DNA by the polymerase chain reaction (PCR), the DNA – protein conjugates can be detected by PCR amplification at levels far below those available for the detection of proteins by conventional antibody-based enzyme-linked immunosorbent assay (ELISA). The combination of the ELISA with the amplification power of the PCR was originally developed by Sano et al.[11] This method, termed immuno-PCR (IPCR), generally allows for an about 1000-fold enhancement of the detection limit of the analogous ELISA.[12] The use of the DNA – STV oligomers **3** as reagents in IPCR leads to an additional about 10-fold sensitivity enhancement compared with the conventional IPCR procedure.[10]

The oligomeric DNA – STV conjugates **3** might also serve as a molecular framework for the generation of DNA-based nanomaterials. For instance, the biotin-binding capabilities can be used for the attachment and positioning of biotinylated macromolecules, such as enzymes and antibodies,[13a,b] low molecular weight peptides and fluorophores,[13d, 16b] and even inorganic metal nanoclusters[14] or polymers.[15] Moreover, due to their size, connectivity and topography, the oligomeric DNA – STV aggregates **3** can be used as a model system for nanostructured particle networks, for instance, serving to establish basic immobilization and characterization techniques as well as novel approaches for the generation of switchable nanoparticle networks.[13g] Since the oligomers **3** are formed by statistical self-assembly, distinct supramolecular species might either be isolated and further functionalized on a preparative scale, or else, obtained by controlling the self-assembly process. As an example of the latter, we have recently established that networks of **3** can be effectively transformed into supramolecular DNA – STV nanocircles **4** by thermal treatment (Scheme 1).[16] Since the endogeneous protein molecule within the DNA – STV nanocircles **4** allows for convenient attachment of other functional molecules, potential applications of such nanostructures include their use as building blocks and templates for nano- and microstructured surface architecture,[5c] their utilization as soft material topographical standards for the development of SFM measurement modes,[13h] and their employment as molecular tools in novel immunological assays.[16b]

In addition, functional analogues of the conjugates **3** and **4** can be obtained from the use of STV covalently functionalized with a single-stranded oligonucleotide. These DNA – STV adducts **6** are synthesized from 5′-thiol-modified oligonucleotides **5** and STV **1** by means of a heterobispecific crosslinker, such as sulfosuccinimidyl-4(*p*-maleimidophenyl)-butyrate (Scheme 1).[6] The covalently bound oligonucleotide moiety in **6** provides a specific recognition domain for the complementary nucleic acid sequence in addition to the four native biotin-binding sites. Thus, **6** can be used as a versatile molecular adaptor in a variety of applications, ranging from the the assembly of nanostructured protein arrays (Scheme 1) to the generation of laterally microstructured protein biochips (Scheme 2). We have demonstrated that **6** can be incorpo-

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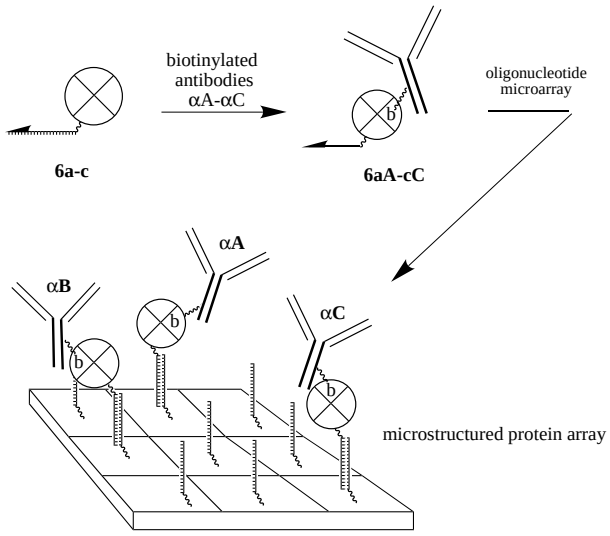

Scheme 2. Schematic drawing of the "DNA-directed immobilization". A set of adducts **6a–c** were functionalized by the coupling of biotinylated antibodies. Capture oligonucleotides immobilized as an array on a solid support are used as positioning elements on a microstructured surface. The various DNA-tagged antibodies are then allowed to bind to their complements by formation of a DNA double helix. Note that due to the enormous specificity of DNA Watson–Crick base pairing, many compounds can be site-specifically immobilized simultaneously in a single step.

rated as building blocks within the self-assembled supramolecules **3**.[13f] This allows for the efficient attachment of functional molecular components, such as antibody molecules, thereby opening novel routes to the utilization of DNA–STV conjugates in life-sciences and nanobiotechnology.

