DNA–Protein Conjugates

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Semisynthetic DNA–Protein Conjugates for Biosensing and Nanofabrication

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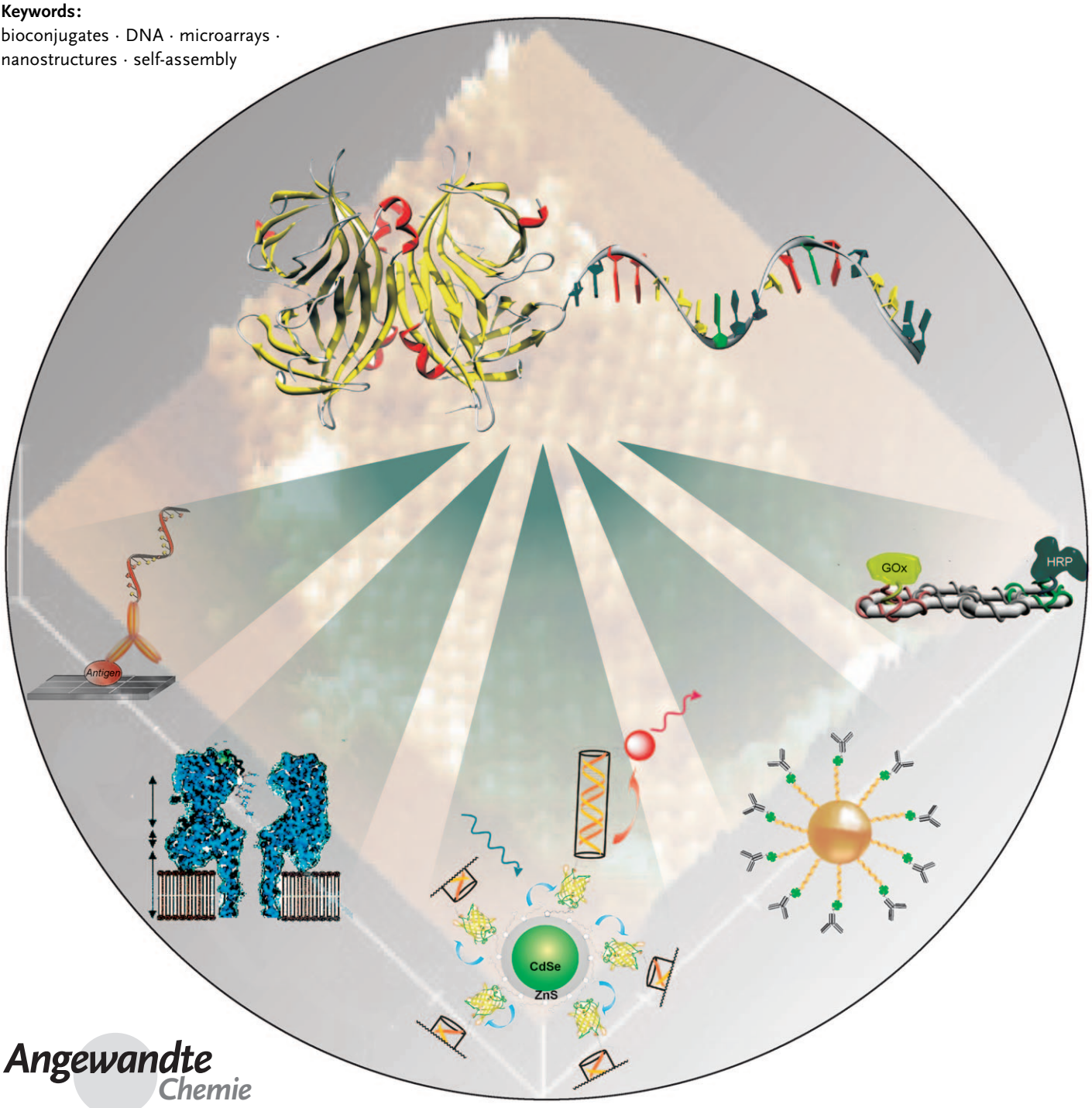

Conjugation with artificial nucleic acids allows proteins to be modified with a synthetically accessible, robust tag. This attachment is addressable in a highly specific manner by means of molecular recognition events, such as Watson–Crick hybridization. Such DNA–protein conjugates, with their combined properties, have a broad range of applications, such as in high-performance biomedical diagnostic assays, fundamental research on molecular recognition, and the synthesis of DNA nanostructures. This Review surveys current approaches to generate DNA–protein conjugates as well as recent advances in their applications. For example, DNA–protein conjugates have been assembled into model systems for the investigation of catalytic cascade reactions and light-harvesting devices. Such hybrid conjugates are also used for the biofunctionalization of planar surfaces for micro- and nanoarrays, and for decorating inorganic nanoparticles to enable applications in sensing, materials science, and catalysis.

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1. Introduction

Nature has evolved numerous highly functional nanomachines comprised of proteins, nucleic acids, and other (macro)molecules to conduct complicated tasks in living systems. Many of these fascinating nanomachines are built by the self-assembly of various protein and nucleic acid components.[1] The ribosome, for example, spontaneously self-assembles from at least three nucleic acid and more than 50 protein building blocks. This self-assembly is driven by an assortment of noncovalent contacts between distinct nucleotides and the phosphate backbone of the ribosomal RNAs with amino acids of the proteins.[2] Such magnificant examples of biological self-assembly have stimulated intensive research on the development of biomimetic, so-called "bottom-up", approaches for the generation of artificial devices with dimensions in the range of 5 to 100 nm, a regime which is difficult to address by conventional microstructuring or chemical approaches.[3] Consequently, early visions on the fabrication of artificial nanometer-scaled objects from biomolecular building blocks (for example, proteins and nucleic acids),[4,5] have in the meantime evolved into a well established research field, which is often referred to as nanobiotechnology.[6,7]

In this bottom-up fabrication of nanoscaled systems, synthetic DNA oligonucleotides have proved to be extraordinarily useful as a construction material.[8–11] The extremely high specificity of Watson–Crick base pairing allows one to readily design artificial receptors by using the predictable adenine–thymine (A-T) and guanine–cytosine (G-C) hydrogen-bonding interaction between complementary nucleic acids. DNA molecules are also readily accessible by synthetic chemical means and they possess high physicochemical stability. Furthermore, the short double helices reveal great mechanical rigidity, and nature provides us with a comprehensive range of highly specific DNA-modifying enzymes, such as ligases and nucleases, which can be used for processing and manipulating the DNA with atomic precision. These features render the genetic material suitable for molecular nanoconstruction.

The generation of semisynthetic DNA–protein conjugates allows the unique structure-directing properties of DNA to be combined with an almost unlimited variety of protein functionality. Proteins have been tailored by billions of years of evolution to perform highly specific functions, such as catalytic turnover, energy conversion, or translocation of other components across membranes. This Review gives an overview on current methods for generating such hybrid DNA–protein conjugates as well as examples of their application in bioanalytics and nanoscience.

2. Synthesis of DNA–Protein Conjugates

Various methods for the chemical coupling of synthetic DNA oligomers with proteins are known, which rely on either covalent or noncovalent strategies. Since comprehensive overviews on the seminal achievements have already been published,[12] this Review will focus in particular on recent developments in this area.

2.1. Noncovalent Coupling

2.1.1. Biotin–(Strept)Avidin Interaction

A frequently applied and simple approach to generate semisynthetic DNA–protein conjugates is based on the remarkable biomolecular recognition of D-biotin (vitamin H) by the homotetrameric proteins avidin or streptavidin (STV). The enormous high affinity constant of the biotin–(strept)-avidin interaction (ca. 10^{14} $dm^3 mol^{-1}$), the extreme chemical and thermal stability of the STV, and the availability of numerous biotin derivatives and mild biotinylation procedures have resulted in biotin–STV conjugates forming the

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basis of many diagnostic and analytical tests.[13,14] Biotinylated oligonucleotides can conveniently be accessed by automated solid-phase phosphoramidite chemistry and are commercially available. It is straightforward to prepare DNA–protein conjugates by simple mixing of biotinylated DNA and the protein-of-interest (POI in Scheme 1 a), conjugated to (strept)-avidin by either a chemical means or by genetic engineering. This approach has indeed been used frequently to generate reagents for bioanalytical assays (see Section 3.1.1) and it also proved feasible in a number of examples concerned with nanofabrication (see Section 3.2.1). The high affinity of biotin–(strept)avidin interactions means that dissociation of the conjugates is usually not an issue; however, intrinsic problems stem from the tetrameric nature of (strept)avidin. This makes it difficult, if not impossible, to control the stoichiometry of the respective DNA–protein conjugates, and can, therefore, represent a severe obstacle for the rational construction of nanodevices. While monobiotinylation of POI can be achieved by molecular biology techniques,[15] genetically engineered conjugation of the POI with streptavidin can also be hampered by the tetrameric nature of the resulting fusion proteins, and in some cases, the bulky STV connector protein may lead to additional obstacles.

2.1.2. Ni-NTA–Hexahistidine Interaction

An alternative way to produce DNA–protein conjugates was developed to avoid the aforementioned problems related to the conjugation of (strept)avidin. This method makes use of the specific interaction of an oligohistidine peptide with a nickel(II) ion complexed by nitrilotriacetic acid (NTA) ligands (Scheme 1 b).[16–19] The conjugation is based on the nickel-mediated interaction between NTA and the POI bearing a hexahistidine (His_6) tag. Two histidine residues together with one NTA molecule can satisfy all six coordination sites of a nickel(II) ion. A His_6 tag is often incorporated at the N or C terminus of a recombinant protein, which can then be purified by affinity chromatography on an NTA-functionalized resin with immobilized nickel(II) ions.[20,21] A recent example of DNA–protein conjugation based on the interaction between Ni-NTA and the His_6 tag was presented by Turberfield and co-workers.[18] They synthesized oligonucleotides containing one, two, or three NTA groups from commercially available amino-modified DNA oligonucleotides (Figure 1). It was previously known that clustering of NTA groups strengthens the interaction to His_6-tagged proteins,[22] and Turberfield and co-workers also observed an increasing stability of the noncovalent DNA–protein conjugates, containing green fluorescent protein (GFP) as the model POI, with increasing numbers of NTA ligands attached to the oligonucleotides. Dissociation constants (K_D) of 120 and 6 nM were determined for the bisNTA– and trisNTA–DNA, respectively. The linkage is site-specific and it can be broken easily by addition of a nickel chelator, such as ethylenediaminetetraacetate (EDTA), or imidazol. The versatility of this approach was demonstrated by tagging a number of DNA nanostructure motifs with His_6-tagged GFP.[18]

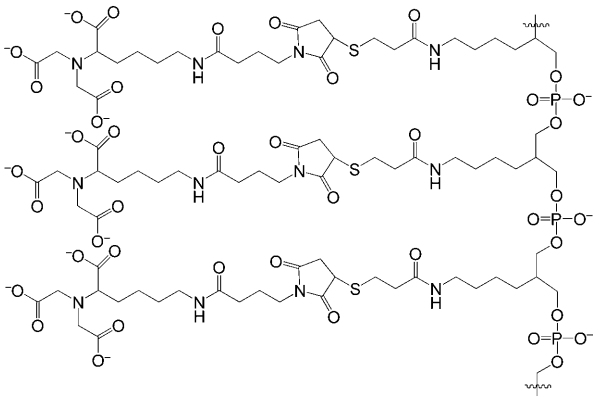

Figure 1. Tris(NTA)-modified DNA. The nucleotide group is omitted.

2.1.3. Antibody–Hapten Interaction

In addition to biotin and NTA ligands, other affinity tags can also be tethered to DNA by solid-phase synthesis. For example, the chromophore fluorescein (Fsc) can be used as a hapten to specifically bind immunoglobulin G (IgG) antibodies raised against it.[23] A large number of examples have been reported where Fsc was attached to DNA oligonucleotides to generate probes for the in vitro detection of complementary nucleic acid sequences, by using anti-Fsc-IgG conjugated to a chromophore, fluorophore, or reporter protein.[24] While such Fsc-IgG binding in analytical set-ups is usually neither characterized on a molecular level nor used for further preparative assembly steps, Mao and co-workers have recently used Fsc-derivatized oligonucleotides to assemble a hapten-modified DNA nanoarray. This array was used as a template to direct antibodies to assemble into very high density nanoarrays with a defined pitch of about 20 nm.[25] Other prominent examples of hapten–antibody pairs which have proven their applicability in various diagnostic assays[26] include IgGs with specificity against biotin, dinitrophenol, digoxigenin, or short peptides, such as the FLAG tag.[27]

2.1.4. Aptamers

The conjugation of nucleic acids with proteins can also be achieved with affinity tags formed by the nucleic acid itself.

