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Developing DNA Aptamer Toolbox for Cell Research

Liang Yue[1], Shan Wang[1], and Weihong Tan[1,2,3]

[1]Hunan University, College of Chemistry and Chemical Engineering, College of Biology, Aptamer Engineering Center of Hunan Province, Molecular Science and Biomedicine Laboratory, State Key Laboratory of Chemo/Biosensing and Chemometrics, 2 Lushan Road, Changsha 410082, China
[2]Chinese Academy of Sciences, Hangzhou Institute of Medicine, The Cancer Hospital of the University of Chinese Academy of Sciences (Zhejiang Cancer Hospital), 150 Dongfang Street Xiasha, Hangzhou 310000, China
[3]Shanghai Jiao Tong University, Shanghai Jiao Tong University School of Medicine, Institute of Molecular Medicine, College of Chemistry and Chemical Engineering, Renji Hospital, 800 Dongchuan RD, Shanghai 200240, China

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1.1 Cells and Their Complexity

Cells are the basic building blocks of life, initially discovered by Robert Hooke in 1665 [1, 2]. Their structural and functional complexity attracts significant research interest due to their intricate composition, abundance, and interactivity [3–5]. Even the simplest unicellular organism comprises various biomolecular components, ranging from nucleic acids, proteins, and lipids to carbohydrates, which are elaborately bonded together. Moreover, cells exhibit remarkable plasticity and can proliferate and differentiate to create distinct cell populations with radically different compositions and interactions, which enables them to implement specific and varied functions [6–8]. The organization of these cell populations yields a highly collaborative living system capable of carrying out all of life's biological processes, where cells execute their functions through molecular interactions with the external environment and other cells [9, 10]. Furthermore, cells are open systems that facilitate the exchange of information, energy, and matter between cells and their external environment [11–14]. The dissipation of energy and matter is a continuous process sustaining cellular functions, which drives the cells to operate far from equilibrium [15, 16]. The nonlinear interactions and reactions of the components further enhance the complexity of cellular systems, revealing extraordinary dynamic behaviors and functions adapting to ever-changing environmental cues [17–19].

To understand cells and their functions in their entirety, it is essential to investigate components that interact to develop and maintain high-order cellular structures and realize complex biochemical activities [20, 21]. Reliable and accurate molecular recognition tools are required to detect specific components from the

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cellular pool [22, 23]. In addition, suitable technologies and methodologies are needed to observe components' dynamics and analyze their nonlinear behaviors in biological processes [24–28]. This field has experienced substantial advancements propelled by breakthroughs in molecular recognition tools and analytical methods. Further, researchers have devoted themselves to developing new and innovative tools to observe, measure, and manipulate cellular components and systems at unprecedented levels of detail. These progresses broaden our understanding of cellular mechanisms and pave the way to develop novel therapies and treatments for diseases associated with cellular dysfunction [29–34].

Functional nucleic acids, as synthetic oligonucleotides with unique chemical-biological properties, sequence programmability, simplicity of modification, and facile functional manipulation, have demonstrated bioactivity, versatility, and *in vivo* bioavailability toward biological and biomedicine applications [35–39]. The rapid progress in DNA nanotechnology has offered remarkable opportunities for tackling the intricate nature of cellular systems [40]. In particular, introducing DNA aptamers with excellent molecular recognition ability into the modular design of molecular tools has opened new avenues in cell research, providing captivating prospects for handling complex molecular systems with precision and accuracy.

1.2 Features and Advantages of DNA Aptamers

DNA aptamers [41], termed "chemical antibodies", are short, single-stranded nucleic acid molecules (20–100 nucleotides) that can fold into complex 3D structures and bind with various targets (e.g. metal ions [42], small organic molecules [43], proteins [44], viruses [45], bacteria [46], cells [47], and even tissues [48]) with specificity and high affinity, Figure 1.1. Compared with natural antibodies, the core competency of DNA aptamers rests with the unique properties of synthetic nucleic acids [41], involving relatively low molecular weight, low-cost standardization synthesis, customized modification, programmable sequences and structures, sequence-dependent multifunctionality, etc. The size of DNA aptamers (10–30 kDa and ~3 nm in diameter) is much smaller than that of natural antibodies (~150 kDa and 10–15 nm in diameter), which endows DNA aptamers with fewer steric hindrance and unimpaired binding affinity in a confined environment, thus rendering a broader application [49]. For instance, Yang et al. reported a straightforward strategy for labeling and manipulating cell surface protein by utilizing DNA aptamers [50]. Specifically, an aptamer-based logic computing reaction is used to recognize selectively and covalently conjugate immune checkpoint antagonizing aptamers (e.g. D-aPDL1, D-Sgc8-PA, and D-Sgc8-99mTc) on the surface of cancer cells, improving the precision and robustness of immune checkpoint blockade therapy.

