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Designing DNA nanodevices for compatibility with the immune system of higher organisms

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Abstract

DNA is proving to be a powerful scaffold to construct molecularly precise designer DNA devices. Recent trends reveal their ever-increasing deployment within living systems as delivery devices that not only probe but also program and reprogram a cell, or even whole organisms. Given that DNA is highly immunogenic, we outline the molecular, cellular and organismal response pathways that designer nucleic acid nanodevices are likely to elicit in living systems. We address safety issues applicable when such designer DNA nanodevices interact with the immune system. In light of this, we discuss possible molecular programming strategies that could be integrated with such designer nucleic acid scaffolds to either evade or stimulate the host response with a view to optimizing and widening their applications in higher organisms.

Deoxyribonucleic acid (DNA) is central to encoding genetic information in eukaryotic and prokaryotic cells, as well as in bacteriophages and viruses. DNA also possesses rich diversity in structure and enzymatic function ¹. It is thus an ideal molecular material in which to integrate versatile chemical functionalities ^{1,2}. DNA is abundant in circulating blood in animals as a result of its release from dying cells; cells therefore constantly come in contact with DNA from extraneous sources (referred to as foreign DNA) that they distinguish as ‘non-self’. This is distinct from endogenous ‘self-DNA’ or the cell’s own genomic DNA present within its nucleus. Mechanisms that differentiate ‘self’ from ‘non-self’ DNA are usually based on simple chemical modifications, for example, cytosine methylation of DNA ³. Exogenous DNA can even function as mobile genetic elements and

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Author contributions

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is responsible for the acquisition of genetic traits 4 . Cells are also capable of recombining non-self DNA with self-DNA. A proportion of non-self DNA is harmful, such as that derived from lytic bacteriophages and viruses, and consequently, mechanisms to rapidly detect and eliminate it have also evolved 5,6 . The interactions of foreign or synthetic DNA with biological systems are therefore multilayered, complex and lead to different outcomes.

DNA has been widely exploited in living systems for bio medical applications as duplex DNA (Fig. 1a) in the form of a synthetic carrier of genetic instructions, such as circularized plasmid DNA (Fig. 1b), and its nanostructured forms (Fig. 1c–g). Nanoparticulate complexes of plasmid DNA with non-immunogenic polymers such as chitosan (Fig. 1c) have been used in vivo for gene delivery 7 . Such nanoparticles are distinct from structural motifs formed by specific sequences of DNA such as the G-quadruplex 8 or i-motif 9 (Fig. 1d). Spherical nucleic acids (SNA; Fig. 1e) represent another distinct class of nanostructured DNA where single-stranded (ss) or double-stranded (ds) DNA are displayed on the surface of inorganic nanoparticles 10 . Importantly, due to its molecular programmability, DNA can be assembled into rationally designed, structurally precise architectures at the nanoscale that are popularly referred to as designer DNA architectures 1 . Designer DNA nanodevices are fabricated using either DNA tiling or DNA origami. DNA tiling exploits hierarchical assembly of structured DNA motifs called tiles using sticky-ended cohesion 1 (Fig. 1f). DNA origami, on the other hand, utilizes multiple, short ‘staple’ strands that hybridize with domains on a large viral-genome-derived ssDNA scaffold, folding it into precise super-architectures 11 (Fig. 1g). Importantly, designer DNA architectures present great potential to probe and program living systems 2 . However, before their successful and wide implementation in higher organisms, it is important to consider the various cellular and systemic responses that such DNA architectures might elicit.

In this Perspective, we discuss examples of DNA nanodevices that have been deployed in multicellular organisms and their modes of introduction. Even though DNA is a natural biopolymer, when present at the wrong place at the wrong time it can elicit a strong inflammatory reaction. We summarize how cells respond to exogenous or endogenous DNA to better understand and possibly anticipate host responses to designer DNA nanodevices. Finally, we outline potential design considerations and immediate challenges that impact the delivery of DNA nanodevices and the corresponding host response with a view to improving tolerance, thereby promoting their wider use.