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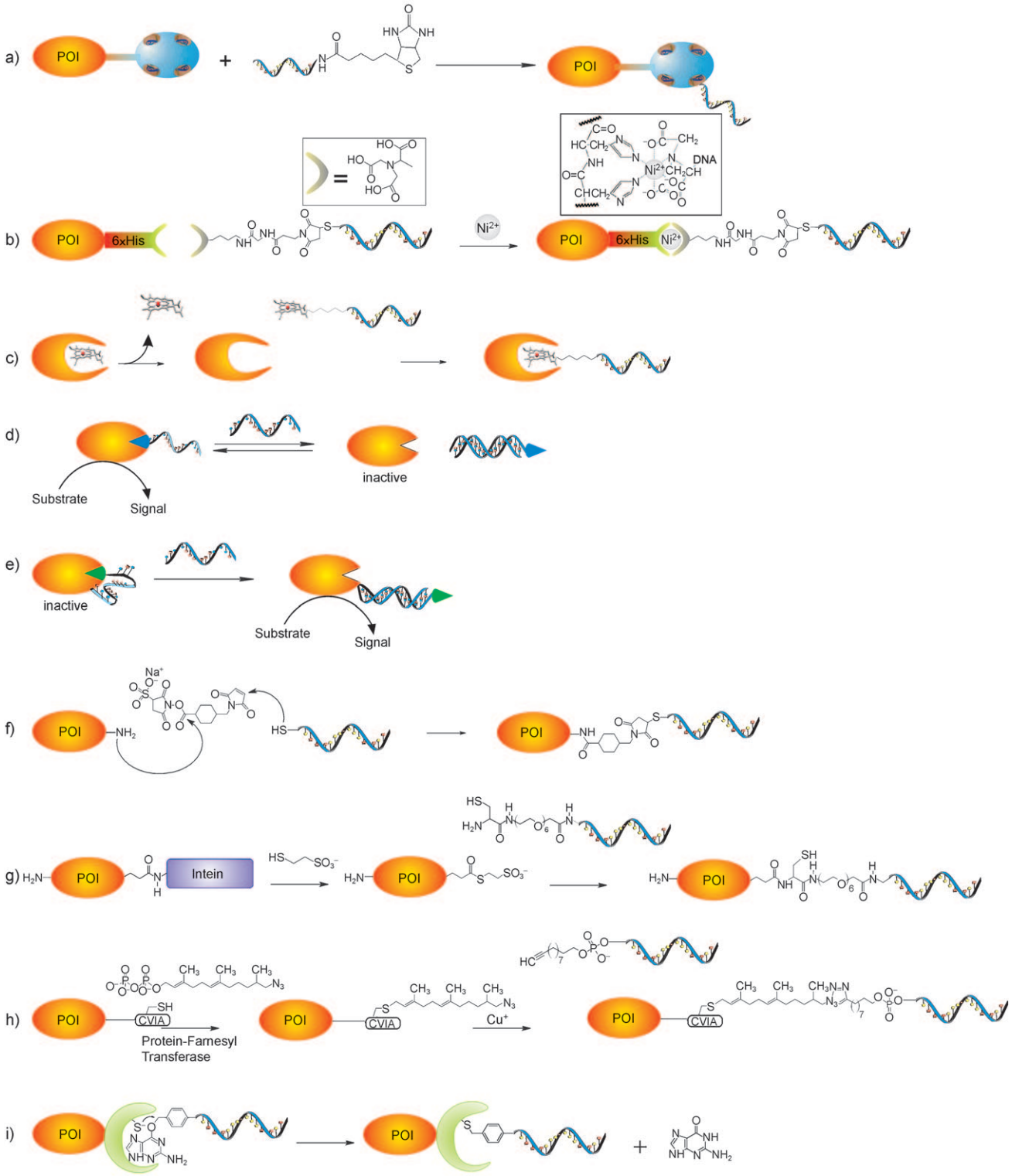

Scheme 1. Strategies for the conjugation of DNA with a protein of interest (POI). a) Biotin–(strept)avidin coupling; b) Ni-NTA-His_6 conjugation; c) cofactor reconstitution of apoenzymes, which can be utilized to switch d) on and e) off enzymatic activity; covalent coupling is often achieved with hetero-dispecific cross-linkers (f; sulfoSMCC shown). An alternative is the formation of fusion proteins using an intein (g), enzymatic modification through farnesyltransferase (h), or application of O^6-alkylguanine-DNA-alkyltransferase (snap tag; i) as the reactive part fused by genetic modification to the POI.

Aptamers are nucleic acid sequences which fold into defined three-dimensional motifs and display high affinity for a given target structure.[28,29] It is thus straightforward to tether an aptamer-forming sequence to the nucleic acid molecule of interest by standard solid-phase synthesis. Although the number of aptamers with specificity against target proteins

is still limited today, which has resulted in the majority of studies being based on the well-characterized anti-thrombin aptamer[30] as a model system, this conjugation principle has been proved to be useful for applications in bioanalytics and nanofabrication (see Section 3.2.2).

2.1.5. Reconstitution of Apoenzymes

A semisynthetic approach to DNA conjugation has been developed for enzymes containing a nondiffusible organic cofactor, often termed a prosthetic group, which is located in the active site and essential for the catalytic activity of the enzyme. Prominent examples of prosthetic groups are porphyrin and flavin derivatives. These cofactors can often be extracted from the protein to yield the respective apoenzyme, which can subsequently be reconstituted with an artificial analogue of the native cofactor. This reconstitution approach has widely been used for applications in structural enzymology and biotechnology,[31] and it offers a facile route to generate hybrid DNA–enzyme conjugates because nucleic acid moieties can readily be conjugated with the cofactor and then reinserted into apoenzymes (Scheme 1 c). This concept was demonstrated by Fruk and Niemeyer, who chemically synthesized DNA conjugates of heme (protoporphyrin IX), which were then used for the reconstitution of apo-myoglobin[32] or apo-horseradish peroxidase.[33] The resulting semisynthetic DNA–enzyme conjugates were found to be fully functional and, as a consequence of the appended DNA moiety, capable of specific hybridization with complementary nucleic acids immobilized on a range of surfaces for applications in sensing[34,35] and biocatalysis (see Section 3.2.3).

Conceptually similar, Simon et al. used a flavin–oligonucleotide conjugate to reconstitute apo-flavin reductase (Scheme 1 d).[36] The cofactor dissociates from the resulting DNA–protein hybrid upon hybridization with complementary oligonucleotides, thereby leading to inactivation of the enzyme. This priciple was used to detect a specific target DNA. Ghadiri and co-workers chemically conjugated protease enzymes with DNA oligonucleotides[37] which also contained a small-molecule inhibitor at the opposite end (Scheme 1 e). The inhibitor spontaneously inserts into the enzyme's active site as long as the DNA is single-stranded, and thus shuts down the catalytic activity. Hybridization with complementary target DNA, which leads to the formation of rigid dsDNA, pulls out the inhibitor, thereby restoring the enzyme's activity. This DNA-directed allosteric activation has been used for the detection of target DNA[37] and the construction of molecular logic devices.[38]

2.2. Covalent Coupling

Covalent coupling is the method of choice to circumvent obstacles which might result from the dissociation of noncovalent, reversible interactions and to ease characterization of DNA–protein conjugates. Seminal work in this area took advantage of stochastic coupling of nucleic acids with proteins by means of either homo-difunctional cross-linkers, such as glutardialdehyde,[39] or else UV-induced cross-linking,[40] to produce probes for nucleic acid hybridization assays. However, these approaches usually do not lead to stoichiometrically defined conjugates.

2.2.1. Disulfide and Maleimide Coupling

Thiol chemistry is the key to many successful approaches for DNA–protein conjugation. For example, genetically engineering cysteine residues in recombinant proteins allows the preparation of DNA conjugates with a defined stoichiometry and regioselectivity at the coupling site. In fact, the pioneering work in the preparation of semisynthetic DNA–protein conjugates, carried out by Corey and Schultz,[41] was based on the formation of a disulfide bond between alkylthiol-modified DNA oligomers and a cysteine residue of staphylococcus nuclease (SN). The oligonucleotide–SN conjugate functioned as a synthetic nuclease because its ssDNA moiety was designed to form specific triple helices at complementary target regions of a plasmid DNA, thus enabling its site-specific cleavage. Later work established that DNA–SN conjugates have enhanced kinetic rate constants for hybridization with double-stranded DNA (dsDNA) targets, probably as a result of coulombic attraction between the basic SN moiety and the target DNA, which leads to an increased effective concentration of the conjugate near its target site.[42,43] Site-specific coupling of thiolated oligonucleotides to proteins has been used to synthesize well-defined DNA–protein conjugates consisting of an individual DNA oligonucleotide covalently attached within the lumen of the α-hemolysin pore. These "DNA nanopores" have been used to realize new sensor elements (see Section 3.2.5).[44,45] Site-specific formation of a disulfide bond was also used in the synthesis of DNA conjugates from recombinant STV,[46] as an alternative to statistical covalent conjugation using heterodispecific cross-linkers (see below).

While the formed disulfide bond is still amenable to cleavage under reductive conditions, irreversible coupling can be achieved by means of cross-linkers bearing a maleimide functionality, such as sulfoSMCC (Scheme 1 f). This molecule is a representative example of the broad class of heterodispecific cross-linkers, which are typically first coupled with the protein to install thiol-reactive maleimido groups. After purification of the maleimido-activated protein, it is then treated with thiol-modified DNA oligonucleotides.[47–49] If a site-specific linkage is required and the POI contains accessible cysteine residues, the coupling order can as well be reversed, by using amino-modified oligonucleotides.[50] The method is versatile because it does not necessarily require genetic engineering of proteins, and even long DNA fragments can be readily coupled, which are accessible by a polymerase chain reaction (PCR) using a thiol-modified primer. However, the method requires extensive purification of the conjugates to remove excessive protein and oligonucleotides after each coupling step.

2.2.2. *Bioorthogonal Chemistry*

Since it is not always possible to install a single chemically accessible cysteine residue into the POI, coupling methods are needed which are orthogonal to the variety of functional groups within native proteins. A representative example is based on the so-called "expressed protein ligation" (EPL)[51,52] of recombinant proteins containing a C-terminal thioester, which spontaneously and selectively couples to N-terminal cysteine conjugates of nucleic acids under mild conditions.[53] As shown in Scheme 1 g, the POI is genetically modified with an intein domain. The fusion protein is expressed in *E. coli*. and bound to an affinity column. Addition of low-molecular-weight thiol compounds, such as mercaptoethanesulfonic acid, effects a reaction with the intein that leads to cleavage of the POI from the column and generation of the C-terminal thioester of the POI, which is then ligated with cysteine–nucleic acid conjugates. The EPL method is advantageous over conventional cross-linking techniques, because it allows PNA– and DNA–protein conjugates to be readily prepared with well-defined stoichiometric composition and regiospecific linkage.[53–55]

Active research is devoted to establish bioorthogonal coupling approaches for the selective functionalization of proteins in vitro and in live cells,[56] and the most prominent examples include the Staudinger ligation of azide- and phosphine-modified components,[57] as well as the copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition of azides and alkynes (CuAAC).[58] More recently, additional alternatives, such as photoinducible 1,3-dipolar cycloaddition, strain-promoted azide–alkyne cycloaddition, cross-metathesis of allyl sulfides, and Diels–Alder reactions of tetrazine and *trans*-cyclooctene, have been developed.[59] Staudinger ligation and Huisgen cycloaddition have both been widely applied in chemical biology for tagging biomolecules to investigate living systems,[60] or for in vitro bioanalytics,[61] and these two methods have also been used recently for the synthesis of DNA–protein conjugates. For example, glycoproteins on the surface of HEK cells were metabolically labeled with azide groups to enable their selective modification with phosphine-derivatized oligonucleotides (Figure 2).[62,63] The DNA-labeled cells were then amenable

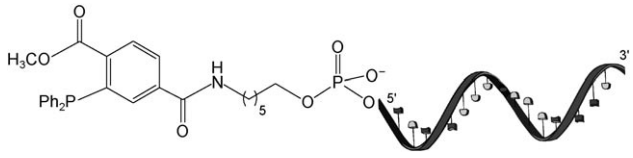

Figure 2. Phosphine-modified oligonucleotide for the synthesis of DNA–protein conjugates through reaction with azido-functionalized glycoproteins.

to micropattering by DNA-directed immobilization[62,63] or investigation of cell adhesion and patterning through atomic force microscopy (AFM).[64] Interestingly, this research group very recently reported the direct covalent coupling of *N*-hydroxysuccinimide (NHS) activated DNA oligonucleotides with native cell-surface proteins.[65] While lacking specificity of conjugation, this approach appears to be rapid, efficient, and widely applicable since it allows virtually any mammalian cell to be tagged with oligonucleotides. Its power has been demonstrated in DNA-directed capture and electrochemical metabolic analysis of single mammalian cells on a microelectrode array[66] and for the programmed in-solution assembly of three-dimensional microtissues with defined cellular connectivity.[67]

CuAAC has already proven to be a versatile method for the modification of synthetic DNA oligonucleotides,[68] and nowadays copper-free methods are also available for this purpose.[69] Distefano and co-workers have recently used copper-catalyzed cycloaddation in combination with enzyme-mediated protein modification to prepare DNA–protein conjugates (Scheme 1 h).[70] They used the enzyme protein-farnesyl transferase (PFTase) to specifically attach an azido-modified isoprenoid to a short recognition sequence that was fused to the POI through genetic modification. After labeling by PFTase, the azido groups were coupled with alkyne-modified oligonucleotides to generate DNA conjugates of fluorescent proteins. The site-selective incorporation of non-natural amino acids bearing chemically addressable alkyne or azide groups[71,72] into recombinant proteins holds great potential for the generation of well-defined DNA–protein conjugates. This approach has already been used for the specific photo-cross-linking of DNA with a protein containing the non-natural amino acid *p*-benzoyl-L-phenylalanine, which was incorporated by genetic modification into the protein in response to an amber nonsense codon by using an orthogonal tRNA/aminoacyl-tRNA synthetase pair.[73]

