RSC Chemical Biology

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: Y. Suzuki, *RSC Chem. Biol.*, 2026, DOI: 10.1039/D6CB00026F.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/rsc-chembio

View Article Online
DOI: 10.1039/D6CB00026F

DNA Origami-Based Drug Delivery and Cell Manipulation: Toward Intelligent Nanomedicine

Yuki Suzuki

[1]Division of Chemistry for Materials, Graduate School of Engineering, Mie University, 1577 Kurimamachiya-Cho, Tsu, Mie, 514-8507, Japan.

E-mail: ysuzuki@chem.mie-u.ac.jp

ABSTRACT

DNA origami has emerged as a versatile platform for constructing nanoscale architectures with precise shape programmability, molecular-level addressability, and dynamic structural reconfigurability. Since its introduction, DNA origami has evolved from a structural design method into a functional nanosystem capable of integrating molecular recognition, logic-gated operations, and mechanical motion, enabling a wide range of biomedical applications. This review outlines recent advances in DNA origami-based drug delivery and the regulation of cellular functions and cell fate. Strategic control over size, shape, and mechanical properties is discussed in the context of cellular uptake, intracellular behavior, and the efficient delivery of small-molecule drugs and nucleic acid therapeutics. Recent progress in dynamic DNA origami nanodevices and nanorobots that respond to molecular, chemical, or physical cues is highlighted, including systems that enable spatiotemporally controlled payload release and the nanoscale organization of membrane receptors to modulate cellular signaling. In addition, key stabilization strategies required for *in vivo* applications, such as covalent linking of constituent DNA strands and surface-coating methods, are summarized. Finally, future challenges and perspectives are discussed, emphasizing the growing role of chemical biology in endowing DNA origami with sensing, decision-making, and adaptive functions for intelligent nanomedicine.

View Article Online
DOI: 10.1039/D6CB00026F

Introduction

The construction of artificial molecular systems that autonomously perform preprogrammed functions in living organisms is one of the ultimate goals in nanomedicine. The realization of such systems, often referred to as nanorobots and molecular robots,[1, 2] requires nanobiocompatible materials with target specificity, spatiotemporal controllability, and high reproducibility for molecular-level manipulation of cellular processes.

Nucleic acids, as representative biomolecules, have attracted considerable attention not only as carriers of genetic information but also as programmable nanomaterials owing to their high molecular recognition capability and self-assembly properties. DNA, in particular, is an excellent building block for constructing nanoscale architectures because it exhibits sequence-complementary interactions based on Watson–Crick base pairing and possesses a well-defined B-form double-helical structure. Research focused on designing and constructing precise artificial nanostructures using DNA as a programmable polymer has evolved into the field of structural DNA nanotechnology.[3]

Among the available approaches, DNA origami has emerged as a highly versatile strategy for fabricating nanostructures with predefined shapes. In DNA origami, a long single-stranded DNA is folded into a predefined shape using numerous short staple strands.[4] In addition to a high degree of designability, DNA origami structures possess intrinsic molecular addressability, enabling the precise positioning of functional molecules at designated sites. Owing to these characteristics, DNA origami has rapidly evolved into a multifunctional nanoplatform capable of integrating diverse molecular functions.[5]

Coupled with developments in nucleic acid and peptide therapeutics, applications of DNA origami have expanded toward the development of molecular devices and systems that function in living cells and *in vivo* environments. Over the past fifteen years, numerous DNA origami-based nanodevices have been reported as drug carriers and as nanotools for controlling cellular functions and cell fate. Cellular uptake efficiency can be modulated through the deliberate design of DNA origami size and shape. Moreover, therapeutic agents such as anticancer drugs can be loaded, and their release behavior can be finely tuned through structural design.[6] In addition, design strategies for dynamic DNA nanostructures that undergo mechanical motion in response to external stimuli, including chemical, photonic, and molecular signals, have advanced considerably. As a result, DNA origami nanostructures have evolved from simple carriers into dynamic nanosystems equipped with logic-gated functions.[7, 8]

In this review, I provide an overview of recent advances in DNA origami-based drug delivery and the regulation of cellular functions and cell fate, with particular emphasis on dynamic DNA nanostructures and stabilization strategies. I further discuss the potential of these technologies to advance intelligent nanomedicine and highlight how the high programmability of DNA origami offers new guiding principles

View Article Online
DOI: 10.1039/D6CB00026F

for artificial molecular systems in biological environments. Here, the term "intelligent nanomedicine" is used in a restricted sense to describe programmable nanosystems that do more than simply carry cargo or passively target tissues. Specifically, these systems integrate the following functions within a single design framework: contextual sensing of molecular, chemical, or physical cues; conditional structural or functional actuation through information processing; and biologically meaningful outputs, such as controlled payload release or receptor reorganization. Under this definition, targeted carriers, stimuli-responsive carriers, and logic-enabled nanodevices are viewed not as equivalent categories but as progressively more sophisticated stages along a continuum toward intelligent nanomedicine.

Fundamentals of DNA Origami

DNA origami, first reported by Rothemund in 2006, is a method in which a long single-stranded DNA (ssDNA) molecule of known sequence, referred to as the scaffold strand, is folded into a designed shape using numerous short ssDNA molecules, referred to as staple strands (**Figure 1a**).[4] Each staple strand connects distant regions of the scaffold strand, typically at three to four positions through half-crossovers. Sequence-specific base pairing between the scaffold strand and the staple strands at their designated positions results in the interconnection of adjacent double helices through multiple crossovers. This leads to the formation of designed DNA nanostructures in which multiple DNA duplexes are arranged in an antiparallel manner. In addition to two-dimensional DNA origami, strategies have been developed to construct three-dimensional[9-14] and wireframe architectures (**Figure 1b**).[15-18] The high structural designability and versatility of the scaffolded DNA origami method have made it one of the most widely used approaches for fabricating custom-shaped DNA nanostructures.

One of the most remarkable features of DNA origami, and a key factor underlying its broad applicability, is its high "addressability", which arises from its construction principle. Because each staple strand has a unique base sequence, specific positions within the assembled structure can be addressed through individual staple strands. During the design process, it is possible to specify whether the termini of individual staple strands are positioned on the front or back surfaces, or on the interior or exterior of the structure. This addressability is a distinctive advantage of DNA origami, enabling the selective incorporation of a wide range of molecules, including proteins, nucleic acids, and organic and inorganic compounds, at designated positions on a specific face of the structure through terminal extensions or chemical modifications of staple strands (**Figure 1a**).

