ACES Asian Chemical Editorial Society
DOI: 10.1002/cnma.201600036
CHEMNANOMAT
Focus Review

DNA Nanotechnology

SPECIAL ISSUE

Progress in Biomedical Applications of Functionalized Nucleic Acid Nanodevices (NANDs)

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Abstract: DNA and RNA are building blocks with excellent programmability for nanoscale assembly. Owing to their appealing properties such as precision in bottom-up fabrication, enhanced stability and cellular internalization, designed nucleic acid nanodevices (NANDs) have been widely explored in biomedical research. Compared with pristine nucleic acids, chemically modified NANDs exhibit a wider spectrum of applicability. In this Focus Review, we highlight recent advances of NANDs functionalized with small molecules, peptides/proteins or inorganic materials, and elucidate their promising future for biomedical application.

1. Introduction

Nucleic acid nanotechnology has garnered widespread attention since its debut in 1980s as a facile and robust method for precise nanoscale fabrication and assembly. First introduced by Nedrian Seeman,[1] the field has seen a boom in the last three decades. By taking advantage of the classic Watson–Crick base-pairing (A-T/U and G-C), numerous tools were developed, for example, tile-based assembly,[2] DNA origami[3] and dynamic strands replacements.[4] Those elaborate nanostructures were not made simply for aesthetic reasons, but also for realistic applications in many other disciplines such as hierarchical assembly,[5] DNA computing[6] and supramolecular chemistry.[7]

Nucleic acids are generally known as the carriers and transporters of genetic information; in designed NANDs, however, nucleic acids are typically recruited as basic building blocks owing to their excellent programmability. Given by the biological nature, it naturally occurs to scientists that NANDs could be applied in biomedical research. As such, one remarkable feature of NANDs over other nanotechnologies is the ability to precisely control the size, shape and surface property of the final products and the spatial arrangement of cargos at molecular level, which is normally difficult to realize in other nanoscale self-assembled systems. Moreover, NANDs such as origami and tetrahedron often show enhanced stability[8] and remarkably prolonged half-life in biological systems as compared to their linear counterparts. Similar to other nanomaterials, NANDs are frequently designed to exhibit favorable properties such as multivalence for specific purposes. For instance, the immunostimulatory effects of non-methylated CpG oligonucleotides were greatly enhanced when they were assembled into nanostructures.[9] Together, these characteristics consolidate the basis of NANDs for biomedical applications. Those advances have been comprehensively discussed in a number of outstanding perspectives and reviews.[10]

Nevertheless, pristine NANDs alone are often insufficient to satisfy the growing needs partially due to the lack of essential functional units, necessitating the modification of NANDs with a vast variety of functional components. In this Focus Review, we highlight recent progress of chemically functionalized NANDs and focus on their biomedical applications. Indeed, functionalization of NANDs allows the installation of targeting ligands (e.g., aptamers[11] and folic acid[12]), stimuli-sensitive modules, therapeutic and diagnostic molecules. These molecules add new weapons to the arsenal of NANDs, drastically enriching their capabilities and extending their applications. Although RNA nanotechnology is rapidly developing and exhibits considerable promise for the future,[13] DNA nanotechnology is currently more mature and will be the major focus of this review.

2. Chemical Functionalization of Nucleic Acids

The chemical functionalization of nucleic acids has been extensively studied. Inasmuch as exhaustively presenting the whole field is beyond the scope of this review, the purpose of this part is to give a general overview of the most widely adapted nucleic acid modifications. For more detailed reactions and strategies, our readers are referred to reviews published earlier.[7,14]

The 3′ and 5′ ends of nucleic acids are the commonest modification sites, in part benefiting from the modular solid-phase synthesis of oligonucleotides and the abundance of end modifications from commercial sources. The modification strategies have been extensively studied[15] and functional groups such as amine, thiol, alkyne, and azide (Figure 1 a) have been routinely used for conjugation of nucleic acid to modules such as chromophores, probes, polymers[16] and proteins.[17]

Modifications of specific nucleotides on both ribose and bases are also growing rapidly. For ribose modifications, the 3′ and 5′ positions of the five-membered sugar ring are less explored likely due to the low yield.[14d] Instead, modifications at the 2′ and 4′ position are relatively easy to achieve and hence frequently used to alter the stability, hydrophobicity and immunogenicity of the products. 2′-methylation and 2′-fluorination are among the most popular modifications (Figure 1 b). Of note, changes on these positions always have an impact on DNA which may stabilize[18] or destabilize[19] the double helix structure. One emblematic example is the locked nucleic acid (LNA)[20] (Figure 1 b) in which the conformation of the five-membered pyranose ring is locked at the 2′-hydroxyl and 4′-carbon by a bridging methylene group. This confined conformation results in higher melting temperature and binding affinity, which has been widely exploited for detection and therapeutic purposes.[21] Other reported examples include pyrenes,[18,22] dansyl[23] and amino linkers[24] etc.

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This manuscript is part of a Special Issue on Nanobiointerfaces. Click here to see the Table of Contents of the special issue.

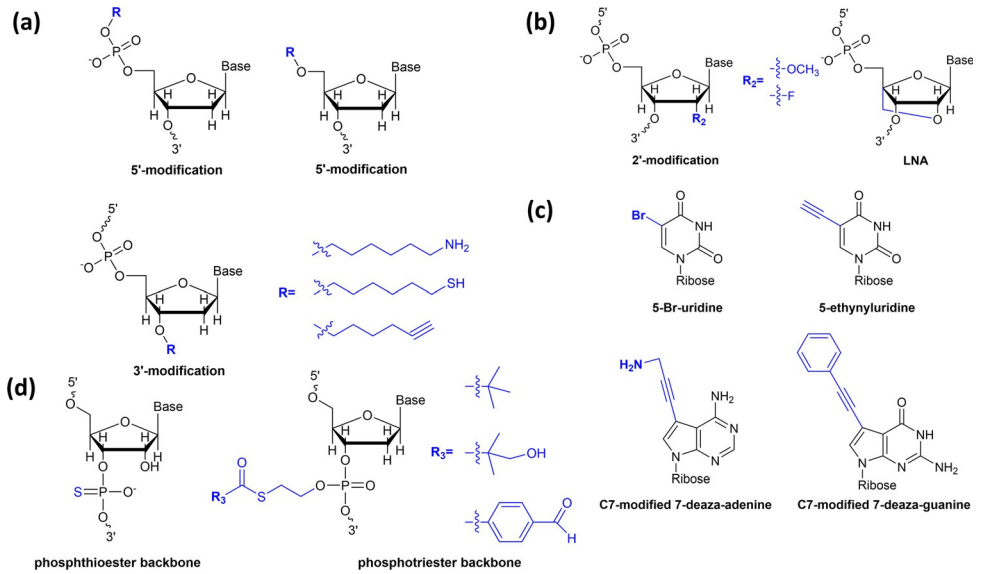

Figure 1. Examples of nucleic acid modifications. (a) Modifications at the 5′ and 3′ ends of nucleic acids; (b) Modifications on ribose. (c) Modified pyrimidines and purines. (d) Phosphodiester modification.

Modifications and replacement of nucleobases have been widely exploited. To maintain the helical structure and the base pairing, pyrimidines are normally modified at the 5-position, and purines are often replaced with 7-deaza-purines to introduce additional modifications at the 7-position[25] (Figure 1 c). Some of the analogues showed excellent biocompatibility and have already been employed as tools for chemical biology (e.g., 5-ethynyluridine (EU) labeling to study RNA transcription and turnover[26]). Introduction of artificial nucleobases furnishes DNA/RNA with additional characters such as fluorescence[27] and can even expand the genetic alphabets.[28] Featured examples include Kool's expanded nucleobases,[29] pyrene and porphyrin as reviewed earlier.[30] Some of these artificial nucleobases show ability of metal ion chelating and have been applied for multimetallic catalysis, sensing, artificial photosynthesis, data storage, nanooptics, and nanoelectronics.[5] The introduction of bioorthogonal groups on nucleobases for subsequent ligation is also frequently reported.[31]

Replacement of the phosphodiester by phosphothioester (Figure 1 d) is adaptable to solid phase synthesis and has been widely used in the antisense oligonucleotides (ASO) industry.[32] Generally speaking, this modification significantly improves the stability against nuclease of oligonucleotides[33] and prolongs their half-life in vivo.[34] Moreover, phosphothioate bond helps elicit plasma protein binding in circulation (which prevents rapid renal clearance) and more efficient RNase-H cleavage inside cells.[35] Recently, Dowdy et al. developed an interesting charge-convertible phosphotriester modification (Figure 1 d) in which the neutral siRNA were designed to restore its negatively charged phosphodiesters upon cleavage of the pendent thioester by intracellular thioesterase.[36]

It should be pointed out that non-covalent modifications such as intercalation were also frequently exploited for DNA functionalization. Intercalation is the insertion of ligand, typically aromatic and planar molecules, into the base-pairing plane that alters the conformation,[37] thermo-stability[38] and surface properties of DNA.[39] Many intercalators were developed for DNA staining (e.g., ethidium bromide) or as antitumor drugs to disrupt the helix structure of DNA, which ultimately leads to replication arrest. The growing understanding of intercalation has shed light on nucleic nanotechnology. For example, Sleiman et al. illustrated that intercalators such as ethidium bromide served as chaperones in DNA self-assem-

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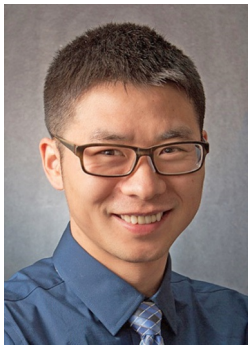

bly.[40] In another context, DNA was designed as the carriers of those therapeutic intercalators (e.g., doxorubicin, Dox).[41] Other common noncovalent nucleic acid modifications include polyamide-based groove binders[42] and cationic polymers for complexing with nucleic acids by electrostatic interactions.