The specific coupling of proteins and DNA has also been achieved by purely enzymatic means. For this purpose, Tominga et al. used microbial transglutaminase (MTG) from *Streptomyces mobaraensis*, an enzyme that specifically catalyzes the acyl transfer reaction between a primary amine and the γ-carboxyamide group of Gln residues in peptides and proteins.[74] This reaction proceeded in good yield, when POIs were fused through genetic modification with a short peptide tag containing the acyl-acceptor Lys residues (Met-Lys-His-Lys-Gly-Ser). The oligonucleotide was modified with *N*-carbobenzyloxyglutaminylglycine (Z-QG), which functions as the acyl donor. It was demonstrated for alkaline phosphatase and enhanced green fluorescent protein (eGFP) that the respective DNA–protein conjugates could be used in DNA-directed immobilization.[74]

Although not yet fully exploited, another approach to generate DNA–protein conjugates takes advantage of specific enzymatic activities which lead to the self-labeling of an enzyme. An early example has been described by Smith et al., who managed to organize several proteins along a one-dimensional dsDNA fragment by utilizing the specific binding of DNA (cytosine-5)-methyltransferases to distinct recognition sequences within dsDNA.[75] Since covalent adducts are formed when the synthetic DNA base analogue 5-fluorocytosine (^{F}C) is present in the recognition site, it was possible to covalently attach two representative methyltransferases, M.HhaI and M.MspI, at their target sites, G^{F}CGC and ^{F}CCGG, respectively. Since the methyltransferases can be

fused through genetic modification with additional binding domains or other POIs, this system holds potential for the assembly of multiprotein aggregates.[75,76]

The human O^6-alkylguanine-DNA-alkyltransferase (hAGT) is another self-labeling protein, also refered to as a "snap tag", which was developed by Johnsson and co-workers.[77,78] The hAGT can be fused by genetic modification to a POI and subsequently treated with benzylguanine-modified DNA oligonucleotides (Scheme 1 i). This approach has the advantage that no purification is required for the POI–snap-tag conjugate, as demonstrated by the attachment of ssDNA to distinct proteins within the crude *E. coli* lysate.[79]

3. Applications

DNA–protein conjugates are nowadays increasingly applied in two areas: bioanalytics and the assembly of biomolecular nanostructures.

3.1. Bioanalytics

3.1.1. Probes for Nucleic Acids and Proteins

The presence of two functional moieties means that DNA–protein conjugates can be used as reagents for the analysis of both nucleic acids and proteins (Figure 3). Early

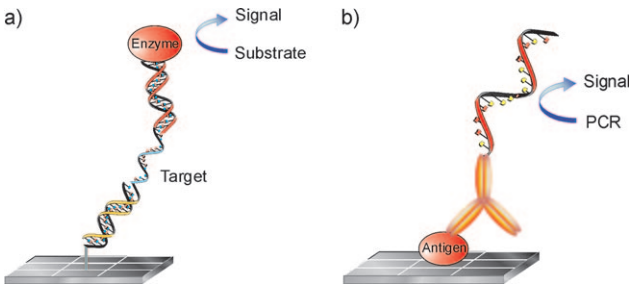

Figure 3. Application of DNA–protein conjugates in analytical systems. Both the nucleic acid and protein portion can be used: a) sandwich hybridization assay, where the tethered protein is an enzyme that functions as the reporter; b) immunopolymerase chain reaction (IPCR), where the protein is an antibody tethered to a DNA that reports binding upon PCR amplification.

applications of these hybrids have focussed on nonradioactive hybridization assays (Figure 3 a). In these, alkaline phosphatase (AP),[80] horseradish peroxidase (HRP),[81] β-galactosidase,[82] lipase,[83] or esterase[84] were used for catalytic amplification of the signal for the detection of nucleic acid analytes. In 1992, Sano et al.,[85] described an innovative assay, the immuno-PCR (IPCR), which enables the ultrasensitive detection of proteins and other antigens by taking advantage of DNA–protein conjugates. As shown in Figure 3 b, IPCR represents a modification of the conventional enzyme-linked immunosorbent assay (ELISA). In IPCR, the DNA moiety of the hybrid is amplified by PCR. The almost exponential amplification power of PCR results in the IPCR method being about 1000–10 000-fold more sensitive than the analogous ELISA system. Thus, this immunoassay is widely applicable in clinical diagnosis and biomedical research.[48,86–88] Recently, proximity ligation, a related technique based on DNA–antibody conjugates was developed by Landegren and co-workers.[89] In this approach, oligonucleotides tethered to two different antibodies are ligated only in the presence of the target protein, thus enabling subsequent amplification of the DNA marker by PCR or rolling circle amplification. This method is also suitable for the detection of protein–protein interactions in whole cells. An efficient coupling of the DNA marker with the antibody is essential for the performance of all these immunoassays. Although covalent conjugation based on maleimide coupling has been described,[48] the signal-generating conjugates are often prepared in situ by means of the (strept)avidin–biotin recognition system.[88]

3.1.2. Biochips

The physicochemical robustness and ready availability of nucleic acid molecules enables DNA microarrays to be fabricated by industrial processes, and today they are established tools in genomics and biomedical research.[90,91] In contrast, the immobilization of microarrays of more delicate proteins (such as receptor and regulator proteins) at chemically activated surfaces is often obstructed by their instability, since they generally reveal a significant tendency to undergo denaturation.[61] DNA-directed immobilization (DDI) of DNA–protein conjugates provides a chemically mild process for the highly parallel binding of multiple delicate proteins to a solid support by using DNA microarrays as immobilization matrices (Figure 4).[12,47] The DDI method takes advantage of specific Watson–Crick base pairing between complementary oligonucleotides, one of which is used as a tag for the protein to be immobilized and the other one is bound to a solid substrate, thus functioning as a specific capture molecule. In DDI applications, the lateral surface structuring is carried out with stable nucleic acids, and thus the DNA microarrays can be stored almost indefinitely. They are functionalized with DNA–POI conjugates immediately prior to use, for example, to generate biochips for immunoassays.[55,92–95] A particular advantage of DDI is that the proteins retain their biological activity because they are attached to the surface through the short double-stranded DNA linker rather than being directly fixed to the surface through multiple covalent or noncovalent contacts which may restrict their conformational freedom and might lead to (partial) denaturation of the tertiary structure. After the assay, the DDI-based arrays can even be regenerated by alkaline denaturation of the double-helical DNA linkers.[35,96,97]

Another advantage of DDI for microarray-based detection of protein–protein interactions stems from the fact that the intermolecular binding of the target antigens by antibodies can be carried out in homogeneous solution, instead of by a less-efficient heterogeneous solid-phase immunosorption. The immuno complexes thus formed are site-specifically captured at the microarray by nucleic acid hybridization.[55,92]

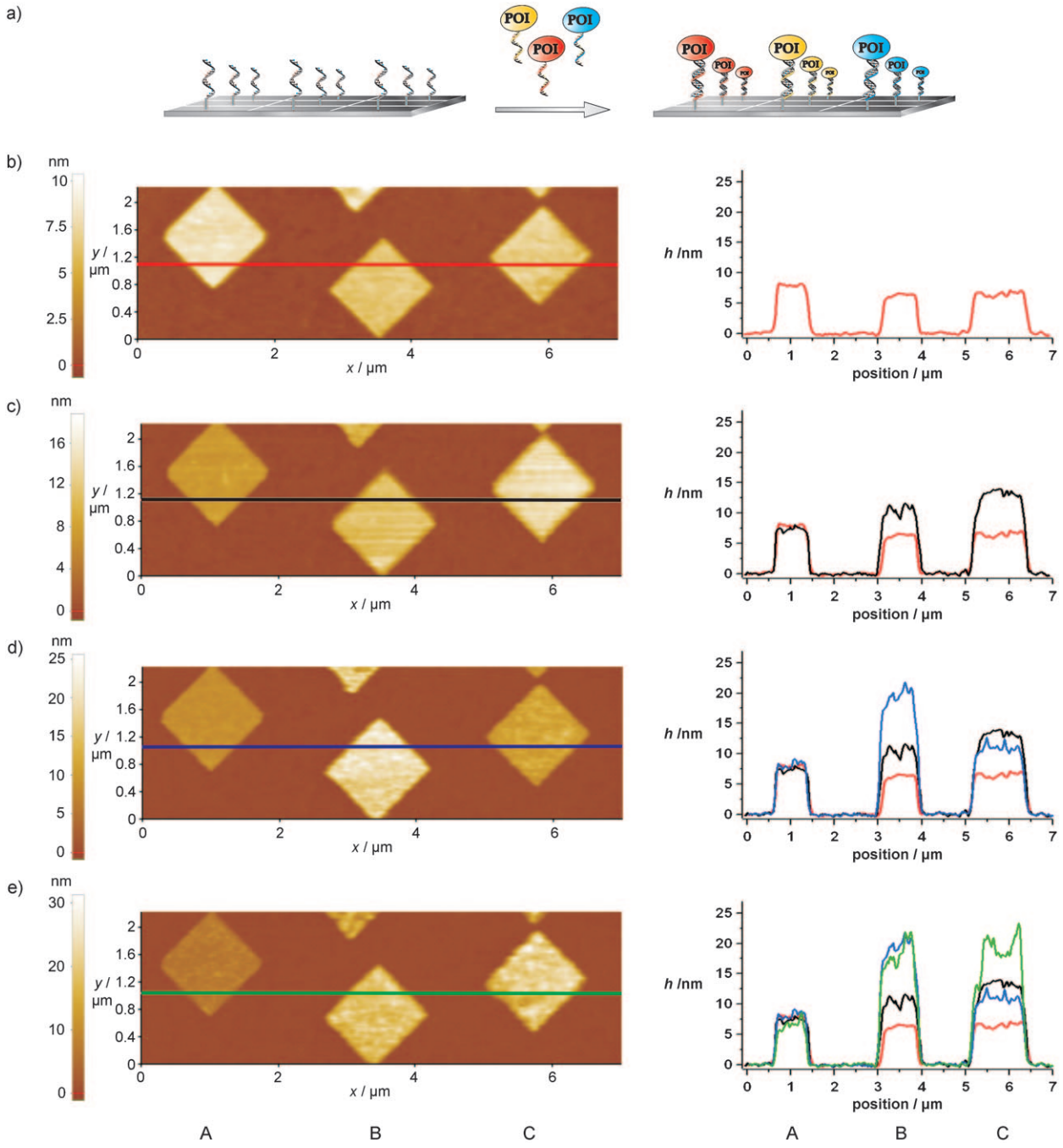

Figure 4. DDI of proteins on surfaces. a) Schematic illustration and b)–e) demonstration of DDI for serum immunodiagnostics by using an array of ssDNA patches prepared by nanografting.[94] The topographic AFM images and corresponding height profiles were obtained from ssDNA nanopatches containing three different oligonucleotides (red line in b), after incubation with a solution containing ssDNA–STV (complementary to patch B) and ssDNA–GOx (complementary to patch C) conjugates (black line profile in c), and subsequent incubation of human serum containing antibodies against STV (blue line in d) and GOx (green line in e). The increase in height indicates specific immobilization for all binding steps. Reprinted from Ref. [94] with kind permission.

This DDI capture principle was also applied in functional proteomics to identify active members of small-molecule libraries which bind to protein targets.[98–103] In this approach, small-molecule inhibitors were conjugated to PNA oligomers. The small-molecule portion of the probe is designed to bind to proteins in a mechanism-dependent manner, thereby differentiating between active and inactive proteins. The PNA portion of the probes functions as a code for the synthetic history of the small-molecule part and, therefore, allows for deconvolution of the probe through hybridization at an oligonucleotide microarray. This method has been validated with specific inhibitor probes for cathepsins[98] and caspase.[99] Furthermore, the approach has been applied to the discovery of new proteolytic activities from dust-mite extracts.[101]

Additional applications of the DDI method concern the area of in vitro protein evolution. Two research groups

established an approach to covalently link nucleic acids in situ with proteins, such as single-chain antibody fragments.[104,105] The principle is based on 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, thus leading to the specific conjugation of the informative (mRNA) with the functional (polypeptide) moiety. This approach can be used for the high-throughput screening of peptide and protein libraries, as well as for generating protein microarrays.[106] Similar, snap-tag coupling has been applied for genotype–phenotype coupling in the context of protein selection from DNA libraries.[107]

The broad scope of the DNA-directed immobilization principle is further emphasized by recent studies on the generation of bioarrays containing living cells,[62–64,108,109] as well as by numerous examples regarding specific immobilization of nonprotein components.[110]

3.2. Nanofabrication

3.2.1. Stochastic Formation of Streptavidin Networks

A simple approach to functional DNA–protein nanostructures is based on the self-assembly of STV and bisbiotinylated dsDNA fragments.[111] These divalent dsDNA molecules interconnect the tetravalent STV, thereby generating networks in which the STV is predominantly conjugated with two dsDNA modules, as indicated by AFM imaging (Figure 5a). Since tri- and tetravalently conjugated STV molecules occur with much lower frequency, these nanostructures have a large residual biotin-binding capacity, which can be utilized for further functionalization with biotinylated components. For example, biotinylated antibodies have been coupled to the nanostructures, thereby generating functional complexes that are highly suitable for use in IPCR assays.[87,88,111] Since the size, connectivity, and topography of these oligomeric networks are comparable to those of DNA-linked nanoparticle networks,[112] the dsDNA–STV oligomers can also serve as model systems to establish fundamental techniques for the immobilization and characterization of such structures.[113] For example, the DNA fragments in the networks are susceptible to external stimuli, thus giving rise to the development of ion-switchable aggregates of nanoparticles[113] (Figure 5a), which can act as model systems for functional nanomaterials with controllable interparticle spacing or in which the DNA is accessible to enzymes.

