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Challenges and opportunities for structural DNA nanotechnology

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Abstract

DNA molecules have been used to build a variety of nanoscale structures and devices over the past 30 years, and potential applications have begun to emerge. But the development of more advanced structures and applications will require a number of issues to be addressed, the most significant of which are the high cost of DNA and the high error rate of self-assembly. Here we examine the technical challenges in the field of structural DNA nanotechnology and outline some of the promising applications that could be developed if these hurdles can be overcome. In particular, we highlight the potential use of DNA nanostructures in molecular and cellular biophysics, as biomimetic systems, in energy transfer and photonics, and in diagnostics and therapeutics for human health.

The field of structural DNA nanotechnology can be traced back to the words written by Nadrian Seeman in 1982: “It is possible to generate sequences of oligomeric nucleic acids which will preferentially associate to form migrationally immobile junctions, rather than linear duplexes, as they usually do.”¹ Seeman had wanted to organize proteins in three-dimensional (3D) crystals so that he could study their structure with X-ray crystallography. Three decades later the field has outgrown its roots in protein crystallography and delivered numerous advances in the control of matter on the nanoscale (Fig. 1). The history and state of the art in structural DNA nanotechnology have been widely reviewed^{2–7}. Here, instead, we seek to stimulate discussions about the future of the field.

Research in structural DNA nanotechnology began with the construction of relatively flexible branched junction structures⁸ and topological structures^{9–16}, progressing to the fabrication of crossover DNA tiles with greater rigidity. These tiles could be used to assemble higher-order periodic and aperiodic lattices^{17–30}, and nanotubes^{31–37}. A landmark

of periodic DNA structure assembly was achieved by Seeman and co-workers³⁸ in 2009 with the formation of 3D DNA crystals from tensegrity triangles³⁹ that diffract X-rays to 4 Å resolution.

One of the most important development in structural DNA nanotechnology since the introduction of the crossover tile has been the use of a 'scaffold' DNA strand for the assembly of aperiodic structures. It had been previously demonstrated that a long single-stranded DNA chain could be used to organize double-crossover tiles into barcode-patterned lattices⁴⁰, and that a 1.7-kb single-stranded DNA chain could serve as a scaffold for the assembly of a 3D wire-frame octahedron⁴¹. The breakthrough came with the concept of 'DNA origami', where a long scaffold strand (single-stranded DNA from the M13 phage genome, ~7,429 nucleotides long) was folded with the help of hundreds of short 'staple' strands into defined two-dimensional (2D) shapes⁴². The scaffold is thought to corral the component strands in a way that leads to high effective concentrations and proper stoichiometry, so that even unpurified oligonucleotides can be used to produce well-formed 2D structures in near-quantitative yields. DNA origami structures can also be used as molecular pegboards with a resolution of 4–6 nm, and they have been widely used in the assembly of heteroelements such as proteins and nanoparticles (see below).

Three general strategies have been explored to extend DNA-origami nanoconstruction to the third dimension. The first relies on folding interconnected individual or continuous DNA origami sheets into hollow 3D cages^{43–46}. The second method builds custom 3D shapes by constraining layers of helices to a honeycomb⁴⁷ or square lattice⁴⁸; the targeted insertion and deletion of base pairs within such rigid 3D blocks allows twisted and curved 3D objects to be made⁴⁹. The third strategy is to stack concentric double-helical circles containing different numbers of turns, and therefore having differing circumferences, to match the rounded contours of a target container shape⁵⁰.

Taking advantage of the sequence specificity and the resulting spatial addressability of DNA nanostructures, many of the DNA nanoarchitectures listed above have been used for the organization of heteroelements such as proteins^{22,29,51–57}, peptides⁵⁸, virus capsids⁵⁹, nanoparticles^{60–74} and carbon nanotubes⁷⁵. And in turn, several of these DNA-directed assemblies have led to unique and improved functional properties, such as increased enzyme-cascade activities^{76,77} due to spatially positioned enzyme pairs, and shifts of surface plasmon resonance controlled by custom arrangement of nanoparticles^{78–80} through DNA-mediated self-assembly.

Nearly 30 years after Seeman's original proposal, scientists now have at their disposal a multitude of designs and techniques with which to devise increasingly complex systems for scientific and technological applications. However, structural DNA nanotechnology is still at a relatively early stage in its development, and here we will discuss the challenges that must be overcome to reach greater levels of control and functionality.

Technical challenges

DNA origami has already provided a spectacular example of the power of static self-assembly as a design paradigm to create custom cookie-cutter shapes each with a mass of ~5 megadaltons (which is twice the mass of a ribosome). Could much more complex DNA-nanostructure designs be made in the future? It is instructive to note that the number of transistors per integrated circuit has doubled every two years for the past four decades — roughly a one-million-fold increase between 1971 and 2011. Such an increase in complexity underlies the difference between a modern-day smart phone and a simple pocket calculator: a comparable example from biology would be the difference between a cell and an individual macromolecular complex (for example, a ribosome). Here we outline two

approaches where an investment of resources and effort may sustain similar exponential growth in the complexity of DNA nanostructures over the next two decades. Two of the most prominent obstacles are the high cost of synthetic DNA and the high error rate of self-assembly.