3. Biomedical Applications of Functionalized NANDs

Functionalized NANDs are promising candidates for biomedical research. In this part, we focus on recent advances in biomedical applications of these materials, typically for therapy and diagnostics. Based on the molecules that were introduced to NANDs, we focus on three types of functional groups: namely small molecules, peptides/proteins and inorganic materials. Other materials such as lipids[43] and block copolymers[21d,44] functionalized NANDs will not be discussed in detail due to limited space.

3.1. NANDs modified with small molecules

Small molecules such as probes, ligands and drugs have been covalently attached or intercalated to NANDs to achieve designed purposes such as imaging, targeting and delivery of therapeutics.

Dox is a well-known chemotherapy drug for various cancers. It works by intercalating DNA and interrupting the transcription process of tumor cells. As such, many drug delivery systems (DDS) have been designed to incorporate Dox to various DNA nanostructures through intercalation to achieve controlled release and reduced systematic toxicity. Ding et al. designed a set of Dox-loaded DNA origami[41,45] (Figure 2a) displaying remarkable efficacy not only to regular but also to Dox-resistant cancer cells in vitro. In vivo studies implied that the Dox-loaded origami accumulated in tumor site and released the cargo in a pH-dependent manner without inducing significant systematic toxicity, underscoring the biocompatibility of the DNA origami in live animals. By using a twisted 12 bp/turn design, Högberg et al.[46] improved the loading efficiency and enhanced the anti-tumor effect of the Dox-loaded DNA origami. Taking advantage of strand displacement, Tan et al. facilely developed a DNA nanotrain for targeted delivery of Dox[47](Figure 2b). The system, composed of only three DNA strands, showed high loading efficiency of Dox and was imparted tumor-targeting function by an aptamer. In other approaches, Dox was attached to the vehicles through covalent bonds. For example, Tan et al. fabricated a series of Dox-aptamer conjugates[48] for targeted delivery. Except of Dox, NANDs were able to carry other payload molecules as well. Very recently, Zhang et al. constructed DNA-drug nanostructures assembled from photolabile DNA-camptothethin amphiphiles which were able to release drug and DNA upon light irradiation[49] (Figure 2c). The nanostructured DNA showed enhanced stability against DNase I and comparable efficacy as free drug to cancer cells.

For imaging purpose, numerous fluorescent and radioactive agents have been attached to NANDs to monitor the activities such as pH fluctuation, metabolism and protein trafficking both in vitro and in vivo.[50] Sando et al. anchored a fluorescent aptamer to astrocytes[51] (Figure 3a) to monitor the real-time release of a adenine-derived gliotransmitter. Chen and Liu et al. developed a DNA-based turn-on fluorogenic probe measuring cellular traction and adhesion with unprecedented resolution[52] in 2014 (Figure 3b). Moreover, fluorophore-modified aptamers also showed promising results for in vivo imaging:[53] as aptamers are rapidly cleared from circulation, the resulting low background ensures better sensitivity in visualizing tumor.

Another unique application of NANDs is harnessing DNA as a template to construct DNA sequence-encoded small-molecule libraries for drug discovery,[54] termed DNA-templated synthesis. Some featured works in this field include those from Liu,[55] Harbury,[56] Neri[57] and Li.[58] The fundamental concept is

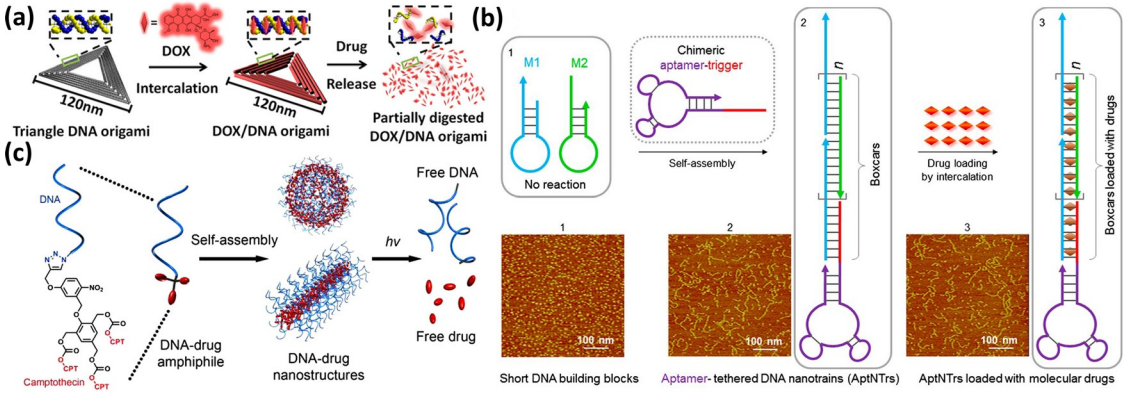

Figure 2. Drug-functionalized NANDs as delivery vehicles. (a) DNA origami for doxorubicin delivery,[45] Dox was loaded to the NANDs via intercalation. Reprinted with permission from ref. [45]. Copyright (2014) American Chemical Society. (b) Self-assembly of DNA nanotrains for the delivery of molecular drugs.[47] The assembly was triggered by a chimeric aptamer, forming the structure where the aptamer serves as the 'locomotive' and the repetitive dsDNA serves as the 'boxcars'; the drug molecules were then loaded to into the 'boxcars' through intercalation and released from the nanotrain upon degradation of dsDNA. Reprinted with permission from ref. [47]. Copyright (2013) National Academy of Sciences. (c) A light-triggered, self-immolative nucleic acid-drug nanostructures,[49] the amphiphilic DNA-camptothecin conjugate initially assembled into micellar nanostructures in an aqueous environment, upon the UV light, cleavage of the 2-nitrobenzyl group led to the release of DNA from the nanostructure and degradation of the prodrug core to generate free camptothecin molecules. Reprinted with permission from ref. [49]. Copyright (2015) American Chemical Society.

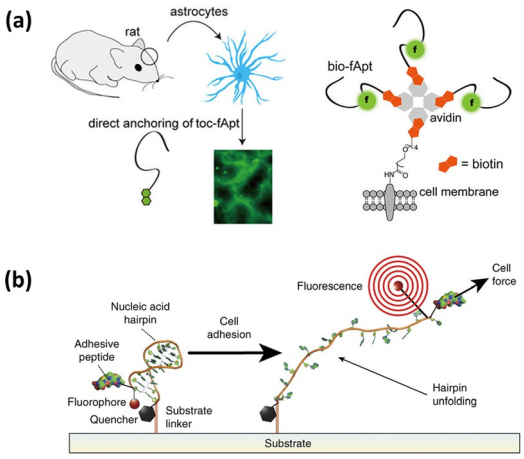

Figure 3. Functionalized NANDs for imaging. (a) The detection of gliotransmitter using modified aptamers;[51] left: a tocopherol and fluorophore modified aptamer (toc-fApt) was directly linked to astrocytes for detection of gliotransmitter; right: illustration of a biotinylated fluorescent aptamer (bio-fApt) anchored on the cell surface with the aid of avidin. Reprinted with permission from ref. [51]. Copyright (2012) American Chemical Society. (b) A DNA-based probe for reversible and optical visualizing of cellular traction forces.[52] Cell adhesion and force unfold the nucleic acid hairpin, restoring the initially quenched fluorescence of the fluorophore. Reprinted with permission from ref. [52]. Copyright (2014) Nature Publishing Group.

