Applied chemistry of natural DNA

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Recently, natural DNA has emerged as an appealing biomacromolecule for functional materials. It is abundant and renewable, and possesses the well known double helix structure that promises many unique properties difficult to find in other polymers. Natural DNA has been applied in electronic, optical and biomaterials, as a catalyst for enantioselective reactions, and as a material for cleaning the environment. Most of the applications are based on combining DNA with other chemicals or nanoparticles by electrostatic binding, intercalation or groove binding. In this *critical review* article, recent developments in utilizing natural DNA are reviewed by focusing on three basic properties of DNA: the electrostatic property as a polyelectrolyte, selective affinity for small molecules, and biocompatibility (128 references).

1. Introduction

DNA is the fundamental hereditary material of living organisms and is widely distributed in the natural world. Studies of DNA have already greatly contributed to the progress of life science, in which the discovery of the DNA double-helical structure more than 50 years ago has established the foundation of molecular biology, making scientists able to understand life on the molecular level in the genome. Based on the knowledge about the genetic code, DNA has been used in genetic engineering, forensics, phylogenesis, history and anthropology.

More recently, the use of DNA as a biomacromolecule has been attracting additional attention of researchers in diverse areas of science. As a result, DNA is regarded as an ideal macromolecule for creating new functional materials. Utilization of DNA has become a lively field in science. Not only have many experimental reports been published in the last two decades, but also DNA research has emerged as an expanding field attractive enough to obtain more attention from scientists and engineers. Although these publications covered many applications of DNA as electronic, optical, and biomaterials, as catalyst, and in environmental protection, separation, they seem to rely on a few fundamental properties of DNA that relate to the famous double helical structure. This review article details such applications based on this double helical structure of DNA, by focusing attention on three basic features of the natural DNA molecule as schematically shown in Fig. 1: being a polyelectrolyte, having selective affinity for small molecules, and its biocompatibility.

Among these characteristics, the electrostatic property of DNA as a highly charged polyelectrolyte is most important, because it has been widely used for exploring various DNA-based applications. A DNA molecule consists of two polynucleotide strands coiled around each other in a helical

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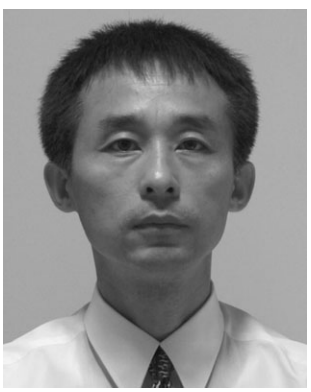

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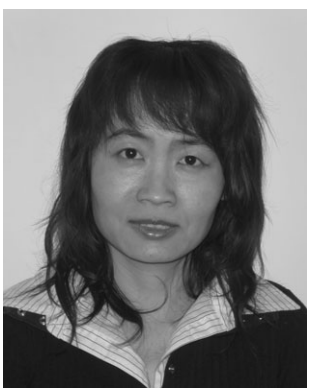

HongYan Diao

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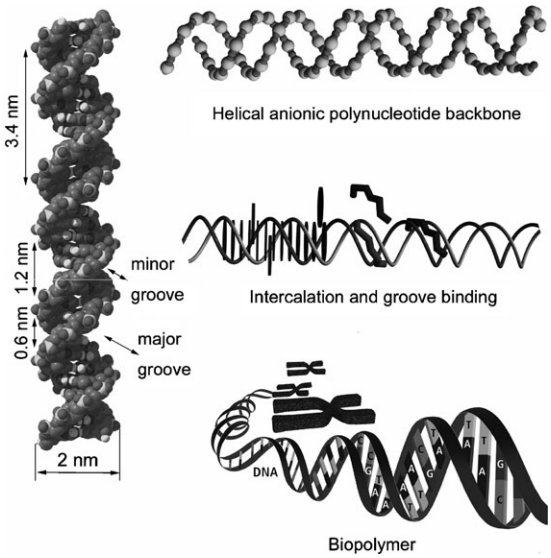

Fig. 1 Basic features of natural DNA.

fashion, with a diameter of approximately 2 nm. Moreover, the double helix chains of DNA are negatively charged by the phosphate groups that are regularly arranged in the two backbones. Therefore, DNA is an ideal template to fabricate highly ordered nanostructures by binding cationic agents such as metal ions, cationic surfactants and polycationic agents. The second feature of DNA is its selective affinity for small molecules. The most common DNA structure is the B-DNA type in which the stacked bases are regularly spaced 0.34 nm along the helix axis, and the helical structure possesses a wide major groove and a narrow minor groove of approximately the same depth. Some small molecules can intercalate into the spaces between the stacked bases, or bind in the grooves between the two backbones. Both of the interaction patterns are highly selective toward the structure of the small molecules. By this special affinity, DNA can be used as an environmental material to selectively remove toxic pollutants, or as a template to arrange functional molecules. Thirdly, DNA is perfectly biocompatible, as it can be found in almost all living organisms. This offers DNA excellent prospects for serving as biomaterials.

The development of DNA utilization depends not only on the excellent properties, but also on its availability. Currently, various DNAs with a designed sequence can be synthesized by automated synthesis systems, and amplified by the PCR method. However, such synthetic DNA is still expensive and not easy to produce in large quantities; therefore, laboratories usually use it at the microgram or milligram level. On the other hand, another type of DNA that is extracted from natural products, referred to natural DNA in this paper, has a large potential for mass-production. In fact, it has had commercial products on sale by a few companies, and can be supplied by the ton. As a substance widely existing in organisms, natural DNA is plentiful in the natural world. A typical case is salmon milt in fishery, which is often used for livestock feedstuff. The salmon milt contains over 10% of dry weight DNA, and its manufacture is very facile. Because salmon fishery is an increasing industry with a worldwide supply exceeding 2.4 million tons per year,[1] it is estimated that about 3000 tons of salmon DNA is available per year. Other resources include herring milt, scallop testis, and even artificial microorganisms produced by biological methods. Moreover, DNA can be efficiently extracted from animal tissues that have a high nucleus to cytoplasmic mass ratio, such as thymus gland and spleen. Undoubtedly, such facile and renewable resources will effectively promote further research of DNA.

In present paper, we have reviewed research that was limited to the investigation of natural DNA, including the marine DNA mentioned above, the calf thymus DNA, and various microbial DNA such as bacteriophage λ-DNA, circular plasmid pBR322 DNA, and others. In these studies, it appears that the parameters of molecular weight, purity, molecular shape (linear or circular), content of the guanine (G) and cytosine (C) bases, even the content of single strand DNA (a byproduct in manufacture), are more important than the base sequence of DNA. However, materials derived from synthetic DNA, which usually self-assembles by a designed sequence, are outside the scope of the present review. Readers who are interested in applications based on designed DNA are referred to recent review articles.[2–7] In this review, articles published within the last decade are reviewed according to the three basic properties of natural DNA shown in Fig. 1. Section 2 outlines DNA complexes with cationic binding agents, stressing particular advantages to form nano-scale ordered materials. Section 3 reviews the research on the selective affinity of DNA such as intercalation and groove binding. Section 4 summarizes several attempts that use DNA as a biomaterial.

Norio Nishi

Norio Nishi received his BS in 1965 and PhD in Polymer Chemistry in 1970 from Hokkaido University. He became a full professor of the Graduate School of Environmental Science, Hokkaido University in 1993 and served as dean in 1998–2002. His research interests include the development of biomass utility, biomolecular materials, and synthesis of peptides. He was a recipient of contribution awards of the Society of Polymer Science of Japan in 2006, for his contributions in novel bimolecular materials.