Biological responses to DNA in higher organisms

Cells protect self-DNA against foreign DNA using surveillance systems that detect non-self DNA and trigger mechanisms for their swift elimination. For example, in bacteria and archaea, sequence-independent and sequence-specific restriction mechanisms 5,6 , and the memory-based adaptable CRISPR/Cas (clustered regularly interspaced short palindromic repeats/CRISPR associated complex) 12 systems direct endonuclease activity against foreign DNA. Thus DNA-based early immune systems evolved from bacteria, diversified in eukaryotes, and are at the heart of mammalian responses to natural DNA.

DNA viruses and bacteriophages are detected by direct binding of their DNA to one or more receptors in the host cell they infect. Therefore when exposed to natural or synthetic nucleic acids, cells respond as they do to viral infection 13 . Eukaryotic cells respond to DNA in two ways; (i) by upregulating interferon (IFN) genes, and (ii) stimulating caspase-1 protease activity through inflammasome signalling pathways 14 . Detection of DNA converges on upregulation and secretion of type I IFNs (IFN- α and IFN- β) 14,15 . Secreted IFNs induce the expression of a large number of IFN-stimulated genes, many of which have broad antiviral and inflammatory roles 15 . DNA-dependent activation of caspase-1 inflammasomes results in the release of cytokines, such as interleukin-1 β and interleukin-18, which have antimicrobial as well as pro-inflammatory properties 16,17 . Defined inflammatory responses to DNA facilitate the long-term adaptive immune response to infection. This feature is exploited in DNA-based vaccines to boost immunity 18 . In other settings, however, chronic expression of IFNs and activation of caspase-1 cause inflammatory tissue damage in the host 19 . Thus, like natural DNA, synthetic DNA nanodevices too are likely to elicit an overall inflammatory and antiviral-like response from the host.

Host cells detect exogenous DNA that is extracellular, vacuolar or cytoplasmic using >20 different receptor proteins 14,19 . The majority elicit IFN secretion, and one (AIM2) directly activates caspase-1 (ref. 20; Fig. 2). Only major DNA receptors are discussed here to provide an overall view of the complex system. For example, dsDNA in the cytosol is bound by receptors such as IFI16, DDX41, cGAS, MRE11 and DNA-PK; all induce IFNs via a protein called STING 14,21 (Fig. 2). On binding cytosolic DNA, cGAS synthesizes a newly identified second messenger — 2',3'-cGAMP (cyclic [G(2',5')pA(3',5')p]) — that allosterically activates STING to enhance IFN responses 22–24 (Fig. 2). Interestingly, A:T-rich dsDNA in the cytosol can be transcribed by RNA polymerase III into 5'PPP-containing RNA that induces IFN expression by binding the RIG-I dsRNA receptor 25 . The protein LRRFIP1 binds cytosolic DNA and induces IFNs via a novel β -catenin pathway 26 . Post endocytosis into vacuoles, dsDNA containing unmethylated CpG motifs is detected by the TLR9 receptor that induces the expression of IFNs independently of STING 14,21 (Fig. 2).

Little is known about nucleotide modifications that affect receptor binding and downstream signalling. One could speculate that self-DNA is protected from cytosolic DNA sensors via compartmentalization into the nucleus, whereas RNA sensors detect specific 'foreign' chemical modifications of RNA distinguishing it from host mRNA. Differential methylation and secondary structures of dsDNA elicit differential responses via the TLR9 receptor. The 2'-deoxyribose sugar is an important determining factor in the context of backbone chemistry (phosphodiester versus phosphorothioate) in two ways. First, ribose sugars are not tolerated on TLR9 ligands. Second, abasic as well as non-CpG-motif-containing 2'-deoxyribose phosphodiester chains activate TLR9, whereas similar phosphorothioate chains inhibit TLR9 27,28 . In the case of the cytosolic AIM2 receptor, sequence-independent detection of ~80 bp dsDNA results in its oligomerization and activation of caspase-1 (ref. 29). In addition, cytosolic DNA receptors might detect oxidation-damaged DNA more readily 30 .