The randomly assembled dsDNA–STV networks can also be converted into well-defined supramolecular DNA–STV nanocircles (Figure 5a) by a simple thermal treatment.[114] Since they are readily available and possess a well-defined stoichiometry and structure, these nanocircles can be further functionalized with biotinylated haptens to produce functional reagents for competitive IPCR assays. Such IPCR assays have an approximately 1000-fold lower detection limit than conventional competitive ELISA.[115] Moreover, STV modules containing covalently bound ssDNA can be incorporated into the networks or nanocircles for another approach to nanofabrication.[12] Such dsDNA–STV nanostructures might be useful for "biomolecular templating", an approach established mainly by Braun and co-workers, whereby the electrostatic and topographic properties of supramolecular DNA–protein complexes are used for the templated growth of metallic nanostructures.[116,117] It should also be noted that the dsDNA–STV nanostructures have proven to be useful as standards for the AFM study of the topography of soft materials,[118] because the two different biopolymers (DNA and proteins) occur in a highly characteristic, well-defined, supramolecular structure. This enables direct comparison of, for example, the deformation properties of the biopolymers depending on the detection mode applied in the AFM study.[118]

3.2.2. Rationally Assembled DNA Nanoarrays

The groundbreaking work of Seeman[5] means that the assembly of DNA superstructures and arrays with well-defined structural features on the nanometer length scale is nowadays well established.[8–11] Much effort is currently underway to extend the scope and applicability of such DNA nanoarrays by adding functionality to the DNA superstructures through decorating them with proteins. While the assembly of linear one-dimensional arrays of DNA–protein conjugates was already demonstrated in the 1990s,[47,119] protein modification of two-dimensional arrays was achieved only recently. In an early demonstration, Yan et al. reported the DNA-templated self-assembly of STV arrays through the use of two-dimensional biotin-modified lattices prepared from so-called 4×4 DNA tiles (Figure 5b).[120] Biotin-coupling was also used in subsequent studies to prepare arrays of STV and STV-modified nanoparticles[121,122] or even finite nanoarrays, which can be used to position individual proteins precisely, much like a molecular pegboard (Figure 5c).[123,124] Since STV shows no functionality other than its high biotin-binding capability, these studies primarily serve as proof-of-concept for the rational approach to DNA nanoengineering.

To extend the functionality and to demonstrate applicability in biosensing, similar assembly strategies were applied by Yan and co-workers to fabricate nanoarrays comprising aptamer motifs. These arrays were capable of selectively capturing target proteins.[125–128] This approach also enabled the generation of nanoarrays containing multiple different proteins, such as thrombin and platelet-derived growth factor.[128] The incorporation of small-molecule recognition groups, such as haptens (see Section 2.1.3)[25] or peptides[129] to recruit antibodies, was also explored. The latter approach was based on site-specific hybridization of DNA–peptide conjugates to nanoarrays containing complementary ssDNA areas, and the resulting architecture was used for the study of protein–protein interactions.[129]

DNA scaffolds generated by the so-called DNA origami technique developed by Rothemund[130] have recently been utilized for the fabrication of protein nanoarrays. For example, Kuzuya et al. used the origami technique to produce punched DNA nanotapes, which contain defined numbers of nanometer-scaled wells of approximately $7\times12\times2$ nm. The presence of two biotin groups attached inside these wells enabled single STV molecules to be captured (Figure 5d).[131]

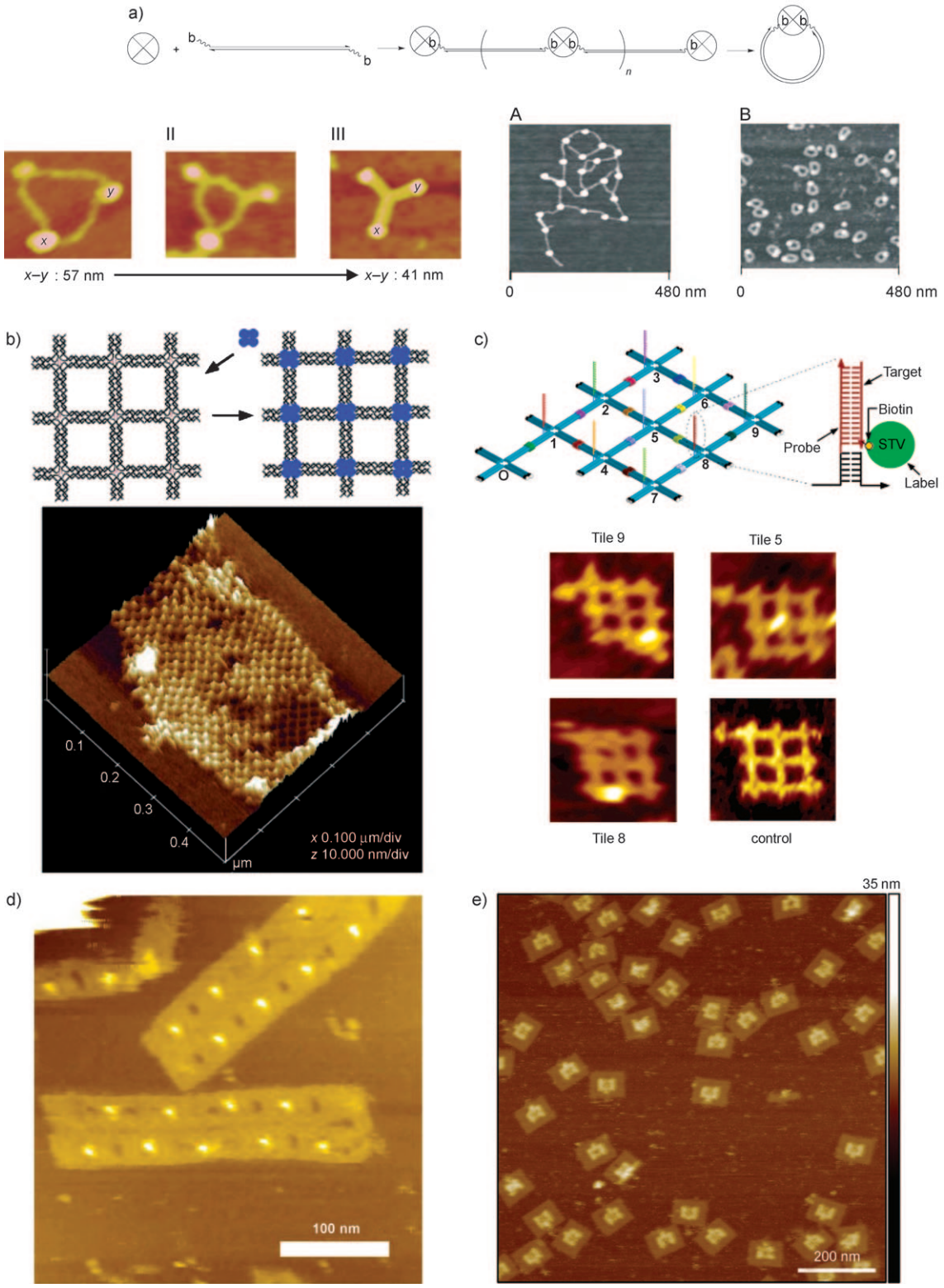

Figure 5. DNA–STV nanostructures. a) Stochastic self-assembly of dsDNA–STV conjugates from 5′,5′-bisbiotinylated DNA and STV; b = biotin. The structure of the conjugates is simplified, since a fraction of the STV molecules function as tri- and tetravalent linker molecules between adjacent dsDNA fragments (see AFM image A). The dsDNA–STV networks can be converted into dsDNA–STV nanocircles by thermal treatment (see AFM image B);[114] the AFM images on the left illustrate structural changes observed for representative DNA_3–STV_3 elements upon increasing the ionic strenght.[113] The relative distance between the protein particles descreases with increased supercoiling of the interconnecting DNA linkers (state I = low salt; II = intermediate; III = high salt). b) 2D nanoarray of oligomerized 4×4 DNA tiles containing biotin groups for the attachment of STV. Adapted from Ref. [120] with kind permission. c) Binding of a single molecule of STV on finite nanoarrays comprised of nine 4×4 tiles. The AFM images indicate successful tagging of arrays at the expected tiles. In the control reaction, the arrays were exposed to biotinylated targets that were not complementary to any tile. Reprinted from Ref. [123] with kind permission. STV-decorated DNA superstructures produced by the origamic technique are shown in (d)[131] and (e).[133]

In comparison to earlier work concerning the similar STV fixation in wells of linear arrays comprised of nine-helix DNA tiles,[132] the origami technique enabled significantly better control over the immobilization.[131] In other recent examples, origami nanoconstructs containing NTA groups were used to immobilize His-tagged fluorescent proteins,[19] and multiple STV molecules were selectively bound to origami structures containing 24 biotinylated staple strands (Figure 5e).[133] DNA nanoarrays have also been modified with biotin-modified polyamides in a sequence-selective manner to position STV molecules at predefined sites on the array.[134,135] This approach should also be suitable for modifying origami structures.

In addition to using DNA nanoarchitectures as templates for positioning proteins, it is also fascinating that proteins can be used to modify a given DNA nanostructure. Besides the aforementioned protection of DNA against metalization (see Section 3.2.1),[117] this approach was used by Seeman and co-workers to construct a nanomechanical device for which the switching stimulus was the protein *E. coli* integration host factor (IHF). The device changed its shape when the DNA-distorting IHF was added.[136] Another impressive example was reported by Turberfield and co-workers, who used RuvA to induce and stabilize conformational isoforms in DNA nanoarrays.[137] RuvA is a protein that binds to the four-stranded DNA structure formed in the Holliday junction—an important intermediate in genetic recombination. Turberfield and co-workers demonstrated by transmission electron microscopy that the addition of RuvA during the self-assembly of two-dimensional nanocrystals completely changes both the lattice symmetry and connectivity.

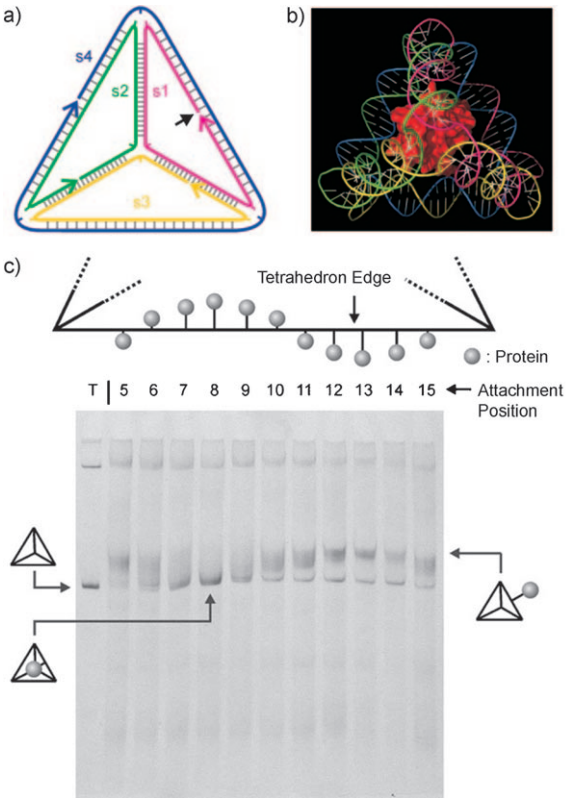

Figure 6. Encapsulation of a single cytochrome *c* protein inside a tetrahedral DNA cage. a) Four oligonucleotides were used for the assembly of the tetrahedron; b) molecular model to illustrate that cytochrome *c* fits inside the tetrahedron; c) gel electrophoretic analysis of encapsulation efficiency. The encaged protein induces higher electrophoretic mobility. Reprinted from Ref. [138] with kind permission.

3.2.3. Adressing and Cascading of Enzymes

DNA nanoarchitectures have also been employed to modify protein function and accessibility to proteins. For example, Turberfield and co-workers encapsulated a single cytochrome *c* protein molecule in a rigid tetrahedral DNA cage.[138] To this end, the protein was covalently attached to one of the four oligonucleotides forming the cage. Very interestingly, the authors were able to demonstrate that successful encapsulation depended on the position of the attachment site along one edge of the tetrahedron (Figure 6). This phenomenon was clearly demonstrated by electrophoretic mobility analysis and is in agreement with the expected helical trajectory of the attachment point along the edge. The authors noted that the use of modified cages whose bars could be extended or broken might allow functional control over the encapsulated protein, thereby, for example, allowing the possibility to initiate an apoptotic protease cascade.[138]

An example of how intrinsic enzyme functionality can be changed by tethering DNA is shown by DNA–protein conjugates prepared by cofactor reconstitution (see Section 2.1.5). Such hybrids prepared from myoglobin (Mb)[32] or horseradish peroxidase (HRP)[33] reveal largely altered catalytic properties compared to the native enzymes. For example, the presence of the bulky and highly charged oligonucleotide in proximity to the active site dramatically increases the catalytic properties of Mb.[33] Interestingly, this effect strongly depends on the sequence of the appended oligonucleotide, thus allowing the activity of such hybrid catalysts to be fine-tuned.[139] Very recently, a synthetic heme derivative containing an oligonucleotide and a photoactivatable Ru^{3+} fragment was used for the reconstitution of Mb.[140] The resulting hybrid enzyme was capable of both catalyzing light-induced peroxidation reactions and specific nucleic acid hybridization, which enabled selective immobilization on solid supports as well as recovery of the catalyst.