Cellular Uptake of DNA Origami and Its Drug Delivery Applications

View Article Online
DOI: 10.1039/D6CB00026F

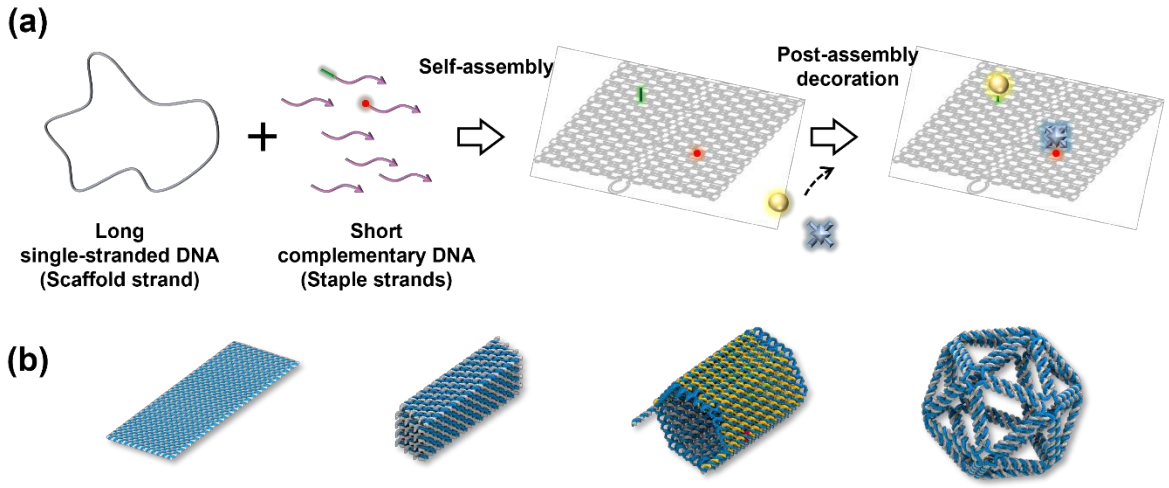

Figure 1. Construction and functionalization of DNA origami nanostructures. (a) A long single-stranded DNA molecule (scaffold strand) is folded into a prescribed shape through sequence-specific hybridization with numerous short deoxyoligonucleotides (staple strands). Chemical modification of the staple strands enables the site-specific introduction of desired moieties and/or additional DNA sequences during the self-assembly process. These modified sites can subsequently serve as anchoring points for post-assembly decoration with various functional molecules, including oligonucleotides, proteins, and inorganic nanoparticles. (b) A wide variety of DNA origami nanostructures with designed geometries can be produced, including two-dimensional sheet-like structures, three-dimensional solid blocks. and wireframe architectures.

Since the establishment of the DNA origami method, considerable efforts have been devoted to exploiting DNA origami nanostructures as novel drug carriers. The uptake of DNA origami structures by cultured cells has been investigated primarily through fluorescent labeling. The studies have shown that internalization efficiency depends on the size and shape of the origami structures, as well as on the cell type.[6] In particular, compact structures are generally internalized more readily than elongated rod-like architectures.

One of the simplest approaches to using DNA origami as a drug carrier involves the intercalation of anticancer agents, such as Doxorubicin (Dox), into the DNA duplexes of the origami structure (**Figure 2a**).[19] DNA origami can accommodate Dox at high density, enabling efficient intracellular delivery and, consequently, pronounced cytotoxic effects in cancer cells.

When three-dimensional DNA origami structures are used as carriers, both the Dox loading capacity and release kinetics can be modulated by controlling the intrinsic twist density of the DNA helices. In a representative study using 18-helix bundle nanotubes, a design with approximately 12.0 bp per turn, corresponding to a globally twisted structure, exhibited approximately 33% higher Dox loading than a conventional design with 10.5 bp per turn (**Figure 2b**)[20]. This twisted structure also showed slower, non-Fickian release kinetics and prolonged intracellular retention, which correlated with enhanced cytotoxic effects compared with free Dox and non-twisted DNA origami carriers.

View Article Online
DOI: 10.1039/D6CB00026F

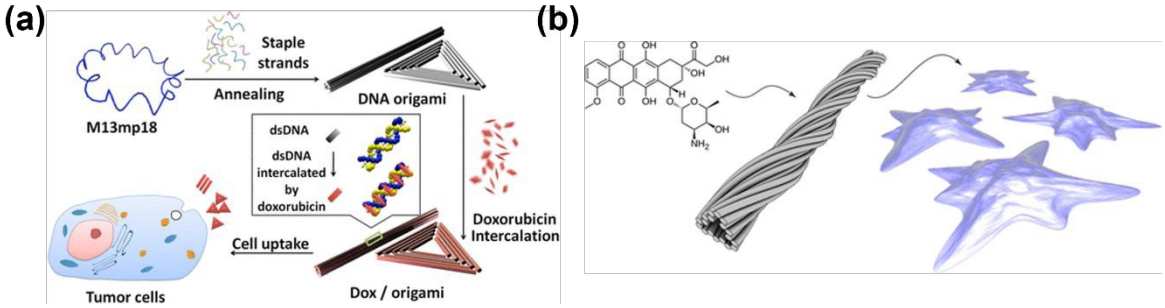

Figure 2. Loading of chemotherapeutic drugs onto DNA origami nanostructures via intercalation and their delivery into cancer cells. (a) Intercalation of doxorubicin (Dox) into the DNA duplexes of DNA origami nanostructures (reproduced with permission from ref. 19; Copyright 2012, American Chemical Society). (b) Dox loading into 18-helix bundle DNA origami nanotubes with different degrees of global twist. By tuning the overall twist of the nanostructure design, both the Dox loading capacity and release kinetics can be systematically controlled (reproduced with permission from ref. 20; Copyright 2012, American Chemical Society).

Beyond *in vitro* cell culture systems, *in vivo* Dox delivery using DNA origami has also been explored. Studies in tumor-bearing mice have shown that DNA origami nanostructures accumulate selectively in tumor tissues.[21] Delivery of Dox to the tumor site resulted in marked antitumor effects.

In addition to small-molecule drugs, DNA origami nanostructures have been investigated as carriers for small interfering RNAs (siRNAs). For example, DNA nanostructures with precisely defined sizes and shapes have been used to systemically deliver siRNA targeting the anti-apoptotic protein Bcl2.[22] In these studies, siRNA-loaded DNA origami nanostructures exhibited efficient cellular uptake mediated by scavenger receptors, leading to potent gene silencing *in vitro*. Importantly, intravenous administration of the siRNA-loaded DNA nanostructures in tumor-bearing mouse models resulted in significant suppression of tumor growth without detectable systemic toxicity, demonstrating therapeutic efficacy *in vivo*.

Beyond chemotherapy and nucleic acid delivery, DNA origami is increasingly being explored as a programmable vaccine scaffold. In particular, recent work has shown that DNA origami can be used not only to co-deliver antigens and adjuvants but also to control their nanoscale spatial arrangement. A notable example is a square-block DNA origami vaccine in which CpG spacing was systematically tuned, revealing that a spacing of 3.5 nm promoted dendritic-cell activation, antigen cross-presentation, Th1-polarized immunity, and durable antitumor responses.[23] These findings extend the utility of DNA origami beyond passive delivery, demonstrating that geometric control itself can serve as an immunological design parameter.

Overall, these studies demonstrate that DNA origami is an excellent molecular delivery platform. Drug loading capacity, release behavior, and intracellular function can be tailored through structural design and chemical modification. However, although key parameters such as structural compactness, aspect ratio,

View Article Online
DOI: 10.1039/D6CB00026F

rigidity, and ligand presentation consistently influence delivery performance, their specific effects remain highly dependent on cell type and administration route. Importantly, cellular uptake should not be conflated with productive delivery, as internalized nanostructures are often sequestered within endolysosomal compartments. Moreover, the structural integrity of internalized DNA origami often remains unresolved because fluorescence-based uptake measurements do not directly indicate whether the nanostructures remain intact after cellular internalization; in principle, fluorescence signals can persist even after structural disassembly.

Recently, a label-free method for quantifying the structural integrity of DNA origami after in vivo administration was reported. This method, termed proximity ligation assay for structural tracking and integrity quantification (PLASTIQ), is based on proximity ligation between ligatable staple pairs (LSPs). The resulting products are quantified by sequencing or qPCR, enabling accurate analysis from as little as 1 μL of blood.[24] Because PLASTIQ can determine whether local helices within a structure remain intact, it may provide useful guidance for the rational design of staple routing and nick positions. Although the current method is limited to evaluating extracellular structures, future adaptations incorporating cell isolation may enable analysis of cell-internalized DNA nanostructures, thereby supporting the rational development of DNA origami-based nanomedicines with more predictable in vivo behavior.