In contrast with the complex modifying process and unmanageable conjugation numbers and sites of natural antibodies [51], DNA aptamers are facile to be synthesized and modified as a consequence of the technological progress in phosphoramidite chemistry-based oligonucleotide synthesis over the past decades [52].

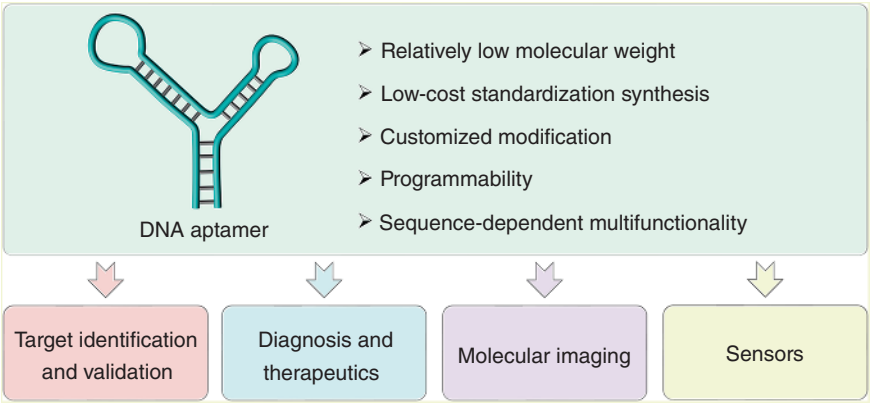

Figure 1.1 Schematic illustration of the DNA aptamer tools for various applications.

With recent advancements in automated modular synthesizers and phosphoramidite chemistry, DNA aptamers can now be conjugated with multiple functional groups at predetermined locations [53]. This approach provides many benefits for practical applications of DNA aptamers, including aptamer–drug conjugates (ApDCs) [54] and artificial nucleobase-expanded aptamers [55].

Furthermore, nucleic acids possess base sequence programmability and modular design characteristics, whereas natural antibodies currently do not. By integrating modular design and programmability into DNA aptamer systems, modular and programmable toolboxes can be created to meet future demands in advanced biological molecular tools and materials. Specifically, DNA aptamers with target recognition abilities can be regarded as functional modules that can be integrated, on demand, into nanostructures [56], nanodevices [57], and other functional systems [58]. These features enable researchers to engineer DNA aptamer-based systems with a high degree of flexibility and precision at the molecular level. Further, the highly predictable base-pairing and structural reconfiguration rules of nucleic acids provide a strong basis for designing programmable DNA structures and controlling DNA dynamics. Through their conjugation with specific functional components, DNA aptamers can respond to various environmental cues [59–61], such as pH, light, and temperature, and numerous biological targets (e.g. DNA/RNA, ATP, enzymes, and metal ions) leading to innovative nanodevices. With these superior merits, DNA aptamer-based systems have been considered promising candidates for various cellular applications.

1.3 On-demand Synthesis and Screening of DNA Aptamers

Highly accurate and pure DNA oligonucleotides are vital for DNA aptamer screening and their practical applications. Since its introduction by Caruthers in 1981 [62], phosphoramidite chemistry has emerged as the gold standard for