Microstructured DNA–Protein Arrays

The covalent DNA–STV conjugates **6** can be utilized to generate laterally microstructured protein arrays. Such devices are currently of tremendous interest due to the demands of high-throughput biomedical analysis and proteom research.[17–19] While comparable microarrays comprised of oligonucleotides[20] can easlily be fabricated by automated deposition techniques, the stepwise, successive immobilization of proteins on chemically activated surfaces is obstructed by the general instability of sensitive biomolecules, often revealing a high tendency for denaturation. DNA-directed immobilization (DDI) provides a chemically mild, site-selective process for the attachment of multiple delicate proteins to a solid support (Scheme 2).[6, 13b] This method uses DNA microarrays as a matrix for the simultaneous, site-selective immobilization of many different DNA-tagged proteins and other molecular compounds. Since the lateral surface patterning is carried out at the level of the physicochemically stable nucleic acid oligomers, the DNA microarrays can be stored almost indefinately, functionalized with proteins of interest by DDI immediately prior to use, and subsequent to hybridization, they can be regenerated by alkaline denaturation of the double helical DNA cross-linkers.[13b]

As an early demonstration of the feasibility of the DDI approach, the "self-sorting immobilization" of a set of antibody molecules, tagged with distinct oligonucleotides by means of the covalent STV conjugates **6**, was demonstrated by the site-selective hybridization to distinct positions of an array, containing the complementary capture oligonucleotides.[6] Later evaluation, carried out with oligonucleotide-modified enzymes, indicated that DDI not only proceeds with a higher immobilization efficiency than conventional immobilization techniques, but is also completely reversible. Series of more than 150 cycles of hybridization and subsequent regeneration were carried out with various DNA-tagged proteins allowing for the reproducible functionalization of sensor-surfaces with adjustable amounts of proteins. As a particular advantage of using the DDI method in immunosensor applications, the intermolecular binding of the antigen target by the antibodies can be carried out in homogeneous solution instead of using a much less efficient heterogeneous solid-phase immunosorption. Subsequently, the immunocomplex formed can be captured by means of DNA-directed immobilization.[21a–c]

These features should allow us to use the DDI technique in a variety of applications ranging from the recovery and reconfiguration of biosensor chips,[13b] the fabrication of mixed arrays containing both nucleic acids and proteins for genome and proteome research,[13c] to the generation of miniaturized multiplex sensor elements.[13a,b, 21, 22] Despite these fascinating biotechnological perspectives, special emphasis has to be given to the chemical and structural features of the nucleic acid constituents employed in the DNA-directed assembly, since they determine the immobilization efficiency of the individual components. As an example, solid-phase hybridization studies have shown that the attachment of the voluminous STV protein to an oligonucleotide leads to up to a fivefold decrease in hybridization kinetics.[13a] This study also revealed that variations in the oligonucleotide sequence induce up to 100-fold changes in the immobilization efficiency, correllating with the presence of nucleic acid secondary structures, such as intramolecular hairpin loops. Solutions to this problem might be obtained from the incorporation of chemical groups into the DNA-tagged bioconjugates to modulate its hybridization characteristics,[13d] or else, from the employment of artificial nucleic acid analogues, such as peptide nucleic acids,[23] which are less susceptible to secondary structure formation.

Nanostructured Protein Assemblies

Besides the lateral functionalization at the micrometer scale, a nanostructuring of surfaces can also be achieved through the DNA-directed immobilization. For this, surface-bound nucleic acids of the appropriate length are used to generate nanostructured complexes from various molecular building blocks, for instance enzymes or other functional proteins. The concept of using DNA as a framework for the precise spatial arrangement of molecular components, initially suggested by Seeman,[4c] was experimentally demonstrated by positioning several of the covalent DNA–STV conjugates **6** along a

single-stranded RNA or DNA carrier molecule that contained a set of complementary sequences (Scheme 1).[6] Owing to the adapter properties of **6**, almost any type of biotinylated compound can be organized within a supramolecular aggregate.[6, 13, 14] As an additional attractive feature, the tetravalency of the streptavidin even allows the DNA – STV conjugates **6** to be functionalized with two different types of biotinlyated molecules.[13d] Subsequent to the coupling of a voluminous macromolecular compound, such as an enzyme, the remaining biotin binding sites are saturated with low molecular weight biotinyl compounds. For instance, a positively charged biotinylated peptide was coupled in order to fine-tune the bioconjugate's nucleic acid hybridization properties. Moreover, the coupling of the biotinylated peptides can be applied to supplement the bioconjugate's functionality, for instance, to enhance the catalytic turnover of enzymatic transformations or to exhibit high sensitivity as a detection tool in biosensor applications.[13d]

