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DNA origami and biotechnology applications: a perspective

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Abstract

The use of DNA as a material has opened up new possibilities in the field of nanobiotechnology. Specifically, DNA origami – a technique in which one long single-stranded DNA scaffold is folded into nanoscale shapes and structures using hundreds of short 'staple' oligonucleotides – has contributed to new innovations within this field. Nanostructures created using DNA origami have found use in applications such as biosensing, triggered drug delivery, enzyme cascades and biomolecular analysis platforms. The unmatched features of DNA nanostructures such as cell permeability, biocompatibility, and spatial positioning have contributed to DNA origami playing an important role in the development of materials for biotechnology applications.

Keywords: DNA origami; DNA self-assembly; nanobiotechnology; DNA nanostructures; structural DNA nanotechnology

Nanotechnology has made a huge impact across several disciplines including chemistry, biology, physics, and medicine and its interdisciplinary nature has given rise to new fields such as nanobiotechnology. In the interface between chemistry and biology, DNA – apart from its biological role as the genetic material – has been shown to be useful for the construction of nanoscale materials using the 'bottom-up' approach.[1] Certain features of the molecule such as the nanoscale size, biocompatibility, specific molecular recognition and control over programmable assembly have made DNA a powerful building block. A variety of DNA materials including two-[2] and three-dimensional lattices,[3–6] topological constructs,[7,8] nanomachines and devices[9–11] and biomolecular analysis platforms[12–15] have successfully been created using the above characteristics. These novel DNA materials provide a natural bridge between nanotechnology and biotechnology, leading to potentially unlimited real-world applications.

The construction of DNA objects and lattices, however, was restricted by the length of the component strands. It was largely dependent on the length of the DNA strand that could be synthesized in a laboratory. The advent of the DNA origami[16] technique has opened up new possibilities to create custom-made DNA nanostructures hundreds of nanometers in size. DNA origami structures are made from a long single-stranded DNA scaffold, which is designed to fold into desired shapes held together by 'staple strands' that bind to complementary regions of the scaffold (Fig. 1). The DNA origami method can be used to create shapes of high complexity[17,18] while also providing a platform for precise arrangements of biomolecular (e.g. proteins)[19] or inorganic components (e.g. gold nanoparticles)[20] on their surfaces, thereby extending their use to biotechnological applications.[21] Construction of nanoscale objects employing this method is highly attractive because of the ease and convenience of design, low production cost, high assembly yield, and unparalleled addressability of the resulting origami structures. DNA nanostructures exhibit several characteristics that make them promising candidates for applications in biotechnology. One such attribute is high cellular permeability[22,23] compared with single-stranded and double-stranded DNA that would be useful for drug delivery applications. In addition, these structures have predictable (designed) size and shape, excellent biocompatibility, and allow functionalization at multiple loci with high site-specificity.

DNA origami nanostructures provide a breadboard to address unique points (nucleotides) on the surface and create predefined arrangements of external guests. This ability allows us to study DNA–protein interaction, ligand binding processes or spatially controlled interactions between different molecules. Such a system of protein interactions has been studied using a DNA origami platform.[24] Glucose oxidase (GOx) and horseradish peroxidase (HRP) were positioned at specific locations on a DNA origami structure and the efficiency of the enzyme cascade was studied based on the diffusion distances between the two proteins (Fig. 2(a)). Programmed placement of desired molecules on DNA origami nanostructures also enables observation of molecular events at the single-molecule level. They have been used as analysis platforms to visualize dynamic motions of molecules using high-speed atomic force microscopy.[25] The orientations of different forms of macromolecules can be controlled by placement into these DNA nanostructures (Fig. 2(b)). Such DNA platforms have been used for controlled regulation of DNA methylation,[26] observation of conformational changes in G-quadruplex structures,[27] and analysis of DNA base-excision repair[14] and site-specific recombination events.[28]

Because of their high cell permeability, DNA architectures can be modified with biologically active molecules on their surfaces to

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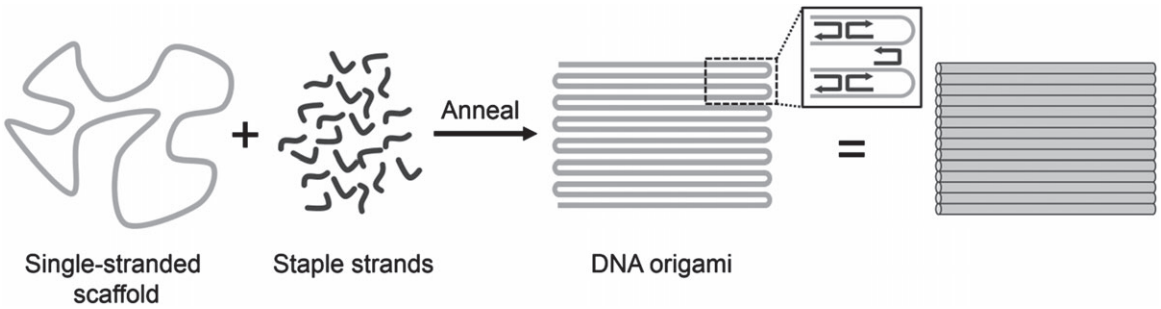

Figure 1. DNA origami construction. An illustration showing the protocol for creating DNA origami structures; a long single-stranded scaffold is folded into desired shapes using hundreds of short staple strands.