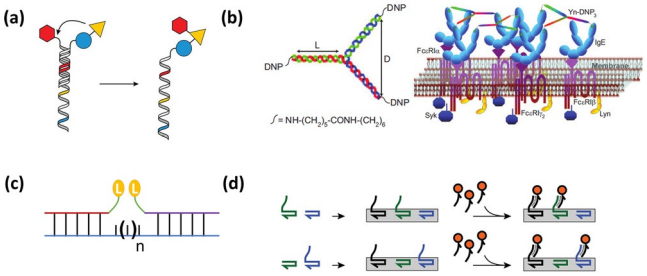

Figure 4. Functionalized NANDs as a tool in drug discovery and chemical biology. (a) Illustration of DNA-templated synthesis.[54] As the product information was encoded in the templated strand, it allows fast and large-scale identification of bioactive lead compounds. Reprinted with permission from ref. [54]. Copyright (2015) American Chemical Society. (b) Y-shaped DNA scaffolds for investigating stimulation pattern of IgE-FcεRI,[61] left: hapten-modified Y-shaped DNA, right: illustration of IgE-FcεRI cross-linked by the rigid scaffold. Reprinted with permission from ref. [61]. Copyright (2007) American Chemical Society. (c) Illustration of the DNA ruler: small-molecule or peptidyl ligands were bivalently presented of on the DNA scaffold with variable distances; the distance of the two ligands for optimal binding affinity was recorded by the DNA template. (d) DNA nanocaliper for spatial control of membrane receptor function.[69] Binding handles were placed at different positions of the nanocaliper by varying their sequences (green and blue) and the complementary strands carrying the protein ligand on their 3′ ends were subsequently attached to the handles, achieving ligands presentation with various distances. Reprinted with permission from ref. [69]. Copyright (2014) Nature Publishing Group.

taking advantage of DNA hybridization to provide spatial proximity and therefore high reactivity (Figure 4a). The hit compounds can be identified by subsequent PCR-amplification and high-throughput DNA sequencing. Over the years, many DNA-encoded chemical libraries were built and held great promise for the discovery of bioactive molecules.[54] Notably, peptide nucleic acids (PNA), a family of synthetic analogues of DNA, were also extensively studied for similar purposes as recently reviewed by Winssinger et al.[59] Beyond this, the DNA-templated synthesis has also been utilized to generate sequence-defined synthetic polymers. One recent elegant example is from Liu and coworkers, who developed a templated synthesis method generating such polymers with backbones unrelated to nucleic acid.[60] It is reasonable to envision that fishing in pools of these unprecedented polymers, one might be able to identify interesting bioactive molecules such as binders and drug candidates.

Taking advantage of the structural rigidity and precision, functionalized NANDs were employed as scaffolds to study how the spatial arrangement of small-molecule ligands affects its receptor binding. Biard et al. utilized a hapten-functionalized Y-shaped DNA nanodevice to crosslink IgE-FcεRI complex on cell surface. The results revealed distance-dependent signaling transduction and stimulation across the cellular membrane due to different level of receptor aggregation[61] (Figure 4b). Seitz et al. presented two copies of the same ligand to estrogen receptor on a double-stranded DNA (dsDNA) for spatial screening.[62] By controlling the distance of the two ligands on the so-called DNA ruler, they observed different binding affinity to the receptor (Figure 4c). This work shed light on optimizing the linker length for those homo-dimeric drug–drug conjugates.

Briefly, NANDs modified with small molecules showed promising effects in DDS, imaging and drug discovery. Small in size, these modifications may impose minimized or well-controlled impact to the fundamental physicochemical properties of NANDs such as hybridization, secondary structure and aqueous solubility. This is critical and beneficial because the original properties of the NANDs are often indispensable in order to yield adducts with desired functions.

3.2. NANDs functionalized with peptides and proteins

The original purpose of DNA nanotechnology, posed by Seeman,[1] is to position macromolecules such as proteins in a spatially controlled manner in order to simplify the crystallization process for structure study. Indeed, the ability to present molecules in a highly ordered and programmable manner makes NANDs one of the most powerful tools for programmed protein assembly[63] and enzyme cascade.[64] Although the original idea is still yet to be fully accomplished,[65] proteins and peptides have been frequently applied in DNA nanotechnology to produce hybrid materials with novel applications.[28,66,71]

The concept of a DNA ruler for interrogation of ligand–receptor interactions was also readily applicable to explore and control protein–peptide or protein–protein interactions.[67] Recently, Seitz and coworkers explored the heterobivalent binding pattern to the endocytic AP-2 adaptor complex by a DNA-based nanodevice.[68] Similarly, Högberg et al. developed a nanocaliper to investigate the spatial arrangement of ephrin ligands (ephrin-A5) for efficient activation of EphA2 receptor (Figure 4d).[69] By adjusting the distance between two ligands with origami, they found that 40 nm is an optimal distance for

stimulation. They further showed that in vitro EphA2 activation in MDA-MB-231 cells by ephrin-A5 nanocalipers decreased cell invasiveness compared to cells treated with empty nanocalipers or ephrin-A5 monomer. This method can be used to uncover the role of the nanoscale spatial distribution of protein ligands in signaling pathways regulating cell–cell communication.

It has been implied that NANDs are excellent materials for DDS as growing evidences[70] demonstrated the efficient uptake by cells despite of their highly negative-charged nature.[71] In 2012, Yan et al. designed a virus-mimicking DNA tetrahedron for antigen presentation[72] (Figure 5 a). Combining antigen and immunostimulatory CpG oligonucleotide in a tetrahedron, they showed a strong and enduring antigen responses in vivo that outperformed antigen alone. The study also inferred that the size, shape, and stability of the NANDs may affect their delivery ability to antigen presenting cells (APCs).

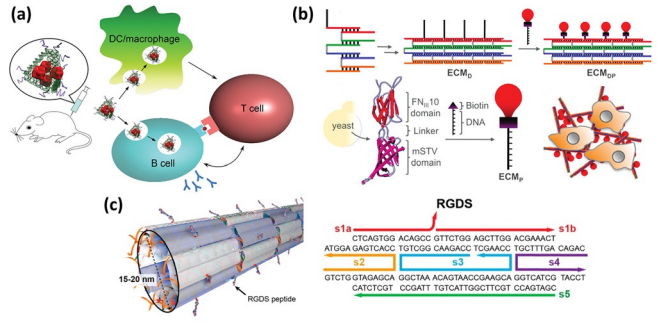

Figure 5. Protein/peptide-functionalized NANDs for delivery and tissue engineering. (a) DNA tetrahedron as a scaffold to assemble streptavidin (model antigen) and CpG oligonucleotides for antigen presenting.[72] Reprinted with permission from ref. [72]. Copyright (2012) American Chemical Society. (b) A DNA/protein-based ECM[73] constructed from a DNA ribbon surface-functionalized with RGD-containing proteins; top: scheme of the fabrication process, proteins were attached on the surface of DNA ribbon through hybridization, ECM_D: DNA ribbon, ECM_{DP}: DNA/protein-based matrix; down: structure of protein-functional group and proposed mechanism; functional protein (ECM_P) contains an $FN_{III}10$ domain, a flexible $(Gly_4Ser)_3$ linker, a monomeric streptavidin (mSTV) domain for conjugation to 5′-biotin DNA, and a His_6 tag. Reprinted with permission from ref. [73]. Copyright (2010) American Chemical Society. (c) Peptide-decorated DNA nanotube for enhanced differentiation of neuron stem cells.[74] The bioactive peptide RGDS was covalently attached to a building block strands (s1a) of the DNA nanotube. Reprinted with permission from ref. [74]. Copyright (2015) American Chemical Society.

Protein/peptide-functionalized NANDs also showed considerable potential in tissue engineering. May et al. designed an artificial extracellular matrix (ECM) based on a DNA-protein hybrid[73] (Figure 5 b), in which the DNA was assembled into a linear ribbon overhanging RGDS peptide. A remarkable cytoskeletal response was achieved by controlling the stiffness of the matrix, which in turn manipulated a number of integrin-mediated processes. Similarly, Stupp et al. periodically attached a RGDS peptide that facilitating cell adhesion on a self-assembled DNA-nanotube (RGDS-NT, Figure 5 c),[74] which showed enhanced ability to induce neural differentiation when compared to RGDS alone. Moreover, RGDS-NT induced a less percentage of astrocytes than the negative control on (3-aminopropyl)triethoxysilane (APTES) coated glass. Though the underlying mechanism is still unclear, it appeared that both the mechanical stiffness of nanotube and the spatial location of cargos play important roles. Liu and Weil et al. designed a DNA-protein hydrogel for the release of functional proteins responding to digestion by DNase I and trypsin,[75] which holds great interest for cell culturing.