The DNA-directed assembly of enzymes can be applied to the construction of artificial multienzyme constructs with a high degree of spatial control. In biological systems, multienzyme complexes have a mechanistic advantage during the multistep catalytic transformation of a substrate because reactions limited by the rate of diffusional transport are accelerated by the immediate proximity of the catalytic centers. This "substrate channeling" of intermediate products also avoids side reactions. An early demonstration of DNA-linked multienzymes was achieved by assembling oligonucleotide-conjugated luciferase and oxidoreductase.[141] The enzymes catalyze the consecutive reactions of flavin mononucleotide reduction and aldehyde oxidation, respectively (Figure 7a). It was clearly observed that the total enzymatic activity depended on the absolute and relative spatial orientation of the two enzymes. More recently, oligonucleo-

tide conjugates of glucose oxidase (GOx) and HRP were used to generate a number of dienzymic complexes with various spatial orientations.[142] Kinetic rate measurements on the coupled reaction of glucose oxidation and peroxidation of the fluorogenic dye Amplex Red revealed a significant increase in the reactivity of those complexes in which GOx and HRP were bound in direct proximity on the DNA carrier strand.

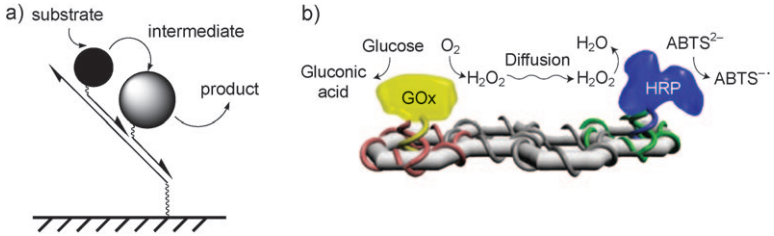

Figure 7. Cascade of consecutive enzyme-catalyzed steps by a DNA–protein complex. a) Surface-attached two-enzyme systems, as studied for oxidoreductase/luciferase,[141] or GOx/HRP combinations;[142] b) directed assembly of the GOx/HRP system on two-dimensional hexagonal DNA nanoarrays[143] revealed significant greater proximity effects than the examples in (a). Reprinted from Ref. [145] with kind permission.

Willner et al. have also investigated the effects of DNA assembly on the dienzymic system of GOx and HRP.[143,144] In one study, the enzymes were assembled on linear DNA carriers prepared by the rolling circle amplification (RCA) process. In this case, the enzyme cascade could not be activated in the absence of the organizing DNA template or in the presence of a foreign DNA.[144] In the other study, the enzymes were tethered to "hinges" of a two-dimensional DNA scaffold made of two or four hexagonal DNA strips (Figure 7b).[143] The enzyme cascade proceeded effectively only in the presence of the DNA template, while it was not observed in diffusion-controlled homogeneous mixtures of the same components or in the presence of noncomplementary calf-thymus DNA. It has previously been noted that studies on enzyme cascades are not only useful in exploring proximity effects in biochemical pathways, they may also allow the development of novel catalysts for enzyme process technology.[12] It is now anticipated that self-assembling, dynamic, and functional biomolecular networks could be made by merging the addressability of DNA structures with the versatile functionality of protein libraries.[145]

3.2.4. *Functionalization of Nanoparticles*

With respect to synthetic nanosystems and materials science, the developments in the DNA-directed organization of semiconductor and metal nanoparticles[3,112,146–148] have stimulated the use of DNA–protein conjugates to decorate and install functional moieties on the particle surface. For example, ssDNA–STV conjugates were bound to biotinylated gold nanoclusters, thereby enabling their hierarchical organization into supramolecular biometallic nanostructures (Figure 8a).[119] The modularity of this assembly system allows functional proteins, such as immunoglobulins, to be conveniently incorporated into the nanostructures for specific binding to complementary antigens.[119] The DNA-directed immobilization of ssDNA-tagged antibodies on DNA-modified gold nanoparticles (DNA–AuNPs) was used to generate reagents for immunoassays.[149,150] In such hybrid particles (Figure 8b) the proteins are separated from the metal surface by a highly hydrated layer of dsDNA. This is advantageous to reduce their general tendency to (partially) denature upon direct contact with surfaces. Moreover, the noncovalent assembly of such particles is highly modular because ssDNA–STV conjugates can be used as universal adaptors to enable ready incorporation of any biotinylated protein. The binding of such particles can easily be visualized since the gold particles catalyze a reductive deposition of silver,[149,150] thereby enabling spa-

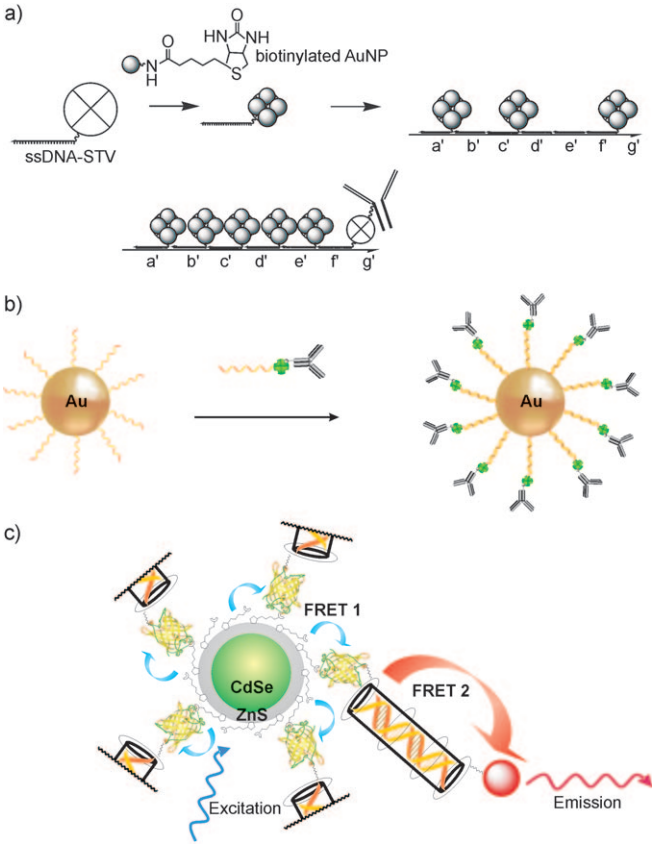

Figure 8. Functionalization of nanoparticles with DNA–protein conjugates: a) hierarchical organization of biotinylated 1.4 nm gold nanoparticles (AuNPs). The AuNPs were coupled by means of covalent ssDNA–STV conjugates and then these AuNP-loaded proteins subsequent assembled into biometallic nanostructures.[119] The construct underneath contains a biotinylated antibody to enable specific binding to antigens. b) DNA-directed immobilization of ssDNA-tagged antibodies on colloidal DNA-modified gold nanoparticles (DNA–AuNPs).[149] The ssDNA–STV conjugates were used in this example to achieve maximum flexibility in the modular assembly of reagents for immunoassays.[95] c) DNA–protein conjugate-based three-chromophore FRET system assembled by electrostatic binding of ssDNA–EYFP and subsequent DNA hybridization with dye-labeled complementary oligonucleotides.[157] For simplicity, only one DNA cylinder is drawn to illustrate that the donor/acceptor separations can be controlled by the helicity of the DNA.

tially addressable detection of very low amounts of chip-immobilized antigens.[95]

The reversible immobilization of DNA–protein conjugates on the surface of DNA–AuNPs[151] was realized by combining the above described strategy for nanoparticle decoration with the concept of strand displacement, originally developed for the generation of nanomechanical DNA devices.[152,153] Covalent conjugates of ssDNA and the fluorescent protein EYFP were used to monitor DNA-directed binding and displacement of the proteins.[151] Both processes could be conveniently followed by observation of the fluorescence emission of EYFP, which was quenched upon its binding to the gold nanoparticles. It should be noted in this context, that DNA conjugates of fluorescent proteins (FPs) are considered as building blocks to fabricate photonic devices, which serve as models to mimic basic parts of the photosynthesis machinery of living cells. The rapid developments in molecular and cell biology has led to a broad range of FPs which cover the entire range of visible light being available nowadays.[154] Covalent tethering of FPs with DNA oligonucleotides enables rational assembly of supramolecular conjugates containing multiple (biological) chromophors with defined spatial orientation to construct systems in which Forster resonance energy transfer (FRET) is possible. These systems can be analyzed by time-resolved and single-molecule fluorescence spectroscopy.[50,155] FPs have also been used to decorate luminescent semiconductor nanoparticles, often referred to as quantum dots (QDs), to give FRET systems that function as photonic devices and for the investigation of basic spectroscopic phenomena.[156,157] An example of how DNA–protein conjugates can be used to investigate QD-FP FRET systems is shown in Figure 8c. In this case, the ssDNA–FYFP conjugate was attached to CdSe/ZnS QDs through electrostatic interactions.[157] Hybridization with complementary oligonucleotides labeled with an organic dye led to FRET systems with three chromophores, in which donor/acceptor separations could be precisely controlled by the helicity of the DNA. This enabled the detailed study of FRET processes by steady-state and time-resolved photoluminescence measurements. It was observed that the supramolecular FP-QD complexes function as powerful donors, which combine the intrinsic advantages of QDs (large and spectrally broad absorption cross-section) and EYFP (high quantum yield) to enable long-distance FRET processes of up to 13 nm.[157] The aforementioned assemblies of DNA-modified FPs may, therefore, be regarded as the first examples of photonic devices.

3.2.5. Miscellaneous

The scope of the applications of DNA–protein conjugates in the nanosciences exceeds the topics summarized above. As noted in Section 2.2.1, DNA oligonucleotides were incorporated as functional moieties into protein nanopores to form nanoscaled devices for nucleic acid sensing (Figure 9).[44,45] The resulting bioconjugates can be incorporated in lipid bilayer membranes, thereby enabling the identification of individual DNA strands with single-base resolution by using patch-clamp techniques.[45] Reagents for the detection of

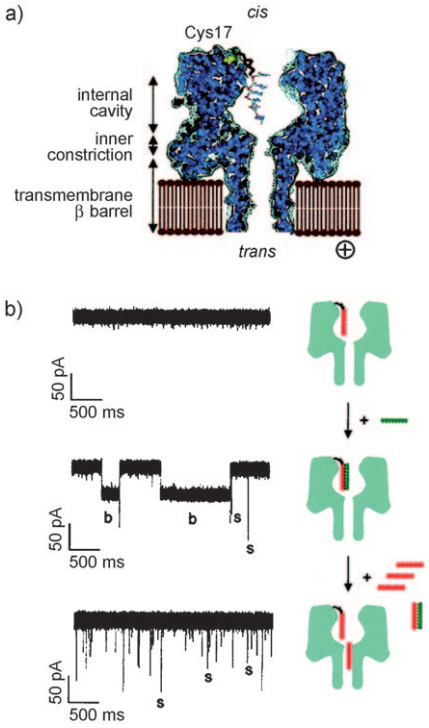

Figure 9. DNA-modified α-hemolysin nanopore (a), which can be used for sensing nucleic acid hybridization (b). A positive electrical potential drives negatively charged molecules from the *cis* to the *trans* side of the bilayer. Negative current deflections in the traces (b) indicate the binding of complementary oligonucleotides (green), while only short downward spikes were observed for the translocation of a noncomplementary oligonucleotide (red). Reprinted from Ref. [45] with kind permission.

proteins have been created by nanoscale assembly of DNA-tagged small molecules in a combinatorial screening format.[158,159] This approach led to the identification of ligands for proteins that have a high-affinity because of polyvalent interactions. This example, together with those of DNA–antibody conjugates (Section 3.1.1), emphasizes the general point that supramolecular constructs containing a DNA structural backbone to control the spatial arrangement of the binding sites allow the specific recognition of the target's topography, even when the individual epitopes are either not nearby and/or individual affinities are only weak. An additional great advantage of nanoscaled DNA–receptor constructs is that the backbone can be modified and detected by enzymatic means; thus, the supramolecular constructs are detectable at extremely low levels and even in rather complex environments as a result of the enormous potential of PCR techniques.[88]

DNA–protein conjugates have also been used to explore strategies for molecular movement on solid supports. For example, Stojanovic and co-workers have reported polycatalytic "spider molecule" assemblies comprised of STV molecules conjugated with biotinylated nucleic acid catalysts (deoxyribozymes) with phosphodiesterase activity.[160] As shown by surface plasmon resonance, these conjugates are capable of diffusing through a hydrogel matrix covered with a high density of oligonucleotide substrates. The substrates

were cleaved at similar rates as in solution. The loss of spider molecules through diffusion outside the matrix was minimal. Since lateral diffusion rates could be controlled through variation of the recognition units and number of catalytic "legs", the authors anticipate that this approach might have potential for drug-release devices.[160] Recently, Hiyama et al. attached DNA oligomers to microtubuli (MT) proteins and showed that the conjugates glide on kinesin motor proteins immobilized on glass surfaces (Figure 10).[161] When the MTs

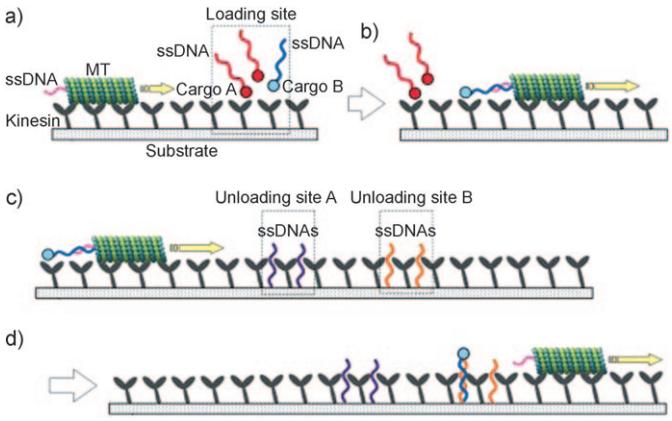

Figure 10. DNA-mediated autonomous loading (a, b), transport (c), and unloading (d) of cargo to/from ssDNA-modified microtubuli proteins (MT), which glide on kinesin-coated glass surfaces. Cargos A (blue) and B (red) contain ssDNA which is complementary or non-complementary to MT-ssDNA, respectively. Reprinted from Ref. [161] with kind permission.

reach a loading side, they can pick up DNA-modified cargo to transport and unload it at designated zones by strand displacement. Experimental realization of this intriguing system was disrupted by nonspecific interactions of the cargo (1 μM diameter avidin-mofified polystyrene beads) with the MTs and the glass surface. Moreover, the biological motor-based motility on the glass surface occurs in a random fashion, and thus directional delivery can not yet be achieved. Nonetheless, the example is fascinating because it impressively demonstrates how the individual properties of nucleic acids and proteins can be synergistically combined to fabricate novel autonomous nanosystems.