DNA synthesis and sequence design

At current prices of about US\$0.10 per base for oligonucleotide synthesis on the 25-nmol scale, the overall material cost for constructing a new M13-based origami is around US\$700. A key technological opportunity is the emerging commercial availability of affordable arrays on which small amounts of each of the tens of thousands of unique oligonucleotide sequences are printed at a current price of less than US\$0.001 per base. If reliable low-cost methods for enzymatic amplification of subsets of strands from these arrays could be further developed^{81,82}, this would raise the possibility of custom-designed DNA nanostructures that are 1 gigadalton in mass (that is, around 100 times as complex as current M13-based origami) for a material cost of ~US\$1,000. Large reductions in cost of enzymatic amplification would also enable production of gram to kilogram quantities of complex DNA nanostructures, which will be important for many but not all applications.

For DNA origami, a current constraint has been the reliance on the 7 kb genome of M13 as the primary source of scaffold. To create larger structures, ideally one could fold either a longer unique scaffold, or as an alternative, multiple scaffolds with distinct sequences. Furthermore, it seems unlikely that M13 encodes the optimal sequence for high-yield folding of all possible DNA nanostructures. Thus, one would want to generate many unique scaffold sequences, each tailored for optimized folding into a particular origami shape, or at least a large number of distinct generic scaffold sequences that can support independent foldings in a single pot. Affordability is a concern, but gene synthesis from array-printed DNA again may provide a solution^{81,82}. Consideration of these issues naturally leads to the question of what the rules are for effective sequence design, and our current ignorance in this area warrants much future work in this direction, involving an interplay between theory and experiment.

Hierarchical and templated assembly

Conventional DNA origami uses a single long scaffold molecule as half the material. Using this approach to build a gigadalton DNA nanostructure, one would need a scaffold over 1 megabase long, approaching the length of the *Escherichia coli* genome (Fig. 2a, top). Such large DNA molecules are mechanically fragile and difficult to synthesize. Instead, we can imagine current origami as 'super-tiles' that can be linked together hierarchically to form larger superstructures^{83,84} (Fig. 2a, bottom). Each super-tile can be made a larger size by changing the design to enable use of a higher ratio of non-scaffold to scaffold-strand mass⁸⁵. The design of super-tile interfaces will need to be optimized to improve yield^{86–88}. Higher-order superstructure can be further enforced by use of a super-scaffold that organizes super-tiles⁸⁹. Both a super-scaffold and algorithmic assembly could be used to organize multiple orthogonal super-tiles in specific patterns within a given larger structure. Also, lithographically etched surfaces could be used to template long-range order on a collection of super-tiles^{90–93}; merging top-down with bottom-up approaches in this way will attract the attention of the semiconductor industry for microfabrication applications.

Finer structure control

Long-term progress towards building large nanostructures will require a mature understanding of the kinetics and thermodynamics of self-assembly within and between DNA building-blocks^{94–96}. One particular area of weakness has been the lack of quantitative tools for analysing defect occurrence in complex DNA nanostructures. Test structures

should be designed that sum or magnify the effect of cumulative small folding errors to produce substantially deviated geometries that are easy to assay using molecular imaging or other higher-throughput methods. In addition, more work is needed to investigate kinetic aspects of assembly, such as the order of association of staple strands to the scaffold in DNA origami.

In addition to building larger and more complex DNA nanostructures, and reducing assembly errors, it is equally important to achieve structural control at the finest possible level in all three dimensions. A lattice-constrained DNA nanostructure is limited in precision to the nanometre scale. However, just as external peptide loops can fine-tune the structure of an antibody or triosephosphate isomerase-barrel protein scaffold, so we can use external forces to tweak the fine structure of a DNA nanoshape. Furthermore, lattice-constrained paradigms can be abandoned altogether at local 'active sites'. Instead, one can substitute binders and catalysts derived from other molecules (for example, single-stranded DNA, single-stranded RNA or protein), in some cases enhanced by new chemical functionalizations.

Precision positioning of heteroelements for functionality

The ability to construct sophisticated machines and actuators is one of the key technical goals of nanotechnology. Although self-assembly of nucleic acids alone provides a rich capacity for driving active or functional behaviour, the introduction of heteroelements such as nanoparticles and proteins can lift DNA nanotechnology into a new dimension of functional potential. One main challenge continues to be efficient integration of heteroelements into DNA structures, especially when precise control over orientation and position of the guests is demanded. On the DNA side, the most popular starting point has been commercially available amino- or thiol-modified oligonucleotides, which then can be converted to different functionalities by reaction with appropriate heterobifunctional crosslinkers. For integration of proteins, a challenge has been the coupling of oligonucleotides to unique positions on the protein, and the subsequent purification of the conjugate⁹⁷ (Fig. 2b). To accommodate the diversity of guest proteins of interest, a suite of effective methods will probably need to be developed. Increasingly exact control over protein orientation could be achieved by multiple attachment to a DNA nanostructure, or by 3D cavities that use steric interactions to constrain the guest. If this challenge could be addressed, the active site of enzymes may, for example, reliably be programmed to face the exit of a molecular cavity or tubular structure.