Proteins are the most intricate machinery created by nature, superior to any artificial nanodevices. Harnessing the power of both protein and DNA nanotechnology, strong synergy in protein/peptide functionalized NANDs have been realized and elaborately controlled. Along this direction, 'the mechanical surgeon inside the blood vessel' predicted by Richard P. Feynman[76] might not just be a fancy dream.

3.3. NANDs functionalized with inorganic materials

Aside from functional molecules mentioned above, inorganic materials such as gold nanoparticles (AuNPs), carbon nanotubes[77] (CNTs) and quantum dots (QDs) were also routinely incorporated to NANDs to generate hybrid materials for biomedical research. The high affinity of DNA (specially ssDNA) to many inorganic materials enables wide applications, ranging from biosensing to targeting delivery.[78]

AuNPs are one of the most-exploited inorganic materials in the context of biomedical research[71,79] exhibiting excellent biocompatibility[80] and tunable cytotoxicity.[81] The fabrication of AuNPs is straightforward and well-documented, allowing fine tuning of their size, shape,[82] and surface properties.[79] Moreover, the unique electronic and optical profiles of AuNPs make them especially attractive for imaging and diagnostics.[83] Through thiol, polyadenine sequence[84] and other conjugation methods,[85] NANDs were easily anchored to the surface of AuNPs, which resulted in higher stability[86] and enhanced cellular uptake.[87] Notably, the positioning of AuNPs arranged by NANDs were exploited to adjust the accumulation and elimination rate.[88]

Spherical nucleic acids (SNAs) are a class of nanostructures arranging nucleic acid on a spherical core like AuNPs or liposomes,[89] as pioneered by Mirkin and coworkers. The extremely high density of nucleic acids was proven a key factor for the rapid cell internalization of SNAs, likely through the scavenger receptor mediated endocytosis.[90] As such, the SNAs have been used for the delivering of nucleic acid warheads including ASOs, siRNA,[91] as well as immunomodulating oligonucleotides (e.g., CpG DNA). For instance, SNAs composed of a AuNP core and a layer of ASO or siRNA shell led to prominent antisense or RNA interference effects.[91] By imparting an antibody-DNA conjugate to the anti-HER2 ASO-shelled SNA by hybridization, cell-selective knockdown of intracellular HER2 mRNA were achieved[92] (Figure 6 a). Beyond those proof-of-concept works, the antisense or RNAi-based SNAs have displayed remarkable efficacy in preclinical research such as reversing impaired wound healing[93] by knocking down ganglioside-monosialic acid 3 (GM3) synthase. With these accumulated developments and advances, the SNA-based systems hold the potential for clinical trials in the near future.[94]

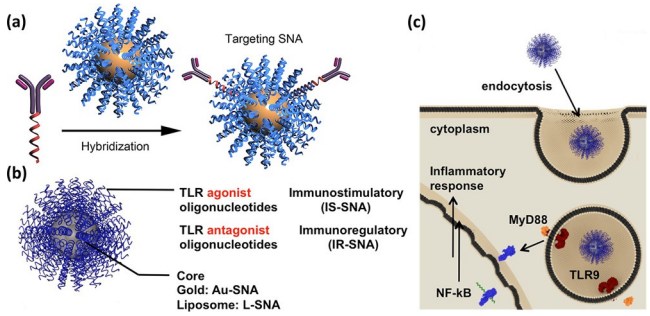

Figure 6. SNAs and their applications in biomedicine. (a) Antibody-linked SNA[92] for cell-selective gene regulation via antisense or RNAi. Reprinted with permission from ref. [92]. Copyright (2012) National Academy of Sciences. (b and c) Immunomodulatory SNA[95] for the modulation of immune responses; TLR agonistic or antagonistic oligonucleotides were assembled on the surface of gold nanoparticles or liposomes. Reprinted with permission from ref. [95]. Copyright (2015) National Academy of Sciences.

More recently, the same group reported a type of immunomodulatory SNA that was able to either stimulate or regulate the immune system, depending on the specific sequence of the attached nucleic acids[95] (Figure 6 b–c). Comprehensive in vitro and in vivo experiments showed that the SNAs are more potent than their single-stranded counterparts. When the immune-stimulatory SNAs were applied to cancer therapy, enhanced in vivo immune responses were detected. Interestingly, the results suggested that it was the spatial orientation and high-density presentation of oligonucleotides instead of the AuNP core that contributed most to the unprecedented properties of SNAs, as they have demonstrated that SNAs with different core materials also showed similar effects, consistent with what they discovered previously.[96]

Integrated NAND and AuNP were explored for DDS[71,97] as well. Jon et al. designed an aptamer-AuNP hybrid where Dox was intercalated into DNA duplex for targeted therapy of prostate cancer.[98] Taking advantage of the photothermal properties of AuNPs, Farokhzad et al. designed near-infrared (NIR)-responsive DNA-AuNP hybrids for Dox delivery[99] (Figure 7 a). Under NIR irradiation, the photothermal property of AuNPs resulted in elevated temperature and DNA denaturation, which in turn triggered the release of Dox. Aside from Dox, Mirkin and Lippard et al. constructed a platinum(IV) prodrug coated DNA-AuNP DDS (Figure 7 b).[100] The DDS were efficiently internalized by cells and showed remarkable cytotoxicity to cancer cell lines comparable to free cisplatin.

Owing to the unique electrical and optical properties of gold, integrated NANDs-gold nanomaterials were commonly designed for biosensing[101] and theranostics.[102] For example, Heeger et al. devised an electrochemical DNA (E-DNA) sensor by immobilizing a stem-loop structure on gold surface in 2003.[103] Upon hybridization to the loop-binding strand, a major conformational switch of the stem-loop structure induced a dramatic distance change between the 5′-terminal ferrocene and the gold surface, which consequently resulted in a measurable change of electrochemical signal. Later, Fan et al. applied the tetrahedral structure to E-DNA sensor (Figure 7 c) enabling detection of DNA[104] and microRNA[105] with higher sensitivity (attomolar) and better specify even in complex biological fluids. Meanwhile, there is a growing tendency using DNAzyme functionalized AuNPs for biosensing.[106] For example, Lu et al. designed a DNAzyme-gold nanoparticle probe for uranyl detection inside living cells[107] (Figure 7 d). In the absence of uranyl, the chromophore was quenched by both the AuNP and quencher; while in the presence of uranyl, the DNAzyme cleaved the substrate strand with fluorescent label, resulting in the detachment of the chromophore with the quencher and hence increased fluorescence. This probe can be efficiently taken up by cells, implying its potential for metal ion sensing in biological systems.

The development of quantitative point-of-care testing enables fast, portable, low-cost and accurate detection of molecule-of-interest in complex biological samples, which offers enormous potential for purposes such as anti-doping drug-testing, early diagnosis and monitoring of patients. Along this direction, Yang et al. have elegantly designed a few systems that transfer the molecular recognitions by aptamers to signals such as pressure and volume changes, which were then quantified by simple tools such as volumetric bar-chart chip or portable pressure meter.[108] In one system, they generated an Au core/Pt shell nanoparticle (Au@PtNP)-encapsulated hydrogel crosslinked by a DNA aptamer and its complimentary strand.[108a] The hydrogel dissolved upon introducing the target molecule of the aptamer, released the Au@PtNP to catalyze the decomposition of H_2O_2 to produce oxygen gas. The pressure generated by the gas was then reflected and measured by the distance traveled of the ink bar of V-Chip (Figure 7 E).

Other inorganic materials including carbon nanomaterials,[109,110] magnetic nanoparticles (MNPs),[109] QDs[110] and mesoporous silica nanoparticles (MSNs)[111] have also been explored to functionalize NANDs to fulfill various biomedical goals. Particularly, the hybrid materials coupling the NANDs with carbon nanomaterials (e.g., single walled nanotube and graphene) and QDs offer good opportunities for sensing and imaging.[112] On the other hand, NAND-MSN hybrids were extensively explored for DDS.[111]

Overall, the physicochemical properties of inorganic materials impart desired complementarity to NANDs. For example, introduction of inorganic materials enabled interactions with external physical signals such as light and temperature, allowing stimuli-sensitive and on-demand controlled release. Moreover, the rapid internalization of these inorganic materials renders them promising candidates for intracellular delivery.

