

Static DNA Nanostructures For Cancer Theranostics: Recent Progress In Design And Applications

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Abstract: Among the various nano/biomaterials used in cancer treatment, the beauty and benefits of DNA nanocomposites are outstanding. The specificity and programmability of the base pairing of DNA strands, together with their ability to conjugate with different types of functionalities have realized unsurpassed potential for the production of two- and three-dimensional nano-sized structures in any shape, size, surface chemistry and functionality. This review aims to provide an insight into the diversity of static DNA nanodevices, including DNA origami, DNA polyhedra, DNA origami arrays and bioreactors, DNA nanoswitch, DNA nanoflower, hydrogel and dendrimer as young but promising platforms for cancer theranostics. The utility and potential of the individual formats in biomedical science and especially in cancer therapy will be discussed.

Keywords: static DNA nanostructures, cancer treatment, biosensing

Introduction

The integration of nanotechnology into other biomedical sciences has been a blessing for all researchers around the world. Various nanocarriers including polymeric nanoparticles such as hydrogels, dendrimers, polymerosomes, lipid nanocarriers such as liposomes, micelles, organic nanoparticles such as carbon nanotubes, fullerenes, and non-organic nanoparticulates of gold, silver, magnetic Fe₃O₄, etc. have often been used for various purposes in the detection and cure of human diseases, in particular, cancer.¹⁻³ Nevertheless, various physiological/physiological barriers still need to be addressed in relation to: (i) tumor cell heterogeneity such as clonal evolution and alteration/loss of tumor cell-specific surface markers, (ii) microenvironment of cancer cell such as the presence of a dense extracellular matrix, poor vascular system, low pH, etc., (iii) presence of different physiological barriers such as plasma/nuclear/organelle membranes, blood-brain barrier (BBB), etc; (iv) properties of nanoparticles, including their safety profile, stability, drug encapsulation efficiency, etc.^{4,5} These barriers have all directed experiments towards the production of the ideal nanoparticle capable of sensing, adapting and reacting to the surrounding microenvironment.

Formulation of such an intelligent and adaptable nanodevice requires a high degree of programmability, a potential, which is hopefully presented by Watson-Crick base pairing principle defined for nucleic acid hybridization. In the first steps of imagining such a fantasy, Seeman used branched DNA with sticky ends to prepare and introduce the first

two-dimensional (2D) DNA crystal structure in 2003.⁶ One year later, Turberfield et al, attempted the one-stage self-assembly of the first DNA cage (DNA tetrahedra) as a typical example of DNA-fabricated nanocarrier (DNA box) for encapsulation of biomolecules.⁷ Later, functionalization strategies, such as the integration of aptamers, allowed construction of more advanced DNA boxes with the controllable opening-closing performance of box/lid to allow for demand-driven freight exposure/release (box opening) for simultaneous biosensing and the triggering of a therapeutic effect only at the desired site (cancer cells). In addition, due to the unique sequence of DNA molecule used in the construction of these finite-sized DNAs, the resulting structures are fully addressable and adaptable in terms of conjugation with a variety of functional units such as small molecules, macromolecules and inorganic nanoparticles that can be easily and accurately (with nanometer precision) placed anywhere on or in such structures (Table 1).

DNA objects can fall in two categories, as static and dynamic formats. While dynamic formats such as walkers, tweezers, rotor/motor, etc. are capable of cycling movements and stepping/walking on defined DNA tracks using energy derived from DNA strand displacement, the use of static formats is widely accepted and further advanced in the context of cancer theranostics and nano drug delivery vehicles for cancer monitoring and treatment. Accordingly, this review aims to provide an overview of the current design formats, benefits and applications of static DNA nanostructures (DNs) including DNA origami nanostructures (DONs), DNA arrays/bioreactors, DNA polyhedra (DNA cage), DNA nanoflower, hydrogel, dendrimer and DNA nanoswitch as emerging promising platforms for cancer treatment in the preclinical settings.

Static DNA Structures For Cancer Theranostics

DNA nanostructures have been extensively used for the study and manipulation of biological processes, which is critical to understand the molecular mechanism underlying pathological/disease-related condition in biomedicine. DN has also found numerous applications in the field of biosensing, detection, treatment, and drug/gene delivery of various cargoes, including nucleic acids, proteins, aptamers, siRNA, miRNAs, and nanoparticles. Accordingly, functionalized DNA structures with theranostic targeting moieties were used for the simultaneous mapping and targeting of various genetic, immunological, infectious, cancer and metabolic diseases. (see Table 1).⁸ In this

section, we will explain the use of static DNAs in biomedicine and oncology practice.

DNA Origami

As a key method for the production of DN, DNA origami objects can be adapted in both static and dynamic forms. Looking at the methods for production of DN, there are the scaffold-free tile assembly method (Figure 1A) and the scaffold DNA origami method (Figure 1B). The multi-strand tile method is a bottom-up approach used in the first preparation of two-directional DNA polyhedra. In short, in this process, each tile is made from several single stranded DNA (ssDNA) with sticky ends, where cohesion between complementary strands from different tiles can lead to the assembly of larger structures such as 2D arrays as well as polyhedra (Figure 1A). In the meantime, DNA origami follows a top-down scaffold-mediated approach in which a long ssDNA usually M13 genomic DNA is forced to fold into DONs using short ssDNA oligonucleotides called staples. What's more, staple act as docking sites for functionalization by nanoparticles and biomolecules. In this way, and with the help of computer programs DNA origami objects can be designed and assembled in almost any form.⁹⁹ These modifications together with biocompatibility and biostability¹⁰⁰⁻¹⁰² have made DNA origami nanostructures suitable platforms for targeted delivery, imaging, and controlled release of therapeutic cargoes.^{14,103-107}

DONs are functional as immune-activating programmable adjuvants.³³ They are also efficient as simultaneous dual-modality bioimaging and cancer targeting nanotools in vivo¹⁴ (Figure 2A). In addition, DONs can be used to enhance the efficacy of chemotherapy (Figure 2B),¹⁰⁸ reduce drug side effects, and bypass drug resistance.^{19,30} Notably, DONs with a different twist allow tuning drug release kinetics of the DNA intercalating Doxorubicin (Dox) for optimum delivery to tumor cells (Figure 2C).^{21,107} Recently, DNA origami ultrasensitive biosensors namely DNA origami nanoantennas have been successfully adapted for biosensing and superresolution microscopy of Zika-virus nucleic acids at the single molecule level.¹⁰⁹

More recently, DONs have been shown to be useful tools for investigating antigen-antibody interactions to determine the strongest binding to antibodies in the immune system affecting antibody development for

Table 1 Potential Of DNA Nanostructures In Biomedicine

Agents	Examples	DNA Nanostructure Formats	Application
Small molecules	Fluorescent	Icosahedral ⁹ Nanoflower ¹⁰	Functional in vivo imaging (organocellular pH sensing) Traceable targeted drug delivery
	Radioisotope	DNA origami ^{11,12}	Treatment and imaging of organ failure (antioxidant potential against acute kidney injury)
	Quantum dots	DNA hydrogel-Dox (cargo) ¹³	Imaging and drug delivery
	Radioisotope/ NIR Fluorescent	Tetrahedral-folic acid (tumor-targeting moiety) ¹⁴	In vivo imaging
	Electrochemiluminescence luminophore	Dendrimer-Dox DNA circuit ¹⁵	ECL biosensor (early diagnosis and prognosis of various diseases)
	Photosensitizer	Aptamer (targeting moiety) G-quadruplex (Dox loading) ^{16,17}	Selective drug delivery
	Mos ₂	Mos ₂ -DNA nanosheets ¹⁸	Combined photothermal and chemotherapy of cancer
	Doxorubicin	DNA origami, ¹⁹⁻²¹ DNA nano clew, ²² Open caged pyramidal DNA ²³ DNA hydrogel + gold nanorods ^{24,25} DNA nanoswitch (Light/pH responsive nanocapsule) ²⁶ DNA nanoswitch (Bioreducible nanocapsule-folic acid (targeting ligand)) ²⁷	Cancer chemotherapy Photothermal and chemotherapy of cancer Drug delivery for targeted cancer therapy Tumor-specific chemotherapy
	Epirubicin	Dendrimer ²⁸	Controlled release delivery of chemotherapeutic to cancer cells
	Cisplatin	Aptamer-based DNA nanoswitch ²⁹	Cancer theranostics (early-stage diagnosis and precise therapy of tumors)
	Daunorubicin	DNA origami ³⁰	Overcoming cancer chemotherapeutic drug resistance
	Actinomycin D	DNA nanopyramid/Au nanoclusters ³¹	Anti-bacterial (simultaneous detection and killing of <i>Escherichia coli</i> and <i>Staphylococcus aureus</i>)
	Coralyne	DNA hydrogel ³²	Controlled drug release
	Oligonucleotide	CpG	Origami, ³³ dendrimer, ³⁴ Tetrahedron, ³⁵ Nanoflower, ³⁶ Au-DNA, ³⁷ Dnanococoon(cage) ³⁸ Tube, ³⁹ Tetrapod-like structured DNA (tetrapodna), Tetrahedron, and Tetragon, ⁴⁰ DNA hydrogel-DOX(cargo) ⁴¹

(Continued)

Table 1 (Continued).