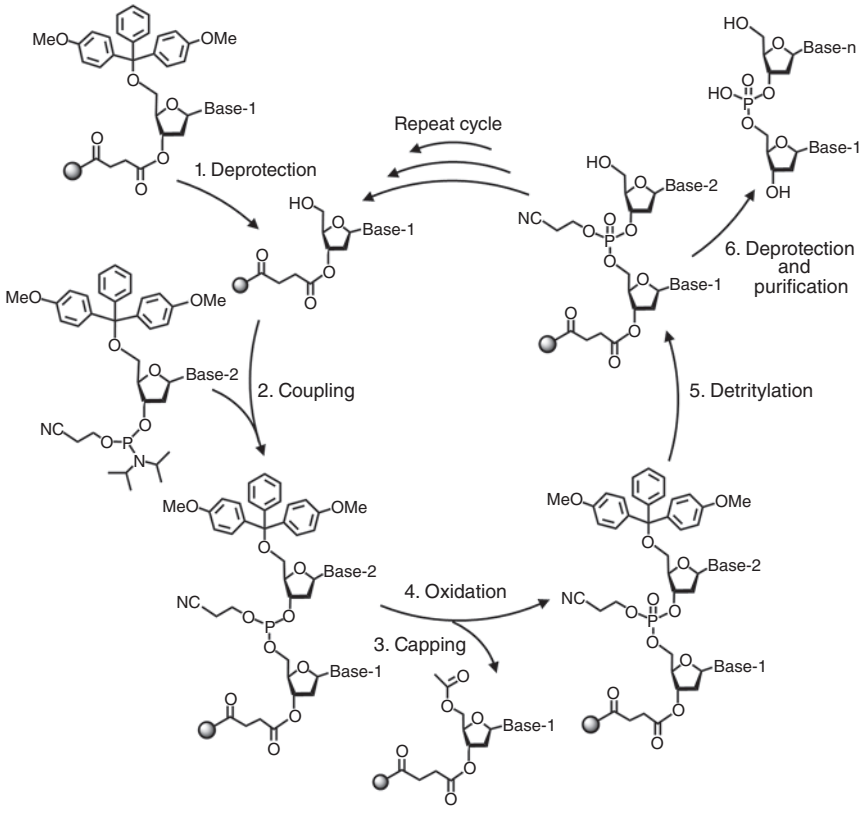

Figure 1.2 Schematic representation of solid-supported oligonucleotide synthesis.

the synthesis of short DNA oligonucleotides (<200 nucleotides). This innovative chemical method has revolutionized the field of DNA synthesis, which allows for the production of DNA sequences with high purity and chemical modifications customized for specific research or practical applications. To grow oligonucleotide chains using phosphoramidite chemistry via a solid-supported oligonucleotide synthesis, the following steps are typically involved, as depicted in Figure 1.2: (1) Deprotection of resin-supported nucleoside: The first nucleoside protected by a dimethoxytrityl (DMT) group is pre-attached to resin. Then, the 5′-DMT protecting group is removed by acid-catalyzed detritylation to expose the 5′-hydroxyl group as a reactive site for the addition of the next nucleotide; (2) Coupling: Taking tetrazole or its derivative as an activator, the diisopropylamino group of the incoming nucleoside phosphoramidite is protonated to enhance its reactivity as a leaving group. The 5′-hydroxyl group of the support-bound nucleoside attacks the phosphorus atom of the activated nucleoside phosphoramidite, resulting in a new phosphorus-oxygen bond; (3) Capping: To prevent the formation of any truncated sequences, a "capping" procedure is introduced after the coupling reaction, which effectively prevents unreacted 5′-hydroxyl groups from participating in subsequent coupling reactions. With all unreacted sites "capped," the oligonucleotide

synthesis proceeds exclusively at full-length oligonucleotides; (4) Oxidation: The phosphite-triester (P^{III}) formed during the coupling step is susceptible to acid and needs to be converted into a stable form (P^{V}) before the implementation of next acidic detritylation step. This can be achieved through iodine oxidation in the presence of water and pyridine. The resulting phosphotriester is, essentially, the DNA backbone and is protected with a 2-cyanoethyl group from undesirable reactions during subsequent synthesis cycles; (5) Detritylation: After the oxidation step, the DMT-protecting group located at the 5′-end of the resin-bound DNA chain is removed for the following nucleotide phosphoramidite. Repeat steps 2 through 5 until the desired oligonucleotide sequence has been synthesized; (6) Deprotection and purification: Finally, all the remaining protecting groups (e.g. the protecting groups from the heterocyclic bases and phosphodiester backbone) are removed, and the oligonucleotide is purified using various methods, such as HPLC or PAGE.

