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Nucleic acid nanotechnology—towards Ångström-scale engineering

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Received 3rd November 2003, Accepted 25th November 2003
First published as an Advance Article on the web 22nd December 2003

Nucleic acids and analogues are suitable building blocks for reliable self-assembly of nanometer-sized two- or three-dimensional materials. In order to mimic or approach nature with respect to size and function, Ångström-scale chemical engineering is emerging as pivotal for future developments. Efforts within nucleic acid nanotechnology will be focussed on generating rigid and stable low nanometer-sized structures carrying functionalities with predictable spatial positioning allowing, by encoded self-assembly, functional nucleic acid architectures to be built towards applications within the biological and material sciences.

Introduction

An increasing number of researchers within nanoscience are using nucleic acids as building blocks in the bottom-up approach towards novel materials.[1–3] This development has mainly been stimulated by the predictable self-assembly of complementary nucleic acids strands by Watson–Crick hybridization, the well-defined dimensions of nucleic acid duplexes, and the opportunity of functionalization by conjugation. This research has led, *e.g.*, to advanced nucleic acid nanostructures and devices,[2,4] semisynthetic DNA–protein conjugates,[5] and efficient assembly of individual oligonucleotide-functionalized nanoparticles into two- and three-dimensional networks.[6] Some of these assemblies have proven useful for diagnostic purposes,[5,6] and construction of nanometer-sized DNA-based nanowires has been accomplished.[7–9] Rapid development will continue towards technologies based on nucleic acid nanowires generated by metallization processes[7–10] or on the use of oligonucleotides to bridge spherical nanoparticles.[6,11,12] As an example of the state of the art, successful self-assembly of DNA-based two-dimensional networks into functional scaffolds for protein arrays and highly conductive nanowires was recently demonstrated (Fig. 1).[9]

† A research center funded by the Danish National Research Foundation for studies on nucleic acid chemical biology.

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The need for Ångström-scale architectures and building blocks

The research focussed on construction of two- or three-dimensional DNA-based assemblies has in general so far resulted in relatively large objects (>50 nm dimensions) without precise control of size and shape and/or without built-in function.[2,3,5,13–18] Thus the necessary resolution to mimic nature-like engineering, *e.g.*, enzyme size (~10 nm dimensions) and function, has in general not yet been achieved. In this article I will focus on the architectural needs, the state of the art, and the immediate challenges for chemists in this area with emphasis on the importance of achieving *Ångström-scale* (1 Å = 0.1 nm) *structural control* with the goal of obtaining *functional low nanometer-sized materials and components*. The current size limit of lithographic techniques is already the impressive 50–100 nm, and nanolithographic and nanomanipulation methods are continuously being further refined.[8,19–21] Therefore, the bottom-up approach should now approach the Ångström-scale in order to take advantage of the power of precise control of the three-dimensional placement of the building blocks and chemical functionalities. The obvious molecules for such architectures are nucleic acids and analogues thereof. These can be foreseen to play an important role towards achieving *nano-sized assemblies* with, *e.g.*, the following *functional characteristics* and applications:

- Receptor function for small and large molecules—towards biosensors and devices for drug delivery
- Function as artificial enzymes—towards nanometer-sized synthesizers
- Triggered conformational mobility—towards nanorobotics
- Optimized charge transport through nucleic acids—towards novel and efficient DNA-based diagnostics with electrical readout
- Function as ligands—towards novel drug classes based on nucleic acid scaffolds

A plethora of chemically modified nucleotides[22,23] and single- or double-stranded oligonucleotides functionalized with

