DNA as a Supramolecular Scaffold

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INTRODUCTION

Since the elucidation of the structure of DNA in 1953, a revolution has occurred in molecular biology that recently culminated in the sequencing of the entire human genome. However, the properties that define DNA as a macromolecule with the most essential role in biology are also of tremendous importance in the design and understanding of DNA as a structural component in nanotechnology. After all, it could be argued that DNA is the ultimate supramolecular architecture. In this article, we describe the structural features of DNA that allow application as what promises to be one of the most fundamental supramolecular scaffolds. In describing this, we discuss the importance of molecular recognition and DNA binding in the design of synthetic DNA architectures, diagnostic agents, and the growth of new DNA-hybrid materials. The understanding of how DNA acts as a template, both to allow its own replication and to assemble cations, is also vital, as is an appreciation of topological concepts in the design of new materials. Finally, we describe how these ideas were applied to the design of DNA-based architectures for nanolithography, DNA machines, molecular wires, and as chiral memory devices.

DNA STRUCTURE

Considering its integral importance to life, the structural components of deoxyribose nucleic acid (DNA) are remarkably simple. Each strand of DNA is a linear polymer comprising a constant backbone of alternating deoxyribose sugars and negatively charged phosphodiesters, and a selection of four heterocyclic bases (Fig. 1). The uniqueness of the DNA strand is defined purely in terms of the sequence of the bases.

Two polymeric nucleic acid strands are held together by Watson–Crick hydrogen bonds (2.8–3.0 Å) between two bases to form a double helix. Adenine and thymine base pairs hydrogen bond together via two interactions, and guanine and cytosine through three. The conformation of the base pairing defines the mutual positions of the two sugar–phosphate strands in DNA. Hydrophobic and van der Waals interactions as well as π stacking between sets of base pairs also contribute to the stability of the overall structure.

Although the individual bases are planar heterocycles, considerable flexibility is conferred upon the entire structure from a number of contributing factors. Base pairing, for example, can deviate from planarity by up to 35° to minimize steric interactions without appreciable loss of hydrogen-bonding energy.[1] Furthermore, there are 12 different torsion angles associated with the sugar–phosphate backbone that can adopt a range of values, and hence, can have significant effects on the overall structure of the oligonucleotide. Linking the bases to the deoxyribose sugars is a glycosidic bond, which can have a torsion angle (χ) dependent on the orientation of the base in relation to the sugar. The sugar has a nonplanar ''puckered'' conformation dependent on its five internal torsion angles (τ_0–τ_4). The relative populations of the conformations arise from the type of base attached, and in solution, it is possible for these to interconvert, which alters the orientation of the substituents and, hence, the backbone of the entire structure. The final six variable torsion angles (α–ζ) define the conformation of the phosphodiester backbone. Steric restrictions prevent large angular variations between 0–360°; however, many possible low-energy conformations of the oligonucleotide exist.[2]

As a function of both flexibility and restraint, the overall structure of DNA can adopt several polymorphs, including left- and right-handed helices. Four major secondary structures of DNA are known; A-DNA, B-DNA, and triple helices are right-handed, and Z-DNA structures are left-handed[3] (Fig. 2). The A-conformer is the most compact, being wider and having a helical rise of 11 bases (25.6 Å). Z-DNA is composed of alternating purine-pyrimidine nucleotides, giving the structure a zigzag appearance and has a rise of 12 bases (37 Å). A triple helix, comprising one purine and two pyrimidine strands, results from a ''base triplet'' hydrogen-bonding system. Its dimensions are similar to those of A-DNA, with the second pyrimidine strand lying in the grooves formed by a pyrimidine and a purine strand.

