Cancer Science
Japanese Cancer Association JCA
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Unsolicited Review Article

DNA origami applications in cancer therapy

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Key words
Cancer, DNA origami, drug delivery, nanotechnology, targeted therapy

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Funding Information
Burapha University, Mahidol University.

Received April 25, 2017; Revised May 23, 2017; Accepted May 24, 2017

Cancer Sci **108** (2017) 1535–1543

doi: 10.1111/cas.13290

Due to the complexity and heterogeneity of cancer, the development of cancer diagnosis and therapy is still progressing, and a complete understanding of cancer biology remains elusive. Recently, cancer nanomedicine has gained much interest as a promising diagnostic and therapeutic strategy, as a wide range of nanomaterials possess unique physical properties that can render drug delivery systems safer and more effective. Also, targeted drug delivery and precision medicine have now become a new paradigm in cancer therapy. With nanocarriers, chemotherapeutic drugs could be directly delivered into target cancer cells, resulting in enhanced efficiency with fewer side-effects. DNA, a biomolecule with molecular self-assembly properties, has emerged as a versatile nanomaterial to construct multifunctional platforms; DNA nanostructures can be modified with functional groups to improve their utilities as biosensors or drug carriers. Such applications have become possible with the advent of the scaffolded DNA origami method. This breakthrough technique in structural DNA nanotechnology provides an easier and faster way to construct DNA nanostructures with various shapes. Several experiments proved that DNA origami nanostructures possess abilities to enhance efficacies of chemotherapy, reduce adverse side-effects, and even circumvent drug resistance. Here, we highlight the principles of the DNA origami technique and its applications in cancer therapeutics and discuss current challenges and opportunities to improve cancer detection and targeted drug delivery.

Conventional chemotherapeutic agents have significant drawbacks, such as low solubility, low stability, and cytotoxicity, which have led to inadequate efficiency of cancer therapy. These obstacles can be minimized by a drug delivery system in which a carrier directly delivers the drug to specific target cells. Nanoparticles are now established and widely used as pharmaceutical delivery systems in the clinic for both diagnostic agents and therapeutic drugs. Various materials have been explored to be utilized for nanocarrier construction, including liposomes,[1] poly lactic-co-glycolic acid,[2] metals, such as gold and silver nanoparticles (AuNPs and AgNPs, respectively),[3] and magnetic nanoparticles.[4] However, these materials still have disadvantages. For instance, construction of liposomal nanoparticles with uniform size, shape, and charge is difficult, and multifunctional modification to some nanoparticles can be laborious and inefficient.

Self-assembly of DNA molecules could be programmed by complementary base pairing interactions. These properties make it a promising candidate as a structural building block for nanoscale construction. Nadrian C. Seeman pioneered the use of DNA to construct nanostructures,[5] that has culminated in the field of "structural DNA nanotechnology." Not only are DNA nanostructures biocompatible and biodegradable, but they can also be modified with a wide range of functional entities, such as aptamers,[6,7] lipids,[8,9] proteins,[10–12] and inorganic nanomaterials,[13–15] making DNA nanostructures an attractive platform for the development of drug delivery systems.

DNA Origami Technique

Based on structural DNA nanotechnology, DNA nanocarriers could be designed and constructed in a controllable manner. However, the size and complexity of DNA nanostructures made by conventional methods are quite limited. In order to construct large DNA nanostructures, the scaffolded DNA origami technique was introduced in 2006 by Paul Rothemund.[16] This technique is based on the folding of a long single-stranded DNA (ssDNA) (scaffold) with the help of hundreds of short ssDNA (staples) to hold the scaffold in place (Fig. 1a,b). This technique has enhanced potential applications of DNA nanotechnology as the construction of larger DNA nanostructures has now become possible with less time and labor. Since then, DNA origami nanostructures in diverse sizes and shapes have been reported, including discrete objects like nanotubes,[21] a dolphin,[22] and a tetrahedron.[23] Later, researchers successfully generated 3D DNA origami nanostructures with multilayers, such as a monolith, a square nut, and a railed bridge (Fig. 1c),[18] and DNA origami with complex curvatures, such as an ellipsoid, a sphere, and a nanoflask.[24]