Regulation of Cellular Functions and Cell Fate Using DNA Origami Nanodevices

Shape-reconfigurable DNA origami nanostructures as platforms for drug delivery

Since the development of the DNA origami method, remarkable progress has been made in the design and construction of dynamic DNA origami nanostructures capable of shape reconfiguration.[25-29] Among these, container-like DNA nanostructures have attracted particular attention because of their broad range of potential applications, and a variety of designs incorporating opening and closing mechanisms have been proposed. Following pioneering studies that led to the development of nanoscale DNA origami boxes[9, 13] and prisms,[12] capsule-like structures responsive to external stimuli, such as pH[30] and light,[31, 32] have been developed using the DNA origami method (**Figure 3a and 3b**).

One representative example for intracellular drug delivery is a DNA origami nanocapsule incorporating azobenzene derivatives,[32] which undergoes reversible opening and closing upon irradiation with ultraviolet (UV) and visible light. After the nanocapsule is introduced into cells, irradiation with laser light of a specific wavelength enables *in situ* control of its state (open or closed). Given that various cargos,

View Article Online
DOI: 10.1039/D6CB00026F

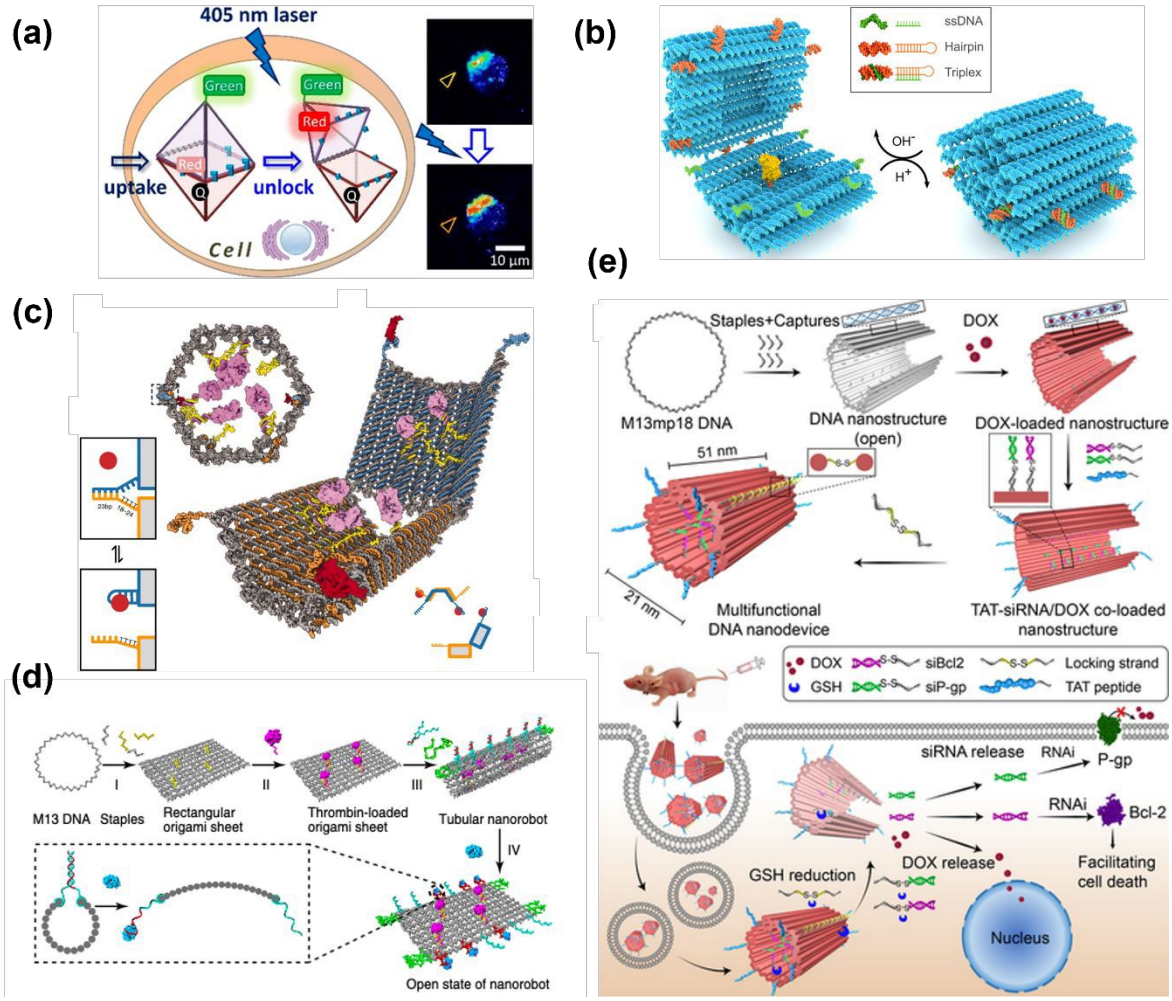

Figure 3. Reconfigurable DNA origami nanostructures for controlled drug delivery. (a) A photoresponsive DNA origami capsule that undergoes structural opening after cellular internalization upon light irradiation (reproduced with permission from ref. 30; Copyright 2019, American Chemical Society). (b) A pH-responsive DNA origami capsule that opens in response to acidic environments (reproduced with permission from ref. 28; Copyright 2019, American Chemical Society). (c) A DNA origami nanorobot designed for molecular delivery to specific target cells through ligand-mediated recognition (reproduced with permission from ref. 7; Copyright 2012, AAAS). (d) A DNA origami nanorobot for targeted thrombin delivery to tumor-associated vasculature in mice (reproduced with permission from ref. 31; Copyright 2018, Springer Nature). (e) A multifunctional DNA origami nanodevice co-loaded with siRNA and a chemotherapeutic drug for combination cancer therapy. The nanodevice undergoes intracellular opening and releases siRNA in response to cleavage of disulfide bonds by intracellular glutathione (GSH), while intercalated doxorubicin (Dox) is released simultaneously (reproduced with permission from ref. 32; Copyright 2021, Wiley-VCH).

including gold nanoparticles and biomolecules, can be loaded into the capsule, this photoresponsive capsule functions as a nanotool capable of releasing encapsulated materials into cells at arbitrary times and at the single-cell level.

The incorporation of aptamers that undergo conformational changes upon recognition of specific

View Article Online
DOI: 10.1039/D6CB00026F

proteins expressed on the cell surface has enabled the construction of more sophisticated DNA origami-based systems. A well-known example is the "DNA nanorobot" developed by Douglas et al. (**Figure 3c**).[7] This nanorobot has a hexagonal tube-like structure with a hinge, and its opening and closing are regulated by conformational changes in DNA aptamers. When a target molecule binds to the DNA aptamers positioned at the termini of the structure, the duplex DNA segments that maintain the tube in a closed state dissociate, causing the structure to open. Antibodies against human leukocyte antigen (HLA) are loaded inside the structure and are exposed to the exterior only upon recognition of the target protein. Furthermore, by incorporating different DNA aptamers that recognize distinct target molecules, unlocking mechanisms equipped with logic-gated functions have been realized. Using such DNA nanorobots, the regulation of target cell proliferation and activation of T cells has been successfully achieved.

DNA origami-based nanorobots that express their functions in response to molecular recognition events hold considerable promise as intelligent *in vivo* drug delivery systems. For example, a nanorobot developed by Li et al. displays DNA aptamers that recognize tumor-associated proteins on its exterior, while thrombin, a blood-coagulation protease, is loaded inside (**Figure 3d**).[33] In this system, binding of the aptamer to the target protein triggers dissociation of the double-stranded DNA, thereby functioning as an unlocking mechanism. As a result, thrombin is exposed only when the nanorobot binds to target tumor cells, enabling localized induction of blood coagulation at the tumor site. In tumor-bearing mouse models, intravenously administered nanorobots selectively delivered thrombin to tumor-associated vasculature, inducing intravascular thrombosis and effectively suppressing tumor growth while promoting tumor necrosis.