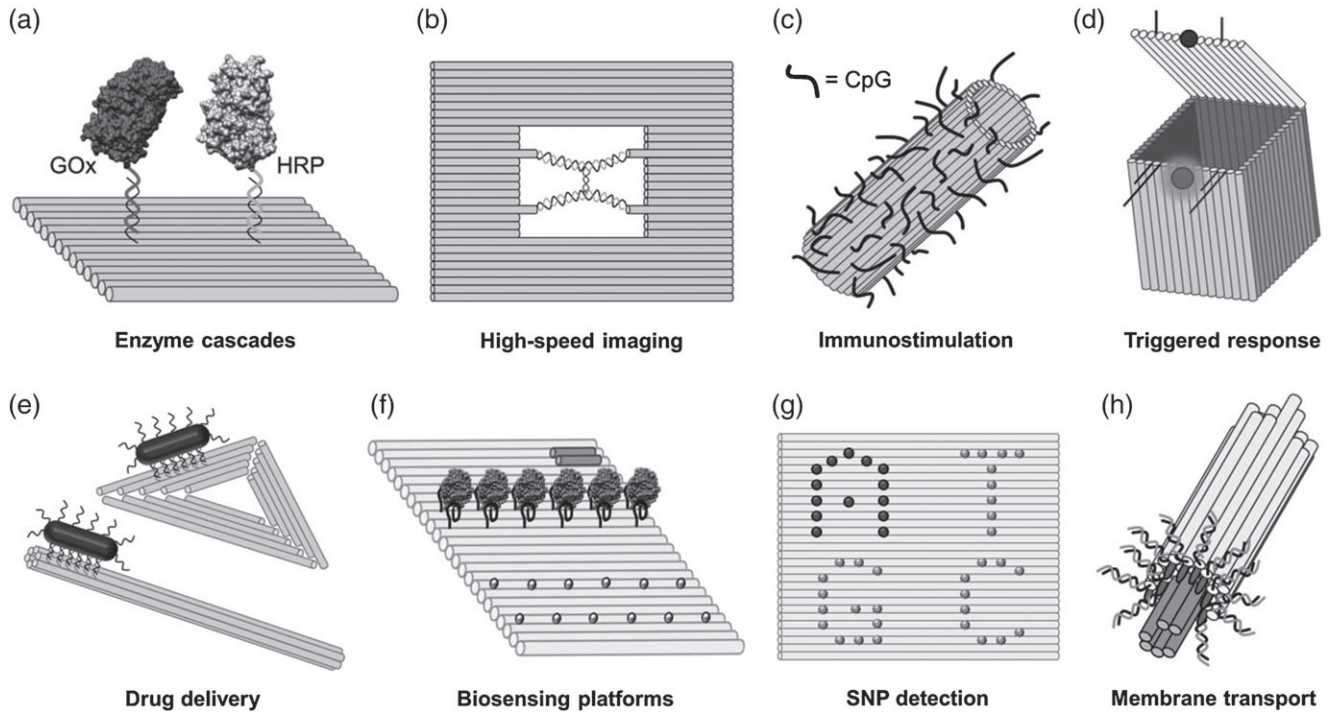

Figure 2. Biotechnology applications of DNA origami. (a) Spatially controlled enzyme cascades involving glucose oxidase (GOx) and horse radish peroxidase (HRP). Image adapted with permission from ref. 24; Copyright © 2012, American Chemical Society (ACS). (b) A DNA-origami frame for fast-scanning AFM analysis of molecular events. Image adapted with permission from ref 25; Copyright © 2014, ACS. (c) A CpG sequence coated DNA origami nanotube for cellular immunostimulation. Image adapted with permission from ref. 29; Copyright © 2011, ACS. (d) A nanoscale DNA box with a controllable lid for targeted drug delivery. Image adapted with permission from ref. 34; Copyright © 2009, Nature Publishing Group. (e) DNA origami-gold nanorod complex for cancer theranostics. Image adapted with permission from ref. 36; Copyright © 2015, Wiley-VCH. (f) Nucleic acid probe tiles for label-free RNA hybridization assays. Image adapted with permission from ref. 37; Copyright © 2008, American Association for the Advancement of Science (AAAS). (g) A DNA-origami visual read-out platform for single nucleotide polymorphism detection. Image adapted with permission from ref. 38; Copyright © 2011, ACS. (h) Synthetic lipid membrane channels formed by DNA origami act as membrane transport channels. Image adapted with permission from ref. 39; Copyright © 2012, AAAS.

