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DNA Block Copolymers

Nucleic Acid/Organic Polymer Hybrid Materials: Synthesis, Superstructures, and Applications

*Minseok Kwak and Andreas Herrmann**

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Extensive efforts have been devoted to the development of hybrid structures consisting of biomacromolecules and organic polymers connected through covalent bonds. While the combination of proteins and peptides with synthetic macromolecules has been explored in depth, far fewer examples of nucleic acid/polymer hybrids are known. In this Review we give selected examples of this exciting class of materials which can be arranged as linear block copolymer architectures, as side-chain polymers, or as cross-linked networks. Emphasis is placed on the fabrication of these materials as well as on their potential applications in nanoscience, diagnostics, and biomedicine.

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1. Introduction

One of the most exciting goals in chemical synthesis is the generation of macromolecules with a well-defined monomer sequence and dispersity that, at the same time, exhibit complex functions. Polymer chemistry can be used for the economic production of synthetic macromolecules. Anionic polymerization provides access to high-molecular-weight products with extremely low dispersities; however, this technique requires the strict exclusion of impurities and demands inert conditions. An alternative to this method is controlled living radical polymerization,[1] which yields macromolecules with comparable dispersities and gives easy access to block copolymers, extending the variety of functional monomers.[2] One group of branched macromolecules with higher structural accuracy and diversity of functional groups are the dendrimers, which display a branched macromolecular architecture.[3,4] Starting from a multivalent core molecule, treelike structures have been generated by iterative synthesis that includes alternating growth and deprotection steps. A multitude of functionalities have been incorporated into the core of these structures,[5] as well as in the dendritic scaffold and the periphery.[6,7] To synthesize polymers with an even greater variety of functional groups one must also rely on an iterative synthetic protocol, in which the macromolecule is built up on a solid support. The first approaches in this direction were described by Merrifield for the generation of oligo- and polypeptides.[8] With this groundbreaking synthetic method it is now possible to synthesize a large variety of peptides up to 50–70 amino acids in length with perfect control over the monomer sequence.[9] To achieve even larger protein structures, several coupling strategies have been elaborated for the covalent linkage of these building blocks through native peptide bonds.[10–13] The principle of solid-phase synthesis was subsequently adapted for the synthesis of other biomacromolecules such as nucleic acids[14–16] and carbohydrates.[17–19] Solid-phase synthesis is amenable to the production or introduction of nonnatural analogues,[20] for example peptide nucleic acids (PNAs)[21–23] and locked nucleic acids (LNAs),[24,25] in addition to the natural monomers to increase functional diversity. Indeed, more recently efforts have intensified to build up macromolecules that have only little structural relationship to their natural counterparts by employing solid supports and automated synthesizers.[26,27] Today, these chemical approaches towards well-defined functional macromolecules compete with techniques from molecular biology. Protein engineering is a powerful tool for expressing natural and de novo designed peptide sequences.[28] It is even possible to add nonnatural monomers to the set of naturally occurring amino acids.[29–31] The same holds true for polynucleic acids.[32] A variety of dye-functionalized nucleotides have been incorporated into DNA, in particular for sequencing. As a result of rapid developments in the field of recombinant DNA technology, a multitude of enzymatic methods are available to modify, manipulate, and amplify DNA. The most important enzymes in this context are kinases, ligases, nucleases, and polymerases. With this set of enzymes in combination with chemical DNA synthesis almost any desired polynucleic acid can be produced. Important techniques include the polymerase chain reaction (PCR) and the production of plasmid DNA in recombinant bacteria. An impressive example is the assembly of a complete infectious viral genome (5386 base pairs (bp)) from chemically synthesized oligodeoxynucleotides (ODNs) utilizing the molecular biology techniques mentioned above.[33]

In addition to improving synthetic methods to increase the size and functional complexity of one type of macromolecule, researchers have developed strategies to combine different classes of biomacromolecules. This includes posttranslational modifications of proteins, for instance the synthesis of glycosylated peptide structures.[34,35] Peptides have also been covalently connected to ODNs.[36] These are usually referred to as peptide–oligonucleotide conjugates (POCs)[37,38] or oligonucleotide–peptide conjugates (OPCs).[39] Only a few of these covalent nucleopeptides are found in nature.[40] The majority of POCs were designed to improve the pharmacokinetic properties of therapeutically useful ODNs.

In contrast to these structures, biomacromolecules have been combined with organic polymers in a covalent fashion.

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The different architectures are distinguished by how the biological moieties are connected to the synthetic macromolecules. The two building blocks can be arranged in an alternating manner leading to linear block copolymer topologies (Figure 1a). Alternatively, several peptide or nucleic acid moieties can be arranged along a polymer backbone leading to side-chain polymer structures (Figure 1b). The opposite case, in which several synthetic polymers are attached to a biomacromolecular backbone, is rarely found (Figure 1c).[41] Peptides were combined with organic polymers already in the mid-1970s.[42,43] A multitude of excellent reviews deal with the synthesis[44–52] and supramolecular organization[53–56] of these hybrid materials.

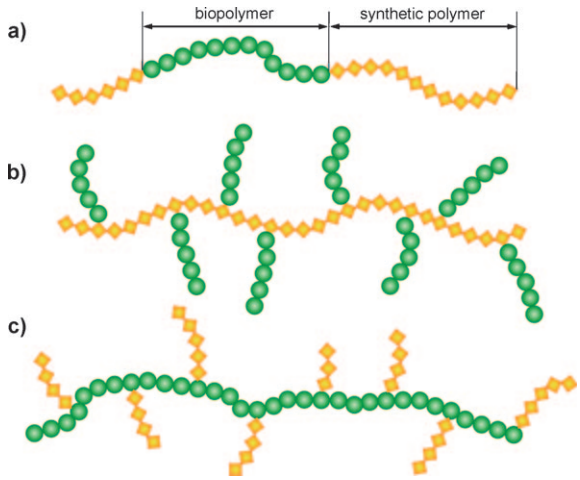

Figure 1. Illustrations of covalently bonded biopolymers (green circles) and synthetic (yellow diamonds) polymers. a) Linear block copolymer composed of biopolymer and synthetic polymer blocks. b) Biomacromolecules grafted onto a synthetic polymer backbone. c) Synthetic polymer side chains on a biomacromolecule backbone.

Much less attention had been paid to the class of bioorganic hybrids consisting of nucleic acids and synthetic polymers.[57] In contrast to research on peptide–polymer hybrids, the first reports of DNA–polymer conjugates go back only to the late 1980s. Antisense ODNs were grafted onto a poly(L-lysine) (PLL) backbone. These nucleic acid/polymer conjugates were employed to inhibit the synthesis of vesicular stomatitis virus proteins acting as antiviral agents.[58–60] Since then, numerous applications of these materials have been realized in addition to gene or ODN delivery. These range from the purification of biomaterials to the detection of DNA. In recent years, instead of hydrophilic polymers like PLL and poly(ethylene glycol) (PEG), hydrophobic polymers have been connected to ODNs to generate macromolecular amphiphiles. Because of microphase separation, these tend to self-assemble into micellar systems with nanometer dimensions. These materials thus have promising potential for applications in the fields of bionanotechnology and nanomedicine. This Review deals with the synthesis and potential applications of DNA block copolymers (DBCs). Different synthetic strategies will be detailed for the production of linear DBCs, DNA side-chain architectures, and DNA/polymer hybrid networks. At the same time, we will highlight the applications of these DBC architectures, primarily in biological and biomedicinal contexts but also in the field of nanoscience. The DBCs in this Review will be discussed in the order of their structural complexity: DBCs of linear topology will be described first, followed by side-chain architectures and cross-linked structures consisting mainly of the latter structure type.

2. Linear DNA Block Copolymers

2.1. Synthesis

Existing preparative methods for the generation of linear DBCs allow for the systematic variation of the length and sequence composition of the nucleic acid segment as well as the nature of the organic polymer block. Grafting strategies have proved to be especially successful. In this approach, prefabricated biological and organic polymer building blocks are coupled through complementary terminal groups. Two synthetic schemes can be distinguished: the nucleic acid blocks can be coupled to the organic polymer segments either in solution or on a solid support.

For the attachment in the aqueous phase, three different types of coupling reactions have been employed (Scheme 1). In the first approach, amino-functionalized ODNs are reacted with carboxylic acid terminated polymers like PEG,[61,62] poly(*N*-isopropylacrylamide) (PNIPAM),[63,64] poly(D,L-lactic-*co*-glycolic acid),[65] and poly(*p*-phenyleneethynyl-

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Andreas Herrmann studied chemistry at the University of Mainz (Germany). In 2000 he completed his graduate studies at the Max Planck Institute for Polymer Research in the group of Professor K. Müllen. After a brief stint as a consultant he returned to academia in 2002 and worked on protein engineering at the Swiss Federal Institute of Technology, Zurich, with Professor D. Hilvert. In 2004 he was appointed head of a junior research group at the Max Planck Institute for Polymer Research. In 2007 he moved to the Zernike Institute for Advanced Materials at the University of Groningen (The Netherlands), where he holds a chair for Polymer Chemistry and Bioengineering.

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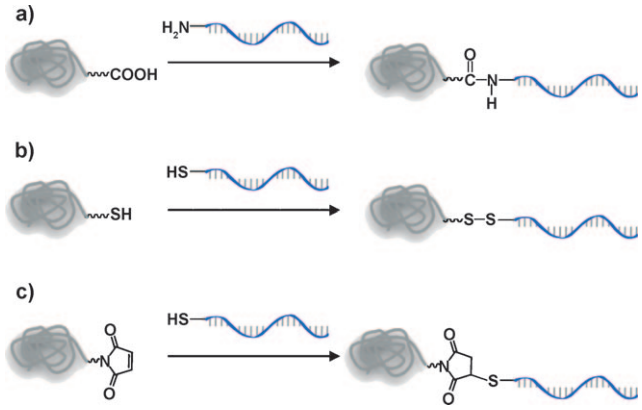
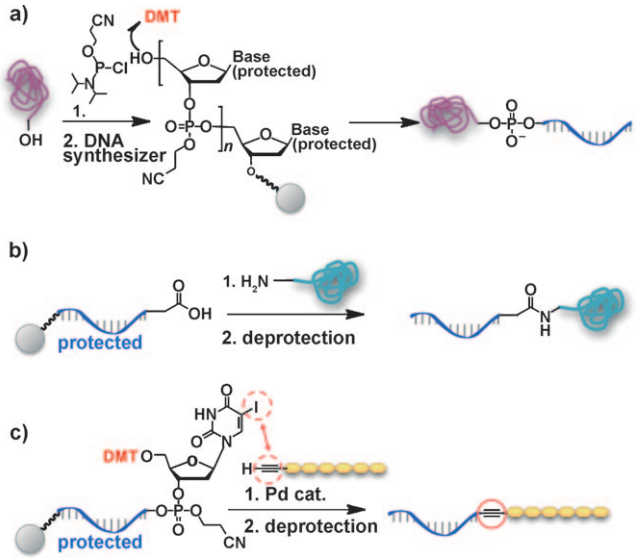


Scheme 1. Coupling methods for the synthesis of DNA block copolymers in solution. a) Carboxylic acid terminated polymer coupled with an amino-functionalyzed ODN. b) Disulfide bond formation between polymer and ODN thiols. c) Michael addition of a terminal maleimide on the polymer and thiol-modified ODN.