B-DNA is the most common polymorph due to its chirality and geometry along the sugar–phosphate backbone. The overall width of the helix is approximately 20 Å and completes one turn every 10 bases (34 Å).[4] As with

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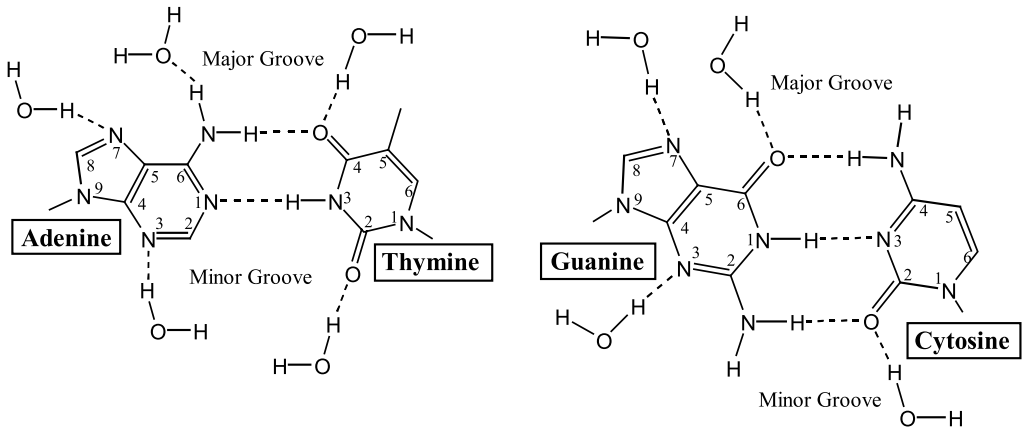


Fig. 1 Base-pairing of DNA nucleotides.

each double helix, the asymmetry of the base pairs results in major and minor grooves along the helical axis, with dimensions that are related to the distance and orientation of the base pairs in relation to the axis. The major groove is approximately 13 Å wide and 8 Å deep, whereas the minor groove is 4.5 Å and 6 Å, respectively. The minor groove has hydrophobic hydrogen atoms of the sugar groups forming its walls. The major groove is richer in base substituents, having the O_6, N_6 atoms of the purine moieties and the N_6, O_4 atoms of pyrimidines available.

DNA BINDING

The two major ways in which DNA interacts with other entities can be classed as nonspecific covalent bonding via either the phosphodiester or sugar moieties or electrostatic dominated noncovalent bonding. Nonreversible covalent interactions are important when considering the cellular processing of many drugs, but it is the ability to form noncovalent interactions that defines DNA as the ultimate supramolecular building block.

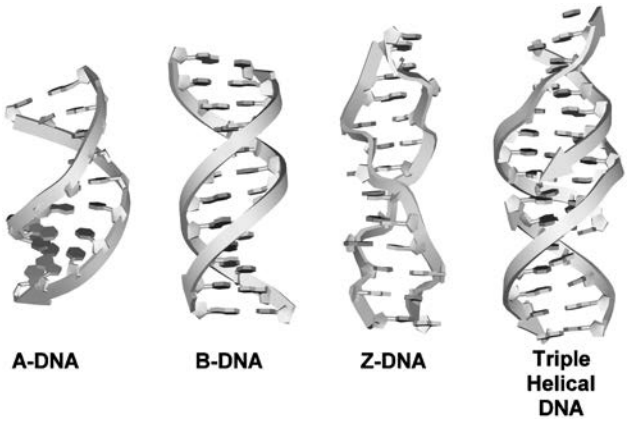


Fig. 2 The common polymorphs of DNA. (*View this art in color at www.dekker.com.*)

Noncovalent interactions with DNA occur via two binding modes: groove binding and intercalation. Much research has been carried out to exploit the properties of DNA in the design of targeted intercalating and groove-binding drugs in such areas as anticancer, antibacterial, and antiviral agents, and in the postgenomic era, the need for a deeper understanding of how DNA interacts with other molecules is becoming more apparent. However, analyses of the integral features of the structure of DNA allow a much more comprehensive view of its noncellular potential.

Conventionally, the structures of the molecules designed for each binding mode followed certain distinctive criteria.[5] Boundaries are now being extended and becoming less clear, and an ever-increasing number of physical and chemical techniques are emerging to aid in the determination of the structures, sequence specificity, energies, and binding stoichiometries of these noncovalent interactions.[6] The specific binding mode of interaction can now be determined by a combination of such techniques as x-ray crystallography, NMR, gel footprinting, circular dichroism, and fluorescence, UV, IR, and mass spectroscopy.