Agents	Examples	DNA Nanostructure Formats	Application
	Antisense peptide nucleic acid	Tetrahedral DNA ⁴²	Anti-bacterial (inhibiting methicillin-resistant <i>Staphylococcus aureus</i>)
		DNA tetrahedron ⁴³	Anti-bacterial (restoring antibiotic drug sensitivity in cefotaxime-resistant <i>Escherichia coli</i>)
	miRNA	DNA shuriken, ⁴⁴ DNA tube, ⁴⁵ Branched DNA, ⁴⁶ Molecular beacons ⁴⁷	Cancer targeting, cancer detection (biosensing)
	SIRNA	Nanoribbon, ⁴⁸ Tetrahedran, ⁴⁹ DNA nanosuitcase (DNA box), ⁵⁰ Nanomotor (RCA wrapped gold nanowires), ⁵¹ Dendrimer, ⁵² Hydrogel, ^{53,54} DNA cage(prism) ⁵⁵	Gene therapy of cancer/genetic disorders, cancer targeting
	CRISPER-Cas9	DNA Nanoclew ⁵⁶	Genome editing, gene therapy
	Aptamer	Pyramidal DNA, ⁵⁷ Isohedran, ⁵⁸ Tetrahedran, ⁵⁹ DNA Dendrimer ⁶⁰	Cancer therapy
		DNA hydrogel ⁶¹	Predictive cancer diagnostics (detection and analysis of rare circulating tumor cells)
		Hydrogel-Au NPs (output) ⁶²	Biosensor (non-enzymatic and visual detection of glucose)
		DNA walker-Au NPs (surface electrode) ⁶³	Signal transduction for protein biosensing
		DNAzyme-based DNA walker ⁶⁴	Cancer therapy (in situ cancer cell growth inhibition)
		DNA walker- Au NPs (surface electrode) ⁶⁵	Biosensing
		DNA walker-Au NPs (surface electrode) ⁶⁶	Multiplexing, SNP detection
		Light-responsive DNA nano switch ⁶⁷	Targeted cancer chemotherapy
	Short hairpin RNA (shRNA)/CpG RNA	Microflower-tumor-specific peptide neoantigens (cargo) ⁶⁸	Nanovaccines (for synergistic delivery to antigen-presenting cells (APCs) in lymph nodes for colorectal cancer immunotherapy)
	Hairpin DNA	DNA circuit-Au NPs (electrode surface) ⁶⁹ DNA walker-gold nanocages@graphene nanoribbons (surface electrode) ⁷⁰	Biomolecule analysis of human serum (nucleic acid, thrombin, and adenosine) (Electrochemical biosensor)

(Continued)

Table 1 (Continued).

Agents	Examples	DNA Nanostructure Formats	Application
	DNA probe	DNA origami-based shape IDs ^{71,72}	Genotyping (Hepatitis B Virus (HBV), single-molecule haplotyping of human genomic DNA)
		DNA hydrogel ^{73,74}	Phenotyping of infective pathogens in microfluidics (ie, Ebola, the Middle East respiratory syndrome (MERS), and others)
		DNA hydrogel ⁷⁵	High yield gene expression (siRNA production inside cells for gene regulation)
		DNAzyme motor ⁷⁶	Biosensing
		DNA tweezer ⁷⁷	Portable POC device capable of SNP detection (health status monitoring)
		Enzyme-powered linear DNA motor ⁷⁸ DNA tweezer ⁷⁹	Drug delivery (intracellular transport) Prognostic device (detection of telomerase activity and its product length distribution)
	DNAzyme	DNA hydrogel ⁸⁰	Controlled release of biocatalysts and activation of enzyme cascades
		DNA hydrogel+ Au nanoparticles (NPs)+fluorescent DNA ⁸¹	Biosensing (multiplex microRNA imaging in living cells)
		DNA tweezer ⁸²	Biosensor (specific multi analysis of miRNAs in biological samples)

(Continued)

Table 1 (Continued).

Agents	Examples	DNA Nanostructure Formats	Application
Proteins	Protein	DNA Cage ⁸³	Gene regulation (delivery of transcription factor)
		DNA hydrogel ⁶⁷	The local treatment of bone diseases (eg, osteoporosis)
		DNA nanoswitch ⁸⁴	Point-of-care (POC) diagnostics to measure antibody titer (eg, HIV+ patients immunized with AT20 therapeutic vaccine)
		DNA origami box ⁸⁵	Synthetic infecting particles (engineering nonpolyhedral, nonprotein synthetic viruses for infecting human cells)
	Peptide	DNA nanotube ⁸⁶	Cell therapy (for preferential differentiation of neural stem cells into neurons and not astrocytes)
		Nanorobot ⁸⁷	Targeted drug delivery
	Antibody (fragments)	CpG oligonucleotide (Nano-cocoon) ⁸⁸	Cancer immunotherapy (inducing a postsurgical inflammatory response in the wound site to prevent cancer relapse)
		DNA cage ⁸⁹	Enhanced catalytic activity and increased anti-protease stability
		Tubular DNA Origami ⁹⁰	Active enzyme delivery to target cells
		DNA tweezer-NAD(+) cofactor ⁹¹	DNA nanoreactor (regulated enzyme activation/inhibition for the design of feedback or feed-forward control loops)
Organic NPs	Graphen (transistor)	DNA tweezer ⁷⁷	SNP genotyping
	Carbon nanotube and polyaniline	DNA hydrogel ⁹²	Biocompatible and implantable energy storage devices for in vivo application

(Continued)

Table 1 (Continued).

Agents	Examples	DNA Nanostructure Formats	Application
Inorganic nanoparticles	Gold nanoparticles (Au NPs)	DNA polyhedron-caged (AuNP), ^{20,93} DNA circuit (Flower-like AuNPs) ⁹⁴ Au-tetrahedral nanostructure probe ⁹⁵	Cancer therapy, biosensing Detection of infectious disease (avian influenza A (H7N9) virus)
	Iron oxide NPs	DNA origami robots conjugated with iron oxide NPs + fluorescent antibody fragments (cargo) ⁹⁶	Diagnosis and therapy of cognitive disorders (schizophrenia, depression, and attention deficits)
	Silver nanocluster	DNA hydrogel ⁹⁷	Wound healing, antimicrobial and fluorescent potential
		DNA hydrogel ⁹⁸	Antioxidant therapy (hydroxyl radical sensing for probing cellular damage, mutagenesis, cancer, and degenerative diseases)

Abbreviations: NIR, Near-infrared fluorescence; SPECT, single-photon emission computed tomography.

cancer immunotherapy and the vaccine development. In this way, DNA origami was used to represent nanoscale patterns of antigens. This is the first report to provide detailed information on how the antigen distances ranging from 3 to 17 nm can affect the binding affinity of mono and bivalent antibodies, as they have been shown to bind strongly to two antigens when a preferable 16 nm antigenic distance is reached.¹¹⁰ Similarly, DNA origami nanoarrays can interrogate the clustering behavior of cell-surface receptors, because DN allows the representation of receptor ligands in an extremely customizable manner, with adjustable factors such as ligand number, spatial localization and, multivalency.¹¹¹ As proof of concept, they are used to study epidermal growth factor (EGF) and integrin signaling with nanoscale spatial resolution. That, using multivalent DNA biochips revealed cooperative work of EGF-integrin in modulating the behavior of melanoma cancer cell in a number and ratio-dependent manner.¹¹² Further examples of DON in cancer therapy and drug delivery can be found in.^{99,113}

DNA Cage: Polyhedra

DNA polyhedra has proven to be a promising class of drug-carriers frequently used for biomedical applications. The advantage of polyhedra compared over other structures such as DNA origami is their small size and cost efficiency. One of the most popular structures in this class is the tetrahedron introduced by Turberfield.¹¹⁴ This specific example can be constructed from only four strands with near-quantitative yields within 30 seconds and is compression-stable enough to be imaged by atomic force microscopy.

Polyhedra represents a porous structure and is suitable for chemical modifications. In addition, it can be reconfigured with a high load capacity by external triggers for on-command drug release. Numerous applications in multi-modality medical diagnostics and treatments (Figure 3) are proposed for these structures.¹¹⁵ DNA polyhedra can be filled with one or more nanoparticles (Figure 3A),^{20,93} proteins (eg, cytochrome C (Figure 3B),¹¹⁶ β -galactosidase,¹¹⁷ catabolite activator protein (CAP),⁸³ nucleic acids (eg, siRNA),⁵⁰ or other biomolecules¹¹⁸ to enable their passive or active on-target delivery. For example, encapsulation affords conditional activation of caged enzymes/transcription factors upon exposure to their specific protein/DNA substrates.⁸³ Alternatively, the device protects its cargo (eg, siRNA) from nuclease degradation and site-specific cleavage.⁵⁰