2. Helical anionic polynucleotide backbone

Compared to other anionic polymers, DNA is distinguished by the highly charged double helical structure, which shows local stiffness in a range of about 50 nm but long-range flexibility in water. These characteristics make DNA feasible to create precisely ordered materials.[8] During the last decade, many novel materials consisting of natural DNA and various cationic substances, including metal ions, nanoparticles, proteins, surfactants, and polycationic macromolecules, have been reported. In this section, these publications are classified into four categories as schematically shown in Fig. 2. These categories are briefly explained below and will be discussed in more detail in the following subsections.

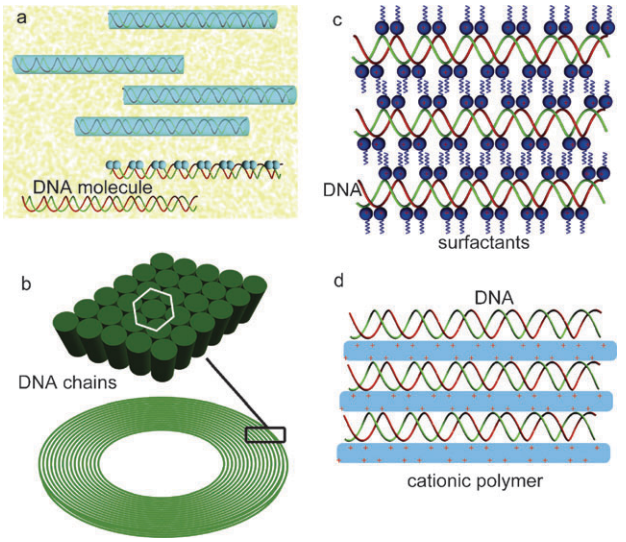

Fig. 2 Schematic representation of the structures of DNA/cation complexes. (a) The complex of linear DNA molecules with cationic ions or nanoparticles. (b) DNA condensation by multivalent cations resulting in a toroidal structure. (c) DNA–cationic surfactant complex. (d) DNA–cationic polymer complex formed by electrostatic layer-by-layer (LbL) deposition.

In dilute solutions (<1 mg ml^{-1}), DNA forms wormlike coils. However, the DNA molecules in dilute solution can be easily stretched to linear templates that can lead to ordered nanostructures[8,9] (Fig. 2(a)). As the DNA is concentrated (>1 mg ml^{-1}), the molecules spontaneously undergo unidirectional ordering and transform into liquid crystals of the 'cholesteric' type.[10] In the presence of multivalent cations such as polyamines, DNA undergoes a dramatic condensation to a compact, usually highly ordered toroidal structure[11] (Fig. 2(b)). When DNA reacts with cationic surfactants such as hexadecyltrimethylammonium chloride, a precipitate is formed, producing a complex that is soluble in common organic solvents, and thus can be easily cast to thin films. The conformation of the DNA–surfactant complex is controllable and often locally ordered (Fig. 2(c)). Like other anionic polymers, DNA reacts with polycationic macromolecules, thus by some common protocols such as the process of electrostatic layer by layer (LbL) deposition, DNA can be made into many useful materials (Fig. 2(d)).

2.1 Interaction of linear DNA with cationic substances

One application for DNA being explored is "nanowires", which were widely recognized as important elements in the development of futuristic nanoscale electro-devices by the bottom-up technique.[9] Because a DNA molecule in aqueous solution usually is disordered as a result of thermal fluctuations, the individual DNA molecules must be separated and stretched before serving as templates for nanowire fabrication. Several methods to stretch and align DNA molecules have been reported, including molecular combing[12,13] (Fig. 3), electrophoretic stretching,[14,15] and hydrodynamic stretching.[16] Following stretching and positioning, the DNA molecules are generally treated with a metal ion solution to bind metal ions to DNA. These metal ions can be reduced to form metal

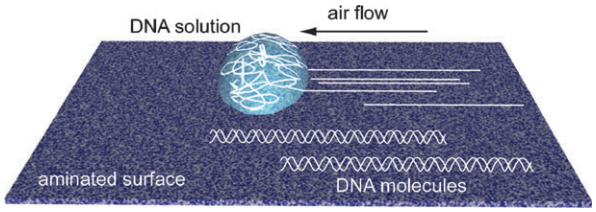

Fig. 3 Schematic mechanism of the DNA combing method. By moving a DNA solution with an air flow over an aminated surface, DNA molecules are stretched to linear templates.

clusters. As the metal seeds on the DNA templates serve as catalysts for further reduction, the clusters keep growing until the reaction is finished. With such an approach, various metallic nanowires have been prepared by the deposition of silver,[17–20] palladium (Fig. 4),[21,22] platinum,[23,24] nickel,[25,26] copper[27,28] and cobalt[29] metal ions on DNA.

Not only metal ions, but also a number of metal clusters with positive charges have been successfully bound to DNA as well. In order to charge the metal clusters, they are commonly treated with reagents that combine two functions: stabilizing the colloidal nanoparticles and positively charging their surface. Cetyltrimethylammonium bromide (CTAB), a cationic and oily surfactant able to form bilayer on the surface of the nanoparticles, is frequently used in these treatments (Fig. 5). For example, it was reported that CTAB binding to the surface of silver clusters (3.5 nm) and gold clusters (18 nm) in aqueous solutions forms a charged shell. After treating template DNA with the charged cluster emulsion, AFM imaging showed that the charged particles were successfully linked to a linear structure.[30] Besides CTAB, charging reagents that do not form bilayers on the surface have been also reported. As shown in Fig. 5, generally, such reagents have two functional groups on each molecular end, one with affinity to the surface of the nanoparticles, such as a thiol group, and the other, commonly a amino/protonated amino/quaternary ammonium group, is to positively charge the surface. For example, Sun *et al.*[31] have successfully fabricated ring-like silver nanostructures by assembling 4-aminothiophenol charged silver clusters on circular templates of plasmid pBR322 DNA. Hong and co-workers[32] have reported a

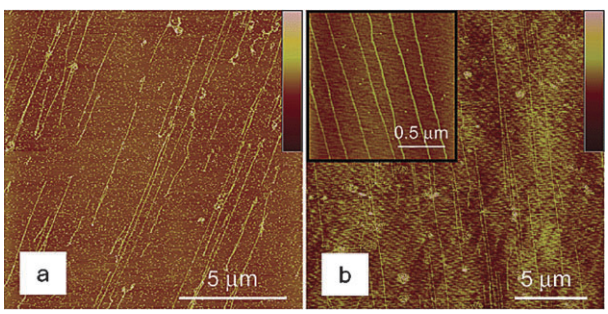

Fig. 4 Atomic force microscope (AFM) images of (a) 1D-aligned Pd nanowires prepared by depositing Pd ions on linear templates of DNA, and (b) the corresponding precursory DNA molecules. The inset in (b) gives a detailed view of the parallel DNA duplexes. Height scale: (a) 15 nm and (b) 1.0 nm. Reprinted with permission from ref. 22. Copyright 2003, American Chemical Society.

