Trends in Biochemical Sciences

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Review

DNA Nanocarriers: Programmed to Deliver

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Simple base-pairing rules of complementarity, perfected by evolution for encoding genetic information, provide unprecedented control over the process of DNA self-assembly. These rules allow us to build exquisite nanostructures and rationally design their morphology, fine-tune their chemical properties, and program their response to environmental stimuli. DNA nanostructures have emerged as promising candidates for transporting drugs across various physiological barriers of the body. In this review, we discuss the strategies used to transform DNA nanostructures into drug delivery vehicles. We provide an overview of recent attempts at using them to deliver small molecule drugs and macromolecular cargoes and present the challenges that lay ahead for these synthetic vectors as they set new paradigms in the field of nanotechnology and medicine.

Highlights

DNA can be used as a building block for creating nanoscale structures.

DNA nanostructures are programmable, offering unique characteristics for use as drug delivery carriers.

DNA nanocarriers can be targeted to specific cells; be triggered using chemical, molecular or environment cues to release drugs; and offer tunable cellular uptake profiles.

DNA nanocarriers are used for a variety of cargos including small molecules, therapeutic oligonucleotides, antibodies, peptides, and photosensitizers.

Nanocarriers can be functionalized to provide a 'see-and-treat' strategy for the creation of theranostics.

DNA Nanostructures and the Fantastic Voyage

In the 1966 movie 'Fantastic Voyage', a submarine is shrunk down to microscopic size and injected into the bloodstream of a scientist to surgically remove a blood clot in his brain. In 2018, we still have no technology that lets us shrink objects. But we do possess technologies to build and deploy nano-sized machines with a variety of arsenals to target various pathologies. These nanovehicles are as good as the submarine in the Hollywood classic. Leading these new generation nanocarriers are the products of DNA nanotechnology.

Rising beyond the constraints of biology, DNA is now recognized as a powerful material for construction of rationally designed nanostructures. Features unique to the molecule have made it an excellent material for programmed bottom-up assembly of designer nanostructures [1,2] with wide ranging applications in biosensing [3,4], biomolecular analysis [5,6], materials science [7,8], and molecular computation [9,10], to name a few. On the biomedical front, DNA nanostructures have potential utility as drug delivery carriers [11]. An ideal nanocarrier of drugs preserves the biological properties of drugs *in vivo*, while helping to decrease pharmaceutical doses and to minimize potential side effects. To achieve this, a drug delivery carrier should possess the following characteristics: good safety profile, ease of cargo loading, target specificity, high cellular uptake, intracellular biostability, triggered release of the cargo, and allowance for additional functionalities. Advantages of using DNA for this purpose stem from its properties. Molecular recognition offered by **DNA base-pairing rules** (see Glossary) allows for unparalleled control in dictating the size and geometry of DNA nanostructures. Programmable interactions of DNA with other molecules offer multiple functionalities, such as imaging agents and receptor targeting ligands that are useful accessories for *in vivo* destinations. Furthermore, DNA nanostructures are biocompatible and stable under physiological conditions. These features are used to control the circulation time, drug release rate, and specificity to a particular target site. Also, chemical synthesis of DNA strands with any desired sequence provides flexibility in design and enables construction of various nanostructures. As a result, DNA

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nanostructures deliver drug molecules to target sites with improved efficiency, enhancing the efficacy of the drug while sparing other vital organs (Figure 1).

Design of DNA Nanostructures: A Throwback

Design of DNA nanostructures plays a vital role in their applications. Ned Seeman, known as the founder of DNA nanotechnology, proposed in the 1980s that DNA could be used as a framework to crystallize guest molecules [12], a notion that spurred the dawn of this now well-established field. Over the past 3 decades, scientists have created a variety of structures using DNA. A majority of DNA self-assembly is based on **sticky-ended cohesion**, where two motifs containing complementary single-stranded extensions can hybridize to form objects or larger arrays. Some of the very first objects assembled were polyhedra (Figure 2A,B) [13,14], followed by tile-based assembly of periodic lattices (Figure 2C) [15]. A major milestone in this area was the first rationally designed 3D DNA crystal, made by Seeman and coworkers [16].

In 2006, Paul Rothemund introduced the DNA origami technique [17], a strategy in which a long single-stranded 'scaffold' DNA is folded by hundreds of short complementary 'staple' strands into arbitrary shapes (Figure 2D). DNA origami achieved a rock star status in the field soon thereafter. Scientists worldwide used this strategy and further developed it to create a multitude of 2D and 3D objects (both solid and hollow) [18]. The scaffold strand, being several thousands of nucleotides long, made DNA origami useful to create large structures compared to the earlier tile-based approach.

Another strategy to create DNA nanostructures is by using single-stranded DNA tiles (Figure 2E). The tile contains four domains and adjacent tiles connect to each other by pairing up with complementary domains, continuing to form DNA lattices composed of parallel DNA helices. Single-stranded DNA tiles enable creation of 2D structures using the 'molecular canvas' strategy [19] and 3D shapes using single-stranded 'bricks' [20].

DNA Nanostructures for Drug Delivery

A variety of drug cargoes have been delivered with different DNA nanostructures, a preview of which is shown in Figure 3. A list of DNA nanostructures and the cargoes delivered using them is shown in Table 1. The size and shape of DNA nanostructures along with their surface charge and properties are important parameters that affect the localization of nanocarriers to specific organs. DNA nanocarriers can be built with well-defined geometries in sizes ranging from a few nanometers to more than 100 nm, a range that can meet the size requirements for targeted delivery to most organs, including the brain (5–100 nm, efficiency decreases with size), lung (<200 nm so carriers are not trapped in lung capillaries), liver (<100 nm can cross liver fenestrae and target hepatocytes), and lymph nodes (6–34 nm for intra-tracheal administration and 80 nm for subcutaneous administration) [21]. The morphology of the nanocarriers is another crucial physical property that controls the extent of delivery of the cargo to different sites (discussed in section on cellular uptake) [22].

Depending on the type of therapy needed, DNA nanostructures are tailored to carry a variety of drug cargoes. For example, in immunotherapy, DNA nanocarriers can carry unmethylated cytosine-phosphate-guanine (CpG) sequences to enhance immune response, or carry monoclonal antibodies and other drugs as immune checkpoint inhibitors to help the immune system recognize and attack cancerous cells. The main challenge in this therapy is that the DNA nanostructures themselves are likely to elicit an immune response [23]. DNA nanostructures allow seamless integration of nucleic acid therapeutics, owing to their similar chemical nature. They are best suited for the delivery of modern macromolecular drugs such as miRNA, **small**

Glossary

Antisense oligonucleotides: antisense strands act as templates for the mRNA that serves as the source for the protein code. When used for therapy, antisense strands with complementary sequences hybridize with the target mRNA, thereby inhibiting the translation of the target protein.
DNA base-pairing rules: the DNA double helix has two strands (Watson and Crick strands) in which complementary nucleotides pair with each other, that is, adenine with thymine (A:T) and cytosine with guanine (C:G).
Endocytosis: an active process of cellular ingestion by which the plasma membrane of eukaryotic cells folds to surround the substances that need to be taken up into the cell from the extracellular medium.
Intercalation: the insertion of planar molecules of an appropriate size and chemical nature in between the base pairs of double-stranded DNA.
Locked nucleic acids: a class of high-affinity RNA analogs in which the ribose ring is 'locked' in the ideal conformation for Watson–Crick binding. A locked nucleic acid oligonucleotide offers substantially increased affinity for its complementary strand, compared to traditional DNA or RNA oligonucleotides.
Micellization: a process where surfactant molecules condense in water to form stable nanosized aggregates called micelles.
Multi-arm DNA junctions: often used as building blocks in the construction of DNA nanostructures. DNA junctions with 3, 4, 5, 6, 8, and 12 arms have been synthesized.
Opsonization: nonspecific interactions of any foreign body including nanoparticles and DNA nanostructures with plasma proteins resulting in the formation of protein corona, a significant hurdle for any nanocarrier.
Rolling-circle amplification: an isothermal process in which DNA or RNA polymerases continuously add single nucleotides to a primer annealed to the circular template. The process results in a long single-stranded DNA that contains multiple repeating copies of the circular template's complementary sequence.

interfering RNA (siRNA), or **antisense oligonucleotides** with great potential in targeting and altering gene expression or protein production. In addition, incorporation of protein components in DNA nanostructures is an area of interest relevant to many fields of research such as vaccine development, biocatalysis, biosensors, and enzyme replacement therapy. Another application of DNA nanocarriers is in photodynamic therapy [24], where DNA nanostructures act as carriers for conventional photosensitizers (typically small molecules) that have the photosensitivity but may lack solubility and stability in biological environment.

Cargo Attachment

The choice of cargo attachment depends on factors such as tunable stability of the drug–carrier complex and responsiveness to internal, environmental or remote triggers for controlled delivery. Attachment and release of the drug from the carrier is restricted by the need to ensure that the activity of the drug be retained or regained after release at the site of delivery. Different strategies are utilized to load cargo molecules into DNA nanocarriers. For small molecules such as anthracyclines (e.g., doxorubicin) [25] and metal complexes [26], **intercalation** within the DNA duplex of the nanostructure is frequently used (Figure 4Ai). DNA polyhedra [27,28], cage-like DNA nanostructures [29] and DNA origami-based structures (such as triangular, tubular, and cylindrical shapes) [25,30–33] are loaded with anthracyclines using this strategy and show improved tumor localization and toxicity compared to free anthracycline molecules. In addition, triangular DNA origami structures are used to deliver a carbazole-derivative photosensitizer 3, 6-bis[2-(1-methylpyridinium) ethynyl]-9-pentyl-carbazole diiodide (BMEPC) that lacks efficiency due to poor solubility in aqueous environment and a tendency to aggregate [34]. Binding to DNA origami reduces aggregation of BMEPC, leading to enhanced therapeutic efficacy.