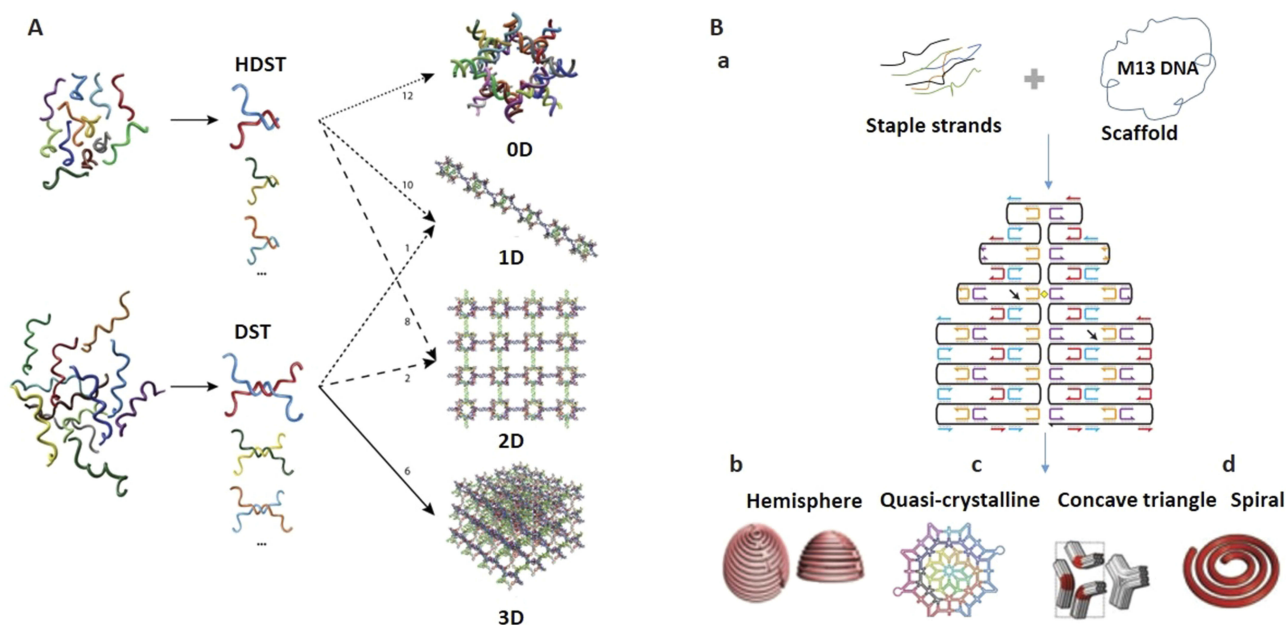


Figure 1 Concept of DNA tile and DNA origami assembly methods. (A) Tile method for mounting DNA grids. Reprinted with permission from Manuguerra I, Grossi G, Thomsen RP, et al. Construction of a polyhedral DNA 12-arm junction for self-assembly of wireframe DNA lattices. *ACS Nano*. 2017;11(9):9041–9047. doi:10.1021/acsnano.7b03538.¹⁶⁵ Copyright (2017) American Chemical Society. (B) a, Principle design of DNA origami. Adapted by permission from Springer Nature: *Nature*, Rothemund PWK. Folding DNA to create nanoscale shapes and patterns. 2006;440:297. doi:10.1038/nature04586.¹⁶⁶ Copyright (2006). b,d, DNA origami objects. b, From Han D, Pal S, Nangreave J, Deng Z, Liu Y, Yan H. DNA origami with complex curvatures in three-dimensional space. *Science*. 2011;332(6027):342–346. doi:10.1126/science.1202998.¹⁶⁷ Reprinted with permission from AAAS. c, DNA origami pattern. Reprinted by permission from Nature Springer: *Nature Nanotechnology*, Zhang F, Jiang S, Wu S, et al. Complex wireframe DNA origami nanostructures with multi-arm junction vertices. 2015;10:779. doi:10.1038/nnano.2015.162.¹⁶⁸ Copyright (2015). d, From Dietz H, Douglas SM, Shih WM. Folding DNA into twisted and curved nanoscale shapes. *Science*. 2009;325(5941):725–730. doi:10.1126/science.1174251.¹⁶⁹ Reprinted with permission from AAAS.

The surface modification of DNA polyhedra with disease-specific markers (eg, folate or oxidized low-density lipoprotein receptor-1” (LOX-1)) not only leads to active targeting but also to more than 30–40-fold internalization efficiency.^{119,120} DNs can encapsulate toxic and hydrophobic agents, such as Dox for efficient chemotherapy of cancer cells while reducing damage to normal cells.¹²¹ The decoration of the Dox-encapsulated DNA cage with tumor-penetrating peptide increases the cellular uptake of DNA tetrahedron for the targeted administration of toxic chemotherapeutic in vitro (Figure 3C).¹²² Dox-loaded DNA nanoblocks are found in the endocytic vesicles after entry into the target cells, where the acidic milieu triggers the release of drugs from these DNA nanostructures and induces selective augmented cytotoxic actions of Dox (>40 times) on folate receptor expressing cancer cells, while promoting the degradation of nanostructures that prevents their accumulation in the cells.¹²⁰

DNA tetrahedra are examples of DNA nanocages for large molecules or as building blocks for more complex nano-devices enabling rapid and reliable assembly with quantitative yields, in vitro and in vivo structural stability, and plasticity.^{7,114,123} As proof of concept, DNA tetrahedra that can easily penetrate mammalian cells have been shown to be stable in cytoplasm.⁵⁹ A recent report also showed that multivalent DNA tetrahedrons

functionalized with CpG motifs are permeable to cells and stimulate an immune response with applications in vaccine development and immunotherapy.³⁵ siRNA administration using DNA tetrahedra was reported as a vehicle for targeted gene therapy. The adaptability of the design permits precise control of nanoparticle size, the spatial orientation, and density of targeting ligands on the nanoparticle surface or the inside, which could lead to enhanced delivery of the siRNAs into cells and efficient gene silencing.¹²⁴ Moreover, DNA polyhedra were used as carriers of chemotherapeutics to overcome multi-drug resistance in cancer cells.¹¹⁸ Similarly, aptamer-decorated DNA icosahedral nanoparticles were used to deliver Dox molecules.⁵⁸ The construction of cage-like DNA structures called nanosuitcases was reported for siRNA loading and on-demand release upon recognition of an oligonucleotide marker (eg, mRNA or a microRNA (miRNA)(Figure 3D).⁵⁰ In addition, the pH-triggered controlled release of Dox-intercalated nanoring functionalized with mucin aptamer (MUC1) was shown to have therapeutic potential for breast cancer.¹²⁵ In another example, hybrid systems such as DNA polyhedron-caged gold nanoparticles (AuNP) were produced for the controlled release of Dox when triggered by pH and DNA enzyme (T7 exonuclease).⁹³ Moreover, DNA tetrahedron structures are used as molecular probes with high-sensitivity for DNA target

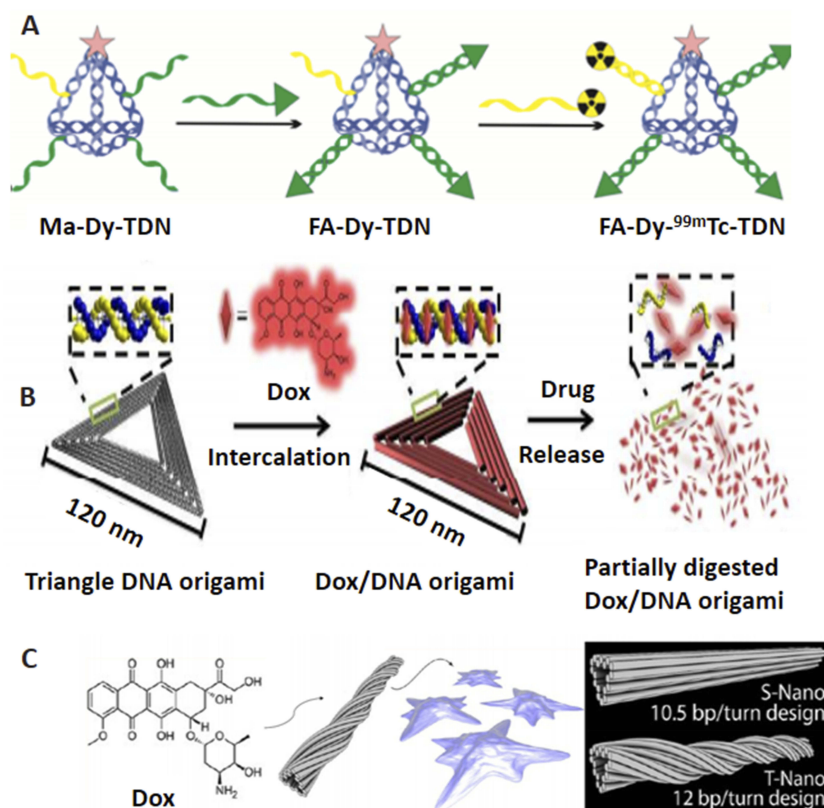


Figure 2 DON for targeted drug delivery. **(A)** Multiple-armed tetrahedral DNA nanostructure (TDNs) for dual-modality in vivo imaging and targeted cancer therapy. Adapted with permission from Jiang D, Sun Y, Li J, et al. Multiple-armed tetrahedral DNA nanostructures for tumor-targeting, dual-modality in vivo imaging. *ACS Appl Mater Interfaces*. 2016;8(7):4378–4384. doi:10.1021/acsami.5b10792.¹⁴ Copyright (2016) American Chemical Society. **(B)** Triangle-shaped DNA origami affords optimum drug internalization for cancer chemotherapy. Adapted with permission from Zhang Q, Jiang Q, Li N, et al. DNA origami as an in vivo drug delivery vehicle for cancer therapy. *ACS Nano*. 2014;8(7):6633–6643. doi:10.1021/nn502058j.¹⁰⁸ Copyright (2014) American Chemical Society. **(C)** DON with a different degree of twist and relaxation achieves tunable drug release kinetics. Adapted with approval from Zhao YX, Shaw A, Zeng X, Benson E, Nystrom AM, Hogberg B. DNA origami delivery system for cancer therapy with tunable release properties. *ACS Nano*. 2012;6(10):8684–8691. doi:10.1021/nn3022662.²¹ Copyright ACS, <https://pubs.acs.org/doi/abs/10.1021%2Fnn3022662>. Further permissions related to the material excerpted should be directed to ACS.