Similar as described for the DDI technique, the chemical and structural features of the nucleic acid constituents employed in the supramolecular assembly of DNA-tagged components are essential for the aggregation efficiency of the individual components. Often the carrier molecule contains stable intramolecular secondary structures, and, thus, an equilibrium is formed that involves both the uncomplexed carrier and the protein – carrier conjugate. This equilibrium can be affected by "helper" oligonucleotides that bind to uncomplexed regions of the carrier, thereby disrupting its secondary structure and shifting the aggregation equilibrium towards completion.[13e, 14] Moreover, it was demonstrated that DNA molecules are superior templates for the supramolecular assembly relative to RNA carriers, because of the lower stability of the intramolecular folding in DNA.[13e] These results again underline the necessity to overcome the problems associated with intramolecular secondary structure, for instance, by either chemical means, such as the employment of synthetic DNA analogues, or else by developing model algorithms that allow for the computational a-priori design of appropriate nucleic acid fragments.

Two key publications describing the assembly of DNA-functionalized gold colloids and nanoclusters have largely spread out the common interest in the nucleic-acid-directed assembly of molecular compounds. The work of Mirkin et al. [24] and Alivisatos et al. [25] was motivated by the requirements of material sciences to generate well-defined superstructures of nanocrystal metal clusters and semiconductor quantum dots, which are investigated for new material properties and potential applications in microelectronics and optics. Meanwhile, both groups have impressively demonstrated the power of the DNA-directed assembly to generate novel, programmed materials, ranging from stoichiometrically defined nanoscale assemblies to macroscopic repetitive DNA – nanocluster aggregates and binary networks, which can even be used for biosensor applications.[26] Stimulated by these advances, the DNA – STV conjugates **6** have also been applied as auxiliaries to organize gold nanoclusters.[14] Subsequent to biotinylation, gold nanoclusters were coupled with **6**, and the cluster-loaded proteins were assembled by nucleic acid hybridization, thereby generating novel biometallic nanostructures such as **7** in Scheme 1. As a particular advantage of this system, the DNA – STV conjugates **6** can be used like components of a molecular construction kit. Thus, functional proteins, such as an immunoglobulin molecule, can be conveniently incorporated into the biometallic nanostructures. The proof of feasibility was achieved by the assembly of construct **7b** (Scheme 1), which is capable of specifically binding to surface-immobilized complementary antigen.[14] Such experiments clearly demonstrate the applicability of DNA-directed assembly to construct novel inorganic/bioorganic hybrid nanomaterials.

Towards Oligofunctional Protein Constructs

The use of specific nucleic acid hybridization opens up a novel, uncomplicated yet powerful strategy for supramolecular synthesis. Various molecular devices are coupled with a distinct oligonucleotide sequence and are subsequently organized on appropriate nucleic acid scaffolds. This should allow even the fabrication of highly complex supramolecular structures by means of a modular construction kit. Seeman and co-workers have impressively demonstrated the power of using DNA in the rational construction of one-, two-, and three-dimensional DNA molecular frameworks.[5a,b] They use branched DNA molecules, "DNA-junctions" or "double-crossover" molecules, that contain three, four, and more double helical arms as modular building blocks to fabricate extended one-dimensional structures, periodic two-dimensional lattice structures, and even complex three-dimensional supramolecules of DNA. For instance, they have fabricated the "truncated octahedron", a DNA polyhedron that contains an individual arm at each of its 24 vertices, which might be used for the selective spatial positioning of proteins, inorganic nanoclusters, or other functional molecular devices.

Although these examples demonstrate the significant progress achieved in the fabrication of artifical DNA nanostructures, the use of such framework to realize dense protein and biochip assemblies, suggested earlier by Seeman,[4c] has not yet been accomplished. However, it should be noted in this regard that Smith and co-workers have reported on the site-selective arrangement of several proteins, DNA (cytosin-5)-methyltransferases, along a one-dimensional double-stranded DNA fragment.[27] Their approach is based on the specific binding of methyltransferases to distinct recognition sequences within double helical DNA and their ability to form covalent adducts if the synthetic DNA base analogue 5-fluorocytosine is present in the recognition site. Interestingly, methyltransferases have also been shown to be capable of modulating the charge transport that can occur along the DNA double helix.[28] Since the methyltransferase extrudes the target base cytosine completely out of the double helix, this local distortion effectively reduces the photoinduced charge transfer through the DNA duplex. These results give rise not only to the development of electrochemical sensors for the study of protein – DNA interactions, but also to the development of novel bioelectronic devices.