Scheme 2. Coupling reactions that can be used to prepare DNA block copolymers on a solid support. a) A hydroxylated polymer was converted into the phosphoramidite and coupled with 5′-OH group of DNA in the last step of the DNA synthesis. DMT = dimethoxytrityl. b) An amino-modified polymer was added to a carboxylic acid modified DNA on a solid support. c) Pd-catalyzed Sonogashira–Hagihara coupling of an acetylene-terminated π-conjugated polymer and a 5-iodouracil nucleobase on the solid phase (adapted from Ref. [73]).

ene)[66] to form an amide bond (Scheme 1a). In a second coupling reaction, disulfide bonds are formed (Scheme 1b).[67] For this procedure, the ODN must be equipped with a terminal sulfhydryl group while the PEG is functionalized at one end with a 2-pyridyl disulfide group. The third ligation procedure relies on the Micheal addition (Scheme 1c). Also in this case thiol-modified ODNs are utilized. The PEGs, however, are modified with either a terminal maleimide or acrylic acid group.[68] All of these coupling schemes are very well suited for the generation of DBCs containing water-soluble organic polymer units. Moreover, since thiol- and amino-modified ODNs are available from commercial sources, the block copolymer architectures can be prepared in a conventional chemical laboratory without expensive DNA-synthesizer equipment.

The preparation of amphiphilic DBC structures containing hydrophobic polymers by coupling in solution is rather difficult because of the different solubility properties of the individual components. For this reason, strategies have been developed for grafting polymers onto DNA on a solid support (Scheme 2). When the ODN is present on the resin, the nucleotides are still protected, in particular at the phosphate groups, such that the ODN is soluble in organic solvents in which the organic polymer moiety can be dissolved as well. Again, as for solution-phase coupling, different reactions have been established for attaching the organic polymer to the ODN. The most straightforward approach is the transformation of the hydrophobic polymer into a phosphoramidite polymer by the reaction of a terminal hydroxy group with phosphoramidite chloride (Scheme 2a). The activated macromolecules are then attached to the detritylated 5′-OH group of the ODN. After incubation with concentrated ammonia for deprotection and release from the solid support, single-stranded (ss) DNA diblock copolymers could be obtained, for example, DNA-*b*-polystyrene (PS)[69] and DNA-*b*-poly(propylene oxide) (PPO).[70] When both termini of the organic polymer were activated as phosphoramidites, triblock architectures of the type DNA-*b*-PEG-*b*-DNA were also accessible.[71] Alternatively, amide bond formation can be utilized to graft poly(butadiene) (PB), which has a terminal amino group, onto immobilized carboxyl-modified DNA (Scheme 2b).[72] For the attachment of poly(*p*-phenyleneethynylene) (PPE), a terminal ethynyl group was coupled with an iodide unit present in the base of the last nucleotide at the 5′-end in a palladium-catalyzed Sonogashira–Hagihara coupling (Scheme 2c).[73]

In all the synthetic routes to DBCs, the nucleic acid segment usually does not exceed a degree of polymerization of 50 nucleotides. How can this synthetic limitation be overcome? The answer is by combining chemical DBC synthesis with methods from molecular biology. To this end, the single-stranded ss DBCs described above were employed as primers for the polymerase chain reaction (PCR) (Figure 2).[74] The utilization of one ss DBC and a conventional ODN primer yielded double-stranded (ds) DNA diblock copolymers with extended nucleic acid blocks of the type A-*b*-dsDNA (Figure 2, strategy 1), where A denotes an organic polymer. Employing two ss DBCs with the same organic block as the forward and backward primers resulted in double-stranded triblock architectures of type A-*b*-ds DNA-*b*-A (Figure 2, strategy 2). It is important to note that the PCR process is not only compatible with hydrophilic polymers like PEG but also tolerates hydrophobic polymers like PPO and PS as well as thermoresponsive polymers like PNIPAM. In an extension of this method, two primer DBCs with different organic blocks led to triblock structures with a topology of A-*b*-ds DNA-*b*-B, where A and B denote different polymers. More complex architectures were realized by employing ss DNA-*b*-A-*b*-ss DNA and ssDNA-*b*-X (A or B polymer) primers in combination within the PCR; this

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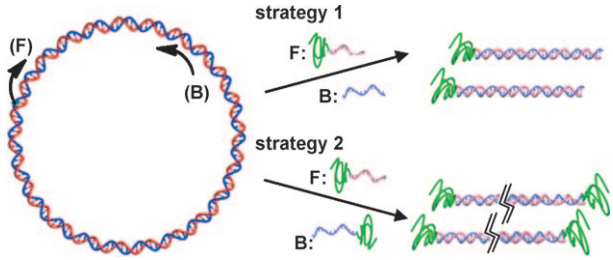

Figure 2. Schematic representation of molecular biological strategies like PCR for the preparation of di- and triblock copolymers with larger DNA units. Strategy 1: DNA block copolymer (forward primer, F) and DNA (backward primer, B) yield PEG-*b*-ds DNA. Strategy 2: Two PEG-*b*-DNA primers produce triblock copolymer PEG-*b*-ds DNA-*b*-PEG (adapted from Ref. [74]).

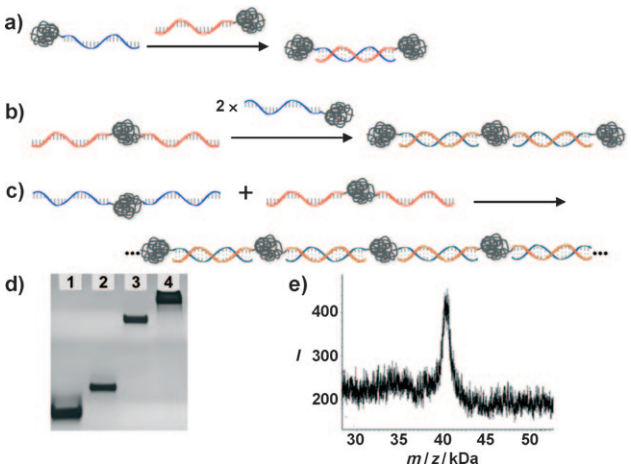

Figure 3. Generation of multiblock structures by DNA hybridization. a) Hybridization of two complementary DNA block copolymers yields a triblock architecture. b) A pentablock architecture composed of triblock and diblock DBCs. c) One-dimensional polymeric assemblies consisting of two complementary triblock copolymers. d) Polyacrylamide gel of triblock and pentablock copolymers; lane 1: ss DNA-*b*-PEG-*b*-ss DNA, lane 2: ds DNA-*b*-PEG-*b*-ds DNA, lane 3: PEG(5 kDa)-*b*-ds DNA-*b*-PEG-*b*-ds DNA-*b*-PEG(5 kDa), lane 4: PEG(20 kDa)-*b*-ds DNA-*b*-PEG-*b*-ds DNA-*b*-PEG(20 kDa). e) MALDI-TOF mass spectrum of the pentablock copolymer with 5 kDa PEGs shown in lane 3 in (d) (adapted from Ref. [71]).

resulted in pentablock structures, X-*b*-ds DNA-*b*-A-*b*-ds DNA-*b*-X.[75] The molecular weights of the nucleic acid segments easily exceed 1 000 000 g mol^{-1} and as a result of the mechanism of the templated polymerization, the products are monodisperse. In this light, the usage of ss DBCs within the PCR process pushes the limits in the synthesis of block copolymers in terms of molecular weight as well as structural accuracy.

2.2. Supramolecular Strucures

In the era of nanoscience, the assembly of structures on the nanoscale and the presence of several levels of organization are important criteria for synthesizing compounds with functional and material properties. A few years ago Seeman and Rothemund reported wholly unprecedented designed self-assemblies of DNA.[76,77] Their groundbreaking work gave birth to an entire field of nucleic acid nanoarchitectures.[78,79] Nanostructures constructed of DBCs offer several unique opportunities in this respect. The aggregation behavior of the bioorganic hybrid structures can be controlled either by the recognition of the nucleic acid moiety or by the interactions of the organic polymer units and combinations thereof.

Supramolecular polymers have gained increasing interest in recent years because of the dynamic properties of these materials resulting from the reversible bonds holding the polymeric chain together.[80] DBCs are an excellent example of this type of material. Well-defined block-type structures can be assembled from ss DBC building blocks (Figure 3). One example is the generation of triblock structures from two ss DBCs with complementary sequences by hybridization and thus duplex formation (Figure 3a).[71] Extension of this concept resulted in pentablock architectures through the assembly of a single-stranded triblock structure of the type DNA-*b*-PEG-*b*-DNA with two equivalents of single-stranded diblock structures of the type DNA-*b*-PEG (Figure 3b). These structures were characterized by PAGE (Figure 3d) and MALDI-TOF mass spectrometry (Figure 3e). Even larger one-dimensional polymer assemblies were achieved when two single-stranded self-complementary triblock structures were employed with a DNA-spacer-DNA topology (Figure 3c).[81] Polymer formation was evident by the increased viscosity of the reaction mixture compared to solutions of nonpolymerizing analogues. Molecular weights can be easily controlled by varying the ratios of DNA hybrid monomers because excess monomer acted as effective "chain terminators".