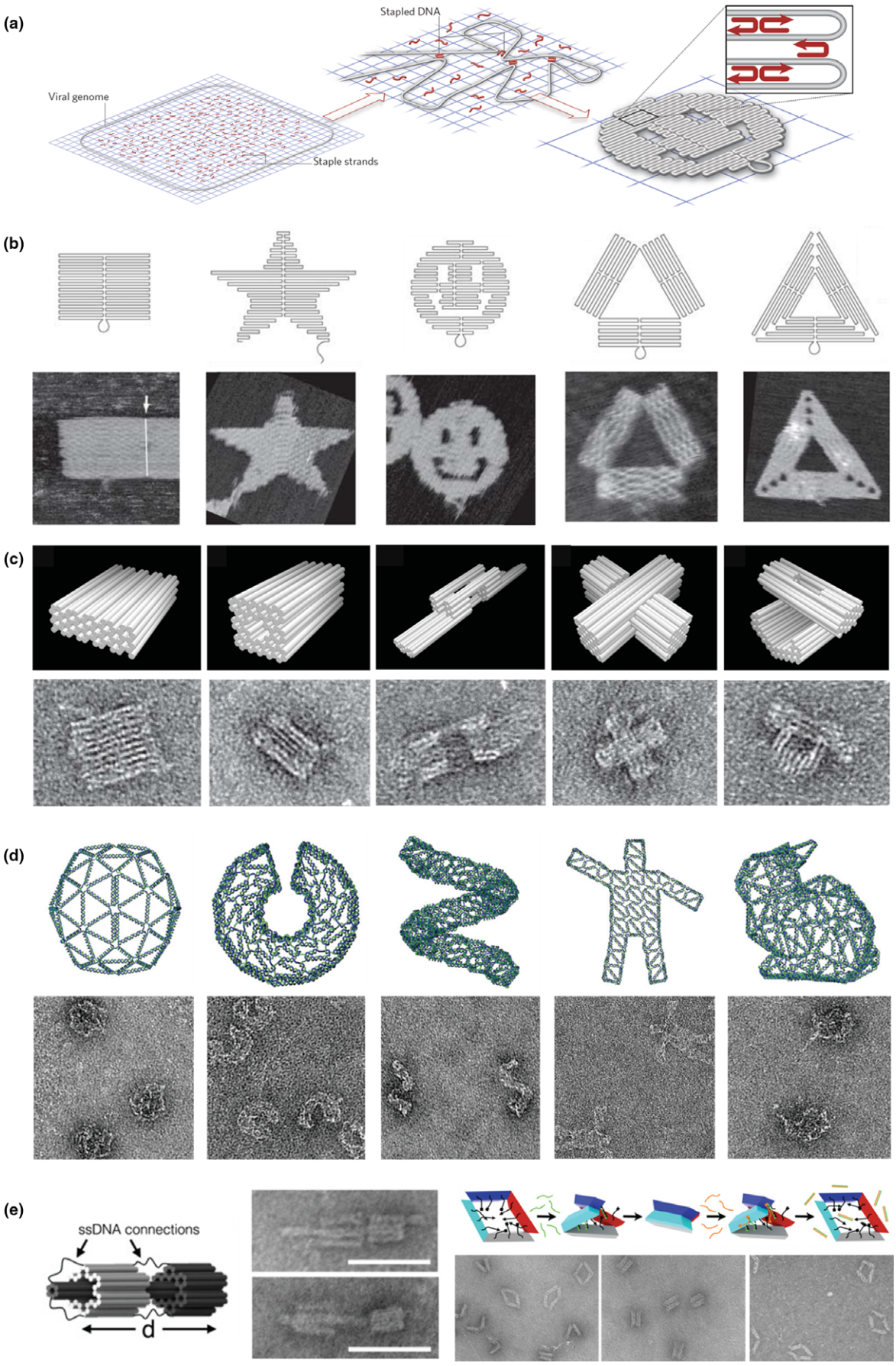

Fig. 1. DNA origami technique and nanostructures. (a) Principles of DNA origami technique. Hundreds of staples (red) fix the scaffold (gray) to create a desired shape. Reproduced from Sandersen (2010), with permission from [Nature Publishing Group].[17] (b) First examples of DNA origami nanostructures from Rothemund. Top panels are the designed shapes and bottom panels are atomic force microscope (AFM) images. Reproduced from Rothemund (2006), with permission from [Nature Publishing Group].[16] (c) Multilayered DNA origami nanostructures. Top panels, designed shapes; bottom panels, AFM images. Reproduced from Douglas *et al.* (2009), with permission from [Nature Publishing Group].[18] (d) Wireframe DNA origami nanostructures. Top panels, designed shapes; bottom panels, AFM images. Reproduced from Benson *et al.* (2015), with permission from [Nature Publishing Group].[19] (e) Movable DNA origami nanostructures. Reproduced from Marras *et al.* (2015), with permission from [US National Academy of Sciences].[20]

Recently, mesh-like, wireframe DNA origami structures have been developed and are reported to be more stable in low magnesium concentrations (Fig. 1d).[19] Moreover, the development of dynamic DNA origami, such as a nanobox,[25] a logic gated nanorobot,[6] a nanocapsule,[26] a movable slider, and an actuator (Fig. 1e),[20] have significantly advanced the scaffold DNA origami technique and hold great promise for highly complex DNA nanostructures.

Design software. Computer programs that could be used to assist researchers in visualizing designed DNA nanostructures in 3D perspectives, such as GIDEON[27] and Nanoengineer-1,[28] have been developed. However, they were not specifically designed for the scaffolded DNA origami technique. According to Rothemund's technique, this origami design process is much easier than the conventional process because the sequences of all DNA strands are already defined by that of the scaffold. However, there are certain steps that computer software could assist to complete complex configurations with less time and less human error. As a result, several computer programs have been developed to facilitate the origami design process and assure correct sequence identification and staple strand alignment.