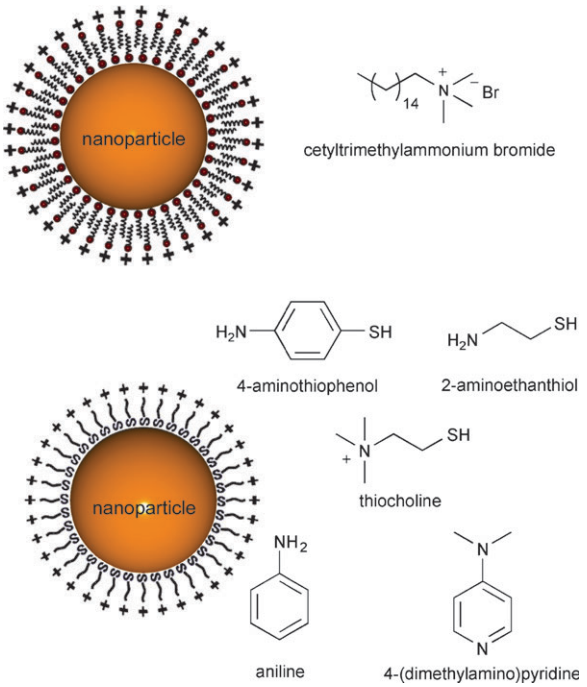

Fig. 5 Reagent examples for charging metal clusters.

facile approach to assemble nanowires by conjugating 2-aminoethanthiol-capped gold nanoparticles (AuNPs) onto DNA molecules that were immobilized on a 3-aminopropyltriethoxysilane-coated Si substrate. Ohtani and co-workers[33] prepared positively charged AuNPs by reducing $HAuCl_4$ with aniline. After attaching the AuNPs to the DNA molecules, AFM results revealed that the AuNPs were successfully assembled on DNA molecules with a long-range order. In Ongaro's work,[34] 4-(dimethylamino)pyridine-stabilized AuNPs could selectively bind to calf thymus DNA that was aligned between gold electrodes, and finally formed a linear nanoparticle array. Warner and Hutchison report[35] that phosphine-stabilized precursor AuNPs with a narrow size distribution underwent a ligand-exchange reaction with thiocholine (see Fig. 5) to replace the phosphine molecules on the surface of the clusters, giving the corresponding positively charged AuNPs. The subsequent electrostatic reaction of those charged AuNPs to the DNA backbone resulted in several desirable architectures, including extended linear chain-like structures, ribbon-like structures composed of parallel nanoparticle chains, and branched structures.

Besides these metal nanoparticles, inorganic nanoparticles such as CTAB-capped cuprous oxide nanoparticles[36] and pyrrolidinone-capped Fe_2O_3[37] have been reported to result in highly ordered structures on the DNA template. Similarly, Nabiev and co-workers[38] proposed a protocol to organize cysteamine-charged fluorescent semiconductor CdSe/ZnS quantum dots or quantum rods on the DNA template to lead to highly ordered quasi-nanowires.

DNA has also been reported to be a guiding template for the polymerization of conductive polymers, such as polypyrrole,[39,40] and polyaniline.[41–43] By the interaction of cationic monomers (or precursors of the monomer) to the backbone of DNA immobilized on a Si surface, the polymers can be synthesized along the DNA templates. This approach has potential for fabrication of high-density conducting polymer nanowires with a predetermined position and orientation on a Si surface. An example[41] of the DNA directed growth of polyaniline is schematically shown in Fig. 6. The DNA templates that were immobilized on a Si surface were incubated in an aniline monomer solution (19 mM, pH 4.0 adjusted by 0.1 M HCl), to emulsify and organize the aniline monomers along the DNA chains. Then, the aligned aniline monomers were polymerized enzymatically by adding horseradish peroxidase (HRP) and H_2O_2 to form polyaniline/DNA nanowires (Scheme A). Because polyaniline structures can be reversibly charged and discharged simply by exposing them to HCl and NH_3 gas, the nanowire conductivity is sensitive to the proton doping–undoping process (Scheme B). Such nanowires hold promise for sensitive chemical sensor applications.

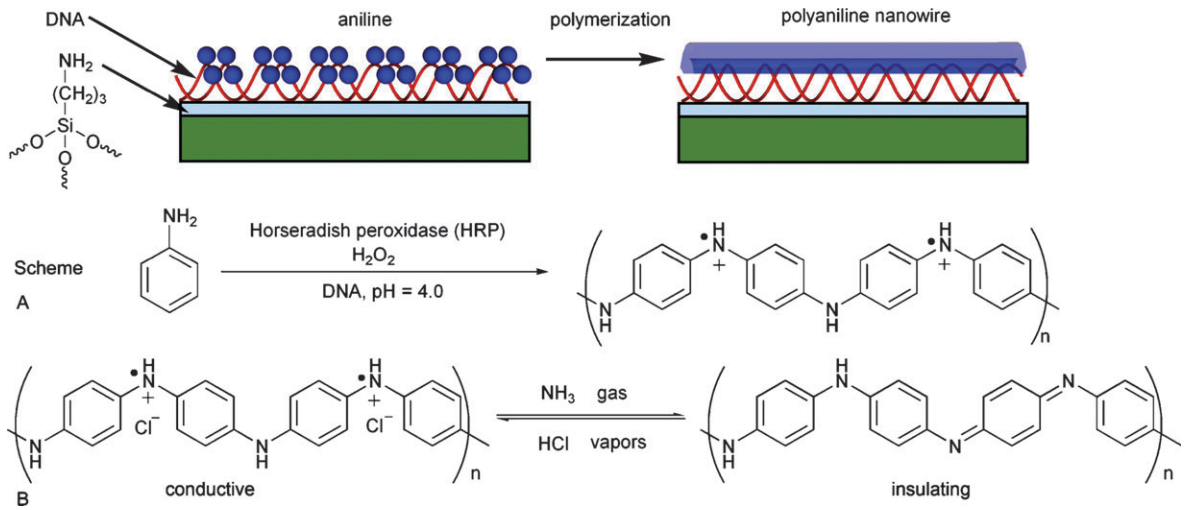

Fig. 6 A polyaniline nanowire polymerized on a Si surface with DNA as a guiding template, and its change in molecular structure by acid–base doping (based on ref. 41).

2.2 DNA condensation

Natural DNA in viruses and biological cells is often highly condensed, tightly packed in a liquid-crystalline fashion, which typically occupies only 0.01 to 0.0001% of the volume of an uncondensed, wormlike DNA coil in aqueous solution.[44,45] These compact states of DNA have been the subject of numerous investigations, because such compact and highly ordered DNA structures may find applications in nanoelectronics, as biosensors, DNA chips and catalysts. Two detailed review articles are available[46,47] about the structures and phase transitions of the condensed DNA in the presence of monovalent ions. Many experimental reports indicate that a number of multivalent cations can facilitate the condensation of DNA in a test tube as well,[11,48] and usually induced a highly ordered toroidal structure, as shown in the schematic model in Fig. 2(b). Most investigators of DNA condensation assume that a hexagonal packing of DNA strands can give the condensate an optimal packing density. Reagents most commonly used in condensation studies are naturally occurring polyamines, such as spermidine and spermine, and $Co(NH_3)_6^{3+}$. Others include cationic polypeptides such as polylysine, and basic proteins such as protamine, histones H1 and H5. The toroidal structures (in some rare cases shaped as rods or spheres) formed when DNA is condensed *in vitro* can vary significantly in morphology by varying the condensation conditions. Their shapes, and especially the size (*i.e.*, 50–300 nm), depend on the solution conditions[49] (*e.g.*, ionic strength and solvent polarity), DNA properties (*e.g.*, length, persistence length, and extent of supercoiling), the nature of the condensing agent[49,50] (*e.g.*, charge density), and even the charged surface of the substrate.[51] The mechanism of DNA condensation has been mainly considered to be a nucleation–growth process, in which the highly ordered toroidal structure starts from a spontaneous nucleation loop of a single DNA molecule as a proto-toroid, followed by the collection of additional DNA leading to growth.[52]

Such ordered structures formed by the DNA condensation have implications for their usability in fabrication of nanostructures. Researchers have begun exploiting this in material applications. In attempting to find a highly stable DNA mesophase, Pillai and co-workers[53] compared the stabilization effects of alkali metal ions Li^+, Na^+, K^+, Rb^+, and Cs^+ on the liquid crystalline organization of high-molecular-weight calf thymus DNA, and found that Li^+ could induce a highly stable biphasic cholesteric-columnar arrangement.