Not only the nucleic acid units but also the organic polymer segments are well suited to inducing superstructure formation. This is especially true for hydrophobic polymer segments, as they tend to undergo microphase separation in aqueous medium. Amphiphilic DBCs form micellar aggregates exhibiting a hydrophobic polymer core and a corona of ss DNA. In the case of ss DNA-*b*-PS, these micelles were formed with the help of a cosolvent, and the average diameters of the aggregates could be adjusted by varying the lengths of the nucleic acid units.[69] The fabrication of micelles from DNA-*b*-PPO was even simpler because the polymer exhibits a low glass transition temperature ($T_G = -70\,^{\circ}C$), such that micelles form after a simple heating and cooling procedure. It should be pointed out that the formation of such DNA/polymer hybrid nanoparticles is experimentally much less demanding than the generation of complex nanostructures exclusively based on nucleic acids.[82,83]

The structural properties of the spherical DBC aggregates can be altered by hybridization. While hybridization of DBC micelles with short ODNs complementary to the corona did not change their spherical shape (Figure 4a), Watson–Crick base-pairing with long templates induced the formation of rodlike aggregates (Figure 4b).[84] The sequence composition of the templates was selected to encode the complement to that of the DNA micelle corona multiple times. During hybridization, a double helix formed from which a PPO

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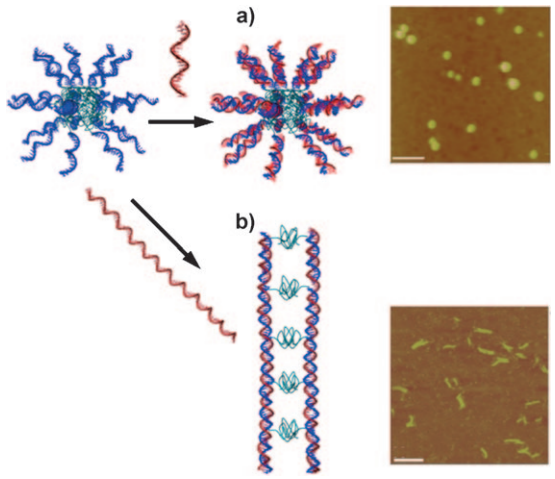

Figure 4. Representation and AFM images of PPO-*b*-DNA aggregates. a) Hybridization of the spherical ss DNA micelle (blue) with the complementary DNA (red) generates ds DNA-*b*-PPO nanostructures. b) Hybridization of the spherical ss DNA micelle with a long template results in rodlike aggregates. Scale bars in images: 200 nm (adapted from Ref. [84]).

moiety projected every 22 nucleotides. This led to the parallel arrangement of two double helices "glued" together by the hydrophobic interactions of the organic polymer units. Even the lengths of the rodlike aggregates could be controlled by the length of the template molecules.

In addition to small micellar structures with diameters of up to 45 nm,[85] DBCs have been used to form larger objects. When 12-mer ODNs were attached to PB, vesicles with an average diameter of 80 nm were found along with higher ordered vesicles exhibiting a closed DBC double-layer membrane.[72] These structures were employed to prepare ODN-modified surfaces on which bacterial biofilm formation was studied.[86]

2.3. Applications and Functions

2.3.1. Nanoscience

Linear DBCs are not only suited for the construction of supramolecular and nanoscopic structures alone; owing to the self-recognition properties and biological role of DNA, the resulting aggregates can also be easily equipped with extra functionality. One example, which makes use of the fact that DBCs are macromolecules with coded information, is DNA-templated chemistry[87] in DBC micelles (Figure 5). For this purpose, ss DBC aggregates were hybridized with reactant DNA bearing functional groups. Owing to the close proximity of these groups within the micellar confinement, several organic reactions like the Michael addition and amide bond formation could be carried out sequence-specifically within the programmable nanoreactors. It is even possible to spatially define the transformations to take place either at the surface of the micelles (Figure 5a) or within the interior of the bioorganic particles shielded from the environment (Figure 5b). The location of the reaction can be controlled by attaching the reactants to either the 5′- or the 3′-ends.

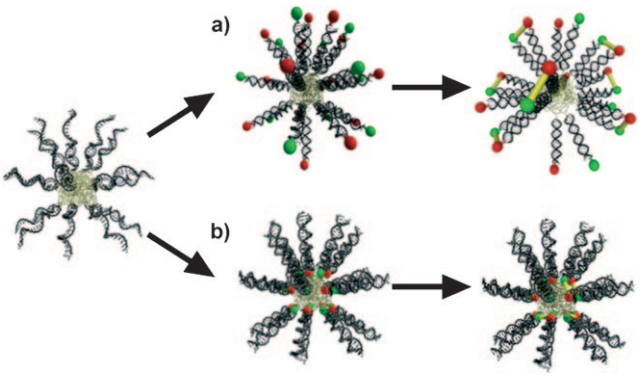

Figure 5. DNA-templated synthesis a) on the surface of a PPO-*b*-DNA micelle and b) in the core of the micelle. Red and green spheres correspond to reacting functional groups, and yellow sticks represent the resulting covalent bonds (adapted from Ref. [70]).

DBC micelles can also be employed as scaffolds for enzymatic conversions. In contrast to the thermostable polymerases described in Section 2.1 for the preparation of ds DBCs, the polymerase terminal deoxynucleotidyl transferase (TDTase) does not need a template for adding any of the four nucleoside triphosphates (dNTPs). DBC micelles were incubated at 37 °C with this enzyme and dTTP. With increasing reaction time, the size of the DNA nanoparticles increased through the addition of polyT segments to the DBC. The diameter of the nanoscale objects could be gradually adjusted from 10 to 23 nm over a reaction time of 16 h. This was the first demonstration that the size of nanoparticles could be increased with the help of an enzyme.[85]

In another example DBCs were employed to generate organic/inorganic particle networks. DNA-*b*-PS micelles were hybridized with DNA-modified gold nanoparticles exhibiting the complementary sequence on the surface.[69] The resulting composites proved that DBCs can be used to generate higher ordered structures through sequence-specific hybridization with other DNA-modified nanomaterials. Moreover, sharp melting temperatures were detected for their disassembly, which was explained by a cooperative melting model.

The Watson–Crick base-pairing of DBCs is not limited to the generation of nanosized systems; the double-helix formation has also been utilized for the preparation of microcapsules. Alternating layers of T_{30}-*b*-PNIPAM and A_{30}-*b*-PNIPAM were assembled on silicon templates. After dissolution of the core and functionalization of the surface with PEG, stable microcapules were obtained that might be exploited for the delivery of siRNA and anticancer drugs.[88]

2.3.2. Diagnostics

When linear DNA block copolymers were utilized for DNA detection, the nucleic acid units were coupled to light-emitting oligomers or polymers. One kind of probe architecture consists of a central fluorene emitter unit onto which two identical ODNs are attached to form a so-called twin probe (Figure 6a). This conjugate was applied in homogeneous DNA detection assays. By a mechanism that relies on the

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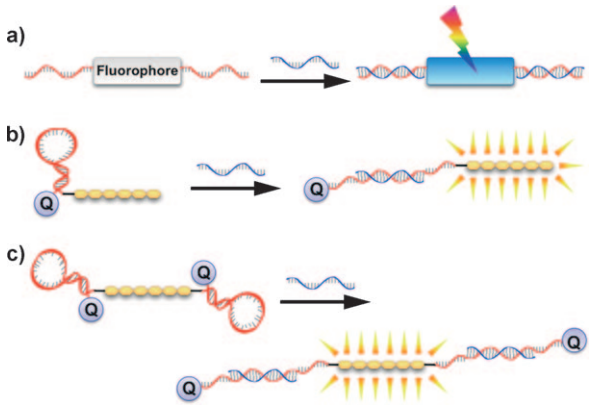

Figure 6. Representation of DBC-based DNA detection. a) Twin-probe reporter with a fluorescence emitter in the middle. b) Molecular beacon with a light-emitting polymer. c) Beacon with a stem–loop DNA structure at either end of the polymer. Q = quencher moiety.

dequenching of the fluorescence upon hybridization, complementary target sequences as well as single-nucleotide polymorphisms (SNPs) could be detected. The selectivity of the twin-probe system is remarkable when one considers that it contains only a single emitting species and does not require dual labeling. DNA detection was realized with concentrations ranging from 10^{-3} to 10^{-8} M.

Other DBC probe architectures, however, rely on labeling with several light-emitting entities. An excellent example is the conjugated polymer molecular beacon. This consists of a ss DNA unit functionalized at one end with a negatively charged, highly emissive PPE, while the other end is attached to a quencher moiety (Figure 6b).[73] The DNA sequence was selected to form a stem–loop structure. In this configuration, the conjugated polymer is in close proximity to the quencher, leading to complete suppression of the emission of the conjugated polymer by an effect referred to as superquenching.[89] In the presence of a complementary target sequence, hybridization results in a separation of the polymer and quencher, and thus to light emission from the conjugated emitter. Unfortunately, this promising probe has not been evaluated with respect to its sensitivity and selectivity. A combination of the twin probe and the conjugated polymer molecular beacon architectures was realized by attaching two stem–loop structures with terminal quenchers to the two ends of a negatively charged PPE polymer (Figure 6c). At target concentrations of 4×10^{-6} M, the fluorescence signal from the conjugated polymer was two orders of magnitude higher than the signal achieved with a noncomplementary sequence. Even SNP detection was possible with such a probe.[66]

2.3.3. *Biomedicine*

In addition to diagnostics, linear DBCs have been successfully applied in the field of biomedicine. Oligonucleotides (mainly aptamers, antisense ODNs, and small interfering RNA (siRNA)) are now under active investigation as new potential drugs because of their extremely high selectivity in target recognition. All of them, however, display the shortcomings of short half-life in vivo because of either low stability towards nucleases, which are present in plasma and inside the cells, or rapid excretion on account of their relatively low molecular weights. Furthermore, their negative charge prevents easy internalization into cells. One way to tackle these problems is to functionalize pharmaceutically active nucleic acids with PEG.[93] Direct coupling of PEG[90] or coupling through a linker molecule[91] has resulted in increased stability of oligonucleotides towards enzymatic degradation, prolonged plasma permanence, and enhanced penetration into cells by masking the negative charges of ODNs. One DBC drug already commercialized in 2004 is a PEGylated 28-mer aptamer, PEG-aptanib, which was approved by the FDA for the treatment of age-related macular degeneration of the retina. This DBC drug consists of a branched PEG (40 kDa) attached to the ODN through a pentylamino linker.[92]