4. Conclusions and Outlook

In this Review, examples are described which concern the artificial combination of biomolecular building blocks of the two large families of biopolymers—nucleic acids and proteins. Chemistry is the key discipline in the development of techniques for both covalent and noncovalent bioconjuation. It is foreseeable that the current establishment of a broader repertoire of methods will continue to meet the demands for the individual modification of specific POIs with synthetic DNA moieties, with precise control over the stoichiometry and site selectivity. The functionality of the building blocks means that applications of nucleic acid–protein hybrids are extremely broad—particularly, because of the myriad of evolutionary tailored functional proteins. It is therefore a challenge to select the appropriate functionality and render the respective native protein suitable for abiotic modification and application, without losing the intrinsic enzymic activity, by taking advantage of modern methods of molecular biology and enzyme process engineering. The establishment and exploitation of DNA–protein conjugates inside living systems represents another challenging goal. DNA–protein conjugates can be used in the areas of sensing, device fabrication, and nanoconstruction. Advanced engineering of more-complex DNA scaffolding (such as three-dimensional and/or dynamic DNA architecture) as well as implementation of DNA-modified nanoparticles with their size-dependent material properties will certainly extend the scope of the DNA–protein hybrids. The future of this class of molecules is bright and most likely not limited to the generation of artificial multienzymes, synthetic antennae and light-harvesting devices, or oligospecific receptor constructs. It seems that future perspectives are only limited by the imagination of creative researchers working at the cross-section of chemistry, biology, and materials science.

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[1] J. M. Berg, J. L. Tymoczko, L. Stryer, *Biochemistry, 5th ed.*, W. H. Freeman, New York, **2002**.
[2] D. M. J. Lilley, *ChemBioChem* **2001**, *2*, 31–35.
[3] C. M. Niemeyer, *Angew. Chem.* **2001**, *113*, 4254–4287; *Angew. Chem. Int. Ed.* **2001**, *40*, 4128–4158.
[4] K. E. Drexler, *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 5275–5278.
[5] N. C. Seeman, *J. Theor. Biol.* **1982**, *99*, 237–247.
[6] C. M. Niemeyer, C. A. Mirkin, *NanoBiotechnology: Concepts, Methods and Applications*, Wiley-VCH, Weinheim, **2004**.
[7] C. A. Mirkin, C. M. Niemeyer, *NanoBiotechnology II: More Concepts and Applications*, Wiley-VCH, Weinheim, **2007**.
[8] N. C. Seeman, *Nature* **2003**, *421*, 427–431.
[9] U. Feldkamp, C. M. Niemeyer, *Angew. Chem.* **2006**, *118*, 1888–1910; *Angew. Chem. Int. Ed.* **2006**, *45*, 1856–1876.
[10] C. Lin, Y. Liu, S. Rinker, H. Yan, *ChemPhysChem* **2006**, *7*, 1641–1647.
[11] C. Lin, Y. Liu, H. Yan, *Biochemistry* **2009**, *48*, 1663–1674.
[12] C. M. Niemeyer, *Nano Today* **2007**, *2*, 42–52.
[13] M. Wilchek, E. A. Bayer, *Methods Enzymol.* **1990**, *184*, 14–45.
[14] M. Wilchek, E. A. Bayer, *Methods Enzymol.* **1990**, *184*, 51–67.
[15] Biotinylation in vivo is a rare and highly specific process, which takes place at distinctive short polypeptides, recognized by biotin–protein ligases (such as BirA). For a review on the in vivo biotinylation of proteins, see A. Chapman-Smith, J. E. Cronan, Jr., *Biomol. Eng.* **1999**, *16*, 119–125. The BirA system is frequently applied to generate monobiotinylated recombinant proteins; for examples, see D. J. Min, J. D. Andrade, R. J. Stewart, *Anal. Biochem.* **1999**, *270*, 133–139; I. Chen, M. Howarth, W. Lin, A. Y. Ting, *Nat. Methods* **2005**, *2*, 99–104.

[16] G. D. Meredith, H. Y. Wu, N. L. Allbritton, *Bioconjugate Chem.* **2004**, *15*, 969 – 982.
[17] J. Shimada, T. Maruyama, T. Hosogi, J. Tominaga, N. Kamiya, M. Goto, *Biotechnol. Lett.* **2008**, *30*, 2001 – 2006.
[18] R. P. Goodman, C. M. Erben, J. Malo, W. M. Ho, M. L. McKee, A. N. Kapanidis, A. J. Turberfield, *ChemBioChem* **2009**, *10*, 1551 – 1557.
[19] W. Shen, H. Zhong, D. Neff, M. L. Norton, *J. Am. Chem. Soc.* **2009**, *131*, 6660 – 6661.
[20] E. Hochuli, H. Dobeli, A. Schacher, *J. Chromatogr.* **1987**, *411*, 177 – 184.
[21] E. Hochuli, *J. Chromatogr.* **1988**, *444*, 293 – 302.
[22] A. N. Kapanidis, Y. W. Ebright, R. H. Ebright, *J. Am. Chem. Soc.* **2001**, *123*, 12123 – 12125.
[23] E. W. Voss, *Fluorescein Hapten: An Immunological Probe*, CRC, Boca Raton, FL, **1984**.
[24] S. S. Kadkol, W. R. Gage, G. R. Pasternack, *Mol. Diagn.* **1999**, *4*, 169 – 183.
[25] Y. He, Y. Tian, A. E. Ribbe, C. Mao, *J. Am. Chem. Soc.* **2006**, *128*, 12664 – 12665.
[26] J. R. Crowther, *ELISA: Theory and Practice*, Humana Press Inc., Totowa, **1995**; *Principle and Practice of Immunoassays* (Eds.: C. P. Price, D. J. Newman), Macmillan, London, **1997**; O. N. Chappey, P. Sandouk, J. M. Scherrmann, *Pharm. Res.* **1992**, *9*, 1375 – 1379.
[27] A. Knappik, A. Plueckthun, *Biotechniques* **1994**, *17*, 745 – 761.
[28] M. Famulok, J. S. Hartig, G. Mayer, *Chem. Rev.* **2007**, *107*, 3715 – 3743.
[29] G. Mayer, *Angew. Chem.* **2009**, *121*, 2710 – 2727; *Angew. Chem. Int. Ed.* **2009**, *48*, 2672 – 2689.
[30] A current search on Pubmed delivers more than 250 entries for applications of the anti-thrombin aptamer, initially described in 1992: L. C. Bock, L. C. Griffin, J. A. Latham, E. H. Vermaas, J. J. Toole, *Nature* **1992**, *355*, 564 – 566.
[31] L. Fruk, C.-H. Kuo, E. Torres, C. M. Niemeyer, *Angew. Chem.* **2009**, *121*, 1578 – 1603; *Angew. Chem. Int. Ed.* **2009**, *48*, 1550 – 1574.
[32] L. Fruk, C. M. Niemeyer, *Angew. Chem.* **2005**, *117*, 2659 – 2662; *Angew. Chem. Int. Ed.* **2005**, *44*, 2603 – 2606.
[33] L. Fruk, J. Müller, C. M. Niemeyer, *Chem. Eur. J.* **2006**, *12*, 7448 – 7457.
[34] L. Fruk, J. Müller, G. Weber, A. Narváez, E. Domínguez, C. M. Niemeyer, *Chem. Eur. J.* **2007**, *13*, 5223 – 5231.
[35] L. Fruk, J. Kuhlmann, C. M. Niemeyer, *Chem. Commun.* **2009**, 230 – 232.
[36] P. Simon, C. Dueymes, M. Fontecave, J.-L. Decout, *Angew. Chem.* **2005**, *117*, 2824 – 2827; *Angew. Chem. Int. Ed.* **2005**, *44*, 2764 – 2767.
[37] A. Saghatelian, K. M. Guckian, D. A. Thayer, M. R. Ghadiri, *J. Am. Chem. Soc.* **2003**, *125*, 344 – 345.
[38] N. C. Gianneschi, M. R. Ghadiri, *Angew. Chem.* **2007**, *119*, 4029 – 4032; *Angew. Chem. Int. Ed.* **2007**, *46*, 3955 – 3958.
[39] M. Renz, C. Kurz, *Nucleic Acids Res.* **1984**, *12*, 3435 – 3444.
[40] J. Czichos, M. Kohler, B. Reckmann, M. Renz, *Nucleic Acids Res.* **1989**, *17*, 1563 – 1572.
[41] D. R. Corey, P. G. Schultz, *Science* **1987**, *238*, 1401 – 1403.
[42] D. R. Corey, *J. Am. Chem. Soc.* **1995**, *117*, 9373 – 9374.
[43] S. V. Smulevitch, C. G. Simmons, J. C. Norton, T. W. Wise, D. R. Corey, *Nat. Biotechnol.* **1996**, *14*, 1700 – 1704.
[44] S. Howorka, S. Cheley, H. Bayley, *Nat. Biotechnol.* **2001**, *19*, 636 – 639.
[45] S. Howorka, L. Movileanu, O. Braha, H. Bayley, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 12996 – 13001.
[46] R. B. Fong, Z. Ding, C. J. Long, A. S. Hoffman, P. S. Stayton, *Bioconjugate Chem.* **1999**, *10*, 720 – 725.
[47] C. M. Niemeyer, T. Sano, C. L. Smith, C. R. Cantor, *Nucleic Acids Res.* **1994**, *22*, 5530 – 5539.
[48] R. D. Joerger, T. M. Truby, E. R. Hendrickson, R. M. Young, R. C. Ebersole, *Clin. Chem.* **1995**, *41*, 1371 – 1377.
[49] For a detailed description on maleimide-based coupling of proteins with oligonucleotides, see F. Kukolka, M. Lovrinovic, R. Wacker, C. M. Niemeyer in *Bioconjugation Protocols: Strategies and Methods* (Ed.: C. M. Niemeyer), Humana, Totowa, NJ, **2004**, pp. 181 – 196.
[50] F. Kukolka, C. M. Niemeyer, *Org. Biomol. Chem.* **2004**, *2*, 2203 – 2206.
[51] R. M. Hofmann, T. W. Muir, *Curr. Opin. Biotechnol.* **2002**, *13*, 297 – 303.
[52] R. S. Goody, K. Alexandrov, M. Engelhard, *ChemBioChem* **2002**, *3*, 399 – 403.
[53] M. Lovrinovic, R. Seidel, R. Wacker, H. Schroeder, O. Seitz, M. Engelhard, R. Goody, C. M. Niemeyer, *Chem. Commun.* **2003**, 822 – 823.
[54] For examples, see M. Lovrinovic, M. Spengler, C. Deutsch, C. M. Niemeyer, *Mol. BioSyst.* **2005**, *1*, 64 – 69; S. Takeda, S. Tsukiji, T. Nagamune, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2407 – 2410; M. Lovrinovic, C. M. Niemeyer, *Biochem. Biophys. Res. Commun.* **2005**, *333*, 943 – 948; I. Burbulis, K. Yamaguchi, A. Gordon, R. Carlson, R. Brent, *Nat. Methods* **2005**, *2*, 31 – 37; M. Lovrinovic, C. M. Niemeyer, *ChemBioChem* **2007**, *8*, 61 – 67; M. Lovrinovic, L. Fruk, C. M. Niemeyer, *Chem. Commun.* **2007**, 353 – 355; S. Takeda, S. Tsukiji, H. Ueda, T. Nagamune, *Org. Biomol. Chem.* **2008**, *6*, 2187 – 2194.
[55] C. F. W. Becker, R. Wacker, W. Bouschen, R. Seidel, B. Kolaric, P. Lang, H. Schroeder, O. Müller, C. M. Niemeyer, B. Spengler, R. S. Goody, M. Engelhard, *Angew. Chem.* **2005**, *117*, 7808 – 7812; *Angew. Chem. Int. Ed.* **2005**, *44*, 7635 – 7639.
[56] E. M. Sletten, C. R. Bertozzi, *Angew. Chem.* **2009**, *121*, 7108 – 7133; *Angew. Chem. Int. Ed.* **2009**, *48*, 6974 – 6998.
[57] M. Köhn, R. Breinbauer, *Angew. Chem.* **2004**, *116*, 3168 – 3178; *Angew. Chem. Int. Ed.* **2004**, *43*, 3106 – 3116.
[58] J. F. Lutz, *Angew. Chem.* **2007**, *119*, 1036 – 1043; *Angew. Chem. Int. Ed.* **2007**, *46*, 1018 – 1025.
[59] T. Kurpiers, H. D. Mootz, *ChemBioChem* **2008**, *9*, 2317 – 2325.
[60] J. A. Prescher, C. R. Bertozzi, *Nat. Chem. Biol.* **2005**, *1*, 13 – 21.
[61] P. Jonkheijm, D. Weinrich, H. Schroeder, C. M. Niemeyer, H. Waldmann, *Angew. Chem.* **2008**, *120*, 9762 – 9792; *Angew. Chem. Int. Ed.* **2008**, *47*, 9618 – 9647.
[62] R. A. Chandra, E. S. Douglas, R. A. Mathies, C. R. Bertozzi, M. B. Francis, *Angew. Chem.* **2006**, *118*, 910 – 915; *Angew. Chem. Int. Ed.* **2006**, *45*, 896 – 901.
[63] E. S. Douglas, R. A. Chandra, C. R. Bertozzi, R. A. Mathies, M. B. Francis, *Lab Chip* **2007**, *7*, 1442 – 1448.
[64] S. C. Hsiao, A. K. Crow, W. A. Lam, C. R. Bertozzi, D. A. Fletcher, M. B. Francis, *Angew. Chem.* **2008**, *120*, 8601 – 8605; *Angew. Chem. Int. Ed.* **2008**, *47*, 8473 – 8477.
[65] S. C. Hsiao, B. J. Shum, H. Onoe, E. S. Douglas, Z. J. Gartner, R. A. Mathies, C. R. Bertozzi, M. B. Francis, *Langmuir* **2009**, *25*, 6985 – 6991.
[66] E. S. Douglas, S. C. Hsiao, H. Onoe, C. R. Bertozzi, M. B. Francis, R. A. Mathies, *Lab Chip* **2009**, *9*, 2010 – 2015.
[67] Z. J. Gartner, C. R. Bertozzi, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 4606 – 4610.
[68] P. M. Gramlich, C. T. Wirges, A. Manetto, T. Carell, *Angew. Chem.* **2008**, *120*, 8478 – 8487; *Angew. Chem. Int. Ed.* **2008**, *47*, 8350 – 8358.
[69] K. Gutsmiedl, C. T. Wirges, V. Ehmke, T. Carell, *Org. Lett.* **2009**, *11*, 2405 – 2408.
[70] B. P. Duckworth, Y. Chen, J. W. Wollack, Y. Sham, J. D. Mueller, T. A. Taton, M. D. Distefano, *Angew. Chem.* **2007**, *119*, 8975 – 8978; *Angew. Chem. Int. Ed.* **2007**, *46*, 8819 – 8822.
[71] L. Wang, P. G. Schultz, *Angew. Chem.* **2005**, *117*, 34 – 68; *Angew. Chem. Int. Ed.* **2005**, *44*, 34 – 66.