Morri *et al.* investigated the DNA molecular orientation in condensed DNA films[54,55] that were prepared by drying a dilute DNA solution. X-Ray diffraction and polarization microscopy revealed that the DNA molecules have been ordered in two patterns in the dried films. In the outer zones, the DNA molecular chains were aligned along the annular curve of the peripheral edge, whereas in the innermost zone, the DNA molecules are radially oriented. Such findings may be important to new electronic or optical applications.

Samoc's group[56] has precisely measured optical parameters of a series of DNA films that were prepared by spin-coating and casting DNA aqueous solutions. They discussed the optical properties with regard to the formation of liquid crystalline phases. However, the values of the refractive indices and birefringence in the DNA films varied considerably, depending on the film fabrication method, solution concentration, drying conditions, and relative humidity (RH) of the environment, which suggest that the structure of the condensed DNA in the films are influencing their optical properties.

Dobashi's group[57] proposed a new method to prepare microspheres of DNA liquid crystalline gels. By dripping salmon milt DNA solution into an aqueous cobalt chloride solution, the DNA molecules are complexed by bivalent cobalt ions, and form a dialytic layer on the surface of the DNA solution droplets. Subsequently, the DNA molecules are cross-linked by the bivalent cobalt ions penetrating into the droplet, producing the water-insoluble gel. These gel microspheres are liquid crystalline, and able to adsorb acridine orange, a cancerogenic intercalator of DNA.

The DNA condensation induced by multivalent metal ions was investigated by mixing concentrated metal ions with DNA solutions. The results revealed that the structure of the DNA complex strongly depends on the ion type.[58] Al^{3+}, Cr^{3+}, Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Y^{3+}, La^{3+}, In^{3+} and Cd^{2+} metal ions at 1 M concentration immediately formed water-insoluble gels when they are mixed with a highly concentrated solution of DNA (10 mg ml^{-1}). As shown in Fig. 7, scanning electron microscope (SEM) images taken from the surface of the condensed DNA materials showed that the DNA–Al^{3+} complex is more regular than the DNA–Cu^{2+} complex. The results from circular dichroism (CD) and infrared (IR) spectra indicated that Cu^{2+} not only strongly binds to the phosphate group but also inserts into the DNA base pairs, leading to the destruction of the B-DNA structure.

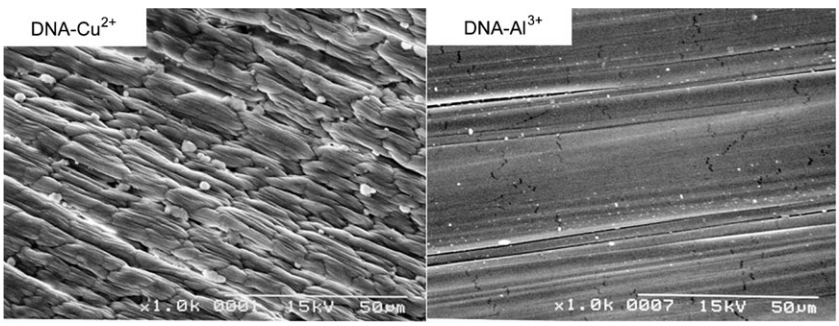

Fig. 7 SEM images taken from the surface of DNA–Cu^{2+} and DNA–Al^{3+} complexes. The surface structure of the DNA–Al^{3+} complex is more regular than the DNA–Cu^{2+} complex. Reprinted with permission from ref. 58. Copyright 2005, Elsevier.

More recently, Chen's group[59] reported a DNA condensation experiment that used a dendrimer as the neutralization reagent. The dendrimer was a second-generation poly(amidoamine), which has 14 tertiary amine groups linked to an ethylenediamine core, and 16 primary amine groups on the surface. The dendrimers reacted with DNA, resulting in a 2-D array nanostructure on a mica substrate. AFM topographic images showed that the DNA chains were highly ordered with an average interhelical distance of about 3.8 nm and a height of 2.6 nm.

2.3 Interaction of DNA with cationic surfactants

Binding cationic surfactants to the polyanionic backbones of DNA leads to a complex that is insoluble in water but soluble in common organic solvents. In a series of reports,[60–63] Okahata *et al.* have described the preparation and characterization of DNA–cationic surfactant complexes. Generally, DNA–surfactant complexes, which are water-insoluble precipitates, can be readily prepared by mixing aqueous solutions of anionic DNA and cationic surfactant. These complexes are often soluble in common organic solvents, and easy to cast to thin films. CD spectra revealed that the DNA molecules in the films retain their double helical structure. Polarized absorption spectra of intercalating dye molecules indicated that the DNA–surfactant complexes allow intercalation even in chloroform solution, despite the fact that intercalation is usually observable only in water. Interestingly, when the DNA–surfactant cast film was stretched to three times its length, it was found that the DNA molecules in the film align along the stretching direction.[60] Recently, this phenomenon was verified again by another group with X-ray diffraction (XRD),[64] and was explained by an elongational flow mechanism.[65]

Moreover, the conformation and even morphology of the DNA–cationic surfactant complexes can be controlled by varying their environmental condition, or by changing the molecular shape of the cationic surfactants. It was demonstrated[64] that hydration and change in temperature could lead to variations of the base-pair stacking within the DNA in the aligned film, as evidenced by the clearly different wide-angle XRD diffraction patterns in a variety of environmental conditions were . On the other hand, the surfactant tail molecular shape/size has been also reported to affect the mesophase structures of the DNA–surfactant complexes.[66,67] As shown in Fig. 8, surfactant tails with rodlike (I), discotic (II) and cubic (III) shapes produce a typical smectic A morphology, double lamellocolumnar liquid crystalline phase, and inverted hexagonal columnar phase, respectively. These controllable DNA hybrid materials may have applications in organic microelectronics. In addition, Chu and co-workers studied the nanostructures of the complexes of calf thymus DNA with the cationic surfactants didodecyldimethylammonium bromide (DDAB) and CTAB, by using synchrotron small-angle X-ray scattering (SAXS).[68] The results showed that the complex of DNA and double-tailed DDAB formed a bilayered lamellar structure, whereas the complex of DNA and single-tailed CTAB preferred the structure of hexagonal packed cylinders. Moreover, the addition of a neutral single-chained

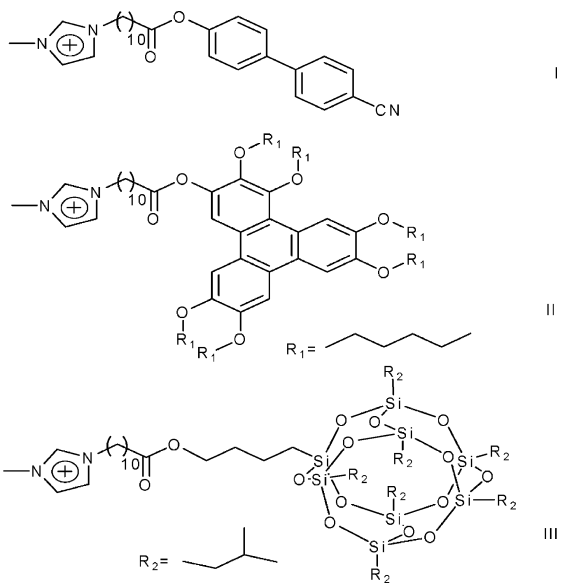

Fig. 8 Cationic imidazolium salts with various molecular shape and size, to form complexes with DNA to control their mesophase structure (based on ref. 67).

surfactant, 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (PHGPC), to the DNA-CTAB complexes can induce a structural transition, in which the hexagonal structure is changed to a multi-bilayered lamellar structure. It was also reported that the pattern depends on the ratios of charged to neutral surfactant, and charged surfactant to DNA.