The advancement of automated oligonucleotide synthesis technologies has significantly improved the synthetic length, speed, cost, and throughput, enabling high-throughput, cost-effective, and large-scale synthesis of DNA aptamers with high efficiency and precision. Because of the precise synthesis capability, the fine control over DNA aptamers is accessible, allowing for their incorporation with different chemical modifications where the position and quantity of modification can be manipulated to alter the properties and functions of DNA aptamers. The ability to engineer the structure and specificity of DNA aptamers through synthesis and chemical modification makes them a highly flexible and customizable tool for a diverse range of biological and biomedical research areas.

Relative to antibodies evolved from the natural immune response, aptamers are produced mainly by an *in vitro* evolution method, the systematic evolution of ligands by the exponential enrichment (SELEX) technology, developed by Szostak and Gold in 1990 [63, 64]. This technology involves iterative rounds of selection and amplification of specific nucleic acid sequences in a large oligo pool, through which various aptamers have evolved for targeting small molecules (e.g. organic dyes [65], ATP [66], cocaine [67], and mycotoxins [68]) or proteins (e.g. thrombin [69] and tumor biomarkers [70]), with specificity and high affinity. Nevertheless, the screened DNA aptamers through SELEX processes may undergo structural reconfiguration and function loss in a physiological environment, which limits their utilization in cell research.

In 2006, our group introduced an extension of the SELEX technology called cell-SELEX [47], aiming to select aptamers against living cells. Since then, the target has been developed into diverse living species [71, 72] (e.g. bacteria, viruses, and disease tissues). For the cell-SELEX technology, a large-capacity (10^{15}–10^{16}) oligonucleotide library needs to be engineered before the screening, and each oligonucleotide comprises random sequence regions in the middle and primer sequence-binding regions at both ends. A typical cell-SELEX process involves a six-step selection and amplification cycle: (i) Incubation of target cells with a library of single-stranded DNAs including a random domain; (ii) Collection of the target cell-bound oligonucleotides; (iii) Amplification of the collected oligonucleotides by PCR; (iv) Counter-selection step by using negative cells to reduce the common

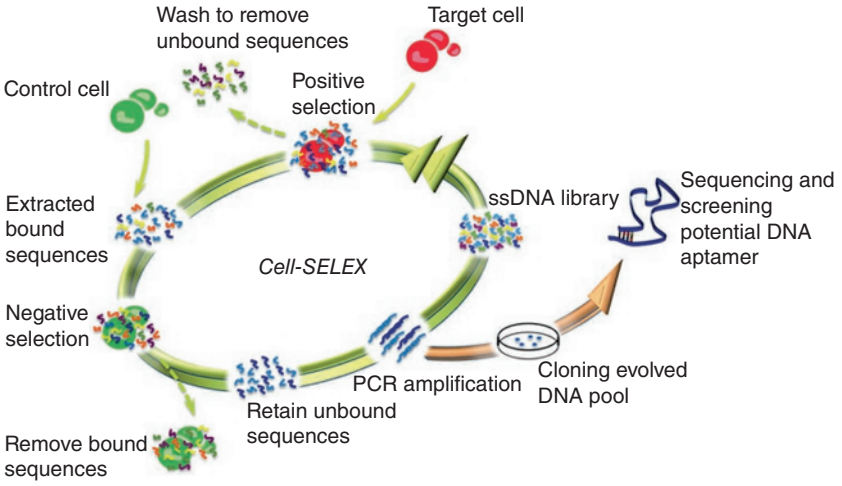

Figure 1.3 Schematic representation of DNA aptamer screening by the cell-SELEX process. Source: Reproduced with permission from Sefah et al. [72]. © 2010 Springer Nature.

sequences that bind with both cells; (v) Retainment of the unbound DNAs from counter-selection process; (vi) Amplification of the unbound DNAs to yield an enriched oligonucleotide pool for the following round selection. The process is iterated until the sequences specifically targeting the cell are highly enriched. The aptamer's affinity to the target molecules in a living cell can be significantly improved after 10–20 rounds of screening. Finally, corresponding pools are sorted and optimized for further cloning and sequencing, as shown in Figure 1.3.