In 2008, Andersen and colleagues demonstrated a software package for designing DNA origami nanostructures, which they used for the construction of a dolphin with a flexible tail.[22] After shape and folding path determination, the software fills in sequences of the M13mp18 DNA into a scaffold path, creates crossover patterns of staple strands, and then generates sequences of all staple strands. However, this program is semi-automated as the process of connecting the staple strands needs to be performed manually. The most popular software developed for DNA origami design called "caDNAno" was launched in 2009.[29] It can be utilized for designing multilayer 3D DNA origami nanostructures where DNA helices can be aligned in two different patterns, a honeycomb lattice and a square lattice. This program also provides a list of scaffolds in different lengths besides M13mp18 that could be selected for origami constructions and then generates a set of staple strands including sequences ready to be synthesized. The simulation software developed for computing 3D DNA origami nanostructures designed by caDNAno is called "Cando."[30] It provides computational analysis for DNA nanostructures such as internal constraints. The latest origami design software, called DNA Origami Sequence Design Algorithm for User-defined Structures or "DAEDALUS," was released in 2016.[31] It can be used for designing arbitrary DNA nanostructures by top-down strategies. The research team claimed that this is a fully automated program that does not require any feedback from users. They also showed that 45 different DNA architectures can be designed and constructed using this program. Collectively, these automated programs effectively facilitate the DNA origami design process, allowing each step to be easier, faster, and more accurate.

Functionalization. In addition to self-assembly properties, DNA origami nanostructures also provide chemical sites for functionalization by a wide range of biomolecules and thus represent a promising candidate to generate multifunctional nanomaterials. Addition of DNA aptamers onto DNA origami can be easily achieved as aptamer sequences can be extended from selected staple strands at predefined positions. With aptamer modification, conformational changes of DNA origami in response to target molecules could be achieved.[6] Moreover, aptamer-modified DNA origami nanostructures could be used as a malaria diagnostic tool (Fig. 2a).[7]

DNA origami nanostructures can also be modified by hydrophobic moieties in order to interact with cell membranes. For instance, cholesterol motifs have been attached onto tube-like[8] and monolith[9] DNA origami nanostructures, which enable these DNA nanostructures to fuse with lipid bilayers, as shown in Figure 2(b). In addition, DNA origami nanostructures that are modified by protein moieties have been used in the regulation of many cellular processes. For example, an attachment of transforming growth factor-β onto a rectangular DNA origami has led to protein translocation into the nucleus.[10] As shown in Figure 2(c), transferrin proteins attached onto DNA origami nanostructures have been confirmed to enhance cellular internalization of these DNA nanostructures into KB cells.[12]

Organic fluorescent moieties, such as Cy3 and Cy5, have also been used to label DNA origami nanostructures by covalently conjugating to staple strands, and can be used in many applications, including cellular uptake experiments.[33–35] Several studies have also used quantum dot (QD)-conjugated DNA origami in bio-imaging and biodistribution studies in animal models.[13–15] Metal nanoparticles have also been incorporated into DNA origami, including AgNPs and AuNPs. With ssDNA-functionalized metal nanoparticles, the immobilization of both AgNP and AuNP onto a triangular DNA origami nanostructure at predefined positions has been demonstrated (Fig. 2d).[32] Interestingly, certain modifications result in stimuli-responsive DNA origami. For example, azo-benzene modification allows DNA origami structures to undergo conformational change following light activation (Fig. 2e).[26,36] Kohman and colleagues also utilized UV light to trigger the release of proteins that were encapsulated inside a DNA origami nanocage.[37]

As promising nanomaterials, many applications have been proposed for DNA origami nanostructures, such as platforms for single-molecule studies,[38] nano-assembly lines,[39] enzymatic studies,[40] and organization of amyloid fibrils.[41] In addition, the DNA origami technique also represents a promising strategy to generate DNA nanostructures for drug carriers and biosensors.

DNA Origami as Drug Delivery Vehicles

In 2006, Erben and coworkers demonstrated that they could encapsulate a single protein, cytochrome c, in a central cavity of a DNA tetrahedron.[42] This work has inspired the idea of using DNA nanostructures as a nanocarrier in a drug delivery system. Several lines of evidence proved that DNA nanostructures possess abilities to enhance efficacies of chemotherapy,