[72] Examples for the site-specific incorporation of alkyne or azide groups into recombinant proteins using non-natural amino acids: Z. Zhang, L. Wang, A. Brock, P. G. Schultz, *Angew. Chem.* **2002**, *114*, 2964 – 2966; *Angew. Chem. Int. Ed.* **2002**, *41*, 2840 – 2842; A. Deiters, T. A. Cropp, M. Mukherji, J. W. Chin, J. C. Anderson, P. G. Schultz, *J. Am. Chem. Soc.* **2003**, *125*, 11782 – 11783; T. Yanagisawa, R. Ishii, R. Fukunaga, T. Kobayashi, K. Sakamoto, S. Yokoyama, *Chem. Biol.* **2008**, *15*, 1187 – 1197; P. R. Chen, D. Groff, J. Guo, W. Ou, S. Cellitti, B. H. Geierstanger, P. G. Schultz, *Angew. Chem.* **2009**, *121*, 4112 – 4115; *Angew. Chem. Int. Ed.* **2009**, *48*, 4052 – 4055; E. Kaya, K. Gutsmiedl, M. Vrabel, M. Müller, P. Thumbs, T. Carell, *ChemBioChem* **2009**, DOI: 10.1002/cbic.200904930.

[73] H. S. Lee, R. D. Dimla, P. G. Schultz, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5222 – 5224.

[74] J. Tominaga, Y. Kemori, Y. Tanaka, T. Maruyama, N. Kamiya, M. Goto, *Chem. Commun.* **2007**, 401 – 403.

[75] 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 – 2167.

[76] E. M. Singer, S. S. Smith, *Nano Lett.* **2006**, *6*, 1184 – 1189.

[77] A. Keppler, S. Gendreizig, T. Gronemeyer, H. Pick, H. Vogel, K. Johnsson, *Nat. Biotechnol.* **2003**, *21*, 86 – 89.

[78] N. Johnsson, K. Johnsson, *ACS Chem. Biol.* **2007**, *2*, 31 – 38.

[79] M. A. Jongsma, R. H. Litjens, *Proteomics* **2006**, *6*, 2650 – 2655.

[80] E. Jablonski, E. W. Moomaw, R. H. Tullis, J. L. Ruth, *Nucleic Acids Res.* **1986**, *14*, 6115 – 6128.

[81] M. S. Urdea, B. D. Warner, J. A. Running, M. Stempien, J. Clyne, T. Horn, *Nucleic Acids Res.* **1988**, *16*, 4937 – 4956.

[82] S. S. Ghosh, P. M. Kao, A. W. McCue, H. L. Chappelle, *Bioconjugate Chem.* **1990**, *1*, 71 – 76.

[83] E. Kynclova, A. Hartig, T. Schalkhammer, *J. Mol. Recognit.* **1995**, *8*, 139 – 145.

[84] Y. Wang, M. Stanzel, W. Gumbrecht, M. Humenik, M. Sprinzl, *Biosens. Bioelectron.* **2007**, *22*, 1798 – 1806.

[85] T. Sano, C. L. Smith, C. R. Cantor, *Science* **1992**, *258*, 120 – 122.

[86] For examples, see J. Ren, Z. Chen, S. J. Juan, X. Y. Yong, B. R. Pan, D. M. Fan, *Cancer* **2000**, *88*, 280 – 285; S. Wiltshire, S. O'Malley, J. Lambert, K. Kukanskis, D. Edgar, S. F. Kingsmore, B. Schweitzer, *Clin. Chem.* **2000**, *46*, 1990 – 1993; H. Liang, S. E. Cordova, T. L. Kieft, S. Rogelj, *J. Immunol. Methods* **2003**, *279*, 101 – 110; M. Adler, R. Wacker, E. Booltink, B. Manz, C. M. Niemeyer, *Nat. Methods* **2005**, *2*, 147 – 149; M. Spengler, M. Adler, A. Jonas, C. M. Niemeyer, *Biochem. Biophys. Res. Commun.* **2009**, *387*, 278 – 282.

[87] C. M. Niemeyer, M. Adler, R. Wacker, *Nat. Protoc.* **2007**, *2*, 1918 – 1930.

[88] For a recent overview on IPCR and related technologies, see M. Adler, R. Wacker, C. M. Niemeyer, *Analyst* **2008**, *133*, 702 – 718.

[89] For examples, see S. Fredriksson, M. Gullberg, J. Jarvius, C. Olsson, K. Pietras, S. M. Gustafsdottir, A. Ostman, U. Landegren, *Nat. Biotechnol.* **2002**, *20*, 473 – 477; M. Gullberg, S. M. Gustafsdottir, E. Schallmeiner, J. Jarvius, M. Bjarnegard, C. Betsholtz, U. Landegren, S. Fredriksson, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 8420 – 8424; S. M. Gustafsdottir, A. Nordengrahn, S. Fredriksson, P. Wallgren, E. Rivera, E. Schallmeiner, M. Merza, U. Landegren, *Clin. Chem.* **2006**, *52*, 1152 – 1160; O. Söderberg, M. Gullberg, M. Jarvius, K. Ridderstrale, K. J. Leuchowius, J. Jarvius, K. Wester, P. Hydbring, F. Bahram, L. G. Larsson, U. Landegren, *Nat. Methods* **2006**, *3*, 995 – 1000; S. M. Gustafsdottir, J. Schlingemann, A. Rada-Iglesias, E. Schallmeiner, M. Kamali-Moghaddam, C. Wadelius, U. Landegren, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 3067 – 3072; E. Schallmeiner, E. Oksanen, O. Ericsson, L. Spangberg, S. Eriksson, U. H. Stenman, K. Pettersson, U. Landegren, *Nat. Methods* **2007**, *4*, 135 – 137; O. Ericsson, J. Jarvius, E. Schallmeiner, M. Howell, R. Y. Nong, H. Reuter, M. Hahn, J. Stenberg, M. Nilsson, U. Landegren, *Nucleic Acids Res.* **2008**, *36*, e45; L. Zhu, H. Koistinen, U. Landegren, U. H. Stenman, *Clin. Chem.* **2009**, *55*, 1665 – 1671.

[90] M. C. Pirrung, *Angew. Chem.* **2002**, *114*, 1326 – 1341; *Angew. Chem. Int. Ed.* **2002**, *41*, 1276 – 1289.

[91] U. R. Mueller, D. V. Nicolau, *Microarray Technology and Its Applications*, Springer, Berlin, **2005**.

[92] C. M. Niemeyer, R. Wacker, M. Adler, *Nucleic Acids Res.* **2003**, *31*, 90e.

[93] For recent examples, see R. Wacker, C. M. Niemeyer, *ChemBioChem* **2004**, *5*, 453 – 459; C. Boozer, J. Ladd, S. Chen, S. Jiang, *Anal. Chem.* **2006**, *78*, 1515 – 1519; J. K. Ng, P. K. Ajikumar, Y. C. Tang, J. Y. Lee, G. Stephanopoulos, H. P. Too, *Electrophoresis* **2007**, *28*, 4638 – 4644; Y. Jung, J. M. Lee, H. Jung, B. H. Chung, *Anal. Chem.* **2007**, *79*, 6534 – 6541; N. Tort, J.-P. Salvador, R. Eritja, M. Poch, E. Martínez, J. Samitier, M. P. Marco, *TrAC Trends Anal. Chem.* **2009**, *28*, 718 – 728.

[94] F. Bano, L. Fruk, B. Sanavio, M. Glettenberg, L. Casalis, C. M. Niemeyer, G. Scoles, *Nano Lett.* **2009**, *9*, 2614 – 2618.

[95] H. Schroeder, M. Adler, K. Gerigk, B. Müller-Chorus, F. Götz, C. M. Niemeyer, *Anal. Chem.* **2009**, *81*, 1275 – 1279.

[96] C. M. Niemeyer, L. Boldt, B. Ceyhan, D. Blohm, *Anal. Biochem.* **1999**, *268*, 54 – 63.

[97] H. Schröder, L. Hoffmann, J. Müller, P. Alhorn, M. Fleger, A. Neyer, C. M. Niemeyer, *Small* **2009**, *5*, 1547 – 1552.

[98] N. Winssinger, J. L. Harris, B. J. Backes, P. G. Schultz, *Angew. Chem.* **2001**, *113*, 3254 – 3258; *Angew. Chem. Int. Ed.* **2001**, *40*, 3152 – 3155.

[99] N. Winssinger, S. Ficarro, P. G. Schultz, J. L. Harris, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11139 – 11144.

[100] N. Winssinger, R. Damoiseaux, D. C. Tully, B. H. Geierstanger, K. Burdick, J. L. Harris, *Chem. Biol.* **2004**, *11*, 1351 – 1360.

[101] J. Harris, D. E. Mason, J. Li, K. W. Burdick, B. J. Backes, T. Chen, A. Shipway, G. Van Heeke, L. Gough, A. Ghaemmaghami, F. Shakib, F. Debaene, N. Winssinger, *Chem. Biol.* **2004**, *11*, 1361 – 1372.

[102] J. J. Diaz-Mochon, L. Bialy, L. Keinicke, M. Bradley, *Chem. Commun.* **2005**, 1384 – 1386.

[103] F. Debaene, N. Winssinger, *Methods Mol. Biol.* **2009**, *570*, 299 – 307.

[104] N. Nemoto, E. Miyamoto-Sato, Y. Husimi, H. Yanagawa, *FEBS Lett.* **1997**, *414*, 405 – 408.

[105] R. W. Roberts, J. W. Szostak, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 12297 – 12302.

[106] M. Kurz, K. Gu, A. Al-Gawari, P. A. Lohse, *ChemBioChem* **2001**, *2*, 666 – 672.

[107] V. Stein, I. Sielaff, K. Johnsson, F. Hollfelder, *ChemBioChem* **2007**, *8*, 2191 – 2194.