Compared with the traditional SELEX strategy, cell-SELEX possesses several superiorities in the complex cellular system: (i) Massive biomolecules exist with different functional states and abundance in the cell. Aptamers produced by cell-SELEX can bind to these biomolecules naturally, conducive to understanding how these molecules interact and realize complex biochemical activities. (ii) It is unnecessary to know and purify target cells' molecular signatures in cell-SELEX since aptamers can target unknown molecules, and it enables the discovery of potential biomarkers. (iii) For cell research, aptamers generated from cell-SELEX are in their active and native states without structural unfolding in a cellular environment. (iv) Accompanied by the occurrence of disease in a living system, a fundamental change at the molecular level takes place, like the emergence of cancer with unique biomarkers. By analyzing the molecular difference between disease cells and normal cells, potential biomarkers and new molecular events can be targeted by cell-SELEX, thus distinguishing disease cells from normal cells. Cell-SELEX has successfully screened out hundreds of aptamers against more than 80 different cell lines, opening up new avenues for cellular applications [73, 74]. For example, Wu et al. reported a DNA aptamer, XQ-2d, which targets the transferrin receptor CD71 with high affinity and specificity [75]. CD71, a membrane glycoprotein and receptor of the iron-transferrin complex, is abundantly expressed in various cancer cells, including those found in the brain, liver, breast, lung, and colon. It is considered a significant independent

prognostic marker and a promising therapeutic target for several cancers. Among 40 clinical samples of pancreatic adenocarcinoma investigated using Aptamer XQ-2d, CD71 is overexpressed in 82.5% of the samples. Furthermore, Aptamer XQ-2d has been used for the targeted delivery of doxorubicin (DOX) to pancreatic cancer cells. The selective delivery of loaded DOX into PDAC cell line PL45 results in a significant antiproliferation effect, implying the XQ-2d aptamer's great potential for targeted pancreatic cancer therapy.

With the advent of chemical synthesis and cell-SELEX technology, DNA aptamers have emerged as a powerful toolbox for the biological and biomedicine fields due to their unique properties, including high binding affinity, specificity, stability, and simplicity of synthesis. DNA aptamers have been widely applied for cell research to identify and validate new targets on the surface of cells [76], to investigate intracellular processes, or to control cell behaviors. Also, they have been employed as biosensors [41] to detect proteins, nucleic acids, or small molecules in real time or as therapeutic agents for targeted drug delivery [57] and gene regulation. Furthermore, DNA aptamers have assisted in the isolation and detection of rare cell populations as well as in cell imaging [56] and molecular diagnostics [77]. Overall, DNA aptamers constitute a versatile tool for cell research in favor of the elucidation of the complicated workings of cells. As research in DNA aptamers progresses, new and exciting applications are anticipated to emerge, which could significantly enhance their utility and impact on cell research. These advancements have the potential to revolutionize the field of biomedicine, resulting in improved patient outcomes.

1.4 Toward a Toolbox of DNA Aptamers for Cellular Applications

As life science continues to develop, there is a growing requirement for molecular tools that enable researchers to understand cellular compositions and precisely control their dynamic behaviors. Recent advances in molecular engineering in DNA aptamers have paved the way toward a DNA aptamer-based toolbox for various cellular applications [39, 78–80]. This toolbox consists of a standardized set of DNA aptamers that have been extensively validated for their specificity, affinity, protocols for synthesis, modification, integration, characterization, and utility in cellular applications. We believe the ongoing development of such a toolbox will unlock the full potential of DNA aptamers for cell research.

To develop and utilize a toolbox composed of a versatile set of DNA aptamers for cell research, here are the necessary protocols we can follow: (i) Identify the tasks and select appropriate aptamers. To reach research goals, we need to identify the target (e.g. biomolecules, viruses, cells, or tissues) and then select a suitable DNA aptamer with high affinity and specificity against the target. (ii) Verification of DNA aptamers: Once the DNA aptamer candidate is selected, it is crucial to verify its binding affinity and specificity by performing enzyme-linked oligonucleotide assay (ELONA) or surface plasmon resonance (SPR) analysis.

