Applied
NANOMEDICINE

2022
2(2), 337

In vivo sojourn of DNA nanodevices: Taking stock of the past and perspective for future challenges & applications

Krupa Kansara[1]*, Ashutosh Kumar[2], Dhiraj Bhatia[1,3*]

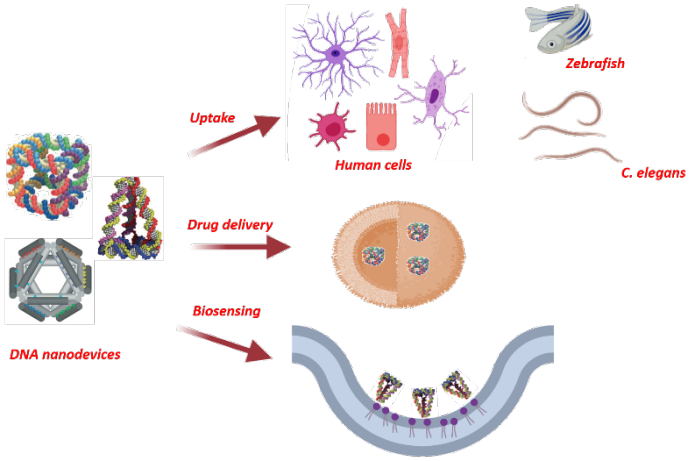
[1]Biological engineering discipline, Indian Institute of Technology Gandhinagar, Palaj 382355, India. [2]Biological and Life Sciences, School of Arts and Science, Ahmedabad University, Navrangpura, Ahmedabad 380009, India. [3]Centre for Biomedical Research, Indian Institute of Technology Gandhinagar, Palaj 382355, India

Submitted on: 17-Mar-2022, Accepted and Published on: 20-June-2022

Review

ABSTRACT

DNA based nanotechnology has witnessed phenomenal advances in biomedical applications such as drug delivery, bio imaging and bio sensing. Designer DNA nanodevices are at the forefront of many technological breakthroughs in modern science. An emerging class of programmable DNA nanodevices offer unique and powerful approaches for targeted drug delivery in vivo with minimal toxicity. The potential advantages of DNA nanostructures have ensured a bright future of DNA based nanodevices for almost all the domains of biomedical applications. This review integrates fundamental aspects of DNA nanotechnology and delineates the recent advances in therapeutics and in vivo targets of designer DNA nanostructures. It specifically discusses the history of the DNA nanotechnology origin, building of DNA nanostructures, their interface with biological systems such as cellular uptake, intracellular fate and in vivo targeting of 3D DNA nanostructures. Finally, it identifies the challenges of DNA nanotechnology and paves the prospective ways for their futuristic transitions into devices in healthcare and bioengineering.



Keywords: DNA nanodevices, in vivo delivery, biosensing and therapeutics, regenerative medicine, biomedical applications

INTRODUCTION

Nanotechnology has emerged as one of the fast growing potential field in past few years from the initial demonstration of metal based nanomaterials to programmable biological materials like DNA nanodevices.[1-3] Much of these developments mainly focus on applications such as targeted drug release, biosensing, bioimaging, synthetic biology, material science, improving cancer detection and treatments.[4, 5] The rapidly growing DNA nanotechnology have potential to achieve more aims as it enables geometry based control and precision of ligand functionalization. Highly sophisticated DNA nanoscale devices are one of the recent advancements of the nanotechnology field. The very specific molecular recognised pattern of DNA molecules allow programmable self-assembly of DNA and known as a DNA nanodevices.[6] Highly distinguished complimentary DNA sequences bind with each other under a controlled mechanism and form stable double helix durable nanodevices which are accurate and distinct designs with size ranges from nanometres to millimetre and capability to pass molecular level information.[3] Basic and important mechanism of DNA nanotechnology includes; understanding of DNA thermodynamics in terms of predicting molecular single stand DNA folding and their network formations, cost and quality of synthesized DNA nanostructure, reduced cellular interference and purity of DNA.[7, 8]

Though discovered in early 1980s, DNA nanotechnology field pioneered by Nadrian Seeman's innovative ideas; the field had not gained attention until early 2000s when three dimension nanodevices started being explored for different biomedical purposes.[9, 10] The advancement of recent studies on nanodevices covers programmable immuno-adjuvants and nanorobots, these nanodevices offers multiple potential applications including bioimaging, biosensing, targeted drug deliveries and disease

*Corresponding Author: Krupa Kansara, Dr. Dhiraj Bhatia
Email: krupa.k@iitgn.ac.in (KS), dhiraj.bhatia@iitgn.ac.in (DB)

URN:NBN:sciencein.nanomed.2022.v2.337

https://pubs.thesciencein.org/nanomed

detections.[11] DNA nanostructures have unique physico-chemical properties as DNA has the more accurate and predictable interactions than any natural and synthetic molecule. DNA offers precise binding specificity with thermodynamic stability and have infinite choices of new sequences formations by complementary binding.[12] Under the conventional condition, it has well defined structure in nanometre scale with length of ~ 50 nm. DNA can be rapidly synthesized and manipulated or modified via various automated methods and DNA acting enzymes can program and tune its structure further. Promising development in the field of DNA nanotechnology has ensured DNA as a potential new biochemical compound with surprising abilities.[12]

In this review, we discuss the advancements of DNA nanodevices that have been utilized in *in vivo* biological systems and their applications. We discuss the cellular uptake and *in vivo* targets of these nanodevices in details. We summarize how different cells and *in vivo* systems respond to these nanodevices. Finally, we outline, the potential challenges of these tiny nanodevices and their future perspectives for wider use and benefits.

NANOCAGES AND MOTIFS IN DNA NANOTECHNOLOGY

Ned Seeman realized the concept of building various nanostructures of DNA using the specific base pairing principle of Watson−Crick model.[9] The sticky ends which are single stranded overhangs of DNA can be connected together via base pairing and form 3D DNA motifs tile. Ned Seeman's group first reported these simple and immobile four arm Holliday junction DNA tile that was constructed by four single stranded DNA.[9] However, these structure had two major limitation; their flexibility and instability. The same research group reported that the Holliday junction could form immobile arms with different sequences and it was lacking the detrimental two-fold symmetry.[13] In early 1990s, the structural flexibility had decreased and 3D DNA cube was made with three connecting arms.[14] The robustness was increased and double cross over (DX), higher order structure were constructed by two strand exchanged of DNA double helices instead of single stranded arms which was involved in the Holliday junctions.[15] Such DX structures provided robustness, rigidity and stability which was required to construct extended DNA nanostructures with offered connectivity, topology and controlled geometry.[16] In fusion with sticky end cohesions, these motifs were used to form 2D DNA crystals which can grow autonomously and characterized by atomic force microscopy. The DX tiles were the pioneer for structural DNA nanotechnology as these tiles were followed by other numerous tile motif structures which were connected by DNA strand exchange. These tile motif was inspiration for other structures such as star motifs, three point, six point and T-junctions.[17-22] Hamblin et al., reported longest single stranded DNA template construction protocol from repetitive motifs recently.[23] These motifs were used to assemble DX tiles into well define DNA structures with precise length and pattern from selected minimum tile units. To enhance the structure stability and reduce distortion in shape, the concept of sequence symmetry in tile motifs was introduced which exhibit the large growth of 2D arrays from minimum strands.

The landscape of DNA nanotechnology entered a new dimension when Paul Rothemund introduced the DNA origami art.[24] DNA origami was a method in which a designer well-constructed DNA objects could be easily constructed from a long and viral scaffold single stranded DNA with the help of multiple short staple strands. Apart from the historic Paul Rothemund's contribution to DNA origami art, previous work done by various researchers to construct of octahedron by folding a continuous DNA strand with the aid of short strands and DNA barcode systems have provided ground breaking inspiration as well.[25, 26] In general the DNA origami approach based on the construction that address nearly 200 points in the area of 8,000–10,000 nm^2 which was unrivalled. DNA origami's unique feature which is simple strands that interact with the scaffold are not purified usually and simplifying the structure. With any arbitrary design and free available artificial interface, we can create robust and efficient designs in laboratory and it has access to any researchers globally. Subsequently, DNA origami approach was extended to construct a pallet of 2D and 3D DNA designs and a single-stranded DNA (ssDNA) tile (SST) was developed to create "DNA bricks".[27] These DNA bricks were further used in designing the 2D and 3D nano constructs as a primary framework. DNA motifs and junctions have been used to construct 3D polyhedral structures and DNA nanodevices have been made and characterized. Some examples of the top-down approach for nanoscale devices are cubes, truncated octahedron, octahedra, tetrahedra, dodecahedra, trigonal bipyramids, icosahedra, prisms and bucky- balls.[28-30] Design a complex and larger structures such as DNA box, platonic solids i.e., buckyballs, octahedra, tetrahedra, cubes, icosahedra, spheres and flasks, DNA origami approach can be the most powerful tool. The unhybridized sequences presence at the junctions and double stranded DNA arms flexibility allow smooth bending to create wireframe polyhedral via multiple and multi arm junctions connections.[31, 32] Additionally, many researchers across the globe have been focused to create 3D polyhedra using DNA origami-based approaches and rigid crossover motifs. Polyhedra or DNA nano cages have been very remunerative areas of DNA nanotechnology as these structures possess internal void which can be used as a host cargo to incorporate any well-known nanostructures and functionalized with any biological tags which can target broad range of proteins, enzymes and antibodies for multiple biological applications. Synthetic DNA nanocages are biocompatible in nature and exhibit the better stability thus these nanocages are ideal candidates to study various biological applications via *in vitro* and *in vivo* assays.[12] Figure 1 demonstrates different forms of DNA assemblies.

CONSTRUCTION OF 3D DNA NANOCAGES

To build 3D DNA nanocages, the basic design rule of DNA motif polymerization applies and it is listed in three different categories: (a) direct assembly through single step in which the nanodevices are constructed by simple mixing and annealing the

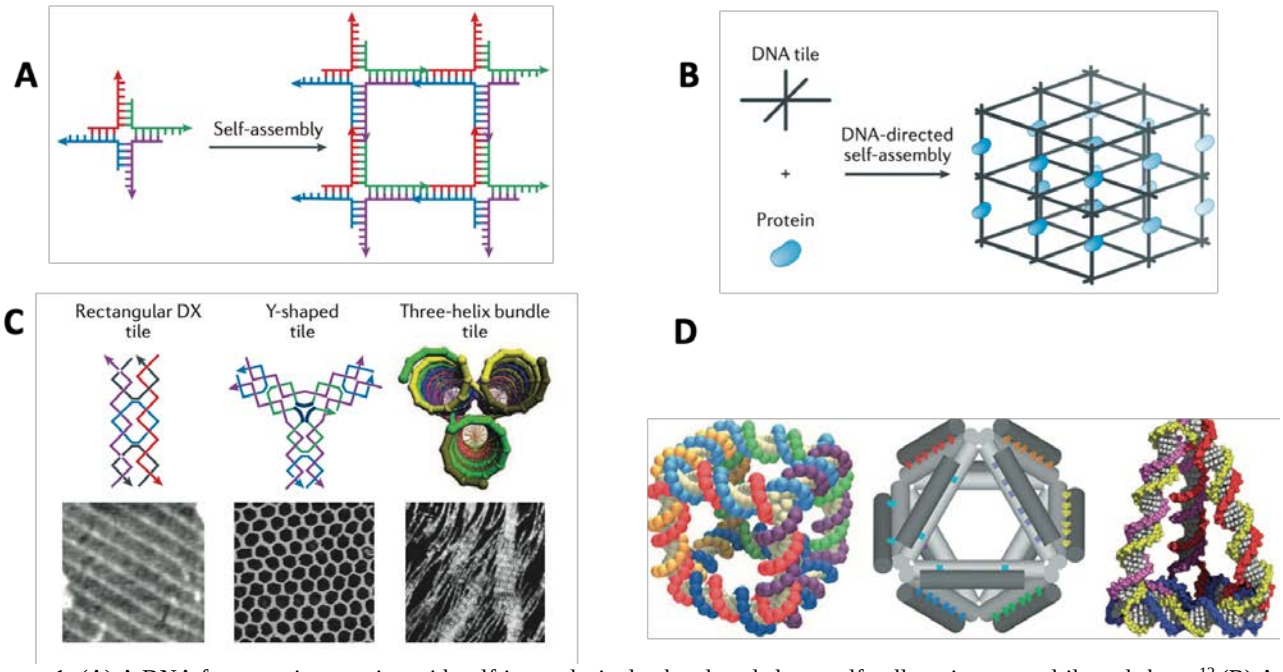


Figure 1: (A) A DNA four-way intersection with self-integral, single-abandoned closes self-collects into a quadrilateral shape.[12] (B) A DNA framework that helps protein (blue) crystallization.[12] (C) DNA nanotechnology themes. The top boards show tile themes in DNA nanotechnology, and the baseboards show AFM pictures of their gatherings into grids. The left boards show an illustration of a double-crossover (DX) tile; the centreboards show an illustration of a Y-molded DNA theme self-gathering into hexagonal 2D cross-sections; and the right boards show an illustration of three-helix bundles.[33] (D) DNA 3D shape developed from associated three-arm junctions.[34] The center board shows an octahedron built from a long DNA strand and five interfacing strands.[26] The right board shows a tetrahedron developed from four DNA single strands.[35] Reproduced with permission from references 12, 33, 34, 35 and 26.