To recruit larger molecules (e.g., streptavidin), their ligands (e.g., biotin) can be chemically conjugated to the nanostructure (Figure 4Aii) [35]. For example, in a DNA tetrahedron-based vaccine complex, the component strands were modified to contain biotin that recruited streptavidin within the carrier. The complex also contained CpG oligonucleotides and showed enhanced cellular uptake and strong immune response *in vivo* via induction of streptavidin-specific memory B cells [35]. By modifying DNA strands that are part of the nanostructure to contain specific ligands, any desired protein could be attached to a DNA nanostructure. Alternatively, the cargo can be covalently linked to component DNA strands and assembled as part of the structure (Figure 4Aiii). For example, a photosensitizer, pyropheophorbide-a (Pyro) was covalently linked at the vertices of DNA tetrahedra and used for photodynamic therapy [36].

For attaching nucleic acid cargoes such as CpG motifs [37] and siRNA [38], the simplest route is by designing single-stranded extensions on the nanostructure that are complementary to the cargo oligonucleotide (Figure 4Aiv). DNA tetrahedra hybridized with siRNAs using such a strategy show >50% decreased expression of firefly luciferase in HeLa cells and >60% reduction of GFP in KB cells [38]. In addition, nucleic acid cargoes can be incorporated into the design of DNA nanocarriers. Antisense sequences incorporated into 3D DNA prisms cause robust gene downregulation in HeLa cells expressing firefly luciferase [39]. Similarly, RNA tiles functionalized with anti-PLK1 RNA domains effectively target and downregulate Polo-like kinase 1, an enzyme necessary to prevent disruption of mitosis and apoptosis in human prostate cancer cells [40]. In other examples, CpG motifs are incorporated into DNA tetrahedra [41], DNA nanoflowers [42], and DNA nanotubes [37,43] by extending the component DNA strands. It is possible to increase the potency of CpG-associated DNA structures by merely modifying the DNA content of the nanostructures as in CpG-coupled polypodna [44] and

Single particle tracking: following and analyzing the position of the particle (e.g., drug molecule) as a function of time using a series of images collected over different time points.

Small interfering RNA: short double-stranded RNA that can bind to and promote the degradation of mRNA, thereby interfering with the translation of specific proteins.

Sticky-ended cohesion: two DNA duplexes containing complementary single-stranded overhangs can bind to each other to form a pseudo-continuous duplex. The local structure of the duplex in the sticky-end region is also B-DNA, like the rest of the structure, thus allowing rational design of arrays.

Thermal ablation: destruction of tissue by extreme temperatures (elevated or depressed). The temperature change is concentrated to a focal zone in and around the tumor.

Toehold-based strand displacement: a process through which two strands with partial or full complementarity hybridize to each other, displacing one or more pre-hybridized strands in the process. The process is initiated at complementary single-stranded domains (toeholds) and progresses through a branch migration process.

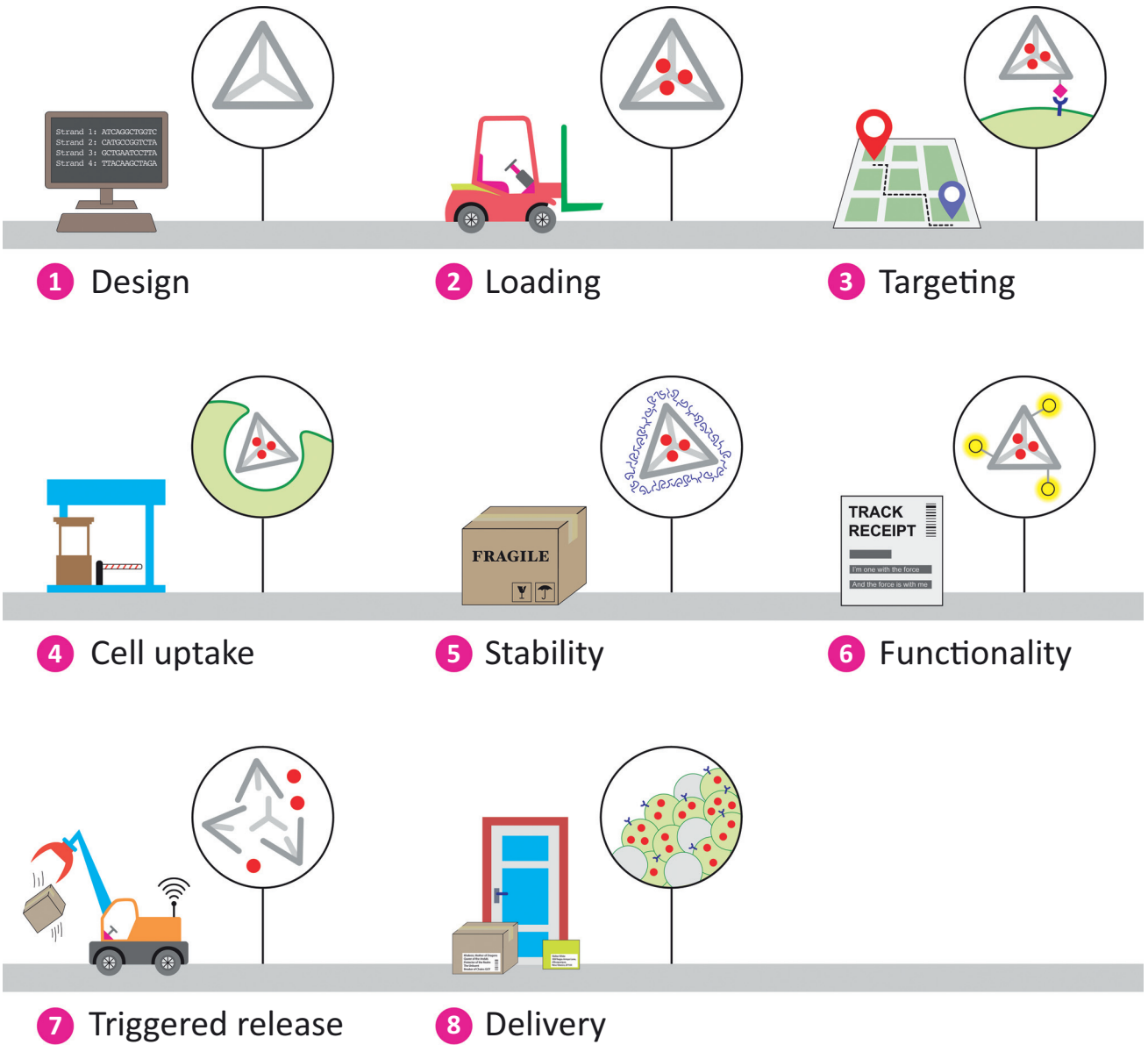

Figure 1. DNA Nanostructures and the Fantastic Voyage. Designed nanostructures are loaded with cargoes and targeted to specific sites where they are taken up by cells. The design and geometry of the carriers are made to be stable in biological conditions and once triggered, the carriers release the cargo. Nanostructures are also designed to perform other functions such as in vivo imaging.

dendrimer structures [45]. Gold nanoparticles or nanorods containing single-stranded DNA tethers can also be attached to DNA nanostructures using this method [46,47]. Triangular and tubular DNA origami structures with gold nanorods are used in photothermal therapy that uses near-IR laser photoabsorbers to generate heat for **thermal ablation** of cancer cells [48].

In cases where covalent linkage or hybridization of the cargo to the carrier nanostructure is not feasible, the cargo can be physically constrained within the nanostructure and could be

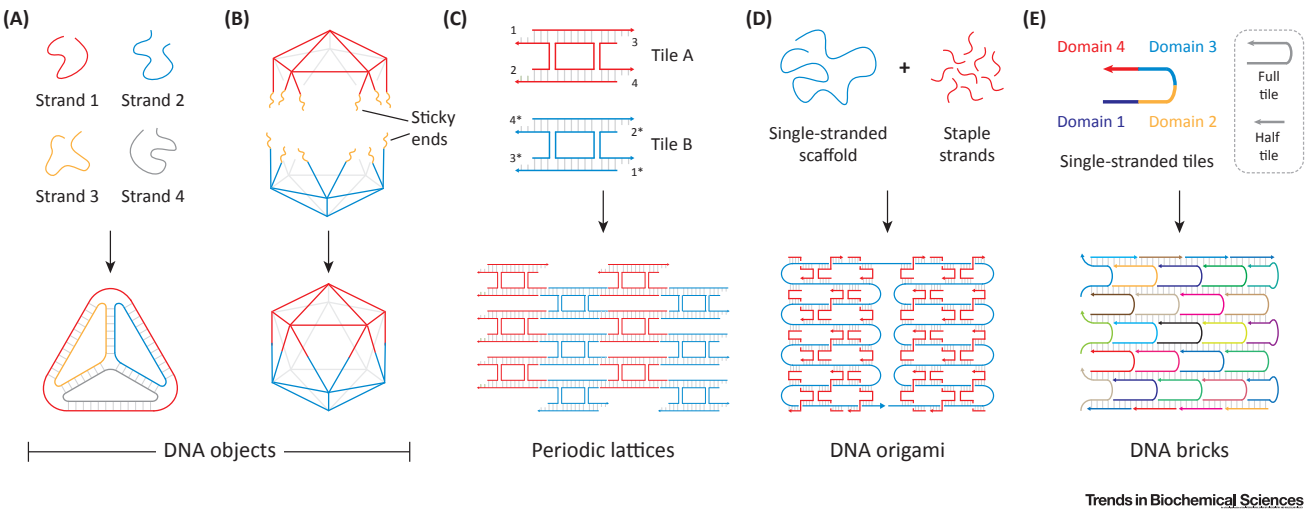

Figure 2. Designing DNA Nanostructures. (A) Cooperative self-assembly of DNA strands into a DNA tetrahedron. (B) Component halves designed from five-arm branched junctions connected via sticky ends to form a DNA icosahedron. (C) Double crossover DNA motifs tailed with sticky ends alternate to form a 2D array (complementary sticky ends are denoted by n-n*). (D) A long scaffold strand is folded into desired shapes by using short complementary staple strands in a method called DNA origami. (E) Single-stranded DNA tiles connect to each other via complementary domains to form a molecular canvas.

delivered to target locations without compromising cargo functionality (Figure 4Av). DNA icosahedra are used to encapsulate gold nanoparticles during assembly by entrapment within the cavity of the nanostructure [49]. Such a strategy is also used in direct synthesis of protein-encapsulated DNA nanoflowers for delivering RNase A and cytochrome *c* [50].