detection and single nucleotide polymorphism (SNP) typing of biological fluids, as well as protein biosensing (Figure 3E) in vitro.¹²⁶

DNA Arrays And Bioreactors

Another advantage of DNA origami which deserves to be discussed in a separate section, is its potential for the fabrication of DNA arrays/ bioreactors, which can be achieved by site-specific functionalization of DNA origami structures with proteins, nanoparticles, antibodies, enzymes, etc. (Figure 4) with well-defined regularity and periodicity is of great interest to the scientific community.¹²⁷ Applications include sensing, catalysis, and highly ordered device fabrication.¹²⁸ The addressability of DNA origami structures enables site-specific localization of several functional groups on their surfaces. Functionality could be achieved by i) covalently bound chemical groups (eg, amine, thiol, and carboxyl groups) or molecules (eg, biotin), or ii) DNA overhangs (eg, aptamers).¹²⁹ In one of these systems, the chemical conjugation of amino-

modified DNA oligonucleotides was demonstrated with chlorohexane or benzylguanine groups as orthogonal tags for the genetic modification of proteins of interest fused with Halo-Tag or Snap-tag, respectively. Authors reported orthogonal modified DNA origami by decorating DNA origami with multiple proteins including mKate-Snap, CCP-Halo, and mSTV, which resemble the characteristic of human face including mouth, eyes, and nose, respectively (Figure 4A).¹³⁰ Another study used DNA-binding potential of zinc finger proteins for site-specific protein positioning on DNA origami.¹³¹ Previous formats of DNA nanoarrays were also attempted in 2005 by DNA hybridization-mediated adhesion of Au nanoparticles of different sizes to prescribed locations on DNA tiles, producing alternating rows of the two components (Figure 4B).¹³² DNA nanotubes conjugated with two enzymes, horseradish peroxidase, and glucose oxidase, demonstrated enzymatic cascade, as an example of the nanoscale DNA-based bioreactor.¹²⁷ Moreover, punched DNA origami assembly was exploited for the design of robust and precisely

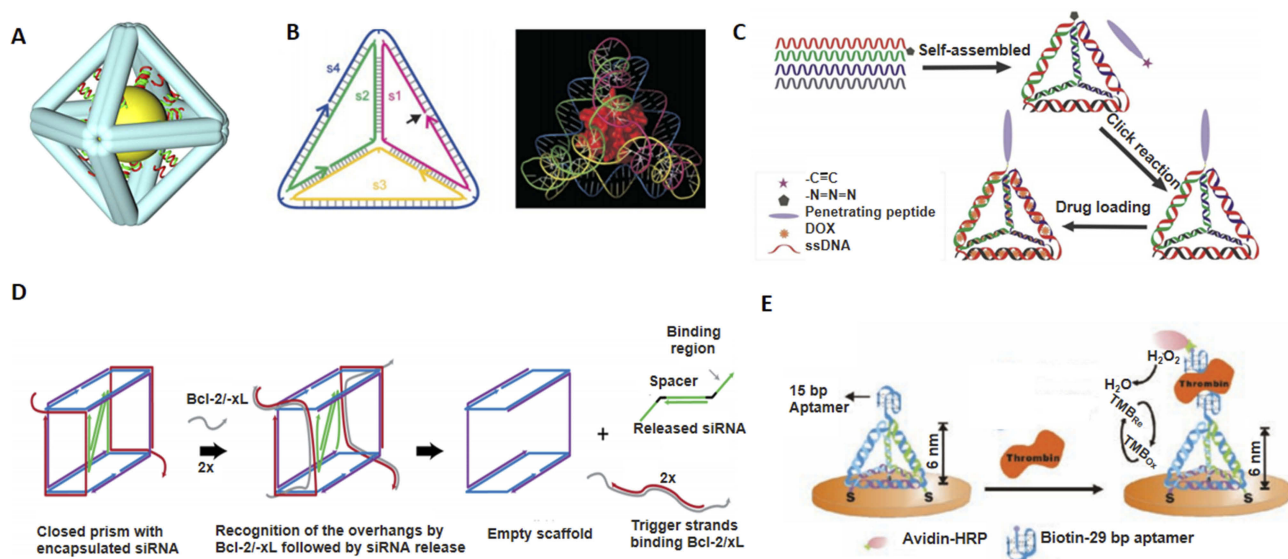


Figure 3 DNA nanocage. (A) Inorganic nanoparticle inside the DNA cage as a hybrid drug delivery system. Reprinted with permission from Zhang C, Li X, Tian C, et al. DNA nanocages swallow gold nanoparticles (AuNPs) to form AuNP@DNA cage core-shell structures. *ACS Nano*. 2014;8(2):1130–1135. doi:10.1021/nn406039p. Copyright (2014) American Chemical Society. (B) Single protein encapsulated in a rigid DNA tetrahedron nanocage. Reprinted with permission from Erben CM, Goodman RP, Turberfield AJ. Single-molecule protein encapsulation in a rigid DNA cage. *Angew Chem Int Ed Engl*. 2006;45(44):7414–7417. doi:10.1002/anie.200603392. John Wiley and Sons. (C) Doxorubicin-loaded tumor-penetrating peptide-modified DNA tetrahedron. Reprinted with permission from Xia Z, Wang P, Liu X, et al. Tumor-penetrating peptide-modified DNA tetrahedron for targeting drug delivery. *Biochemistry*. 2016;55(9):1326–1331. doi:10.1021/acs.biochem.5b01181. Copyright (2016) American Chemical Society. (D) DNA nanosuitcases for encapsulation and conditional release of siRNA. Reprinted with permission from Bujold KE, Hsu JCC, Sleiman HF. Optimized DNA. “Nanosuitcases” for encapsulation and conditional release of siRNA. *J Am Chem Soc*. 2016;138(42):14030–14038. doi:10.1021/jacs.6b08369. Copyright (2016) American Chemical Society. (E) DNA tetrahedron structured probe (TSP) for protein biosensing. Adapted with permission from Pei H, Lu N, Wen Y, et al. A DNA nanostructure-based biomolecular probe carrier platform for electrochemical biosensing. *Adv Mater*. 2010;22(42):4754–4758. doi:10.1002/adma.201002767. John Wiley and Sons.

programmed 2D streptavidin nanoarrays by the periodical embedding of biotinylated nanowells in DNA origami structures (Figure 4C).¹³³

DNA Nanoflowers

Long, single-stranded DNA and pyrophosphate released from (in vitro) polymerase reactions can form dense, compact organic-inorganic composite nanomaterials, known as nanoflowers (NFs).¹³⁴ In contrast to the key role of DNA base pairing in DNA nanostructures formation, NF assembly is independent of DNA hybridization. In addition, unlike other DNs, the shape of nanoflowers cannot be precisely programmed (Figure 5).¹³⁵

NF can be assembled using DNA building blocks by a PCR reaction such as rolling circle amplification (RCA). This reaction requires only a primer and template DNA to control over size, construct and functionalization of nanoflowers. This means that the rational design of any RCA-suitable templates allows the elongated DNA building blocks to carry a large number of concatemer functional or structural moieties, which further condense into NFs.¹³⁶ Due to the dense DNA packaging, NFs resist to nuclease degradation, dissociation or denaturation. Recently, the NF blooming (formation) principle has been used to design a simple and

sensitive electrochemical biosensing system. In this preparation, the NF blooming in the nanochannels has been tailored to occur upon binding of target miRNA, followed by binding of a circle template DNA and a specific primer to the captured miRNA, triggering RCA and NF blooming in the nanochannels. NF formation increased steric hindrance in the channels, which reduced the anodic current of potassium ferricyanide ($K_3[Fe(CN)_6]$), thus producing enhanced electrochemical detection signals (Figure 5A).¹³⁵

In addition to excellent biostability, NFs exhibit excellent photostability. In this sense, construction of aptamer conjugated multicolor fluorescence resonance energy transfer (FRET)-NFs is reported with applications in multiplexed cellular bioimaging and drug screening, in which NF were integrated with three different fluorophores (FAM, Cy3, and ROX), therapeutic drug payloads (Dox), and two aptamers (sgc8 and MUC 1). In addition, single wavelength excitation leads to multi-fluorescence emissions of NF, allowing monitoring of drug transport for targeted drug delivery (Figure 5B).¹⁰ Moreover, immuno-nanoflowers have been developed for nuclease resistance and efficient CpG delivery.³⁶ CpG NFs were found to be potent immunostimulators capable of triggering the proliferation of macrophage-like cells that stimulated the secretion of immunostimulatory