oligonucleotides together; (b) component based modular self-assembly, where initial modules are prepared through hybridization method and later synthesized into higher order nanodevices; (c) DNA origami approach, in which the nano constructs are synthesized using a long ssDNA scaffold and multiple short complementary staple strands (Figure 2).[36]

Direct assembly through single step

Direct assembly through single step reaction involves the direct hybridization of element strands into the final DNA nanostructures. DNA tetrahedron is the ideal example of direct assembly, it was first assembled via direct hybridization of four oligonucleotides in equimolar ratio which was very fast and simple technique. Additionally, complex 3D polyhedral structure such as cages was fabricated through one pot assembly which was robust in nature.[37, 38] The major drawback of this technique is incomplete hybridization and secondary structures in DNA elements may form as it involves multiple large structures of long strands with single annealing step. However, this direct assembly approach follows a simple procedure for fabrication of 3D nanocages. Mao et al., has fabricated DNA prisms using two element strands through single step direct assembly which showing the robust and simplicity of this method.[39]

Component based modular self-assembly

In 1991, the first DNA cage was built and it was catenae-like structure resembling a cube. The first DNA cage was fabricated via DNA specific arm assembly into half structures and step wise folding into a cube like geometry.[14] In the component based modular self-assembly approach, various components are first self-assembled via motif formation or single DNA hybridization. The assembly of motifs are further assemble into a full architecture and called as a self-assembly.[40, 41] Octahedra and icosahedron are the examples of the modular self-assembly approaches. Additionally, the different strategy such as three point star motifs were constructed form DNA that could modulate oligomerize of 3D nanocages with various sizes and topologies.[41] Mao's group has achieved different constructs such as tetrahedron, dodecahedron, icosahedron, and buckyball by modulating the concentration and component tiles.[29] To build half icosahedra in 1:5 stoichiometry, a DNA five way junctions (5WJ) was designed to self-assemble with various 5WJ. To assemble one DNA icosahedron, two half icosahedra with complementary ssDNA overhang association in 1:1 is required.[41] DNA icosahedron can act as a cargo which can encapsulate nanomaterials and other smaller biological or chemical materials. Recently, our group have successfully demonstrated the gold nanomaterials and quantum dots encapsulation by mixing in excess in ratio of 1:1 of half icosahedra (VU_5 and VL_5). Researchers also demonstrated that metal DNA junctions could also self-assemble into 3D nanocages with different shapes and topologies such as DNA icosahedron.[42, 43]

DNA origami approach

DNA origami gives rise to level of complexity as it has been explored to fold the large single stranded genome of virus into two different constructs of tetrahedron via staple strands.[44, 45] Such an approach has been made to build DNA box as this structure can assemble form six DNA sheets that are connected

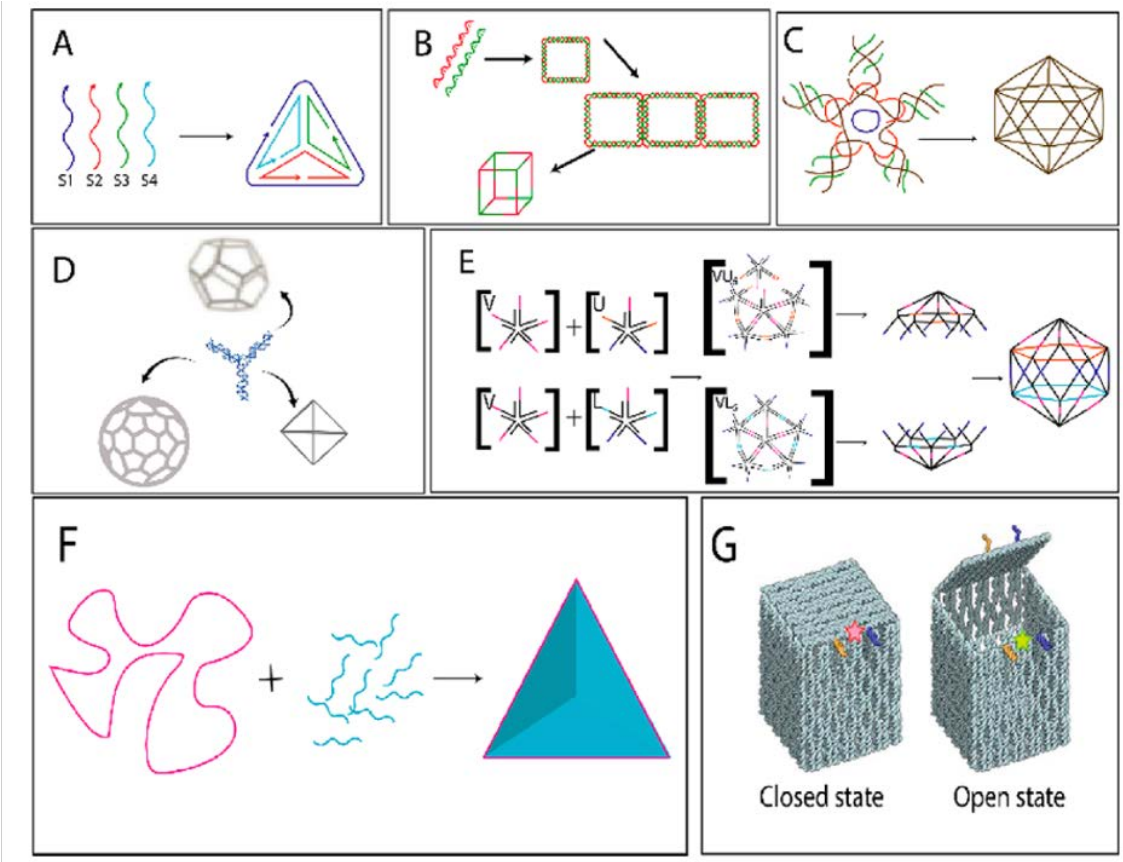


Figure 2. Various techniques for self- assembly of DNA nanocages including.[36] (A) one-pot gathering of DNA tetrahedron, (B) measured assembly of a DNA block from parts, (C) modified self-gathering of 5-equipped DNA vertices into an icosahedron, (D) different gathering courses for tetrahedra, dodecahedra and Buckyball from 3-point-star theme, (E) icosahedron by particular self-gathering strategy, (F) DNA origami-based tetrahedron, (G) DNA origami box with lid.[48] Reproduced with permission from references 36 and 48.

together.[46] The lock and key mechanism of DNA box is based on the toehold-mediated strand displacement in which the lid of the DNA box was modified. These brilliant DNA nanocages can be used as a potential cargos to encapsulate the choice of biological molecules for various biological applications in controlled environment.[47]

CELLULAR UPTAKE AND FATE OF DESIGNER DNA NANODEVICES

Cellular uptake of designer DNA nanodevices

Most of the FDA approved drugs suffer from cellular targeting due to non-specific and multiple routes of entry into the various cells; raising major concern regarding toxicity and lesser therapeutic efficacy.[49, 50] Modern medicine is facing the challenge of an effective and safe delivery of drugs into targeted manner.[50] One approach to overcome the efficacy and safety issue is to use various nanostructure as a delivery agents and enable active transportation of drugs. Different compositions and types of nanomaterials have been explored as a drug delivery agent such as liposomes, metallic nanomaterials, organic polymers and inorganic materials.[51-53] However, it is difficult to control size, shape and surface charge of nanomaterials precisely which hinders the delivery performance and cellular uptake investigation systematically. As a rescue of such challenges, DNA nanotechnology has demonstrated unprecedented abilities to synthesize uniform DNA nanostructures with prescribed size, shape, surface functionality, the number and location of chemical modifications.[44, 54-56] DNA nanostructures are biocompatible in nature and naturally enter into the mammalian cells without triggering any immunogenicity or toxicity.[57] In this section we discuss the cellular uptake, mechanism and fate of various DNA nanostructures such as tile based structures and origami based structures.

Recent advancements have been made in the optimizing in the tile-based nanostructures for higher cellular uptake. One study demonstrated the uptake of TDNs by mouse head and neck cancerous cells and they have reported the smaller size of TDNs (~ 9 nm) uptake was significantly higher than larger size of TDNs (~ 25 nm).[58] In 2017, Rahman et al. investigated the DNA nano-rectangles and 3D nanotubes uptake in DMS53 cancer cells based on their size and shapes.[59] They synthesized an array of 2D nano-rectangles of different lengths and widths and 3D nanotubes of the same lengths and widths as 2D nano-rectangles. They showed higher fraction of cellular uptake of nano-rectangles and 3D nanotubes of the smallest size (32 nm × 12 nm) than the larger (64 nm × 24 nm) ones. However, the uptake difference was statistically uncertain in this case. Mou et al., synthesized fluorescently labelled various DNA nanostructures such as

TDNs, dodecahedra, and buckyballs to investigate their cellular uptake in HeLa cells.[60] They observed that the DNA buckyball with ~ 84 nm of diameter enter HeLa cells in larger fractions than the other two nanostructures with the diameter of ~ 16nm of TDN and ~ 55 nm of dodecahedron based on their confocal imaging and flow cytometry analysis. Such study indicates that there is no concrete conclusion on cellular uptake pattern based on size and shape of tile-based structure. Additionally, ligands modified tile-based DNA nanostructures for targeting specific cellular receptors is in emerging trend for cellular uptake and targeted delivery approach. Li et al., demonstrated that the anticancer aptamer AS1411 decorated TDNs uptake in MCF-7 breast cancer cells was four folds higher than TDNs without aptamers.[61] In a similar way, Raniolo et al., conjugated octahedral DNA nanocages with folic acid and investigate their uptake in HeLa cells that overexpress α-folate receptor.[62] The researchers showed that the uptake of nanocages conjugated with folic acid was 40 folds higher than α-folate receptor negative A431 cells after 24h incubation. These data provide insights into tile-based nanostructures cellular uptake for intracellular applications.

Recent studies provide the insight into size and shape based cellular uptake by DNA origami structures. In 2018, Wang et al.,

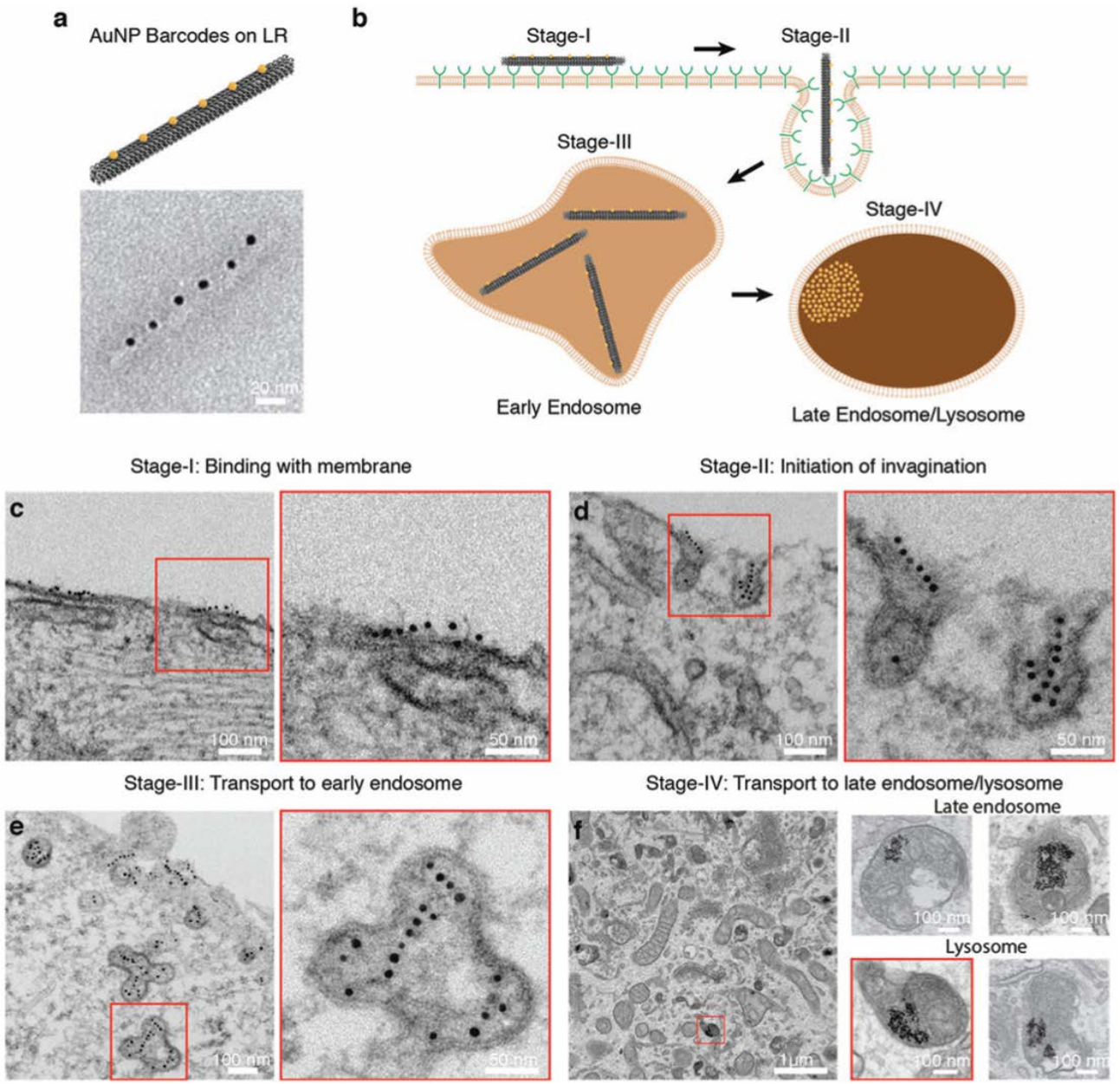


Figure 3. Representation of receptor-interceded DNA origami rod take-up by H1299 cells through transmission electron microscopy. (a) Schematic portrayal and comparing TEM picture of a DNA origami rod discretely marked with AuNPs. (b) Depiction of four phases (Stages I, II, III, and IV) by which cell take-up of the DNA origami pole was thought to happen. (c-f) TEM representation of DNA nanostructures going through Stages I through IV, respectively.[50] Reproduced with permission from reference 50.

reported the size and shape based cellular uptake of DNA origami structures on two different lung cancerous cell lines i.e. H1299 and DMS53.[50] The authors have synthesized fluorescently tagged mixture of two tetrahedron with edge length of 11 and 47 nm; square rods with 32nm × 4nm and 127nm × 8nm base length and width respectively. Cellular uptake analysis by flow cytometry revealed that the uptake of larger tetrahedron and square rods was significantly higher than their smaller counterparts in H1299 cells. In brief, there is a higher fraction of cellular uptake by larger nano constructs through stronger interaction between cells and nano constructs (Figure 3).