Targeting Strategies

One of the main characteristics of a good drug carrier is its ability to reach the specific target site. Active targeting is possible by integrating specific recognition elements such as antibodies, peptides, or proteins that direct the nanocarriers to cell receptors on the target cells. For example, DNA tetrahedron containing cetuximab antibodies recognize epidermal growth factor receptors, often overexpressed on the surface of several types of cancerous cells [51], while those containing a tumor-penetrating peptide (specific to transmembrane glycoprotein neuropilin-1) show enhanced uptake in glioblastoma cells [52]. DNA origami structures containing the protein transferrin showed increased intracellular uptake by 22-fold in tumor cells compared to nanostructures that do not contain this protein [53].

Aptamers are other elements used to direct drug carriers to target locations. Cross-linked DNA networks formed from **multi-arm DNA junctions** containing doxorubicin are delivered specifically to leukemia cells by attaching tumor-targeting aptamer sgc8 on the nanocarrier [54]. Aptamer-tethered DNA nanotrains [55] and DNA nanoflowers [56] also deliver doxorubicin specifically to tumor cells and induce target-specific cytotoxicity. In another example, tubular DNA nanorobots containing thrombin are directed to tumor sites to activate coagulation [57]. Nucleolin-specific aptamers embedded on the exterior surface of the nanorobots provide both targeting and molecular triggering for the mechanical opening of the DNA construct.

Folate receptors overexpressed in tumor cells to sustain uncontrolled growth are also frequent targets for site-specific drug delivery. Conjugation of folate to siRNA-containing DNA

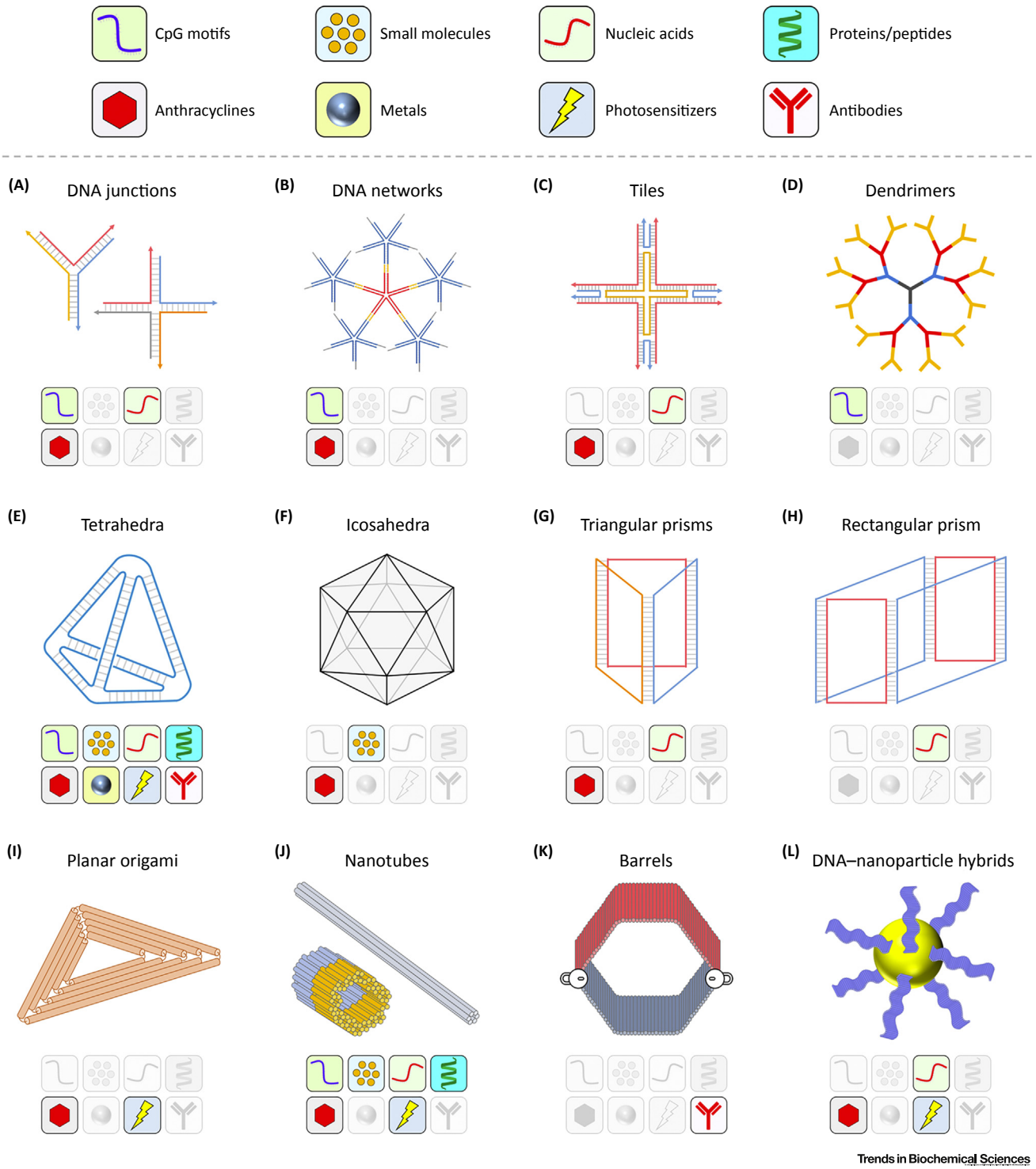

Figure 3. DNA Nanostructures Used for Different Types of Cargo. The icons on the top represent different cargos. Different DNA nanostructures shown in (A)–(L) are used for different types of cargoes, indicated by 'active' icons below each structure. CpG, cytosine-phosphate-guanine sequences.

Table 1. DNA Nanocarriers for Different Types of Cargo

DNA nanostructure	Cargo	*In vitro/in vivo*	Stability	Use	Refs
Tetrahedron	Doxorubicin	EGFR-overexpressing breast cancer cells, MCF-7/ADR breast cancer cells, Intracranial human primary glioblastoma	<12 h in FBS	Cancer therapy	[51,52,27]
	siRNA	KB tumor cells (xenografts)	λ = 24 min	Gene silencing	[38]
	CpG	Murine macrophage-like RAW264.7 cells	Fully intact for 8 h	Immunostimulation	[41]
	Streptavidin–CpG complex	Murine macrophage-like RAW264.7 cells	5 h in FBS at RT	Immunostimulation	[35]
	Methylene blue	SCC7 cells		Photodynamic therapy	[67]
	Pyro	SMMC-7721 cells		Photodynamic therapy	[36]
Icosahedron	Doxorubicin	MUC1 tumor surface marker on epithelial cancer cells	30 min in culture medium at 37°C	Cancer therapy	[28]
Pyramidal nanostructure	Doxorubicin	Breast MDA-MB-231, hepatic HepG2, and colon LoVo and LoVo-R cancer cell lines	λ = 34.5 h under physiological conditions	Cancer therapy	[29]
Triangular prisms	Firefly luciferase antisense strands	HeLa-firefly luciferase cells	λ = 7 h	Gene silencing	[39]
	Doxorubicin	MCF-7 breast cancer cells		Cancer therapy	[71]
Rectangular prisms	siRNA	HeLa human breast cancer cells	12 h in 10% FBS in DMEM	Cancer therapy	[82]
Nanotubes	CpG	Murine macrophage-like RAW264.7 cells		Immunostimulation	[43]
	Streptavidin-modified Lucia luciferase	HEK293 cells		Enzyme replacement therapy	[91]
Nanoribbons	CpG	Antigen-presenting cells (with TLR-9)		Immunostimulation	[123]
Crosslinked junctions	Doxorubicin	T cell acute lymphoblastic leukemia cells	24-h stability with DNase I (2 units/ml)	Cancer therapy	[54]
	Antisense oligonucleotides	T cell acute lymphoblastic leukemia cell line (CCRF-CEM), human Burkitt lymphoma cells (Ramos), drug-resistant myelogenous leukemia line (K562/D)	24-h stability with DNase I (2 units/ml)	Cancer therapy	[54]
Dendrimers	CpG	Macrophage RAW264.7 cells		Immunostimulation	[45]
Polypods	CpG	Murine macrophage-like RAW264.7 cells	22 h in 20% mouse serum at 37°C	Immunostimulation	[44]
Concatamers	Doxorubicin	T cell acute lymphocytic leukemia cells		Cancer therapy	[55]

Table 1. (continued)

