2nd Edition

Nanoscience & Nanotechnology Series

Bionanodesign

Old Forms for New Functions

Maxim Ryadnov

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Bionanodesign

Old Forms for New Functions

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Nanoscience & Nanotechnology Series

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Preface to the First Edition

The progress of today's science and technology encounters an increasing demand for finer and more efficiently performing materials with properties superior to those of current and hence ageing devices. Whether this concerns electronics or drug delivery, cancer diagnostics or alternative energy sources the search for means of miniaturising the existing materials or devising fundamentally new components with higher capacities appears to be relentless.

A saving solution to this is widely proposed as the design and fabrication of nanostructures, molecular architectures with dimensions featured below 100 nm.

By convention, and as originally formulated by Richard Feynman, the challenge of constructing macroscopic structures through the manipulation of individual molecules or even atoms prompted the emergence of a rapidly evolving field – nanotechnology. By definition, nanotechnology mirrors complex organisation at the nanoscale and is underpinned by a variety of related physical events that are combined into one universal process – molecular self-assembly.

The phenomenon of self-assembling molecules is attractive from both academic and application perspectives. However, preferential attention is being given to approaches whereby nanostructured materials or their components can be produced, moreover, produced at whim; that is, designed.

The pursuit for routes that can lead to rational or at least predictable design strategies invoked the main objective of this publication – to bring together contemporary approaches for designing nanostructures that employ naturally derived self-assembling motifs as synthetic platforms.

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Entitled *bioinspired nanoscale design* or *bionanodesign* the book is written in the shape of a review, referenced as fully as permissible within the context of biomolecular recognition and self-assembly, which forms a general trend throughout.

The volume is composed of three core chapters focusing on three prominent topics of applied nanotechnology where the role of nanodesign is predominant. Specific applications that arise from designed nanoscale assemblies as well as fabrication and characterisation techniques are of a much lesser focus and whenever they appear serve as progress and innovation highlights.

In this sense, the book takes a nonstandard approach in delivering the material of this kind. It does not lead straight to applications or methods as most nanotechnology titles tend to do, but instead it admits the initial and primary stress on "nano" rather than on "technology". The task is significantly eased by the cohort of brilliant bioinspired designs reported to date and complicated by the volume they create almost on a weekly basis. For this reason, the author apologises for the inevitable, but not necessarily deliberate, omission of examples, many of which may prove to be equally if not more influential in bionanodesign.

Maxim Ryadnov

Preface to the Second Edition

Biomolecular structures self-assembled from naturally occurring motifs provide access to a wide variety of nanomaterials. Perfected in biology, such motifs support functions as diverse as molecular transport and tissue repair, while being used to construct nearly any conceivable nanomaterial form.

This book sets out to bring together contemporary approaches developed to purpose and re-purpose self-assembling motifs for nanomaterials with desired properties. The volume continues the course taken in the first edition, focusing on *bioinspired nanoscale designs*, but extends the scope of existing and emerging designs to functions which are atypical for well-established nanoscale shapes and forms. Therefore, emphasis is placed on adapting known or "old" forms, be these virus-like particles, DNA double helix or fibrillar matrices, for functions which are "new" to these forms.

The book samples most recent and background literature striving to be referenced as fully as permissible within the context of biologically relevant nanostructures and materials and comprises three core chapters, with each discussing one prominent topic of biological function defined at the nanoscale. Each chapter details structure–function relationships used to inform design rules for functional nanomaterials, giving examples of specific applications that inspire or were inspired by specific designs. Fabrication and characterization methodologies are also described to highlight progress in the field.

The book takes an unconventional approach in that it does not lead straight to applications or focus exclusively on the art of nanoscale design as most nanotechnology titles tend to do, but instead accepts the primary role of "nano" in defining "technology" leaning towards challenges where nano can introduce new solutions. This task finds support from the growing

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number of excellent bioinspired nanoscale designs sourced in the literature and is continuously challenged by the volume they create almost on a weekly basis. For this reason, the author apologises for the inevitable, but not deliberate, omission of many examples which may prove to be equally if not more influential for bionanodesign.