Intracellular fate of designer DNA nanodevices

In this section we discuss the studies on the fate of the designer DNA nanodevices. Post uptake into cells, most nanomaterials are targeted to early endosomes followed by late endosomes and lysosomes through endolysosomal pathway.[63] Based on the properties and size of the nanomaterials, few of them experience the complete degradation through cellular enzymatic activities.[64] Biocompatible coatings of lipids and polymers on nanomaterials enhance their retention period and reduce their degradation inside the cells and to achieve specific goals inside cells, attachment of various aptamers should also encouraged.[65]

In 2016, Vindigni et al., investigated the accumulation of DNA octahedron nanocages in COS fibroblast (Figure 4).[66] The authors assessed immunofluorescent post 2h and 5h incubation and reported accumulation of DNA octahedron nanocages inside lysosomes however they did not quantify the amount of accumulation of nanocages between the time points of two treatments and accumulation in lysosomes relative to entire cell. In 2017, Kang's group synthesized two different sized of mirrored TDNs and assessed their accumulation in HeLa cells through live imaging.[67] The major finding of the study was the smaller TDNs with edge length of 9 nm started co-localized in lysosomes within a minutes after the exposure however the larger TDNs with edge length of 25 nm appeared in lysosomes after 30 minutes of exposure. The authors elucidated that the uptake and accumulation of smaller TDNs enter rapidly through their cellular entry. Xia group's assessed the accumulation and trafficking of fluorescent labelled TDNs with chemical stain LysoTracker in three cell lines including HeLa cells, BEAS-2B and RAW264.7 cells for 12 h exposure.[68] The study revealed that TDNs larger fraction was accumulated in lysosomes of different cells and pH of the lysosomes did not change through TDNs exposure. In such case, DNA origami-based structures showed the same fate as DNA tile-based structure in the cells. Few studies reported DNA origami-based structures accumulated in lysosomes. Shen et al., synthesized DNA nanotubes with weakly fluorescent cyanine dye and assessed the route of trafficking for these nanotubes.[69] The authors stained the lysosomes and demonstrated the accumulation of nanotubes in lysosomes after 12 h of exposure. The authors showed disassembly of nanotubes via decreased fluorescent intracellular signals as they removed the nanotubes containing media and replenish cells with new media without nanotubes over 60 h of exposure. Halley et al., fabricated daunorubicin decorated DNA nanorods as an anticancer therapeutic agent and examined their uptake in multidrug resistant leukaemia cells.[70] Study showed fast accumulation of nanostructures within lysosomes only after one hour of incubation and biocompatibility of nanorods. These studies collectively show the route of trafficking of various DNA nanostructures among the different cell types.

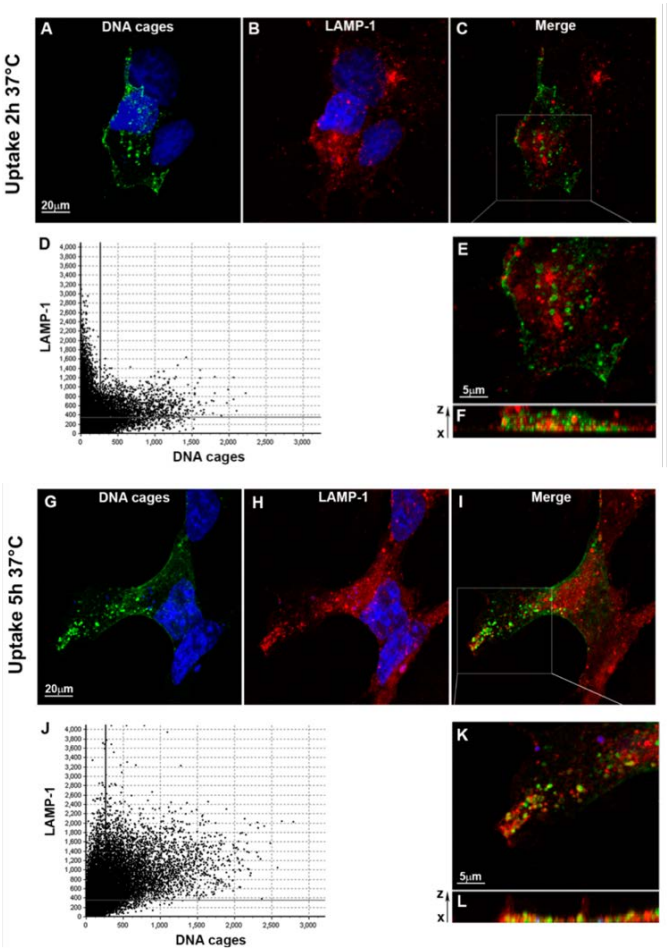


Figure 4. Co-restriction examination of DNA nanocages and lysosomes. Twofold immunofluorescence of LOX-1-V5-communicating COS cells was exposed to DNA cages at 37 °C for 2 h (A−F) and 5 h (G−L). Biotinylated cages were identified by utilizing streptavidin−FITC (A and G), and lysosomes were quantified involving mouse monoclonal anti-LAMP-1 antibody as primary antibody and Rhodamine Red-X-conjugated donkey anti-mouse IgG as the secondary antibody (B and H). The nuclei were stained with DAPI. Higher amplification of the combined pictures (C and I) is displayed in boards E and K. Boards F and L address XZ stack pictures. Co-confinement investigation was performed utilizing IMARIS software, and scatter plots are represented in boards D and J. Scale bar: 20 μm.[66] Reproduced with permission from references 66.

In most cases, the entry of any oligonucleotides based constructs into cells is extremely inefficient due to negative charge on the plasma membrane.[71] Therefore, DNA nanotechnology has been considered a paradigm shift as through endocytosis mechanism DNA nanostructures can enter into cells.[72, 73] Few studies have reported the contradictory results on endocytic mechanism and cellular uptake of such

nanostructures.[71, 74] Lack of studies led to uncertainty about the internalization of bare DNA nanostructures in various cells via any endocytosis approach without cell specific ligands. In most studies, tetrahedron, cube, prism, DNA bricks and origami DNA nanostructures internalization through clathrin and/or caveolin dependent endocytosis pathway have been reported.[50, 66, 71, 75-80] One of the approach to see the internalization and progress of DNA nanostructures across the cell membrane and into the cells is decorate them with cyanine 3 (Cy3) or cyanine 5 (Cy5) fluorescent dye and can visualize through live imaging.[50, 66, 71, 75-80] Another popular approach employs DNA nanostructures labelled with biotin–streptavidin in which streptavidin is fluorescently-tagged. These approaches have raised the concern about the unaccounted cell behaviour that influence the outcome. Studies revealed that HepG2 and HeLa cells uptake phosphate analogues of Cy3 or Cy5 through endocytosis directly and these studies sheds the important finding about cyanine dye use to track DNA nanostructures inside the cells as it may produce a false positive signals for DNA nanostructure cellular uptake.[81] Detailed analytically approaches and inclusion of more controls needs to adopted to track DNA nanostructures cellular uptake through endocytosis pathway for clearer understanding.

IN VIVO TARGETS OF DNA NANODEVICES

A variety of DNA nanostructures have been used in biomedical applications such as drug delivery cargo and diagnosis probes in the higher order living systems. The applications of designer DNA nanodevices in *in vivo* or higher order organism has just emerged however several nanodevices have been used for 2D cell cultures.[57, 63, 70, 79, 81] The limited research of DNA nanodevices in *in vivo* living models is due to their molecular barrier including (i) site of interest for targeting and efficient delivery of DNA nanostructures (ii) stability of DNA nanostructures that introduced externally and (iii) lack of toxicity assessment in *in vivo* models. However the limited literature of the in vivo targets of DNA nanodevices, tetrahedron and icosahedron make strong case studies to discuss the inside molecular mechanism and barriers.[82, 83]

DNA nanodevices delivery strategies depend on the direct injection of specific nanodevices to target cell types for *in vivo* model systems. Currently, *Caenorhabditis elegans* is one of the popular model systems to understand the molecular mechanism of designer DNA nanodevices. The first study of pH sensitive DNA nanodevice has been conducted in *C. elegans* in which the researchers microinjected I-switch nanodevices into *C. elegans* to target specific scavenger cells (Figure 5).[84] These scavenger cells represented anionic ligand-binding cellular surface receptors. The results demonstrated that I-switch nanodevices initiated endosomal maturation in *C. elegans* which enhanced the uptake and internalisation. Similarly, cargo loaded icosahedron have been introduced to specific scavenger cells; in which the integrity and functionality of cargo loaded icosahedron were stable post-delivery. Surana and co-workers investigated stability and clearance of DNA nanodevices by lysosomal degradation in *C. elegans* through targeting the scavenger cells.[85] The group synthesized the DNA nanodevices with two distinct single strand domains that showed 8 hours of half-life in *in vivo* which increased to 11 hours after reducing the number of single stranded domains. In contrast, DNA icosahedron without the free termini was intractable to lysosomal degradation even after 24 hours post exposure. Lower amount of magnesium ions (Mg^{+2}) is required to maintain the structural integrity of icosahedron and tetrahedron which corresponds to normal physiological

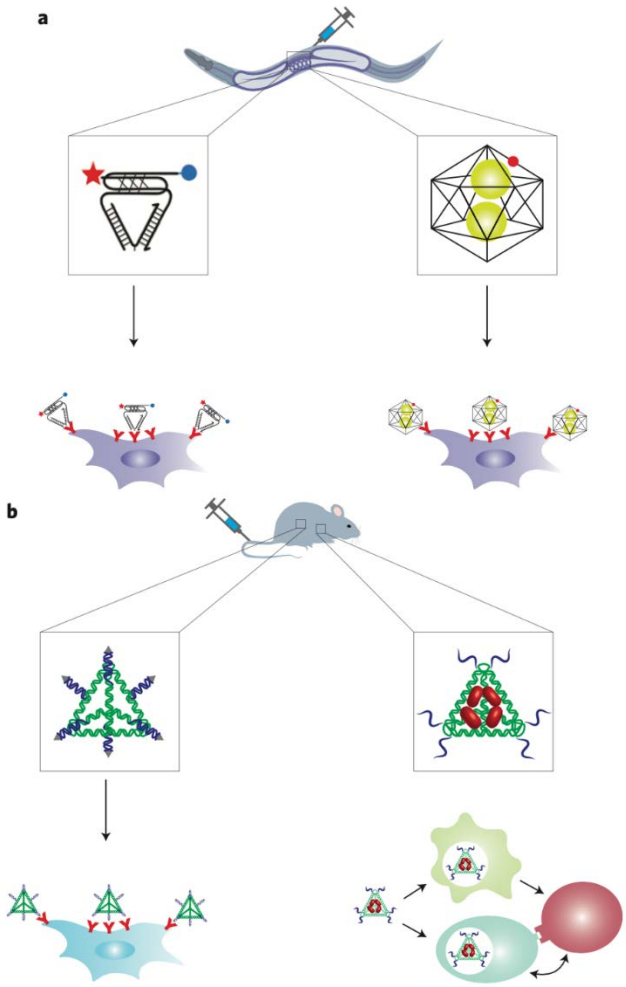


Figure 5. Targeted delivery of DNA nanodevices *in vivo*. a, Microinjection-intervened presentation of a pH- sensitive DNA nanostructures (left) and a cargo stacked DNA icosahedron (yellow circles; right) in *C. elegans* utilizes the anionic ligand-restricting receptors (red, base) to accomplish cell-explicit focusing on. The red star and red and blue circles address fluorophores. [84, 87] b, The DNA tetrahedron, bearing folate moieties (grey triangles) and siRNA (purple duplex) on its surface (left), was designated to murine cancers overexpressing the folate receptor (red). The DNA tetrahedron was likewise utilized as a framework to show streptavidin (red ovals) as an antigen and single-abandoned CpG oligonucleotides (purple strands) as an adjuvant (right), which was brought through venous infusions into the mouse circulation system. Assimilation of the DNA tetrahedron into B-cells (blue) and macrophages (green) prompts downstream enactment of T cells (red), which thusly actuate manufactured immune response creation against streptavidin by B cells.[83 88] Reproduced with permission from references 83, 84, 87 and 88.

concentration of Mg^{+2} thus it increases the stability of these nanodevices in *in vivo* models.[41, 86] DNA nanodevices are destabilized when physiological Mg^{+2} concentrations are decreased to tenfold lower in biological systems.