DNA nanostructure	Cargo	*In vitro/in vivo*	Stability	Use	Refs
Nanoflowers	Doxorubicin	Human breast cancer cells, MUC1-positive human breast cancer cell line	24 h with DNase I (5 units/ml), high temperature, 5 M urea	Cancer therapy	[56,106]
	CpG	Murine macrophage-like RAW264.7 cells		Cancer therapy, immuno-stimulation	[42]
	RNase A, cytochrome *c*	HUVECs	24 h in FBS at 37°C	Bioimaging, biocatalysis	[50]
Cocoon	Doxorubicin	MCF-7 breast cancer cell lines	2 h at physiological pH	Cancer therapy	[100]
Triangular origami	Doxorubicin	Human MDA-MB-231-GFP orthotopic breast tumor cells, MCF-7 breast cancer-resistant cells	24 h in DMEM with 10% FBS at 37°C	Cancer therapy	[30,31]
	Gold nanorod	MCF-7/ADR-resistant breast cancer cells		Photodynamic therapy	[46,47]
	BMEPC	MCF-7 breast cancer cells		Photodynamic therapy	[34]
Rectangular origami	Doxorubicin	Human MDA-MB-231-GFP orthotopic breast tumor cells	24 h in DMEM with 10% FBS at 37°C	Cancer therapy	[31]
Origami nanotubes	Doxorubicin	Breast cancer cell lines: MDA-MB-231, MDA-MB-468, MCF-7	24 h in DMEM and 48 h in 10% FBS, at 37°C, in pH ranging from 4 to 10	Cancer therapy	[25,30,31]
	CpG	Murine splenic cells (antigen-presenting cells, dendritic cells, macrophages, B cells, T cells)	4 h in FBS at 37°C	Immuno-stimulation	[37]
	Thrombin	Tumor-associated endothelial cells	24 h in BSA or FBS	Cancer therapy	[57]
	Gold nanorod	MCF-7 breast cancer cells		Photodynamic therapy	[46]
Origami nanorods	Daunorubicin	HL-60 human acute-promyelocytic leukemia-resistant cells	24 h in culture medium with 20% FBS at 37°C	Cancer therapy	[33]
Origami-nanoparticle superstructures	Doxorubicin	U87 multiple glioblastoma cells		Cancer therapy	[32]
Origami hexagonal barrel	Gold nanoparticles and Fab antibody fragments	Burkitt lymphoma cells (Ramos), AML cells (Kasumi-1), acute T cell leukemia (Jurkat), acute lymphoblastic leukemia (CCRF-CEM), neuroblastoma cells (SH-SY5Y)		Cancer therapy, immuno-stimulation	[103]

AML, acute myeloid leukemia; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; EGFR, epidermal growth factor receptor; FBS, fetal bovine serum; HUVEC, human umbilical vein endothelial cell; λ, half-life; MUC1, mucin 1; RT, room temperature; TLR, Toll-like receptor.

tetrahedron shows gene silencing effects both *in vitro* and *in vivo* [38]. Similarly, metastatic cancer cells are actively targeted by a multi-module packaging RNA nanoparticle functionalized with folic acid [58]. Figure 4B illustrates the targeting strategies used for delivering DNA nanostructures to specific locations.

Cellular Uptake

The cellular uptake profile of a drug carrier needs to be studied to effectively design the nanostructure and deliver cargo molecules to target locations. Some studies show that DNA nanostructures are internalized by cells effectively via **endocytosis** without the need for transfection agents [59]. **Single particle tracking** suggests uptake of tetrahedral DNA nanostructures via receptor-mediated endocytosis, with active transport to lysosomes in HeLa cells [60], while pinocytosis pathways (specifically caveolae-mediated endocytosis and macropinocytosis) are indicated in other studies [27]. In one example, spherical nucleic acids, with high affinity toward class A scavenger receptors, are shown to get internalized by endocytosis via a lipid-raft-dependent, caveolae-mediated pathway [61]. The cellular fate of DNA nanostructures can be decided by modifying them with suitable ligands that target select receptors on cells. Pristine octahedral DNA nanocages are selectively internalized in cells expressing oxidized low-density lipoprotein receptor-1 (LOX-1), through a receptor-mediated endocytosis mechanism [62]. However, DNA nanocages functionalized with folate cause them to be internalized by α folate receptor (αFR)-overexpressing cells [63]. These studies showed an efficiency that is 30 times higher than that observed in cells not expressing these receptors. In addition, DNA nanostructures localize in different intracellular compartments with largely different half-lives depending on the receptors involved in these cellular pathways. DNA nanostructures specific to LOX-1 are delivered to lysosomes via a clathrin-independent and dynamin-dependent internalization process [64] and are thus rapidly degraded inside cells [65]. In contrast, DNA nanostructures are stable and slowly accumulate in αFR-overexpressing cells via a clathrin-independent and dynamin-independent endocytosis mechanism [66].

DNA nanocarriers, in general, provide higher cellular uptake of drug cargoes compared to drug molecules alone. Methylene blue, an effective photosensitizer, limited by its poor tissue penetration and low stability, shows significantly improved cellular uptake when loaded into a DNA tetrahedron [67]. Metal complexes such as ruthenium polypyridyl (RuPOP) complexes loaded into biotin-conjugated DNA tetrahedra show enhanced internalization at the level of the cell nucleus where the nanostructure is cleaved by DNases, resulting in specific, triggered release of contents [26]. When tested against HepG2 cells, the DNA cages encapsulating RuPOP complexes demonstrated effective loading capacity and cytotoxicity for cancer cells. Transportation of DNA nanocarriers into cells can be further improved by mixing them with virus capsid proteins [68] or cationic polymers [69], or by using lactose-containing [70] or lipid-modified [71] hybrid structures. Modifying the surface properties of DNA origami with specific DNA intercalators also improves the transfection rates [72].

As with other kinds of nanocarriers, the extent of cellular uptake is influenced by the size and shape of the nanostructure. Although, DNA nanotubes with high aspect ratio are found to be taken up more by HeLa cells [73], a recent study comparing the cellular uptake properties of DNA origami structures of different geometries underplays the need for high aspect ratio [22]. In this study, compactness of the oligolysine/polyethylene glycol (PEG)-coated DNA origami blocks is found to be of significance. Compared to hollow structures such as a cylindrical barrel or octahedron, solid blocks with no hollow interiors are taken up preferentially by the cells. While morphology influenced the magnitude of uptake, the cell type determined the

uptake kinetics. Those specialized for endocytosis (bone marrow-derived dendritic cells) internalized the nanostructures for longer periods compared to the endothelial cells and epithelial cells [human umbilical vein endothelial cells (HUVECs) and human embryonic kidney 293 cells (HEK293)]. Large rods and tetrahedron-like origami constructs showed better transfection profiles compared to constructs with similar structures but smaller in size, in H1299 cells [74]. More contact with the cell surface was believed to aid the uptake. Thus, both size and shape of the nanostructures affect the internalization into the target cells. In addition to these considerations, the design of nanocarrier must consider other factors such as the site of delivery, preferred route of administration, pharmacokinetics, mechanism of release trigger, and functionalization.

Biostability/Biocompatibility

DNA nanocarriers are affected by cation concentration, nuclease activity, and the innate immune response of the host. Most DNA origami structures, where bundles of duplexes are packed close together, require Mg^{2+} concentrations to be 1–2 orders greater than that in physiological conditions [17]. Structures such as DNA tetrahedra and icosahedra, in contrast, are not significantly affected [49,59]. Stability in DNA origami constructs can be enhanced by changing the arrangements and length of staple strands [75]. For instance, wireframe triangular truss structures (with fewer contacts between double helices compared to bundles in DNA origami structures) fold at biologically relevant Mg^{2+} concentrations (~2–4 mM) [76].

DNA nanocarriers are also challenged by the sustained onslaught of nucleases present in the blood and extracellular milieu and in the lysosomal compartments [77]. Compact 3D nanostructures, where the close-packed duplexes occlude access to hydrolytic enzymes, are more resistant to degradation by nucleases than linear double-stranded DNA or plasmid DNA [78,79]. Presence of single-stranded segments, internal nicks, and regions with greater conformational flexibility make DNA nanostructures vulnerable to attack from nucleases [78]. Enzymatic ligation to reduce the number of free termini (Figure 4Ci) is one effective way to improve the stability of DNA tetrahedra [13,78], DNA nanotubes [80], and DNA prisms [81].

Chemical modifications to DNA backbone and nucleobases can improve the inherent stability of nanostructures (Figure 4Cii). **Locked nucleic acid** inserts in rectangular DNA prisms increased stability in reduced salt and increased temperature conditions [82]. Another strategy to improve stability is by crosslinking the helices within nanostructures by azide-alkyne click chemistry reactions [83], or photochemical crosslinking using 8-methoxypsoralen [84] or 3-cyanovinylcarbazole [85] nucleosides (Figure 4Ciii). Such modifications increase stability at low salt concentrations and high temperatures along with improved resistance to nucleases. Other modifications such as hexaethylene glycol [81], hexanediol [81], and hydrophilic/hydrophobic dendritic chains [86] introduced in component DNA strands increase the lifetime of DNA nanostructures in serum (Figure 4Civ) [81]. Stability of DNA nanostructures against nucleases can also be enhanced by encapsulating them in PEG-based protective layers (Figure 4Cv) [69,87,88] or **micellization** [89]. Enzymes encapsulated in hollow origami nanocages [90] and hexagonal DNA origami tubes [91] are protected against proteolytic degradation and show better-preserved activity compared to free enzymes in biological environments.

It is important that nanocarriers evade immune surveillance and avoid adsorption of serum opsonin proteins [92]. Nonspecific interactions with plasma proteins resulting in the formation of protein corona are substantial hurdles for any nanocarrier. Minimizing the risk of

opsonization and subsequent degradation is an important criterion while designing DNA nanostructures. In addition to influencing the biodistribution and decreasing the rate of clearance of the nanovehicle, it would also help improve targeted delivery of the cargo [92,93]. DNA nanocages targeted to cells expressing LOX-1 or αFR receptors did not seem to be affected by the protein corona [65]. In other structures, modifying DNA nanocarriers with dendritic alkyl chains enables the nanostructures to escape opsonization by complexation with the plasma protein human serum albumin [94]. In addition, protein-dendron conjugates (with bovine serum albumin or class II hydrophobin) [95] and viral capsid proteins (e.g., from cowpea chlorotic mottle virus) [68] are also effective coatings to enhance stability of DNA nanostructures (Figure 4Cv).