4. Conclusions

To summarize, we have witnessed the prosperity of functional NANDs for biomedical applications such as diagnostics, drug delivery and immunomodulation in the past decade. Because the functions of pristine NANDs are limited, chemical modification thus emerged as a powerful strategy to circumvent this limitation. The development of advanced synthetic methodologies (in particular various bioorthogonal reactions) coupled with the maturation of DNA/RNA nanotechnology have collectively enabled the facile synthesis of more complex structures

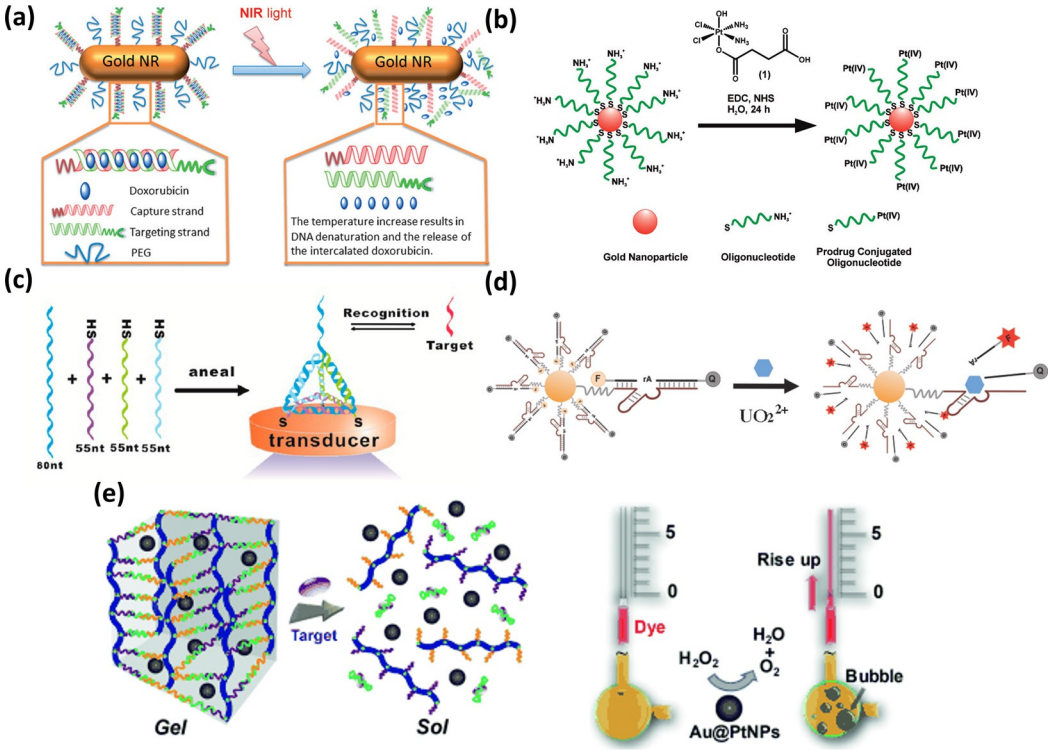

Figure 7. DNA-AuNP hybrids for drug delivery and biosensing. (a) A NIR-responsive DDS,[99] under NIR irradiation; the photothermal property of AuNPs resulted in elevated temperature and DNA denaturation which in turn led to the release of Dox. Reprinted with permission from ref. [99]. Copyright (2012) John Wiley and Sons. (b) A DNA-AuNP hybrid for platinum prodrug delivery;[100] multiple copies of Pt^{IV} prodrug were covalently linked to the outer layer of the DNA-AuNP hybrid. Reprinted with permission from ref. [100]. Copyright (2009) American Chemical Society. (c) A tetrahedron-based DNA probe;[104] the probe was attached to the transducer through three thiol groups and the presence of a target strand led to a electrochemical signal detectable by the transducer. Reprinted with permission from ref. [104]. Copyright (2010) John Wiley and Sons. (d) A DNAzyme-gold nanoparticle probe for uranyl detection;[107] in the absence of uranyl, the chromophore was quenched by the gold surface and quencher; once in the presence of uranyl, the chromophore-labeled strand was cleaved, resulting in the turn-on of fluorescence. Reprinted with permission from ref. [107]. Copyright (2013) American Chemical Society. (e) An Au@Pt nanoparticle encapsulated DNA-crosslinked hydrogel responsive to target molecules.[108a] In the presence of target molecules, the hydrogel immediately dissolved to release the Au@Pt nanoparticles, which then catalyzed the decomposition of H_2O_2, generating large amount of oxygen gas whose volume were readable via the ink bar. Reprinted with permission from ref. [108a]. Copyright (2014) John Wiley and Sons.

for sophisticated applications. This review summarizes recent progress in functional NANDs modified with small molecules, proteins and inorganic materials, with emphasis on their biomedical applications.

Despite these achievements, there is no royal road to advance the NANDs from bench to bedside. One major and practical problem is the cost of generating the functionalized NANDs.[10b] Moreover, although NANDs have substantially improved the stability and cell permeability of nucleic acids, the in vivo pharmacokinetics and potency are still suboptimal. Finally, as exciting results are accumulating rapidly in animal models, more evidences endorsing the benefits of functionalized NANDs in human patients will be immensely appreciated. To address these unmet needs and translate those promising systems to real clinical applications, close collaborations that integrate the state-of-the-art technologies and insights from multiple disciplines including biology, chemistry, nanotechnology, biomedicine and engineering are of great importance.

Acknowledgements

This work is supported by grants from National Natural Science Foundation of China (NSFC21474004) and State High-Tech Development Program of China (863 Program No. 2015AA020941). H.L. thanks the support from the Youth Thousand-Talents Program of China. We gratefully thank Prof. Hao Yan for his kind suggestions during the writing.

Keywords: assembly · DNA nanotechnology · nanodevices · nucleic acids

[1] N. C. Seeman, *J. Theor. Biol.* **1982**, *99*, 237 – 247.
[2] C. Lin, Y. Liu, S. Rinker, H. Yan, *ChemPhysChem* **2006**, *7*, 1641 – 1647.
[3] a) P. W. Rothemund, *Nature* **2006**, *440*, 297 – 302; b) K. E. Dunn, F. Dannenberg, T. E. Ouldridge, M. Kwiatkowska, A. J. Turberfield, J. Bath, *Nature* **2015**, *525*, 82 – 86.
[4] F. Wang, C. H. Lu, I. Willner, *Chem. Rev.* **2014**, *114*, 2881 – 2941.
[5] F. A. Aldaye, A. L. Palmer, H. F. Sleiman, *Science* **2008**, *321*, 1795 – 1799.
[6] Y. Amir, E. Ben-Ishay, D. Levner, S. Ittah, A. Abu-Horowitz, I. Bachelet, *Nat. Nanotechnol.* **2014**, *9*, 353 – 357.
[7] T. J. Bandy, A. Brewer, J. R. Burns, G. Marth, T. Nguyen, E. Stulz, *Chem. Soc. Rev.* **2011**, *40*, 138 – 148.