Designer DNA nanostructures have been delivered intravenously in mammalian systems. A general strategy to deliver nanostructure is targeting the specific site of interest that enables to bind specific cellular receptor resulting the sufficient cellular internalization. DNA tetrahedron nanostructures linked with folate moieties and siRNA were studied in nude mice xenografted tumour model.[83] The results demonstrated significant uptake of DNA nanostructures in tumour environment that overexpress folate receptor however the some DNA nanostructures distributed in non-cancerous cells bearing folate receptors and showing even distribution in tissues and siRNA reduced the expression level of targeted gene. Importantly, this approach hints to target and deliver drug molecules in selected malignancies. Liu and co-workers engineered DNA tetrahedron linked with streptavidin (model antigen) and CpG deoxy-oligonucleotides (adjuvant) to produce antibodies in mouse model.[88] These DNA nanostructures produced antibodies against streptavidin by circulating macrophages and dendritic cells in the bloodstream.

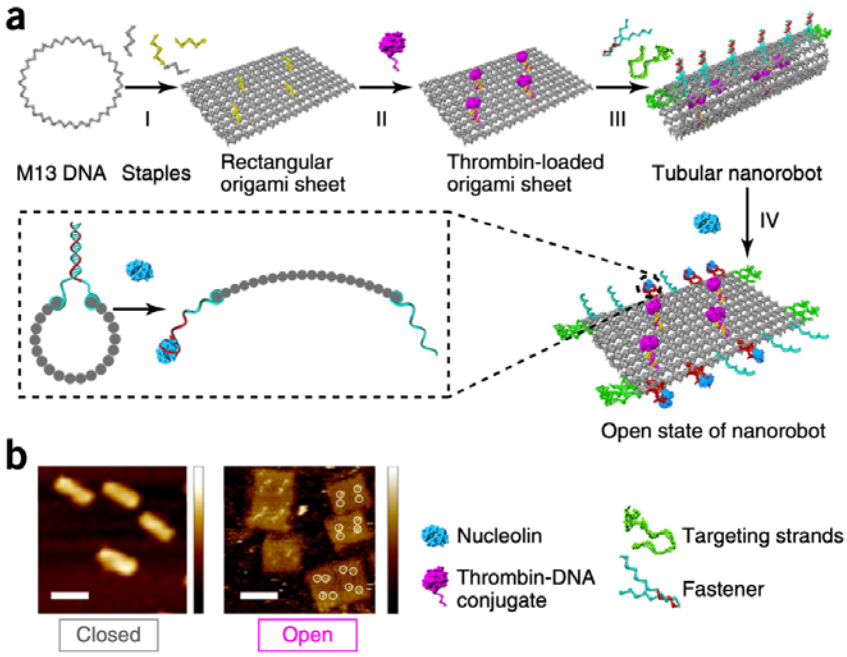


Figure 6. Plan and portrayal of thrombin-functionalized DNA nanorobot. (a) Schematic delineation of the development of thrombin-stacked nanorobot by DNA origami, and its reconfiguration into a rectangular DNA sheet in light of nucleolin restricting. (a, I) Single-abandoned M13 phage genomic DNA is connected by predesigned staple strands, prompting the arrangement of a rectangular DNA sheet. (a, II) Thrombin is stacked onto the outer layer of the DNA sheet structure by hybridization of poly-T oligonucleotides formed to thrombin particles with poly-A arrangements that stretch out from the outer layer of the DNA sheet. (a, III) Addition of the clasp and focusing on strands brings about the development of thrombin-stacked, rounded DNA nanorobots with extra focusing on aptamers at the two finishes. (a, IV) The tube nanocarrier opens because of the presence of nucleolin to uncover the epitomized thrombin. (b) DNA nanorobots were inspected by AFM and representative pictures of shut (left) and opened states (right) are shown. The four brilliant spots showed on the outer layer of the origami sheet address the thrombin particles (thrombin molecules on the four DNA-sheet-thrombin gatherings on the right of open states picture are featured by white circles). The AFM pictures are illustrative of three free examinations. Scale bars, 100 nm.[90] Reproduced with permission from reference 90.

One of the earliest study by Bachelet's group that investigated the biocomputing application of barrel like DNA nano robots in *Blaberus discoidalis*, living insect.[89] The group constructed aptamer conjugated DNA nano robot that can mimic logic gates such as CNOT, NOT, NAND, OR, XOR and AND. These DNA nano robots decoded Boolean computations in living *Blaberus discoidalis* when vascular endothelial growth factor and platelet-derived growth factor were conjugated with DNA nano robots. Recently, these group has demonstrated that these nano robots can be controlled by human thoughts inside the cockroaches. They analysed the cognitive state related electroencephalogram with manipulated electromagnetic field. The cascade sequence was thermally induced and nano robots' configuration was changed after adding of metal nanoparticles to these nano robots' locks.

Interestingly one of the recent study by Li and co-workers on DNA nano robots covered the anticancer therapeutic application against several tumour-bearing mouse models such as human ovarian cancer xenograft tumour model (SK-OV3), mouse melanoma cancer model (B16-F10), mouse lung cancer model (doxycycline-inducible Kras mutation) and human breast cancer xenograft tumour model (MDA-MB-231).[90] The group synthesized self-assembled DNA nano robots based on origami approaches with various functional elements and conjugated with thrombin and AS1411 aptamer (Figure 6). These intelligent nano robots were programmed to transport cargoes which specifically target tumours. These nano robots were conjugated outside with AS1411 aptamer which sense, bind and recognise nucleolin protein which specifically expressed in tumour associated epithelial cells. The blood protease thrombin was placed in inner cavities of these nano robots which activate coagulation at tumour site. DNA nano robot's reconfirmation occurred when aptamer AS1411 recognized the nucleolin protein on the proliferating tumour vascular endothelial cells. The open reconfigured robots deliver thrombin molecules to tumour blood vessels, activated coagulation at specific sites, inducing intravascular thrombosis resulting tumour necrosis and restricted further tumour growth. These DNA nano robots exhibited the inhibition and blockage of blood supply to developing tumour and tumour growth. These intelligent nano robots may inspire the

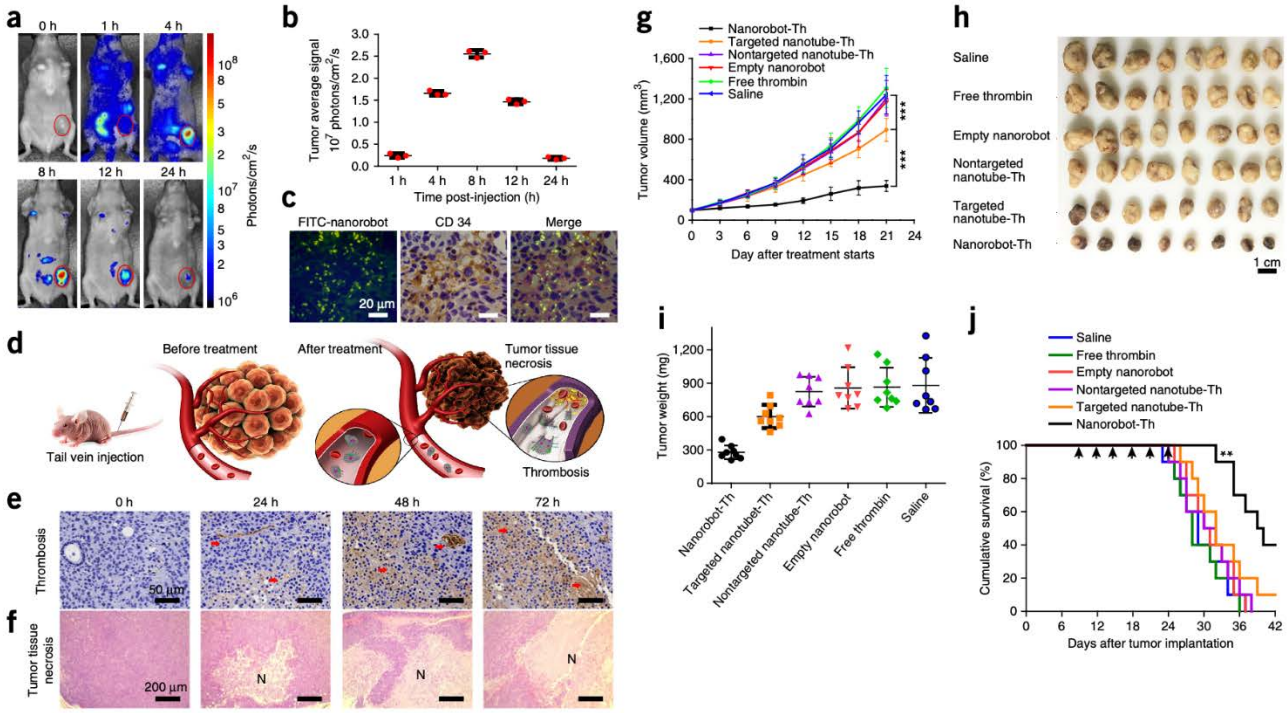


Figure 7. DNA nanorobots target cancers, actuate apoplexy in cancer vessels and hinder cancer development in vivo. (a) Optical imaging of a MDA-MB-231 mouse bearing a human breast cancer when an intravenous infusion of Cy5.5-labelled nanorobot. An extreme focus fluorescent sign was recognized exclusively in the cancer area of mice 8 h after infusion. 0 h = before injection. The pictures are illustrative of three autonomous trials. (b) In vivo fluorescence intensity at the tumour sites was estimated at the demonstrated time focuses after administration of the nanorobots. Error bars address the mean ± s.d. of three individual tests. (c) FITC-tagged nanorobots were infused intravenously into mice bearing MDA-MB-231 cancers. Tumours were reaped 8 h later, and tumour segments were stained with anti-CD34 antibody and inspected by confocal microscopy. The nanorobot (green) shows up in the vein rich locales (against CD34; brown). Cores are demonstrated in blue. The pictures are illustrative of three autonomous examinations. Scale bars, 20 μm. (d) Schematic portrayal of the remedial component of nanorobot-Th inside growth vessels. DNA nanorobot-Th was administrated to breast cancer xenografted mice by tail vein infusion and designated cancer-related vessels to convey thrombin. The nanorobot-Th ties to the vascular endothelium by perceiving nucleolin and opens to uncover the epitomized thrombin, which initiates restricted apoplexies, cancer dead tissue and cell putrefaction. (e) MDA-MB-231 tumour harvested at 24, 48 or 72 h after administartion of nanorobot-Th were immunostained for CD41 (actuated platelets) to identify thrombosis (brown, showed by red bolts). The pictures are illustrative of three free tests. Scale bars, 50 μm. (f) Tumors collected previously and 24, 48 or 72 h after treatment with nanorobot-Th were stained with H&E. Necrotic tissues are meant by N. Scale bars, 200 μm. Information are illustrative of three free examinations. (g-I) MDA-MB-231 growth bearing mice were treated on day 0 with saline, free thrombin, designated void nanorobot, nontargeted nanotube-Th, designated nanotube-Th or nanorobot-Th. Growth volumes up to day 21 (g, n = 8 naturally free, nanorobot-Th versus designated nanotube-Th, P = 0.00016; designated nanotube-Th versus saline, P = 0.00031). Agent photos of the cancers (h) and normal growth loads (I, mistake bars address the mean ± s.d. of 8 growths) of the showed gatherings of mice. (j) Cumulative endurance of MDA-MB-231 growth bearing mice (n = 10) thought about utilizing Mantel-Cox Log-rank test, P = 0.0048. Dark bolts show the infusion time points.[90] Reproduced with permission from reference 90.

design of various other designer nanodevices that can be used as a potential anti-cancer therapeutics (Figure 7). The current approach may be useful to treat for the other diseases through structural modifications of such DNA nanodevices with various targeting groups and different cargoes.

APPLICATION OF DESIGNER DNA NANODEVICES

Last two decades have witnessed the rapid growth of DNA nanotechnology in the fields of biosensing, synthetic biology, clinical diagnosis and drug delivery.[46] DNA nanotechnology has advantage including predictability, high rigidity, stability and programmability; through self-assembly and conjugation with several nanomaterials such as quantum dots, polymers, gold nanomaterials and carbon based nanomaterials stable and biocompatible DNA nanodevices can be constructed.[91, 92] In this section we summarizes the application and advantages of various DNA nanodevices for drug delivery, biosensing and bioimaging.