DOI: 10.1039/b313986g

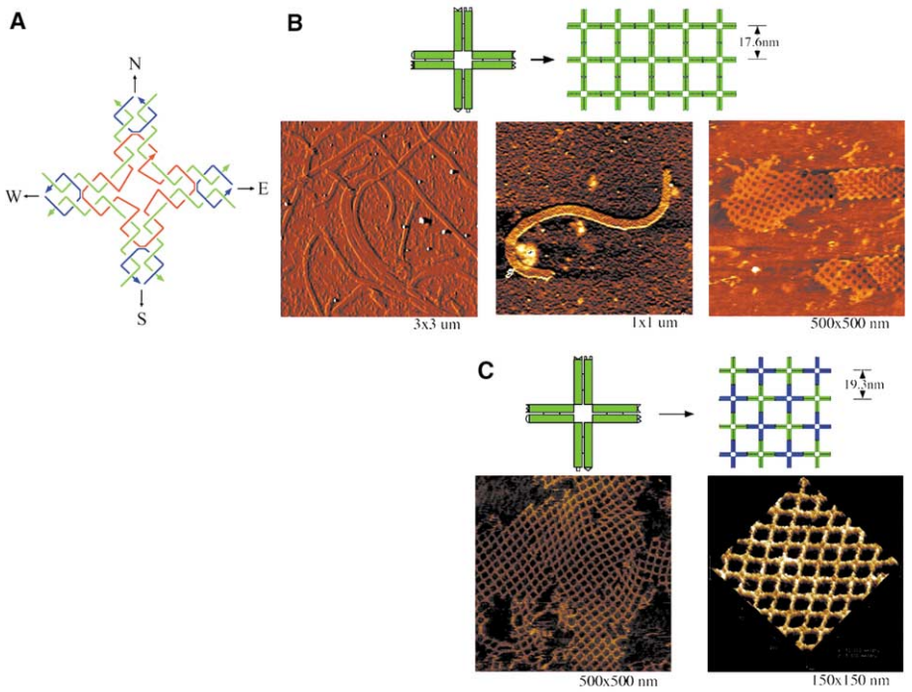

Fig. 1 Self-assembly of DNA nanoribbons and nanogrids. (**A**) The tile strand structure composed of nine oligonucleotides. (**B**) Self-assembled nanoribbons. (**C**) Self-assembled nanogrids. Protein arrays and conductive nanowires were similarly assembled using biotin-functionalized or metallized nanoribbons, respectively.[9] Reprinted with permission from *Science*, 2003, **301**, 1882–1884. Copyright 2003 AAAS.

lipophilic groups or peptides[24,25] have been intensively studied in an attempt to optimize the biological activity of antisense oligonucleotides. Out of this contemporary research, many chemical developments and nucleotide modifications have emerged that will be pivotal for future progress within nucleic acid nanotechnology. As an example, efficient conjugation of carbon nanotubes to DNA and PNA has been achieved, and the ability of these covalently bonded adducts to hybridize to nucleic acid complements has been verified.[26,27] However, also this important and innovative research has resulted in relatively large non-uniform systems.

Towards Ångström-scale engineering

Controlled self-assembly of mono-functionalized nucleic acid strands

Structural control has been obtained for systems assembled by hybridization of functionalized oligonucleotides, typically monofunctionalized, with complementary target strands used as scaffolds or templates. Niemeyer *et al.* successfully demonstrated the viability of this strategy by organization of several streptavidin-modified oligonucleotides by hybridization onto complementary strands.[28] A similar strategy was more recently used by Matsuura *et al.* to organize site-specifically and periodically glycosylated oligonucleotides,[29] an approach that can be used to probe and utilize the multivalent nature of many carbohydrate-binding proteins, and to generate new nano-sized gluco-clusters. Further examples along the same line include self-assembly of oligonucleotides derivatized with cyclobutadiene(cyclopentadienyl)cobalt complexes or phenyleneethylene units into oligomers,[30] and the formation of multicomponent dendrimers by hybridization between oligodeoxynucletides end-conjugated with polyester moieties.[31]

Template-directed synthesis

The known concept of DNA-templated synthesis has been explored by Liu and co-workers for encoded generation of small molecules in solution.[32,33] Impressive results have been obtained taking advantage of preorganization by hybridization betweeen two oligonucleotides functionalized with electrophilic and nucleophilic reaction partners, respectively,[32] and even multistep small-molecule synthesis has been achieved.[33] This work extends DNA-templated linking of oligonucleotide strands by non-natural linkages,[34,35] and its considerable scope is underlined by a recent report on DNA-templated copper-catalyzed ester hydrolysis.[36] In a longer perspective self-assembling nano-synthesizers can be envisioned that carry out multi-step combinatorial syntheses encompassing preorganization, catalysis, purification, trafficking of products, *etc.*

Assembly of artificial receptors

Generation of receptors by hybridizing individual oligonucleotide receptor units in the presence of a ligand of choice is an appealing concept, and the first efforts in this direction have been published. Stojanovic *et al.* have published ligand-driven assembly of heterodimeric receptors for small molecules with the individual units of the receptor being non-functionalized oligonucleotides.[37] Metal ion chelation has been achieved based on metal-ion mediated enhanced cooperativity of binding of two oligonucleotides onto an oligonucleotide template. Thus, the application of two salicylaldehyde-conjugated oligonucleotides, a DNA template, ethylenediamine and Mn^{2+} or Ni^{2+} led to the formation of metallosalen–DNA complexes (Fig. 2).[38] In