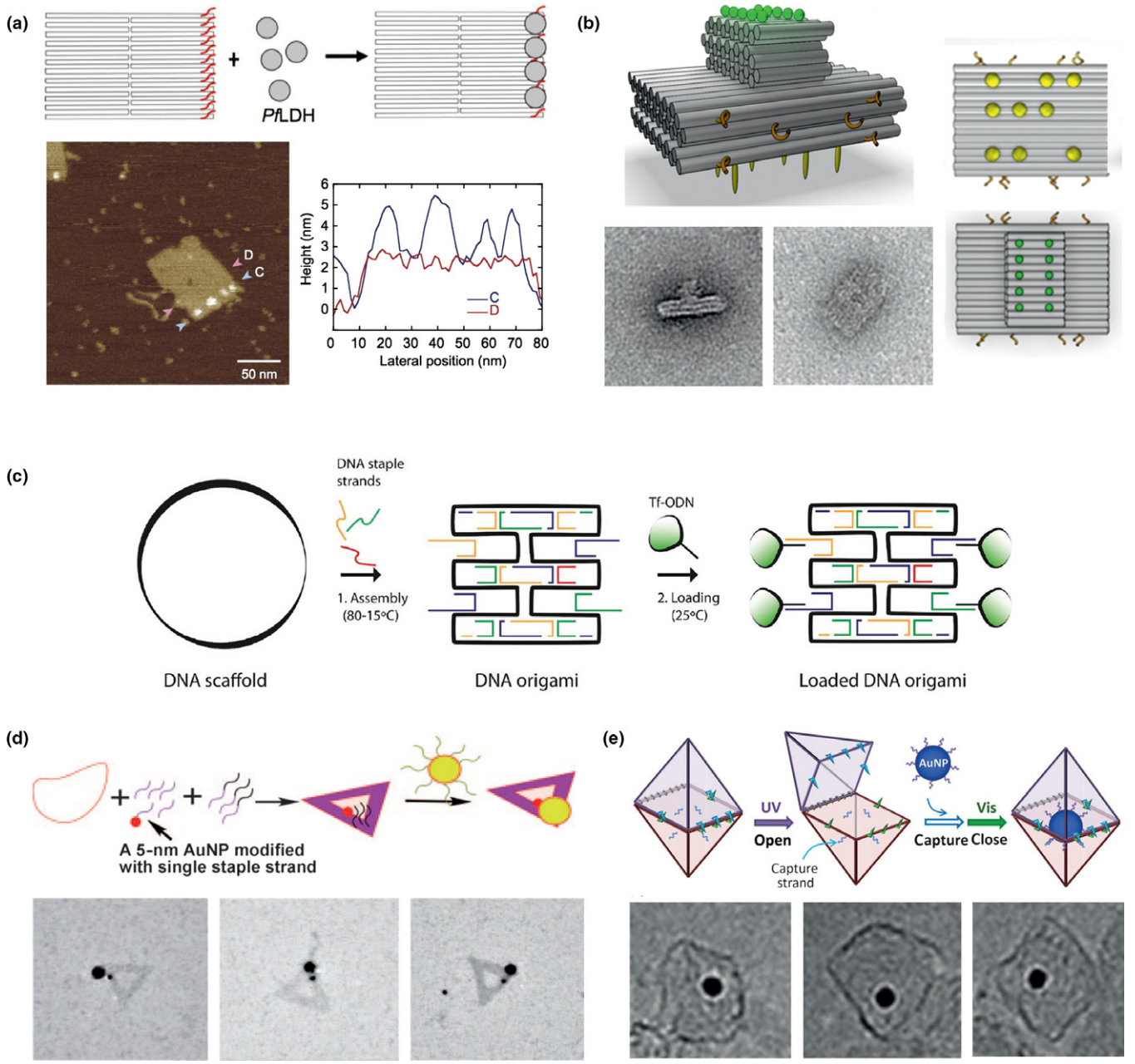

Fig. 2. Functionalized DNA origami nanostructures. (a) Anti-*Pf*LDH aptamer-modified DNA origami rectangles as a diagnostic tool for malaria. Reproduced from Godonoga *et al.* (2012), with permission from [Nature Publishing Group].[7] (b) DNA origami monoliths modified with cholesterols (yellow) and fluorescent molecules (green). Reproduced from Czogalla *et al.*, with permission from [John Wiley and Sons].[9] (c) Transferrin-modified DNA origami rectangles for enhanced cellular internalization. Reproduced from Schaffert *et al.* (2016), with permission from [John Wiley and Sons].[12] (d) Silver nanoparticles (AgNP) (yellow) and gold nanoparticles (AuNP) (red) precisely organized onto DNA origami triangles. Reproduced from Pal *et al.* (2010), with permission from [John Wiley and Sons].[32] (e) Azo-benzene modified DNA origami nanocapsules which their conformational changes could be controlled by light. Reproduced from Takenaka *et al.* (2014), with permission from [John Wiley and Sons].[26]

reduce adverse side-effects, and even circumvent drug resistance. Several studies have reported that DNA origami nanostructures of various sizes and shapes showed no significant cytotoxicity either *in vitro* or *in vivo*.[35,43–45] For use in a biological system, DNA origami nanostructures have to meet certain requirements.