Drug delivery

In earlier section we have discussed of intracellular fate of DNA nanodevices and the mechanism of DNA nanostructures accumulation inside the cells. Through programming and modification DNA nanostructures can specifically enter into the target cells via endocytosis through clathrin- or caveoleo-mediated pathway. DNA nanostructures can be programmed in such a way that they can directly bypass the route of lysosomal degradation and enter into targeted subcellular compartment. For drug delivery applications, DNA nanochannels can be synthesized to mimic the natural membrane pores for specific and

targeted drug molecule cargo transportation in plasma membrane.[93]

Doxorubicin (Dox) has been widely used as one of the most effective FDA approved chemotherapeutic drug to treat wide range of cancers including breast cancer and head and neck cancer.[94] The mechanism of action of Dox molecule is, it inhibits the macromolecular synthesis. Dox molecule adversely affect the normal cells and toxicity ratio is higher in non-cancerous organs.[95] In the past few years the attempts have been made to conjugate Dox drug with liposomes, various nanomaterials and micelles to reduce the toxicity and improve the drug efficacy.[96-98] To overcome such limitations, programmable DNA nanostructure has been developed as a carrier for Dox molecule for targeting delivery as Dox molecule can easily reside itself in DNA through GC rich regions of DNA double helices. Dox decorated various DNA origami structures to target breast cancer cells have been constructed by the Högberg and co-workers.[99] These designer DNA nano constructs showed sophisticated relaxation in the double helices of DNA and various degree twist in their structure. By controlling the design, dox release rate and encapsulation in origami structure the group demonstrated the increased cytotoxic behaviour and reduced elimination rate. Högberg's group confirmed the increased apoptotic population in treated breast cancer cells through flow cytometry analysis which further confirmed the efficacy of nano constructs. This group demonstrated that the controlled drug release mechanism is possible through programming the twist degrees of nano constructs. Such programmable nanodevices are efficient for targeted drug delivery agents which shows higher penetration, lesser elimination and increased target specific toxicity in various cancerous cell lines. Jiang et al., synthesized Dox conjugated 2D and 3D origami structures with higher Dox loading efficacy. The higher degree of cellular uptake by these nanostructures have been found in human breast adenocarcinoma cancer cells (MCF-7).[100] These nanostructures exhibited cellular toxicity to normal MCF-7 and more importantly to Dox-resistant MCF-7 cells due to their targeted cellular uptake and proper distribution via DNA origami structures. Similar research project was carried out by the same group and they demonstrated the more therapeutic efficacy of Dox conjugated DNA origami nanostructures in *in vivo* system.[101] The research was carried out in nude mice bearing orthotopic breast tumours and after observing fluorescence imaging and other safety based experiments, they observed that Dox conjugated DNA origami nanostructures had potential to reduce tumour size significantly without any specific system toxicity. These findings strongly recommend that designer DNA nanostructures could provide efficient and safe platform for targeted drug delivery in both *in vitro* and *in vivo* systems.

DNA nanostructures especially DNA origami are generally complicated and expensive to synthesize, to overcome of these challenges. Yan and co-workers designed a novel strategy to synthesize 3D gold nanoparticle-DNA superstructure.[10] These nanostructures have been constructed by growing and folding origami DNA simultaneously on gold nanoparticles. The strategy employs the combination of flexibility and self-assembly of DNA nanostructures with rigidity and efficacy of gold nanoparticles. These 3D superstructures have a high drug/molecule loading capacity and high efficacy for targeting drug delivery and cellular imaging. Additionally, self-assemble DNA nanodevices were studied as nano cargo for Dox mediated delivery. Tan et al., fabricated multifunctional aptamer based DNA nano assembly (AptNA) for cancer therapeutics.[102] These assembly was self-assemble in Y- shaped domain having different DNA subunits with different functions such as encapsulated anticancer drugs, targeted aptamers, antisense oligonucleotides with therapeutic efficiency. To generate unique building block, these assemblies were conjugated with X-shaped connectors via hybridization. These blocks/units were further conjugate with hundreds of same units through photo-cross-linkage to synthesize aptamer based programmable and multifunctional nano assemblies. Tan et al., calculated Dox loading sites in these assemblies and reported that each aptamer base assembly contains various 100-200 building units and each unit could provide more than 220 Dox loading sites which was remarkable. Additionally, they incorporated therapeutic antisense oligonucleotides which has potential to inhibit drug efflux pump to increase the ejection of anticancer drugs via P-gp inhibition resulting enhanced drug retention and efficiency. In addition, these aptamer based nano assemblies were more stable in the physiological environment and exhibited excellent integrity.[102] Such qualities hint that aptamer based assemblies could be innovative platforms for drug delivery without leaking of intercalated drug molecules.

Recently, Tan et al., engineered DNA nanoflowers with multifunctional properties against multidrug resistant cancer cell lines and chemo sensitive cells.[103] The group reported that these nanoflowers contained multifunctional domains such as aptamers for specific recognition of cancer cells; Dox conjugated DNA for drug delivery and fluorophores for imaging. These nanoflowers showed high loading capacity of 71.4% wt/wt and densely packed with drug conjugated motifs. These Dox decorated nanoflowers were programmable for drug release under both acidic and basic conditions and stable in physiological environment. More complex, sophisticated and layer by layer self-assembled stacked Molybdenum disulphide linked DNA (MoS_2-DNA) nano super structure was engineered by Li et al., for efficient and targeted Dox mediated delivery in cancer cells (Figure 8).[104] Dox molecules intercalated in the DNA neighbouring Molybdenum disulphide nanosheets in these super structure. The Dox molecules were released through these nano constructs via disassembling of MoS_2-DNA due to stronger binding of ATP with linking aptamers as high level of ATP molecules were present in many cancer cells. MoS_2 nanosheets provide protective shell to DNA and reduced the chance of nuclease digestion which further enhanced targeted Dox mediated drug delivery response.

Small interfering RNA (siRNA) works within the fundamental RNA interference (RNAi) in eukaryotic cells and regulates the expression of genes by inducing cleavage and targeting few complementary mRNA.[105] They are generally 20-24 bases in length similarly as miRNA and produced by Dicer enzymes in cells.[106] Synthetic or chemically produced siRNA can bind with complementary mRNA to induce a cellular RNAi process.[107, 108]

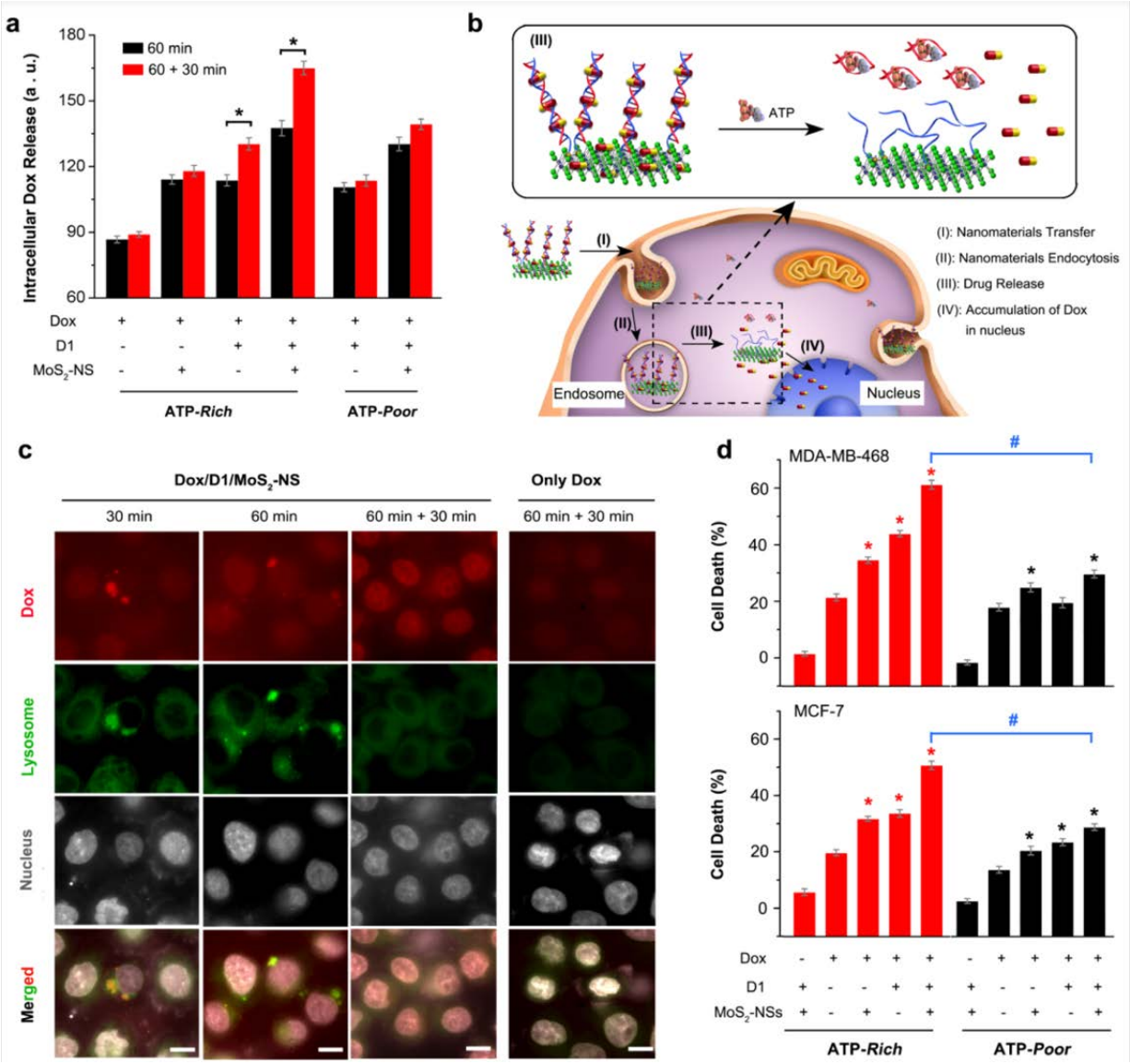


Figure 8. Intracellular Dox targeted delivery and anti-cancerous impact. (a) Dox release in MDA-MB-468 cells. The cells were first precultured in either Hglucose (ATP-rich) or L-glucose (ATP-poor) media for 8 h and afterwards incubated with different Dox-composite groups at 37 °C for 60 min under ATPrich and ATP- poor circumstances, separately. The treated examples were hence hatched with new ATP-rich and ATP-poor media for extra 30 min in the wake of eliminating the overabundance Dox/transporters. (b) Schematic outline showing intracellular cycles of medication conveyance and ATP-incited discharge from DOX/D1/MoS2-NS. The inset demonstrated the job of endosomes in the conveyance of Dox/D1/MoS2-NS to the core. (c) Intracellular conveyance of Dox/D1/MoS2-NS for various hatching times saw by fluorescence microscopy. The nuclei (grey) were stained by Hoechst 33342, and the late endosomes and lysosomes (green) were stained by LysoTracker Green. Scale bar: 10 μm. (d) In vitro cytotoxicity impact of Dox/D1/MoS2-NS on MDA-MB-468 and MCF-7 under intracellular ATP-rich and ATP-poor circumstances. Information is implied ± SD, n = 3, Student's t-test, $p < 0.05$, *significant when contrasted with the Dox-possibly bunch, # huge when contrasted with the relating ATP-poor group.[104] Reproduced with permission from reference 104.

These chemically synthesized siRNA are a major part of nucleic acid based drug candidates for RNAi therapies. New methodology have been invented to deliver siRNAs conjugated with nanomaterials, polymers and lipids however these conventional systems cannot be controlled for specific sizes, compositions and surface chemistry which leads to non-specific toxicity, lack of selectivity and inferior performance.[109-111] To avoid such challenges, DNA nanodevices with well define controllable size, and flexible component strands can be easily synthesized to carry siRNA with DNA-RNA hybridization. Lee and co-workers fabricated DNA tetrahedrons for siRNA delivery to tumour through silencing target genes.[83] DNA tetrahedron size was specifically controlled around 30 nm to avoid renal filtration which is a normal elimination step of siRNA. They formulated tetrahedron with spatial orientation of cancer targeting ligand molecules such as peptides and folate, the density of peptides and

folate was optimized on the DNA tetrahedron surfaces as well. The result demonstrated that at least three molecules of folate per tetrahedron was required to deliver siRNA for targeted delivery in cancer cells and appropriate spatial orientations of ligands were required for gene silencing. Targeted gene silencing was achieved when tetrahedron was conjugated with three folate molecules and the local density was maximized otherwise the gene silencing was disappeared. The researcher observed that higher density of folate decorated tetrahedron enhance the intracellular uptake in the cells.

DNA nanodevices can act as a protective shell to siRNA during the delivery process as siRNA does not degrade by nuclease and these devices provide a biocompatible platform to the cellular environment. Sleiman et al., assembled gene silencing 3D DNA prisms with antisense therapeutic oligonucleotides against mammalian cells.[112] The results demonstrated that antisense strands shackled on nano prisms and induced gene silencing in cells, additionally gene knockdown was maintained effectively as DNA prisms enhanced stability of antisense oligo units. The same research group developed DNA nano suitcase that had ability to encapsulate siRNA and controllable release of siRNA with oligonucleotide trigger and mRNA/microRNA provided as a trigger in this case. These nano suitcases are potentially stable in biological conditions and protect siRNA cargos.