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CHAPTER 1

Introductory Notes

The last decade has witnessed considerable progress in the development of technologies pertinent to the life sciences. This progress has encountered an increasing demand for finer materials with properties superior to those of current devices.[1] Performance efficacy, functional versatility and ease of use are amongst the major factors behind the choice of components and fabrication methodologies for next-generation materials. In the search for suitable and most effective approaches, molecular self-assembly has emerged as a strategy meeting all the current requirements.[2] As the progress continues, the strategy also reveals the pros and cons of technological developments, revising them and revealing new criteria for improvements. Perfected by Nature, the processes of molecular self-assembly are being successfully adapted by design providing reliable rules for the assembly of diverse architectures whose structure and function are defined at the nanoscale. Naturally occurring systems continue to be a major source of inspiration for self-assembly motifs and nanoscale designs. Principally, artificial designs are mimetics of native biology, aiming to devise desired functions with more effective outcomes. Cross-validation of motifs, which are unknown for one molecular class but common for another, also takes place in the design process, adding to the growing toolbox of construction components. The same can be said about functions. Each function has a precedent in natural systems, but not all systems can be readily adapted by design. Therefore, target functions can be atypical for chosen molecular forms. For example, a virus capsid is a recognizable form in biology, that is, an old form, but if designed to destroy bacterial cells on contact it delivers an atypical function, that is, a new function.[3] Another example is DNA. It is a universal, old, genetic material for all forms of life, but if assembled into a nanoscale vault as a reactor for enzymatic reactions,[4] it becomes assigned to an atypical, new function, Figure 1.1.

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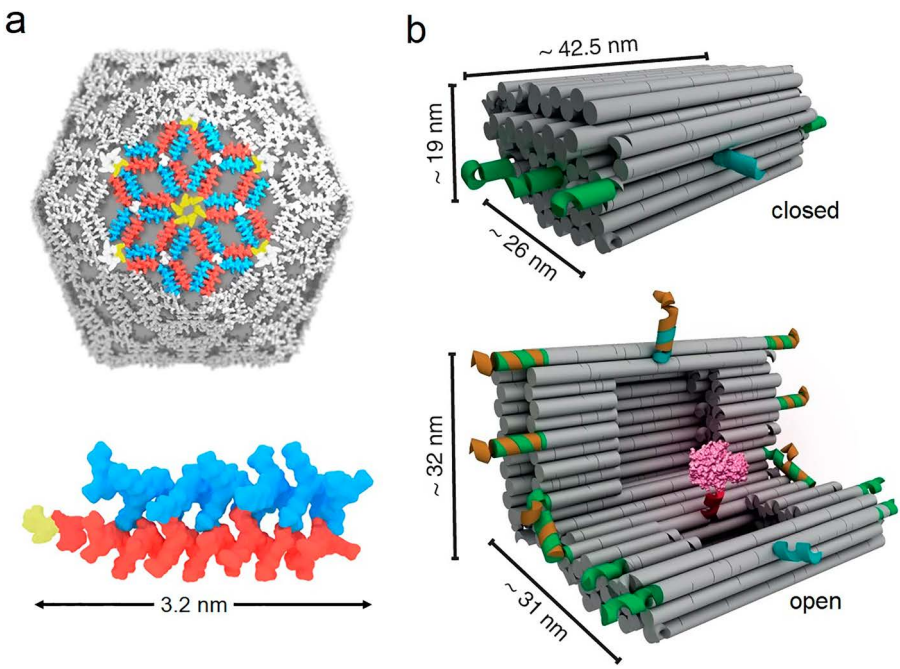

Figure 1.1 Old forms for new functions. (a) An artificial protein capsid designed to kill bacteria on contact by lysing their cell membranes. The capsid incorporates antimicrobial sequences (blue) and counterpart sequences (red) mediating the assembly. Reproduced from ref. 3, https://doi.org/10.1038/s41467-017-02475-3, under the terms of the CC BY 4.0 license https://creativecommons.org/licenses/by/4.0/. (b) A DNA assembly designed as a nanoscale vault hosting an enzyme molecule (pink) in the closed (top) and open (bottom) states. Reproduced from ref. 4, https://doi.org/10.1038/s41467-017-01072-8, under the terms of the CC BY 4.0 license https://creativecommons.org/licenses/by/4.0/.