[8] a) Q. A. Mei, X. X. Wei, F. Y. Su, Y. Liu, C. Youngbull, R. Johnson, S. Lindsay, H. Yan, D. Meldrum, *Nano Lett.* **2011**, *11*, 1477 – 1482; b) J. W. Conway, C. K. McLaughlin, K. J. Castor, H. Sleiman, *Chem. Commun.* **2013**, *49*, 1172 – 1174.
[9] a) J. Li, H. Pei, B. Zhu, L. Liang, M. Wei, Y. He, N. Chen, D. Li, Q. Huang, C. H. Fan, *ACS Nano* **2011**, *5*, 8783 – 8789; b) K. Mohri, E. Kusuki, S. Ohtsuki, N. Takahashi, M. Endo, K. Hidaka, H. Sugiyama, Y. Takahashi, Y. Takakura, M. Nishikawa, *Biomacromolecules* **2015**, *16*, 1095 – 1101; c) K. Mohri, M. Nishikawa, N. Takahashi, T. Shiomi, N. Matsuoka, K. Ogawa, M. Endo, K. Hidaka, H. Sugiyama, Y. Takahashi, Y. Takakura, *ACS Nano* **2012**, *6*, 5931 – 5940; d) V. J. Schüller, S. Heidegger, N. Sandholzer, P. C. Nickels, N. A. Suhartha, S. Endres, C. Bourquin, T. Liedl, *ACS Nano* **2011**, *5*, 9696 – 9702.
[10] a) F. C. Simmel, *Nanomedicine* **2007**, *2*, 817 – 830; b) D. Smith, V. Schuller, C. Engst, J. Radler, T. Liedl, *Nanomedicine* **2013**, *8*, 105 – 121; c) Y. J. Chen, B. Groves, R. A. Muscat, G. Seelig, *Nat. Nanotechnol.* **2015**, *10*, 748 – 760; d) S. Surana, A. R. Shenoy, Y. Krishnan, *Nat. Nanotechnol.* **2015**, *10*, 741 – 747.
[11] S. M. Douglas, I. Bachelet, G. M. Church, *Science* **2012**, *335*, 831 – 834.
[12] H. Lee, A. K. Lytton-Jean, Y. Chen, K. T. Love, A. I. Park, E. D. Karagiannis, A. Sehgal, W. Querbes, C. S. Zurenko, M. Jayaraman, C. G. Peng, K. Charisse, A. Borodovsky, M. Manoharan, J. S. Donahoe, J. Truelove, M. Nahrendorf, R. Langer, D. G. Anderson, *Nat. Nanotechnol.* **2012**, *7*, 389 – 393.
[13] a) P. X. Guo, *Nat. Nanotechnol.* **2010**, *5*, 833 – 842; b) W. W. Grabow, L. Jaeger, *Acc. Chem. Res.* **2014**, *47*, 1871 – 1880.
[14] a) R. Varghese, H. A. Wagenknecht, *Chem. Commun.* **2009**, 2615 – 2624; b) A. J. A. Cobb, *Org. Biomol. Chem.* **2007**, *5*, 3260 – 3275; c) M. Endo, H. Sugiyama, *ChemBioChem* **2009**, *10*, 2420 – 2443; d) S. H. Weisbrod, A. Marx, *Chem. Commun.* **2008**, 5675 – 5685.
[15] Y. C. Dong, D. S. Liu, Z. Q. Yang, *Methods* **2014**, *67*, 116 – 122.
[16] T. Schnitzler, A. Herrmann, *Acc. Chem. Res.* **2012**, *45*, 1419 – 1430.
[17] B. Saccà, C. M. Niemeyer, *Chem. Soc. Rev.* **2011**, *40*, 5910 – 5921.
[18] J. Skov, T. Bryld, D. Lindegaard, K. E. Nielsen, T. Hojland, J. Wengel, M. Petersen, *Nucleic Acids Res.* **2011**, *39*, 1953 – 1965.
[19] K. Kawai, K. Kawabata, S. Tojo, T. Majima, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2363 – 2366.
[20] S. K. Singh, P. Nielsen, A. A. Koshkin, J. Wengel, *Chem. Commun.* **1998**, 455 – 456.
[21] a) W. P. Kloosterman, E. Wienholds, E. de Bruijn, S. Kauppinen, R. H. A. Plasterk, *Nat. Methods* **2006**, *3*, 27 – 29; b) R. N. Veedu, J. Wengel, *Chem. Biodiversity* **2010**, *7*, 536 – 542; c) R. H. Wijdeven, J. Neefjes, H. Ovaa, *Trends Cell Biol.* **2014**, *24*, 751 – 760; d) A. M. Rush, D. A. Nelles, A. P. Blum, S. A. Barnhill, E. T. Tatro, G. W. Yeo, N. C. Gianneschi, *J. Am. Chem. Soc.* **2014**, *136*, 7615 – 7618.
[22] a) K. Yamana, H. Zako, K. Asazuma, R. Iwase, H. Nakano, A. Murakami, *Angew. Chem. Int. Ed.* **2001**, *40*, 1104 – 1106; *Angew. Chem.* **2001**, *113*, 1138 – 1140; b) V. A. Korshun, D. A. Stetsenko, M. J. Gait, *J. Chem. Soc. Perkin Trans. 1* **2002**, 1092 – 1104.
[23] A. Misra, S. Mishra, K. Misra, *Bioconjugate Chem.* **2004**, *15*, 638 – 646.
[24] S. Sitaula, S. M. Reed, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 850 – 855.
[25] S. Jäger, G. Rasched, H. Kornreich-Leshem, M. Engeser, O. Thum, M. Famulok, *J. Am. Chem. Soc.* **2005**, *127*, 15071 – 15082.
[26] C. Y. Jao, A. Salic, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 15779 – 15784.
[27] M. Nakamura, Y. Fukunaga, K. Sasa, Y. Ohtoshi, K. Kanaori, H. Hayashi, H. Nakano, K. Yamana, *Nucleic Acids Res.* **2005**, *33*, 5887 – 5895.
[28] D. A. Malyshev, K. Dhami, T. Lavergne, T. J. Chen, N. Dai, J. M. Foster, I. R. Correa, F. E. Romesberg, *Nature* **2014**, *509*, 385 – 388.
[29] A. T. Krueger, H. G. Lu, A. H. F. Lee, E. T. Kool, *Acc. Chem. Res.* **2007**, *40*, 141 – 150.
[30] E. T. Kool, *Acc. Chem. Res.* **2002**, *35*, 936 – 943.
[31] a) M. Fischler, U. Simon, H. Nir, Y. Eichen, G. A. Burley, J. Gierlich, P. M. E. Gramlich, T. Carell, *Small* **2007**, *3*, 1049 – 1055; b) H. Rao, A. A. Tanpure, A. A. Sawant, S. G. Srivatsan, *Nat. Protoc.* **2012**, *7*, 1097 – 1112; c) M. Fischler, A. Sologubenko, J. Mayer, G. Clever, G. Burley, J. Gierlich, T. Carell, U. Simon, *Chem. Commun.* **2008**, 169 – 171.
[32] A. De Mesmaeker, K. H. Altmann, A. Waldner, S. Wendeborn, *Curr. Opin. Struct. Biol.* **1995**, *5*, 343 – 355.