Integration of inorganic nanoparticles into nanostructures has also received considerable attention. Metallic nanoparticles (in particular gold and silver) have led the way owing to their simple functionalization with oligonucleotides⁹⁸. Conversely, reports of quantum dot self-assembly on DNA nanostructures are scarce^{67,71,74}. Functionalization of quantum dots presents several difficulties, owing to the reduced stability of thiol-based conjugation, along with aqueous and salt incompatibility, and thus requires more unconventional approaches⁷⁴. Functionalization and organization of DNA structures with single-walled carbon nanotubes⁷⁵ and fullerene molecules⁹⁹ is also in its early stages and will require attention in the near future. Turning finally to DNA metallization^{100,101}, which could have great potential for the fabrication of nanomaterials for electronics and photonics, current techniques do not yet allow the synthesis of homogeneous wires, although the complexity of metallized architectures achieved has been increasing over the years^{102,103}.

Active self-assembly

Structural DNA nanotechnology might be incorrectly regarded as static, as much of its most striking progress thus far has been in the creation of end-point structures. But a similar

misconception could arise if one were given only a frozen snapshot of the cytoskeleton as a permanent framework that enforces cellular shape and structural integrity. In fact, cytoskeletal elements in a cell undergo continual rearrangement, mediated in part by the action of some of the most complex molecular motors in nature. This dance, far from equilibrium, underlies remarkable cellular behaviour such as neutrophil transmigration, where these leukocytes morph and squeeze their way through small gaps in the epithelial cell layer lining a blood vessel. A goal is to develop DNA-based devices that rival the power of active self-assembly found in cells.

Research into reconfigurable DNA nanostructures has been active for over a decade^{104–115}. An important development has been stepwise kinetic-control over self-assembly by means of the hybridization chain reaction¹¹⁶. Here an initiator strand triggers the opening of a hairpin strand, thereby creating a new free end that can act to open the next hairpin; these sequential cascades proceed autonomously, powered by an overall increase in base pairing after each step. A similar design was later conceived to create polymers that grow by insertion at the interface with a catalyst¹¹⁷. This study was inspired by the actin-polymerization-based propulsion of *Listeria* within the cytoplasm. At present, these DNA-based devices are less complex, less robust and orders of magnitude slower than their natural counterparts, so great demand exists for devices designed to operate at higher rates and efficiencies. One possible route is to explore other energy sources, such as ATP hydrolysis or photon absorption, that have been successfully exploited by nature to create fast machines.

A particularly popular target has been the molecular walker^{110,111}. DNA-based walkers have been demonstrated to walk autonomously along paths, carry cargo or act as assembly lines. Further advances will require increased speed and robustness, as discussed above. Future avenues for research include programming direction in multiple path systems, or inducing state changes that 'drive' the walker in real time along a chosen path (Fig. 2c). DNA walkers could also be used for the assembly of other structures by means of a programmed recognition–activation–reaction pathway. Mastery over communication between multiple walkers is likely to be critical for efficient productive capability.

Expression and assembly *in vivo*

Of great relevance to many cell biologists is the biocompatibility of DNA nanostructures and their potential for function in cells. Naturally this raises the question of whether such nanostructures can be genetically encoded for intracellular expression and assembly. Thus far, it has been shown that DNA nanostructures encoded as long single strands, designed by taking advantage of the paranemic crossover motif, can be amplified by polymerases *in vitro* or *in vivo*^{41,118–120}. It remains to be shown that increasingly complex DNA nanostructures can be folded efficiently within a cell. The emerging field of RNA nanotechnology¹²¹ might seem more promising in this regard because RNA is readily transcribed into a single strand in cells, which can be directly folded into a programmed nanostructure; DNA can be made in a single-stranded form in cells as well, although less commonly, using rolling-circle or else reverse-transcription-based methods. Even though few rules are known for the reliable design of RNA nanostructures, recent work by Aldaye and co-workers¹²² represents an important step in engineering RNA molecules to assemble into predefined discrete 1D and 2D structures *in vivo*. More importantly, DNA and RNA nanotechnology have enormous synergistic potential, where the predictability of DNA folding can be coupled to the diversity of RNA functionality¹²³.

DNA origami structures show surprising stability in a cell lysate¹²⁴ and against digestion by purified nucleases¹²⁵, which suggests that biostructural DNA nanotechnology is limited more by the ability to assemble nanostructures *in vivo* and less by their lack of stability.