trigger cellular mechanisms. DNA origami-based structures have been shown to be biologically-active carrier systems for CpG sequences (Fig. 2(c)). Hosting CpG sequences on these DNA nanostructures was found to elicit a high level of immune response in mammalian cells.[29] Since DNA oligonucleotides can be modified with a wide variety of biomolecules, this approach could be extended in combination with viral components to generate vaccines and adjuvants with precisely tailored surfaces. More intensive research is needed to analyze the cytotoxicity, immunological behavior, and the general biocompatibility of DNA constructs, but they have so far exhibited unprecedented advantages over current strategies for use as intelligent drug carriers. The occurrence of different forms of DNA,[30,31] environment-dependent inter- and intramolecular complexes[32] and toe-hold mediated strand displacement process[33] have led to the construction of dynamic DNA nanostructures. One such example is the DNA origami box with a controllable lid (Fig. 2(d)) that can be locked or opened using external stimuli (in this case, additional DNA strands).[34] Another example is a 'clam-shell'-like DNA nanostructure that contains two aptamers specific to two different receptors on the cell membrane.[35] These DNA containers have been successfully used for targeted release of proteins upon binding to cancer cells, which effectively induced apoptosis. DNA origami nanostructures also serve as templates for the integration of multiple functional elements into a unified platform. Gold nanorods functionalized on DNA origami triangles and nanotubes (Fig. 2(e)) have been used for

photothermal cancer therapy *in vitro* and *in vivo*.[36] The dual functionality of gold nanorods and DNA origami provided improved cellular uptake and these structures exhibited enhanced antitumor efficacy compared with bare gold nanorods.

Since DNA nanostructures are completely water-soluble, anchored molecular probes can be designed to interact with their targets in homogeneous solution rather than at a heterogeneous interface. A DNA origami-based platform was used to create a 'molecular chip' through site-specific anchoring of DNA probes.[37] This solution-phase DNA array was used for label-free RNA hybridization assays (Fig. 2(f)). DNA origami platforms were also used as a biosensor for detecting single nucleotide polymorphisms (SNPs).[38] In this case, the platform was designed to contain hairpin extensions arranged in the shapes of the alphabet letters corresponding to nucleotides (A, T, C, G) and used as a visual read-out of SNP detection using atomic force microscopy (Fig. 2(g)). The ability to functionalize DNA strands with a variety of moieties is an important feature that makes the use of DNA as a material more attractive. Cholesterol-tagged DNA origami nanopore structures have been used to mimic transmembrane channels in lipid bilayers (Fig. 2(h)); these structures showed a response similar to natural ion channels.[39] Such DNA origami nanopores have also been used to control DNA translocation.[40] Tailored pore size in these structures was used to control the folding of dsDNA molecules while specific binding sites in the DNA origami nanopore allowed selective detection of single-stranded DNA as a function of the DNA sequence.

In summary, DNA origami-based nanostructures have been used as molecular chips for label-free RNA detection, for triggered cargo release, immunostimulation and as platforms for spatially-controlled enzyme cascades and analysis of dynamic molecular events. DNA origami nanostructures of varying shapes and sizes have been shown to be stable in cell lysates obtained from various normal and cancerous cell lines.[41] The size of DNA origami nanostructures, the existence of well-developed chemical and enzymatic methods to modify their nucleotides and functionalities, and their biocompatibility make them versatile nanostructures with widespread biological applications. However, the major bottleneck in the creation of DNA origami structures is the limited availability of suitable ssDNA scaffolds. DNA origami designs are constrained to discrete dimensions due to the use of entire genomes as scaffolds (for example, the frequently used M13). Recently developed 'molecular canvas' strategy[42] and single-stranded 'DNA bricks'[43] provide alternative routes to the creation of nanometer-scale structures. Other constraints such as DNA geometry and sense/antisense pairing necessitate manual adjustment in designing complex structures using DNA origami. The DNA origami technique, however, has been enhanced by recent improvisations such as automated routing algorithms,[44] analysis of efficient folding pathways[45] and the fabrication of finite-size wireframe nanostructures with high complexity and programmability.[46]

Despite the limitations in size and availability of scaffold sequences, the ease of preparation of DNA origami makes it a widely used procedure for spatial arrangement of molecules at the nanoscale. In fact, the size range (hundreds of nanometers to micron-scale) of DNA origami structures makes it suitable for developments in two primary areas of applications: nanomedicine and solid state devices. While the former application requires compact nanostructures, the latter requires nanometer to micrometer scale structures that can be combined with top-down approaches for spatial patterning. Recent advancements in the field of nanobiotechnology highlight the possibility of exploiting the unique properties and biocompatibility of DNA nanostructures for *in vivo* applications.[36,47] Applications in nanomedicine will be aided by further research in the encapsulation of a variety of drugs and biomolecules, and triggered responses based on external and environmental stimuli.[48] The use of DNA origami to pattern micrometer-scale structures created by lithography[49,50] holds promise in the development of solid state devices with precise and programmable surface interactions.

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