Stability. The stability of DNA nanostructures in a physiological environment is an essential criterion. It has been shown that DNA nanostructures have higher stability than ssDNA and normal DNA duplexes in nuclease-containing conditions.[46] This stability might be the case that unusual shapes and structures of the DNA nanostructures possess physical complexities and hinder the accessibility and functioning of nucleases. Different shapes of DNA origami nanostructures have been shown to remain intact in cell lysates at room temperature for 12 h.[46] Consistently, other reports showed that DNA nanotubes,[43,47] DNA triangles,[15] and rod-like DNA nanostructures[48] were stable under various biological conditions. In contrast, Hahn and co-workers found that the stability of different DNA nanostructures in very low Mg^{2+} concentrations or

in the presence of nuclease might be dependent on structural design and incubation time length.[44] Later, Halley and colleagues also confirmed that structural design might be another key factor to the stability of DNA nanostructures in biological environments.[48] However, some DNA origami nanostructures have been utilized in *in vivo* experiments and the results have confirmed their adequate stability. By i.v. injection into mice, DNA origami could be transported to tumor sites through the bloodstream.[15, 49–51] DNA origami nanostructures have also been investigated inside living insects, *Blaberus discoidalis*, by hemocoel injection and the results showed that these DNA nanorobots could properly function inside living systems.[52,53]

To use as drug delivery vehicles, DNA origami nanostructures must be designed to have optimal stability in cellular conditions. Perrault and Shih showed that, after i.v. injection into mice, a lipid bilayer-encapsulated nanostructure remained in blood circulation significantly longer than the free form (Fig. 3a).[49] Additionally, spermidine-stabilized DNA origami nanostructures have been recently reported to be more stable in cell lysates than plain structures.[54] Therefore, it has been shown that extensive investigations into structural design and modifications have significantly improved the stability of DNA nanostructures and strengthened their potential for use in biological settings.

Drug loading and release. Drug loading and releasing capabilities of DNA origami nanostructures can vary between different shapes and, thus, can play an important role in structural design for nanocarriers. Unmethylated cytosine-phosphate-guanine