Furthermore, biological systems can be used to select active or biocompatible structures (Fig. 2d). Just as with DNA-based aptamers¹²⁶, it is possible to create a population of structures with variable segments for downstream selection according to desired function or even overall structure. The development of methods to evolve single-stranded DNA structures is undoubtedly a challenge worth pursuing. Liu and co-workers recently reported a phage-assisted continuous evolution technique in which the directed evolution of gene-encoded molecules can be linked to protein production in *E. coli*¹²⁷. Adaptation of this technique would present an excellent starting point for the evolution of DNA nanostructures towards creation of bioactive and compatible structures.

Future applications for structural DNA nanotechnology

Here we outline a few applications of DNA nanostructures that will become increasingly feasible as the technical hurdles listed above are overcome.

Molecular and cellular biophysics

Seeman's original goal of hosting guest proteins in designed DNA crystals for high-resolution structure determination by X-ray diffraction remains an important one¹. But DNA nanotechnology can be expected to contribute to macromolecular structure determination in other ways. Detergent-resistant liquid crystals of DNA nanotubes have allowed weak-alignment NMR studies of membrane proteins¹²⁸. This tool proved its worth in the recent *de novo* NMR structure determination of UCP2 — a 33 kDa six-transmembrane helix inner mitochondrial membrane protein¹²⁹. We can expect many more NMR structures of small to medium-sized alpha-helical membrane proteins to be solved in a similar manner over the next decade. Hosting weakly aligned macromolecules at high density within pores of a 2D DNA crystal may be useful for accelerating cryoelectron microscopic data collection¹³⁰.

DNA nanostructures are primed to make an impact on studies in single-molecule biophysics, both as aids for imaging and as tools for constraining multiple macromolecules simultaneously^{125,131–133} (Fig. 3). DNA frames hosting macromolecules have been imaged by fast-scanning atomic force microscopy to enable the real-time observation of G-quadruplex formation¹³⁴ and enzyme-catalysed DNA methylation¹³⁵. Analogous strategies can be conceived for the study of any protein with a DNA-binding domain (for example, a protein–DNA conjugate).

Biomimetic systems

Feynman famously wrote on a blackboard: “What I cannot create, I do not understand.” Biomimetic systems can serve as simple models for more complex systems, but also as a foundation for inspiring development of useful materials and devices. A long-term challenge for the DNA-nanostructure field is to generate an artificial cell in which most of the functional behaviour is provided by DNA (Fig. 4). This lofty goal will not be achieved any time soon, but mimics of various natural macromolecular machines represent tractable targets in the near term. One attractive idea is to couple DNA nanostructures to protein-based ATPases for faster performance than DNA-only systems have been able to provide.

DNA nanostructures could be designed as bio-inspired nanopores, and the functionalization of oligonucleotides with hydrophobic molecules may permit the incorporation of such structures into lipid bilayers. The shape and diameter of the nanopores could dictate a cut-off for the diffusion of macromolecules; such nanopores might offer improvements over the current state of the art for nano-pore-based DNA sequencing. In the more distant future, nanopores and ATPase–DNA hybrid structures could be combined to create artificial channels for active transport.

Energy transfer and photonics

Photosynthesis, the basis of all life on Earth, boasts the remarkable ability to transform solar energy into chemical energy, and has driven chemists to design artificial systems that mimic its every aspect¹³⁶. In particular, supramolecular chemistry has contributed greatly to the design of artificial light harvesting, energy transfer and charge separation complexes^{137,138}. The main drawback to traditional approaches is the need for extensive organic synthesis efforts, leading to two extreme situations: small constructs with two to five functional units and ångström-level spatial control, or longer constructs with many repeating units, but reduced control over the overall shape and size.

The bottom-up assembly of organic suprastructures affords spatial control at the ångström level; DNA nanostructures can be used as the interface between molecular entities to provide nanometre-scale precise junctions to attach different molecular entities. For example, light-harvesting complexes can be put in close contact with charge transfer units in a modular fashion, using DNA as a molecular pegboard. This might constitute a fresh approach for the construction of 'artificial leaf' systems (Fig. 5). The often water-insoluble systems can be placed side by side with proteins or other biomolecules. Furthermore, increasing knowledge of the functionalization of nanoparticles with oligonucleotides encourages the use of DNA nanostructures as 'motherboards' for many potential applications. The ability of DNA to transport charge over considerable distances along its bases is a consequence of oxidation^{139,140}, a process that can compromise the integrity of the strand or even the structure. Thus, double-stranded DNA^{141–143} and DNA origami¹⁴⁴ have been used as scaffolds for dye-based photonic wires, where energy is transferred in a linear fashion over tens of nanometres. DNA nanostructures are intrinsically more rigid than double-stranded DNA and can be used to build longer photonic wires, and further, the unique 2D and 3D spatial arrangements allow the construction of branched paths for energy transfer. Combining plasmonic nanostructures¹⁴⁵, semiconductors and proteins in complex networks leads to the concept of molecular circuits, where photons and chemical and electrical potential can be interconverted. DNA boards may also be used to merge bottom-up and top-down methods for the organization of chemically synthesized inorganic nanowires¹⁴⁶.