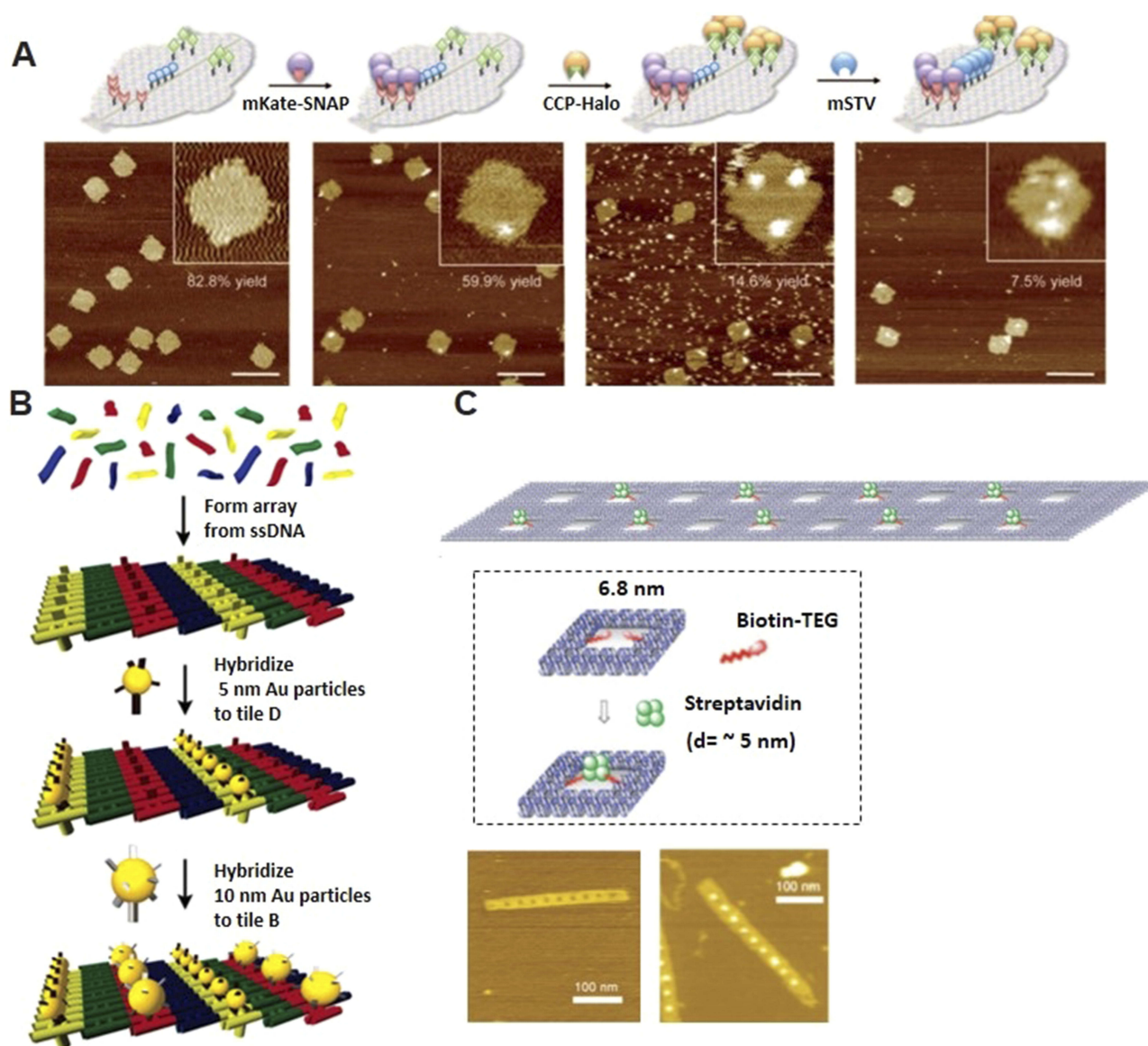


Figure 4 DNA origami arrays and bioreactors. **(A)** multi-protein decoration of DNA origami structures (arrays) resembling a human face. Adapted with permission from Sacca B, Meyer R, Erkelenz M, et al. Orthogonal protein decoration of DNA origami. *Angew Chem Int Ed Engl.* 2010;49(49):9378–9383. doi:10.1002/anie.201005931. John Wiley and Sons.¹³⁰ **(B)** Assembly steps for the 2D nanocomponent arrays. Adapted with permission from Pinto YY, Le JD, Seeman NC, Musier-Forsyth K, Taton TA, Kiehl RA. Sequence-encoded self-assembly of multiple-nanocomponent arrays by 2D DNA scaffolding. *Nano Lett.* 2005;5(12):2399–2402. doi:10.1021/nl0515495.¹³² Copyright (2005) American Chemical Society. **(C)** 2D streptavidin nanoarrays on rectangular DNA origami surface. Adapted with permission from Kuzuya A, Kimura M, Numajiri K, et al. Precisely programmed and robust 2D streptavidin nanoarrays by using periodical nanometer-scale wells embedded in DNA origami assembly. *ChemBiochem.* 2009;10(11):1811–1815. doi:10.1002/cbic.200900229. John Wiley and Sons.¹³³

cytokines that induced apoptosis and necrosis of cancer cells. Moreover, pH-responsive multifunctional DNA NFs containing cancer-targeting aptamer, fluorophores, and Dox molecules were used to prevent drug efflux and improve drug retention in MDR cells, thus bypassing MDR and reducing side effects (Figure 5C).^{136,137} In addition, using microflower nanosystem, synergistic nanovaccines were constructed for effective immunotherapy of cancer. The

NFs were formulated to comprise intertwining DNA-RNA nanocapsules (iDR-NCs) conjugated with DNA CpG, STAT3 short hairpin RNA (shRNA) adjuvants, as well as tumor-specific peptide neoantigens. shRNA in iDR-NCs synergistically activated antigen-presenting cells (APCs), primed CD8⁺ T cells, induced T cell memory, and markedly suppressed the progression of neoantigen-specific colorectal tumors (Figure 5B).⁶⁸

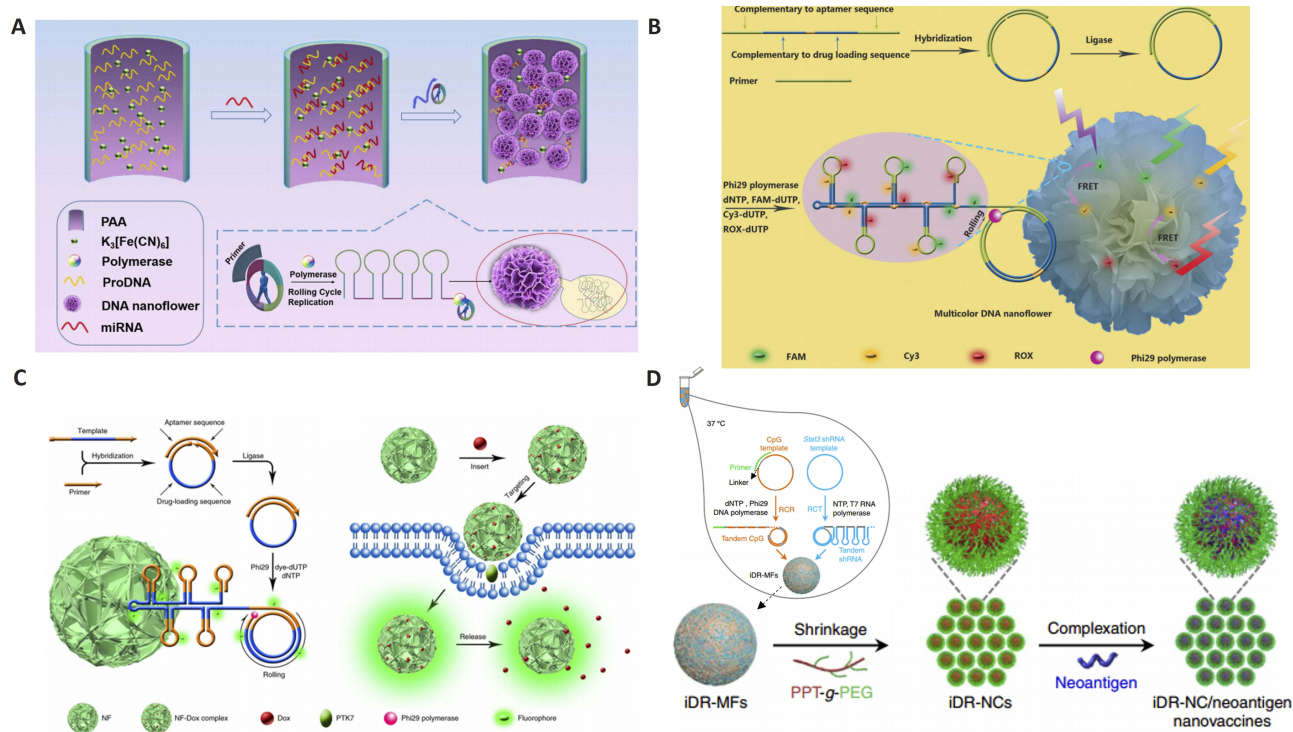


Figure 5 DNA nanoflower preparation and its biomedical applications. **(A)** miRNA initiated the growth and blooming of DNA nanoflower in nanochannel. Adapted with permission from Shi L, Mu C, Gao T, et al. DNA nanoflower blooms in nanochannels: a new strategy for miRNA detection. *Chem Commun.* 2018;54(81):11391–11394. doi:10.1039/c8cc05690k. Permission conveyed through Copyright Clearance Center, Inc.¹³⁵ **(B)** DNA nanoflowers for multiplexed cellular imaging and traceable targeted drug delivery. Adapted from Hu R, Zhang X, Zhao Z, et al. DNA nanoflowers for multiplexed cellular imaging and traceable targeted drug delivery. *Angew Chem Int Ed Engl.* 2014;53(23):5821–5826. doi:10.1002/anie.201400323. John Wiley and Sons.¹⁰ **(C)** RCR-based assembly of NFs loaded with chemotherapeutics for targeted drug delivery. Reprinted by permission from Springer Nature: Lv Y, Hu R, Zhu G, et al. Preparation and biomedical applications of programmable and multifunctional DNA nanoflowers. *Nat Protoc.* 2015;10(10):1508–1524. doi:10.1038/nprot.2015.078.¹³⁶ Copyright (2015). **(D)** Synergistic immunotherapy of cancer by DNA-RNA microflowers (iDR-MFs) loaded with tumor neoantigens. Adapted with permission from Zhu G, Mei L, Vishwasrao HD, et al. Intertwining DNA-RNA nanocapsules loaded with tumor neoantigens as synergistic nano-vaccines for cancer immunotherapy. *Nat Commun.* 2017;8(1):1482. doi:10.1038/s41467-017-01386.⁶⁸