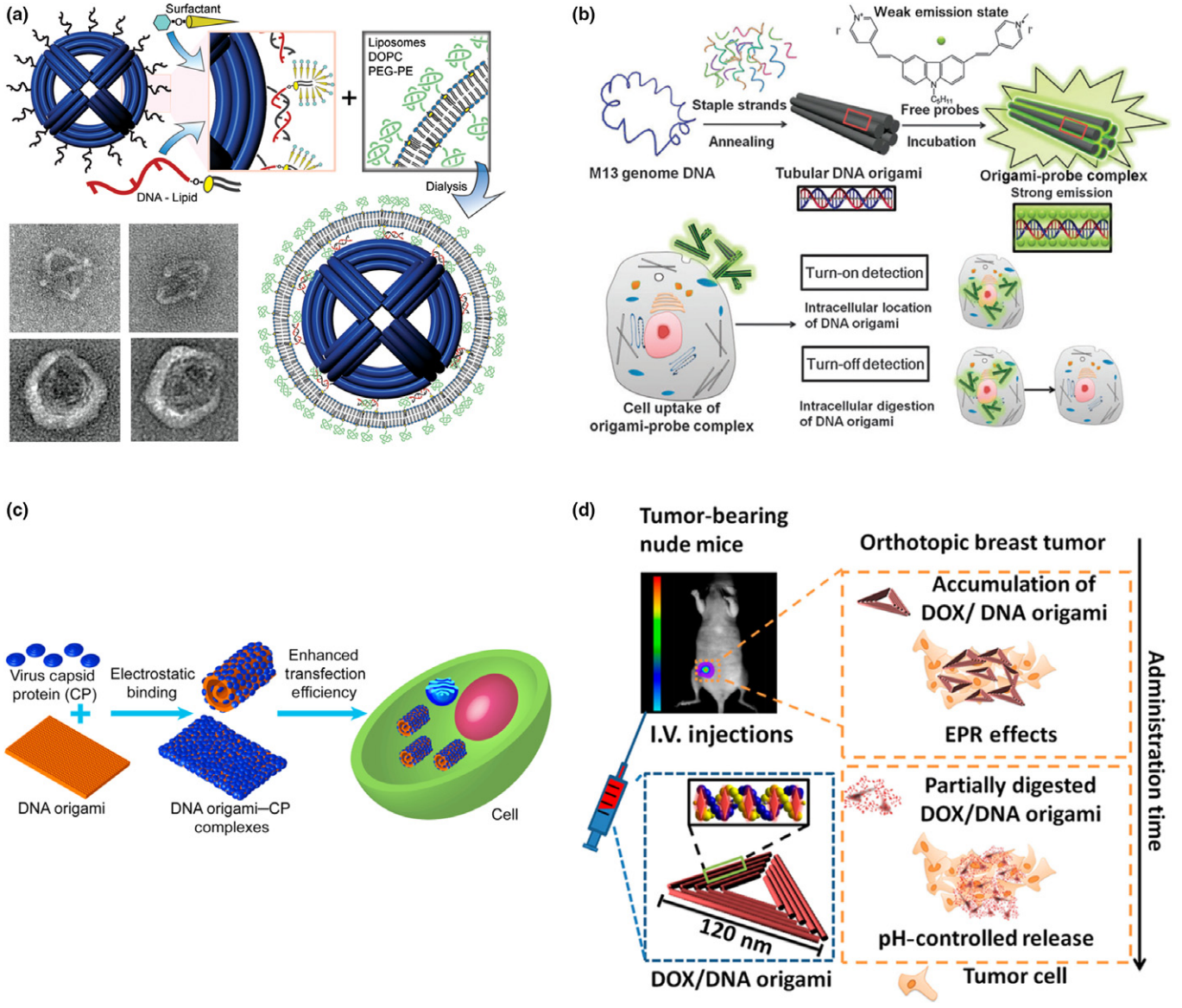

Fig. 3. DNA origami nanostructures as drug carriers. (a) DNA octahedron (blue) encapsulated inside lipid bilayer. Top panels, transmission electron microscopy images of free octahedrons; bottom panels, transmission electron microscopy images of lipid encapsulated octahedrons. Reproduced from Perrault and Shih (2014), with permission from [American Chemical Society].[49] DOPC, 1,2-dioleoyl-sn-glycero-3- phosphocholine; PEG-PE, polyethylene glycol- phosphatidylethanolamine. (b) Fluorescently labeled DNA origami tubes for cellular tracking. Reproduced from Shen *et al.* (2012), with permission from [American Chemical Society].[47] (c) Virus capsid protein (CP; blue) covered DNA origami rectangles (orange). Reproduced from Mikkila *et al.* (2014), with permission from [Royal Society of Chemistry].[35] (d) Doxorubicin (DOX)-containing DNA origami triangles showing enhanced permeability and retention (EPR) effects. Reproduced from Zhang *et al.* (2014), with permission from [American Chemical Society].[15]

(CpG) sequences, which can trigger immune response, have been used as a model cargo and loaded onto DNA nanocarriers by covalent attachment.[34,55] Encapsulation of a Fab fragment,[6] AuNPs,[6] and active enzymes[56,57] inside a cavity of DNA origami nanostructures have been reported. Data showed that these DNA nanostructures allowed the cargo and enzymes to be more stable, more catalytically active, and more resistant to protease digestion. In addition to internal loading, gold nanorods (AuNRs) were functionalized onto a surface of DNA origami nanostructures and were i.v. injected into mice for photothermal therapy applications.[13,50]

Several DNA origami nanostructures have been used for doxorubicin delivery experiments such as a triangle,[15,45] a tube,[43] and ribbon.[58] Previous reports showed that more drugs could be loaded into 3D DNA origami but released faster from 2D structures. Additionally, Zhao and co-workers reported that DNA nanostructures can be designed to vary in their encapsulation abilities and release rate depending on the amounts of relaxation in the DNA double-helix structures.[43] In addition, other intercalating agents have also been tested with DNA origami, for instance, daunorubicin[48] and 3, 6-bis[2-(1-methylpyridinium) ethynyl]-9-pentyl-carbazole diiodide (BMEPC).[59]

Cellular internalization. Larger size and stronger compactness of DNA origami nanostructures have been shown to allow more efficient internalization than less compact structures or individual ssDNA.[34] Ouyang and colleagues constructed DNA nanoribbons in various sizes and found that the DNA nanostructures with high length-to-width ratio were preferentially internalized by cells.[55] Shen and co-workers used carbazole-based cyanine, which shows a strong fluorescent signal when binding to DNA helices, to visualize DNA nanotubes after internalization into MCF-7 cells (Fig. 3b).[47] Confocal microscopy studies showed that the intact DNA nanotubes were internalized into cells and aggregated in lysosomes. In addition, Chopra and colleagues reported that spermidine-modified DNA nanostructures can be delivered into cells through electroporation.[54]

To improve cellular uptake efficiency, DNA nanostructures have been modified with targeting ligands such as folate,[33] cell-penetrating proteins,[58] and transferrin.[12] It has been shown that after functionalization with viral capsid proteins, the cellular internalization of DNA origami nanostructures was enhanced (Fig. 3c).[35] Recent data for DNA nanoribbons proved that, by using inhibitors of specific internalization pathways, the structures were internalized into the cytoplasm of H460 cells by clathrin- and lipid raft-mediated endocytosis.[60] Moreover, these nanoribbons exhibited endosomal escape abilities after 2 h of incubation.

DNA origami nanostructures also showed enhanced permeability and retention (EPR) effects. Passive accumulation of DNA origami in three different shapes, triangle, rectangle, and tube, have been investigated using QD labelling after i.v. injection into tumor-bearing mice (Fig. 3d).[15] The triangles were shown to accumulate at the tumor site at higher levels than the tubular nanostructures 24 h after injection. Consistent with previous results, AuNR-modified triangles exhibited better internalization into MCF-7 cells than AuNR-modified tubes.[50] These consistent results seem to confirm that the cellular uptake effects are dependent on the size and shape of the nanostructures.[45] However, it remains inconclusive which structural design of DNA origami nanostructures is most preferable for cellular internalization.