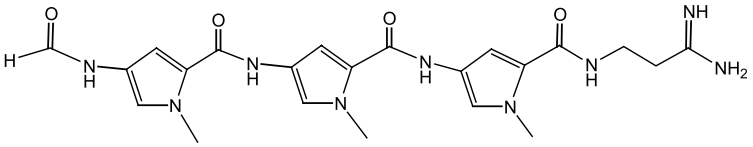
In the biological environment, it is important to understand the processing of the genetic code stored in the DNA. The ability to stimulate or prevent this processing is of great importance in terms of molecular biology and drug design, and also in terms of supramolecular chemistry, as the noncovalent interactions involved in such ''switching'' mechanisms are of great interest. These mechanisms involve a variety of molecular tools in order to recognize the genetic code in a sequence-specific manner. Biology achieves this through the surface motifs of proteins, which are too large to fit into the minor

groove and instead interact in or around the major groove.[7] This acts as a particularly attractive macroreceptor site for DNA recognition agents, as the dimensions of the major groove are dependent to a large extent on the base sequence and so can be seen as a molecular ''lock'' that is activated by a highly specific recognition ''key.'' For example, distortion of the DNA backbone is activated by transcription regulators that incorporate cylindrical binding units such as α helices or zinc fingers that insert into the major groove.

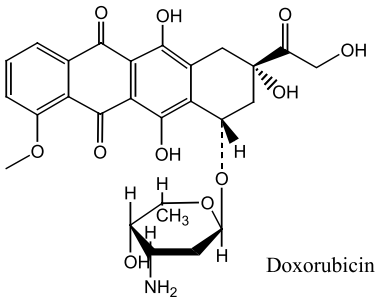
It is possible to mimic the biological mechanisms used to bind DNA. For example, oligonucleotides of natural and synthetic origins are capable of forming triplexes by sequence-specific binding to the major groove, and the area of metallosupramolecular assembly can provide cationic charge around the metallocenters, which confer a substantial electrostatic contribution by interactions with the anionic sugar–phosphate backbone.

Such large arrays of macromolecular recognition contrast sharply with the mode of DNA binding of small molecule recognition agents. Typically, cationic at physiological pH and much smaller than proteins, these agents are able to bind preferentially to the minor groove, where the electrostatic potential is negative, displacing the hydrogen-bonded water molecules. Strong electronic interactions along with the formation of significant van der Waals contacts make the complex stable, with dissociation constants in the order of 10^{-5} M^{-1}. The natural antibiotic distamycin A (Fig. 3) and its derivatives are well-established reversible minor-groove binding agents. It incorporates an oligopeptidic pyrrolic framework with a terminal amidino moiety and is highly selective for A·T-rich regions containing more than four A·T base pairs. Typical minor-groove binders include an extended ring system with unfused rings, although recent developments in sequence-dependent DNA binding included ligands with polyaromatic nuclei. These conjugated systems form strong hydrophobic contacts with the sugar-containing walls of the minor groove. Increased interactions can be introduced through hydrogen-bonding moieties to improve the affinity of the groove binders for G·C-rich regions. Derivatives of such groove binders as distamycin A can be used for alkylating and nonalkylating agents in anticancer therapeutics.[8] It is believed that the ability of small molecule recognition agents to alkylate DNA in a sequence-specific manner and modify the function of nucleic acids irreversibly will increase the specificity for target cells.