This view conforms to the mission of bioinspired nanoscale design, or *bionanodesign*, which seeks to exploit engineering principles of biology to build up nanostructures, *i.e.* molecular architectures with regular periodicities featured below 100 nm. As formulated by Richard Feynman,[5] the challenge of understanding matter by constructing macroscopic structures from the bottom up prompted the field of nanotechnology. Self-assembling molecules lend themselves as primary components for nanostructures, and together with the rules of their assembly provide necessary tools towards the production of desired materials and away from alternative, dysfunctional forms.

The hierarchical nature of self-assembly is what reserves predictability and reproducibility in the assembly of resultant materials. In particular, this is the case for biopolymers, such as polypeptides and nucleic acids, which determine the morphological diversity of biological structures and can serve as ready-to-use design templates. However, additional constraints are also

required to maximize the accuracy of a target nanoscale assembly from a chosen biopolymer type. This is addressed by the intrinsic propensity of self-assembly for autonomous control over supramolecular propagations of individual molecules.[6] These involve molecularly encoded folding, which correlates each hierarchical level with a self-assembly pattern, thus allowing biopolymer building blocks to assemble with a precision of a single nanometre.

Our ability to reproduce such a state of control and prediction has improved considerably over the last decade, although it is limited owing to an incomplete understanding of molecular self-assembly processes. More insight gained into biomolecular hierarchies will lead to new molecular models and designs with new properties, which may be perceived as unachievable today. Therefore, reviewing available guidance for the fabrication of functional nanostructures remains of paramount importance.

Replicating Nature's designs faithfully reproduced over millions of years has proven to be the most straightforward route to success. Nature offers a wealth of structural motifs capable of defining nanoscale forms, including DNA topologies, protein polyhedra, multicomponent extracellular matrices and biological membranes. Despite their diversity, all such motifs point to a robust design rationale for the broadest spectrum of possible nanostructures – but how to extract such a rationale for engineering artificial systems?

Of different types as well as within every type, Nature's designs are individually unique as required by functions that they carry or are assigned to. On the one hand, this underpins conserved templates adaptable for synthetic designs. On the other hand, biopolymers obey the same assembly principle: they adopt secondary structures to build functional quaternary systems, *i.e.* natural nanoscale objects. Synthetic designs reported to date take both routes. Protein or DNA structures based on pre-assembled native folds as well as systems designed from scratch, but inspired by native structures, are peers. Thus, a general approach to tackle the problem may focus on the assimilation of Nature's way in creating macromolecular assemblies by employing the structure–assembly relationships of existing examples. Eventually, this may reveal the essence of that structure-based strategy, which will allow us to exploit biomolecular recognition for nanoscale designs. Indeed, nanostructured systems shown as more advanced tend to assemble from better understood assembly elements. For instance, designs derived from DNA offer precision and control to match, while polypeptides offer the richest repertoire of self-assembly motifs.

However, irrespective of chemistry or assembly class, the synthesis of a discrete system that would span nano- to microscale dimensions is never a trivial task. Monodispersity, an ability to maintain the internal order and morphology of resulting assemblies and reliability of prescribed assembly modes remain the hurdles to be overcome towards functionally reproducible nanostructures.

Naturally occurring systems appear to be free of such barriers. They are highly conserved sequential couplings of exquisitely fitted subunits that use spatially self-maintained molecular arrangements. Emulating natural

self-assembly patterns should be beneficial for engineering bioinspired systems. Nanobjects generated in this way can lead to materials with predictable and tuneable properties that are frequently referred to as "smart" materials. However, this hardly proves to be the case, in particular for *de novo* nanodesigns, which, despite their impressive numbers, remain short of original examples.

Indeed, whereas the total number of particular designs may have exceeded hundreds, unique designed forms are limited to just a few. In part, this is determined by applications, but possibly to a larger extent by the synthetic inaccessibility of large biomolecular subunits of natural assemblies. Thus, the success of extracting one robust rationale for artificial designs continues to be hampered by the need to find efficient ways which would allow for control over the assembly of smaller, simpler, albeit more entropy-dependent, self-assembling motifs. Given Nature's preference for biopolymer precursors, a set of requirements can be identified for a potential self-assembling candidate as follows.

First, it must be synthetically accessible in a monodisperse form. This requirement is limiting for any type of intended nanostructures, and also directly relates to the autonomous control of the nanoscale assembly.

Second, it has to adopt a recognition pattern ensuring a minimized impact of entropy factors (*e.g.* inter- and intramolecular dynamics) on the assembly, which ensures the hierarchical order of the assembly and consequently presents a major morphology-defining parameter.