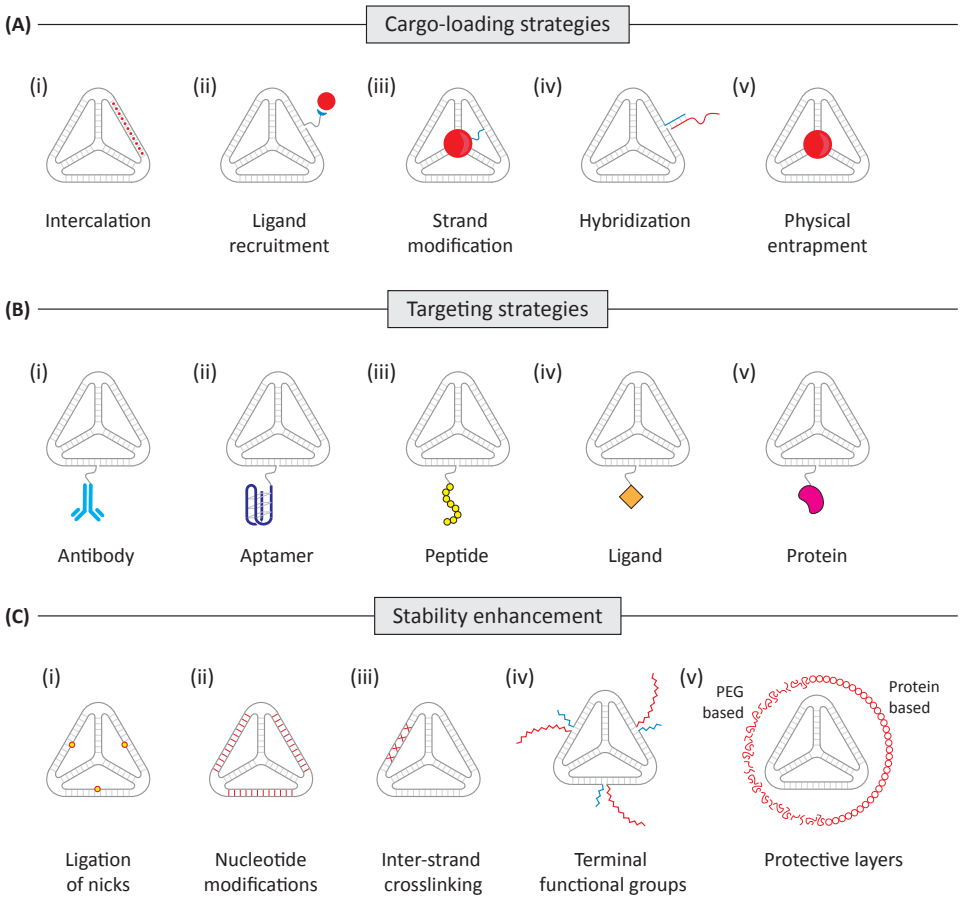

Figure 4. Features of DNA Nanostructures for Drug Delivery. (A) Drug-loading strategies. Cargo molecules can be encapsulated in the nanostructure by (i) intercalation, (ii) ligand recruitment, (iii) strand modification, (iv) hybridization, and (v) entrapment. (B) Targeting strategies. Drug-loaded nanostructures can be designed to reach specified locations by using cell-specific antibodies, aptamers, peptides, ligands, or receptor-specific proteins. (C) Strategies to improve biostability. Modifications to improve the stability of DNA nanostructures include (i) ligation of nicks, (ii) nucleotide modifications, (iii) inter-strand crosslinking, (iv) functional groups such as hexaethylene glycol and hexane diol, and (v) polyethylene glycol (PEG)-based or protein-based protective layers.

Triggered Release

DNA nanocarriers that reach their destination need to be able to release the cargo they carry based on molecular or environmental cues. For example, nanoparticles loaded within DNA scaffolds are released by altering the pore size of the scaffold using a complementary DNA strand (Figure 5A) [96]. Similarly, pore size of hairpin-containing DNA polyhedra can be

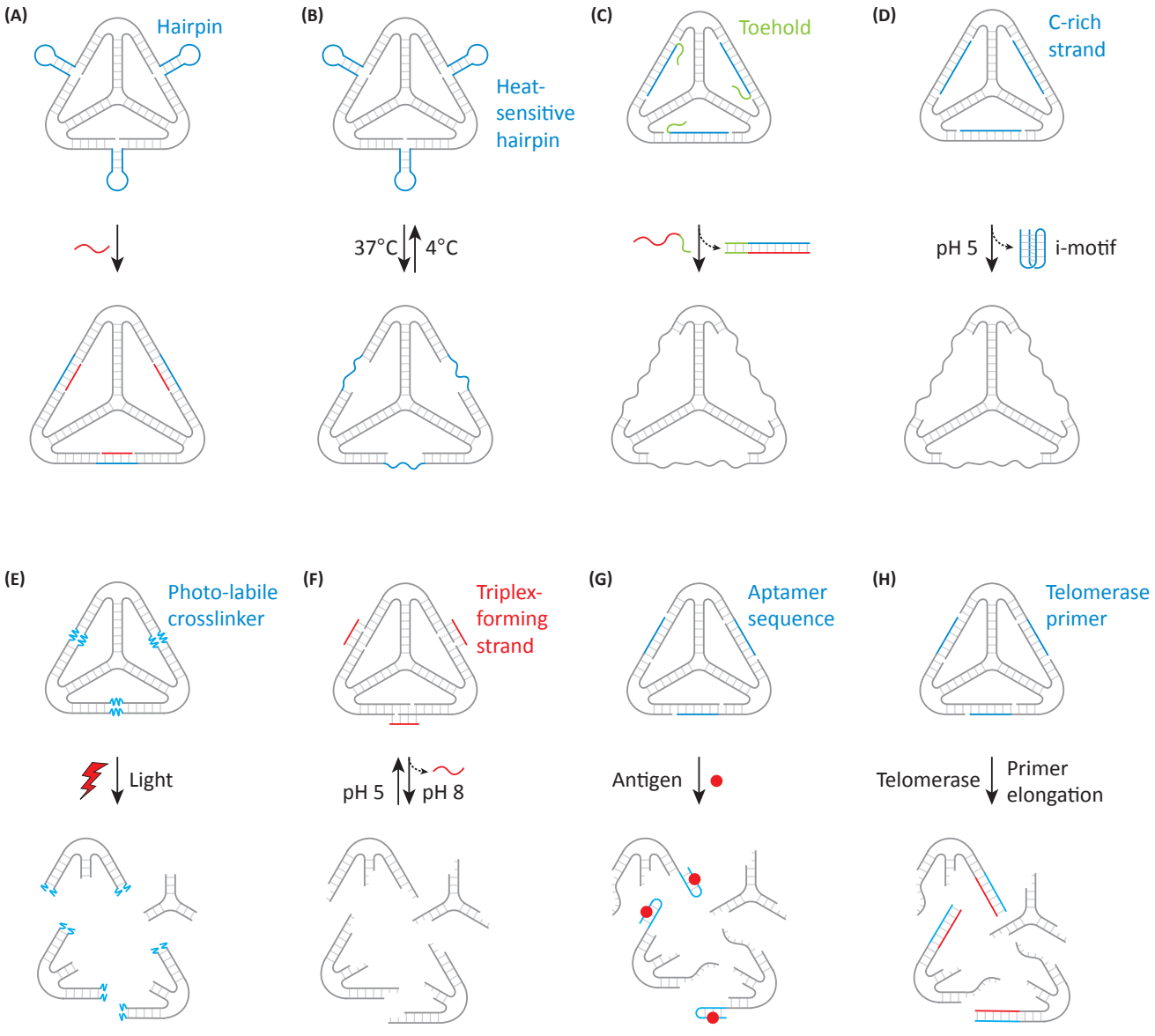

Figure 5. Reconfigurable DNA Carriers. The nanostructures can be triggered to release the cargo once they reach the target location by (A) an oligonucleotide that is complementary to a hairpin region of the nanocarrier, thus expanding the structure; (B) temperature-triggered expansion of the nanostructure; (C) toehold-mediated strand exchange, resulting in single-stranded regions destabilizing the nanocarrier; (D) cytosine-rich strand forms an i-motif at low pH, thereby destabilizing the carrier; (E) nanostructures stabilized by triplex forming oligonucleotides dissociate on pH change; (F) dissociation of nanocarriers due to aptamer remodeling of sequences in sticky ends on recognizing antigens; and (G) primer elongation of strands at sticky ends due to telomerase activity resulting in dissociation of the carrier. Note: Modifications are shown only on the front-faced edges of the tetrahedra for clarity.

controlled by heat; the polyhedron can be expanded at 37°C, thereby internalizing drug molecules and is closed at 4°C, retaining the drug. The drug can be released by increasing the temperature (Figure 5B) [97].