For the successful delivery of antisense DNA, ODNs were likewise conjugated with PEG. However, for successful delivery they were complexed with positively charged polyelectrolytes. As a result, so-called polyion complex micelles[67,68] and the synonymous polyelectrolyte complex micelles[61,94] were obtained with a core–shell structure. The nucleic acid moiety and the positively charged polyelectrolyte formed an inner core through ionic interactions, while the PEG chains constituted the shell. For effective delivery in HuH-7 cells, the DBCs were designed to contain a disulfide linkage between the ODN and the PEG. This linker resulted in better antisense effects than the utilization of an acid-cleavable connecting element. Another important parameter is the type of polyelectrolyte. Poly(L-lysine)-containing micelles were shown to be less efficient than poly(ethyleneimine) (PEI), which indicated that the DNA-*b*-PEG was successfully transported from the endosomal compartment into the cytoplasm thanks to the buffering effect of PEI. Upon cleavage of the disulfide linkage hundreds of active antisense ODNs were released into the cell interior in response to high glutathione levels in the cytoplasmatic compartment.[67] In a similar set of experiments, the internalization of the polyion complex micelles into HuH-7 cells was promoted by the attachment of a lactose targeting unit at the other terminus of the PEG block, that is, opposite to the ODN; this modification led to increased antisense activity.[68]

Besides antisense delivery in vitro, polyelectrolyte complex micelles were successfully employed to suppress oncogenes in test animals. A c-raf antisense ODN formulation, which was intravenously administered to nude mice bearing human lung cancer tumors, exhibited significant antitumor activity. Moreover, the DBC-containing micelles showed higher accumulation levels than the non-polymer-modified ODN in the solid tumor region.[61] In previous experiments the same group demonstrated a significant reduction in tumor growth when c-raf antisense ODNs were employed in polyelectrolyte complex micelles injected directly into the tumor region.[94]

More recently the same electrostatically induced DBC aggregates were utilized for the therapeutic delivery of siRNA.[62,95] In the context of anti-angiogenic gene therapy, PEG-conjugated vascular endothelial growth factor (VEGF) siRNA complexed with PEI was shown to inhibit VEGF

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expression in the tumor tissue and suppress tumor growth in an animal model without any detectable inflammatory response.[95] The micelles were administered to mice intravenously and intratumorally. After the intranvenous injection, enhanced accumulation of the nanosized DBC particles was detected in the tumor region. This study demonstrated the feasibility of using DBC micelles as a potential carrier for therapeutic siRNA in local and systemic treatment of cancer.

As with DBC nanoparticles assembled by means of electrostatic interactions, amphiphilic DBCs that form nanoparticles by microphase separation have also been employed for drug-delivery purposes. In this case the DNA did not serve any biological function but instead facilitated the extremely convenient generation of multifunctional particles. These nanoscale objects were equipped with targeting units that recognize cell surface receptors of Caco-2 cells, a cancer cell line (Figure 7). Specifically, folic acid was coupled to a DNA sequence complementary to that of the micelle corona, such that these functionalities could be introduced by a simple hybridization procedure (Figure 7a). In a second step a

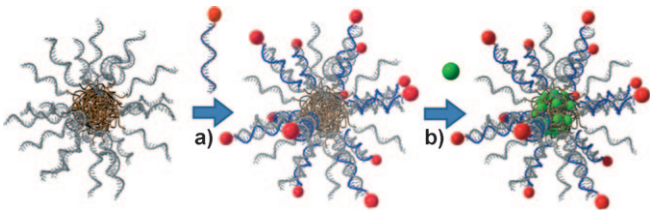

Figure 7. Diagram of the drug-delivery system based on DNA block copolymer micelles. a) The micelle made up of single-stranded DNA is hybridized with the folic acid conjugated to complementary DNA (red sphere, blue strand) to equip the nanoparticle surface with targeting units. b) The hydrophobic anticancer drug, doxorubicin (green sphere), is loaded into the PPO core of the micelle (reprinted with permission from Ref. [96].)

hydrophobic anticancer drug, doxorubicin, was loaded in the hydrophobic core of the DBC micelles (Figure 7b). With both units acting in concert, the cancer cells were efficiently killed.[96] Prior to the drug-delivery experiment it was demonstrated that an increasing number of targeting units on the micelle surface facilitate internalization of the DBC nanoparticles by receptor-mediated endocytosis. In contrast, when the folic acid moieties were directed into the interior of the nanosized objects, they were not recognized by the cells. The internalization process was monitored by confocal laser scanning fluorescence microcopy with DBC particles that were fluorescently labeled by the same convenient hybridization procedure as that for introduction of the targeting units. Since it is easy to functionalize DBC micelles, these materials qualify to be used as a combinatorial platform for the testing and high-throughput screening of drug-delivery vehicles.

The nondirected internalization of DBC systems, that is, without external targeting units, was studied as well. In particular, the question of whether the shape of the nanoscale objects influences uptake was addressed. For that purpose, Caco-2 cells were incubated with ss and ds spherical DBC particles as well as with the rodlike particles described in Section 2.2 (Figure 4). Although all the particles were composed of the same DBC materials, the rodlike structures were internalized one order of magnitude more efficiently than their spherical counterparts.[97] Such experiments are important because they suggest that the shape of a nanoparticle might be an additional important design criterion for drug-delivery vehicles.

3. DNA Side-Chain Polymers

As mentioned in the introduction, two different classes of DNA side-chain polymers are known. The structures that will be discussed first consist of a ds DNA backbone onto which organic polymers are grafted. The first structures with such an architecture consisted of bacteriophage T4 (166 kbp) or λ phage DNA (48.5 kbp) onto which polyacrylamide or poly(*N*-isopropylacrylamide) (PNIPAM) were attached.[98,99] The DNA and the polymer were connected by an intercalator that consisted of a phenazinium derivative functionalized with acrylic acid. The graft architecture was then obtained by polymerization of acrylamide or *N*-isopropylacrylamide and the polymerizable intercalator as co-monomer in the presence of DNA. However, this strategy does not lead to the covalent attachment of the biological and the organic segments. For this purpose, psoralen was investigated; this intercalator is known to undergo cycloaddition with nucleobases. When a psoralen-containing vinyl monomer was employed under experimental conditions similar to those described above, covalent coupling of the acrylic acid polymers and the DNA was realized upon irradiation with UV light.[100,101] Subsequently this synthetic strategy was slightly refined. Instead of the incorporation of numerous intercalating moieties along the main chain of the synthetic polymer unit, semitelechelic PNIPAMs terminated with a psoralen group were generated. This made it possible to prefabricate the organic polymer building block, and it was subsequently grafted onto the ds DNA by irradiation with UV light.[102]

Like the linear DNA-*b*-PNIPAM polymers,[101] these graft architectures were employed for thermoprecipitation (Figure 8). In this application the DNA backbone acted as the recognition site while the PNIPAM side chains induced thermoresponsive properties. One conjugate was utilized for the capture of ethidium: about 95% of this DNA-binding genotoxin could be removed from a highly dilute solution (3 ppm). Besides the thermally induced affinity separation of small molecules, such graft architectures were used to isolate DNA-binding proteins. Hind III, an endonuclease, was successfully coprecipitated as a DNA-PNIPAM conjugate by the addition of glycerin, which decreases the lower critical solution temperature (LCST: the critical temperature below which a mixture is miscible in all proportions).[99] It should be noted that this experiment was carried out in Mg^{2+}-free buffer. In the absence of this cofactor, Hind III binds DNA strongly but does not show any nuclease activity. A DNA-PNIPAM conjugate that worked even more efficiently was based on a DNA-block-brush architecture (Figure 8).[41] It

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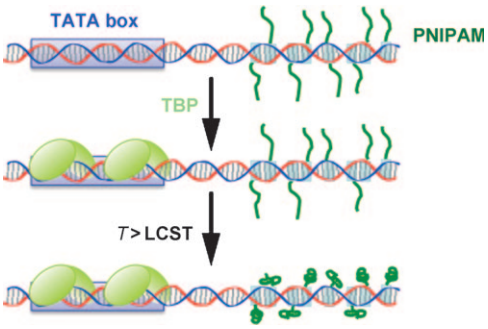

Figure 8. Diagram of affinity precipitation and separation of the DNA binding protein (TBP) using a thermoresponsive polymer. After the binding of TBP on the TATA box, the solution was heated to precipitate the assembled aggregate that formed as a result of the thermally induced phase transition of PNIPAM side chains (adapted from Ref. [41]).

consisted of one nucleic acid segment with PNIPAM attached as side chains and another segment of pristine ds DNA encoding the so-called TATA box, which is involved in the initiation of transcription in eukaryotes. When a mixture of TATA box binding protein (TBP) and bovine serum albumin (BSA) that does not show any affinity to DNA was incubated with such a block conjugate, rapid and selective precipitation of bound TBP was achieved through temperature modulation. The TBP purified under homogenous conditions exhibited a purity of more than 90% as estimated from SDS-PAGE analysis.