In addition to protein- and antibody-based cargos, DNA origami nanodevices have also been extended to the co-delivery and coordinated release of nucleic acid therapeutics and small-molecule drugs. A representative example is a tubular DNA origami nanodevice designed to facilitate the combined delivery of siRNA and chemotherapeutic agents. Specifically, siRNAs are spatially organized within the inner cavity of the device, whereas doxorubicin is intercalated into the DNA duplex (**Figure 3e**).[34] Redox-responsive disulfide locks enable autonomous structural opening in the reductive intracellular environment of tumor cells, thereby triggering the release of siRNAs and chemotherapeutic agents in a spatiotemporally controlled manner. This multifunctional nanodevice achieved efficient gene silencing, enhanced cytotoxicity, and pronounced tumor growth suppression *in vivo* without observable systemic toxicity, demonstrating how dynamic DNA origami architectures can integrate sensing, decision-making, and payload release within a single nanosystem.

Spatial Control of Receptor Signaling using DNA Origami

View Article Online
DOI: 10.1039/D6CB00026F

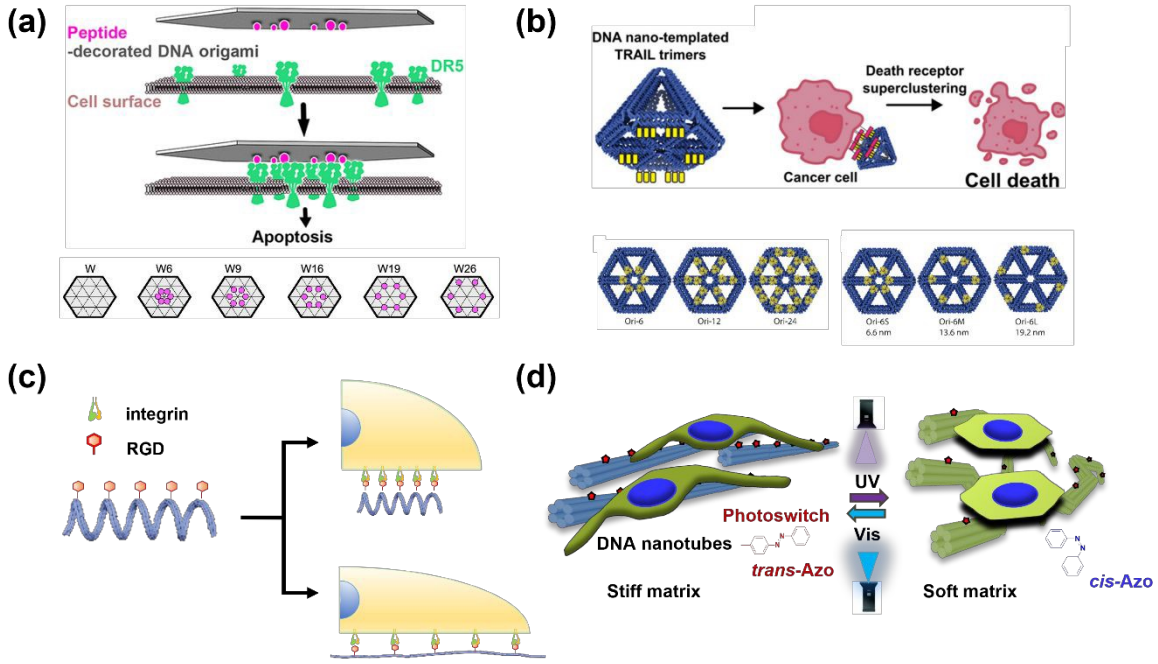

Figure 4. Clustering of membrane proteins via ligand-patterned DNA origami nanostructures. (a) Clustering of death receptor 5 (DR5) induces cell death using a peptide-patterned DNA origami sheet. The number and spacing of ligand peptides can be tuned in a prescribed manner (reproduced with permission from ref. 34; Copyright 2021, American Chemical Society). (b) Superclustering of DR5 induced by DNA-origami-templated peptide trimers (reproduced with permission from ref. 35; Copyright 2025, American Chemical Society) . (c) Schematic illustration of changes in the HeLa cell morphology induced by RGD-modified DNA-origami nanosprings carrying i-motif-forming sequences. (d) Schematic illustration of cell morphology changes induced by photocontrollable DNA origami nanotubes. A stiff nanoscaffold containing fibrous DNA nanotubes and a soft nanoscaffold containing flexible DNA nanotubes are reversibly interconverted by UV/visible (VIS) light irradiation (reproduced with permission from ref. 40; Copyright 2023, Royal Society of Chemistry).

Beyond their use as molecular carriers, recent studies have demonstrated that DNA origami nanostructures can be employed to precisely control the nanoscale clustering of membrane proteins on living cells. This strategy has been pursued primarily for two purposes: to elucidate how the spatial arrangement of target membrane proteins influences downstream signaling pathways and to establish methodologies for artificially controlling such signaling-related cellular events by engineering the spatial organization of proteins of interest.

Fang et al. used DNA origami to investigate how the spatial organization of programmed death-ligand 1 (PD-L1), the ligand for the programmed death-1 (PD-1) receptor, affects T-cell activation.[35] Using wireframe DNA origami sheets displaying PD-L1 at defined spacings, they demonstrated that ligand spacing critically influences the degree of T-cell signaling suppression. Specifically, arrangements with a spacing of approximately 200 nm, but not more densely packed patterns, induced a greater reduction in interleukin-2 (IL-2) expression. These findings provided a new design principle for immunomodulatory

View Article Online
DOI: 10.1039/D6CB00026F

nanoplatforms.

DNA origami has also been used to induce apoptosis by clustering death receptor 5 (DR5). In a study by Wang et al., DNA origami structures decorated with tumor necrosis factor–related apoptosis-inducing ligand (TRAIL)-mimicking peptides that bind to DR5 were constructed (**Figure 4a**).[36] Using a series of constructs using different peptide numbers and interligand separations, the authors found that a hexagonal arrangement with an interligand distance of approximately 5 nm was optimal for inducing receptor clustering and apoptosis. In a subsequent study, Aba et al. patterned multivalent single-chain TRAIL (sc-TRAIL) trimers on hexagonal DNA origami nanopyramids and achieved efficient cancer cell killing in tumor organoids using the DNA origami-templated TRAIL trimers (**Figure 4b**).[37] Together, these studies demonstrate that precise control over ligand valency and spatial arrangement using DNA origami provides a powerful platform for dissecting and engineering apoptosis pathways through nanoscale organization, thereby offering promising strategies for the development of effective cancer therapies.

From a chemical biology perspective, these studies are particularly compelling not simply because DNA origami serves as a scaffold, but because it provides a modular platform for integrating functionally selected and chemically defined ligands, including peptide ligands, aptamers, and synthetic mimics of bioactive molecules, whose interactions with target receptors can be systematically examined. Many of these functional moieties are themselves products of chemical biology approaches, such as molecular selection, rational design, chemical synthesis, and site-specific modification. DNA origami enables these ligands to be displayed with nanometer-scale control over spacing, valency, orientation, and dynamic presentation. In this context, DNA origami functions as a molecularly precise perturbation tool, allowing researchers not only to test causal hypotheses about how nanoscale ligand geometry governs receptor signaling but also to evaluate how the molecular identity and mode of presentation of chemically engineered ligands shape biological responses. This capability distinguishes receptor-organization studies from conventional delivery applications and underscores their importance as a conceptual bridge between structural DNA nanotechnology and quantitative chemical biology.

Chemo-Mechanical Regulation via Integrin Clustering

The studies described above used static DNA origami nanostructures to organize ligands of target proteins with defined numbers and spatial arrangements. More recent work has extended this concept to dynamic DNA origami nanostructures that manipulate membrane protein clustering in response to external stimuli or environmental cues.