In addition, water remediation –using magnetite nanoparticles that absorb and remove heavy metals– can be achieved by hybrid nano-flowers consisting of single-crystalline petals coated with magnetite nanoparticles. Additionally, hybrid nanoflowers (hNFs) consisting of organic (enzymes)-inorganic (metal ions) particles is reported to enhance catalytic activities and stability of proteins under various experimental conditions.^{138,139}

DNA Dendrimers

As relatively complicated DNs, DNA dendrimer with a branched configuration can be developed by the facile modular assembly. DNA dendrimers are layer-by-layer self-assembled functional branched DNA units (eg, Y-shaped building blocks) made through predesigned base-pairing hybridization. These somewhat bulkier DNs form promising scaffolds for versatile biomedical applications (Figure 6). For example, they can accommodate more docking sites for loading drugs or other functional materials.

Moreover, by implementing functional DNA units, these structures can be constructed into targeted and stimuli-responsive drug delivery systems. Also, sterically crowded conformations, homogeneous controlled sizes, and structural stability are additional advantages of dendrimeric nanostructures. Furthermore, they can be used as biosensing devices. For instance, DNA dendrimers coupled with DNA nanomachines (DNA circuits) has realized an ultrasensitive biosensor with a detection limit of 0.0661 pg/mL and 0.062 nM for laminin¹⁵ and KRAS gene fragment (Figure 6A),¹⁴⁰ respectively. Tailoring the capture and reporter DNA/protein units can extend the prognostic value of such dendrimer-based self-amplification system for detection of a different disease-related species with high sensitivity in biological samples. Moreover, achieving a therapeutic effect, catalytically self-assembled siRNA-based gene silencing via functionalized DNA dendritic complexes was constructed. Incorporated sgc8c aptamers to DNA dendrimers enabled targeting of overexpressed protein tyrosine

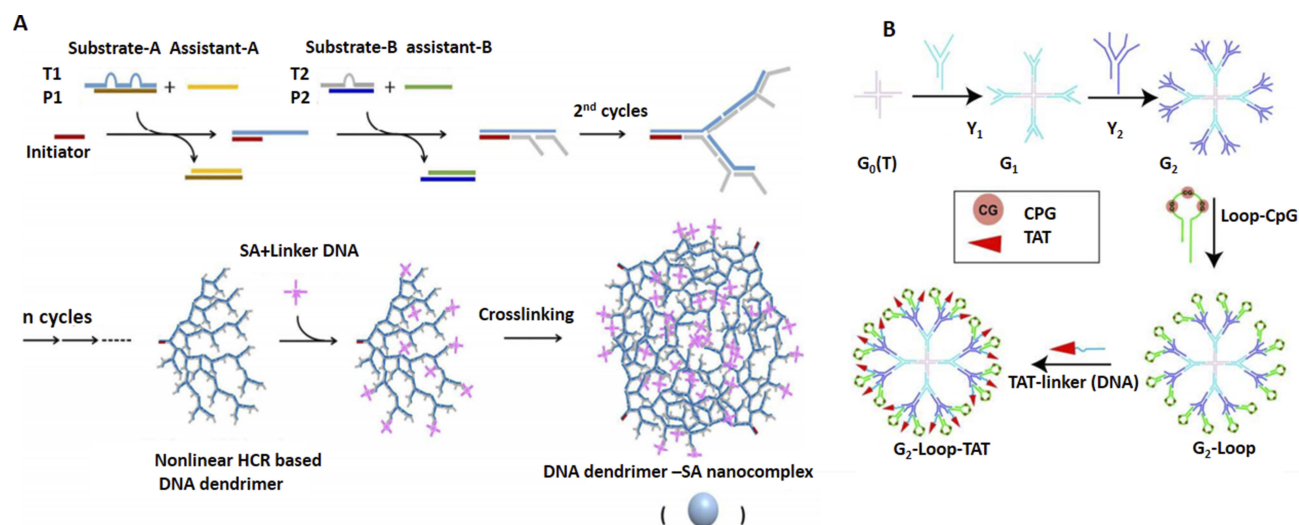


Figure 6 Design and application of DNA dendrimer in biomedicine. **(A)** DNA dendrimer-streptavidin for efficient signal amplification for biosensing. Adapted with permission from Zhao Y, Hu S, Wang H, et al. DNA dendrimer-streptavidin nanocomplex: an efficient signal amplifier for construction of biosensing platforms. *Anal Chem.* 2017;89(12):6907–6914. doi:10.1021/acs.analchem.7b01551.¹⁴⁰ Copyright (2017) American Chemical Society. **(B)** Multifunctional DNA dendrimers. Adapted with permission from Qu Y, Yang J, Zhan P, et al. Self-assembled DNA dendrimer nanoparticle for efficient delivery of immunostimulatory CpG motifs. *ACS Appl Mater Interfaces.* 2017;9(24):20324–20329. doi:10.1021/acsami.7b05890.³⁴ Copyright (2017) American Chemical Society.

kinase-7 (PTK7) receptor on tumor cells and led to higher gene silencing efficiency with lower cytotoxicity when compared to the commercial cationic lipid transfection agents.¹⁴¹ Another study demonstrated that the formulation of dendrimeric siRNA improves condensation, stability, and gene silencing efficiencies.⁵² Moreover, branched DNA constructs –containing anti-miRNA– have been used for oncomiRNA targeting of cancer. In another report, a multifunctional dendrimeric-based system composing of tumor-targeting ligands –such as MUC1, AS1411, and ATP aptamers– was used for high loading and controlled delivery of an anthracycline drug called “epirubicin” to cancer cells. MUC1 and AS1411 aptamer were coated on the surface of dendrimer to assist crossing the cellular and nuclear membranes, respectively. Meanwhile, ATP aptamer incorporated in the building blocks directed dendrimer to the ATP-enriched lysosomes where more dendrimer disassembly can occur.²⁸ Furthermore, ligase-independent efficient self-assembly of DNA dendrimer is reported by annealing DNA units with elongated adhesive ends. These structures were used for delivery of immunostimulatory CpG DNA encoding tumor necrosis factor- α to immune cells.¹⁴² Programmable DNA dendrimers coated with CpG-containing hairpin-loops were found to trigger stronger immune responses than those conjugated with linear CpG. Further surface functionalization of DNA dendrimer with TAT –a classic cell-penetrating peptide– enhanced cell internalization and cytokines production (Figure 6B).³⁴

Possessing a similar mesh-like structure formed out of branched building blocks, DNA hydrogels with even broader biomedical as well as bioelectronic applications are developed, which we discuss in the next chapter.

DNA Hydrogel

A further advantage of DNA as a natural polymer is its capability to act as an irreplaceable building block for the construction of swollen networks of cross-linked DNA in an aqueous solution called DNA hydrogels.¹⁴³ DNA can be the only component of a hydrogel, the backbone or cross-linker, which connects the main building blocks (hybrid hydrogels) through physical entanglement or chemical reactions (Figure 7).^{144,145}

Hybrid hydrogels can lead to many preferred properties that would otherwise not be achievable by other nanostructures. These properties include biocompatibility, mass transport of ions, nutrients, proteins, drug, etc., biodegradability, mimicking dynamic nature of surrounding microenvironment (eg, extracellular matrix (ECM)), mechanical properties (porosity, stiffness, adhesion, swelling and phase transition (sol-gel) and stimuli-responsiveness.¹⁴⁵ These smart polymers afford controlled disruption/deformation of the DNA network at the desired microenvironment to achieve on-target cargo release for a therapeutic effect (Figure 7A).¹⁴⁶ From this point of view, tissue engineering and cell therapy for regenerative medicine can benefit from the soft nature of DNA hydrogels