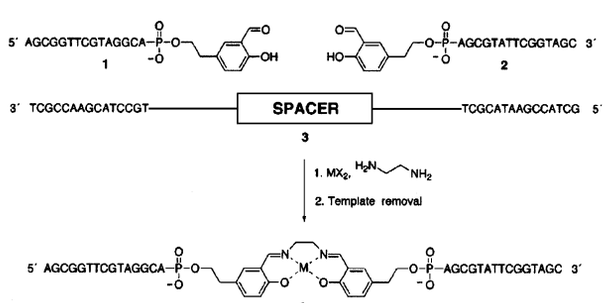

Fig. 2 Nucleic acid template-directed assembly of metallosalen–DNA complexes.[38] Reprinted with permission from *J. Am. Chem. Soc.*, 2001, **123**, 8618–8619. Copyright 2001 American Chemical Society.

another example, similar template-mediated preorganization by hybridization of two oligonucleotides conjugated with iminodiacetic acid at the 3′- and 5′-end, respectively, induced Gd^{3+} binding.[39]

In a similar direction, we have recently introduced oligonucleotide azacrown ether conjugates as molecular building blocks for self-assembling molecular receptors. Thus, the thermal stability of duplexes between two complementary oligonucleotides containing juxtaposed triaza crown ether units was increased by the presence of selected alkanediamine ligands.[40] A similar effect was recently reported by metal chelation.[41] Further development elaborating on ligand-driven assembly of oligonucleotide-based receptors can be foreseen, *e.g.*, with the inclusion of more radically functionalized oligonucleotide receptor units,[42] possibly utilizing combinatorial strategies. A similar need for additional functionality has been underlined in the closely related area of nucleic acid aptamers.[43]

Charge transfer in DNA and metal chelation

The transformation of nucleic acids themselves into a conducting material, by other means than metallization processes,[7–10] is currently being intensively studied and explored.[44–46] Efforts are directed towards the construction of double strands with metals ions placed on top of each other in the middle of the duplex taking advantage of metal binding nucleobases or base-pairing mediated by metal chelation.[47–49] It was recently shown that consecutive incorporations of several of the base pairs shown in Fig. 3 within a DNA : DNA duplex allowed the chelated Cu^{2+} ions to interact with each other.[49] In an alternative approach, formation of complexes between metal ions and nucleic acid duplexes is being studied and explored for the generation of nano-circuits by self-assembly.[50]

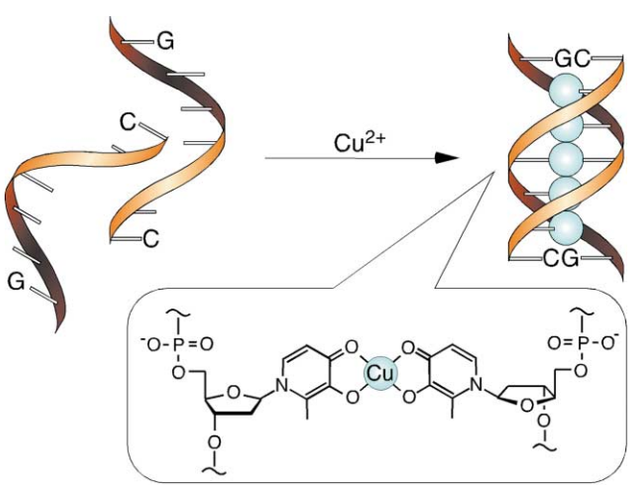

Fig. 3 Self-assembly of a metal array in artificial DNA.[49] Reprinted with permission from *Science*, 2003, **299**, 1212–1213. Copyright 2003 AAAS.

Chemical modification of the nucleobase or carbohydrate moieties of nucleic acids is another possible approach towards enhanced charge transport in DNA. The use of the redox-active flavin molecule instead of the adenine nucleobase has been proposed[45] together with the use of a linear pyranosyl pairing system,[51] and synthetic procedures in this direction have been published.[52] Alternatively, conformational restriction and increased nucleobase stacking could improve charge transport, and a conformationally restricted 1′-*C*,2′-*O*-methylene-linked RNA-like nucleotide has been studied in this context.[53] The outcome of these experiments has underlined the importance of nucleobase stacking and duplex conformations for charge transfer, and also the need for further chemical exploration to improve the charge transfer properties of nucleic acid derivatives.

Extensively functionalized nucleic acid architectures

The research described above involving metal binding nucleobases exemplifies the prospects of Ångström-scale molecular engineering towards functional assemblies within nucleic acid nanotechnology. In fact, the substitution of the nucleobase stack with other types of stacking molecular entities is a very active research area in which various systems are being studied for other purposes than diagnostic applications.[54–57]

Another approach for high-resolution entities is functionalization of the individual nucleotide building blocks, either in the nucleobase, the internucleoside linkage or the carbohydrate moiety. It should be stressed that maintenance of hybridization strength and fidelity irrespective of functionalization is a prerequisite, a fact that potentially becomes a limitation when considering the option of introducing multiple functionalities. The knowledge obtained from the research on antisense oligonucleotides is important in this context, as illustrated by two recent reports on extensive functionalization of the carbohydrate moiety.