This approach has been applied, in particular, to control cell morphology and motility by modulating the clustering and de-clustering of integrins, which mediate cell adhesion to the extracellular matrix (ECM).

View Article Online
DOI: 10.1039/D6CB00026F

Integrins bind to arginyl–glycyl–aspartic acid (RGD) motifs in the ECM, and integrin clustering activates downstream signaling pathways that regulate cellular mechanical properties, including migration and protrusive activity. Based on this mechanism, stimuli-responsive artificial ECMs have been developed in which RGD motifs are incorporated into thermoresponsive polymers[38] or signal-responsive DNA polymers[39, 40] to modulate cell mechanics.

DNA origami nanostructures capable of contraction and relaxation are particularly well suited to this application because they can present multiple RGD peptides at precisely defined positions, while dynamically altering interligand spacing through actuator-like motions triggered by environmental changes or external stimuli, including pH variations,[41] nucleic acid strands,[8] and light (**Figure 4d and 4e**).[42] As a result, the clustering state of integrin molecules can be modulated at the nanoscale, enabling regulation of cell morphology and migration. More recent designs have incorporated microRNA(miRNA)-responsive logic gates, such that spatial rearrangement of RGD ligands–and consequently integrin clustering or declustering–occurs only in the presence of specific combinations of miRNAs.[8] This strategy enables cell-selective control of motility through programmable molecular cues.

Taken together, these studies demonstrate that DNA origami is not merely a platform for molecular organization but has evolved into the basis of dynamic nanosystems that integrate nucleic acid and peptide modules for molecular recognition, logic-gated operation, and mechanical motion within a single architecture. This high degree of programmability and modularity represents a key advantage for the realization of intelligent nanomedicine. In both drug delivery and cell manipulation, the central challenge is not simply to bring a functional molecule into contact with a biological target, but to control when, where, and in what structural context that interaction occurs. Drug delivery places greater emphasis on the transport and conditional release of therapeutic cargo, whereas cell manipulation focuses on the spatial and temporal organization of bioactive signals at the cell surface or within intracellular environments. Nevertheless, both rely on the same core capabilities of DNA origami: programmable control over molecular positioning, valency, responsiveness, and stability in biological media. From this perspective, cell manipulation should be viewed not as a separate direction from drug delivery, but as an extension of the same design principles toward more active regulation of cellular behavior and fate.

Strategies to Enhance Stability in Biological Environments

To translate DNA origami nanostructures effectively into biomedical applications, structural stability under physiological conditions must be improved.[43] Because DNA origami employs numerous staple strands as structural elements, the assembled structures inevitably contain a large number of nicks. These discontinuities can serve as initiation sites for DNA degradation, and considerable efforts have therefore

View Article Online
DOI: 10.1039/D6CB00026F

been devoted to improving stability by connecting staple-strand termini after assembly.

Two main approaches have been developed to seal backbone nicks: enzymatic ligation and chemical ligation (**Figure 5a**). In enzymatic ligation, commercially available enzymes such as T4 DNA ligase are used, and this strategy has been demonstrated to be effective for two-dimensional and wireframe DNA origami structures.[44] However, successful ligation requires physical access of the enzyme to the nick sites, which limits its applicability to densely bundled three-dimensional DNA origami structures in which DNA helices are tightly packed.

Chemical ligation represents a promising alternative, particularly for three-dimensional DNA origami. Reagents such as cyanogen bromide (CNBr)[45] and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)[46, 47] have been used to catalyze the sealing of backbone nicks in DNA origami structures. These treatments confer enhanced resistance to thermal denaturation, nuclease-mediated degradation, and destabilization in cell lysates.[45] It should be noted that both enzymatic and chemical ligation generally require phosphorylation of the 5′ termini of DNA strands–particularly the staple strands in DNA origami–to serve as reactive substrates. This requirement can be met either by enzymatic phosphorylation using kinases or by employing pre-phosphorylated oligonucleotides.

In addition to enzymatic and chemical ligation, photochemical cross-linking strategies have been explored to reinforce DNA origami structures. One early example involves photo-cross-linkable molecules such as 8-methoxypsoralen, which forms covalent adducts with pyrimidine bases upon irradiation with ultraviolet (UV) light.[48] When DNA origami structures treated with such photosensitizers are exposed to UV light, interstrand covalent linkages are generated, increasing thermal stability relative to untreated structures.

Sequence-programmable covalent cross-linking approaches have also been developed, in which closely positioned bases within the origami design are connected through controlled photochemical reactions. In one such method, thymidine residues are positioned to form cyclobutane pyrimidine dimers upon UV irradiation, creating additional covalent connections that reinforce the internal structure of the origami (**Figure 5b**).[49] These photo-cross-linked structures exhibit enhanced resistance not only to elevated temperatures but also to other destabilizing environments, demonstrating that photochemical stabilization can effectively complement ligation-based approaches.

Beyond strategies that connect internal nicks, surface coating methods using lipids or polymeric materials have also been explored. For example, coating an octahedral wireframe DNA origami nanostructure with a lipid bilayer containing PEG-modified lipids has been shown to improve nuclease resistance and reduce immune activation in mice (**Figure 5c**).[50] Notably, these lipid-coated structures exhibited prolonged circulation in the bloodstream compared with uncoated counterparts.

View Article Online
DOI: 10.1039/D6CB00026F

Coating with cationic polymers represents another effective stabilization strategy that preserves the structural integrity of DNA origami under low-salt and cell-culture conditions. Initial studies employing poly(2-dimethylaminoethyl methacrylate) (PDMAEMA)-based polymers demonstrated that electrostatic coatings can be applied to three-dimensional DNA origami nanostructures with negligible cytotoxicity.[51] These studies also suggested that the polymer layer can modulate the activity of encapsulated enzymes, plausibly by restricting substrate diffusion. Subsequently, coating with PEG–poly(L-lysine) was introduced to form DNA origami polyplex micelles (DOPMs), which exhibited strong resistance to DNase