The cellular responses to DNA also depend on the cell type involved; plasmacytoid dendritic cells (pDCs), myeloid DCs (mDCs) and macrophages and T- or B-lymphocytes express different sensors for DNA and thus respond differently 31 . For example, in the case of TLR9, pDCs secrete large amounts of IFN- α when exposed to CpG oligonucleotides, which spontaneously form nano-particles as a result of G-tetrad formation (called Class A CpG) 28,30 . TLR9 activation of B cells by CpG oligonucleotides devoid of complex secondary structures (Class B CpG) leads to cell proliferation 28 . Further, mDCs that detect non-CpG dsDNA via TLR9 trigger suppressor T-cell responses 32 . CpG DNA when presented in synthetic motifs such as SNAs (Fig. 1e) are far more potent stimulators of TLR9 responses than the corresponding ssDNA. This underscores the interplay of DNA structure and cell type in generating an activating or suppressive biological response 33 .

Homeostatic mechanisms prevent unnecessary activation of DNA responses by prompt disposal of cellular DNA at the end of its life cycle 34,35 (Fig. 2). The importance of DNases is revealed in hereditary autoinflammatory syndromes where high levels of circulating DNA are seen, such as systemic lupus erythematosus 36 , Aicardi–Goutières syndrome 37 and rheumatoid arthritis 38 . Mutations in DNase I (encoded by the gene DNASE1) and DNase III (encoded by TREX1) result in increased circulating DNA and IFN expression 37,39,40 . DNase I, a secreted enzyme, degrades DNA from ingested food as well as that in blood. Reduced DNase I activity can result in lupus, a disease that shows features of increased IFN production 41,42 . Similarly, loss of TREX1 results in the accumulation of ~60 b long ssDNA, which drives IFN-dependent pathology 37,39 . Likewise, DNase II (encoded by Dnase2a) deficiency in mice results in IFN-driven autoimmunity, which is reversible if mice are also deficient in IFN receptors or Sting 43,44 . Therefore successful DNA scaffolds must not only fly under the radar to avoid activating antiviral-like inflammatory responses, but must also be amenable to natural and safe disposal from biological systems post-deployment. Thus DNA nanodevices need to incorporate design principles to navigate the multilayered detection and defense machinery of higher organisms.

Designer DNA nanodevices in vivo

A selection of designer DNA nanodevices have been used either as drug-delivery vehicles or diagnostic probes in living systems 2 . Although several nanodevices have been applied to cells in culture, their application in multicellular organisms has only just emerged. This is primarily due to major molecular barriers faced in vivo such as (i) efficient delivery and targeting to the site of interest, (ii) stability of externally introduced DNA nanodevices, and (iii) their potential toxicity in the host organism. Of the limited number of nanodevices deployed in vivo, the DNA icosahedron and tetrahedron present good case studies to discuss these molecular barriers 45,46 .

Currently, in vivo delivery strategies predominantly rely on injections to target DNA nanodevices to specific cell types. The first study of a designer DNA architecture in a multicellular living organism used a pH-sensitive DNA nanodevice, the I-switch, which was microinjected into *Caenorhabditis elegans*; post-injection, the I-switch was targeted to specific scavenger cells that displayed cell-surface anionic ligand-binding receptors (Fig. 3a;

left panel). Once internalized, it was shown that the nanodevice could probe endosomal maturation 47 . This same strategy was exploited to introduce a cargo-loaded DNA icosahedron to scavenger cells, where both cargo functionality and device integrity were quantitatively preserved post-delivery 45,48 (Fig. 3a; right panel).

In mammalian systems, DNA architectures have been delivered intravenously. A general design principle for targeting the nanostructure to the site of interest exploits the display of specific ligands on the nanodevice that enables its binding to a cell-specific endogenous receptor, leading to its cellular uptake. By exploiting the overexpression of the folate receptor on cancerous cells, it was possible to target tetrahedral DNA nanoparticles bearing folate moieties and short interfering RNA (siRNA) to xenograft tumours in nude mice 46 (Fig. 3b; left panel). Post-internalization, the siRNA reduced the expression level of a target gene 46 . However, despite their enrichment in the tumour, some DNA nanoparticles could also be uptaken by non-cancerous cells bearing the folate receptor, given its ubiquitous expression across tissues 49 . Nevertheless, this strategy seems promising to target selected malignancies. Another example is the in vivo delivery of a tetrahedral DNA device displaying antigens and adjuvants to produce antibodies 50 . A model antigen (streptavidin) and adjuvant (CpG deoxyoligonucleotides) were assembled on a DNA tetrahedron and injected into a mouse. Identification of the DNA–streptavidin–CpG complex by circulating macrophages and dendritic cells in the bloodstream led to the production of anti bodies against streptavidin 50 (Fig. 3b; right panel).