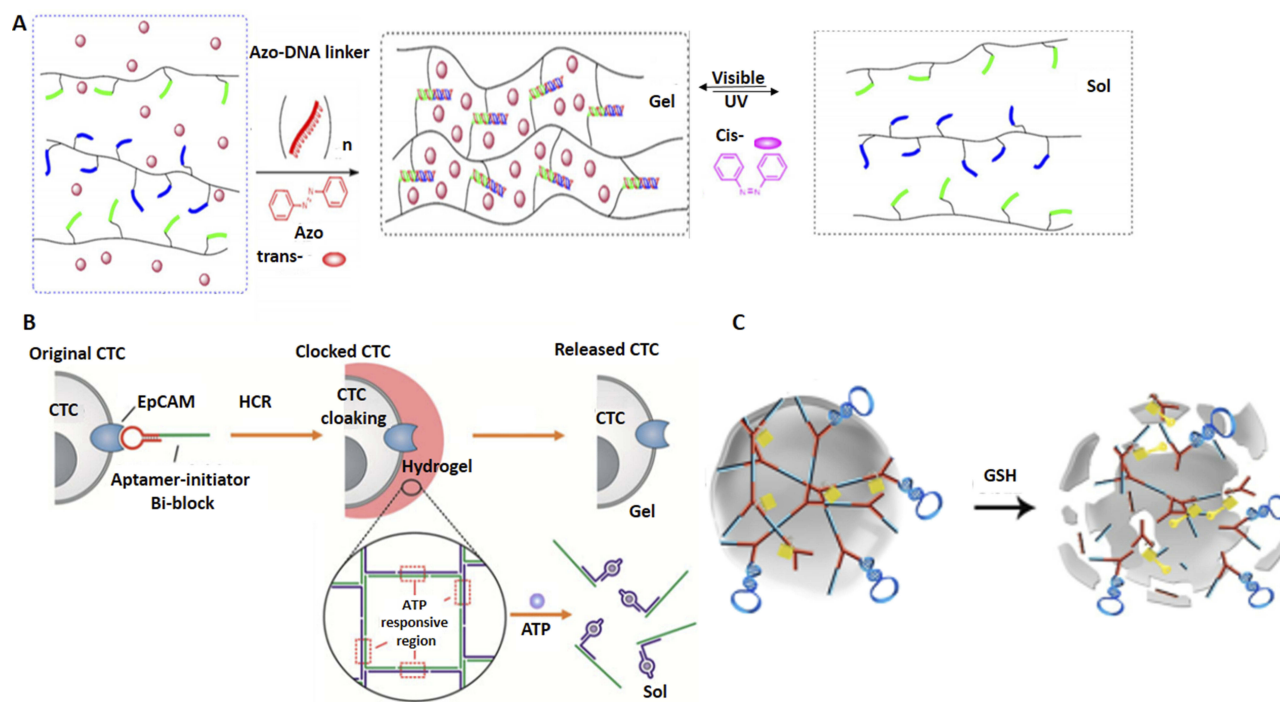


Figure 7 Design and application of DNA hydrogels in biomedicine. **(A)** Schematic representation of the photo-responsive properties of DNA hydrogels for on-demand cargo release of the payloads. Adapted with permission from Kang H, Liu H, Zhang X, et al. Photoresponsive DNA-cross-linked hydrogels for controllable release and cancer therapy. *Langmuir*. 2011;27(1):399–408. doi:10.1021/la1037553.¹⁴⁶ Copyright (2011) American Chemical Society. **(B)** Apatmer (EpCAM) binding-mediated catalysis of HCR control gel-sol state of ATP-responsive DNA hydrogel, resulting in cloaking (gel state) and releasing (sol state) of circulating tumor cells. Adapted with permission from Song P, Ye D, Zuo X, et al. DNA hydrogel with aptamer-toeholdbased recognition, cloaking, and decloaking of circulating tumor cells for live cell analysis. *Nano Lett*. 2017;17(9):5193–5198. doi:10.1021/acs.nanolett.7b01006.⁶¹ Copyright (2017) American Chemical Society. **(C)** Self-assembly of aptamer-based nanohydrogel using Y-shaped building blocks for targeted cancer gene therapy. Adapted with permission from Li J, Zheng C, Cansiz S, et al. Self-assembly of DNA nanohydrogels with controllable size and stimuli-responsive property for targeted gene regulation therapy. *J Am Chem Soc*. 2015;137(4):1412–1415. doi:10.1021/ja512293f.⁵⁴ Copyright ACS, <https://pubs.acs.org/doi/abs/10.1021%2Fnn3022662>. Further permissions related to the material excerpted should be directed to ACS.

allowing potential imitation of the physiological environment and living matrices, while porous structure mainly benefits accommodation of therapeutic payloads.¹⁴⁷

Different types of DNA building blocks can be used to produce these hydrogels, including single-stranded DNA branched double-stranded DNA, or Y-shaped DNA or X-shaped DNA through intermolecular i-motif structures, enzyme ligation, DNA hybridization, or enzyme polymerization. Although the use of DNA hydrogels in the clinic is not yet practiced, nonetheless, they already find numerous application in drug delivery,¹⁴⁶ sensing,¹⁴⁸ tissue engineering,^{149,150} 3D cell culture,¹⁴⁹ providing a template for nanoparticle synthesis,^{92,151} and cell transplant therapy,¹⁵² among others (see for review).¹⁵³

Hydrogels are popular nanostructures for tissue engineering because they can be tailor-made as biomimetics of ECM. DNA hydrogels are applicable for wound dressing with fluorescent and anti-bacterial potential. Due to their multifunctionality, they can simultaneously perform bio-sensing, and bioimaging tasks in living cells, as well.^{97,98,154}

In terms of their biomedical applications, DNA gels nanostructures can be used for in situ encapsulation and preservation by protecting their cargoes from degradation, as reported for camptothecin (CPT), a DNA-binding drug, as well as porcine insulin.¹⁵⁵ Interestingly, the formation of target-triggered DNA hydrogel has been harnessed as an effective tool for live single-cell isolation by liquid biopsy.⁶¹ For example, a DNA staple strand with aptamer-toehold biblocks is shown to specifically recognize EpCAM, a cell surface biomarker expressed by circulating tumor cells (CTCs). Binding of the aptamer to EpCAM triggers subsequent aptamer-triggered hybridization chain reaction (HCR) via toehold-initiated branch migration for simultaneous capturing and cloaking of live single/clustered CTCs in porous DNA hydrogel with minimal cell damage. Moreover, controlled decloaking of CTCs was affordable using defined chemical stimuli to release intact living CTCs for subsequent culture and live cell analysis (Figure 7B).⁶¹

Interestingly, Dgel made of branched cross-linked DNA has found application for integration of plasmid-coding genes to envision a cell-free gene/protein synthesis system

for production of different types of proteins/genes with extremely high production yields.¹⁵⁶ In comparison to a linear plasmid, a high yield of over 300 times¹⁵⁷ and ~50 times is reported for protein and mRNA expression, respectively.⁷⁵ In view of the high RNA productivity of the Dgel, a recent study has reported a DNA hydrogel that can be used as RNA-producing machinery for gene regulation inside live cells. For this purpose, a plasmid carrying the gene transcribing siRNA against a target gene (green fluorescent protein (GFP)) was introduced into the gel scaffold. Such DNA hydrogel was capable to effectively shut down fluorescent protein expression in live cells.⁷⁵

Another study, reported an albumin-DNA hybrid hydrogel for the controlled release of Rho-inhibiting C3 toxin for targeted inhibition of osteoclast formation and activity and possible application in local treatment of bone disease such as osteoporosis. This system is desirable with regard to its self-healing and injectable nature, DNA hybridization-mediated rapid gelation under physiological conditions, and the possibility to be loaded with specific cargoes (active proteins) and promote their spatiotemporal controlled release.⁶⁷

With the aim of cancer-targeting, multifunctional and programmable aptamer-based DNA nanoassembly (AptNA) is reported using three Y-shaped building units; each incorporating different functional elements including targeting aptamer (sgc8), a therapeutic antisense oligonucleotide (MDR1 siRNA), and intercalated anticancer drug (Dox). This DNA nanohydrogel is a promising anti-cancer material due to its modularity, multifunctionality, programmability, excellent biocompatibility, and biostability. It also provides active targeting and controllable transportation of drug to the on-target cells. Moreover, using such a system drug resistance was reduced due to inhibition of P-gp expression –the anti-cancer drug efflux pump receptor–by the MDR1 siRNA.¹⁵⁸ Similarly, catalytic hybridization was used for assembly of aptamer-based nanohydrogels (Y-gel-Apt), by employing two Y-shaped monomers (YMA, YMB) and a DNA linker (LK) bearing disulfide linkages and sticky ends. YMA contained a DNAzyme sequence for targeting MMP-9 and antisense oligonucleotide sequence in LK that enabled targeting of c-raf-1 – a tumor proliferation marker. Sticky ends were used for hybridization-mediated self-assembly whereas disulfide linkages led to the rapid fragmentation of nanohydrogels under cancer reductive environment, enabling a stimuli-responsive effective gene therapy (Figure 7C).⁵⁴ More examples of aptamer-incorporated hydrogels for theranostics applications are overviewed elsewhere.