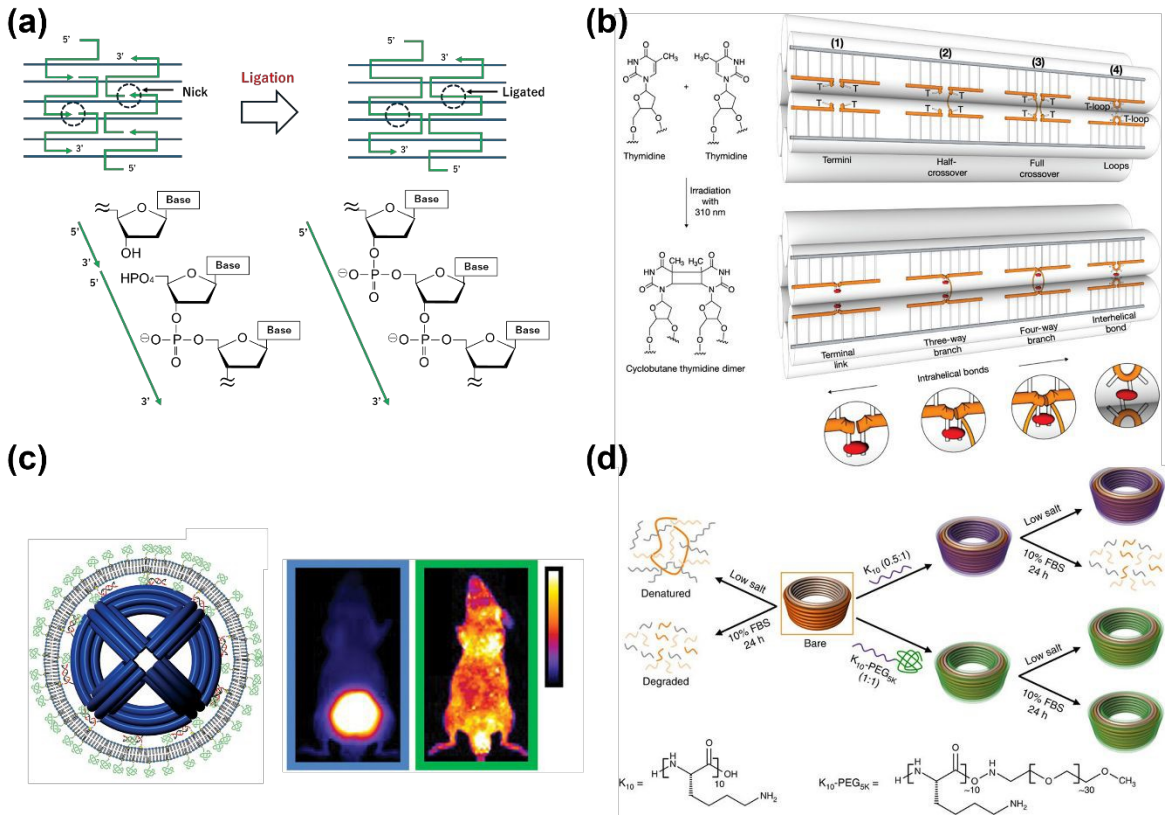

Figure 5. Strategies for stabilizing DNA origami nanostructures in biological environments. (a) Post-assembly enzymatic or chemical ligation of staple strands to seal nicks within DNA origami structures. Phosphorylation of the 5' termini of staple strands is required for efficient ligation. (b) Photochemical cross-linking of DNA strands within DNA origami nanostructures through the formation of cyclobutane thymidine dimers upon ultraviolet (UV) irradiation (reproduced with permission from ref. 47; Copyright, 2018, AAAS). (c) Surface coating of DNA origami nanostructures with a lipid bilayer containing PEGylated lipids. Fluorescence imaging of mice shows that lipid-encapsulated origami is distributed throughout the body (right), whereas non-encapsulated origami accumulates predominantly in the bladder (left) (reproduced with permission from ref. 48; Copyright 2014, American Chemical Society). (d) Electrostatic coating of DNA origami nanostructures with oligolysine and oligolysine-polyethylene glycol (PEG) copolymers (reproduced with permission from ref. 51; Copyright 2017, Springer-Nature).

View Article Online
DOI: 10.1039/D6CB00026F

I digestion, serum-containing media, and Mg^{2+}-free buffers while remaining compatible with diverse surface functionalizations.[52] Systematic studies using oligolysine and oligolysine–PEG copolymers further demonstrated substantial improvements in nuclease resistance, with up to a tenfold increase in resistance to DNase I digestion using oligolysine alone and up to three orders of magnitude greater resistance to serum nucleases when PEGylated oligolysines were employed (**Figure 5d**).[53] Importantly, oligolysine–PEG–coated DNA nanostructures were shown to survive endosomal uptake and exhibited a modest but reproducible increase in pharmacokinetic bioavailability in mouse models. These findings highlight a promising strategy for protecting DNA origami nanostructures intended for *in vivo* applications.

Collectively, these stabilization strategies are indispensable for transforming DNA origami from shape-defined nanostructures into functional nanomedicines capable of operating reliably in living organisms.

Future Challenges and Perspectives

As outlined in this review, the potential of DNA origami in biomedical applications expanded rapidly, particularly in drug delivery and the regulation of cellular functions. These advances have been enabled by the high degree of shape programmability, molecular-level addressability, and controlled structural reconfigurability of DNA origami. Nevertheless, several challenges must be addressed before these attractive properties can be fully exploited *in vivo* with high stability and reproducibility.

Foremost among these challenges is the need to maintain both structural stability and functional integrity in biological environments. Degradation by the diverse nucleases present in vivo is a fundamental issue common to DNA nanostructures and is therefore not unique to DNA origami. To date, a variety of stabilization strategies, including enzymatic and chemical ligation, photo-cross-linking, and surface coating with lipids or polymers, have been proposed. However, these treatments may hinder subsequent functional modifications or impair the activity of incorporated molecular components. In particular, for dynamic DNA origami nanostructures that undergo conformational changes, stabilization must be achieved without compromising the flexible DNA segments required for motion. DNA domains responsible for signal and stimulus responsiveness, such as toeholds,[54] aptamers,[55] photoresponsive elements,[56-58] and triplex- or quadruplex-forming sequences,[59] must also remain intact. Future efforts will require rational design principles and practical methodologies, such as site-selective ligation and localized coating, that reinforce vulnerable regions while preserving programmed dynamics and functionality.

Another important issue is immunogenicity, which may either be detrimental or advantageous depending on the intended application. In drug delivery, unintended activation of innate immune pathways may alter pharmacokinetics, biodistribution, and safety profiles, whereas in vaccination and immunotherapy, controlled immunostimulation may be desirable. Accumulating evidence indicates that

View Article Online
DOI: 10.1039/D6CB00026F

immune recognition depends on multiple factors, including DNA sequence motifs, nanostructure geometry, dose, degradation behavior, and surface chemistry. Accordingly, future design principles should aim not only to improve stability and targeting but also to rationally tune immunocompatibility or immunostimulatory properties in an application-specific manner.

DNA nanodevices and nanorobots reported to date have demonstrated the successful integration of molecular modules for target recognition, therapeutic action, logic-gated operations, and mechanical motion within a single DNA origami nanostructure. Current efforts are shifting toward the development of more intelligent and multiresponsive platforms capable of expressing distinct functions sequentially or cooperatively in response to different stimuli or combinations thereof. Particularly in complex biological environments, a key challenge will be selecting appropriate target molecules and designing sensors for these targets and their combinations during the design stage, so that the intended tasks can be executed reliably while minimizing unintended side effects.

In addition to the DNA strands used to control structural reconfiguration, a wide variety of nucleic acids have been employed as functional modules. These include DNA and RNA aptamers, small interfering RNAs (siRNAs), and CpG motifs, which enable target recognition and trigger signal transduction in and on living cells. In this context, achieving multifunctionality requires careful consideration of sequence orthogonality and the suppression of unintended crosstalk among these nucleic acid components. At the structural design stage, sequence orthogonality must be ensured not only among these functional modules, but also with the constituent strands of the DNA origami architecture, namely the scaffold and staple strands.

To date, M13mp18 and its derivatives have been widely used as scaffold strands for DNA origami construction. More recently, alternative scaffold strands with sequences orthogonal to M13-based scaffolds have been designed, synthesized, and made commercially available.[60] This expansion of material-level options is expected to provide a foundation not only for implementing multifunctionality within a single DNA origami nanostructure, but also for enabling the coordinated operation of multiple DNA origami-based nanorobots and nanodevices.

Over the past two decades, DNA origami nanostructures have evolved into versatile platforms for constructing programmable nanosystems with prescribed biological and therapeutic functions. Recent work suggests that their scope is expanding beyond that of conventional drug delivery vehicles. For example, a reversibly gated DNA membrane channel that enables stimulus-controlled transport of folded proteins across lipid bilayers demonstrates that programmable DNA nanostructures can be engineered not only as carriers, but also as dynamic transmembrane devices for regulating molecular exchange.[61] Such systems may open new possibilities as alternative strategies for the intracellular delivery of therapeutic

View Article Online
DOI: 10.1039/D6CB00026F

proteins.