The other type of DNA side-chain polymer consists of a synthetic polymer backbone onto which ODNs are grafted (Figure 9). These bioorganic hybrid structures are accessible by two general synthetic strategies. The first relies on the functionalization of a prefabricated synthetic polymer backbone with ODNs (Figure 9a), while the second is based on attaching ODNs to a polymerizable unit, which is subsequently incorporated into the polymer main chain by copolymerization (Figure 9b). With regard to the first approach, a multitude of synthetic procedures have been elaborated to attach the ODN to the polymer backbone. A copolymer of polyacrylic acid (PAA) could be modified with

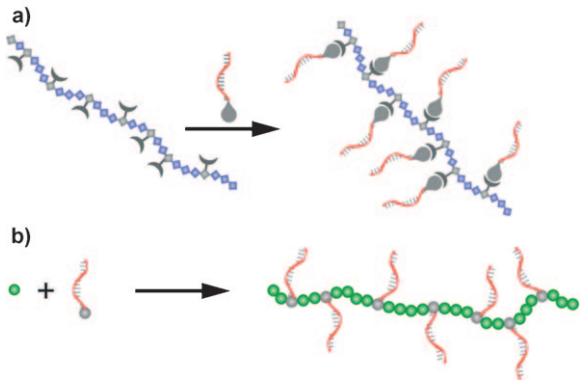

Figure 9. Preparation of side-chain polymers with grafted DNA. a) DNAs are attached to the functional groups along the polymer backbone. b) Copolymerization of a monomer and a nucleic acid monomer.

amino-terminated ODNs as side chains by amide bond formation employing 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as the coupling reagent.[103] In a similar approach amino-functionalized ODNs were reacted with a poly(*N*,*N*-dimethylacrylamide) copolymer that contained activated ester groups to establish stable amide bridges between the polymer backbone and the nucleic acid units.[104] RNA units could be coupled to a poly(L-lysine) backbone through the formation of *N*-morpholine rings. This procedure included periodic acid oxidation and cyanoborohydride reduction of the 3′-end ribose unit.[105] In addition to approaches that rely on coupling the two segments in solution, synthetic routes have been elaborated that rely on grafting onto the solid support. For instance, after their solid-phase synthesis the ODNs were left on the solid support. The detritylated 5′-OH groups of the ODNs were reacted with an organic polymer carrying multiple phosphoramidite groups along its backbone.[106,107] This approach was also used to prepare block-brush copolymers since the main chain was built up by sequential ring-opening metathesis polymerization (ROMP) of differently functionalized norbornene monomers. Another possibility to generate DNA side-chain polymers on the solid phase relied on immobilizing the synthetic polymer backbone on glass beads.[108] The hydroxy groups present on the controlled pore glass surface were reacted with alternating copolymers containing maleic anhydride units so that a covalent ester bond between the support and the polymer formed. The remaining anhydride structures were utilized to bind a 5′-trityl-protected mononucleotide carrying an aminoalkyl spacer by means of bond formation that acted as an initiator of ODN synthesis. Because the ester and the amide connections have different stabilities, the DNA side-chain polymers could be selectively cleaved from the solid support after completion of the DNA synthesis by treatment with base. These two strategies lead to drastically different grafting densities: Performing the grafting reaction as the final step of conjugate synthesis resulted in an average of five ODNs per polymer backbone,[107] while DNA synthesis on the immobilized polymer initiators yielded up to 109 nucleic acid units per polymer backbone.[109] It should be mentioned that in these solid-phase approaches the ODNs have different attachment sites. While the first route leads to conjugates with the ODN connected to the polymer chain at the 5′-end, the latter procedure results in hybrids with the ODN attached at the 3′-end.

A conceptually different approach to generating DNA-polymer hybrids with ODNs as side chains consists of the copolymerization of ODN macromonomers. For this purpose, the ODN was functionalized with a polymerizable group either by coupling amino-terminated ODNs to methacrylic acid in solution[110] or by attaching an acrylamide-functionalized phosphoramidite to the ODN sequence on the solid phase.[111,112] A variety of vinyl ODN macromonomers underwent radical polymerization with acrylamide and *N*-isopropylacrylamid to yield the corresponding side-chain polymers.[112–115] Moreover, a variety of ODN-containing side-chain polymers were generated for electrochemical and optical sensors. However, these structures were poorly soluble and they could not be thoroughly characterized.[116–120]

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The arrangement of several ODN units along a single polymer chain has important consequences for base-pairing properties when two of these architectures with complementary sequences are hybridized. It has been found that the melting curves are sharper than those of structures obtained from the hybridization of complementary untethered ODNs in solution. Furthermore, the melting temperatures are shifted to higher values. This characteristic melting behavior was the subject of several physical studies.[121,122] Most important is that these properties, which originate from multimerization of ODNs along a polymer chain, could be exploited for highly selective DNA detection systems. This is especially true for the sharp melting curves that allow resolution enhancement for the detection of single base mismatches in other nanoparticle systems like DNA-modified gold particles.[123–125]

A similar system was realized with ss DNA side-chain polymers linked to a PNIPAM backbone.[126] When the dissolved materials were heated above T_{LCST}, they tended to form core–shell particles with the PNIPAM in the interior and the nucleic acid units at the surface. When the particles were hybridized with the complementary sequences, the colloidal stability of the particles decreased, leading to the formation of particle aggregates at defined salt concentrations (Figure 10a). This aggregation was monitored by measuring the absorbance at 500 nm. Since in this case the particles were not connected by means of bridging ODNs, the mechanism of aggregation was different from that of ODN-functionalized gold nanoparticles. One drawback of the DNA-PNIPAM side-chain polymer system was the high detection limit, in the μM range. Much more sensitive were the detection systems based on block graft architectures with a polynorbornene backbone.

When, in addition to ODNs, ferrocene units, were attached to the polymer main chain, the resulting conjugates could be employed in a three-component sandwich-type electrochemical detection strategy (Figure 10b).[107] After immobilization of a probe sequence, the electroactive DNA side-chain polymer and the target DNA exhibiting complementarity to both sequences were added and the redox signal was measured. The sensitivity of that signal was as low as 100 pM.

In a different approach, not one but two side-chain polymers were employed in a sandwich-based DNA detection assay (Figure 10c). One DNA side-chain polymer was employed as a capture ODN, and after the addition of the target DNA the second side-chain architecture was used as the detection polymer. The fluorescence intensity after an enzyme-catalyzed conversion of a fluorogenic coumarin derivative served as the final readout of the assay. It was demonstrated that the utilization of DNA side-chain architectures induced a 50-fold increase in sensitivity over that obtained with the nontethered pristine ODNs used as a reference. The detection limit of these assay configurations lies around 0.1 pM.[127,128]

For DNA detection purposes, DNA side-chain polymers with a conjugated backbone have also been employed.[117] This experimental setup was special in that the poly(oxadiazole–fluorene) derivatives, which served as the backbone of the DNA side-chain architectures, were immobilized on a glass surface. Light-directed synthesis of the probe DNA was performed directly on the chip, that is, on the conjugated polymer coating (Figure 10d). For the design of the conjugated polymer, special attention was paid to the stability of the material because of the harsh conditions required in DNA synthesis. For the DNA detection assay, a dye-labeled target was hybridized on the chip. The fluorescence energy transfer from the conjugated polymer to the dye was measured as a read-out. The detection limit of these conjugated polymer–DNA chips was 100 pM.

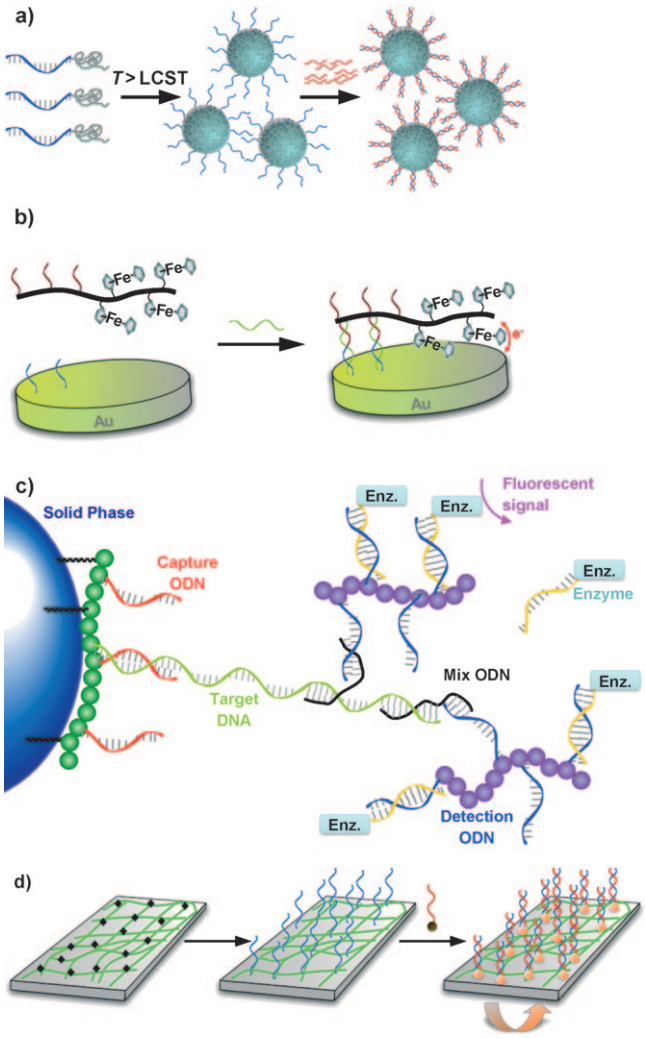

Figure 10. Examples of DNA detection methods based on DNA–polymer conjugates. a) Hybridization of ss DNA–PNIPAM aggregates reduces their colloidal stability. Detection of particle aggregation by optical absorption (adapted from Ref. [126]). b) Electrochemical detection of DNA employing a DNA–ferrocene–polymer hybrid (adapted from Ref. [107]). c) Sandwich method of enzyme-linked oligosorbent assay. The enzymatic reaction induces the fluorescent signal (adapted from Ref. [127]). d) DNA detection using a light-emitting polymer immobilized on a chip surface (adapted from Ref. [117]) Black diamonds: amino groups as attachment points for ODNs; orange spheres: acceptor dyes that act in a FRET process with the conjugated polymers as donors.

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DNA side-chain polymers have been employed extensively as antisense reagents as well. The advantage of coupling ODNs to a positively charged PLL backbone is that the hybrid can be more easily introduced into intact cells than pristine DNA. A 15-mer ODN-PLL side-chain architecture complementary to the initiation region of the mRNA of vesicular stomatitis virus (VSV) N-protein specifically inhibited expression of VSV proteins. Moreover, antiviral activity was exhibited when the DNA side-chain polymer was added in cell culture medium at doses as low as 100 nM.[58] Antiviral activity against human immunodeficiency virus type 1 (HIV-1) was evaluated through a similar approach.[129] Two regions of the viral RNA were selected as targets for antisense ODNs and incorporated into side-chain architectures. These experiments demonstrated that the first step of the viral infection, reverse transcription and the synthesis of proviral DNA, could be prevented in cell culture.