Future applications of DNA-directed protein organization will include the manufacturing of stoichiometrically and

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spatially defined aggregates from multiple enzymes and antibody fragments. As an example, the rational assembly of oligospecific antibodies is highly attractive, since the presence of multiple binding domains leads to an enhanced affinity for the target structure. Supramolecular constructs that contain a DNA structural backbone to control the spatial arrangement of the binding sites should allow for specific recognition of the target's topography, even when the individual epitopes are either not nearby or reveal only weak antibody-antigen interactions. These type of applications will require a stringent regioselective control over the positioning of the binding domains, which should be obtainable by the use of single-chain antibody fragments[29] or aptamers.[30] Both types of receptor molecules are small enough to ensure that the distance between the supramolecule's binding sites is predominantly determined by the structure of the DNA backbone. An additinal great advantage of DNA-based, nanoscaled receptor constructs is that the backbone can be detected or modified by enzymatic means. For example, the supramolecular constructs are traceable at extremely low levels and even in rather complex environments owing to the enormous detection potential of PCR techniques.[10] Moreover, enzymatic or cytotoxic groups might be incorporated to allow for therapeutic applications.[31]

Spatially arranged multienzyme constructs, which are not accessible by conventional chemical cross-linking, are another class of attractive targets. In biological systems, multienzyme complexes reveal mechanistic advantages during the multistep catalytic transformation of a substrate, since reactions limited by the rate of diffusional transport are accelerated by the immediate proximity of the catalytic centers. Furthermore, the "substrate-channeling" of intermediate products avoids side reactions. Besides serving to explore proximity effects in biochemical pathways, artifical multienzymes will allow the development of novel catalysts for enzyme process technology that are capable of regenerating cofactors, or else to perform the multistep chemical transformation of cheap precursor molecules into drugs and fine chemicals.[32]

Conclusion

In conclusion, it seems likely that DNA – protein conjugates and nucleic-acid-based molecular fabrication of nanostructured elements will contribute to the rapid establishment of a novel discipline termed as "biomolecular nanotechnology", or perhaps "nanobiotechnology". The supramolecular arrangements may find applications as biological encapsulation and drug-delivery systems, artificial multienzymes, or light-harvesting devices. Moreover, nucleic-acid-based conjugates might be utilized for the self-assembling nanoscale fabrication of technical elements and hybrid composites, such as dense arrays of ultra fast molecular switches, transistors, or logical elements,[33] as well as chimeric hybrid devices for applications in material sciences, biomedical diagnostics, and interface structures between electronic and living systems. However, a variety of serious technical obstacles remain to be solved, ranging from the currently limited availability of large amounts of synthetic DNA fragments to the refinement of appropriate analytical techniques. Nevertheless, it seems safe to predict that besides electrophoresis, optical spectroscopy, and nuclear magnetic resonance, in particular the rapid improvements of scanning probe microscopy[34] and real-time affinity measurements[35] will soon allow for the comprehensive understanding of biomolecular structure and reactivity of synthetic nucleic-acid-based supramolecular components. Thus, the examples presented above give the promise of a rapid and highly exciting development of DNA-based technology.[36]

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[1] a) J.-M. Lehn, *Supramolecular Chemistry*; *Concepts and Perspectives*, VCH, Weinheim, **1995**; b) *Comprehensive Supramolecular Chemistry* (Eds.: J.-M. Lehn, J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vögtle), Elsevier, Oxford, **1996**; c) F. Vögtle, *Supramolecular Chemistry*, Wiley, Chichester, **1991**.

[2] R. P. Feynman in *Miniaturization* (Ed.: H. D. Gilbert), Reinhold, New York, **1961**, pp. 282 – 296.