[33] G. D. Hoke, K. Draper, S. M. Freier, C. Gonzalez, V. B. Driver, M. C. Zounes, D. J. Ecker, *Nucleic Acids Res.* **1991**, *19*, 5743 – 5748.
[34] R. S. Geary, D. Norris, R. Yu, C. F. Bennett, *Adv. Drug Delivery Rev.* **2015**, *87*, 46 – 51.
[35] C. F. Bennett, E. E. Swayze, *Annu. Rev. Pharmacol.* **2010**, *50*, 259 – 293.
[36] B. R. Meade, K. Gogoi, A. S. Hamil, C. Palm-Apergi, A. van den Berg, J. C. Hagopian, A. D. Springer, A. Eguchi, A. D. Kacsinta, C. F. Dowdy, A. Presente, P. Lonn, M. Kaulich, N. Yoshioka, E. Gros, X. S. Cui, S. F. Dowdy, *Nat. Biotechnol.* **2014**, *32*, 1256-U1122.
[37] L. S. Lerman, *Proc. Natl. Acad. Sci. USA* **1963**, *49*, 94 – 102.
[38] A. Mukherjee, R. Lavery, B. Bagchi, J. T. Hynes, *J. Am. Chem. Soc.* **2008**, *130*, 9747 – 9755.
[39] J. Brglez, P. Nikolov, A. Angelin, C. M. Niemeyer, *Chem. Eur. J.* **2015**, *21*, 9440 – 9446.
[40] A. A. Greschner, K. E. Bujold, H. F. Sleiman, *J. Am. Chem. Soc.* **2013**, *135*, 11283 – 11288.
[41] Q. Jiang, C. Song, J. Nangreave, X. W. Liu, L. Lin, D. L. Qiu, Z. G. Wang, G. Z. Zou, X. J. Liang, H. Yan, B. Q. Ding, *J. Am. Chem. Soc.* **2012**, *134*, 13396 – 13403.
[42] P. B. Dervan, *Bioorg. Med. Chem.* **2001**, *9*, 2215 – 2235.
[43] a) Z. Cao, R. Tong, A. Mishra, W. Xu, G. C. Wong, J. Cheng, Y. Lu, *Angew. Chem. Int. Ed.* **2009**, *48*, 6494 – 6498; *Angew. Chem.* **2009**, *121*, 6616 – 6620; b) S. D. Perrault, W. M. Shih, *ACS Nano* **2014**, *8*, 5132 – 5140; c) Y. R. Wu, K. Sefah, H. P. Liu, R. W. Wang, W. H. Tan, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 5 – 10; d) C. C. Wu, T. Chen, D. Han, M. X. You, L. Peng, S. Cansiz, G. Z. Zhu, C. M. Li, X. L. Xiong, E. Jimenez, C. J. Yang, W. H. Tan, *ACS Nano* **2013**, *7*, 5724 – 5731.
[44] a) A. M. Rush, M. P. Thompson, E. T. Tatro, N. C. Gianneschi, *ACS Nano* **2013**, *7*, 1379 – 1387; b) C. Zhang, L. L. Hao, C. M. Calabrese, Y. Zhou, C. H. J. Choi, H. Xing, C. A. Mirkin, *Small* **2015**, *11*, 5360 – 5368.
[45] Q. Zhang, Q. Jiang, N. Li, L. R. Dai, Q. Liu, L. L. Song, J. Y. Wang, Y. Q. Li, J. Tian, B. Q. Ding, Y. Du, *ACS Nano* **2014**, *8*, 6633 – 6643.
[46] Y. X. Zhao, A. Shaw, X. H. Zeng, E. Benson, A. M. Nystrom, B. Hogberg, *ACS Nano* **2012**, *6*, 8684 – 8691.
[47] G. Z. Zhu, J. Zheng, E. Q. Song, M. Donovan, K. J. Zhang, C. Liu, W. H. Tan, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 7998 – 8003.
[48] a) Y. F. Huang, D. H. Shangguan, H. P. Liu, J. A. Phillips, X. L. Zhang, Y. Chen, W. H. Tan, *ChemBioChem* **2009**, *10*, 862 – 868; b) G. Z. Zhu, S. Cansiz, M. X. You, L. P. Qiu, D. Han, L. Q. Zhang, L. Mei, T. Fu, Z. Chen, W. H. Tan, *Npg Asia Materials* **2015**, *7*, e169.
[49] X. Y. Tan, B. B. Li, X. G. Lu, F. Jia, C. Santori, P. Menon, H. Li, B. H. Zhang, J. J. Zhao, K. Zhang, *J. Am. Chem. Soc.* **2015**, *137*, 6112 – 6115.
[50] Y. Krishnan, F. C. Simmel, *Angew. Chem. Int. Ed.* **2011**, *50*, 3124 – 3156; *Angew. Chem.* **2011**, *123*, 3180 – 3215.
[51] T. Tokunaga, S. Namiki, K. Yamada, T. Imaishi, H. Nonaka, K. Hirose, S. Sando, *J. Am. Chem. Soc.* **2012**, *134*, 9561 – 9564.
[52] B. L. Blakely, C. E. Dumelin, B. Trappmann, L. M. McGregor, C. K. Choi, P. C. Anthony, V. Duesterberg, B. M. Baker, S. M. Block, D. R. Liu, C. S. Chen, *Nat. Methods* **2014**, *11*, 1229 – 1232.
[53] a) J. Charlton, J. Sennello, D. Smith, *Chem. Biol.* **1997**, *4*, 809 – 816; b) B. J. Hicke, A. W. Stephens, T. Gould, Y. F. Chang, C. K. Lynott, J. Heil, S. Borkowski, C. S. Hilger, G. Cook, S. Warren, P. G. Schmidt, *J. Nucl. Med.* **2006**, *47*, 668 – 678; c) B. Tavitian, F. Duconge, R. Boisgard, F. Dolle, *Methods Mol. Biol.* **2009**, *535*, 241 – 259.
[54] R. M. Franzini, D. Neri, J. Scheuermann, *Acc. Chem. Res.* **2014**, *47*, 1247 – 1255.
[55] Z. J. Gartner, D. R. Liu, *J. Am. Chem. Soc.* **2001**, *123*, 6961 – 6963.
[56] a) D. R. Halpin, P. B. Harbury, *PLoS Biol.* **2004**, *2*, 1015 – 1021; b) D. R. Halpin, P. B. Harbury, *PLoS Biol.* **2004**, *2*, 1022 – 1030; c) D. R. Halpin, J. A. Lee, S. J. Wrenn, P. B. Harbury, *PLoS Biol.* **2004**, *2*, 1031 – 1038.
[57] S. Melkko, J. Scheuermann, C. E. Dumelin, D. Neri, *Nat. Biotechnol.* **2004**, *22*, 568 – 574.
[58] a) Y. Z. Li, P. Zhao, M. D. Zhang, X. Y. Zhao, X. Y. Li, *J. Am. Chem. Soc.* **2013**, *135*, 17727 – 17730; b) P. Zhao, Z. T. Chen, Y. Z. Li, D. W. Sun, Y. Gao, Y. Y. Huang, X. Y. Li, *Angew. Chem. Int. Ed.* **2014**, *53*, 10056 – 10059; *Angew. Chem.* **2014**, *126*, 10220 – 10223.
[59] C. Zambaldo, S. Barluenga, N. Winssinger, *Curr. Opin. Chem. Biol.* **2015**, *26*, 8 – 15.
[60] J. Niu, R. Hili, D. R. Liu, *Nat. Chem.* **2013**, *5*, 282 – 292.
[61] D. Sil, J. B. Lee, D. Luo, D. Holowka, B. Baird, *ACS Chem. Biol.* **2007**, *2*, 674 – 684.