In general, groove binding effects only subtle changes in the overall structure by bending or kinking the DNA. Intercalation, on the other hand, has a much more dramatic structural effect in terms of helical twist and compression. Intercalators are typically polycyclic aromatic compounds that bind reversibly to the DNA double-helical structure by inserting into the central hydrophobic region of the helix between the stacked base pairs. These chromophores are conventionally two or three six-membered rings, for example, doxorubicin (Fig. 3), of approximately the same size as a base pair. This means that, in addition to π-stacking interactions between the intercalator and the adjacent base pairs, strong van der Waals contacts further contribute to the stability of the complex. There are also a number of intercalating agents containing side chains that play vital roles in both the stablization and sequence specificity of interactions with DNA by binding in the minor groove.[9]



Distamycin A



Doxorubicin

Fig. 3 Minor groove binder distamycin A and intercalator doxorubicin.

The process of intercalation demands an alteration of the α–ζ torsion angles along the sugar–phosphate backbone in order to accommodate the aromatic ring system. Rotation around these torsion angles results in a separation of the base pairs and a subsequent extension of the helical axis (approximately 3.4 Å per bound intercalator) and ''unwinding'' of the two DNA strands. The extent of the unwinding is dependent on the individual intercalator. Polyintercalators are well known,[10] containing two or more aromatic ring systems bridged by appropriate linker chains that facilitate simultaneous intercalation along the DNA strands and the potential for increased sequence specificity.

DNA AS A TEMPLATE

DNA as a helical anion can be viewed as a macromolecular template. The fact that DNA is comprised of two self-complementary strands of base pairs is responsible for its ability to replicate upon dissociation of these strands. In fact, the replication of DNA via a templating effect to form a complementary strand aided by Watson–Crick base pairing is linked to the central dogma of molecular biology.[11] By utilizing a self-complementary and ''self-templating'' molecule such as DNA, the emergence of life and the possibility for evolution was realized. This is because the use of a double helix of DNA, containing two identical copies of genetic information, allowed the development of an exquisite DNA repair mechanism. Furthermore, the supramolecular coiling of DNA in the chromosomes of a cell is the key to cellular development via protein expression; this is thought to occur through a kind of unwrapping process in which DNA is the key molecule.

The anionic nature of DNA also has important implications in the construction of virus capsids, in which the protective capsid is either assembled around the DNA from a large number of repeating protein building blocks or preformed for DNA insertion. This self-assembly process is partly responsible for the fast spread of viral infection, as many viruses can spontaneously assemble from a soup of DNA and protein-based building blocks.[12] The ability to manipulate the structure of the DNA duplex by altering the code should therefore affect the ability of DNA to coil in chromatin formation as well as gene therapy vector design and in the manipulation of virus structure. It was shown that DNA can also direct the assembly of small molecular units that can undergo a template-driven polymerization process.[13] The covalent casting of noncovalent architectures serves to determine large molecular constructs that express well-defined modes of aggregation. In the case of one-dimensional hydrogen-bonding motifs, covalent casting yields molecular strands that adopt a duplex mode of aggregation.

It is well known that nucleic acids are capable of promoting chemical reactions, and catalytic nucleic acids were found in nature or evolved through in vitro selection processes. Interestingly, it appears that the DNA acts in a similar manner to enzymes and promotes chemical reactions through spatial alignment of the reaction partners; i.e., via a template effect.[14] Recent reports reveal new facets of nucleic acid promoted chemistry and indicate that DNA strands are able to promote chemical reactions by bringing reaction partners into close proximity of each other rather than by precisely aligning the reactive groups. Therefore, it appears that DNA-templated synthesis is a phenomenon that applies to a remarkable variety of chemical reactions (Fig. 4). The ease of automated DNA synthesis, predictable binding, and established methods for nucleic acid amplification and sequence analysis render DNA a promising template for future developments.[15]

DNA TOPOLOGY

Research into the application of noncovalent metal–ligand interactions to direct self-assembly resulted in a number of interesting topological structures.[16] It is well established that DNA topology is a crucial factor in many biological processes, such as topoisomerism and DNA supercoiling. Highly ordered DNA topology is also of considerable interest with respect to the