Therapeutic efficacy. Nanocarrier properties such as optimal stability, low cytotoxicity, high loading, and releasing capability collectively lead to high efficacy in cancer therapy. Several studies showed that DNA origami nanostructures enhanced anticancer activities and circumvented drug resistance. Jiang and colleagues reported that triangular and tubular DNA origami nanostructures with doxorubicin resulted in increased apoptosis of doxorubicin-resistant breast cancer cells,[45] dependent on the length of incubation time and drug concentration (Fig. 4a). Interestingly, when loaded in a DNA origami structure, doxorubicin was retained inside DNA nanostructures and gradually diffused out, causing slower cellular elimination rates, whereas free drugs have faster cellular elimination rates.[43] This property resulted in higher numbers of apoptotic cells in the drug–DNA nanotube-treated group compared to the free drug-treated group.

DNA nanocarriers have been reported to reduce the side-effects of chemotherapeutic drugs. Zhang and co-workers showed that doxorubicin-containing DNA triangles effectively decreased tumor size while mice showed no weight loss when compared to those in the free drug group, indicating that doxorubicin-loaded DNA nanocarriers were less toxic.[15] More results showed that DNA nanostructures can enhance therapeutic efficiency. After loaded with doxorubicin, DNA nanoribbons functionalized onto AuNPs exhibited higher antitumor efficiency compared to free doxorubicin.[58] These data indicate that DNA nanoparticles can be internalized by cancer cells and prolong the effects of therapeutic drugs and significantly enhance drug efficacy with fewer side-effects.

Photodynamic therapy. Photodynamic therapy (PDT) is a cancer treatment that uses photosensitizers along with light to kill cancer cells. A number of photosensitizers for PDT exist, including porphyrins, aminolevulinic acid, and silicon phthalocyanine Pc 4. However, some agents suffer from limitations such as weak absorption, rapid clearance, and poor solubility, which consequently result in inadequate therapeutic efficacy.[61] DNA origami nanostructures have also been used as nanocarriers of photosensitizer agents for applications in PDT. For instance, AuNRs have been used as a photosensitizer functionalized onto DNA origami.[50,51] After attachment of AuNRs, these AuNR-modified nanosystems were injected into nude mice and examined for both cellular imaging and photothermal therapy purposes (Fig. 4b). The results indicated that DNA nanocarriers successfully delivered AuNRs into the tumor region and caused tumor-specific damage following near-infrared irradiation laser treatment. Tumor cell viability was significantly lower in mice treated with AuNR-modified DNA nanocarriers compared to those treated with free AuNRs. In addition to AuNRs, the biomedical efficiency of BMEPC-loaded DNA triangles as cellular imaging and PDT has been reported.[59] These examples indicate that DNA nanoparticles can also be used in combination with other cancer therapeutic systems and effectively reduce adverse side-effects due to increased specificity and unique carrier properties.

Detection. In addition to drug delivery, nanoparticles can be used in cellular and molecular imaging for cancer detection.[62] The development of targeted cancer therapy, as well as the advances of nanomaterials suitable for biomedical sciences, gives rise to the need for powerful imaging tools to probe molecular and microenvironmental changes that are associated with cancer progression. By chemical modification with fluorescent molecules, DNA nanostructures could be used as imaging agents for cellular detection. Fluorescent probes such as cyanine dye molecules can be covalently incorporated into the strands of DNA origami and used as a means of direct visualization in live cells.[47,59] DNA origami nanostructures were successfully conjugated with infrared-emitting QDs and