The hybridization feature of DNA is another route to controllably close or open DNA cages. For example, the surface porosity of DNA tetrahedra is controlled using **toehold-based strand displacement** (Figure 5C) [98]. pH changes can also be used to reconfigure nanostructures. In one example, DNA boxes have lids that are closed or opened using pH changes and the resulting i-motif interactions (Figure 5D) [99]. Another example is a DNA cocoon (long-chain single-stranded DNA complexed with polymeric nanogel-coated DNase I) that intercalates doxorubicin [100]. In an acidic environment, the polymeric shell degrades, thus activating the DNase I, resulting in cocoon-like self-degradation of the DNA and release of doxorubicin. Cargo molecules can also be attached to DNA nanostructures via photo-labile crosslinkers that can be cleaved by exposure to light, thus releasing the cargo (Figure 5E) [101]. In addition, DNA tetrahedra stabilized by triplex formation at pH 5 [102] can be dissociated by changing the pH to 8 (Figure 5F). In another study, hexagonal barrel-shaped DNA origami nanorobots containing antibody fragments as cargoes were 'locked' by aptamers that recognize target cell surface proteins [103]. Binding of the aptamer to the cell surface receptor triggers a conformational change in the lock (aptamer reconfiguration on binding the antigen), thus opening the robot and releasing the payload (Figure 5G). Recently, DNA icosahedra containing platinum nanoparticles released the drugs in response to telomerase in tumor cells [104]. The two halves of the icosahedron are connected by sticky ends that contained telomerase primer and telomeric repeats. In the presence of telomerase, primer elongation results in dissociation of the icosahedron into its two constituent halves through strand displacement, leading to release of the nanoparticles (Figure 5H).

Functionalization

The versatility of DNA nanostructures allows tagging of these nanocarriers with functional molecules [105]. DNA strands bearing fluorescent dyes can be used to track the cellular uptake of nanostructures [49]. A wide range of fluorescent probes can be readily coupled on DNA stands and are used to visualize or trace the pathways of the delivery vehicle. For example, aptamer-conjugated DNA nanoflowers decorated with fluorophores for multi-color fluorescence resonance energy transfer (FRET) imaging demonstrate the potential of incorporating multiple functionalities into DNA nanostructures [106]. In another example, rectangular DNA prisms are labeled with FRET dyes to provide a signal once the cargo is deployed [82]. Methods such as single particle tracking [60] or fluorescence microscopy [107] allow investigation of cell entry pathways of nanostructures and study of the pharmacokinetics and biodistribution patterns of the nanocarriers *in vivo*. This type of functionality also provides the ability to test the stability of these carriers and to monitor the release of payloads once they reach their destination [108].

Concluding Remarks and Future Perspectives

Currently, there exists many opportunities for nanomedicine to address issues that limit conventional medicine from meeting critical medical needs. Shortcomings such as low biological solubility and lack of target specificity can be overcome using nanostructure-based drug carriers. DNA nanocarriers are useful not only for drug delivery but also for non-invasive and real-time monitoring of the therapy. Post-assembly functionalization of DNA nanostructures is possible through sequence-specific recognition [109] and provides the means to unobtrusively implement additional characteristics to the nanocarrier. Furthermore, extrinsic control, such as

Outstanding Questions

How do DNA nanostructures affect cellular nucleic acids? Should we be mindful of the sequences, which are usually generated randomly, to ensure that interactions, if any, with genomic DNA or RNA are minimal?

Although alternatives have been suggested, use of viral DNA scaffold still forms a major part of DNA origami literature. In the context of drug delivery, how safe is the use of viral DNA?

How do we rationally design DNA nanocarriers for a specific route of administration? The route of administration and location of the target tissue determine the various physiological barriers that the nanocarriers would encounter. There is a lack of design principles that allow us to predict the suitability of nanostructures of different morphologies for different routes of administration.

As informational molecules, molecular computation is one of the unique strengths of DNA nanostructures compared to other materials. Naturally, this feature has been utilized to program the delivery of drugs *in vivo* using the levels of different biomarkers as inputs. However, there still exists scope for building multifunctional nanodevices or nanorobots that carry multiple drugs, and release quanta of the appropriate drugs in response to dynamic changes in the levels of biomarkers at the target site.

How can the stability of DNA nanostructures be improved further? While strategies have been developed to hold the nanostructures intact under the mild physiological conditions, and to render them resistant to attack from enzymes, there is still a requirement for ways to keep the DNA-based nanocarriers stable and safe for longer periods under more extreme and adverse conditions in the body.

by light, of duplex stability and conformation in the nanostructures provides spatial and temporal regulation of these nanocarriers in living systems. DNA sequence-encoded instructions can be used to assign tasks such as localization of the delivery vehicle and loading and unloading of cargo and report cellular activities before and after drug release, an aspect termed intelligent delivery. The true potential of DNA-based nanocarriers lies in the ability to couple these features together and control where, when, and how the cargo is delivered through complex logic operations. There are a few instances where molecular circuitry constructed using DNA is used to program delivery of drugs: by AND logic-gated aptamer reconfiguration [103] or by interaction of different nanobots to perform AND, OR, XOR, NAND, and CNOT logical operations [110]. More complex DNA nanorobots are designed to be triggered in living cockroaches even by physiological (or pathological) signals derived from brain activity of a human subject [111]. In this case, an algorithm recognizes patterns of electroencephalogram recorded in human subjects and turns on a radio frequency-induced electromagnetic field that then causes nanoparticles tethered to DNA nanobots to heat up, leading to opening up of the carrier and escape of the cargo held within. DNA-based nanostructures have the potential to process various kinds of diagnostic inputs to deliver specific and programmed output in the form of drug release. Such autonomous robots can respond to dynamic changes in the body by migrating to specific sites on sensing certain biochemical changes that the structure is programmed to respond to. We now have nanorobots and dynamic DNA nanostructures that move on DNA origami-based platforms (e.g., DNA spiders [112]), making them capable of 'walking' on diverse platforms. Teaching them to hitch a ride with migrating cells by adhering to them will get us closer to developing molecular surgeons. From improving biostability of the DNA nanobots to ensuring compatibility of molecular circuitry of the nanodevice within complex mammalian hosts, translation of these into functional nanoscopic medical devices for humans is likely to be challenging. However, autonomous and dynamic control of multi-drug release through physiological outputs and brain–computer interface holds tremendous promise for DNA-based nanocarriers.

For DNA nanostructures to be used widely as drug carriers, large-scale production is still an important challenge. This challenge is recently addressed using biotechnological processes for scalable preparation of DNA nanostructures [113,114]. Understanding the kinetics and thermodynamics of DNA nanostructure assembly [115], controlling folding pathways [116,117], and achieving highly purified constructs [118] are all crucial for large-scale preparation of DNA nanostructures as potential drug carriers. Recent advances in the creation of gigadalton-size assemblies [119,120] address the size limitation of DNA-based constructs. Custom DNA origami single-stranded scaffolds (other than the frequently used M13 viral genome) also help this cause [121]. Aided by cheap production of oligonucleotides with any desired sequence [122], DNA nanocarriers are well poised to be useful in clinical settings.

One other major disadvantage to DNA carrier systems developed thus far is their often complicated designs. With higher level organization and functionality comes more complex scaffolds and annealing protocols. Researchers have looked at ways to simplify DNA nanostructures and their programmability to more easily utilize these delivery systems. **Rolling-circle amplification**-based DNA nanostructures are less complex and thus more cost-effective alternatives to previously proposed DNA carrier systems [123]. This approach involves only a few staple strands and produces self-assembled DNA nanoribbons that are readily internalized at the cell membrane. Another concern is how DNA nanostructures interact with genomic DNA or intracellular nucleic acids and its effect on the maintenance of a certain total amount of DNA in a cell [124]. While off-target effects associated with DNA nanocarriers are as

yet unknown, the success of DNA nanostructure self-assembly in living bacteria [125] indicates that the entry of such designer structures does not cause any harmful effects. Advancements in RNA nanotechnology [126] could be an alternate way to deliver drugs without the side effects that are caused by DNA nanostructures (see Outstanding Questions).

Specific nanocarriers will require different sets of preclinical and clinical considerations than those for its traditional counterparts. Further research in a clinical setting will benefit from tracking drug release and penetration within tumors, imaging-guided focal therapy, and monitoring of therapeutic responses. With modern nanomedicine focused on tailored regimens for individual patients, DNA nanostructures will play a major role in translating lab-based research to clinical settings and take these scientific advances from the bench to the bedside. It will be a fantastic voyage.

Author Contributions

All authors contributed to original writing of the manuscript; B.R.M. and A.R.C. edited and revised the manuscript; A.R.C. created all the figures.