Diagnostics and therapeutics for human health

Probably the most seductive prospect for DNA nanotechnology is as effective drug-delivery nanovehicles for fighting disease. To illustrate a toy version of this concept, a hollow DNA-based box was assembled with a lid that could be opened by strand displacement with a specific oligonucleotide key⁴³. The implication is that responsive nanoboxes could be programmed to deliver their toxic cargoes in a specific fashion, thereby maximizing potency while minimizing side effects. In reality, efficient drug delivery through systemic administration remains an extremely difficult task, as multiple barriers must be overcome before the nanoparticles can come close to specific release of cargo to the intended molecular target¹⁴⁷. Nanoparticles need to avoid clearance by macrophages in the liver and spleen while efficiently penetrating the tissue in the targeted area. For some diseases, leaky vasculature can be targeted passively by nanoparticles simply by virtue of their being small, but for others, active penetration through the endothelial lining of blood vessels is required. Even for solid tumours, where nanoparticles can accumulate passively by enhanced permeation and retention, diffusion beyond the periphery of the tumour mass can be limited. And even if the nanoparticles reach the diseased tissue, a robust method for breaching the plasma membrane of cellular targets is required. Uptake of nanoparticles by endocytosis or pinocytosis by itself is not sufficient, as these compartments are topological equivalents of the outside of cells.

How can DNA-based nanoparticles help to overcome these myriad barriers? DNA nanostructures offer unprecedented control over shape, size, mechanical flexibility and anisotropic surface modification. Clearly, proper control over these aspects can increase circulation times by orders of magnitude, as can be seen for long-circulating particles such as erythrocytes and various pathogenic particles evolved to overcome this issue. But our current knowledge of the proper recipe for long circulation times is limited, so investigation in this area will be important. Surface display of the appropriate ligands can also mediate targeting to and passage through endothelial barriers to diseased tissues, as well as accelerating cellular uptake at the desired target. Mimesis of viral strategies for escaping endosomal compartments into the cytoplasm can in principle be addressed with the additional control over surface functionalization afforded by DNA nanostructures.

The surfaces of DNA nanocontainers are fully addressable, allowing for the incorporation of multiple ligands, labels for bioimaging, antibodies, hormones and so forth that might be used for efficient and site-specific drug delivery and release. Advances in DNA computing may produce systems in which the simultaneous detection of multiple cancerous markers in diseased as opposed to healthy cells would be computed in a programmed fashion, to control and modulate cargo release. Increasing the specificity of tissue targeting permits the use of more potent drugs that would otherwise present problematic toxicity issues. Although several materials for smart drug delivery and bioimaging have been reported, including liposomes, polymers, micelles, nanoparticles and antibodies (for a review see ref. 148), none yet present the level of modular design afforded by DNA nanotechnology. For example, the DNA box could be expanded to create a cluster of nanocontainers, each with its own drug and programmed set of inputs for drug release (Fig. 6). These sense-compute-actuate molecular units could ultimately be prototypical building blocks for the creation of artificial immune systems. General principles of DNA molecular circuitry may be applied in such systems to compute physiological medium inputs, and output state changes that lead to drug release in a series of cascade reactions. A hybridization chain reaction system where cancer cells were transfected with RNA transducers that could recognize specific cancerous markers and induce PKR-mediated cell death was recently reported¹⁴⁹. Adapting a series of dynamic structures (metastable DNA hairpin motifs¹⁵⁰, DNAzymes, entropy driven strand displacement, to name a few) to create DNA-based molecular circuitries for point-of-care diagnosis is a pressing — and exciting — challenge.

When combined with protein engineering, DNA nano-technology could also be used to generate new classes of artificial extracellular matrices: *ex vivo* cellular scaffolding to which human cervical cancer cells adhere strongly, survive and grow with high migration rates has recently been demonstrated¹⁵¹. The use of DNA in DNA/protein-based matrices makes these structures inherently amenable to structural tunability. More research in this direction will certainly be developed, making DNA a promising biomaterial in tissue engineering.

Bright future of structural DNA nanotechnology

We have outlined several key challenges for advancing the field of structural DNA nanotechnology, and have suggested a few potential routes to meeting these milestones. Nature has developed sophisticated and complex behaviour at the nanoscale through millions of years of cellular evolution; we will need an aggressive pursuit of bold and forward-looking ideas if we are to catch up over a mere few decades. Along this trajectory of development, let us hope that the advances of structural DNA nanotechnology can be used by researchers in other fields, who will contribute with new approaches, techniques and expertise. Indeed, structural DNA nanotechnology has already become an interdisciplinary research field, with researchers from chemistry, materials science, computer science, biology and physics coming together to tackle important problems. As the field is

progressing rapidly, we believe that exciting new directions will emerge well beyond the limited set described here.