Third, its assembly should obey the chosen mode of a hierarchical order encoded in primary sequences. This requirement is intrinsic to biopolymers but can be waived for certain molecular mimetics that preferentially lean on bulk forces supporting self-assembly, *e.g.* the hydrophobic effect.

There are several biomolecular motifs that can meet such design criteria. With their encoding traits established empirically, all manifest strong correlations between the chemistry and assembly. However, of notable advantage are those represented by two main classes: nucleic acids and polypeptides. Other motifs developed and used over the course of recent decades can be seen as derivatives or supplements of these two. Exemplified by just these two classes, the key factors underlying the functions of native nanostructures, including monodispersity, consensus folding and environmental responsiveness, provide impact on the choice of artificial designs. In conjunction with the growing body of synthetic designs and improving analytical techniques, the motifs stimulate the search for compatibility marks between structural principles of native assemblies and desired synthetic nanostructures. An attempt to address this or at least to touch upon some of the most design-responsive points in the prescriptive self-assembly is made in this volume.

The objective of this book is to outline developing approaches in the design of nanostructures, which employ bioinspired self-assembling motifs. Entitled *Bioinspired Nanoscale Design* or *Bionanodesign*, the book is written in the shape of a review referenced as fully as permissible within the context of biomolecular design, which forms a general trend throughout.

The volume is composed of three core chapters focusing on three predominant topics of applied nanotechnology where the role of nanodesign is prominent. Specific applications that arise from designed nanoscale assemblies as well as fabrication and characterization techniques are of lesser weight and whenever they appear serve as progress highlights for new functions.

The book places emphasis on "nano" rather than on "technology", providing recent examples of functions exhibited by nanodesigns, but which are not typical for naturally occurring analogues. This task is significantly eased by brilliant bioinspired designs reported to date and is complicated by the large volume of information created almost on a weekly basis. Therefore, the author apologises for the inevitable, but not deliberate, omission of examples many of which may prove to be equally if not more influential in bionanodesign.

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CHAPTER 2

DNA as a Self-programming Module for Nanodesigns

2.1 Introduction

Five research papers published within a span of 3 months[1–5] defined the way in which biology is taught today. The importance of solving the molecular structure of nucleic acids has been discussed in numerous reviews and books. There is not a biologically relevant discipline which does not benefit from the knowledge of the DNA structure. However, none is probably more dependent on the accuracy with which the geometry and spatial organization of DNA are predicted and described than bioinspired nanotechnology. Over a few decades, DNA has emerged as a leading tool in nanodesign.[6–11] This chapter gives an overview of design principles and relevant examples using DNA, starting from design topologies,[8] algorithmic self-assembly[12] and DNA origami.[13]

DNA can be viewed as a repository of the information that genes carry and require passing to offspring. Invariably, this function determines the choice of the structural parameters and folding paths of the molecule. These are conserved and need to accommodate a faithfully reproducible mode of self-replication which can be translated into the building material of life, *i.e.* proteins. Rules to replicate life are expressed in the genetic code *via* the assignment of a unique codon, a triplet of nucleotides, to each of the 20 proteinogenic amino acids.[14] Strictly, there is more than one genetic code[15,16] and also more than one mode of DNA base paring.[17] However, those are particular cases and have yet to find evidence of impact within the context of DNA nanotechnology. More important in this regard are the facts that (1) only a part of genetic information is encoded by the code and (2) each human

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cell type (apart from stem cells) expresses only one set of genes despite having the full copy of the genome. Furthermore, the genome is believed to contain the so-called "pseudogenes",[18] inactive and non-expressible parts of the genome that are often thought of as an evolutionary artefact or "junk" with no functional purpose.[19] The term is debatable as "non-coding" DNA accounts for about 90% of the human genome and can be a stored material with an unidentified function or a result of competing chromosomes.[20–22] Either way, this may prove to be important for nanodesign as the functional uncertainty of junk DNA as opposed to translated DNA can relate to structural alleviations observed for non-coding DNA structures, that is, the requirement for protein-coding DNA, which is read from one end, to be a linear molecule can be waived for non-coding DNA.

This implies that DNA architecture is intrinsically amenable to different topologies and shapes, the repertoire of which is inexhaustible. Whether natural or artificial, DNA designs consider the detailed understanding of DNA chemistry in addition to the visionary acceptance of the structural hierarchy of DNA.