[3] The structure of the 50S ribosomal subunit has recently been resolved at 2.4 Å resolution: a) N. Ban, P. Nissen, J. Hansen, P. B. Moore, T. A. Steitz, *Science* **2000**, *289*, 905; the atomic structures of this subunit and its complexes with two substrate analogues revealed that the ribosome is in fact a ribozyme: b) P. Nissen, J. Hansen, N. Ban, P. B. Moore, T. A. Steitz, *Science* **2000**, *289*, 920; the atomic structure of the 30S subunit has been resolved at 5 Å resolution: c) W. M. Clemons, Jr., J. L. May, B. T. Wimberly, J. P. McCutcheon, M. S. Capel, V. Ramakrishnan, *Nature* **1999**, *400*, 833; the 8 Å crystal structure of the 70S ribosome revealed a double-helical RNA bridge between the 50S and the 30S subunit: d) G. M. Culver, J. H. Cate, G. Z. Yusupova, M. M. Yusupov, H. F. Noller, *Science* **1999**, *285*, 2133.

[4] a) K. E. Drexler, *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 5275; b) K. E. Drexler, *Nanosystems: Molecular Machinery, Manufacturing, and Computation*, Wiley, New York, **1992**; c) N. C. Seeman, *J. Theor. Biol.* **1982**, *99*, 237.

[5] For reviews, see: a) N. C. Seeman, *Trends Biotechnol.* **1999**, *17*, 437; b) N. C. Seeman, *Angew. Chem.* **1998**, *110*, 3408; *Angew. Chem. Int. Ed.* **1998**, *37*, 3220; c) C. M. Niemeyer, *Curr. Opin. Chem. Biol.* **2000**, *4*, 609.

[6] C. M. Niemeyer, T. Sano, C. L. Smith, C. R. Cantor, *Nucleic Acids Res.* **1994**, *22*, 5530.

[7] A pioneering work reported on the synthesis of a oligonucleotide – staphylococcus-nuclease conjugate as a synthetic nuclease: a) D. R. Corey, P. G. Schultz, *Science* **1987**, *238*, 1401; the conjugates reveal enhanced kinetic rate constants for the hybridization with double-stranded target DNA: b) D. R. Corey, *J. Chem. Am. Soc.* **1995**, *117*, 9373; oligonucleotide – enzyme conjugates have been used as hybridization probes: c) S. S. Ghosh, P. M. Kao, A. W. McCue, H. L. Chappelle, *Bioconjugate Chem.* **1990**, *1*, 71; covalent conjugates of single- and double-stranded DNA fragments and an antibody molecule were used as probes in immuno-PCR: d) E. R. Hendrickson, T. M. Hatfield, T. M. Truby, R. D. Joerger, W. R. Majarian, R. C. Ebersole, *Nucleic Acids Res.* **1995**, *23*, 522; an oligonucleotide – asialoglycoprotein conjugate for antisense applications: e) S. B. Rajur, C. M. Roth, J. R. Morgan, M. L. Yarmush, *Bioconjugate Chem.* **1997**, *8*, 935; f) see also the approaches described in [19] and [27]; for oligonucleotide-streptavidin conjugates see references [6, 13 – 16].

[8] P. C. Weber, D. H. Ohlendorf, J. J. Wendoloski, F. R. Salemme, *Science* **1989**, *243*, 85.

[9] M. Wilchek, E. A. Bayer, *Methods Enzymol.* **1990**, *184*, 51.