When instead of PLL, PNIPAM was employed as the synthetic polymer backbone of DNA side-chain polymer architectures, the antisense activity of these conjugates could be controlled by temperature. In initial experiments it was demonstrated that DNA-PNIPAM side-chain polymers bind complementary RNA sequence specifically at 27 °C, which is below their T_{LCST} (33 °C). In contrast, at 37 °C, which is still below the ODN melting temperature, binding to the complementary sequences was significantly reduced, probably because of the restricted spatial accessibility of the ODNs induced by the coil to globule transition.[115] This characteristic recognition behavior of the ODN side-chain polymer was utilized in an in vitro transcription translation system for the stimulus-responsive regulation of gene expression. At 27 °C expression levels were significantly reduced, similar to that when pristine antisense ODN was employed. Above T_{LCST} the presence of the antisense DNA side-chain polymer did not affect expression levels, whereas the control ODN induced a significant antisense effect.[113] Moreover, it was demonstrated that the antisense activity of the DNA-PNIPAM conjugate was regulated rapidly as a result of the conformational change of the PNIPAM backbone. The globule state of the DNA side-chain polymer increases stability against nucleases. At 37 °C complete resistance against nuclease S1 was observed.[114]

4. Cross-Linked Networks of DNA and Polymers

DNA side-chain polymers can be transformed into cross-linked structures by two different methods. When two of these conjugates with complementary sequences are hybridized, networks can be obtained.[104] The second strategy employs cross-linking DNA. To this end, a DNA side-chain polymer was mixed with an ODN that twice encoded the complementary sequence of the nucleic acid/polymer hybrid.[104] Why are such cross-linked polymer architectures or networks important? In recent years hydrogels have attracted considerable attention because they can react to changes in their environment. This stimulus responsiveness, which in the context of polymer networks is primarily associated with swelling and deswelling of the material, can be elicited by changes in temperature, pH, or photon flux. In the case of the DNA hydrogels mentioned above, thermoreversible materials were obtained. While at low temperatures highly swellable networks were produced, at elevated temperatures the networks disaggregated and dissolved.[104] In other hydrogel systems stimuli-induced phase transitions have been exploited, for example, for controlled drug release from nanosized[130] and macroscopic networks.[131] A more specific trigger to induce changes in the physicochemical properties of hydrogels is the recognition of biomolecules. Polymer networks containing a covalently incorporated antibody Fab fragment showed reversible volume changes upon incubation with the antigen fluorescein.[132] Another antibody-containing hydrogel, when applied as a membrane, exhibited pulsatile permeation of a macromolecular model drug in response to stepwise changes in the antigen concentration.[133] Similar to the specific recognition of antibodies and antigens, DNA hydrogels react to base-pairing of complementary sequences. Both shrinking and swelling of DNA hybrid networks were achieved by the addition of an ODN. Inspired by molecular beacons, Maeda and Murakami incorporated stem–loop structures as cross-links into a polyacrylamide matrix. It must be pointed out that the 3′- as well as the 5′-ends were connected to the synthetic polymer.[134] When these networks were hybridized with DNA sequences complementary to the loop structure, the hydrogels expanded. This volume increase can be explained by the concerted disintegration of the loop structure during the hybridization event and the associated longitudinal extension of the cross-links. In contrast, when the sequence incorporated into the hydrogel was designed so as to contain no secondary structure, addition of the complementary DNA sequence led to contraction of the hydrogel. This deswelling is due to the fact that ds DNA is generally shorter than ss DNA. It is important to note that the sequence responsiveness of these networks is sensitive even to single base mismatches. In a similar approach the same group demonstrated the shrinking of semi-interpenetrating networks of DNA–polymer hybrids.[135]

While these volume changes are irreversible, DNA hydrogels with convertible mechanical states induced by hybridization can be generated as well. The basis for this switching process relies on DNA sequences that have been developed in the context of DNA-fueled machines.[136,137] Translated to polymer networks, the DNA side-chain polymers were hybridized with a cross-link strand (Figure 11). The special feature of this cross-link sequence was that besides encoding the complement of the DNA–polymer hybrids, it contained an additional sequence. In the course of cross-linking by hybridization an overhang or so-called "toehold" was generated. When the full complement to the cross-linking strand including the toehold, that is, the removal strand, was added, the cross-links disintegrated.[112] This represents a rather significant step forward in the area of stimulus-responsive hydrogels since the reversal of cross-linking is induced simply by the addition of DNA without the need to change external parameters like temperature, pH, or salinity. Because of the attractiveness of such a mild stimulus for drug delivery, Simmel and co-workers demonstrated the controlled trapping and release of quantum dots in these DNA hydrogel systems by employing single-molecule fluorescence micros-

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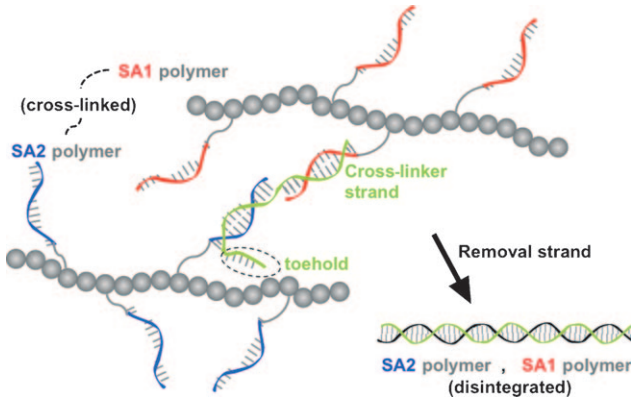

Figure 11. Schematic diagram of reversible DNA cross-linked polyacrylamide hydrogel. The overhang of the cross-linker DNA enables the reversible disintegration of the network by the hybridization of removal strand, which is complementary to the cross-linker strand in full length (adapted from Ref. [112]).

copy and fluorescence correlation spectroscopy.[138] A similar hybridization strategy was employed to reversibly change the mechanical properties of a DNA hybrid network without fully disintegrating the gel. In this case not two but three pristine DNA strands were needed. First, a cross-linking DNA of greater length (80 bases) than that used in the previous example was added to the DNA side-chain polymer (20-mer ODN grafts at both ends) to form the network. Then a so-called fuel strand (50 bases) was introduced that transformed the middle single-stranded region of the cross-linking strand into ds DNA and at the same time generated a 10-base toehold. As a result of hybridization, the cross-link stiffened and compressive forces were generated leading to tension in the polymer chains. When the removal strand was added, it fully hybridized with the fuel strand beginning at the single-stranded toehold and the gel reverted to its initial state without disintegration of the network architecture. In this manner, changes in stiffness greater than a factor of three were observed.[111]

But such hybrid networks are not the only DNA-containing hydrogels known; networks consisting exclusively of DNA have also been produced. This was done by chemical cross-linking of salmon testes DNA. As a cross-linking agent ethylene glycol diglycidyl ether was employed.[139] Characteristic of these gels was a discontinuous volume transition upon addition of acetone to the aqueous DNA hydrogel. At 63 % acetone composition 15-fold shrinking of the material was observed relative to its initial volume.[139] The dependence of the volume transition of such hydrogels on sodium and calcium ions was studied. The gel volume at high Na^+ and low Ca^{2+} concentrations decreased with increasing Ca^{2+} uptake.[140]

An alternative method for generateing cross-linked DNA networks is PCR. In a two-step process two Y-shaped trisoligonucleotides in combination with a double-stranded template were transformed into hydrogels with segment lengths between the branching points ranging from 70 to 1062 nucleotides.[141]

Yet another technique to prepare all-DNA hydrogels consists of exploiting enzyme-catalyzed assembly.[142] The building blocks of this type of hydrogel were branched DNA molecules consisting of three or four interpenetrating nucleic acid strands and containing complementary sticky ends. The X-, Y-, and T-shaped units were hybridized, and covalent phosphodiester bonds between them were realized with T4 DNA ligase. During the process of gel formation, which was carried out under physiological conditions, drugs and even live mammalian cells could be encapsulated. Applications for these hydrogels were proposed including controlled drug delivery, tissue engineering, 3D cell culture, and cell transplant therapy. In a continuation of this work, networks were produced with a whole gene bridging the DNA branching points. Functional proteins were produced within the DNA hydrogels with an in vitro transcription/translation system. Remarkably, the production efficiency was 300 times higher than that of current solution-based approaches.[143]

Because of their C_3-symmetrical structure and low number of constituents (three ODNs), Y-shaped building blocks have been further exploited to form responsive DNA-based hydrogels.[144] A modified three-armed DNA structure can quickly and reversibly form a hydrogel upon a simple change of pH, which induced interlocking terminal cyrosine-rich DNA stretches.

5. Conclusions

The combination of precise control over structure, molecular weight, and chemical function has long been a major goal in chemical synthesis and materials chemistry. Block copolymers, especially those containing biological macromolecules, represent an important step towards achieving this goal. Peptide/organic polymer hybrids have already been widely explored for these purposes, but the relatively recent development of DNA block copolymer materials holds even greater promise. DNA itself is among the most extensively studied biomacromolecules, and a broad range of biological and chemical techniques can be used to generate monodisperse DNA segments of virtually any length and sequence composition. As elaborated above, numerous methods exist for coupling this molecule to hydrophilic and hydrophobic polymers, both in solution and on a solid support. The unique self-recognition properties of DNA allow direct application, for instance in antisense RNA delivery and sensitive DNA detection, as well as specific and facile functionalization for templated reactions and particle recognition. Likewise, DNA hybridization can be harnessed to elaborate more complicated structures, from multiblock copolymers to multilayered vesicles and reversible hydrogels. Without question, the potential applications for this exciting class of materials are many, whether in drug delivery or the build-up of well-defined nanostructures. However, much more investigation is needed. Moreover, further synthetic strategies should be explored to expand the variety of available hybrids and architectures. In light of the significant progress that has been made already in the past decade, practical applications for these materials are likely already on the horizon.