[62] F. Abendroth, A. Bujotzek, M. Shan, R. Haag, M. Weber, O. Seitz, *Angew. Chem. Int. Ed.* **2011**, *50*, 8592 – 8596; *Angew. Chem.* **2011**, *123*, 8751 – 8755.
[63] a) Y. R. Yang, Y. Liu, H. Yan, *Bioconjugate Chem.* **2015**, *26*, 1381 – 1395; b) C. M. Niemeyer, W. Burger, J. Peplies, *Angew. Chem. Int. Ed.* **1998**, *37*, 2265 – 2268; *Angew. Chem.* **1998**, *110*, 2391 – 2395; c) C. X. Lin, Y. Liu, H. Yan, *Nano Lett.* **2007**, *7*, 507 – 512.
[64] a) O. I. Wilner, Y. Weizmann, R. Gill, O. Lioubashevski, R. Freeman, I. Willner, *Nat. Nanotechnol.* **2009**, *4*, 249 – 254; b) C. M. Niemeyer, J. Koehler, C. Wuerdemann, *ChemBioChem* **2002**, *3*, 242 – 245.
[65] a) D. A. Rusling, A. R. Chandrasekaran, Y. P. Ohayon, T. Brown, K. R. Fox, R. J. Sha, C. D. Mao, N. C. Seeman, *Angew. Chem. Int. Ed.* **2014**, *53*, 3979 – 3982; *Angew. Chem.* **2014**, *126*, 4060 – 4063; b) R. J. Sha, J. J. Birktoft, N. Nguyen, A. R. Chandrasekaran, J. P. Zheng, X. S. Zhao, C. D. Mao, N. C. Seeman, *Nano Lett.* **2013**, *13*, 793 – 797.
[66] a) F. Diezmann, O. Seitz, *Chem. Soc. Rev.* **2011**, *40*, 5789 – 5801; b) L. Röglin, O. Seitz, *Org. Biomol. Chem.* **2008**, *6*, 3881 – 3887.
[67] H. Eberhard, F. Diezmann, O. Seitz, *Angew. Chem. Int. Ed.* **2011**, *50*, 4146 – 4150; *Angew. Chem.* **2011**, *123*, 4232 – 4236.
[68] F. Diezmann, L. von Kleist, V. Hauckeb, O. Seitz, *Org. Biomol. Chem.* **2015**, *13*, 8008 – 8015.
[69] A. Shaw, V. Lundin, E. Petrova, F. Fordos, E. Benson, A. Al-Amin, A. Herland, A. Blokzijl, B. Hogberg, A. I. Teixeira, *Nat. Methods* **2014**, *11*, 841 – 846.
[70] a) K. E. Bujold, J. Fakhoury, T. G. W. Edwardson, K. M. M. Carneiro, J. N. Briard, A. G. Godin, L. Amrein, G. D. Hamblin, L. C. Panasci, P. W. Wiseman, H. F. Sleiman, *Chem. Sci.* **2014**, *5*, 2449 – 2455; b) A. S. Walsh, H. F. Yin, C. M. Erben, M. J. A. Wood, A. J. Turberfield, *ACS Nano* **2011**, *5*, 5427 – 5432.
[71] J. W. de Vries, F. Zhang, A. Herrmann, *J. Controlled Release* **2013**, *172*, 467 – 483.
[72] X. W. Liu, Y. Xu, T. Yu, C. Clifford, Y. Liu, H. Yan, Y. Chang, *Nano Lett.* **2012**, *12*, 4254 – 4259.
[73] F. A. Aldaye, W. T. Senapedis, P. A. Silver, J. C. Way, *J. Am. Chem. Soc.* **2010**, *132*, 14727 – 14729.
[74] N. Stephanopoulos, R. Freeman, H. A. North, S. Sur, S. J. Jeong, F. Tantakitti, J. A. Kessler, S. I. Stupp, *Nano Lett.* **2015**, *15*, 603 – 609.
[75] Y. Wu, C. Li, F. Boldt, Y. Wang, S. L. Kuan, T. T. Tran, V. Mikhalevich, C. Fortsch, H. Barth, Z. Yang, D. Liu, T. Weil, *Chem. Commun.* **2014**, *50*, 14620 – 14622.
[76] R. P. Feynman, *Caltech Engineering and Science* **1960**, *23*, 22 – 36.
[77] W. Cheung, F. Pontoriero, O. Taratula, A. M. Chen, H. X. He, *Adv. Drug Delivery Rev.* **2010**, *62*, 633 – 649.
[78] N. Chen, J. Li, H. Y. Song, J. Chao, Q. Huang, C. H. Fan, *Acc. Chem. Res.* **2014**, *47*, 1720 – 1730.
[79] H. Liang, X. B. Zhang, Y. F. Lv, L. Gong, R. W. Wang, X. Y. Zhu, R. H. Yang, W. H. Tan, *Acc. Chem. Res.* **2014**, *47*, 1891 – 1901.
[80] R. Shukla, V. Bansal, M. Chaudhary, A. Basu, R. R. Bhonde, M. Sastry, *Langmuir* **2005**, *21*, 10644 – 10654.
[81] a) E. E. Connor, J. Mwamuka, A. Gole, C. J. Murphy, M. D. Wyatt, *Small* **2005**, *1*, 325 – 327; b) S. Vijayakumar, S. Ganesan, *Toxicol. Environ. Chem.* **2013**, *95*, 277 – 287.
[82] T. J. Song, L. H. Tang, L. H. Tan, X. J. Wang, N. S. R. Satyavolu, H. Xing, Z. D. Wang, J. H. Li, H. J. Liang, Y. Lu, *Angew. Chem. Int. Ed.* **2015**, *54*, 8114 – 8118; *Angew. Chem.* **2015**, *127*, 8232 – 8236.
[83] X. Huang, P. K. Jain, I. H. El-Sayed, M. A. El-Sayed, *Nanomedicine* **2007**, *2*, 681 – 693.
[84] H. Pei, F. Li, Y. Wan, M. Wei, H. J. Liu, Y. Su, N. Chen, Q. Huang, C. H. Fan, *J. Am. Chem. Soc.* **2012**, *134*, 11876 – 11879.
[85] J. X. Li, B. Q. Zhu, Z. Zhu, Y. C. Zhang, X. J. Yao, S. Tu, R. D. Liu, S. S. Jia, C. Y. J. Yang, *Langmuir* **2015**, *31*, 7869 – 7876.
[86] D. S. Seferos, A. E. Prigodich, D. A. Giljohann, P. C. Patel, C. A. Mirkin, *Nano Lett.* **2009**, *9*, 308 – 311.
[87] a) D. A. Giljohann, D. S. Seferos, P. C. Patel, J. E. Millstone, N. L. Rosi, C. A. Mirkin, *Nano Lett.* **2007**, *7*, 3818 – 3821; b) D. A. Giljohann, D. S. Seferos, W. L. Daniel, M. D. Massich, P. C. Patel, C. A. Mirkin, *Angew. Chem. Int. Ed.* **2010**, *49*, 3280 – 3294; *Angew. Chem.* **2010**, *122*, 3352 – 3366.
[88] L. Y. T. Chou, K. Zagorovsky, W. C. W. Chan, *Nat. Nanotechnol.* **2014**, *9*, 148 – 155.
[89] J. I. Cutler, E. Auyeung, C. A. Mirkin, *J. Am. Chem. Soc.* **2012**, *134*, 1376 – 1391.
[90] P. C. Patel, D. A. Giljohann, W. L. Daniel, D. Zheng, A. E. Prigodich, C. A. Mirkin, *Bioconjugate Chem.* **2010**, *21*, 2250 – 2256.
[91] D. A. Giljohann, D. S. Seferos, A. E. Prigodich, P. C. Patel, C. A. Mirkin, *J. Am. Chem. Soc.* **2009**, *131*, 2072 – 2073.
[92] K. Zhang, L. L. Hao, S. J. Hurst, C. A. Mirkin, *J. Am. Chem. Soc.* **2012**, *134*, 16488 – 16491.
[93] P. S. Randeria, M. A. Seeger, X. Q. Wang, H. Wilson, D. Shipp, C. A. Mirkin, A. S. Paller, *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 5573 – 5578.
[94] R. F. Service, *Science* **2015**, *349*, 1150 – 1151.
[95] A. F. Radovic-Moreno, N. Chernyak, C. C. Mader, S. Nallagatla, R. S. Kang, L. L. Hao, D. A. Walker, T. L. Halo, T. J. Merkel, C. H. Rische, S. Anantatmula, M. Burkhart, C. A. Mirkin, S. M. Gryaznov, *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 3892 – 3897.
[96] K. L. Young, A. W. Scott, L. L. Hao, S. E. Mirkin, G. L. Liu, C. A. Mirkin, *Nano Lett.* **2012**, *12*, 3867 – 3871.
[97] A. J. Mieszawska, W. J. M. Mulder, Z. A. Fayad, D. P. Cormode, *Mol. Pharm.* **2013**, *10*, 831 – 847.
[98] D. Kim, Y. Y. Jeong, S. Jon, *ACS Nano* **2010**, *4*, 3689 – 3696.
[99] Z. Y. Xiao, C. W. Ji, J. J. Shi, E. M. Pridgen, J. Frieder, J. Wu, O. C. Farokhzad, *Angew. Chem. Int. Ed.* **2012**, *51*, 11853 – 11857; *Angew. Chem.* **2012**, *124*, 12023 – 12027.
[100] S. Dhar, W. L. Daniel, D. A. Giljohann, C. A. Mirkin, S. J. Lippard, *J. Am. Chem. Soc.* **2009**, *131*, 14652 – 14653.
[101] S. F. Torabi, Y. Lu, *Curr. Opin. Biotechnol.* **2014**, *28*, 88 – 95.
[102] H. Pei, X. L. Zuo, D. Zhu, Q. Huang, C. H. Fan, *Acc. Chem. Res.* **2014**, *47*, 550 – 559.
[103] C. H. Fan, K. W. Plaxco, A. J. Heeger, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 9134 – 9137.
[104] H. Pei, N. Lu, Y. L. Wen, S. P. Song, Y. Liu, H. Yan, C. H. Fan, *Adv. Mater.* **2010**, *22*, 4754 – 4758.
[105] a) Z. L. Ge, M. H. Lin, P. Wang, H. Pei, J. Yan, J. Y. Sho, Q. Huang, D. N. He, C. H. Fan, X. L. Zuo, *Anal. Chem.* **2014**, *86*, 2124 – 2130; b) Y. Wen, H. Pei, Y. Shen, J. Xi, M. Lin, N. Lu, X. Shen, J. Li, C. Fan, *Sci. Rep.* **2012**, *2*, 867.
[106] Y. Xiang, P. W. Wu, L. H. Tan, Y. Lu in *Biosensors Based on Aptamers and Enzymes* (Eds.: M. B. Gu, H.-S. Kim), Springer, **2014**, pp. 93 – 120.
[107] P. W. Wu, K. V. Hwang, T. Lan, Y. Lu, *J. Am. Chem. Soc.* **2013**, *135*, 5254 – 5257.
[108] a) Z. Zhu, Z. C. Guan, S. S. Jia, Z. C. Lei, S. C. Lin, H. M. Zhang, Y. L. Ma, Z. Q. Tian, C. J. Yang, *Angew. Chem. Int. Ed.* **2014**, *53*, 12503 – 12507; *Angew. Chem.* **2014**, *126*, 12711 – 12715; b) Z. Zhu, Z. C. Guan, D. Liu, S. S. Jia, J. X. Li, Z. C. Lei, S. C. Lin, T. H. Ji, Z. Q. Tian, C. Y. J. Yang, *Angew. Chem. Int. Ed.* **2015**, *54*, 10448 – 10453; *Angew. Chem.* **2015**, *127*, 10594 – 10599.
[109] a) L. H. Reddy, J. L. Arias, J. Nicolas, P. Couvreur, *Chem. Rev.* **2012**, *112*, 5818 – 5878; b) T. Chen, M. I. Shukoor, R. W. Wang, Z. L. Zhao, Q. Yuan, S. Bamrungsap, X. L. Xiong, W. H. Tan, *ACS Nano* **2011**, *5*, 7866 – 7873.
[110] A. Samanta, Z. T. Deng, Y. Liu, H. Yan, *Nano Res.* **2013**, *6*, 853 – 870.
[111] a) E. Ruiz-Hernández, A. Baeza, M. Vallet-Regi, *ACS Nano* **2011**, *5*, 1259 – 1266; b) Q. Yuan, Y. F. Zhang, T. Chen, D. Q. Lu, Z. L. Zhao, X. B. Zhang, Z. X. Li, C. H. Yan, W. H. Tan, *ACS Nano* **2012**, *6*, 6337 – 6344; c) C. E. Chen, J. Geng, F. Pu, X. J. Yang, J. S. Ren, X. G. Qu, *Angew. Chem. Int. Ed.* **2011**, *50*, 882 – 886; *Angew. Chem.* **2011**, *123*, 912 – 916.
[112] a) H. Xing, N. Y. Wong, Y. Xiang, Y. Lu, *Curr. Opin. Chem. Biol.* **2012**, *16*, 429 – 435; b) J. J. Lia, J. J. Zhu, *Analyst* **2013**, *138*, 2506 – 2515.

Manuscript received: January 29, 2016
Final Article published: April 5, 2016