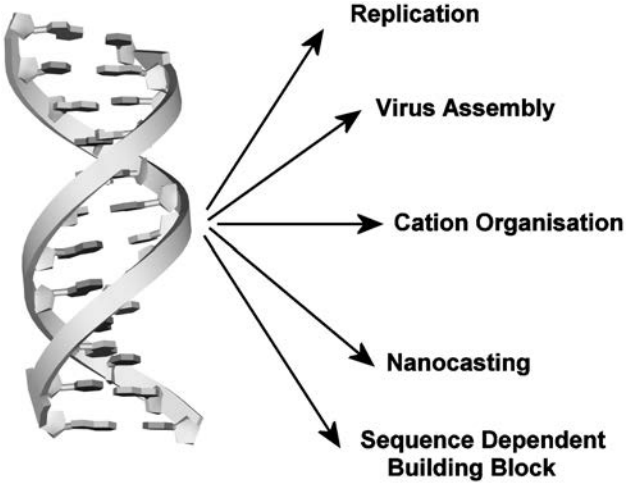


Fig. 4 Examples of DNA chemical reactions. (*View this art in color at www.dekker.com.*)

design of sequence-specific DNA binding agents, allowing targeted recognition and precise regioselectivity along the polymeric strands. The crystallographic motivation for such developments lies in the desire to organize DNA in a periodic array in order to provide a scaffold on which other macromolecules, which are difficult to crystallize by conventional methods, may be arranged.

The first-known topological polymorph of DNA, discovered in 1967,[17] was the DNA catenane consisting of two interlocked rings of circular DNA. It was recognized that these structures had important implications in the cellular functioning of DNA. If, during the replication of circular DNA, a catenane structure is formed upon the interlocking of parent and daughter molecules, continuation of the replication process is impeded. Topoisomerases are a class of enzymes capable of changing the topology of DNA by an incision and repair mechanism. Once the DNA strand is broken, another strand may pass through the rupture, and then repair mechanisms reseal the original strand. Type I topoisomerases effect topological transformations during the disruption of one DNA strand, and Type II topoisomerases are capable of breaking two strands.[18] The design of