Aptamers are short single stranded DNA or RNA molecules that recognise and selectively bind to wide ranges of specific targets including small molecules, peptides, proteins and carbohydrates. Aptamers are versatile, highly specific, selective and tendency to form various shapes due to their tendency to form single stranded loops and helices. Aptamers can be incorporated into DNA nanodevices to assist them to target specific cells for drug delivery.[113] Aptamers are smart drug carriers as they can sense and recognise the suitable cellular environment to deliver drug molecules via DNA nanodevices. DNA nanodevices can make aptamers more stable and resistant to nuclease degradation and enhance the specific drug efficiency.[93] Aptamers based therapeutics are advantageous than protein therapeutics as it offers flexibility in terms of size, synthetic production and modification is possible by different chemistry. Aptamer linked DNA nanodevices have been synthesized and studied by various researchers across the globe to assist targeted drug delivery.[114, 115] One of the most popular aptamer is AS1411 and it has been used as a cancer targeting ligand.[116] AS1411 has nucleolin receptor which is present in several cancer cells and glycoprotein upregulated on the plasma membrane in cancer cells.[117-119] Bermudez et al., synthesized multiple AS1411 linked pyramidal DNA nano constructs and investigated cellular uptake and efficacy in HeLa cells.[79] The results demonstrated that significant cellular uptake and growth retardation was observed by AS1411-DNA pyramids in HeLa cells in absence of transfection reagents. Additionally, the aptamer linked DNA nano constructs was more stable against nuclease degradation compare to bare aptamers. To maximize the drug efficacy, aptamers have been generally co-delivered with various drug molecules. Huang and co-workers engineered aptamer linked Dox-intercalated icosahedron against MUC1 positive cells.[120] The MUC1 is a class of surface marker that is abundantly expressed in cancer cells and this aptamer targets MUC1 marker. The confocal imaging results demonstrated that aptamer linked DNA nanostructure was significantly internalized by MUC1 positive cells and Dox molecules distribution killed cancer epithelial cells.

These studies and observations strongly stipulated that DNA nanostructures can be fabricated with various drug molecules, siRNA and aptamers to enhance physiological specificity and it widely covers the most demanding cancer therapeutics.

Biosensing

In the field of biological detection, researchers across the globe have developed methods to detect biomolecules, proteins and nucleic acid. However, the major challenges are associated with these methods due to their poor specificity, cost and false positive indications.[121] Polymerase chain reaction (PCR) is widely used technique to analyse nucleic acid however the requirements and cost of the instruments are the limiting factors for their use in clinical applications.[122] Similarly, western blot technique is considered as a standard method to detect proteins though it is challenging to perform *in situ* protein localization and sensitivity of this technique is not satisfactory.[121, 123, 124] To overcome these challenges researchers have been dedicated themselves to invent and develop new techniques such as DNA nanotechnology and DNA nanodevices has grown rapidly for biosensing applications from past two decades.[93]

Circulating tumour DNA (ctDNA) is found in peripheral blood stream that comes from cancerous cells or circulating tumour, it is a generally single or double stranded DNA fragment.[125] ctDNA is a potential biomarker for cancer progression and any changes in the ctDNA content correlates to recurrence, drug treatment and metastasis which make it an attractive marker for the tumour evaluation.[126] DNA sequencing and PCR are common techniques to detect ctDNA however chemical factors that are involved in PCR technique may interfere and resulting false positive/negative results and DNA sequencing is expensive technique and need longer detection time hence the DNA nanotechnology provides a novel detection method for ctDNA.[127, 128] Li and co-workers synthesized doxorubicin linked tetrahedron gold nanomaterial (DOX@TDN-Au) via rolling circle amplification (RCA) method.[129] These nanodevice worked as an electrochemical indicator to develop a multi-legged, highly integrated DNA roller electrochemical biosensor and capture probes and ctDNA template.

During the tumour metastasis, few tumour cells are survived in the blood circulatory system and known as circulatory tumour cells (CTC) and due to diagnosis, treatment and metastasis stages, these CTC are released in the peripheral blood circulation.[130] These CTC circulates throughout the body via blood and form secondary tumours if the phenotype of CTC is aggressive therefore, it is important to know the status, presence and nature of CTC in cancer patients. However CTC are low in number thus difficult to track and capture to analyse in cancer patients.[130, 131] Miao's group has fabricated very sensitive multipedal DNA walker for CTC detection and results demonstrated that these nanodevices capture CTC faster than

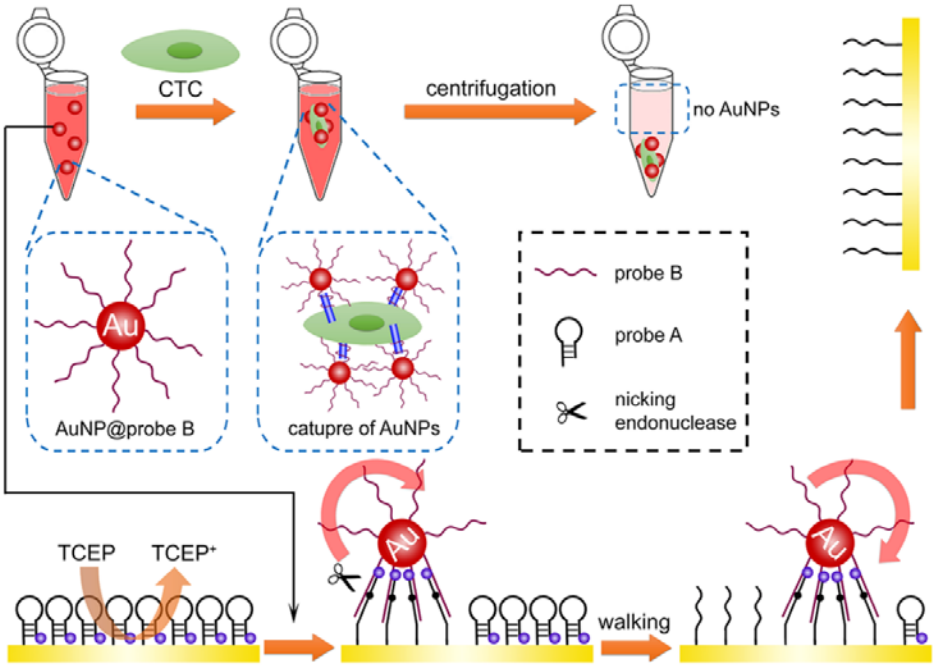


Figure 9. Representation of the Cytosensor Based on Multipedal DNA Walking Strategy ultrasensitive recognition of CTC. AuNPs are utilized to stack different "legs" for DNA strolling, and electrochemical intensification by TCEP is utilized to improve the signal.[132] Reproduced with permission from reference 132.

other conventional techniques (Figure 9). The researchers have compared variation of UV–vis absorbance and electrochemical responses to acknowledge the ultra-sensitive CTC detection via DNA walker.[131, 132]

CONCLUSIONS, CHALLENGES AND FUTURE PERSPECTIVES

Recent advancements and developments in DNA nanotechnology have clearly demonstrated the drug carrier potential of DNA nanodevices. The natural biocompatibility and biodegradability of DNA is suitable for DNA nanodevices thus it is highly recommended targeted drug delivery carrier. The dynamic and well define 2D and 3D nanodevices have been fabricated to deliver specific cargos at specific sites by active or passive release. The highly programmable DNA nanodevices can be modified chemically with various functional groups result in fabrication of flexible and convertible DNA nanostructures. For example, gold nanoparticles and lipid molecules can be linked with thiolated end labelling DNA for the assembly of DNA nanostructure-gold nanoparticles and DNA nanostructure-liposomes.[133-136]

Various designing strategies such as origami based approaches, tile based approaches, nanoparticles conjugations, RCA and metal assisted have been used to synthesize flexible DNA nanodevices to enhance their cellular uptake, retention time, circulation time, change their surface charges, chemical compositions, mechanical properties and additional functions to recognise and responding capacity to specific environments. The unique physico-chemical properties of DNA nanodevices based drug delivery systems is the fabrication and flexibilities that the various ligands and drug molecules can be precisely controlled at a nanoscale with desired shape and size. The flexibility and tunability of DNA nano devices provide a robust platform to bring multiple characteristics in a programmable and controllable manner for biological applications. Last two decades have witnessed the remarkable transition of DNA nanodevices from *in vitro* to *in vivo* environments. DNA nanodevices structures such as origami based, tetrahedron and octahedron have been synthesized to carry small molecules, aptamers, antibodies, nanoparticles, siRNA and drug molecules for different biological applications such as biosensing, bio imaging and drug delivery. A reasonable understanding and efforts on applying DNA nanotechnology for biological applications will have a huge impact in healthcare sectors in the near future as this nascent and developing field has much more to offer.

Given the current knowledge and advantages of DNA nanotechnology there are certain hindrance that need to be overcome for wholesome understanding of the behaviour of DNA nanodevices applications in healthcare sectors.

- DNA nanodevices are competitive with other delivery cargos including liposomes, viruses and bio-polymers. The pharmacokinetics such as adsorption, distribution, circulation and excretion of DNA nanostructures is not well understood. It has been reported that origami-based DNA constructs hold the structural integrity in cell lysate for the longer time however, changes in chemical and physical properties, size and surface charges, modification in oligonucleotides and base combinations is unclear which directly affect the bioavailability and pharmacokinetics. Blood brain barrier and cellular plasma membrane restrict the entry of DNA nanostructures. A majority of literature still covers the cellular uptake of various DNA nanodevices however only few studies focused for penetration of DNA nanodevices in blood brain barrier. Recent studies focused on the insufficient information of cellular uptake through endocytosis or pinocytosis pathways for DNA nanodevices thus it is important to invent new designing approaches to fabricate DNA nanodevices which specifically target organs and reduce the nonselective uptake via normal cells or organs. Additionally, the top qualities such as self-assembly, programable and controllable makes DNA nanodevices superior candidate to overcome such challenges.

- Biosafety is another obstacle for DNA nanotechnology as DNA is biocompatible and biodegradable however these properties may change when DNA is linked or conjugated with another molecules and become DNA nanostructures. The long-term effects and systematically investigation of the potential immunostimulatory response generated by DNA nanostructure should be addressed before using DNA nanostructures for clinical applications. To date, majority of studies primly focused on the well performance of DNA nanostructures in biological applications for example, Anderson et al., investigated that DNA tetrahedron can successfully deliver siRNA into cells and silence target genes in tumour.[83] Additionally, in vivo mouse model did not show any antibody generation response against DNA tetrahedron. However, few critical questions remain unanswered; What would be the potential effects in renal filtration system that exposed by DNA nanostructures? What would be the final cellular fate of nanoparticle conjugated DNA nanodevices as DNA is biocompatible an biodegradable material but nanoparticles do not exhibit such properties; Synthetic DNA sequences may cause adverse gene recombination when conjugated with DNA nanostructures? We assume that these questions related to biosafety should be addressed in futuristic developments of DNA nanotechnology.
- The synthesis, production cost and low yield are major concerns for DNA nanotechnology. The DNA nano constructs with high purity at a gram scale is a minimal requirement for biological applications. Few groups have synthesized and purified DNA nano constructs with cost effective and convenient manner at laboratory scale however these protocols at larger scale has not been reported.[137] Shin et al., demonstrated the purification of high yielded DNA origami nanostructures based on agarose gel separation method.[137] Lin and co-workers developed the contamination free and scalable means to concentrate DNA origami nano constructs via ultracentrifugation.[138] However, we are still far to fill the big gap to produce and purify the DNA nanostructures at larger scale that can cost less.

The cellular environment evolved to manipulate DNA in a certain way and strictly maintain the total amount of DNA at a certain level.[139-141] The penetration of DNA nano constructs temporary increase the DNA levels in cells resulting unexpected cellular changes. DNA nanostructures can be replaced with RNA nanostructures to carry and deliver bio molecules, drugs, polymers, and nanoparticles into cells as an alternative approach. RNA is DNA's biological counterpart and more versatile; researchers have already fabricated RNA nanostructures that designed based on DNA nanotechnology principles and used for targeted drug delivery.[142] As an alternative approach of DNA nanotechnology and detailed understanding of RNA nanotechnology is worthy to explore for biomedical applications, especially for clinical research and healthcare sectors.[143, 144]

The designer DNA nano constructs have been fabricated and studies in various fields such as biology, material science, chemistry, physics and computer science to address unexplored important questions for living systems. DNA nanotechnology's interdisciplinary research will continuously grow to tackle new ideas to broaden the DNA nano constructs engineering and applications. DNA nanotechnology will provide the detailed information to understand the interactions at nanoscale-molecular level which ultimately useful for biomedical sectors.

ACKNOWLEDGEMENTS

Authors sincerely thank all the members of DB group for critically reading the manuscript and their valuable feedback. KK thanks SERB-DST GoI for the National postdoctoral fellowship (NPDF). DB thanks SERB, GoI for Ramanujan Fellowship, IITGN, for the startup grant, and DBT-EMR, Gujcost-DST, GSBTM and BRNS-BARC for research grants.

Conflict of interest: Authors declare no conflict of interest.