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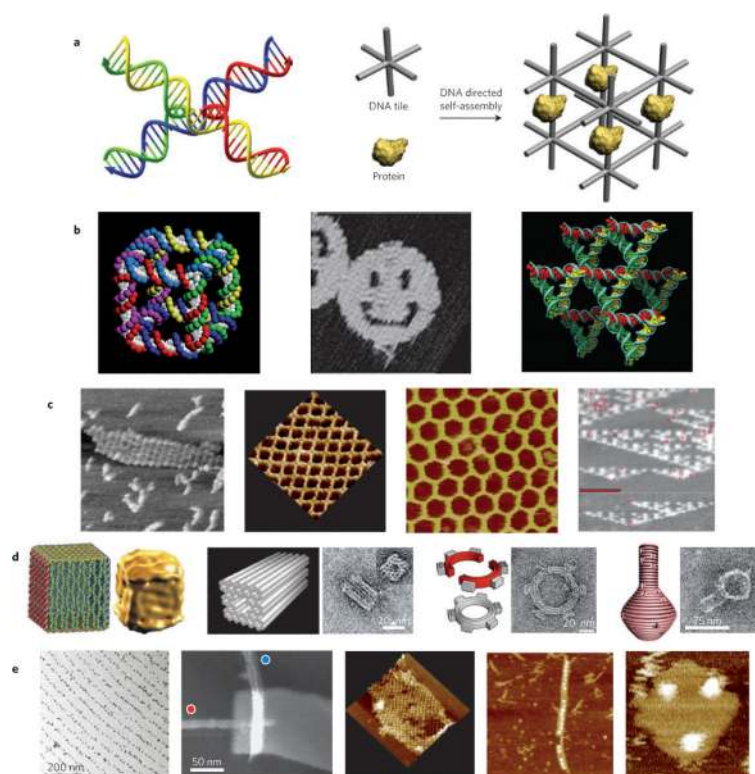


Figure 1. Examples of structural DNA nanotechnology

a, Seeman's original proposal consisted of using immobile DNA junctions (left) to build 3D scaffolds that could be used to organize proteins (right)¹. **b**, Important milestones in structural DNA nanotechnology: the first wireframe 3D cube¹⁰ (left), DNA origami (centre) and a 3D periodic structure composed of tensegrity triangles (right). **c**, DNA periodic arrays composed of double-crossover tiles (left), 4×4 tiles (centre left), three-point star tiles (centre right) and double-crossover-tile-based algorithmic assembly of Sierpinski triangles (right). **d**, Three-dimensional DNA origami: a hollow box (left pair of images), a multi-layer square nut (centre left pair), a square-toothed gear (centre right pair) and a nanoflask (right pair). **e**, DNA nanostructure-directed patterning of heteroelements: double-crossover tiles for the organization of gold nanoparticle arrays (left), DNA origami for the assembly of carbon nanotubes (centre left), biotin-streptavidin protein patterning of 4×4 tiles (centre), aptamer-directed assembly of thrombin arrays on triple crossover tiles (centre right), and Snap-tag and His-tag mediated orthogonal decoration of DNA origami (right). Figures reproduced with permission from: **b**, Nadrian C. Seeman (left), ref. 42, © 2006 NPG (centre), ref. 38, © 2009 NPG (right); **c**, ref. 19, © 1998 NPG (left), ref. 22, © 2003 AAAS (centre left), ref. 24, © 2005 ACS (centre right), ref. 30, courtesy of P. Rothmund (right); **d**, ref. 43, © 2009 NPG (left), ref. 47, © 2009 NPG (centre left), ref. 49, © 2009 AAAS (centre right), ref. 50, © 2011 AAAS (right); **e**, ref. 62, © 2004 ACS (left), ref. 75, © 2010 NPG (centre left), ref. 22, © 2003 NPG (centre), ref. 53, © 2005 Wiley (centre right), ref. 57, © 2010 Wiley (right).