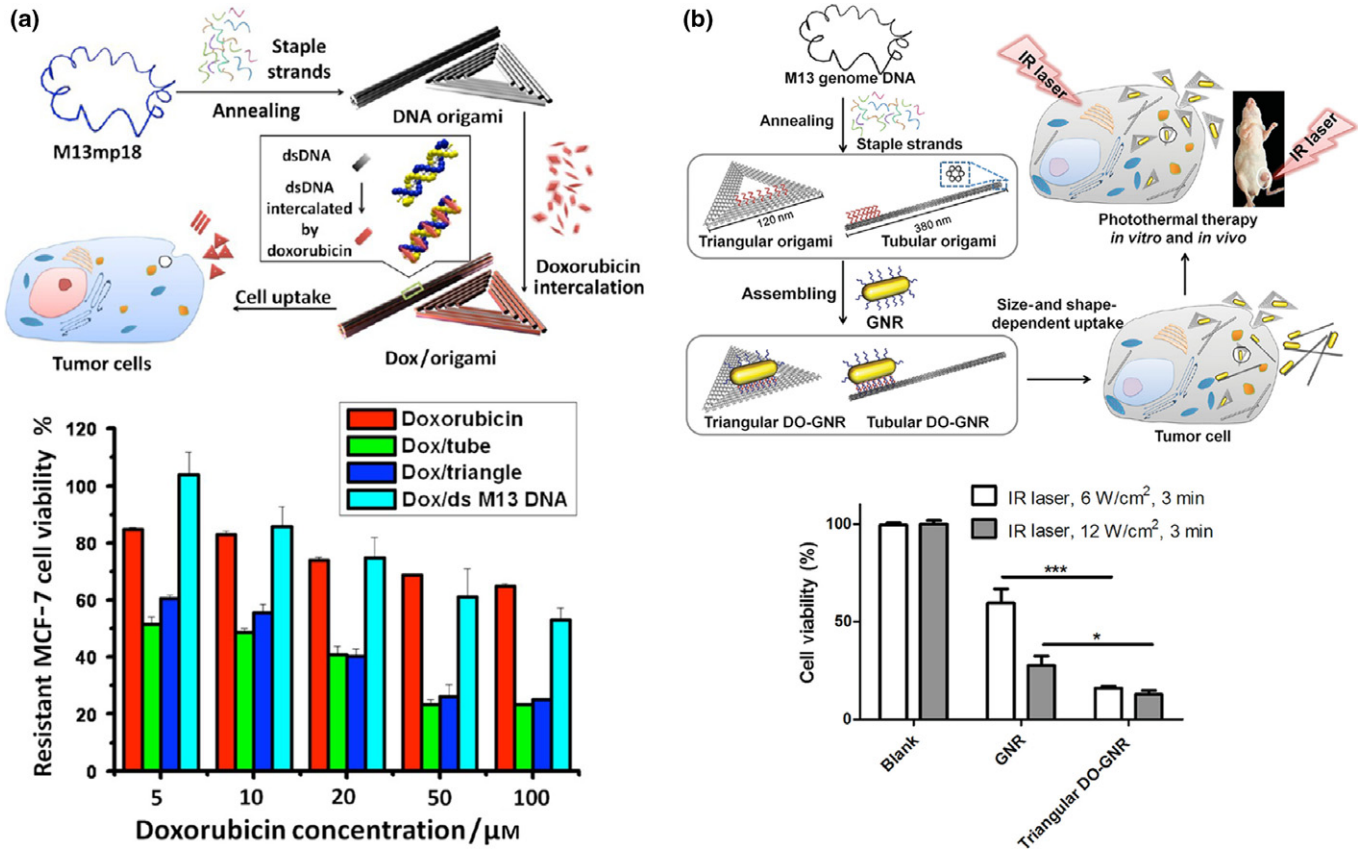

Fig. 4. Additional applications of DNA origami nanocarriers. (a) Doxorubicin (Dox)-loaded DNA origami tubes and triangles exhibited drug resistance circumvention when treated with resistant MCF-7 cells. Reproduced from Jiang (2012), with permission from [American Chemical Society].[45] (b) Gold nanorod (AuNR)-functionalized DNA origami tubes and triangles used in photothermal dynamic therapy showed lower percentage cell viability of tumor cells in mice. Reproduced from Jiang *et al.* (2015), with permission from [John Wiley and Sons].[50] DO-GNR, DNA origami-gold nanorod; dsDNA, double-stranded DNA; GNR, gold nanorod; IR, infrared.

remained stable at high salt concentrations.[14] Moreover, rectangular DNA origami nanostructures have been developed as microRNA analysis tools used for heart failure diagnosis.[63]

Summary and Perspectives

Over 35 years, tremendous progress in structural DNA nanotechnology has been made, and a wide range of medical applications are now obvious to researchers and clinicians. In addition to DNA origami, other DNA nanostructures have been investigated as nanocarriers for drug delivery systems. For example, DNA hydrogels have been used to deliver drug molecules like camptothecin and insulin,[64] doxorubicin,[65] CpG motifs,[66] and siRNA,[67] which could be loaded inside the porous cavity. Nanopores constructed by a DNA origami technique could be used to regulate the entry of therapeutic drugs into cancer cells.[8,68,69]

The DNA origami technique also plays a key role in accelerating the advances in this research field. Although many challenging tasks have been overcome, it is still too early for DNA origami to be used as a drug carrier system in clinical trials. The stability of these macromolecules in physiological conditions is one of the most essential criteria to be considered. Previous results revealed that DNA nanocarriers can survive long enough to reach the target site and complete their functions. Also, DNA is a biomolecule found in living organisms that should not exhibit any cytotoxic effects; however, pharmacokinetic and pharmacodynamic studies of these DNA nanostructures in living animals need to be explored further. Long-term cytotoxicity of DNA nanocarrier use is also essential for future clinical trials. In addition, various therapeutic molecules have been tested as cargo for drug loading and releasing capacities of DNA nanocarriers. Some results showed that loading and releasing capabilities could be tuned by structural design. Even though these DNA origami nanostructures show drug resistance circumvention, the internalization pathway of the DNA nanocarriers should be thoroughly investigated. The ultimate goal of targeted drug delivery is to deliver a drug to a specific site in the body, which results in the requirement of small doses and, therefore, minimal side-effects to normal tissues. *In vivo* targeted delivery by DNA origami nanostructures using the EPR effect as a passive targeting method has been largely proved, but an active targeting method by targeting ligand modification onto origami nanostructures still remains elusive. To increase the therapeutic efficiency, selectivity, and specificity of the nanocarriers are key parameters that should be carefully designed and further examined.

Acknowledgments

This work was supported by Mahidol University (T.K.) and Burapha University (A.U.).

Disclosure Statement

The authors have no conflict of interest.

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