In one report, Seeman and co-workers synthesized single-stranded 2′-thio-DNA containing di-functionalized 2′-*S*-(amino)alkyl or 2′-*S*-(carboxy)alkyl substituents. Subsequent internucleotide amide bond formation led to DNA strands with a covalently stitched nylon lining (Fig. 4).[58] Duplex formation was not evaluated but can be envisioned as shown schematically in Fig. 5, as can also extensive or selected interstrand cross-linking (a hypothetical example is shown in Fig. 4). Because rigidification and structural stabilization thereby will be obtained, implementation of covalent intra- and interstrand cross-linking strategies will become very important and generally applicable in the area of nucleic acid nanotechnology.

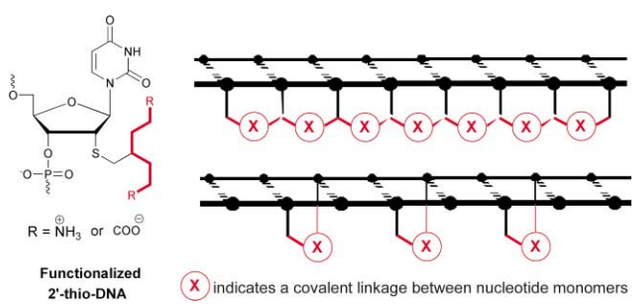

Fig. 4 Cross-linking using functionalized 2′-thio-DNA monomers.[58] Extensive intrastrand cross-linking using di-functionalized 2′-*S*-alkyl arms,[58] and hypothetical controllable interstrand cross-linking using mono-functionalized 2′-*S*-alkyl arms is illustrated.

In another report, we recently demonstrated the versatility of the 2′-nitrogen atom of 2′-amino-LNA monomers for functionalization of high-affinity oligonucleotides, *e.g.*, by studies involving the pyrenylmethyl monomer shown in Fig. 5.[59] Multi-functionalized oligonucleotides were able to hybridize very efficiently with complementary strands, and interstrand

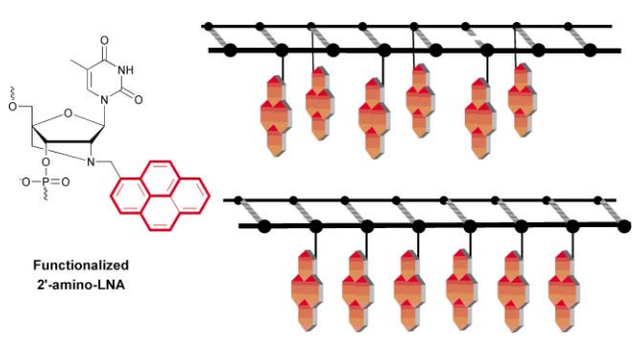

Fig. 5 Functionalized high-affinity duplexes and interstrand and intrastrand communication using 2′-*N*-(pyrenylmethyl)-2′-amino-LNA monomers.[59]

communication was documented by strong pyrene–pyrene eximer band formation (schematically illustrated in Fig. 5). This architecture appears well suited for organizing functionalities at the brim of the minor groove of A-type nucleic acid helices without interfering with base pairing.[59]

Outlook

Nucleic acids and analogs are well suited as scaffolds for design and construction of self-assembling functional entities with low-nanometer dimensions. Needed for future developments is reliable assembly of two- and three-dimensional rigid and stable structures carrying functionalities with predictable spatial positioning. In efforts to increase the stability, to extensively functionalize, and to modulate the conformation of the structures, an important role will be played by nucleotide modifications, *e.g.*, HNA[60,61] (hexitol nucleic acid; stabilizes A-type duplexes), LNA[62] (locked nucleic acid; stabilizes A-type duplexes), α-L-LNA[63] (*α*-L-*ribo* configured LNA; stabilizes B-type duplexes), PNA[64] (peptide nucleic acid), pyranosyl-RNA[51] (induces and stabilizes linear duplexes), and xDNA[65] (containing expanded size base pairs; widening and stabilization of duplexes). With their use, in combination with functionalized nucleotide monomers, and encoded self-assembly by duplex, triplex or quadruplex formation, the time has come for chemists to explore true Ångström-scale engineering to mimic nature and to generate unique functional nucleic acid architectures towards applications related to, *e.g.*, drug discovery, drug delivery, nanorobotics, nanoelectronics and nanocomputing.

Acknowledgements

The Danish National Research Foundation is gratefully acknowledged for financial support. Dr. Poul Nielsen, Department of Chemistry, University of Southern Denmark, is thanked for valuable comments on the manuscript of this article.

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