Another work on DNA–surfactant complexes was reported by Wong and his co-workers,[69] who found that the DNA molecule structural features can be imprinted into CdS nanostructures. The protocol is schematically shown in Fig. 9. The DNA was first treated with a mixed solution of cationic surfactant (dioleoyltrimethylammonium propane) and neutral surfactant, to prepare DNA–surfactant multilamellar structures in which DNA chains are inserted between stacked surfactant sheets. Then Cd^{2+} ions were added, and were organized along the DNA chains in the multilamellar structure surface by electrostatic interaction. Subsequently, the organized Cd^{2+} ions reacted with H_2S to form CdS nanorods.

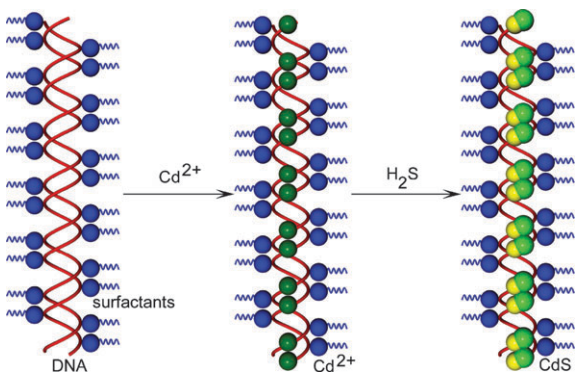

Fig. 9 Schematic representation of DNA–lipid complex membrane template organization. The Cd^{2+} ions (green balls) are organized by DNA strands (red) in the lamellar complexes and subsequently react with H_2S (yellow balls) to form CdS nanostructures (based on ref. 69).

SAXS data revealed that the CdS nanorods had grown along the DNA strands and had formed a highly ordered nanoscale structure. The lamellar spacing remains approximately constant and this suggests that the biomolecular structure is robust during the templating process. In addition, the widths of the nanorods can be tailored by adjusting the surfactant mixture. This technique may lead to custom-designed crystals with useful electronic, magnetic or optical properties.

2.4 Interaction of DNA with cationic polymers

A number of cationic polymers including some proteins have been reported to give DNA complexes. For example, reaction with collagen has been reported to result in an orderly structured complex. Collagen is a rigid helical protein, which consists of three polypeptide chains arranged in a parallel triple-helix with a diameter of 1.5 nm and a length of about 300 nm for a single collagen molecule. Usually, transmission electron microscope (TEM) images of native collagen show a 67 nm banded pattern. The first morphological investigation concerning DNA–collagen complexes using TEM was published in 1997.[70] The banded pattern of the collagen fibrils which are formed in the presence of DNA was more regular than that formed by collagen alone, and the width of the fibrils was larger than that of the fibrils without DNA. This phenomenon has been examined in more detail by the following studies.[71] It was demonstrated that the presence of linear DNA strongly promotes fibril formation, whereas circular closed supercoiled DNA shows an opposite effect. In another report, it was found that the phosphate groups of DNA interact with the positive charges in collagen, and play key roles in the collagen fibril formation.[72] Moreover, Mrevlishvili's group proposed a model (see Fig. 10) for the DNA–collagen complex,[73,74] that represents the DNA helical structure becoming more rigid due to the rigid rod-like structure of the triple-helix collagen (300 nm) overlapping and enveloping the backbone of the DNA.

DNA has also been used as a template to guide a sol–gel polycondensation of a silane coupling reagent to fabricate highly ordered inorganic materials.[75] Shinkai's group successfully constructed tiny silicate structures by using a plasmid DNA extracted from bacteria as the template, which assume either rod-like or circular shapes. In their report, the DNA was first complexed with a special cationic reagent that has two functional groups, ammonium and guanidinium, on each end of the molecule. Thus the DNA complex became positively charged. Subsequently, these aminated DNA complex templates were treated with a silane coupling reagent, tetraethoxysilane, resulting in a silica layer surrounding the DNA template. The obtained nanoparticles were of the same shape as the DNA templates, and their dimensions were uniform. The rod-like structures had a uniform length of about 1 μm, and the diameters of the toroidal-shaped structures were in the range of 500–800 nm.

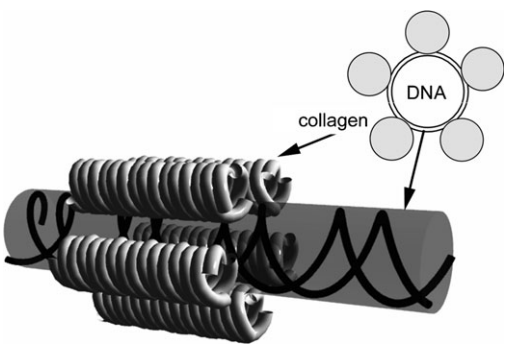

Fig. 10 Schematic model of the collagen–DNA complex (based on ref. 73).

The layer-by-layer (LbL) deposition protocol is a main strategy to fabricate DNA-based complexes with cationic polymers. Dong and co-workers[76] have incorporated silver nanoparticles in a DNA multilayer film. In their experiments, the phosphate groups of DNA were first partially complexed with Ag^+, then the resulting DNA–Ag^+ complex underwent the LbL process with poly(diallyldimethylammonium chloride), a cationic polymer, to form a multilayer film. Finally, the Ag^+ ions bound on the DNA were electrochemically reduced to silver clusters in the interlayers of the two polymers. The nanoparticles formed in the layers were of uniform size, with diameters just under 40 nm. Such silver nanoparticles are useful in chemistry, biology and materials science. Furthermore, in Gu and Hasebe's recent report,[77] a membrane composed of DNA–Cu(II) complex was successfully coated on a glassy carbon electrode by treating DNA with poly(allylamine). The modified electrode was reported to be highly sensitive for detecting H_2O_2. In another example, Lang and Lin[78] fabricated DNA films by LbL assembly with poly(allylamine). The resulting films were water-insoluble, and could readily adsorb water-soluble dyes, such as ethidium bromide, 5,10,15,20-tetrakis(4-*N*-methylpyridyl)-porphine tetra(*p*-toluenesulfonate), and 9-aminoacridine hydrochloride, which are intercalators of DNA. The LbL protocol was also used to prepare DNA-based biomaterials, the details of which will be discussed in section 4.