[10] C. M. Niemeyer, M. Adler, B. Pignataro, S. Lenhert, S. Gao, L. F. Chi, H. Fuchs, D. Blohm, *Nucleic Acids Res.* **1999**, *27*, 4553.
[11] T. Sano, C. L. Smith, C. R. Cantor, *Science* **1992**, *258*, 120.
[12] C. M. Niemeyer, M. Adler, D. Blohm, *Anal. Biochem.* **1997**, *246*, 140.
[13] a) C. M. Niemeyer, W. Bürger, R. M. J. Hoedemakers, *Bioconjugate Chem.* **1998**, *9*, 168; b) C. M. Niemeyer, L. Boldt, B. Ceyhan, D. Blohm, *Anal. Biochem.* **1999**, *268*, 54; c) C. M. Niemeyer, L. Boldt, B. Ceyhan, D. Blohm, *Biol. Chem.* **1999**, S182; d) C. M. Niemeyer, B. Ceyhan, D. Blohm, *Bioconjugate Chem.* **1999**, *10*, 708; e) C. M. Niemeyer, L. Boldt, B. Ceyhan, D. Blohm, *J. Biomol. Struct. Dyn.* **1999**, *17*, 527; f) C. M. Niemeyer, M. Adler, S. Gao, L. F. Chi, *Bioconjugate Chem.* **2001**, *12*, in press; g) C. M. Niemeyer, M. Adler, S. Lenhert, S. Gao, H. Fuchs, L. F. Chi, *ChemBioChem* **2001**, *2*, 260; h) S. Gao, L. F. Chi, S. Lenhert, B. Anczykowsky, C. M. Niemeyer, M. Adler, H. Fuchs, *ChemPhysChem* **2001**, in press.
[14] C. M. Niemeyer, W. Bürger, J. Peplies, *Angew. Chem.* **1998**, *110*, 2391; *Angew. Chem. Int. Ed.* **1998**, 37, 2265.
[15] R. B. Fong, Z. Ding, C. J. Long, A. S. Hoffman, P. S. Stayton, *Bioconjugate Chem.* **1999**, *10*, 720.
[16] a) C. M. Niemeyer, M. Adler, S. Gao, L. F. Chi, *Angew. Chem.* **2000**, *112*, 3183; *Angew. Chem. Int. Ed.* **2000**, *39*, 3055; b) C. M. Niemeyer, R. Wacker, M. Adler, *Angew. Chem.* **2001**; *Angew. Chem. Int. Ed.* **2001**, in press.
[17] For reviews on proteome research, see: a) M. J. Dutt, K. H. Lee, *Curr. Opin. Biotechnol.* **2000**, *11*, 176; b) F. Lottspeich, *Angew. Chem.* **1999**, *111*, 2630; *Angew. Chem. Int. Ed.* **1999**, *38*, 2476; c) A. Dove, *Nat. Biotechnol.* **1999**, *17*, 233.
[18] Microarrays containing dsDNA probes can be used for the detection of DNA-binding proteins by means of fluorescence [a) M. L. Bulyk, E. Gentalen, D. J. Lockhart, G. M. Church, *Nat. Biotechnol.* **1999**, *17*, 573] or mass-spectrometry analysis [b) E. Nordhoff, A. M. Krogsdam, H. F. Jorgensen, B. H. Kallipolitis, B. F. Clark, P. Roepstorff, K. Kristiansen, *Nat. Biotechnol.* **1999**, *17*, 884]; antibody micorarrays are highly attractive as multianalyte immunosensors in clinical diagnostics [c) C. A. Borrebaeck, *Immunol. Today* **2000**, *21*, 379], since the miniaturization of ligand binding assays not only reduces costs by decreasing reagent consumption, but can simultaneously exceed the sensitivity of macroscopic techniques [d) J. W. Silzel, B. Cercek, C. Dodson, T. Tsay, R. J. Obremski, *Clin. Chem.* **1998**, *44*, 2036]; the immobilization of immunoglobulins to glass surfaces has been achieved by photolithographical techniques [e) L. F. Rozsnyai, D. R. Benson, S. P. A. Fodor, P. G. Schultz, *Angew. Chem. Int.* **1992**, *104*, 801; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 759], and, more recently, robotic deposition of multiple proteins was used to manufacture protein microarrays: f) D. Guschin, G. Yershov, A. Zaslavsky, A. Gemmell, V. Shick, D. Proudnikov, P. Arenkov, A. Mirzabekov, *Anal. Biochem.* **1997**, *250*, 203; g) L. G. Mendoza, P. McQuary, A. Mongan, R. Gangadharan, S. Brignac, M. Eggers, *Biotechniques* **1999**, *27*, 778; h) C. A. Rowe, L. M. Tender, M. J. Feldstein, J. P. Golden, S. B. Scruggs, B. D. MacCraith, J. J. Cras, F. S. Ligler, *Anal. Chem.* **1999**, *71*, 3846; protein biochips were applied to determine G protein activation by receptors [i) C. Bieri, O. P. Ernst, S. Heyse, K. P. Hofmann, H. Vogel, *Nat. Biotechnol.* **1999**, *17*, 1105], for the high-throughput gene expression and antibody screening [j) A. Lueking, M. Horn, H. Eickhoff, K. Bussow, H. Lehrach, G. Walter, *Anal. Biochem.* **1999**, *270*, 103], for screening of antibody-antigen interaction [k) R. M. de Wildt, C. R. Mundy, B. D. Gorick, I. M. Tomlinson, *Nat. Biotechnol.* **2000**, *18*, 989], and for the high-throughput identifying of the protein targets of small molecules [l) G. MacBeath, S. L. Schreiber, *Science* **2000**, *289*, 1760].
[19] Two groups reported on a novel approach to the generation of covalent nucleic acid-protein conjugates with potential impact on the fabrication of protein microarrays: a) N. Nemoto, E. Miyamoto-Sato, Y. Husimi, H. Yanagawa, *FEBS Lett.* **1997**, *414*, 405; b) R. W. Roberts, J. W. Szostak, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 12297; the principle is based on the in vitro translation of mRNA, covalently modified with a puromycin group at its 3′-end. The peptidyl-acceptor antibiotic puromycin covalently couples the mRNA with the polypeptide chain grown at the ribosome particle, leading to the specific conjugation of the informative (mRNA) with the functional (polypeptide) moiety.