In parallel, advances in related nucleic-acid nanostructures, such as single-stranded RNA (ssRNA) origami[62, 63], further underscore that the biomedical potential of programmable nucleic acid architectures extends beyond DNA origami, particularly in the context of immunotherapy. Recent studies have shown that ssRNA origami can remodel macrophages, promote natural killer (NK) cell recruitment, and synergize with chemotherapy in vivo, highlighting the potential of nucleic acid nanostructures to reprogram the tumor microenvironment through defined immunological mechanisms.[64]

In this transition, chemical biology has played an increasingly important role by enabling the creation, selection, and chemical modification of functional nucleic acid modules, such as aptamers, antisense oligonucleotides, and stimulus-responsive oligonucleotides, which endow DNA origami with molecular recognition, sensing, and shape reconfigurability. Advances in peptide design and synthesis technologies are also noteworthy. Emerging work on peptide-coated DNA nanostructures has shown that rationally designed surface chemistries can modulate lysosomal pH, membrane integrity, immune signaling, and cell fate in distinct ways,[65] highlighting the possibility of using DNA-based materials to directly control organelle function rather than merely escape endolysosomal sequestration. More broadly, recent perspectives have framed such approaches as part of an emerging paradigm of "organelle interference," in which DNA nanostructures are designed to target and regulate specific intracellular compartments, including lysosomes, mitochondria, nuclei, and other membrane-bound organelles.[66]

The continued integration of the molecular-level systems engineering principles cultivated in structural DNA nanotechnology with the analytical, synthetic, and selection-based methodologies of chemical biology is expected to accelerate progress toward intelligent nanomedicine, in which DNA origami-based nanosystems operate as adaptive, information-processing entities within complex biological environments.

AUTHOR CONTRIBUTIONS

Writing − original draft: Y.S.
Writing − review & editing: Y.S

CONFLICTS OF INTEREST

There are no conflicts to declare.

DATA AVAILABILITY

No primary research results, software, or code have been included, and no new data were generated or analyzed as part of this review.

View Article Online
DOI: 10.1039/D6CB00026F

ACKNOWLEDGMENTS

Y.S. thanks Ibuki Kawamata and Masayuki Endo for providing graphic models of DNA origami nanostructures.

REFERENCES

1. M. Hagiya, A. Konagaya, S. Kobayashi, H. Saito and S. Murata, *Accounts Chem Res*, 2014, **47**, 1681-1690.
2. S. Murata, T. Toyota, S. M. Nomura, T. Nakakuki and A. Kuzuya, *Adv Funct Mater*, 2022, **32**, 2201866.
3. N. C. Seeman and H. F. Sleiman, *Nat Rev Mater*, 2018, **3**, 17068.
4. P. W. K. Rothemund, *Nature*, 2006, **440**, 297-302.
5. P. F. Zhan, A. Peil, Q. Jiang, D. F. Wang, S. Mousavi, Q. C. Xiong, Q. Shen, Y. X. Shang, B. Q. Ding, C. X. Lin, Y. G. Ke and N. Liu, *Chem Rev*, 2023, **123**, 3976-4050.
6. M. M. C. Bastings, F. M. Anastassacos, N. Ponnuswamy, F. G. Leifer, G. Cuneo, C. X. Lin, D. E. Ingber, J. H. Ryu and W. M. Shih, *Nano Lett*, 2018, **18**, 3557-3564.
7. S. M. Douglas, I. Bachelet and G. M. Church, *Science*, 2012, **335**, 831-834.
8. D. Karna, S. Watanabe, G. Sharma, A. Sharma, Y. R. Zheng, I. Kawamata, Y. Suzuki and H. B. Mao, *ACS Nano*, 2025, **19**, 8058-8069.
9. E. S. Andersen, M. Dong, M. M. Nielsen, K. Jahn, R. Subramani, W. Mamdouh, M. M. Golas, B. Sander, H. Stark, C. L. P. Oliveira, J. S. Pedersen, V. Birkedal, F. Besenbacher, K. V. Gothelf and J. Kjems, *Nature*, 2009, **459**, 73-76.
10. H. Dietz, S. M. Douglas and W. M. Shih, *Science*, 2009, **325**, 725-730.
11. S. M. Douglas, H. Dietz, T. Liedl, B. Högberg, F. Graf and W. M. Shih, *Nature*, 2009, **459**, 414-418.
12. M. Endo, K. Hidaka, T. Kato, K. Namba and H. Sugiyama, *J Am Chem Soc*, 2009, **131**, 15570-15571.
13. A. Kuzuya and M. Komiyama, *Chem Commun*, 2009, 4182-4184.
14. D. R. Han, S. Pal, J. Nangreave, Z. T. Deng, Y. Liu and H. Yan, *Science*, 2011, **332**, 342-346.
15. D. R. Han, S. Pal, Y. Yang, S. X. Jiang, J. Nangreave, Y. Liu and H. Yan, *Science*, 2013, **339**, 1412-1415.
16. E. Benson, A. Mohammed, J. Gardell, S. Masich, E. Czeizler, P. Orponen and B. Högberg, *Nature*, 2015, **523**, 441-444
17. F. Zhang, S. X. Jiang, S. Y. Wu, Y. L. Li, C. D. Mao, Y. Liu and H. Yan, *Nat Nanotechnol*, 2015,

View Article Online
DOI: 10.1039/D6CB00026F

10, 779-784.

18. E. Benson, A. Mohammed, A. Bosco, A. I. Teixeira, P. Orponen and B. Högberg, *Angew Chem Int Edit*, 2016, **55**, 8869-8872.
19. Q. Jiang, C. Song, J. Nangreave, X. W. Liu, L. Lin, D. L. Qiu, Z. G. Wang, G. Z. Zou, X. J. Liang, H. Yan and B. Q. Ding, *J Am Chem Soc*, 2012, **134**, 13396-13403.
20. Y. X. Zhao, A. Shaw, X. H. Zeng, E. Benson, A. M. Nyström and B. Högberg, *ACS Nano*, 2012, **6**, 8684-8691.
21. Q. Zhang, Q. Jiang, N. Li, L. R. Dai, Q. Liu, L. L. Song, J. Y. Wang, Y. Q. Li, J. Tian, B. Q. Ding and Y. Du, *ACS Nano*, 2014, **8**, 6633-6643.
22. M. A. Rahman, P. F. Wang, Z. X. Zhao, D. S. Wang, S. Nannapaneni, C. Zhang, Z. J. Chen, C. C. Griffith, S. J. Hurwitz, Z. G. Chen, Y. G. Ke and D. M. Shin, *Angew Chem Int Edit*, 2017, **56**, 16023-16027.
23. Y. C. Zeng, O. J. Young, C. M. Wintersinger, F. M. Anastassacos, J. I. MacDonald, G. Isinelli, M. O. Dellacherie, M. Sobral, H. Bai, A. R. Graveline, A. Vernet, M. Sanchez, K. Mulligan, Y. Choi, T. C. Ferrante, D. B. Keskin, G. G. Fell, D. Neuberg, C. J. Wu, D. J. Mooney, I. C. Kwon, J. H. Ryu and W. M. Shih, *Nat Nanotechnol*, 2024, **19**, 1055-1065.
24. Y. Wang, I. Rocamonde-Lago, J. Waldvogel, B. Shen, Y. C. Wu, J. Zhu, S. Zang, Y. Jia, I. Baars, A. Kloosterman, I. T. Hoffecker, M. R. Wu, Q. He and B. Hogberg, *Nat Nanotechnol*, 2026, **21**, 268-276.
25. A. Kuzuya, Y. Sakai, T. Yamazaki, Y. Xu and M. Komiyama, *Nat Commun*, 2011, **2**, 449.
26. A. E. Marras, L. F. Zhou, H. J. Su and C. E. Castro, *Proc Natl Acad Sci USA*, 2015, **112**, 713-718.
27. T. Gerling, K. F. Wagenbauer, A. M. Neuner and H. Dietz, *Science*, 2015, **347**, 1446-1452.
28. S. Nummelin, B. X. Shen, P. Piskunen, Q. Liu, M. A. Kostiainen and V. Linko, *ACS Synth Biol*, 2020, **9**, 1923-1940.
29. A. Mills, N. Aissaoui, J. Finkel, J. Elezgaray and G. Bellot, *Adv Biol*, 2023, **7**, 2200224.
30. H. Ijäs, I. Hakaste, B. X. Shen, M. A. Kostiainen and V. Linko, *ACS Nano*, 2019, **13**, 5959-5967.
31. T. Takenaka, M. Endo, Y. Suzuki, Y. Y. Yang, T. Emura, K. Hidaka, T. Kato, T. Miyata, K. Namba and H. Sugiyama, *Chem-Eur J*, 2014, **20**, 14951-14954.
32. T. Tohgasaki, Y. Shitomi, Y. H. Feng, S. Honna, T. Emura, K. Hidaka, H. Sugiyama and M. Endo, *Bioconjugate Chem*, 2019, **30**, 1860-1863.
33. S. P. Li, Q. Jiang, S. L. Liu, Y. L. Zhang, Y. H. Tian, C. Song, J. Wang, Y. G. Zou, G. J. Anderson, J. Y. Han, Y. Chang, Y. Liu, C. Zhang, L. Chen, G. B. Zhou, G. J. Nie, H. Yan, B. Q. Ding and Y. L. Zhao, *Nat Biotechnol*, 2018, **36**, 258-264.