3. Intercalation and groove binding

Besides the electrostatic interaction with anionic DNA, as shown in Fig. 11, small molecules can interact with DNA in two other ways: groove binding and intercalation, both excellently selective. As implied by their names, some molecules shaped like the DNA groove can adhere to the edges of base pairs in both the major and minor grooves, and molecules with a planar structure are able to intercalate into the spaces between stacked base pairs. Such selective affinity for small molecules has been exploited for various applications. Here, we will describe them in each subsection below.

3.1 Removing harmful substances with DNA

Generally, compounds that can interact with DNA are often toxic or carcinogenic.[79,80] For example, polycyclic aromatic hydrocarbons (PAHs), aromatic amines, and some endocrine disruptors, are significant pollutants generated from incomplete combustion, often found in coal tar, automobile exhaust, or cigarette smoke. They are known or suspected to exhibit strong carcinogenicity. For removing such harmful chemicals before they interact with intracellular DNA, natural DNA has been considered as a favorable candidate for an adsorbent.

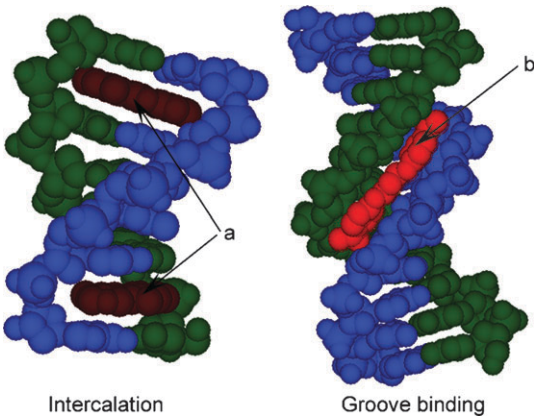

Fig. 11 Schematic representation of intercalation and groove binding of DNA (according to the data in the Protein Data Bank (PDB)): (a) intercalators (PDB ID: 1Z3F); (b) groove binder (PDB ID: 102D).

Contrasting with the common adsorbents such as activated carbon and alumina, DNA-based adsorbents have the advantage of molecular selectivity. Unfortunately, DNA is water-soluble and biodegradable by nuclease enzymes, which makes DNA disadvantageous for environmental water-treatment. However, a number of reports have proposed several solvent methods and claimed that these obstacles have been cleared.

Yamada *et al*. first reported a water-insoluble DNA film prepared by UV light irradiation,[81,82] and indicated that UV light irradiating on a cast film of DNA can force the DNA molecules to cross-link. Due to the cross-linked 3D DNA structure, such UV-irradiated DNA films became water-insoluble and nuclease resistant. In subsequent reports, this UV irradiation method has been used for coating porous glass beads[83] and nonwoven cellulose fabric.[84] These resulting DNA based materials accumulate compounds with a planar structure, for example, dibenzo-*p*-dioxin, dibenzofuran, biphenyl, benzo[*a*]pyrene and ethidium bromide. However, bisphenol A and diethylstilbestrol, which are nonplanar, do not bind to the DNA-based materials as well. Fig. 12 represents the molecular structures of the harmful chemicals that have been examined, and Table 1 shows the results of the selective adsorption.

Table 1 Relative accumulation of harmful compounds by UV-irradiated DNA films (I), DNA-immobilized porous glass beads (II) and DNA-immobilized columns (III) (based on ref. 83)

		Removal (%)		
Harmful compound	Conc./μM	I	II	III
Dibenzo-*p*-dioxin	0.67	65	74	87
Dibenzofuran	1.1	60	69	83
Biphenyl	1.4	50	77	97
Benzo[*a*]pyrene	1.4	45	67	36
Bisphenol A	23	0	0	0
Diethylstilbestrol	2.1	0	0	0

[a] Each value represents the mean of three separate determination.

Table 2 Relative accumulation of harmful and nutritional compounds by the columns of DNA hydrogel beads (I), activated carbon (II) and alumina (III) (based on ref. 85)

		Removal (%)		
Compound	Conc./μM	I	II	III
Dibenzo-*p*-dioxin	0.65	95	98	97
Dibenzofuran	0.60	95	96	95
Biphenyl	0.60	93	96	94
Vitamin B2	0.75	13	93	91
Vitamin B12	0.75	11	95	91

Subsequently, immobilizing DNA in a semi-interpenetrating polymer network (semi-IPN) polyacrylamide hydrogel was proposed.[85] The semi-IPNs can be described as cross-linked polymer networks holding another linear polymer by permanent entanglements. Salmon milt DNA was immobilized in hydrogel beads by an inverse suspension polymerization of acrylamide in a continuous phase of cyclohexane. As shown in Table 2, although activated carbon and alumina adsorb more toxins than the DNA hydrogel beads, the DNA matrix was very selective. Vitamin B2 and B12 remained in the mixed solution but the toxic compounds were effectively removed. This selectivity makes DNA based materials effective for removing harmful chemicals from a complex mixture containing useful compounds.

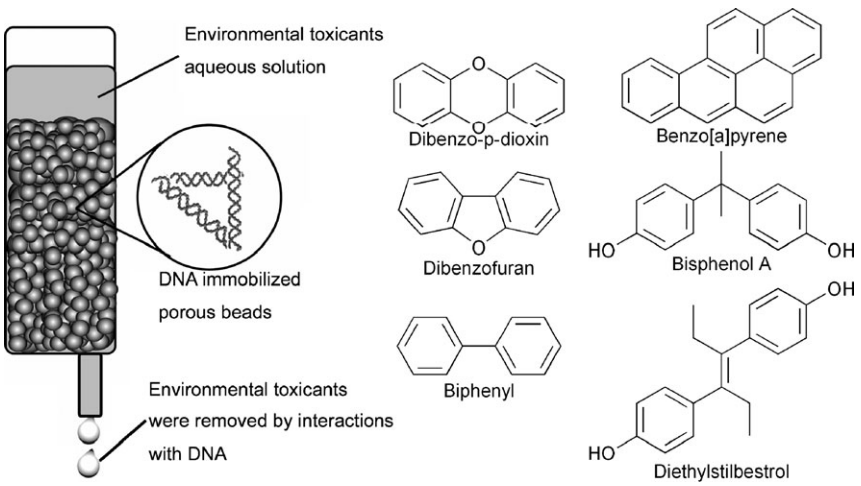

Fig. 12 Schematic representation of a DNA containing column to remove toxins (a) and examples of the examined toxins (ref. 83).

In addition, silica–DNA composites and DNA-loaded polysulfone (PSf) microspheres were fabricated and investigated for environmental applications.[86–88] Results show that both hybrid materials effectively accumulate harmful DNA-intercalating pollutants such as acridine orange. Following these reports using insoluble DNA, DNA aqueous solutions were used directly. A dialytic method was proposed to remove harmful DNA-intercalating pollutants from water,[89] as the large DNA molecules can be kept inside of the dialysis membrane, and accumulate the intercalators which permeated from the outside. As a model experiment, a dilute solution (0.5 ppb) containing three dioxin derivatives, dibenzo-*p*-dioxin, dibenzofuran and biphenyl, was treated with this dialytic method. After dialysis these chemicals could be concentrated to about 200 times their initial concentration. Moreover, the polluted DNA solutions are renewable by removing the intercalators with hexane. Recently, a new application of DNA solution in environmental remediation was evaluated.[90] Aqueous DNA was used as a solubilizing agent to remove common PAHs such as anthracene, phenanthrene and pyrene from contaminated soil. Comparative tests showed that DNA solution is more effective than methyl-β- and γ-cyclodextrins and Tween 80 for removing pyrene.