Among diverse delivery modes, oral administration is popular in various model organisms, yet surprisingly has not been adopted thus far for DNA nanostructures, probably due to the high susceptibility of DNA to acid-catalysed depurination and nucleases, and its low efficiency in traversing tissue barriers. Often, therapeutic proteins or peptides are packaged within liposomes, nano particles, dendrimers or micelles to protect against proteolysis and low gastrointestinal pH 51,52 . Intranasal delivery presents exciting possibilities. The high surface area and permeability of the nasal endo thelial membrane promote rapid absorption and decreased metabolism of the applied nanoparticles 53 . Further, intranasal administration is user friendly and relatively non-invasive. It also provides access to the mammalian central nervous system, through mechanisms that are not yet well understood 54 . For example, plasmid DNA coated with a polycationic lysine derivative has been delivered intranasally in rats where it led to green fluorescent protein expression in the brain 55 . Other routes to access a specific tissue involve direct injections at the target site, most notably injections of chitosan–DNA nanoparticles into the mouse eye 56,57 and lung 58 as well as the rat brain 59 and liver 60 . Direct tissue injections present good potential as they provide high local concentrations and therefore minimize toxicity, but are invasive and require special expertise. Importantly, regardless of the delivery route, DNA nanostructures are likely to elicit an immune response that would need to be either exploited or mitigated depending on the functionality of the architecture — for example, vaccine or therapeutic cargo.

Without robust targeting technology, there is a risk of nonspecific nanodevice delivery to undesired sites. Cell-specific targeting of DNA nanodevices has been achieved using specificligand–receptor pairs, where the DNA architecture enters cells via receptor-mediated

endocytosis 61 . Newer strategies that leverage cancer cell surface-expressed biomarkers 62 , endogenously expressed tissue-specific receptors 45,47 , synthetic recombinant antibodies 63 or aptamers 64 need to be devised to achieve efficient targeting. For example, the ERBB receptor could be a promising route for anti-cancer DNA nanodevices in the future 62 . The anionic ligand-binding receptors that have high affinity for DNA present another viable option. Synthetic protein–DNA partners also present exciting possibilities. For example, a recombinant antibody fused to Furin, a trans-Golgi network trafficking protein, has been used to achieve organelle targeting in cells 63 . This recombinant antibody recognized a specific 8-mer dsDNA sequence on the nanostructure and was able to target it to the trans-Golgi network 63 . Although mechanisms of cytosolic delivery of DNA via receptor-mediated endocytosis, as seen with lipid-complexed DNA 65 , remain unclear, these could be leveraged in future to deliver DNA nanodevices cytoplasmically. Nanodevices enter cells through different endocytic mechanisms, and how these different entry mechanisms could affect efficacy and stability of DNA devices remains to be addressed.

Given that there are several mechanisms that dispose of superfluous DNA to maintain homeostasis, the efficacy and stability of the DNA nanodevice need to match the application for which it is designed. Systemic circulatory stability depends on complex factors such as digestion by extracellular DNases, size and possibly shape of DNA nanodevices, tissue uptake and removal from circulation by the kidneys and liver. While it is known that size or shape of nanostructured gold, carbon, silica, dendrimers, quantum dots, liposomes and polymeric particles affect their in vivo clearance times 52 and uptake 66 , analogous studies on designer DNA nano structures remain to be performed. Cellular stability of DNA nanodevices could be altered by tuning delivery pathways that circumvent lysosomal delivery 61 . Further, partially dissociated nanostructures could trigger stronger immune responses, greater off-target effects and elevated toxicities. Thus nanostructure stability and nanodevice uptake pathways will cumulatively impact their bioavailability.