2.2 Chemistry of DNA

Functional DNA is a monodisperse polymer composed of three types of repeating units – carbohydrate (deoxyribose, pentose monosaccharide); a heterocyclic base, which can be one of four: adenine (A), cytosine (C), guanine (G) or thymine (T); and phosphate – which together make up one DNA monomer, a nucleotide, Figure 2.1. Therefore, polynucleotide is an alternative name for DNA, commonly used as its chemical definition. The sequence of phosphates and carbohydrates coupled alternately constitutes a polynucleotide backbone which is decorated with heterocyclic bases or just basis linked to the first carbon atoms of the five-membered pentose rings, Figure 2.1.

Importantly, a phosphodiester bond lying between two carbohydrates is asymmetric as the bond links different (third and fifth) carbon atoms of the two. This renders a polynucleotide directional and confers the signature of DNA – the memory of chain direction. For example, an individual polynucleotide chain is a single-stranded DNA (ssDNA) that can form relatively flexible structures. However, these are thermodynamically unstable, and tend to intertwine with other ssDNA structures or themselves. A classical double-helix DNA (double-stranded DNA, dsDNA) is an antiparallel assembly of two independent polynucleotide strands oriented opposite to one another. The shape and stability of the structure depend on the extent of inter-strand interactions, which are provided by hydrogen bonding between the bases of the opposite strands, normally referred to as base pairings. The complementary base pairing as postulated by Watson and Crick[3] involves highly specific A–T and G–C interactions, which have proved to be sufficient for programming a large and diverse set of polynucleotide sequences confirming the robustness of this type of binding. The donor–acceptor patterns of hydrogen bonds are

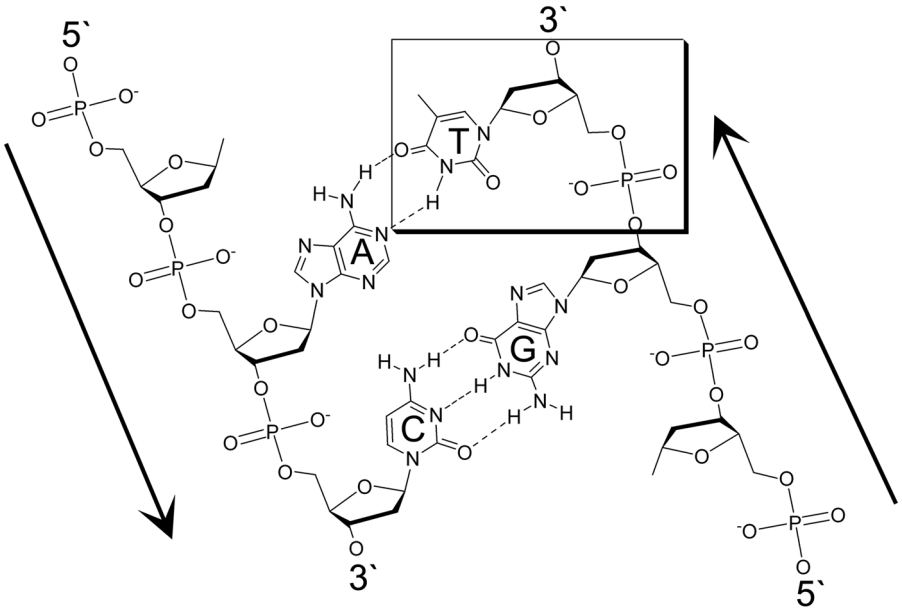

Figure 2.1 Chemical structure of DNA. Two polynucleotide chains run antiparallel to form one double-stranded DNA *via* Watson–Crick base pairing, A–T and C–G. For simplicity, only two pairs are shown. Dashed lines indicate hydrogen bonds. The square highlights a DNA monomer – nucleotide monophosphate, consisting of a base (T), a five-carbon pentose ring, deoxyribose and a phosphate group linking two pentoses. Arrows point in the 5′ → 3′ direction of the asymmetric ends.

not identical for the two base pairs and differ in geometry and the number of bonds per pair. The A–T pair is formed by two bonds, whereas the more stable G–C uses three bonds. G and A belong to a heterocyclic family of purines, which are larger molecules than pyrimidine derivatives C and T. Owing to the size and the geometry of binding, purines can only marry pyrimidines and *vice versa*. These two parameters sum up a simple mechanism regulating appropriate pairings along polynucleotide sequences, Figure 2.1.