(iii) Aptamers modification: To meet the needs in the specific application scenario, it is necessary to modify them with appropriate functional groups. This process can be realized using various chemical or integrative approaches involving post-SELEX chemical modification, DNA nanotechnology, or supramolecular self-assembly. (iv) Application and optimization of DNA aptamer tools: After we have delivered DNA aptamer tools into cells for various applications, it is vital to evaluate their performance, optimize them based on feedback, and create new tools to fill any gaps in the toolbox when confronted with new tasks. By following these protocols and preparing for challenges, researchers can efficiently develop and utilize DNA aptamer tools in cell research.

In this context, we discuss recent advances in engineering DNA aptamers as targeting and regulating modules and integrating them with versatile modules for a multifunctional toolbox, which allows researchers to choose the desired aptamer tools and use them for specific applications without further screening and examination. We introduce the molecular engineering approaches of DNA aptamers, mainly categorized as chemical modification and DNA nanotechnology. We highlight some examples of DNA aptamers engineered for specific cellular applications, including biological regulation, targeted delivery of therapeutic agents, biosensing, cell imaging, and biomimicry.

1.4.1 Chemical Modifications via Solid-Supported Synthesis Strategy

DNA aptamers offer promising molecular recognition tools for cells. However, their full potential in life science has been limited by several challenges, such as poor chemical and biological stability and a narrow range of chemical diversity. To address these limitations, chemical modification of aptamers through a solid-supported synthesis strategy has been exploited to unleash their potential as molecular tools.

Solid-phase synthesis technology is a highly controllable and automated molecular synthesis method that can efficiently produce nucleic acids from individual phosphoramidite building blocks. This technology offers a sequence-predesigned DNA synthesis platform enabling the direct introduction of chemical groups into oligonucleotides in a controlled manner, which significantly enhances the aptamers' biostability and versatility [81–83]. Throughout the chemical synthesis process, aptamers can be modified at nucleotide components, including bases, sugars, or backbone groups, as shown in Figure 1.4. For instance, the backbone modifications on non-bridging oxygen of the canonical phosphodiester linkage with a methyl or ethyl group would provide uncharged backbones for minimizing the electrostatic repulsion between nucleic acid strands [84, 85]. The sugar group of the nucleotides can be modified by introducing a 2′-fluoro or 2′-*O*-methyl group and the locked nucleic acid [86]. It has been demonstrated that 2′-amino and 2′-fluoro substitutions can significantly increase the half-life of aptamers in human serum, extending from mere seconds (eight seconds) up to 86 hours. Furthermore, recent advances in nucleic acid chemistry have promoted the development of threose nucleic acid, peptide nucleic acid, and chirally inverted mirror-image nucleic acids (L-DNA) with unique structures different from natural nucleic acids [87, 88].

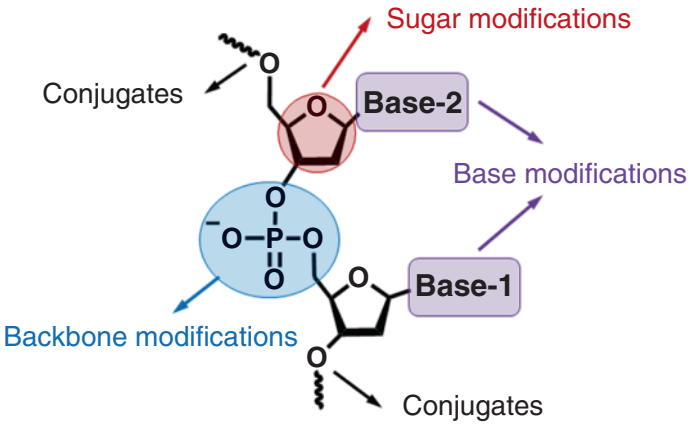

Figure 1.4 Schematic representation of common chemical modifications used in aptamers.

These artificial nucleic acids have also been explored as a promising category of nuclease-resistant and biostable aptamers that cannot be degraded by human blood and serum nucleases. As mentioned, these DNA aptamers can be readily synthesized by automated oligo synthesizer with commercially available materials, facilitating practical applications in diagnostics and therapeutics.