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[1] T. E. Patten, K. Matyjaszewski, *Adv. Mater.* **1998**, *10*, 901.
[2] N. Hadjichristidis, S. Pispas, G. Floudas, *Block Copolymers: Synthetic Strategies, Physical Properties, and Applications*, Wiley, New York, **2002**.
[3] G. R. Newkome, C. N. Moorefield, F. Vögtle, *Dendrimers and Dendrons—Concepts, Syntheses, Applications*, Wiley-VCH, Weinheim, **2001**.
[4] U. M. Wiesler, T. Weil, K. Müllen, *Top. Curr. Chem.* **2001**, *212*, 1.
[5] A. Herrmann, T. Weil, V. Sinigersky, U. M. Wiesler, T. Vosch, J. Hofkens, F. C. De Schryver, K. Müllen, *Chem. Eur. J.* **2001**, *7*, 4844.
[6] T. Weil, U. M. Wiesler, A. Herrmann, R. Bauer, J. Hofkens, F. C. De Schryver, K. Müllen, *J. Am. Chem. Soc.* **2001**, *123*, 8101.
[7] T. Weil, E. Reuther, K. Müllen, *Angew. Chem.* **2002**, *114*, 1980; *Angew. Chem. Int. Ed.* **2002**, *41*, 1900.
[8] R. B. Merrifield, *J. Am. Chem. Soc.* **1963**, *85*, 2149.
[9] W. C. Chan, P. D. White, *Fmoc Solid Phase Peptide Synthesis—A Practical Approach*, Oxford University Press, Oxford, **2000**.
[10] P. E. Dawson, S. B. H. Kent, *Annu. Rev. Biochem.* **2000**, *69*, 923.
[11] M. Köhn, R. Breinbauer, *Angew. Chem.* **2004**, *116*, 3168; *Angew. Chem. Int. Ed.* **2004**, *43*, 3106.
[12] G. Casi, D. Hilvert, *Curr. Opin. Struct. Biol.* **2003**, *13*, 589.
[13] R. Serwa, I. Wilkening, G. Del Signore, M. Muhlberg, I. Claussnitzer, C. Weise, M. Gerrits, C. P. R. Hackenberger, *Angew. Chem.* **2009**, *121*, 8382; *Angew. Chem. Int. Ed.* **2009**, *48*, 8234.
[14] M. H. Caruthers, G. Beaton, J. V. Wu, W. Wiesler, *Methods Enzymol.* **1992**, *211*, 3.
[15] M. H. Caruthers, *Acc. Chem. Res.* **1991**, *24*, 278.
[16] M. H. Caruthers, A. D. Barone, S. L. Beaucage, D. R. Dodds, E. F. Fisher, L. J. McBride, M. Matteucci, Z. Stabinsky, J. Y. Tang, *Methods Enzymol.* **1987**, *154*, 287.
[17] O. J. Plante, E. R. Palmacci, P. H. Seeberger, in *Combinatorial Chemistry, Part B, Vol. 369*, **2003**, pp. 235.
[18] P. H. Seeberger, D. B. Werz, *Nat. Rev. Drug Discovery* **2005**, *4*, 751.
[19] P. H. Seeberger, D. B. Werz, *Nature* **2007**, *446*, 1046.
[20] N. M. Bell, J. Micklefield, *ChemBioChem* **2009**, *10*, 2691.
[21] K. E. Lundin, L. Good, R. Stromberg, A. Graslund, C. I. E. Smith, *Adv. Genet.* **2006**, *56*, 1.
[22] P. E. Nielsen, G. Haaima, *Chem. Soc. Rev.* **1997**, *26*, 73.
[23] E. Uhlmann, A. Peyman, G. Breipohl, D. W. Will, *Angew. Chem.* **1998**, *110*, 2954; *Angew. Chem. Int. Ed.* **1998**, *37*, 2796.
[24] J. S. Jepsen, M. D. Sorensen, J. Wengel, *Oligonucleotides* **2004**, *14*, 130.
[25] M. Petersen, J. Wengel, *Trends Biotechnol.* **2003**, *21*, 74.
[26] H. M. König, R. Abbel, D. Schollmeyer, A. F. M. Kilbinger, *Org. Lett.* **2006**, *8*, 1819.
[27] L. Hartmann, E. Krause, M. Antonietti, H. G. Börner, *Biomacromolecules* **2006**, *7*, 1239.
[28] J. Sambrook, D. W. Russel, *Molecular Cloning*, Cold Spring Harbour Laboratory Press, New York, **2001**.
[29] L. Wang, P. G. Schultz, *Angew. Chem.* **2005**, *117*, 34; *Angew. Chem. Int. Ed.* **2005**, *44*, 34.
[30] T. Hohsaka, M. Sisido, *Curr. Opin. Chem. Biol.* **2002**, *6*, 809.
[31] R. E. Connor, D. A. Tirrell, *Polym. Rev.* **2007**, *47*, 9.
[32] S. Jäger, M. Famulok, *Angew. Chem.* **2004**, *116*, 3399; *Angew. Chem. Int. Ed.* **2004**, *43*, 3337.
[33] H. O. Smith, C. A. Hutchison, C. Pfannkoch, J. C. Venter, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 15440.
[34] S. I. van Kasteren, H. B. Kramer, H. H. Jensen, S. J. Campbell, J. Kirkpatrick, N. J. Oldham, D. C. Anthony, B. G. Davis, *Nature* **2007**, *446*, 1105.
[35] B. G. Davis, *Chem. Rev.* **2002**, *102*, 579.
[36] O. Seitz in *Organic Synthesis Highlights V* (Eds.: H. G. Schmalz, T. Wirth) Wiley-VCH, Weinheim, **2003**, p. 230.
[37] N. Venkatesan, B. H. Kim, *Chem. Rev.* **2006**, *106*, 3712.
[38] T. S. Zatsepin, J. J. Turner, T. S. Oretskaya, M. J. Gait, *Curr. Pharm. Des.* **2005**, *11*, 3639.
[39] C. H. Tung, S. Stein, *Bioconjugate Chem.* **2000**, *11*, 605.
[40] T. J. Kelly, M. S. Wold, J. Li, *Adv. Virus Res.* **1988**, *34*, 1.
[41] N. Soh, D. Umeno, Z. L. Tang, M. Murata, M. Maeda, *Anal. Sci.* **2002**, *18*, 1295.
[42] B. Perly, A. Douy, B. Gallot, *C. R. Seances Acad. Sci. Ser. C* **1974**, *279*, 1109.
[43] Y. Yamashita, Y. Iwaya, K. Ito, *Makromol. Chem.* **1975**, *176*, 1207.
[44] T. J. Deming, *Adv. Drug Delivery Rev.* **2002**, *54*, 1145.
[45] H. A. Klok, *Angew. Chem.* **2002**, *114*, 1579; *Angew. Chem. Int. Ed.* **2002**, *41*, 1509.
[46] D. Löwik, L. Ayres, J. M. Smeenk, J. C. M. Van Hest, *Adv. Polym. Sci.* **2006**, *202*, 19.
[47] T. J. Deming, *Adv. Polym. Sci.* **2006**, *202*, 1.
[48] J. C. M. Van Hest, *Polym. Rev.* **2007**, *47*, 63.
[49] H. G. Börner, *Macromol. Chem. Phys.* **2007**, *208*, 124.
[50] H. G. Börner, H. Schlaad, *Soft Matter* **2007**, *3*, 394.
[51] J. F. Lutz, H. G. Börner, *Prog. Polym. Sci.* **2008**, *33*, 1.
[52] M. A. Gauthier, H. A. Klok, *Chem. Commun.* **2008**, 2591.
[53] H. Schlaad, *Adv. Polym. Sci.* **2006**, *202*, 53.
[54] H. A. Klok, S. Lecommandoux, *Adv. Polym. Sci.* **2006**, *202*, 75.
[55] H. G. Börner, *Prog. Polym. Sci.* **2009**, *34*, 811.
[56] C. M. Niemeyer, *Angew. Chem.* **2010**, *122*, 1220; *Angew. Chem. Int. Ed.* **2010**, *49*, 1200.
[57] F. E. Alemdaroglu, A. Herrmann, *Org. Biomol. Chem.* **2007**, *5*, 1311.
[58] M. Lemaitre, B. Bayard, B. Lebleu, *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 648.
[59] J. P. Leonetti, G. Degols, P. Milhaud, C. Gagnor, M. Lemaitre, B. Lebleu, *Nucleosides Nucleotides* **1989**, *8*, 825.
[60] J. P. Leonetti, B. Rayner, M. Lemaitre, C. Gagnor, P. G. Milhaud, J. L. Imbach, B. Lebleu, *Gene* **1988**, *72*, 323.
[61] J. H. Jeong, S. H. Kim, S. W. Kim, T. G. Park, *Bioconjugate Chem.* **2005**, *16*, 1034.
[62] M. Oishi, Y. Nagasaki, K. Itaka, N. Nishiyama, K. Kataoka, *J. Am. Chem. Soc.* **2005**, *127*, 1624.
[63] Y. G. Takei, T. Aoki, K. Sanui, N. Ogata, T. Okano, Y. Sakurai, *Bioconjugate Chem.* **1993**, *4*, 42.
[64] R. B. Fong, Z. L. Ding, C. J. Long, A. S. Hoffman, P. S. Stayton, *Bioconjugate Chem.* **1999**, *10*, 720.
[65] J. H. Jeong, T. G. Park, *Bioconjugate Chem.* **2001**, *12*, 917.
[66] K. Lee, L. K. Povlich, J. Kim, *Adv. Funct. Mater.* **2007**, *17*, 2580.
[67] M. Oishi, T. Hayama, Y. Akiyama, S. Takae, A. Harada, Y. Yarnasaki, F. Nagatsugi, S. Sasaki, Y. Nagasaki, K. Kataoka, *Biomacromolecules* **2005**, *6*, 2449.
[68] M. Oishi, F. Nagatsugi, S. Sasaki, Y. Nagasaki, K. Kataoka, *ChemBioChem* **2005**, *6*, 718.
[69] Z. Li, Y. Zhang, P. Fullhart, C. A. Mirkin, *Nano Lett.* **2004**, *4*, 1055.
[70] F. E. Alemdaroglu, K. Ding, R. Berger, A. Herrmann, *Angew. Chem.* **2006**, *118*, 4313; *Angew. Chem. Int. Ed.* **2006**, *45*, 4206.
[71] F. E. Alemdaroglu, M. Safak, J. Wang, R. Berger, A. Herrmann, *Chem. Commun.* **2007**, 1358.