REFERENCES

1. P. Patel, N. Meghani, K. Kansara and A. Kumar, *Current drug metabolism*, 2019, **20**, 430-445.
2. K. Kansara, S. Bolan, D. Radhakrishnan, T. Palanisami, H. Ala'a, N. Bolan, A. Vinu, A. Kumar and A. Karakoti, *Environmental Pollution*, 2021, 118726.
3. K. Kansara, A. Kumar and A. S. Karakoti, *Science of the Total Environment*, 2020, **698**, 134133.
4. M. R. Jones, N. C. Seeman and C. A. Mirkin, *Spherical Nucleic Acids*, 2020, 167-197.
5. K. N. Ganesh and Y. Krishnan, *J. Org. Chem.*, 2013, **78**, 12283-12287.
6. N. C. Seeman, *Nature*, 2003, **421**, 427-431.
7. G. C. Pugh, J. R. Burns and S. Howorka, *Nature Reviews Chemistry*, 2018, **2**, 113-130.
8. P.-S. Huang, S. E. Boyken and D. Baker, *Nature*, 2016, **537**, 320-327.
9. N. C. Seeman, *Journal of theoretical biology*, 1982, **99**, 237-247.
10. J. Yan, C. Hu, P. Wang, B. Zhao, X. Ouyang, J. Zhou, R. Liu, D. He, C. Fan and S. Song, *Angewandte Chemie International Edition*, 2015, **54**, 2431-2435.
11. W. Ma, Y. Zhan, Y. Zhang, C. Mao, X. Xie and Y. Lin, *Signal transduction and targeted therapy*, 2021, **6**, 1-28.
12. N. C. Seeman and H. F. Sleiman, *Nature Reviews Materials*, 2017, **3**, 1-23.
13. N. R. Kallenbach, R.-I. Ma and N. C. Seeman, *Nature*, 1983, **305**, 829-831.
14. J. Chen and N. C. Seeman, *Nature*, 1991, **350**, 631-633.
15. T. J. Fu and N. C. Seeman, *Biochemistry*, 1993, **32**, 3211-3220.
16. X. Li, X. Yang, J. Qi and N. C. Seeman, *J. Am. Chem. Soc.*, 1996, **118**, 6131-6140.
17. E. Winfree, F. Liu, L. A. Wenzler and N. C. Seeman, *Nature*, 1998, **394**, 539-544.
18. Y. He, Y. Chen, H. Liu, A. E. Ribbe and C. Mao, *J. Am. Chem. Soc.*, 2005, **127**, 12202-12203.
19. Y. He, Y. Tian, A. E. Ribbe and C. Mao, *J. Am. Chem. Soc.*, 2006, **128**, 15978-15979.
20. X. Wang, R. Sha, M. Kristiansen, C. Hernandez, Y. Hao, C. Mao, J. W. Canary and N. C. Seeman, *Angewandte Chemie Int. Ed.*, 2017, **56**, 6445-6448.
21. D. Liu, M. Wang, Z. Deng, R. Walulu and C. Mao, *J. Am. Chem. Soc.*, 2004, **126**, 2324-2325.
22. S. Hamada and S. Murata, *Angewandte Chemie*, 2009, **121**, 6952-6955.
23. G. D. Hamblin, J. F. Rahbani and H. F. Sleiman, *Nature communications*, 2015, **6**, 1-8.
24. P. W. Rothemund, *Nature*, 2006, **440**, 297-302.
25. H. Yan, T. H. LaBean, L. Feng and J. H. Reif, *Proceedings of the National Academy of Sciences*, 2003, **100**, 8103-8108.
26. W. M. Shih, J. D. Quispe and G. F. Joyce, *Nature*, 2004, **427**, 618-621.
27. B. Wei, M. Dai and P. Yin, *Nature*, 2012, **485**, 623-626.

28. F. A. Aldaye and H. F. Sleiman, *J. Am. Chem. Soc.*, 2007, **129**, 13376-13377.
29. Y. He, T. Ye, M. Su, C. Zhang, A. E. Ribbe, W. Jiang and C. Mao, *Nature*, 2008, **452**, 198-201.
30. C. Tian, X. Li, Z. Liu, W. Jiang, G. Wang and C. Mao, *Angewandte Chemie*, 2014, **126**, 8179-8182.
31. E. Benson, A. Mohammed, J. Gardell, S. Masich, E. Czeizler, P. Orponen and B. Högberg, *Nature*, 2015, **523**, 441-444.
32. F. Zhang, S. Jiang, S. Wu, Y. Li, C. Mao, Y. Liu and H. Yan, *Nature nanotechnology*, 2015, **10**, 779-784.
33. C. Lin, Y. Liu, S. Rinker and H. Yan, *ChemPhysChem*, 2006, **7**, 1641-1647.
34. Y. Zhang and N. C. Seeman, *J. Am. Chem. Soc.*, 1994, **116**, 1661-1669.
35. R. P. Goodman, R. M. Berry and A. J. Turberfield, *Chemical Communications*, 2004, 1372-1373.
36. A. Rajwar, S. Kharbanda, A. R. Chandrasekaran, S. Gupta and D. Bhatia, *ACS Applied Bio Materials*, 2020, **3**, 7265-7277.
37. Z. Nie, X. Li, Y. Li, C. Tian, P. Wang and C. Mao, *Chemical communications*, 2013, **49**, 2807-2809.
38. Y. Yu, B. Jin, Y. Li and Z. Deng, *Chemistry–A European Journal*, 2019, **25**, 9785-9798.
39. Z. Liu, Y. Li, C. Tian and C. Mao, *Biomacromolecules*, 2013, **14**, 1711-1714.
40. M. B. Scheible, L. L. Ong, J. B. Woehrstein, R. Jungmann, P. Yin and F. C. Simmel, *Small*, 2015, **11**, 5200-5205.
41. D. Bhatia, S. Mehtab, R. Krishnan, S. S. Indi, A. Basu and Y. Krishnan, *Angewandte Chemie Int. Ed.*, 2009, **48**, 4134-4137.
42. F. A. Aldaye and H. F. Sleiman, *Angewandte Chemie International Edition*, 2006, **45**, 2204-2209.
43. F. A. Aldaye and H. F. Sleiman, *J. Am. Chem. Soc.*, 2007, **129**, 4130-4131.
44. D. Han, S. Pal, J. Nangreave, Z. Deng, Y. Liu and H. Yan, *Science*, 2011, **332**, 342-346.
45. Y. Ke, J. Sharma, M. Liu, K. Jahn, Y. Liu and H. Yan, *Nano letters*, 2009, **9**, 2445-2447.
46. R. M. Zadegan and M. L. Norton, *Int. j. mol. sci.*, 2012, **13**, 7149-7162.
47. E. S. Andersen, M. Dong, M. M. Nielsen, K. Jahn, R. Subramani, W. Mamdouh, M. M. Golas, B. Sander, H. Stark and C. L. Oliveira, *Nature*, 2009, **459**, 73-76.
48. K. Jahn, R. Subramani, W. Mamdouh, M. Golas, B. Sander, H. Stark and C. Oliveira, *Nat. Lett*, 2009, **459**, 73.
49. F. Hong, F. Zhang, Y. Liu and H. Yan, *Chemical reviews*, 2017, **117**, 12584-12640.
50. P. Wang, M. A. Rahman, Z. Zhao, K. Weiss, C. Zhang, Z. Chen, S. J. Hurwitz, Z. G. Chen, D. M. Shin and Y. Ke, *J. Am. Chem. Soc.*, 2018, **140**, 2478-2484.
51. H. Lv, S. Zhang, B. Wang, S. Cui and J. Yan, *Journal of controlled release*, 2006, **114**, 100-109.
52. N. L. Rosi, D. A. Giljohann, C. S. Thaxton, A. K. Lytton-Jean, M. S. Han and C. A. Mirkin, *Science*, 2006, **312**, 1027-1030.
53. O. Farokhzad, *Nat. Nanotechnol*, 2007, **2**, 751.
54. P. Wang, T. A. Meyer, V. Pan, P. K. Dutta and Y. Ke, *Chem*, 2017, **2**, 359-382.
55. H. Dietz, S. M. Douglas and W. M. Shih, *Science*, 2009, **325**, 725-730.
56. S. M. Douglas, H. Dietz, T. Liedl, B. Högberg, F. Graf and W. M. Shih, *Nature*, 2009, **459**, 414-418.
57. Y. T. E. Chiu, H. Li and C. H. J. Choi, *Small*, 2019, **15**, 1805416.
58. K.-R. Kim, H. Y. Kim, Y.-D. Lee, J. S. Ha, J. H. Kang, H. Jeong, D. Bang, Y. T. Ko, S. Kim and H. Lee, *J. Controlled Release*, 2016, **243**, 121-131.
59. M. A. Rahman, P. Wang, Z. Zhao, D. Wang, S. Nannapaneni, C. Zhang, Z. Chen, C. C. Griffith, S. J. Hurwitz and Z. G. Chen, *Angewandte Chemie Int. Ed.*, 2017, **56**, 16023-16027.
60. Q. Mou, Y. Ma, G. Pan, B. Xue, D. Yan, C. Zhang and X. Zhu, *Angewandte Chemie*, 2017, **129**, 12702-12706.
61. Q. Li, D. Zhao, X. Shao, S. Lin, X. Xie, M. Liu, W. Ma, S. Shi and Y. Lin, *ACS applied materials & interfaces*, 2017, **9**, 36695-36701.
62. S. Raniolo, G. Vindigni, A. Ottaviani, V. Unida, F. Iacovelli, A. Manetto, M. Figini, L. Stella, A. Desideri and S. Biocca, *Nanomedicine: Nanotechnology, Biology and Medicine*, 2018, **14**, 1181-1190.
63. S. Behzadi, V. Serpooshan, W. Tao, M. A. Hamaly, M. Y. Alkawareek, E. C. Dreaden, D. Brown, A. M. Alkilany, O. C. Farokhzad and M. Mahmoudi, *Chemical Society Reviews*, 2017, **46**, 4218-4244.
64. S. D. Perrault and W. M. Shih, *ACS nano*, 2014, **8**, 5132-5140.
65. Y. Tian, Y. Huang, P. Gao and T. Chen, *Chemical Communications*, 2018, **54**, 9394-9397.
66. G. Vindigni, S. Raniolo, A. Ottaviani, M. Falconi, O. Franch, B. R. Knudsen, A. Desideri and S. Biocca, *ACS nano*, 2016, **10**, 5971-5979.
67. J. H. Kang, K.-R. Kim, H. Lee, D.-R. Ahn and Y. T. Ko, *Colloids and Surfaces B: Biointerfaces*, 2017, **157**, 424-431.
68. K. Xia, H. Kong, Y. Cui, N. Ren, Q. Li, J. Ma, R. Cui, Y. Zhang, J. Shi and Q. Li, *ACS applied materials & interfaces*, 2018, **10**, 15442-15448.
69. X. Shen, Q. Jiang, J. Wang, L. Dai, G. Zou, Z.-G. Wang, W.-Q. Chen, W. Jiang and B. Ding, *Chemical communications*, 2012, **48**, 11301-11303.
70. P. D. Halley, C. R. Lucas, E. M. McWilliams, M. J. Webber, R. A. Patton, C. Kural, D. M. Lucas, J. C. Byrd and C. E. Castro, *Small*, 2016, **12**, 308-320.
71. S. Raniolo, G. Vindigni, V. Unida, A. Ottaviani, E. Romano, A. Desideri and S. Biocca, *Nanoscale*, 2018, **10**, 12078-12086.
72. J. Li, C. Fan, H. Pei, J. Shi and Q. Huang, *Advanced materials*, 2013, **25**, 4386-4396.
73. H. Pei, X. Zuo, D. Pan and J. Shi, *Acc. Chem. Res*, 2014, **47**, 550-559.
74. D. S. Lee, H. Qian, C. Y. Tay and D. T. Leong, *Chemical Society Reviews*, 2016, **45**, 4199-4225.
75. K.-R. Kim, S. J. Kang, A.-Y. Lee, D. Hwang, M. Park, H. Park, S. Kim, K. Hur, H. S. Chung and C. Mao, *Biomaterials*, 2019, **195**, 1-12.
76. S. Raniolo, S. Croce, R. P. Thomsen, A. H. Okholm, V. Unida, F. Iacovelli, A. Manetto, J. Kjems, A. Desideri and S. Biocca, *Nanoscale*, 2019, **11**, 10808-10818.
77. L. Liang, J. Li, Q. Li, Q. Huang, J. Shi, H. Yan and C. Fan, *Angewandte Chemie International Edition*, 2014, **53**, 7745-7750.
78. J. W. Keum, J. H. Ahn and H. Bermudez, *Small*, 2011, **7**, 3529-3535.
79. P. Charoenphol and H. Bermudez, *Molecular pharmaceutics*, 2014, **11**, 1721-1725.
80. H. Pei, L. Liang, G. Yao, J. Li, Q. Huang and C. Fan, *Angewandte Chemie International Edition*, 2012, **51**, 9020-9024.
81. A. Lacroix, E. Vengut-Climent, D. de Rochambeau and H. F. Sleiman, *ACS Central Science*, 2019, **5**, 882-891.
82. D. Bhatia, S. Surana, S. Chakraborty, S. P. Koushika and Y. Krishnan, *Nature communications*, 2011, **2**, 1-8.
83. H. Lee, A. K. Lytton-Jean, Y. Chen, K. T. Love, A. I. Park, E. D. Karagiannis, A. Sehgal, W. Querbes, C. S. Zurenko and M. Jayaraman, *Nature nanotechnology*, 2012, **7**, 389-393.
84. S. Surana, J. M. Bhat, S. P. Koushika and Y. Krishnan, *Nature communications*, 2011, **2**, 1-7.
85. S. Surana, D. Bhatia and Y. Krishnan, *Methods*, 2013, **64**, 94-100.
86. A. S. Walsh, H. Yin, C. M. Erben, M. J. Wood and A. J. Turberfield, *ACS nano*, 2011, **5**, 5427-5432.
87. D. Bhatia, S. Surana, S. Chakraborty, S. Koushika and Y. Krishnan, *Journal*, 2011.
88. X. Liu, Y. Xu, T. Yu, C. Clifford, Y. Liu, H. Yan and Y. Chang, *Nano letters*, 2012, **12**, 4254-4259.
89. S. Arnon, N. Dahan, A. Koren, O. Radiano, M. Ronen, T. Yannay, J. Giron, L. Ben-Ami, Y. Amir and Y. Hel-Or, *PloS one*, 2016, **11**, e0161227.
90. S. Li, Q. Jiang, S. Liu, Y. Zhang, Y. Tian, C. Song, J. Wang, Y. Zou, G. J. Anderson and J.-Y. Han, *Nature biotechnology*, 2018, **36**, 258-264.
91. C. Wang, H. Zhang, D. Zeng, L. San and X. Mi, *Chinese Journal of Chemistry*, 2016, **34**, 299-307.
92. C. M. Green, D. A. Hastman, D. Mathur, K. Susumu, E. Oh, I. L. Medintz and S. A. Díaz, *ACS nano*, 2021, **15**, 9101-9110.
93. Q. Hu, H. Li, L. Wang, H. Gu and C. Fan, *Chemical reviews*, 2018, **119**, 6459-6506.