[20] For recent reviews, see: a) D. Gerhold, T. Rushmore, C. T. Caskey, *Trends Biochem. Sci.* **1999**, *24*, 168; b) C. M. Niemeyer, D. Blohm, *Angew. Chem.* **1999**, *111*, 3039; *Angew. Chem. Int. Ed.* **1999**, *38*, 2865; c) E. M. Southern, K. Mir, M. Shchepinov, *Nat. Genet.* **1999**, *21*, 5; see also the series of review articles published in the supplement of *Nat. Genet.* and in the January issue of *Curr. Opin. Chem. Biol.* **2001**, *5*.
[21] The DDI technique was recently adapted using synthetic DNA analogues, pyranosyl-RNA [a) A. Eschenmoser, *Orig. Life Evol. Biosph.* **1997**, *27*, 535], as the recognition element for the addressable immobilization of antibodies and peptides [b) N. Windhab, C. Miculka, H.-U. Hoppe, DE 197 41 716 A 1 **1999** [*Chem. Abstr.* **1999**, *130*, 249 124x]]; this approach makes use of the electric field directed nucleic acid hybridization [c) C. F. Edman, D. E. Raymond, D. J. Wu, E. Tu, R. G. Sosnowski, W. F. Butler, M. Nerenberg, M. J. Heller, *Nucleic Acids Res.* **1997**, *25*, 4907]; the DNA-directed immobilization of hapten groups was used for the immunosensing of pesticides: d) F. F. Bier, F. Kleinjung, E. Ehrentreich-Forster, F. W. Scheller, *Biotechniques* **1999**, *27*, 752.
[22] The DNA-directed immobilization can also be applied to inorganic components, such as gold nanoparticles, to fabricate surface-bound supramolecular architecture: a) T. A. Taton, R. C. Mucic, C. A. Mirkin, R. L. Letsinger, *J. Am. Chem. Soc* **2000**, *122*, 6305; b) C. M. Niemeyer, B. Ceyhan, S. Gao, L. F. Chi, S. Peschel, U. Simon, *Colloid Polym. Sci.* **2001**, *279*, 68. This approach has great potential for analytical methods. It can be used for the highly sensitive scanometric detection of nucleic acids in DNA-chip analyses based on the gold particle promoted reduction of silver ions: c) T. A. Taton, C. A. Mirkin, R. L. Letsinger, *Science* **2000**, *289*, 1757. The use of colloidal gold nanoparticles also allows for the study of chip surface topography [d) R. Möller, A. Csaki, J. M. Köhler, W. Fritzsche, *Nucleic Acids Res.* **2000**, *28*, E91], the signal enhancement in the DNA hybridization detection using quartz crystal microbalance [e) F. Patolsky, K. T. Ranjit, A. Lichtenstein, I. Willner, *Chem. Commun.* **2000**, 1025], and surface plasmon resonance [f) 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]. In the last technique, an about 1000-fold improvement in sensitivity was reported, suggesting that the detection limit of surface plasmon resonance might soon reach that of traditional fluorescence-based DNA hybridization detection.
[23] P. E. Nielsen, *Acc. Chem. Res.* **1999**, *32*, 624.
[24] C. A. Mirkin, R. L. Letsinger, R. C. Mucic, J. J. Storhoff, *Nature* **1996**, *382*, 607.
[25] 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.
[26] J. J. Storhoff, C. A. Mirkin, *Chem. Rev.* **1999**, *99*, 1849.
[27] S. S. Smith, L. M. Niu, D. J. Baker, J. A. Wendel, S. E. Kane, D. S. Joy, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2162.
[28] S. R. Rajski, S. Kumar, R. J. Roberts, J. K. Barton, *J. Am. Chem. Soc.* **1999**, *121*, 5615.
[29] J. Hanes, A. Pluckthun, *Curr. Top Microbiol. Immunol.* **1999**, *243*, 107.
[30] M. Famulok, G. Mayer, M. Blind, *Acc. Chem. Res.* **2000**, *33*, 591.
[31] For reviews, see: a) A. B. van Spriel, H. H. van Ojik, J. G. van De - Winkel, *Immunol. Today* **2000**, *21*, 391; U. Chattopadhyay, *Immunol. Today* **1999**, *20*, 480.
[32] For a review on cofactor regeneration for enzyme-catalyzed synthesis, see: a) H. K. Chenault, E. S. Simon, G. M. Whitesides, *Biotechnol. Genet. Eng. Rev.* **1988**, *6*, 221; Fessner and co-workers have reported on several examples of artifical metabolism, using a mixture of enzyme catalysts, for instance, see: b) O. Eyrisch, W.-D. Fessner, *Angew. Chem.* **1995**, *107*, 1738; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1639.
[33] DNA computing [a) L. M. Adleman, *Science* **1994**, *266*, 1021] has recently been adapted to combinatorial libraries of DNA molecules attached to surfaces. Enzymatic manipulation of the surface-bound oligonucleotides allow for logical operations [b) Q. Liu, L. Wang, A. G. Frutos, A. E. Condon, R. M. Corn, L. M. Smith, *Nature* **2000**, *403*, 175]. A recent publication indicates that the one-dimensional algorithmic self-assembly of DNA triple-crossover molecules can be used to execute a logical operation on a string of binary bits: c) C. Mao, T. H. LaBean, J. H. Reif, N. C. Seeman, *Nature* **2000**, *407*, 493.
[34] Examples for the characterization of biomolecules by AFM: Review: a) H. G. Hansma, K. J. Kim, D. E. Laney, R. A. Garcia, M. Argaman, M. J. Allen, S. M. Parsons, *J. Struct. Biol.* **1997**, *119*, 99; enzymatic processing of nucleic acids: b) S. Kasas, N. H. Thomson, B. L. Smith,