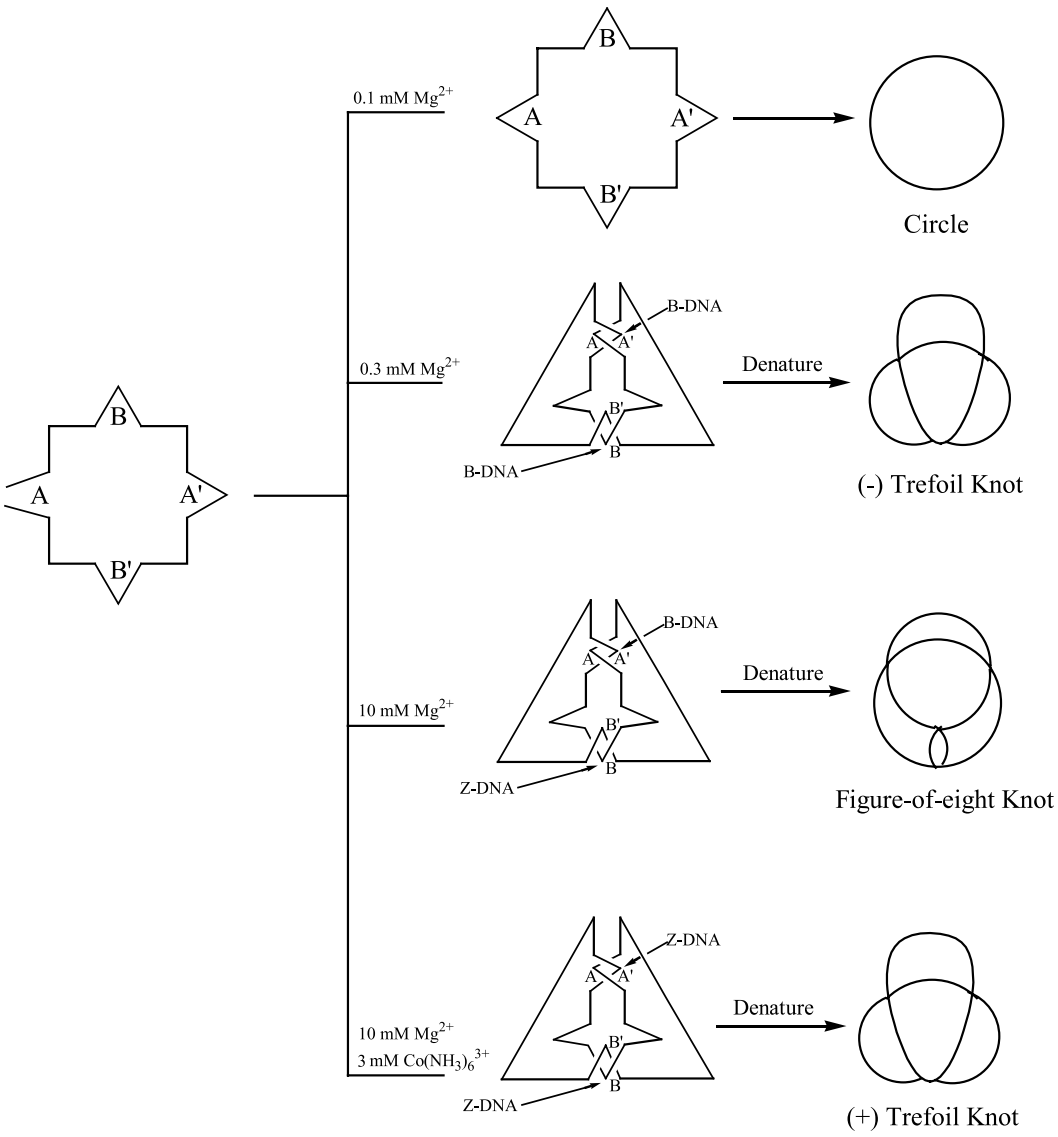


Fig. 5 Seeman's synthesis of DNA knots. (From Du, S.M.; Stollar, B.D.; Seeman, N.C. A synthetic DNA molecule in three knotted topologies. J. Am. Chem. Soc. **1995**, *117*, 1194–1200.)

topoisomerase-inspired synthetic agents could provide a much desired control of highly ordered topology in the area of nanotechnology.

The replication of DNA is controlled by its topology in another vital process known as supercoiling. Topological isomers of circular double-stranded DNA can be made by incision of the duplex and twisting of the strands through 360° before repairing the incision. The overall three-dimensional structure of the DNA undergoes a significant change in the number of helical twists, and a compression along the helical axis in comparison to the uncomplexed B-DNA is observed.

In addition to naturally occurring complex helical DNA topologies, it is also possible to design topologically interesting structures with linear DNA. The differences in chirality between right-handed B-DNA and left-handed Z-DNA duplex formation as well as engineered effects of specific base-pair complimentarity facilitate the formation of a number of molecular knots from a single-stranded DNA oligomer as a function of salt concentration (Fig. 5).[19,20]

Other remarkable complex structures were assembled from both single strands, for example, the DNA ''padlock,''[16] and multiple strands, such as the DNA cube[21] and a truncated octahedron.[22] The formation of a linear double-helical structure from two strands of oligodeoxyribonucleotides is the most energetically favorable, and as such, it would appear that other configurations are unlikely. However, the principles of genetic recombination, a biological process forming new genetic material from the interaction of two pieces of DNA, can be applied to generate novel, topologically interesting architectures. A key structural intermediate in the recombination mechanism is the Holliday junction,[23] which joins four double helices in a branched molecule. The sequence of nucleotides that converge at the junction is generically twofold symmetric, which allows isomerization of the branch point to the region of symmetry. This is not, however, crucial to the formation of DNA branched molecules, and so it is possible to develop junction structures with fixed branch points.