94. C. Carvalho, R. X. Santos, S. Cardoso, S. Correia, P. J. Oliveira, M. S. Santos and P. I. Moreira, *Current medicinal chemistry*, 2009, **16**, 3267-3285.
95. O. Tacar, P. Sriamornsak and C. R. Dass, *Journal of pharmacy and pharmacology*, 2013, **65**, 157-170.
96. T. Yang, B. Li, S. Qi, Y. Liu, Y. Gai, P. Ye, G. Yang, W. Zhang, P. Zhang and X. He, *Theranostics*, 2014, **4**, 1096.
97. G. Unsoy, R. Khodadust, S. Yalcin, P. Mutlu and U. Gunduz, *European Journal of Pharmaceutical Sciences*, 2014, **62**, 243-250.
98. X.-Y. Ke, V. W. L. Ng, S.-J. Gao, Y. W. Tong, J. L. Hedrick and Y. Y. Yang, *Biomaterials*, 2014, **35**, 1096-1108.
99. Y.-X. Zhao, A. Shaw, X. Zeng, E. Benson, A. M. Nystrom and B. r. Högberg, *ACS nano*, 2012, **6**, 8684-8691.
100. Q. Jiang, C. Song, J. Nangreave, X. Liu, L. Lin, D. Qiu, Z.-G. Wang, G. Zou, X. Liang and H. Yan, *Journal of the American Chemical Society*, 2012, **134**, 13396-13403.
101. Q. Zhang, Q. Jiang, N. Li, L. Dai, Q. Liu, L. Song, J. Wang, Y. Li, J. Tian and B. Ding, *ACS nano*, 2014, **8**, 6633-6643.
102. C. Wu, D. Han, T. Chen, L. Peng, G. Zhu, M. You, L. Qiu, K. Sefah, X. Zhang and W. Tan, *Journal of the American Chemical Society*, 2013, **135**, 18644-18650.
103. L. Mei, G. Zhu, L. Qiu, C. Wu, H. Chen, H. Liang, S. Cansiz, Y. Lv, X. Zhang and W. Tan, *Nano research*, 2015, **8**, 3447-3460.
104. B. L. Li, M. I. Setyawati, L. Chen, J. Xie, K. Ariga, C.-T. Lim, S. Garaj and D. T. Leong, *ACS applied materials & interfaces*, 2017, **9**, 15286-15296.
105. G. J. Hannon, *nature*, 2002, **418**, 244-251.
106. E. Bernstein, A. A. Caudy, S. M. Hammond and G. J. Hannon, *Nature*, 2001, **409**, 363-366.
107. A. Reynolds, D. Leake, Q. Boese, S. Scaringe, W. S. Marshall and A. Khvorova, *Nature biotechnology*, 2004, **22**, 326-330.
108. K. A. Whitehead, R. Langer and D. G. Anderson, *Nature reviews Drug discovery*, 2009, **8**, 129-138.
109. J.-S. Lee, J. J. Green, K. T. Love, J. Sunshine, R. Langer and D. G. Anderson, *Nano letters*, 2009, **9**, 2402-2406.
110. M. E. Davis, J. E. Zuckerman, C. H. J. Choi, D. Seligson, A. Tolcher, C. A. Alabi, Y. Yen, J. D. Heidel and A. Ribas, *Nature*, 2010, **464**, 1067-1070.
111. S. C. Semple, A. Akinc, J. Chen, A. P. Sandhu, B. L. Mui, C. K. Cho, D. W. Sah, D. Stebbing, E. J. Crosley and E. Yaworski, *Nature biotechnology*, 2010, **28**, 172-176.
112. K. E. Bujold, J. C. Hsu and H. F. Sleiman, *Journal of the American Chemical Society*, 2016, **138**, 14030-14038.
113. A. D. Keefe, S. Pai and A. Ellington, *Nature reviews Drug discovery*, 2010, **9**, 537-550.
114. W. J. Kang, J. R. Chae, Y. L. Cho, J. D. Lee and S. Kim, *Small*, 2009, **5**, 2519-2522.
115. J. W. Kotula, E. D. Pratico, X. Ming, O. Nakagawa, R. L. Juliano and B. A. Sullenger, *Nucleic acid therapeutics*, 2012, **22**, 187-195.
116. P. J. Bates, E. W. Choi and L. V. Nayak, in *Gene Therapy of Cancer*, Springer, 2009, pp. 379-392.
117. S. Soundararajan, L. Wang, V. Sridharan, W. Chen, N. Courtenay-Luck, D. Jones, E. K. Spicer and D. J. Fernandes, *Molecular pharmacology*, 2009, **76**, 984-991.
118. F. Mongelard and P. Bouvet, *Current opinion in molecular therapeutics*, 2010, **12**, 107-114.
119. E. M. Reyes-Reyes, Y. Teng and P. J. Bates, *Cancer research*, 2010, **70**, 8617-8629.
120. M. Chang, C.-S. Yang and D.-M. Huang, *ACS nano*, 2011, **5**, 6156-6163.
121. S. Yu, T. Chen, Q. Zhang, M. Zhou and X. Zhu, *Analyst*, 2020, **145**, 3481-3489.
122. Y. Zhao, F. Chen, Q. Li, L. Wang and C. Fan, *Chemical reviews*, 2015, **115**, 12491-12545.
123. R. Lund, R. Leth-Larsen, O. N. Jensen and H. J. Ditzel, *Journal of proteome research*, 2009, **8**, 3078-3090.
124. Y. Shi, R. Xiang, C. Horváth and J. A. Wilkins, *Journal of proteome research*, 2005, **4**, 1427-1433.
125. M. Stroun, J. Lyautey, C. Lederrey, A. Olson-Sand and P. Anker, *Clinica chimica acta*, 2001, **313**, 139-142.
126. T. N. Beck, A. J. Chikwem, N. R. Solanki and E. A. Golemis, *Physiological genomics*, 2014, **46**, 699-724.
127. C. Alix-Panabières, H. Schwarzenbach and K. Pantel, *Annual review of medicine*, 2012, **63**, 199-215.
128. J. Das, I. Ivanov, E. H. Sargent and S. O. Kelley, *Journal of the American Chemical Society*, 2016, **138**, 11009-11016.
129. D. Li, Y. Xu, L. Fan, B. Shen, X. Ding, R. Yuan, X. Li and W. Chen, *Biosensors and Bioelectronics*, 2020, **148**, 111826.
130. M. Yu, S. Stott, M. Toner, S. Maheswaran and D. A. Haber, *Journal of Cell Biology*, 2011, **192**, 373-382.
131. B. Hong and Y. Zu, *Theranostics*, 2013, **3**, 377.
132. P. Miao and Y. Tang, *Analytical Chemistry*, 2019, **91**, 15187-15192.
133. C. A. Mirkin, R. L. Letsinger, R. C. Mucic and J. J. Storhoff, *Nature*, 1996, **382**, 607-609.
134. R. C. Mucic, J. J. Storhoff, C. A. Mirkin and R. L. Letsinger, *J. Am. Chem. Soc.*, 1998, **120**, 12674-12675.
135. S. J. Park, A. A. Lazarides, C. A. Mirkin and R. L. Letsinger, *Angewandte Chemie International Edition*, 2001, **40**, 2909-2912.
136. S.-J. Park, A. A. Lazarides, J. J. Storhoff, L. Pesce and C. A. Mirkin, *The Journal of Physical Chemistry B*, 2004, **108**, 12375-12380.
137. G. Bellot, M. A. McClintock, C. Lin and W. M. Shih, *Nature methods*, 2011, **8**, 192-194.
138. C. Lin, S. D. Perrault, M. Kwak, F. Graf and W. M. Shih, *Nucleic acids research*, 2013, **41**, e40-e40.
139. A. Mirsky and H. Ris, *The Journal of general physiology*, 1951, **34**, 451.
140. T. Cavalier-Smith, *Journal of cell science*, 1978, **34**, 247-278.
141. T. R. Gregory, *Biological reviews*, 2001, **76**, 65-101.
142. B. Schwanhäusser, D. Busse, N. Li, G. Dittmar, J. Schuchhardt, J. Wolf, W. Chen and M. Selbach, *Nature*, 2011, **473**, 337-342.
143. K. A. Afonin, E. Bindewald, A. J. Yaghoubian, N. Voss, E. Jacovetty, B. A. Shapiro and L. Jaeger, *Nature nanotechnology*, 2010, **5**, 676-682.
144. W. W. Grabow and L. Jaeger, *Accounts of chemical research*, 2014, **47**, 1871-1880.

AUTHORS BIOGRAPHIES

Ashutosh Kumar works as an Associate Professor in the Division of Biological and Life Sciences, School of Arts and Sciences, Ahmedabad University, Gujarat, India. His current research areas include nanomedicines for cancer and arthritis therapy, DNA Biochip for pathogen detection, nano-based drug and gene delivery, nanoemulsions for food industries, and environmental nanotechnology. His group works at the nano-bio interface and is passionate about understanding nanotechnology's effects on human life and the environment at large. His deep understanding of the environment, combined with his skills in nanotechnology and his versatile analytical knowledge of different animal systems, allowed him to make significant seminal contributions to environmental nanotechnology. His group is a pioneer in understanding the fate of new chemical entities (NCEs) in aquatic systems by analyzing their interaction with biotic and abiotic factors. He has published more than 60 research papers, three books, and 16 chapters in internationally reputed peer-reviewed journals. He has received several national and international awards, including the “Indian National Science Academy Medal for Young Scientist 2014” in

Health Sciences, National Academy of Sciences, India -Young Scientist Platinum Jubilee Award (2015) in the field of Bio-medical, Molecular Biology and Biotechnology for his scientific contributions.

Krupa Kansara works as a National Postdoctoral Fellow (SERB-NPDF) at the Indian Institutes of Technology Gandhinagar (IITGN). She obtained her master's and Doctorate degrees from Ahmedabad University, India. She has been involved in studying the potential effect of nanoparticles on human cells and aquatic organisms including zebrafish and Tetrahymena. Her understanding of the role of abiotic factors in influencing the nanotechnology outcomes of the biotic community is outstanding and by far an exemplar in the field. Her study makes a significant contribution to understanding the possible negative effects of novel technological solutions and the ways to mitigate them. She has published more than 17 research papers including three book chapters in internationally reputed peer-reviewed journals.

Dr. Dhiraj Bhatia received his B.Sc. in Chemistry from KTHM College Nashik, University of Pune in 2005, followed by M.Sc. in Organic Chemistry from Department of Chemistry, University of Pune in 2007 both with rank 1. He then moved to NCBS-TIFR Bangalore for his PhD under the supervision of Prof. Yamuna Krishnan in the field of Structural DNA Nanotechnology. He received Best PhD thesis award from Lily foundation and TAA-Zita Lobo award in 2013. He then moved to Institut Curie in Paris, France to pursue his postdoctoral research in the department of Chemical and Cell Biology with Prof. Ludger Johannes initially as Curie Postdoctoral fellow and later as Human Frontiers Science Program HFSP fellow. He was also selected as Young Scientist to attend 65th Lindau Nobel Laureates Meeting in Germany in 2015. In 2018 he moved back to India to join Indian Institute of Technology Gandhinagar as Assistant Professor and Ramanujan fellow. At IIT Gandhinagar, his lab focusses on the exploration of DNA based nanodevices for translational and biomedical applications. He leads the team of 7 PhD students, 4 postdocs, 1 MTech and 1 JRF. He and his students have received numerous awards and fellowships over last 4 years and the team has published more than 40 international publications in journals like Nanoscale, Traffic, ACS Nano, ACS Biomaterials Sci and Engg, ACS Chemical Neuroscience, Soft Matter, etc. Along with this, Dhiraj also serves as reviewer and member for multiple journals and committees for PhD students, labs, new courses at IITGN and many other institutes across India.