DNA Nanoswitch

Polymeric nanostructures such as hydrogels, liposomal/micellar nanocarriers or DNA-based hydrogels/nanostructures equipped with DNA switches such as azobenzene (Figure 8A)¹⁵⁹/disulfide bonds are examples of static nanoswitches utilized for selective delivery of chemotherapeutics to cancer cells in response to environmental triggers such as hypoxia/pH/redox actuators.¹⁴⁷ For example, disulfide-cross-linked nanohydrogels intercalated with Dox undergo fragmentation upon exposure to reducing agents dithiothreitol (DTT) or glutathione (GSH). These redox/pH dual stimuli-responsive nanohydrogels make promising candidates for delivering anti-cancer drugs because of their biocompatibility, adequate drug loading capacity, biodegradability, controlled yet fast drug release potential (Figure 8B).¹⁶⁰ Moreover, bioreducible nanohydrogels-like nanocapsules (NCs) were reported to actively target cancer cells coated with folic acid.²⁷

Light-switchable aptamer-based nanoconstructs were used for targeting and release of chemotherapeutics within the cancerous milieu. These complexes consisted of lipidated versions of a hepatocyte growth factor receptor (cMet) binding aptamer and a separate lipidated GC-rich DNA hairpin motif bearing intercalated Dox. Multiple 2',6'-dimethylazobenzene moieties were incorporated into the Dox-binding motif to trigger the release of the chemotherapeutics by photoisomerization. The combined features of these nanocarriers increased serum nuclease resistance, favored their import into cells, allowed the selective photo-induced release of the chemotherapeutic into the targeted cells, and enhanced cell mortality.⁷⁴ In addition, pH-responsive and light-responsive DNA microcapsules that encapsulated different loads including Dox-modified dextran, tetramethylrhodamine-modified dextran, CdSe/ZnS quantum dots, MP-11 and microperoxidase-11 has been described for controlled cargo release and cancer therapy *in vitro*.²⁶ The pH-responsive microcapsules composed of a cytosine-rich layer cross-linked by nucleic acid bridges and the light-responsive microcapsules were made of photocleavable *o*-nitrobenzyl-phosphate-modified DNA shells.²⁶

In another study, reduction-responsive hydrogel nanoparticles are utilized for siRNA delivery and targeted gene silencing.⁵³ Furthermore, near-infrared light-responsive hybrid drug delivery platform composed of Au–Ag

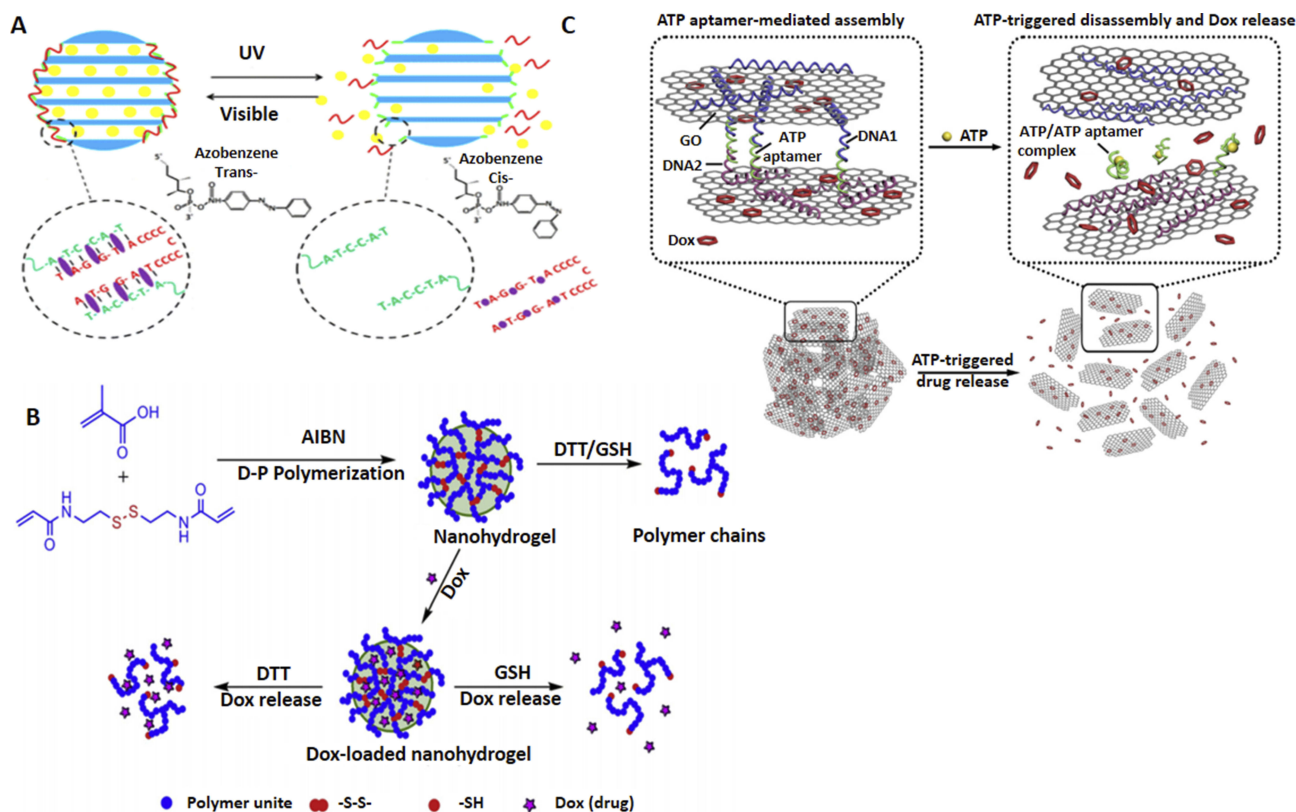


Figure 8 DNA nanoswitch systems as cancer theranostics. (A) Azobenzene-integrated photo controlled drug release from DNA/mesoporous silica. Adapted with permission from Yuan Q, Zhang Y, Chen T, et al. Photon-manipulated drug release from a mesoporous nanocontainer controlled by azobenzene-modified nucleic acid. *ACS Nano*. 2012;6(7):6337–6344. doi:10.1021/nl3018365. Copyright (2012) American Chemical Society. (B) Redox stimuli-responsive drug release of the PMAA nanohydrogels. Adapted with permission from Pan Y, Chen Y, Wang DR, et al. Redox/pH dual stimuli-responsive biodegradable nanohydrogels with varying responses to dithiothreitol and glutathione for controlled drug release. *Biomaterials*. 2012;33(27):6570–6579. doi:10.1016/j.biomaterials.2012.05.062, copyright (2012), with permission from Elsevier. (C) Aptamer-based DNA nanoswitch for controlled drug release. Adapted with approval of Mo R, Jiang T, Sun W, Gu Z. ATP-responsive DNA-graphene hybrid nanoaggregates for anticancer drug delivery. *Biomaterials*. 2015;50:67–74. doi:10.1016/j.biomaterials.2015.01.053, copyright (2015) with permission from Elsevier.

nanorods (Au–Ag NRs) and DNA cross-linked polymeric shell was reported in which near infra-red (NIR) irradiation induce photothermal effect of the Au–Ag NRs which leads to rapid heating of the nanogel, and promote programmed and swift release of captured drugs into the target environment.¹⁶¹ Likewise, reversible DNA hybridization allows specific DNA oligonucleotides to act as triggers for autonomous switching of nanostructures to load or release the therapeutic targets. For example, aptamer-cross linked hydrogels were utilized to control the release of drugs and nanoparticles in response to binding of the DNA aptamer to small metabolites such as adenosine, AMP, and ATP (Figure 8C).^{162,163}

Conclusion And Outlook

Since its inception in the early 1980s and the invention of DNA origami in 2006, DNA nanotechnology has made remarkable strides towards a variety of applications. In view of our interest in the era of cancer research, we provided an overview of the

advances in DNA nanotechnology at the interface of static DNA-based devices and their broad applications in the field of bio/nanomedicine (Table 1). Compared to other nanomaterials, such as polymeric, organic, metallic, and lipid-based nanoparticles, etc., the beauty of DN can be attributed to the simple principle of base pairing that allows construction of an unlimited number of nanostructures with different sizes, shapes, charges, and modularity to serve for a specific purpose. For example, DNA polyhedra (DNA box), and hydrogel are both porous structures that can accommodate therapeutic agent inside while functional ligands/moieties can be coated on the surface of DN. DNA box toolkits are meant for on-command opening (provided by aptamer) and exposure of cloaked cytotoxic drugs, when only the correct trigger (eg, ATP) is available, allowing effective on-target drug delivery. In addition to its function as a drug carrier, the hydrogel structure offers an additional advantage as it can undergo reversible changes of shape (gel/sol state), size (shrunken/swollen) and charge (deprotonated/protonated). Such potential to capture and

adapt to changes in the microenvironment is important for the design of nanocarriers taking into account the dynamic and heterogeneous microenvironment of cancer cells. For example, pH-responsive hydrogel (provided by azo-linker) allows the shrunk hydrogel to enter the endo-lysosomes where the size expansion occurs within the low pH endo-lysosomal vesicles, resulting in a larger pore size and thus the leakage of cytotoxic drugs to induce cancer cell apoptosis. Meanwhile, acid-triggered protonated hydrogel that results in size expansion and electrostatic repulsion of DN promotes endo-lysosomal burst release of hydrogel into the cytosol. Under normal pH, the hydrogel can shrink back to its original size, and its subsequent transport outside the dead cell starts another cycle of penetration into the nearby cancer cells.¹⁶⁴ This way, recycling hydrogel can penetrate into the deeper regions of the tumor by peeling off tumor layers one after another to envision an enhanced therapeutic index for cancer therapy.

With regard to the nature of DNA, the advantages of DNA based architectures including a rapid and facile self-assembly method, forming rigid yet complex structures, automation, and self-amplification potential makes it a suitable and addressable material with unlimited applications in biology, medicine and especially preclinical cancer research and therapy. However, for DNA nanotechnology to reach its full potential, several limitations should be addressed, including simplified and automated design platforms, straightforward and economical production methods, scale-up strategies for size expansion, efficient chemical functionality, enhanced stability to survive harsh fabrication environments, minimizing immunogenicity and assembly defects, and finally improving their penetration and cellular uptake through physiological barriers.

Together, the facile assembly of DNA structures to well-defined architectures capable of crossing physiological barriers, being stable in biological fluids inside the body plus potential for biosensing and performing tasks on target cells with nanometer precision, if achieved, can deliver an unprecedented opportunity for timely cancer detection and therapy at the level of one single cell.

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Disclosure

The authors report no conflicts of interest in this work.

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