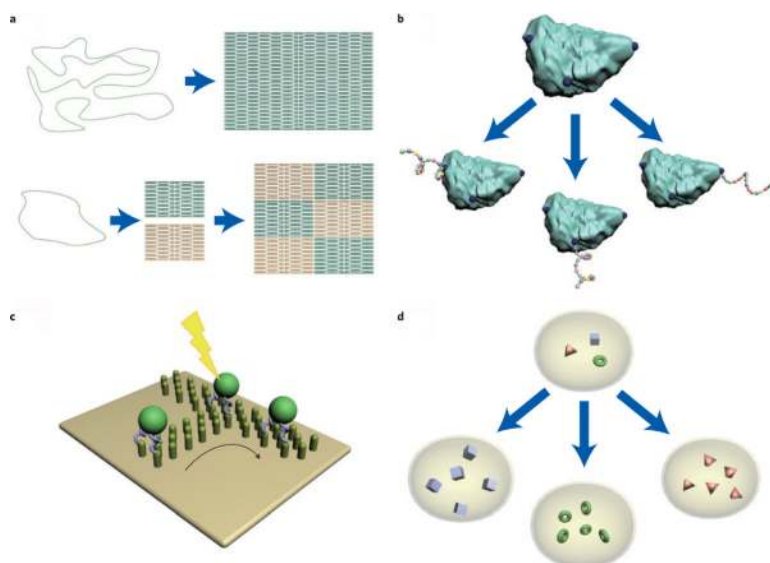


Figure 2. Challenges for DNA nanostructures

a, Expanding size and complexity. Two main approaches are being explored to overcome the current dependence of the structural DNA nanotechnology community on the viral M13 genome: the use of longer DNA scaffold strands (top left) to fold larger structures (top right), or the assembly of pre-formed structures for the constructions of supramolecular assemblies (bottom). **b**, New functional nanostructures. The functionalization of specific protein surface residues (dark blue circles on the light blue proteins) with oligonucleotides, and subsequent purification, would allow for an extra dimension of positioning control of the protein into a DNA template. **c**, New generation of DNA walkers (green spheres with purple legs) with programmable routines and/or sensitive to state changes, such as light, for the selection of routes in multi-path systems. **d**, *In vivo* selection and amplification of DNA nanostructures. Creating procedures for the selection and evolution of biocompatible/bioactive shapes through environmental conditioning, or using cellular replication machinery for the high-throughput production of DNA structures, should lead to new applications of DNA nanotechnology.

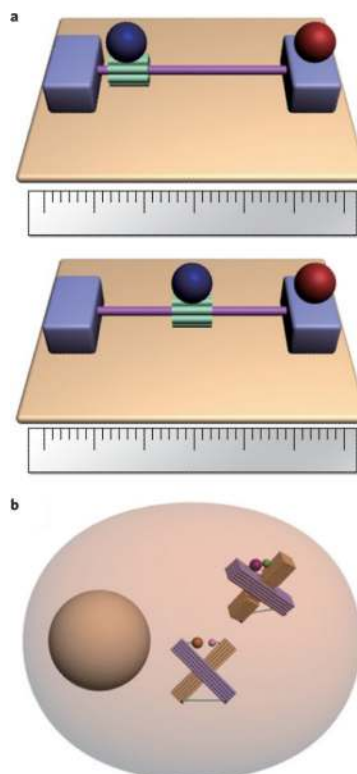


Figure 3. DNA nanotechnology for biophysical studies

a, DNA origami can act as fully addressable molecular pegboards that can be used as molecular rulers for the organization of heteroelements (blue and red spheres). The purple and green blocks can be any DNA structure that directs the sphere position along a platform. A particularly interesting application is the spatial arrangement of enzyme components of cascade reactions. The relative positions of components can be designed with nanometre accuracy, possibly allowing biochemists to suppress diffusion-dependent effects in cascade reactions. This would open classic biochemical systems to new functional properties, and potential improved performances, distinct from bulk reaction measurements. Moreover, such assemblies could be used as models of intracellular compartmentalization or *in vivo* clustering. **b**, When current real-time measurement tools are employed, many *in vivo* interactions elude detection. Fluorescence, and in particular Förster resonance energy transfer, or single-dye fluorescent markers, yield narrow snapshots of *in vivo* reality. DNA scissors, tweezers or tensegrity structures (shown as cross-like structures within translucent pink oval, which represents a cell) may be used for real-time and dynamic measurement of target protein activity, or the specific detection and size estimation of protein complexes required for cellular functions. The DNA nanostructure switches conformation to accompany changes in the shape and size of target structures in their native medium: this allows them to serve as relays between the length scales associated with interactions between protein constructs such as DNA-promoter complexes (~10s of nanometres) and those associated with fluorescence reporting (a few nanometres or less). Two such structures are shown here.