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[72] F. Teixeira, Jr., P. Rigler, C. Vebert-Nardin, *Chem. Commun.* **2007**, 1130.
[73] C. Y. J. Yang, M. Pinto, K. Schanze, W. H. Tan, *Angew. Chem.* **2005**, *117*, 2628; *Angew. Chem. Int. Ed.* **2005**, *44*, 2572.
[74] M. Safak, F. E. Alemdaroglu, Y. Li, E. Ergen, A. Herrmann, *Adv. Mater.* **2007**, *19*, 1499.
[75] F. E. Alemdaroglu, W. Zhuang, L. Zophel, J. Wang, R. Berger, J. P. Rabe, A. Herrmann, *Nano Lett.* **2009**, *9*, 3658.
[76] N. C. Seeman, *Nature* **2003**, *421*, 427.
[77] P. W. K. Rothemund, *Nature* **2006**, *440*, 297.
[78] U. Feldkamp, C. M. Niemeyer, *Angew. Chem.* **2006**, *118*, 1888; *Angew. Chem. Int. Ed.* **2006**, *45*, 1856.
[79] C. Lin, Y. Liu, H. Yan, *Biochemistry* **2009**, *48*, 1663.
[80] T. F. A. de Greef, E. W. Meijer, *Nature* **2008**, *453*, 171.
[81] E. A. Fogleman, W. C. Yount, J. Xu, S. L. Craig, *Angew. Chem.* **2002**, *114*, 4198; *Angew. Chem. Int. Ed.* **2002**, *41*, 4026.
[82] Z. X. Deng, S. H. Lee, C. D. Mao, *J. Nanosci. Nanotechnol.* **2005**, *5*, 1954.
[83] N. C. Seeman, *Mol. Biotechnol.* **2007**, *37*, 246.
[84] K. Ding, F. E. Alemdaroglu, M. Börsch, R. Berger, A. Herrmann, *Angew. Chem.* **2007**, *119*, 1191; *Angew. Chem. Int. Ed.* **2007**, *46*, 1172.
[85] F. E. Alemdaroglu, J. Wang, M. Börsch, R. Berger, A. Herrmann, *Angew. Chem.* **2008**, *120*, 988; *Angew. Chem. Int. Ed.* **2008**, *47*, 974.
[86] N. Cottenye, F. Teixeira, A. Ponche, G. Reiter, K. Anselme, W. Meier, L. Ploux, C. Vebert-Nardin, *Macromol. Biosci.* **2008**, *8*, 1161.
[87] X. Y. Li, D. R. Liu, *Angew. Chem.* **2004**, *116*, 4956; *Angew. Chem. Int. Ed.* **2004**, *43*, 4848.
[88] F. Cavalieri, A. Postma, L. Lee, F. Caruso, *ACS Nano* **2009**, *3*, 234.
[89] C. H. Fan, S. Wang, J. W. Hong, G. C. Bazan, K. W. Plaxco, A. J. Heeger, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 6297.
[90] A. Jaschke, J. P. Furste, D. Cech, V. A. Erdmann, *Tetrahedron Lett.* **1993**, *34*, 301.
[91] C. J. Langer, *Expert Opin. Invest. Drugs* **2004**, *13*, 1501.
[92] T. E. S. Group, *Retina* **2002**, *22*, 143.
[93] For a recent review on PEG in drug delivery, see: K. Knop, R. Hoogenboom, D. Fischer, U. S. Schubert, *Angew. Chem.* **2010**, *122*, 6430; *Angew. Chem. Int. Ed.* **2010**, *49*, 6288.
[94] J. H. Jeong, S. W. Kim, T. G. Park, *J. Controlled Release* **2003**, *93*, 183.
[95] S. H. Kim, J. H. Jeong, S. H. Lee, S. W. Kim, T. G. Park, *J. Controlled Release* **2008**, *129*, 107.
[96] F. E. Alemdaroglu, C. N. Alemdaroglu, P. Langguth, A. Herrmann, *Adv. Mater.* **2008**, *20*, 899.
[97] F. E. Alemdaroglu, C. N. Alemdaroglu, P. Langguth, A. Herrmann, *Macromol. Rapid Commun.* **2008**, *29*, 326.
[98] K. Minagawa, Y. Matsuzawa, K. Yoshikawa, Y. Masubuchi, M. Matsumoto, M. Doi, C. Nishimura, M. Maeda, *Nucleic Acids Res.* **1993**, *21*, 37.
[99] M. Maeda, C. Nishimura, A. Inenaga, M. Takagi, *React. Polym.* **1993**, *21*, 27.
[100] M. Maeda, C. Nishimura, D. Umeno, M. Takagi, *Bioconjugate Chem.* **1994**, *5*, 527.
[101] D. Umeno, M. Maeda, *Anal. Sci.* **1997**, *13*, 553.
[102] D. Umeno, M. Kawasaki, M. Maeda, *Supramol. Sci.* **1998**, *5*, 427.
[103] S. Taira, K. Yokoyama, *Biotechnol. Bioeng.* **2004**, *88*, 35.
[104] S. Nagahara, T. Matsuda, *Polym. Gels Networks* **1996**, *4*, 111.
[105] G. Degols, J. P. Leonetti, C. Gagnor, M. Lemaitre, B. Lebleu, *Nucleic Acids Res.* **1989**, *17*, 9341.
[106] K. J. Watson, S. J. Park, J. H. Im, S. T. Nguyen, C. A. Mirkin, *J. Am. Chem. Soc.* **2001**, *123*, 5592.
[107] J. M. Gibbs, S. J. Park, D. R. Anderson, K. J. Watson, C. A. Mirkin, S. T. Nguyen, *J. Am. Chem. Soc.* **2005**, *127*, 1170.
[108] C. Chaix, C. Minard-Basquin, T. Delair, C. Pichot, B. Mandrand, *J. Appl. Polym. Sci.* **1998**, *70*, 2487.
[109] C. Minard-Basquin, C. Chaix, C. Pichot, *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 895.
[110] T. Mori, D. Umeno, M. Maeda, *Biotechnol. Bioeng.* **2001**, *72*, 261.
[111] D. C. Lin, B. Yurke, N. A. Langrana, *J. Mater. Res.* **2005**, *20*, 1456.
[112] D. C. Lin, B. Yurke, N. A. Langrana, *J. Biomech. Eng.* **2004**, *126*, 104.
[113] M. Murata, W. Kaku, T. Anada, Y. Sato, M. Maeda, Y. Katayama, *Chem. Lett.* **2003**, *32*, 986.
[114] M. Murata, W. Kaku, T. Anada, Y. Sato, T. Kano, M. Maeda, Y. Katayama, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3967.
[115] M. Murata, W. Kaku, T. Anada, N. Soh, Y. Katayama, M. Maeda, *Chem. Lett.* **2003**, *32*, 266.
[116] D. J. Caruana, A. Heller, *J. Am. Chem. Soc.* **1999**, *121*, 769.
[117] K. Lee, J.-M. Rouillard, T. Pham, E. Gulari, J. Kim, *Angew. Chem.* **2007**, *119*, 4751; *Angew. Chem. Int. Ed.* **2007**, *46*, 4667.
[118] H. Korri-Youssoufi, F. Garnier, P. Srivastava, P. Godillot, A. Yassar, *J. Am. Chem. Soc.* **1997**, *119*, 7388.
[119] T. Livache, A. Roget, E. Dejean, C. Barthet, G. Bidan, R. Teoule, *Nucleic Acids Res.* **1994**, *22*, 2915.
[120] T. Livache, B. Fouque, A. Roget, J. Marchand, G. Bidan, R. Teoule, G. Mathis, *Anal. Biochem.* **1998**, *255*, 188.
[121] A. Kudlay, J. M. Gibbs, G. C. Schatz, S. T. Nguyen, M. O. de La Cruz, *J. Phys. Chem. B* **2007**, *111*, 1610.
[122] S. Y. Park, J. M. Gibbs-Davis, S. T. Nguyen, G. C. Schatz, *J. Phys. Chem. B* **2007**, *111*, 8785.
[123] J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin, R. L. Letsinger, *J. Am. Chem. Soc.* **1998**, *120*, 1959.
[124] R. C. Jin, G. S. Wu, Z. Li, C. A. Mirkin, G. C. Schatz, *J. Am. Chem. Soc.* **2003**, *125*, 1643.
[125] N. L. Rosi, C. A. Mirkin, *Chem. Rev.* **2005**, *105*, 1547.
[126] T. Mori, M. Maeda, *Polym. J.* **2002**, *34*, 624.
[127] C. Minard-Basquin, C. Chaix, C. Pichot, B. Mandrand, *Bioconjugate Chem.* **2000**, *11*, 795.
[128] C. Minard-Basquin, C. Chaix, F. D'Agosto, M. T. Charreyre, C. Pichot, *J. Appl. Polym. Sci.* **2004**, *92*, 3784.
[129] B. Bordier, M. Peralaheape, G. Degols, B. Lebleu, S. Litvak, L. Sarihcottin, C. Helene, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 9383.
[130] S. Nayak, L. A. Lyon, *Angew. Chem.* **2005**, *117*, 7862; *Angew. Chem. Int. Ed.* **2005**, *44*, 7686.
[131] N. A. Peppas, J. Z. Hilt, A. Khademhosseini, R. Langer, *Adv. Mater.* **2006**, *18*, 1345.
[132] Z. R. Lu, P. Kopeckova, J. Kopecek, *Macromol. Biosci.* **2003**, *3*, 296.
[133] T. Miyata, N. Asami, T. Uragami, *Nature* **1999**, *399*, 766.
[134] Y. Murakami, M. Maeda, *Biomacromolecules* **2005**, *6*, 2927.
[135] Y. Murakami, M. Maeda, *Macromolecules* **2005**, *38*, 1535.
[136] B. Yurke, A. J. Turberfield, A. P. Mills, F. C. Simmel, J. L. Neumann, *Nature* **2000**, *406*, 605.
[137] L. P. Feng, S. H. Park, J. H. Reif, H. Yan, *Angew. Chem.* **2003**, *115*, 4478; *Angew. Chem. Int. Ed.* **2003**, *42*, 4342.
[138] T. Liedl, H. Dietz, B. Yurke, F. Simmel, *Small* **2007**, *3*, 1688.
[139] T. Amiya, T. Tanaka, *Macromolecules* **1987**, *20*, 1162.
[140] F. Horkay, P. J. Basser, *Biomacromolecules* **2004**, *5*, 232.
[141] S. Keller, J. Wang, M. Chandra, R. Berger, A. Marx, *J. Am. Chem. Soc.* **2008**, *130*, 13188.
[142] S. H. Um, J. B. Lee, N. Park, S. Y. Kwon, C. C. Umbach, D. Luo, *Nat. Mater.* **2006**, *5*, 797.
[143] N. Park, S. H. Um, H. Funabashi, J. F. Xu, D. Luo, *Nat. Mater.* **2009**, *8*, 432.
[144] E. J. Cheng, Y. Z. Xing, P. Chen, Y. Yang, Y. W. Sun, D. J. Zhou, L. J. Xu, Q. H. Fan, D. S. Liu, *Angew. Chem.* **2009**, *121*, 7796; *Angew. Chem. Int. Ed.* **2009**, *48*, 7660.

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