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References

1. Jones, M.R. *et al.* (2015) Programmable materials and the nature of the DNA bond. *Science* 347, 1260901
2. Xavier, P.L. and Chandrasekaran, A.R. (2018) DNA-based construction at the nanoscale: emerging trends and applications. *Nanotechnology* 29, 062001
3. Chandrasekaran, A.R. *et al.* (2018) Detection of cellular microRNAs with programmable DNA nanoswitches. *bioRxiv* http://dx.doi.org/10.1101/334631 334631
4. Chandrasekaran, A.R. *et al.* (2016) Programmable DNA nanoswitches for detection of nucleic acid sequences. *ACS Sens.* 1, 120–123
5. Endo, M. *et al.* (2010) A versatile DNA nanochip for direct analysis of DNA base-excision repair. *Angew. Chem. Int. Ed.* 49, 9412–9416
6. Takeuchi, Y. *et al.* (2016) Single-molecule observations of RNA-RNA kissing interactions in a DNA nanostructure. *Biomater. Sci.* 4, 130–135
7. Sun, W. *et al.* (2014) Casting inorganic structures with DNA molds. *Science* 346, 1258361
8. Tian, C. *et al.* (2017) DNA nanostructures-mediated molecular imprinting lithography. *ACS Nano* 11, 227–238
9. Chandrasekaran, A.R. *et al.* (2017) Addressable configurations of DNA nanostructures for rewritable memory. *Nucleic Acids Res.* 45, 11459–11465
10. Chandrasekaran, A.R. (2018) Reconfigurable DNA nanoswitches for graphical readout of molecular signals. *ChemBioChem* 19, 1018–1021
11. Chandrasekaran, A.R. and Levchenko, O. (2018) DNA nanocages. *Chem. Mater.* 28, 5569–5581
12. Seeman, N.C. (1982) Nucleic acid junctions and lattices. *J. Theor. Biol.* 99, 237–247
13. Goodman, R.P. *et al.* (2005) Rapid chiral assembly of rigid DNA building blocks for molecular nanofabrication. *Science* 310, 1661–1665
14. Bhatia, D. *et al.* (2009) Icosahedral DNA nanocapsules by modular assembly. *Angew. Chem. Int. Ed.* 48, 4134–4137
15. Winfree, E. *et al.* (1998) Design and self-assembly of two-dimensional DNA crystals. *Nature* 394, 539–544
16. Zheng, J. *et al.* (2009) From molecular to macroscopic via the rational design of a self-assembled 3D DNA crystal. *Nature* 461, 74–77
17. Rothemund, P.W.K. (2006) Folding DNA to create nanoscale shapes and patterns. *Nature* 440, 297–302
18. Chandrasekaran, A.R. *et al.* (2016) Beyond the fold: emerging biological applications of DNA origami. *ChemBioChem* 17, 1081–1089
19. Wei, B. *et al.* (2012) Complex shapes self-assembled from single-stranded DNA tiles. *Nature* 485, 623–626
20. Ke, Y. *et al.* (2012) Three-dimensional structures self-assembled from DNA bricks. *Science* 338, 1177–1183
21. Hu, Q. *et al.* (2018) DNA nanotechnology-enabled drug delivery systems. *Chem. Rev.* Published online February 21, 2018. http://dx.doi.org/10.1021/acs.chemrev.7b00663
22. Bastings, M.M.C. *et al.* (2018) Modulation of the cellular uptake of DNA origami through control over mass and shape. *Nano Lett.* 18, 3557–3564
23. Lee, D.S. *et al.* (2016) Cellular processing and destinies of artificial DNA nanostructures. *Chem. Soc. Rev.* 45, 4199–4225
24. Dougherty, T.J. (2002) An update on photodynamic therapy applications. *J. Clin. Laser Med. Surg.* 20, 3–7
25. Zhao, Y.X. *et al.* (2012) DNA origami delivery system for cancer therapy with tunable release properties. *ACS Nano* 6, 8684–8691
26. Huang, Y. *et al.* (2016) A multifunctional DNA origami as carrier of metal complexes to achieve enhanced tumoral delivery and nullified systemic toxicity. *Biomaterials* 103, 183–196
27. Kim, K.R. *et al.* (2013) Drug delivery by a self-assembled DNA tetrahedron for overcoming drug resistance in breast cancer cells. *Chem. Commun.* 49, 2010–2012
28. Chang, M. *et al.* (2011) Aptamer-conjugated DNA icosahedral nanoparticles as a carrier of doxorubicin for cancer therapy. *ACS Nano* 5, 6156–6163
29. Kumar, V. *et al.* (2016) Enhanced chemotherapeutic behavior of open-caged DNA@doxorubicin nanostructures for cancer cells. *J. Cell. Physiol.* 231, 106–110
30. Jiang, Q. *et al.* (2012) DNA origami as a carrier for circumvention of drug resistance. *J. Am. Chem. Soc.* 134, 13396–13403
31. Zhang, Q. *et al.* (2014) DNA origami as *in vivo* drug delivery vehicle for cancer therapy. *ACS Nano* 8, 6633–6643
32. Yan, J. *et al.* (2015) Growth and origami folding of DNA on nanoparticles for high-efficiency molecular transport in cellular

imaging and drug delivery. *Angew. Chem. Int. Ed.* 54, 2431–2435

33. Halley, P.D. *et al.* (2015) Daunorubicin-loaded DNA origami nanostructures circumvent drug-resistance mechanisms in a leukemia model. *Small* 12, 308–320
34. Zhuang, X. *et al.* (2016) A photosensitizer-loaded DNA origami nanosystem for photodynamic therapy. *ACS Nano* 10, 3486–3495
35. Liu, X. *et al.* (2012) A DNA nanostructure platform for directed assembly of synthetic vaccines. *Nano Lett.* 12, 4254–4259
36. Chen, N. *et al.* (2016) "Sense-and-treat" DNA nanodevice for synergetic destruction of circulating tumor cells. *ACS Appl. Mater. Interfaces* 8, 26552–26558
37. Schuller, V.J. *et al.* (2011) Cellular immunostimulation by CpG-sequence-coated DNA origami structures. *ACS Nano* 5, 9696–9702
38. Lee, H. *et al.* (2012) Molecularly self-assembled nucleic acid nanoparticles for targeted *in vivo* siRNA delivery. *Nat. Nanotechnol.* 7, 389–393
39. Fakhoury, J.J. *et al.* (2014) Development and characterization of gene silencing DNA cages. *Biomacromolecules* 15, 276–282
40. Stewart, J.M. *et al.* (2016) Programmable RNA microstructures for coordinated delivery of siRNAs. *Nanoscale* 8, 17542–17550
41. Li, J. *et al.* (2011) Self-assembled multivalent DNA nanostructures for noninvasive intracellular delivery of immunostimulatory CpG oligonucleotides. *ACS Nano* 5, 8783–8789
42. Zhang, L. *et al.* (2015) Self-assembled DNA immunonanoflowers as multivalent CpG nanoagents. *ACS Appl. Mater. Interfaces* 7, 24069–24074
43. Sellner, S. *et al.* (2015) DNA nanotubes as intracellular delivery vehicles *in vivo*. *Biomaterials* 53, 453–463
44. Mohri, K. *et al.* (2012) Design and development of nanosized DNA assemblies in polypod-like structures as efficient vehicles for immunostimulatory CpG motifs to immune cells. *ACS Nano* 6, 5931–5940
45. Qu, Y. *et al.* (2017) Self-assembled DNA dendrimer nanoparticle for efficient delivery of immunostimulatory CpG motifs. *ACS Appl. Mater. Interfaces* 9, 20324–20329
46. Jiang, Q. *et al.* (2015) Self-assembled DNA origami-gold nanorod complex for cancer theranostics. *Small* 11, 5134–5141
47. Song, L. *et al.* (2017) DNA origami/gold nanorod hybrid nanostructures for the circumvention of drug resistance. *Nanoscale* 9, 7750–7754
48. Shanmugam, V. *et al.* (2014) Near-infrared light-responsive nanomaterials in cancer therapeutics. *Chem. Soc. Rev.* 43, 6254–6287
49. Bhatia, D. *et al.* (2011) A synthetic icosahedral DNA-based host–cargo complex for functional *in vivo* imaging. *Nat. Commun.* 2, 339
50. Kim, E. *et al.* (2017) One-pot synthesis of multiple protein-encapsulated DNA flowers and their application in intracellular protein delivery. *Adv. Mater.* 29, 1701086
51. Setyawati, M.I. *et al.* (2016) DNA nanostructures carrying stoichiometrically definable antibodies. *Small* 12, 5601–5611
52. Xia, Z. *et al.* (2016) Tumor-penetrating peptide-modified DNA tetrahedron for targeting drug delivery. *Biochemistry* 55, 1326–1331
53. Schaffert, D.H. *et al.* (2016) Intracellular delivery of a planar DNA origami structure by the transferrin-receptor internalization pathway. *Small* 12, 2634–2640
54. Wu, C. *et al.* (2013) Building a multifunctional aptamer-based DNA nanoassembly for targeted cancer therapy. *J. Am. Chem. Soc.* 135, 18644–18650
55. Zhu, G. *et al.* (2013) Self-assembled, aptamer-tethered DNA nanotrains for targeted transport of molecular drugs in cancer theranostics. *Proc. Natl. Acad. Sci. U. S. A.* 110, 7998–8003
56. Zhu, G. *et al.* (2013) Noncanonical self-assembly of multifunctional DNA nanoflowers for biomedical applications. *J. Am. Chem. Soc.* 135, 16438–16445
57. Li, S. *et al.* (2018) A DNA nanorobot functions as a cancer therapeutic in response to a molecular trigger *in vivo*. *Nat. Biotechnol.* 36, 258–264
58. Shu, D. *et al.* (2011) Thermodynamically stable RNA three-way junction for constructing multifunctional nanoparticles for delivery of therapeutics. *Nat. Nanotechnol.* 6, 658–667
59. Walsh, A.S. *et al.* (2011) DNA cage delivery to mammalian cells. *ACS Nano* 5, 5427–5432
60. Liang, L. *et al.* (2014) Single-particle tracking and modulation of cell entry pathways of a tetrahedral DNA nanostructure in live cells. *Angew. Chem. Int. Ed.* 53, 7745–7750
61. Choi, C.H. *et al.* (2013) Mechanism for the endocytosis of spherical nucleic acid nanoparticle conjugates. *Proc. Natl. Acad. Sci. U. S. A.* 110, 7625–7630
62. Vindigni, G. *et al.* (2016) Receptor-mediated entry of pristine octahedral DNA nanocages in mammalian cells. *ACS Nano* 10, 5971–5979
63. Raniolo, S. *et al.* (2018) Selective targeting and degradation of doxorubicin-loaded folate-functionalized DNA nanocages. *Nanomedicine* 14, 1181–1190
64. Murphy, J.E. *et al.* (2008) Oxidised LDL internalisation by the LOX-1 scavenger receptor is dependent on a novel cytoplasmic motif and is regulated by dynamin-2. *J. Cell Sci.* 121, 2136–2147
65. Raniolo, S. *et al.* (2018) Entry, fate and degradation of DNA nanocages in mammalian cells: a matter of receptors. *Nanoscale* 10, 12078–12086
66. Maldonado-Báez, L. *et al.* (2013) Clathrin-independent endocytosis: a cargo-centric view. *Exp. Cell Res.* 319, 2759–2769
67. Kim, K.R. *et al.* (2016) Nano-formulation of a photosensitizer using a DNA tetrahedron and its potential for *in vivo* photodynamic therapy. *Biomater. Sci.* 4, 605–609
68. Mikkilä, J. *et al.* (2014) Virus-encapsulated DNA origami nanostructures for cellular delivery. *Nano Lett.* 14, 2196–2200
69. Kiviaho, J.K. *et al.* (2016) Cationic polymers for DNA origami coating – examining their binding efficiency and tuning the enzymatic reaction rates. *Nanoscale* 8, 11674–11680
70. Sut, P.A. *et al.* (2016) Lactose-modified DNA tile nanostructures as drug carriers. *J. Drug Target.* 24, 709–719
71. Chan, M.S. *et al.* (2016) Mitochondrial delivery of therapeutic agents by amphiphilic DNA nanocarriers. *Small* 12, 770–781
72. Brglez, J. *et al.* (2015) Designed intercalators for modification of DNA origami surface properties. *Chem. Eur. J.* 21, 9440–9446
73. Hamblin, G.D. *et al.* (2012) Rolling circle amplification-templated DNA nanotubes show increased stability and cell penetration ability. *J. Am. Chem. Soc.* 134, 2888–2891
74. Wang, P. *et al.* (2018) Visualization of the cellular uptake and trafficking of DNA origami nanostructures in cancer cells. *J. Am. Chem. Soc.* 140, 2478–2484
75. Ramakrishnan, S. *et al.* (2016) Structural stability of DNA origami nanostructures in the presence of chaotropic agents. *Nanoscale* 8, 10398–10405
76. Matthies, M. *et al.* (2016) Design and synthesis of triangulated DNA origami trusses. *Nano Lett.* 16, 2108–2113
77. Hahn, J. *et al.* (2014) Addressing the instability of DNA nanostructures in tissue culture. *ACS Nano* 8, 8765–8775
78. Keum, J.W. and Bermudez, H. (2009) Enhanced resistance of DNA nanostructures to enzymatic digestion. *Chem. Commun.* 7, 7036–7038
79. Mei, Q. *et al.* (2011) Stability of DNA origami nanoarrays in cell lysate. *Nano Lett.* 11, 1477–1482
80. O'Neill, P. *et al.* (2006) Sturdier DNA nanotubes via ligation. *Nano Lett.* 6, 1379–1383
81. Conway, J.W. *et al.* (2013) DNA nanostructure serum stability: greater than the sum of its parts. *Chem. Commun.* 49, 1172–1174
82. Bujold, K.E. *et al.* (2016) Optimized DNA nanosuitcases for encapsulation and conditional release of siRNA. *J. Am. Chem. Soc.* 138, 14030–14038