[108] H. Schroeder, B. Ellinger, C. F. W. Becker, H. Waldmann, C. M. Niemeyer, *Angew. Chem.* **2007**, *119*, 4258 – 4261; *Angew. Chem. Int. Ed.* **2007**, *46*, 4180 – 4183.

[109] R. C. Bailey, G. A. Kwong, C. G. Radu, O. N. Witte, J. R. Heath, *J. Am. Chem. Soc.* **2007**, *129*, 1959 – 1967.

[110] Selected examples of DDI of DNA-modified carbohydrates: Y. Chevolot, C. Bouillon, S. Vidal, F. Morvan, A. Meyer, J. P. Cloarec, A. Jochum, J. P. Praly, J. J. Vasseur, E. Souteyrand, *Angew. Chem.* **2007**, *119*, 2450 – 2454; *Angew. Chem. Int. Ed.* **2007**, *46*, 2398 – 2402; J. Zhang, G. Pourceau, A. Meyer, S. Vidal, J. P. Praly, E. Souteyrand, J. J. Vasseur, F. Morvan, Y. Chevolot, *Biosens. Bioelectron.* **2009**, *24*, 2515 – 2521; inorganic nanoparticles: T. A. Taton, R. C. Mucic, C. A. Mirkin, R. L. Letsinger, *J. Am. Chem. Soc.* **2000**, *122*, 6305 – 6306; D. Gerion, W. J. Parak, S. C. Williamson, D. Zanchet, C. M. Micheel, A. P. Alivisatos, *J. Am. Chem. Soc.* **2002**, *124*, 7070 – 7074; C. M. Niemeyer, B. Ceyhan, P. Hazarika, *Angew. Chem.* **2003**, *115*, 5944 – 5948; *Angew. Chem. Int. Ed.* **2003**, *42*, 5766 – 5770; *Angew. Chem.* **2003**, *115*, 5944 – 5948; B. Zou, B. Ceyhan, U.

Simon, C. M. Niemeyer, *Adv. Mater.* **2005**, *17*, 1643 – 1647; Z. Zhang, Q. Cheng, P. Feng, *Angew. Chem.* **2009**, *121*, 124 – 128; *Angew. Chem. Int. Ed.* **2009**, *48*, 118 – 122; or lipid vesicles: C. Yoshina-Ishii, S. G. Boxer, *J. Am. Chem. Soc.* **2003**, *125*, 3696 – 3697; S. Svedhem, I. Pfeiffer, C. Larsson, C. Wingren, C. Borrebaeck, F. Hook, *ChemBioChem* **2003**, *4*, 339 – 343; I. Pfeiffer, F. Hook, *J. Am. Chem. Soc.* **2004**, *126*, 10224 – 10225.

[111] C. M. Niemeyer, M. Adler, B. Pignataro, S. Lenhert, S. Gao, L. F. Chi, H. Fuchs, D. Blohm, *Nucleic Acids Res.* **1999**, *27*, 4553 – 4561.

[112] N. L. Rosi, C. A. Mirkin, *Chem. Rev.* **2005**, *105*, 1547 – 1562.

[113] C. M. Niemeyer, M. Adler, S. Lenhert, S. Gao, H. Fuchs, L. F. Chi, *ChemBioChem* **2001**, *2*, 260 – 265.

[114] C. M. Niemeyer, M. Adler, S. Gao, L. F. Chi, *Angew. Chem.* **2000**, *112*, 3183 – 3187; *Angew. Chem. Int. Ed.* **2000**, *39*, 3055 – 3059.

[115] C. M. Niemeyer, R. Wacker, M. Adler, *Angew. Chem.* **2001**, *113*, 3262 – 3265; *Angew. Chem. Int. Ed.* **2001**, *40*, 3169 – 3172.

[116] E. Braun, Y. Eichen, U. Sivan, G. Ben-Yoseph, *Nature* **1998**, *391*, 775 – 778.

[117] K. Keren, M. Krueger, R. Gilad, G. Ben-Yoseph, U. Sivan, E. Braun, *Science* **2002**, *297*, 72 – 75.

[118] B. Pignataro, L. F. Chi, S. Gao, B. Anczykowsky, C. M. Niemeyer, M. Adler, H. Fuchs, *Appl. Phys. A* **2002**, *74*, 447 – 452.

[119] C. M. Niemeyer, W. Bürger, J. Peplies, *Angew. Chem.* **1998**, *110*, 2391 – 2395; *Angew. Chem. Int. Ed.* **1998**, *37*, 2265 – 2268.

[120] H. Yan, S. H. Park, G. Finkelstein, J. H. Reif, T. H. LaBean, *Science* **2003**, *301*, 1882 – 1884.

[121] H. Li, S. H. Park, J. H. Reif, T. H. LaBean, H. Yan, *J. Am. Chem. Soc.* **2004**, *126*, 418 – 419.

[122] J. Sharma, Y. Ke, C. Lin, R. Chhabra, Q. Wang, J. Nangreave, Y. Liu, H. Yan, *Angew. Chem.* **2008**, *120*, 5235 – 5237; *Angew. Chem. Int. Ed.* **2008**, *47*, 5157 – 5159.

[123] K. Lund, Y. Liu, S. Lindsay, H. Yan, *J. Am. Chem. Soc.* **2005**, *127*, 17606 – 17607.

[124] Y. Liu, Y. Ke, H. Yan, *J. Am. Chem. Soc.* **2005**, *127*, 17140 – 17141.

[125] Y. Liu, C. Lin, H. Li, H. Yan, *Angew. Chem.* **2005**, *117*, 4407 – 4412; *Angew. Chem. Int. Ed.* **2005**, *44*, 4333 – 4338.

[126] C. Lin, E. Katilius, Y. Liu, J. Zhang, H. Yan, *Angew. Chem.* **2006**, *118*, 5422 – 5427; *Angew. Chem. Int. Ed.* **2006**, *45*, 5296 – 5301.

[127] C. Lin, Y. Liu, H. Yan, *Nano Lett.* **2007**, *7*, 507 – 512.

[128] R. Chhabra, J. Sharma, Y. Ke, Y. Liu, S. Rinker, S. Lindsay, H. Yan, *J. Am. Chem. Soc.* **2007**, *129*, 10304 – 10305.

[129] B. A. R. Williams, K. Lund, Y. Liu, H. Yan, J. C. Chaput, *Angew. Chem.* **2007**, *119*, 3111 – 3114; *Angew. Chem. Int. Ed.* **2007**, *46*, 3051 – 3054.

[130] P. W. K. Rothemund, *Nature* **2006**, *440*, 297 – 302.

[131] A. Kuzuya, M. Kimura, K. Numajiri, N. Koshi, T. Ohnishi, F. Okada, M. Komiyama, *ChemBioChem* **2009**, *10*, 1811 – 1815.

[132] A. Kuzuya, K. Numajiri, M. Komiyama, *Angew. Chem.* **2008**, *120*, 3448 – 3450; *Angew. Chem. Int. Ed.* **2008**, *47*, 3400 – 3402.

[133] A. Kuzyk, K. T. Laitinen, P. Torma, *Nanotechnology* **2009**, *20*, 235305.

[134] J. D. Cohen, J. P. Sadowski, P. B. Dervan, *Angew. Chem.* **2007**, *119*, 8102 – 8105; *Angew. Chem. Int. Ed.* **2007**, *46*, 7956 – 7959.

[135] J. D. Cohen, J. P. Sadowski, P. B. Dervan, *J. Am. Chem. Soc.* **2008**, *130*, 402 – 403.

[136] W. Shen, M. F. Bruist, S. D. Goodman, N. C. Seeman, *Angew. Chem.* **2004**, *116*, 4854 – 4856; *Angew. Chem. Int. Ed.* **2004**, *43*, 4750 – 4752.

[137] J. Malo, J. C. Mitchell, C. Vénien-Bryan, J. R. Harris, H. Wille, D. J. Sherratt, A. J. Turberfield, *Angew. Chem.* **2005**, *117*, 3117 – 3121; *Angew. Chem. Int. Ed.* **2005**, *44*, 3057 – 3061.

[138] C. M. Erben, R. P. Goodman, A. J. Turberfield, *Angew. Chem.* **2006**, *118*, 7574 – 7577; *Angew. Chem. Int. Ed.* **2006**, *45*, 7414 – 7417.

[139] M. Glettenberg, C. M. Niemeyer, *Bioconjugate Chem.* **2009**, *20*, 969 – 975.

[140] C.-H. Kuo, L. Fruk, C. M. Niemeyer, *Chem. Asian J.* **2009**, *4*, 1064 – 1069.

[141] C. M. Niemeyer, J. Koehler, C. Wuerdemann, *ChemBioChem* **2002**, *3*, 242 – 245.

[142] J. Müller, C. M. Niemeyer, *Biochem. Biophys. Res. Commun.* **2008**, *377*, 62 – 67.

[143] O. I. Wilner, Y. Weizmann, R. Gill, O. Lioubashevski, R. Freeman, I. Willner, *Nat. Nanotechnol.* **2009**, *4*, 249 – 254.

[144] O. I. Wilner, S. Shimron, Y. Weizmann, Z. G. Wang, I. Willner, *Nano Lett.* **2009**, *9*, 2040 – 2043.

[145] C. Lin, H. Yan, *Nat. Nanotechnol.* **2009**, *4*, 211 – 212.

[146] J. J. Storhoff, C. A. Mirkin, *Chem. Rev.* **1999**, *99*, 1849 – 1862.

[147] M. C. Daniel, D. Astruc, *Chem. Rev.* **2004**, *104*, 293 – 346.

[148] E. Katz, I. Willner, *Angew. Chem.* **2004**, *116*, 6166 – 6235; *Angew. Chem. Int. Ed.* **2004**, *43*, 6042 – 6108.

[149] C. M. Niemeyer, B. Ceyhan, *Angew. Chem.* **2001**, *113*, 3798 – 3801; *Angew. Chem. Int. Ed.* **2001**, *40*, 3685 – 3688.

[150] P. Hazarika, B. Ceyhan, C. M. Niemeyer, *Small* **2005**, *1*, 844 – 848.

[151] P. Hazarika, F. Kukolka, C. M. Niemeyer, *Angew. Chem.* **2006**, *118*, 6981 – 6984; *Angew. Chem. Int. Ed.* **2006**, *45*, 6827 – 6830.

[152] B. Yurke, A. J. Turberfield, A. P. Mills, Jr., F. C. Simmel, J. L. Neumann, *Nature* **2000**, *406*, 605 – 608.

[153] F. C. Simmel, W. U. Dittmer, *Small* **2005**, *1*, 284 – 299.

[154] R. Y. Tsien, *Angew. Chem.* **2009**, *121*, 5721 – 5736; *Angew. Chem. Int. Ed.* **2009**, *48*, 5612 – 5626.

[155] F. Kukolka, B. K. Mueller, S. Paternoster, A. Arndt, C. M. Niemeyer, C. Braeuchle, D. C. Lamb, *Small* **2006**, *2*, 1083 – 1089; F. Kukolka, O. Schoeps, U. Woggon, C. M. Niemeyer, *Bioconjugate Chem.* **2007**, *18*, 621 – 627; G. Heiss, V. Lapiene, F. Kukolka, C. M. Niemeyer, C. Braeuchle, D. C. Lamb, *Small* **2009**, *5*, 1169 – 1175.

[156] Examples: V. R. Hering, G. Gibson, R. I. Schumacher, A. Faljoni-Alario, M. J. Politi, *Bioconjugate Chem.* **2007**, *18*, 1705 – 1708; A. M. Dennis, G. Bao, *Nano Lett.* **2008**, *8*, 1439 – 1445; J. Irrgang, J. Ksienczyk, V. Lapiene, C. M. Niemeyer, *ChemPhysChem* **2009**, *10*, 1483 – 1491; V. R. Hering, T. E. Faulin, E. R. Triboni, S. D. Rodriguez, D. L. Bernik, R. I. Schumacher, V. P. Mammana, A. Faljoni-Alario, D. S. Abdalla, G. Gibson, M. J. Politi, *Bioconjugate Chem.* **2009**, *20*, 1237 – 1241; K. Boeneman, B. C. Mei, A. M. Dennis, G. Bao, J. R. Deschamps, H. Mattoussi, I. L. Medintz, *J. Am. Chem. Soc.* **2009**, *131*, 3828 – 3829; for a recent review on QD-based FRET systems, see I. L. Medintz, H. Mattoussi, *Phys. Chem. Chem. Phys.* **2009**, *11*, 17 – 45.

[157] H. Lu, O. Schoeps, U. Woggon, C. M. Niemeyer, *J. Am. Chem. Soc.* **2008**, *130*, 4815 – 4827.

[158] S. Melkko, J. Scheuermann, C. E. Dumelin, D. Neri, *Nat. Biotechnol.* **2004**, *22*, 568 – 574.

[159] K. Gorska, K. T. Huang, O. Chaloin, N. Winssinger, *Angew. Chem.* **2009**, *121*, 7831 – 7836; *Angew. Chem. Int. Ed.* **2009**, *48*, 7695 – 7700.

[160] R. Pei, S. K. Taylor, D. Stefanovic, S. Rudchenko, T. E. Mitchell, M. N. Stojanovic, *J. Am. Chem. Soc.* **2006**, *128*, 12693 – 12699.

[161] S. Hiyama, T. Inoue, T. Shima, Y. Moritani, T. Suda, K. Sutoh, *Small* **2008**, *4*, 410 – 415.