DNA, MOLECULAR ARCHITECTURE, AND NANOTECHNOLOGY

A significant challenge faced in the use of nanoscale building blocks lies in developing parallel methods for interconnecting and patterning assemblies of the individual components. Molecular scaffolds based on the structure of DNA have the potential of preparing closely spaced, specifically arranged nanoscale assemblies. The ease in which DNA can be synthesized and manipulated means that it is an ideal building block for nanostructured materials.[24] Furthermore, the fact that DNA can interact with other molecular assemblies in a sequence-specific manner means that DNA is a perfect functional scaffold for application in nanotechnology as a molecular device.

The ability to cause DNA to branch is being used in the design of new molecular devices and materials. Such DNA branch junctions are often found in biology; they occur as ephemeral structures in cellular DNA metabolism, including the processes of replication and repair. A particularly significant structure in such systems is the four arm branched Holliday intermediate crucial to the process of genetic recombination.[23] It is fairly simple to design sequences of DNA molecules that lead to stable synthetic variants of Holliday junctions, branched molecules with varied numbers of arms, as well as more complex motifs; it is also elementary to synthesize their constituent strands and engineer subsequent self-assembly of target systems. The use of branched intermediates allows us to make *N*-connected objects from DNA as well as periodic and aperiodic arrays. Similarly, branched DNA motifs were the basis for several nanomechanical devices.[25]

Further applications of the DNA scaffold are as molecular wires in nanoelectronic circuits, facilitated by

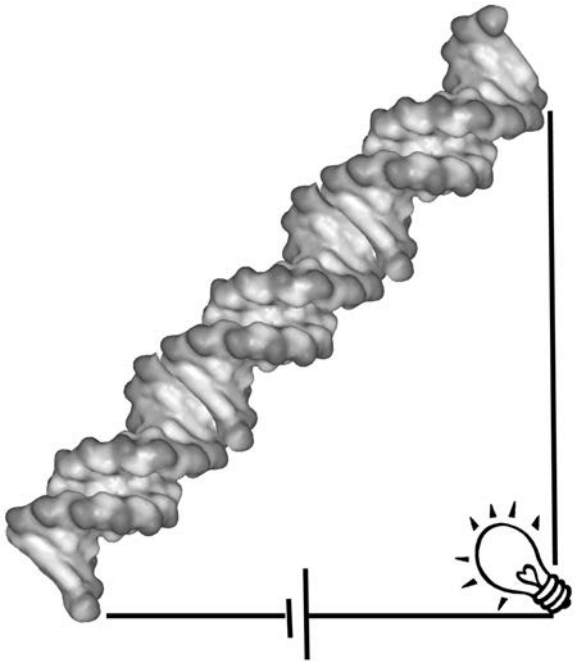


Fig. 6 DNA as a conducting molecular wire. (*View this art in color at www.dekker.com.*)

the fact that electron-transfer processes appear to be mediated by DNA via the π–π-stacked arrangements of the base pairs.[26] This idea, although still the subject of controversy, appears to be limited by recent observations that such events can only occur over small distances.[27] However, DNA has now been used as a template to provide a surface that can be coated with metal atoms to form a conducting or semiconducting sheath.[28] Therefore, should the conducting properties of the polymeric DNA be proven to be insignificant, it appears possible to use the linear framework as a scaffold to assemble or grow a conducting sheath (Fig. 6).

DNA can also be used as a template for the assembly of extended, close-packed nanoparticle structures. Electrostatic binding of ligand-stabilized nanoparticles to the DNA backbone results in extended linear polymers of ribbon-like structures composed of parallel nanoparticle chains and branched structures. Furthermore, the functionalization of DNA with gold nanoparticles led to the design of a DNA array detection method.[29] As such, DNA shows tremendous potential for a variation of biomolecular nanolithography, and the arrangement of nanoscale building blocks on biomolecular scaffolds is a viable approach to interconnecting individual devices into extended, close-packed assemblies.[30]

CONCLUSION

Perfected by nature, the cellular functioning of DNA is an important model for the design of binding agents and therapeutics. However, the potential of such a splendid example of supramolecular architecture as a prototype for the future development of advanced and functional supramolecular scaffolds is now being realized.

ARTICLES OF FURTHER INTEREST

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