One of the earliest studies that addressed DNA nanodevice stability in a multicellular organism exploited the scavenger cells of *C. elegans* to investigate DNA nanostructure clearance by lysosomal degradation 48 . Here, a DNA duplex displaying two single-stranded domains showed an in vivo half-life of 8 hours. Reducing the number of single-stranded domains on the nanostructure increased its half-life to 11 hours. On the other hand, an architecture such as a DNA icosahedron, devoid of free termini, was recalcitrant to lysosomal degradation over 24 hours of investigation 48 . It has also been shown that fluorescently labelled DNA tetrahedra remained stable up to 48 hours post-transfection in cultured human embryonic kidney (HEK) cells 67 . One of the reasons for increased in vivo stability of architectures such as the icosahedron and tetrahedron is that the Mg²⁺ requirement 67,68 for their structural integrity is relatively low and corresponds to physiological concentrations (1–2 mM Mg²⁺) 69 . In this case, the Mg²⁺ is needed to primarily stabilize their constituent N-way junctions. However, this is not the case for most DNA origami-based structures, where phage ssDNA is folded into DNA helices that are packed lengthwise 11 . Such packing requires high concentrations (10–80 mM) of divalent cations, such as Mg²⁺, to lower the Debye screening length and stabilize the overall architecture in vitro 70,71 . When introduced into biological systems, where physiological Mg²⁺ concentrations are at least tenfold lower 69 , these architectures are destabilized. As

shown by Hahn et al., DNA origami-based octahedra, nanotubes and nanorods show low stability in fetal bovine serum (FBS) as well as in cell lines such as mouse 3T3 fibroblasts, HEK-293 and human H441 adenocarcinoma 72 . Further, the low yields of complex origami-based nanostructures results in molecularly heterogeneous populations in bulk 73,74 and pose additional impediments to more widespread applications in vivo. Importantly, increased stability has been demonstrated by incorporating a surface lipid coating on DNA origami-based octahedra in a mouse model 75 , highlighting a strategy to increase the in vivo stability of DNA origami-based nano devices (Fig. 4(i)).

The current knowledge of host responses to dsDNA can be leveraged to design DNA nanodevices that either evade or activate the immune response by tuning their in vivo stability. Fully ligated architectures, with minimal single strands and free termini, might show enhanced stability and resistance to cellular nucleases (Fig. 4(i)). This was borne out in vitro on ligated polyhedra 76 and later confirmed in vivo 48 . Second, within a given architecture, the spatial position and accessibility of a sequence governs its vulnerability to nuclease digestion. It has been shown that sequences placed near three-way junctions in a tetrahedron were more resistant to nucleases 77 . This implies that the stability of a nanostructure can be tuned by varying the degree of accessibility of its vulnerable sites to either enhance its in vivo half-life or to promote its degradation to facilitate a payload release (Fig. 4(ii); blue region). Third, chemical modifications of the DNA scaffold could also make it recalcitrant to degradation. For instance, the stability of a triangular DNA prism in FBS could be enhanced using hexaethylene glycol- and hexanediol- modified phosphoramidites at the 5' and 3' termini of component oligonucleotides 78 . In addition, a covalently linked base pair between 5-formyluracil and 5-aminocytosine via Schiff base formation has been developed (Fig. 4(iii); top chemical structure). DNA duplexes containing this base pair are stable under denaturing conditions and dissociate only at high temperatures 79 . Another novel strategy that crosslinks duplex DNA to enhance structural stability utilizes an aniline-derived DNA heterobase and (hydroxy)benzaldehyde. This is similar to an A–T base pair that can be reversibly crosslinked (Fig. 4(iii); bottom chemical structure) and was used to enhance the half-life of a DNA duplex in vitro 80 .

Another strategy to evade enzymatic cleavage of DNA nano-structures is to use unnatural nucleic acid analogues that have modified chirality such as L-DNA 81 (Fig. 4(iv); left chemical structure), modified backbones such as peptide nucleic acid 82 (Fig. 4(iv); right chemical structure; right chemical structure), or modified sugar residues such as locked nucleic acids (LNA), morpholinos and bridged nucleic acids (PNA) 83 . These unnatural analogues are not substrates for endogenous nucleases and could prolong nanodevice lifetimes, yet affording essentially identical functionality. For instance, it has been shown that the incorporation of 2' fluoro RNA in the viral phi29 DNA-packaging RNA sequence yields an RNA device that was stable in RNase A as well as in FBS over 36 hours and, further, could effectively gear the phi29 motor to package the viral DNA and produce infectious particles 84 . Chimeric nucleic acid duplexes, such as PNA–DNA 85 , RNA–DNA 86 or LNA–DNA 87 , can be further used to fabricate architectures with enhanced stabilities. Such approaches however, are yet to be embraced widely in the field given the prohibitively high cost of their syntheses.