  0947-6539/01/0715-3194 $ 17.50+.50/0 *Chem. Eur. J.* **2001**, *7*, No. 15

H. G. Hansma, X. S. Zhu, M. Guthold, C. Bustamante, E. T. Kool, M. Kashlev, P. K. Hansma, *Biochemistry* **1997**, *36*, 461; mobility of DNA molecules on solid supports: c) M. Argaman, R. Golan, N. H. Thomson, H. G. Hansma, *Nucleic Acid Res.* **1997**, *25*, 4379; crystallization of transfer-RNA: d) J. D. Ng, Y. G. Kuznetsov, A. J. Malkin, G. Keith, R. Giege, A. McPherson, *Nucleic Acid Res.* **1997**, *25*, 2582; mechanical manipulations and force spectroscopy: e) A. Engel, H. E. Gaub, D. J. Muller, *Curr. Biol.* **1999**, *9*, 133; characterization of DNA nanostructures: f) X. Yang, L. A. Wenzler, J. Qi, X. Li, N. C. Seeman, *J. Am. Chem. Soc.* **1998**, *120*, 9779; g) E. Winfree, F. Liu, L. A. Wenzler, N. C. Seeman, *Nature* **1998**, 394, 539; for the characterization of synthetic supramolecular DNA-protein conjugates see references [10, 16]; improved resolution of the AFM might be attained by using carbon nanotubes as tips [h) H. Dai, J. H. Hafner, A. G. Rinzler, D. T. Colbert, R. E. Smalley, *Nature* **1996**, *384*, 147] that can be functionalized by covalent methods [i) S. S. Wong, E. Joselevich, A. T. Woolley, C. L. Cheung, C. M. Lieber, *Nature* **1998**, *394*, 52] to obtain specific chemical and biological discrimination during the imaging.

[35] 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; b) C. Williams, *Curr. Opin. Biotechnol.* **2000**, *11*, 42; c) R. L. Rich, D. G. Myszka, *Curr. Opin. Biotechnol.* **2000**, *11*, 54.

[36] DNA can been utilized to construct nanomechanical devices, for instance, based on the ion-dependent B-to-Z conformational transition of a short DNA double helix: a) C. Mao, W. Sun, Z. Shen, N. C. Seeman, *Nature* **1999**, *397*, 144; for ionic switching of nanoparticles by means of DNA supercoiling see reference [13g]; an array of cantilevers was used to translate DNA hybridization into a nanomechanical response: b) 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; a recent work concerned a molecular machine which is both comprised of DNA and also fuelled with DNA. For this, a DNA molecule was designed to function as a tweezer which can be opened and closed by the addition of single-stranded DNA: c) B. Yurke, A. J. Turberfield, A. P. Mills, Jr., F. C. Simmel, J. L. Neumann, *Nature* **2000**, *406*, 605.