View Article Online
DOI: 10.1039/D6CB00026F

34. Z. R. Wang, L. L. Song, Q. Liu, R. Tian, Y. X. Shang, F. S. Liu, S. L. Liu, S. Zhao, Z. H. Han, J. S. Sun, Q. Jiang and B. Q. Ding, *Angew Chem Int Edit*, 2021, **60**, 2594-2598.
35. T. Fang, J. Alvelid, J. Spratt, E. Ambrosetti, I. Testa and A. Teixeira, *ACS Nano*, 2021, **15**, 3441-3452.
36. Y. Wang, I. Baars, F. Fördös and B. Högberg, *ACS Nano*, 2021, **15**, 9614-9626.
37. G. Aba, S. Abudukelimu, M. de Winter, G. Collu, E. Bos, S. M. W. R. Hamers, L. J. A. C. Hawinkels, N. van Montfoort, F. A. Scheeren and T. H. Sharp, *Nano Lett*, 2025, **25**, 6310-6317.
38. Z. Liu, Y. Liu, Y. Chang, H. R. Seyf, A. Henry, A. L. Mattheyses, K. Yehl, Y. Zhang, Z. Q. Huang and K. Salaita, *Nat Methods*, 2016, **13**, 143-146.
39. K. X. Zhang, R. J. Deng, Y. P. Sun, L. Zhang and J. H. Li, *Chem Sci*, 2017, **8**, 7098-7105.
40. S. Sethi, K. Hidaka, H. Sugiyama and M. Endo, *Angew Chem Int Edit*, 2021, **60**, 20342-20349.
41. D. Karna, M. Stilgenbauer, S. Jonchhe, K. Ankai, I. Kawamata, Y. X. Cui, Y. R. Zheng, Y. Suzuki and H. B. Mao, *Bioconjugate Chem*, 2021, **32**, 311-317.
42. S. Sethi, T. Emura, K. Hidaka, H. Sugiyama and M. Endo, *Nanoscale*, 2023, **15**, 2904-2910.
43. J. Hahn, S. F. J. Wickham, W. M. Shih and S. D. Perrault, *ACS Nano*, 2014, **8**, 8765-8775.
44. A. Rajendran, K. Krishnamurthy, A. Giridasappa, E. Nakata and T. Morii, *Nucleic Acids Res*, 2021, **49**, 7884-7900.
45. K. Krishnamurthy, A. Rajendran, E. Nakata and T. Morii, *Small Methods*, 2024, **8**, 2300999.
46. M. Kramer and C. Richert, *Chem Biodivers*, 2017, **14**, e1700315.
47. N. Weizenmann, G. Scheidgen-Kleyboldt, J. J. Ye, C. B. Krause, D. Kauert, S. Helmi, C. Rouillon and R. Seidel, *Nanoscale*, 2021, **13**, 17556-17565.
48. A. Rajendran, M. Endo, Y. Katsuda, K. Hidaka and H. Sugiyama, *J Am Chem Soc*, 2011, **133**, 14488-14491.
49. T. Gerling, M. Kube, B. Kick and H. Dietz, *Sci Adv*, 2018, **4**.
50. S. D. Perrault and W. M. Shih, *ACS Nano*, 2014, **8**, 5132-5140.
51. J. K. Kiviaho, V. Linko, A. Ora, T. Tiainen, E. Jarvihaavisto, J. Mikkila, H. Tenhu, Nonappa and M. A. Kostiainen, *Nanoscale*, 2016, **8**, 11674-11680.
52. N. P. Agarwal, M. Matthies, F. N. Gür, K. Osada and T. L. Schmidt, *Angew Chem Int Edit*, 2017, **56**, 5460-5464.
53. N. Ponnuswamy, M. M. C. Bastings, B. Nathwani, J. H. Ryu, L. Y. T. Chou, M. Vinther, W. A. Li, F. M. Anastassacos, D. J. Mooney and W. M. Shih, *Nat Commun*, 2017, **8**.
54. B. Yurke, A. J. Turberfield, A. P. Mills, F. C. Simmel and J. L. Neumann, *Nature*, 2000, **406**, 605-608.

View Article Online
DOI: 10.1039/D6CB00026F

55. J. H. Zhou and J. Rossi, *Nat Rev Drug Discov*, 2017, **16**, 181-202.
56. Y. Yoshimura and K. Fujimoto, *Org Lett*, 2008, **10**, 3227-3230.
57. K. Fujimoto, A. Yamada, Y. Yoshimura, T. Tsukaguch and T. Sakamoto, *J Am Chem Soc*, 2013, **135**, 16161-16167.
58. Y. Kamiya and H. Asanuma, *Accounts Chem Res*, 2014, **47**, 1663-1672.
59. F. Wang, X. Q. Liu and I. Willner, *Angew Chem Int Edit*, 2015, **54**, 1098-1129.
60. F. A. S. Engelhardt, F. Praetorius, C. H. Wachauf, G. Brüggenthies, F. Kohler, B. Kick, K. L. Kadletz, P. N. Pham, K. L. Behler, T. Gerling and H. Dietz, *ACS Nano*, 2019, **13**, 5015-5027.
61. S. Dey, A. Dorey, L. Abraham, Y. Xing, I. Zhang, F. Zhang, S. Howorka and H. Yan, *Nat Commun*, 2022, **13**, 2271.
62. C. Geary, P. W. Rothemund and E. S. Andersen, *Science*, 2014, **345**, 799-804.
63. E. Poppleton, N. Urbanek, T. Chakraborty, A. Griffo, L. Monari and K. Gopfrich, *RNA Biol*, 2023, **20**, 510-524.
64. X. Tu, L. Yu, L. Zhao, X. Qi, Y. Xu and H. Yan, *JACS Au*, 2025, **5**, 5758-5769.
65. P. Elblova, M. Lunova, S. J. W. Henry, X. Tu, A. Cale, A. Dejneka, J. Havelkova, Y. Petrenko, M. Jirsa, N. Stephanopoulos and O. Lunov, *Chem Eng J*, 2024, **498**, 155633.
66. P. Elblova, J. Anthi, M. Liu, M. Lunova, M. Jirsa, N. Stephanopoulos and O. Lunov, *JACS Au*, 2025, **5**, 1591-1616.

View Article Online
DOI: 10.1039/D6CB00026F

DATA AVAILABILITY

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.