The conformational preference of the backbone of dsDNA is that of a polymer chain with conformational freedom considerably restricted by the regular stacking of sugar moieties. dsDNA folds as a right-handed helix with two distinctive grooves, major and minor, ~2.2 and ~1.2 nm wide, respectively, Figure 2.2. This so-called B conformation is the most common of three, which also include A,[5] a more compact or dehydrated form of B, and Z,[23,24] a transient left-handed zigzag structure. These structural criteria favour a predominant dsDNA conformation, the antiparallel B-form, Figures 2.1 and 2.2. An ingenious consequence of this form is that it endows dsDNA with remarkable stability and reconstruction properties, allowing it to function in cellular environments.

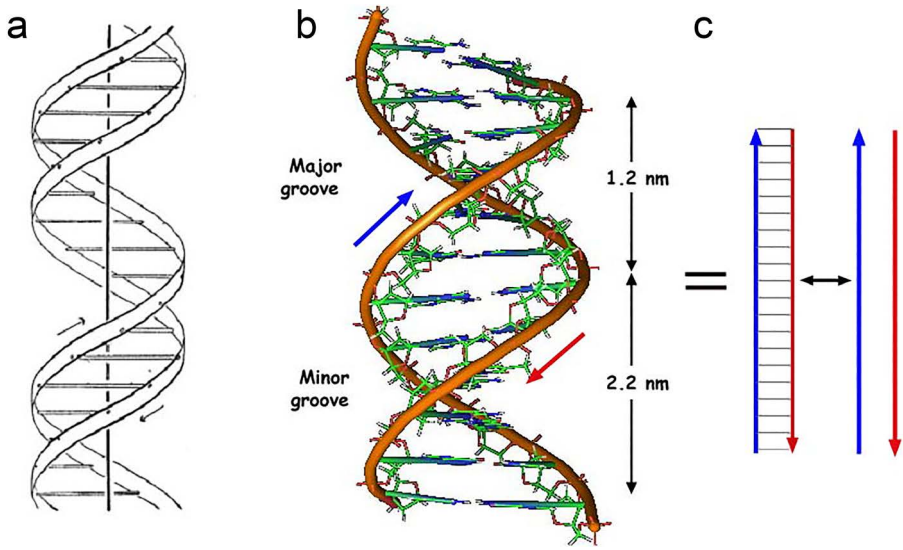

Figure 2.2 DNA double helix. (a) As originally proposed by Watson and Crick.[1] Reproduced from ref. 1 with permission from Springer Nature, Copyright 1953. (b) B-form of DNA as idealized from the PDB. (c) 2D ladder-like structures with arrows denoting 5′ → 3′ strands, with base pairs depicted as ladder steps (left) and, as equally used, omitted (right).

This is indeed a very stable molecule, but its stability carries a cost of maintaining complementarity, a constraint, which ensures the reconstruction of dsDNA from one of its strands. Rendering DNA materials self-replicating and self-repairable significantly increases the probability of error-free designs, which is consecutively supported by another strikingly simple mechanism. The binding energy of two strands can be approximated to k_BT per base pair, where k_B is Boltzmann's constant and T is a variable, *i.e.* temperature.[25–27] Given that under physiological conditions thermal changes exceeding $30k_BT$ are not common, double helices with more than 30 base pairs resist temperature-induced separations.[25,26] Restrictive endonucleases, or restrictases, target DNA, with each restrictase having a specific DNA cleavage site. Therefore, by cleaving DNA the enzymes reproducibly generate fragments of known sizes and sequences irrespective of DNA origin. This property offers tremendous potential for programming single-molecule constructions across length scales, which is one of the main reasons for the popularity and success of DNA nanotechnology. The property is inherent to DNA and remains true for any topology into which the double helix can be shaped. DNA is adaptable to virtually any supramolecular topologies and architectures provided that these are applicable to lengths exceeding the persistence length of the helix, estimated to be 150 base pairs or 50 nm.[28,29] Below this size, DNA is considered to be rod-rigid and inflexible, *i.e.* straight. However, owing to complementarity and the considerable flexibility of ssDNA,