3.2 Applying DNA in stereochemistry

DNA is chiral as a result of the asymmetric centers in the ribose units and due to its helix structure. Therefore, DNA is an attractive material for use in stereochemistry. Natural DNA has been investigated for enantioselection because of the crucial importance of enantioselection in various fields such as drug and food analysis, biochemistry or clinical pharmacology. Chaires and co-workers[91] reported a dramatic experiment of structural selectivity of DNA binding for the naturally occurring anticancer agent (+)-daunorubicin and its (−)-enantiomer. Their results indicated that (+)-daunorubicin binds selectively to right-handed DNA, whereas the (−)-enantiomer binds selectively to left-handed DNA.

The efficient preparation of enantiopure compounds is of primary importance for the production of pharmaceuticals, vitamins, agrochemicals and flavorants. Synthesizing compounds with high enantioselectivity is the main goal of modern organic chemists. Roelfes and Feringa[92,93] introduced a novel DNA-based asymmetric catalysis concept, which is able to yield very high enantioselectivities up to 99% (see Fig. 13). Such high enantioselectivity is attributed to a catalytically active copper complex anchored on the DNA molecule through a 2,2-bipyridine unit intercalating into the DNA double helix. It was reported that the DNA is the source of chirality in the Cu(II) catalyzed Diels–Alder reactions. When the Diels–Alder reaction was catalyzed by the Cu complex alone without DNA, both *endo* and *exo* cycloaddition products were formed. Although there is a preponderance of the *endo* product is of, both essentially were generated as racemic mixtures. As the active Cu(II) center is brought into proximity of the chiral environment of the DNA double helix, it allows for a transfer of chirality from DNA to the reaction product. The reactions performed in water allowed virtually complete regioselectivity (up to 99% *endo*) and excellent enantioselectivity (up to 99% ee). In following studies, a high enantioselectivity was also found in the Diels–Alder reaction of α,β-unsaturated-2-acyl imidazoles[94] and a electrophilic fluorination[95] of beta-keto esters.

3.3 Enhancing photonic devices with DNA

Recently, DNA has been investigated as a material for fabricating photonic devices[96] because some lumophores intercalated in the DNA double helix can greatly enhance the efficiency of light emission. Such exciting properties were firstly reported by Ogata and his co-workers,[97] who demonstrated that the fluorescent dye Rhodamine 6G (Rh6G) intercalated in DNA cause amplified spontaneous emission from the Rh6G-DNA–surfactant complex films (Fig. 14). The experimental result in the subsequent reports revealed that a similar enhancement effect could be found from other dyes. 4-[4-(Dimethylamino)styryl]-1-docosylpyridinium bromide (DMASDPB), a dye well known as a nonlinear optical material, has been reported[98,99] to result in an enhanced efficiency similar to Rh6G. Spectroscopic analysis revealed that the DMASDPB intercalates into DNA, and thus can be oriented in the anisotropic DNA–surfactant films. Similarly, it has been reported[100] that incorporating sulforhodamine (SRh), a laser dye, into the DNA–surfactant films results in an amplified photoluminescence. The intensity is significantly higher than that of incorporating SRh into poly(methyl methacrylate) (PMMA), a typically transparent polymer host. Moreover, enhanced photoluminescence was also confirmed in the case of 4-(4-[bis(2-chloroethyl)amino]styryl)-1-methylpyridinium tosylate (BASPT),[101] a highly multiphoton-active lasing dye. These publications have adequately shown the feasibility of DNA as a photonic material with enhanced properties.[96]

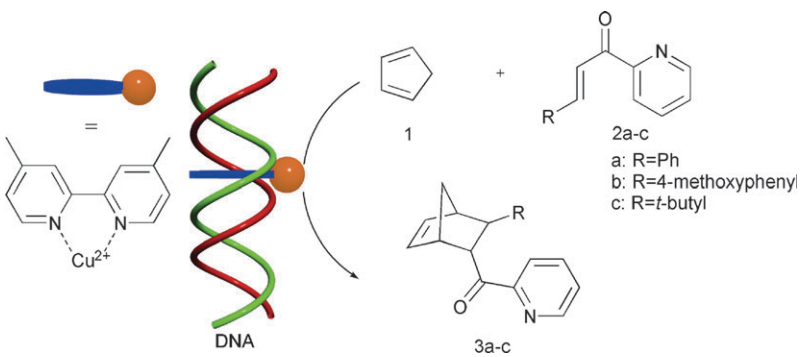

Fig. 13 Schematic representation of the asymmetric Diels–Alder reaction catalyzed by copper complexes intercalated in DNA (based on ref. 93).

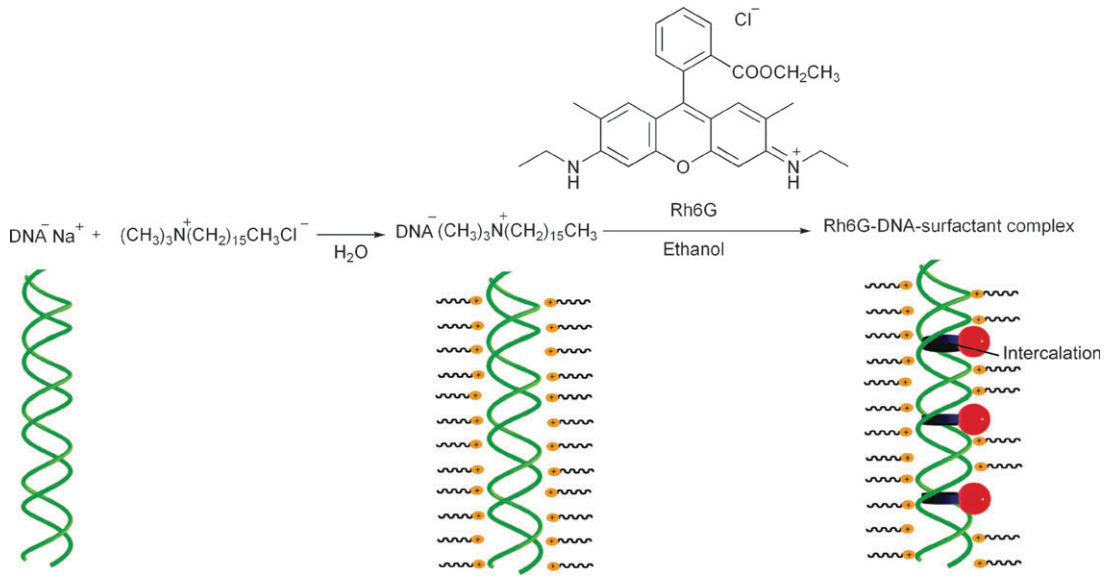

Fig. 14 Schematic diagram for the preparation of the Rhodamine 6G–DNA surfactant complex. The fluorescent dye intercalated in DNA causes an amplified spontaneous emission (based on ref. 97).

Furthermore, promising DNA based photonic devices with significant improvement in performance have been reported.[102,103] Steckl and co-workers have investigated the utilization of DNA thin films as electron blocking layers (EBL) in photonic devices, indicating that the DNA layer exhibits significant enhancements in luminance and luminous efficiency compared to conventional (organic light emitting diodes) OLEDs without the DNA layer. Although the exploitation of DNA in photonic applications is in an initial stage, it is expected that more interesting and innovative developments will appear in the future.