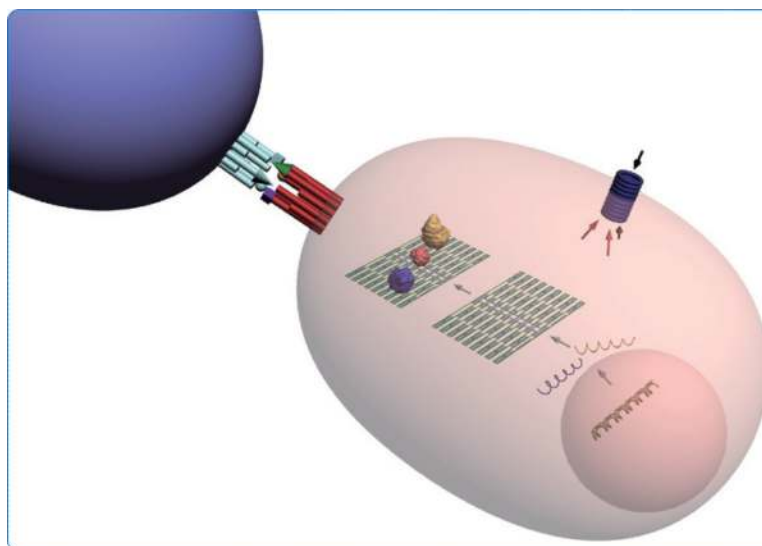


Figure 4. DNA nanostructures as biomimetic and *in vivo* active systems

Aldaye and co-workers recently reported the assembly of two enzymes of a hydrogen-production cascade reaction using RNA arrays, which led to improved yields¹²². *In vivo* replication of complex DNA structures allows intracellular components (blue, pink and yellow objects) to be organized with tighter and more complex spatial control for the study of cellular properties or new capabilities due to the cytosol clustering effect. Conversely, DNA structures can be designed and 'expressed' that fold into biomimetic structures, such as DNA-based nanopores, channels or pumps, introducing artificial layers of cell communication and interaction with its external medium. Also, DNA nanostructures can induce immune responses and actively modulate cell-cell communication on clustering and spatial organization of membrane protein markers, or, in a more abstract concept, acting as specific cell-cell glue (here shown as light blue and red rods connecting the dark blue and pink cells).

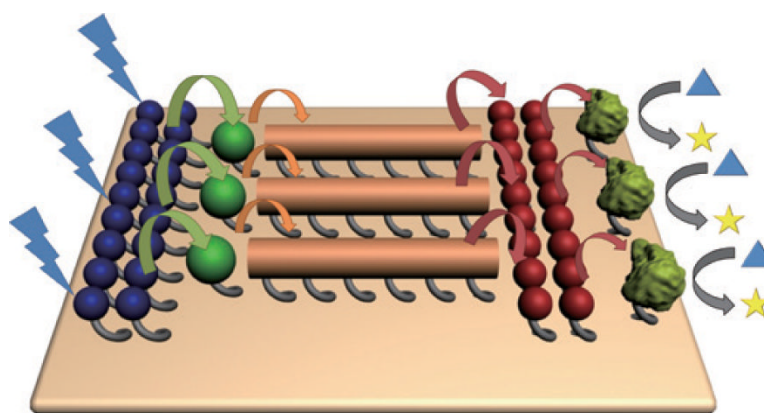


Figure 5. DNA nanotechnology for energy transfer and photonics

DNA nanostructures provide a useful tool for the organization of photonic components in a linear fashion or in branched networks. The modularity of assembly, along with the plethora of DNA functionalization of photonic components, allows for the construction of photonic molecular circuits. Light-harvesting complexes can be spatially clustered and aligned, where sequential energy or charge-transfer processes lead to optimized channelling efficiency, to create a new generation of photonic wires, plasmonic or conducting devices (blue, green and red spheres and orange rods represent photonic components that can serve as light-harvesting and energy-transfer materials). Enzymes or membrane complexes (uneven green spheres) can be used as final energy or electron acceptors, acting as molecular transducer units, where light is transformed into chemical potential (represented by the transformation of substrate (triangles) into a higher-energy product (stars)). Physical separation of photonic components creates a new layer of spectral separation, allowing the construction of larger and more complex photonic circuitry.

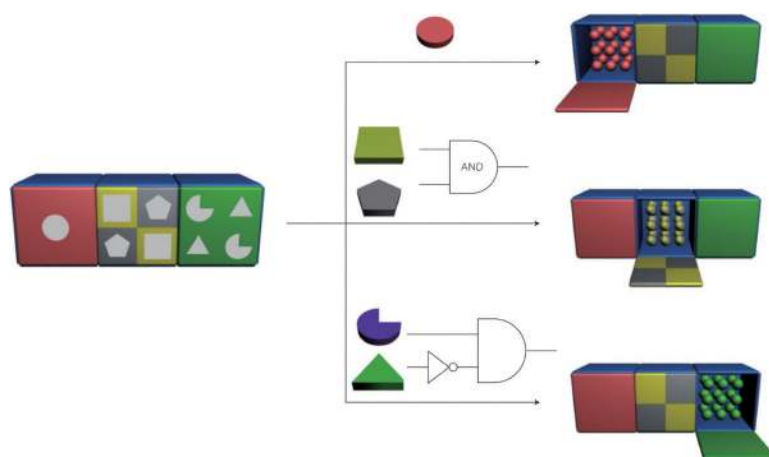


Figure 6. Structural DNA nanotheranostics

DNA structures can be used to build disease-targeting units for diagnostics and therapeutics (or 'theranostics'). Hollow structures are designed in a modular fashion, where multiple pharmacologically active species can be caged into different compartments. Advances in DNA computing may allow the detection of several disease markers (such as interaction between aptamers and membrane receptors, or hormone-activated switches) that are input into a programmed response. The use of multiple input stimuli for the controlled release of drugs may increase drug delivery specificity. This way, the presence of pathogens or multiple cancer markers, for example, can be simultaneously analysed, triggering suitable therapeutics. The magnitude and duration of the response can also be programmed, from continuous cargo release to threshold-controlled dumping. Such a system might be regarded as a platform model of an artificial immune system.