83. Cassinelli, V. *et al.* (2015) One-step formation of "chain-armor"-stabilized DNA nanostructures. *Angew. Chem. Int. Ed.* 54, 7795–7798
84. Rajendran, A. *et al.* (2011) Photo-cross-linking-assisted thermal stability of DNA origami structures and its application for higher-temperature self-assembly. *J. Am. Chem. Soc.* 133, 14488–14491
85. Gerrard, S.R. *et al.* (2012) A new modular approach to nano-assembly: stable and addressable DNA nanoconstructs via orthogonal click chemistries. *ACS Nano* 6, 9221–9228
86. Bujold, K.E. *et al.* (2014) Sequence-responsive unzipping DNA cubes with tunable cellular uptake profiles. *Chem. Sci.* 5, 2449–2455
87. Ke, Y. *et al.* (2012) Two design strategies for enhancement of multilayer-DNA-origami folding: underwinding for specific intercalator rescue and staple-break positioning. *Chem. Sci.* 3, 2587–2597
88. Perrault, S.D. and Shih, W.M. (2014) Virus-inspired membrane encapsulation of DNA nanostructures to achieve *in vivo* stability. *ACS Nano* 8, 5132–5140
89. Agarwal, N.P. *et al.* (2017) Block copolymer micellization as a protection strategy for DNA origami. *Angew. Chem. Int. Ed.* 56, 5460–5464
90. Zhao, Z. *et al.* (2016) Nanocaged enzymes with enhanced catalytic activity and increased stability against protease digestion. *Nat. Commun.* 7, 10619
91. Ora, A. *et al.* (2016) Cellular delivery of enzyme-loaded DNA origami. *Chem. Commun.* 52, 14161–14164
92. Salvati, A. *et al.* (2013) Transferrin-functionalized nanoparticles lose their targeting capabilities when a biomolecule corona adsorbs on the surface. *Nat. Nanotechnol.* 8, 137–143
93. Okholm, A.H. and Kjems, J. (2016) DNA nanovehicles and the biological barriers. *Adv. Drug Deliv. Rev.* 106, 183–191
94. Lacroix, A. *et al.* (2017) Development of DNA nanostructures for high affinity binding to human serum albumin. *J. Am. Chem. Soc.* 139, 7355–7362
95. Auvinen, H. *et al.* (2017) Protein coating of DNA nanostructures for enhanced stability and immunocompatibility. *Adv. Healthc. Mater.* 6, 1700692
96. Lo, P.K. *et al.* (2010) Loading and selective release of cargo in DNA nanotubes with longitudinal variation. *Nat. Chem.* 2, 319–328
97. Juul, S. *et al.* (2013) Temperature-controlled encapsulation and release of an active enzyme in the cavity of a self-assembled DNA nanocage. *ACS Nano* 7, 9724–9734
98. Zhang, C. *et al.* (2012) Reversibly switching the surface porosity of a DNA tetrahedron. *J. Am. Chem. Soc.* 134, 11998–12001
99. Burns, J.R. *et al.* (2018) DNA origami inside-out viruses. *ACS Synth. Biol.* 7, 767–773
100. Sun, W. *et al.* (2014) Cocoon-like self-degradable DNA nanoclew for anticancer drug delivery. *J. Am. Chem. Soc.* 136, 14722–14725
101. Kohman, R.E. *et al.* (2016) Light-triggered release of bioactive molecules from DNA nanostructures. *Nano Lett.* 16, 2781–2785
102. Liu, Z. *et al.* (2013) A smart DNA tetrahedron that isothermally assembles or dissociates in response to the solution pH value changes. *Biomacromolecules* 14, 1711–1714
103. Douglas, S.M. *et al.* (2012) A logic-gated nanorobot for targeted transport of molecular payloads. *Science* 335, 831–834
104. Ma, Y. *et al.* (2018) A telomerase-responsive DNA icosahedron for precise delivery of platinum nanodrugs to cisplatin-resistant cancer. *Angew. Chem. Int. Ed.* 57, 5389–5393
105. Valsangkar, V. *et al.* (2017) Click-based functionalization of a 2′-*O*-propargyl-modified branched DNA nanostructure. *J. Mater. Chem. B* 5, 2074–2077
106. Hu, R. *et al.* (2014) DNA nanoflowers for multiplexed cellular imaging and traceable targeted drug delivery. *Angew. Chem. Int. Ed.* 53, 5821–5826
107. Jiang, D. *et al.* (2016) Multiple-armed tetrahedral DNA nanostructures for tumor-targeting, dual-modality *in vivo* imaging. *ACS Appl. Mater. Interfaces* 8, 4378–4384
108. Shen, X. *et al.* (2012) Visualization of the intracellular location and stability of DNA origami with a label-free fluorescent probe. *Chem. Commun.* 48, 11301–11303
109. Chandrasekaran, A.R. and Rusling, D.A. (2018) Triplex-forming oligonucleotides: a third strand for DNA nanotechnology. *Nucleic Acids Res.* 46, 1021–1037
110. Amir, Y. and et al (2014) Universal computing by DNA origami robots in a living animal. *Nat. Nanotechnol.* 9, 353–357
111. Arnon, S. and et al (2016) Thought-controlled nanoscale robots in a living host. *PLoS One* 11, e0161227
112. Lund, K. and et al (2010) Molecular robots guided by prescriptive landscapes. *Nature* 465, 206–210
113. Praetorius, F. and et al (2017) Biotechnological mass production of DNA origami. *Nature* 552, 84–87
114. Stahl, E. and et al (2014) Facile and scalable preparation of pure and dense DNA origami solutions. *Angew. Chem. Int. Ed.* 53, 12735–12740
115. Wei, X. and et al (2014) Uncovering the self-assembly of DNA nanostructures by thermodynamics and kinetics. *Acc. Chem. Res.* 47, 1861–1870
116. Dunn, K.E. and et al (2015) Guiding the folding pathway of DNA origami. *Nature* 525, 82–86
117. Wah, J.L.T. and et al (2016) Observing and controlling the folding pathway of DNA origami at the nanoscale. *ACS Nano* 10, 1978–1987
118. Mathur, D. and Medintz, L. (2017) Analyzing DNA nanotechnology: a call to arms for the analytical chemistry community. *Anal. Chem.* 89, 2646–2663
119. Ong, L.L. and et al (2017) Programmable self-assembly of three-dimensional nanostructures from 10,000 unique components. *Nature* 552, 72–77
120. Wagenbauer, K.F. and et al (2017) Gigadalton-scale shape-programmable DNA assemblies. *Nature* 552, 78–83
121. Chandrasekaran, A.R. and et al (2016) Evolution of DNA origami scaffolds. *Mater. Lett.* 170, 221–224
122. Marchi, A.N. and et al (2014) Toward larger DNA origami. *Nano Lett.* 14, 5740–5747
123. Ouyang, X. and et al (2013) Rolling circle amplification-based DNA origami nanostructrures for intracellular delivery of immunostimulatory drugs. *Small* 9, 3082–3087
124. Gregory, T.R. (2001) Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. *Biol. Rev.* 76, 65–101
125. Elbaz, J. and et al (2016) Genetic encoding of DNA nanostructures and their self-assembly in living bacteria. *Nat. Commun.* 7, 11179
126. Jasinski, D. and et al (2017) Advancement of the emerging field of RNA nanotechnology. *ACS Nano* 11, 1142–1164