3.4 Other applications of DNA affinity

As mentioned above, DNA has the unique feature to bind small molecules with excellent selectivity, and DNA itself can be ordered by various simple methods.[54–56,104] Therefore, it is conceivable that DNA could be used as a carrier to arrange small functional molecules. This concept was demonstrated by Morii *et al.*,[105] who aligned several familiar DNA-binding dyes such as acridine orange, ethidium bromide, and Hoechst 33 258 in a specific orientation, by mixing them with DNA and subsequently drying the solution in a horizontal magnetic field. Furthermore, using DNA as a carrier to arrange porphyrins, which have large π-conjugated systems, and which have been investigated for photochemical and electronic applications, has been reported.[106] The experimental results revealed that the flat porphyrins interact with DNA and the primary binding mode is intercalation. The porphyrin-containing DNA films are expected to find applications as molecular devices with photonic functions in nanotechnology.

Moreover, chemicals that are in part planar can intercalate to DNA and thus have been used to immobilize DNA on certain surfaces. Shimomura *et al.* succeeded in immobilizing DNA on a Langmuir–Blodgett monolayer at the air–water interface[107] by using octadecyl acridine orange, an amphiphilic intercalator. Later, Nakamura and his co-workers reported a protocol for immobilizing DNA on a monolayer containing anthryl groups.[108] Maeda and co-workers introduced DNA intercalators into vinyl derivatives, and immobilized DNA in hydrogels by a general polymerization.[109,110] Recently, Kelley and co-workers[111] described a successful DNA-surface immobilization achieved by an intercalation between DNA and ethidium derivative linkers on a film surface.

4. Biocompatibility

Natural DNA has several suitable characteristics for biomaterials. Biocompatibility with humans is the primary advantage of natural DNA in biomedical applications. Because the molecular structure of DNA in vertebrate species is homogenous,[112] unlike other biopolymers such as proteins and sugars, it has no or low immunogenic properties, so that may limit both innate and acquired immune responses[113] in the human body. Second, DNA has the advantage in its capability of binding small molecules, which means that some pharmacological molecules can be attached to DNA *via* electrostatic interaction, groove binding and intercalation. This characteristic has made DNA-based materials beneficial for loading drugs. Third, DNA is degradable in the human body, thus greatly increasing its serviceability for medical devices and controlled drug-release systems. Actually, natural DNA combined with other materials has been tried for several medical applications, including improved compatibility of medical devices, dental and medical implantology, and cell cultures.

For example, DNA has been used to modify PSf membranes by blending or immobilizing DNA on its surface.[114,115] Blood compatibility is a common problem of PSf when it is used as a hollow fiber in hemodialysis. However, by combining with salmon DNA, it was reported that the surface of the hybrid membrane became more hydrophilic. A blood-adsorption experiment showed that the number of platelets adhered on the surface of the DNA-blended PSf membranes was reduced

as compared to that on the PSf membrane alone, suggesting that the DNA–hybrid membrane has better blood compatibility.

Furthermore, using DNA coatings to improve the biocompatibility of biomaterial surfaces has also been explored by Jansen and co-workers, who fabricated multilayered DNA-coatings by LbL self-assembly, and using poly-D-lysine or poly(allylamine hydrochloride) as the cationic counterparts.[116] In order to evaluate the biocompatibility of the DNA coatings in implantology, both *in vitro* and *in vivo* experiments were carried out. The *in vitro* experiments of rat primary dermal fibroblasts (RDF) revealed that the presence of multilayered DNA-coatings do not affect RDF cell viability and morphology but increase proliferation.[116,117] Further, the results of *in vivo* rat model experiments revealed that the presence of the multilayered DNA-coating did not induce any adverse effects in terms of inflammation and healing of wounds.[117,118] Additionally, when the multilayered DNA coatings were immersed in simulated body fluids (SBF), it was observed that the deposition of calcium phosphate was enhanced on multilayered DNA coatings as compared with non-coated controls, and it was found that the SBF-pretreated DNA coatings affected the differentiation of osteoblast-like cells through an increased deposition of osteocalcin.[119] Furthermore, these DNA coatings can be functionalized by biological active reagents, as was done in the following two cases: bone morphogenetic protein 2 (an osteo-inductive factor[120]), and vascular endothelial growth factor (which affects endothelial cells through positive effects on proliferation, migration, tubule formation, survival, and integrin expression[121]). In other group's works,[122] multilayered DNA and poly-D-lysine complex films were reported to be biodegradable. About 90% of DNA within the film was released after an enzymatic incubation with chymotrypsin in PBS for 35 h.

DNA–chitosan bilayer membranes were investigated for treatment of wounds, and the results showed that the membranes will adhere to rabbit peritoneum tissue.[123] Another study demonstrated that DNA–chitosan complexes exhibit no cytotoxicity for MG-63 osteoblast-like cells *in vitro*, and cause only a mild tissue response when the complexes were implanted in rats.[124] Furthermore, DNA–cationic surfactant films[125] were reported to have positive biological properties as well. When the DNA–surfactant films were implanted in rats, there were no inflammatory reactions or inhibition of new tissue formation.

Finally, given that DNA is digestible in the small and large intestines but not in the human stomach, using natural DNA to protect orally administered drugs from gastric acidity has been explored.[126,127] Lactic acid-producing bacteria, which are very sensitive to gastric acidity, were mixed into a complex gel system containing DNA, gelatin and carrageenan. Due to the electrostatic interaction of DNA and the amino groups of gelatin, this gel system was not damaged in simulated gastric juice but was destroyed in simulated intestinal juice. As a result, large numbers of bacteria survived after an incubation in simulated human gastric juice. This gel system thus provided an effective oral delivery method with a high protective capability.

5. Conclusions

In this review, we have presented an overview of the advancement in the utilization of natural DNA. DNA is a well-known biopolymer serving as a carrier of genetic information in most living organisms, and is a biomacromolecule with a special double helix structure. Although natural DNA is abundant in nature, its utilization has not been researched thoroughly. Many DNA resource materials are simply used as feed or waste because the potential of DNA is poorly understood. Research on the utility of natural DNA started in the mid-1990s,[60,128] and a tremendous progress in this new field has been achieved. Currently, natural DNA has been applied in electronic, optical and biomaterials, as a catalyst for enantioselective reactions, and material for cleaning the environment, and a wide variety of papers have been published. These applications appear to depend on a few fundamental properties of DNA: the electrostatic property that makes DNA as a helical linear polymer, the selective adsorption of small molecules, and biocompatibility. On the other hand, the use of natural DNA can also be summarized according to several basic concepts, including (i) using DNA as a template to fabricate highly ordered nano-scale structures, such as DNA-based nanowires,[25,28,29] and DNA–cationic surfactant films;[60,61] (ii) using DNA as an adsorbent to remove harmful chemicals that have an affinity to DNA;[83,89,90] and (iii) using DNA to improve biocompatibility.[114,117,119,121,127] Besides, there are two new concepts implied in recent publications, which perhaps can open new avenues for further applications of natural DNA. The first is using DNA as an asymmetric catalyst to synthesize compounds with high enantioselectivity.[92–94] It is expected that this concept can be expanded to a wide range of reactions. The second is using DNA to fabricate photonic devices with enhanced properties.[96,100–103] It has been found that the combination of natural DNA and an intercalated dye enhances light emission which has extremely interesting potential for photonic applications.

In conclusion, such recent developments in this field offer promising new opportunities. It is still a great challenge to develop practical uses of natural DNA. We hope that this article will stimulate future research into the use of natural DNA for various new applications.

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