An important property of nanostructured DNA scaffolds is their propensity to induce antibody responses. An example is the use of a tetrahedral DNA nanodevice as an adjuvant in vertebrates that produced antibodies against an antigen of choice 50 . Another example is a 30-helix, hollow DNA origami tube, which when coated with CpG motifs potently activated immune cells in a TLR9-dependent manner 88 . This has also been observed with CpG-motifs presented on SNA that elicited better antibody responses and tumour regression in mice compared with their ssDNA counterparts 33 . Given that such immunostimulatory or immunoregulatory DNA nanodevices outperform unstructured or polymer-coated stimulatory DNA, they could become important in prophylaxis, therapeutics and possibly even transform vaccine biology 89 . Conversely, DNA nanodevices could also be designed to potentially suppress immune responses, which would be useful in contexts such as uncontrolled inflammation. Regulatory T cells are suppressor cells that dampen immune responses and can be elicited using optimized CpG-free, polymer-complexed DNA nanostructures 32,90 . In addition, single-stranded phosphorothioate oligonucleotides with four TTAGGG motifs can directly bind AIM2 and IFI16 and prevent inflammasome signalling, and may be a starting point to find specific inhibitors of these pathways 91 .

While the above applications leverage immune responses in complex mammalian systems, simpler invertebrate models offer the power of genetics for more fundamental studies on the interaction of nanostructured DNA scaffolds with the innate immune system. For example, *C. elegans* or *Drosophila melanogaster*, which lack acquired immunity and some of the DNA receptors found in mammals but maintain humoral and innate immune responses, are excellent test beds to apply DNA nanodevices as probes and tools 92,93 . However, the genetically tractable zebrafish (*Danio rerio*), which harbours both an innate and adaptive immune response as well as the IFN-response system, may eventually prove to be a better model system 94,95 . Importantly, it is also known that not all DNA sequences are equally immunogenic. Therefore, screens to identify sequences of low (or enhanced) immunogenicity should better inform nanostructure design to fine-tune immune responses (Fig. 4(v),(vi)). Further, immune responses are likely to be species-specific, as has been observed in the case of differential immunomodulatory effects of DNA on human versus mouse cells 28 .

Outlook and summary

We envision that the next generation of biologically smart DNA nano devices would need to incorporate an additional layer of design. These would leverage size and shape information to achieve precise targeting and clearance properties, exploiting sequences with custom-designed host immune responses. Such nanodevices could act as smart cargo carriers that, as a function of molecular logic, can either be destabilized or remodelled to release their cargo. Although there are rare examples of conditional cargo release from DNA nano-devices albeit in vitro 96–98 , there is paucity of information on the effects of DNA sequence, nanostructure size and shape on their immunogenicity and in vivo clearance of the resultant DNA nanostructures in any organism. These data would improve our understanding of basic DNA sequence requirements to develop design criteria that can rationally tune biological nanodevice functionality such as stability or immunogenicity. Even the latest sequence design programmes 99,100 consider only architectural shape and flexibility of DNA

nanodevices. Such next-generation designer DNA nanodevices with customized host responses would find tantalizing applications in drug delivery. Given that responses of individual subjects to DNA differ greatly, one can envisage DNA nanodevices engineered for personalized immunotherapy. The capacity to cell-specifically deliver DNA architectures and/or achieve molecular-logic-guided cargo release could enable cellular programming and reprogramming. More excitingly, in the longer term, the delivery of DNA or RNA cargo encoding transcription programs, which bypass the immune system, could be used to remodel synapses to alter learning and memory, or for tissue regeneration, or building organoids from single cells. To fully harness the potential of the DNA scaffold in vivo, the next layer of design in DNA nanodevices needs strategies to leverage endogenous surveillance mechanisms to maximally tap nano device functionality.

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Figure 1.

DNA and its various nanostructured forms. a, Duplex DNA is widely exploited in therapeutics and biomedical applications in the form of diverse nanostructures. b, Circularized elements such as plasmids are routinely used for gene expression. c, Plasmids are often complexed with non-immunogenic polymers such as chitosan or polyethylene glycol (green) to enhance gene delivery. d, Structural motifs such as G-quadruplexes are formed by specific sequences of DNA in response to chemical triggers such as ions and pH. e, Spherical nucleic acids are fabricated by immobilizing single-stranded or duplex DNA (green strands) on the surface of inorganic nanoparticles (brown core). f, Designer DNA nanodevices are formed by assembling rationally designed DNA motifs with sticky ends (top). The DNA buckyball structure (bottom) is ~80 nm in diameter. g, DNA origami-based nanodevices use several staple strands (blue and orange) to fold a large viral genome-derived DNA strand (grey) into defined super-architectures that can be used to deliver molecular payloads (yellow and pink). Figure reproduced with permission from: d, ref. 8, Nature Publishing Group; e, ref. 10, American Chemical Society; f, ref. 101, Nature Publishing Group; g, ref. 96, AAAS.

Figure 2.

Natural mechanisms to detect and dispose of foreign DNA by host cells. Several cytosolic receptors, such as IFI16, MRE11, DNAPK, DDX41 and cGAS, detect DNA in the cytosol, directly bind duplex DNA (dsDNA) and trigger transcription of type I interferons (IFNs), which in turn upregulate various interferon-stimulated genes (ISGs). These cytosolic receptors induce IFNs via the protein STING. cGAS binds DNA and synthesizes a novel second messenger — 2',3'-cGAMP (cGAMP) — that activates STING. RNA polymerase III (POL III) transcribes DNA into RNA that binds the RNA receptor RIG-I, and induces IFNs

independently of STING. LRRFIP1 receptor induces IFNs via β -catenin (β -cat). The AIM2 receptor binds DNA and assembles inflammasome platforms activating caspase-1 (CASP1). Caspase-1 controls the release of proinflammatory cytokines and triggers cell death. dsDNA can also be detected within endosomes (pink filled circles) by TLR9, which activates IFN expression. dsDNA is disposed of extracellularly by DNases (such as DNase I) or within lysosomes, for example by TREX1.

Figure 3.

Targeting and delivery of DNA nanostructures in vivo. a, Microinjection-mediated introduction of a pH-sensitive DNA nanodevice (left) and a cargo-loaded DNA icosahedron (yellow spheres; right) in *C. elegans* uses the anionic ligand-binding receptors (red, bottom) to achieve cell-specific targeting. The red star and red and blue circles represent fluorophores. b, The DNA tetrahedron, bearing folate moieties (grey triangles) and siRNA (purple duplex) on its surface (left), was targeted to murine tumours overexpressing the folate receptor (red). The DNA tetrahedron was also used as a scaffold to display streptavidin (red ovals) as an antigen and single-stranded CpG oligonucleotides (purple strands) as an adjuvant (right), which was introduced via venous injections into the mouse bloodstream. Internalization of the DNA tetrahedron into B-cells (blue) and macrophages (green) leads to downstream activation of T cells (red), which in turn activate synthetic antibody production against streptavidin by B cells. Figure reproduced with permission from: a(left), ref. 47, Nature Publishing Group; a(right), ref. 45, Nature Publishing Group; b(left), ref. 46, Nature Publishing Group; b(right), ref. 50, American Chemical Society.

Figure 4.

Strategies to tune stability of designer DNA nanodevices to tailor host immune responses. DNA architectures devoid of nicks or single-stranded domains show increased stability in vivo, which can be enhanced by encapsulation in non-immunogenic polymers (i). Positioning nuclease-sensitive sequences (blue region; ii) near N-way junctions decreases susceptibility to nucleases (blunt blue arrows). Incorporation of modified, chemically crosslinkable nucleobases (green region; iii) could enhance duplex stability in vivo. Top and bottom chemical structures show examples of crosslinkable nucleobases 79,80. Non-natural nucleic acid analogues (red; iv), such as L-DNA (left chemical structure) and peptide nucleic acid (right chemical structure), are not substrates for endogenous nucleases. Immunogenicity of a designer DNA nanodevice could be lowered by structurally shielding sequences of high immunogenicity (magenta regions; v), and exposing sequences of low immunogenicity (orange regions; vi) to the external milieu. Part (i) reproduced with permission from ref. 102, AAAS.