Meanwhile, this innovative approach enables the efficient synthesis of ApDCs using oligonucleotide synthesizers while preserving the biological activity of the included drugs. In 2014, Tan and coworkers developed the first ApDC phosphoramidite module, which incorporated an anticancer drug moiety and a photocleavable linker [53]. Upon the application of this module, automated and modular synthesis of ApDCs has been realized, allowing for the effective introduction of multiple drugs at predetermined positions. These ApDCs display specific targeting toward cancer cells and can be photoactivated to release drugs in a precise and controlled manner. Subsequently, various ApDCs have been exploited by using an automated DNA synthesizer. For example, Lv et al. reported a Sgc8-5FU ApDC by modifying DNA Aptamer Sgc8 with 5-fluorouracil (5-FU). The internalization and subsequent transportation pathways of the ApDC were investigated by the single-particle tracking (SPT) technique [89]. The results reveal that the ApDC predominantly entered the cells via caveolin-mediated endocytosis, similar to the pure DNA aptamer. Besides, Xuan et al. incorporated bioorthogonal chemistry with prodrug design to develop a novel aptamer prodrug conjugate (ApPdC) [90]. In the designed ApPdC, the hydrophilic DNA aptamer functions as a tumor-targeting subunit, whereas the hydrophobic prodrug promotes the self-assembly of ApPdC and acts as a free radical generator to enhance the efficacy of chemodynamic therapy.

Another approach involves the introduction of nucleotide analogs like Z:P, which mimic the structures and functions of natural nucleotides, into DNA aptamers to expand their chemical composition [91]. Artificial nucleotide bases promise to improve the diversity, properties, and functionalities of aptamers, making them more practical for various application scenarios. Our group proposed the concept of

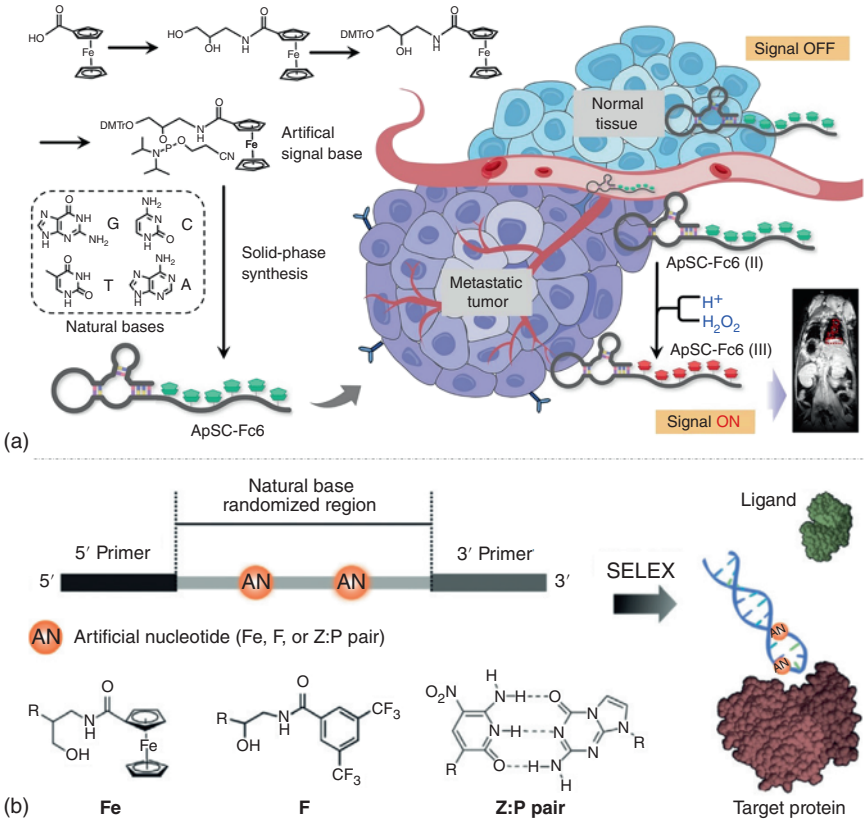

Figure 1.5 Schematic representation of (a) the logic-gated aptamer-signal base conjugate for targeted molecular imaging of metastatic cancer. Source: Reproduced with permission from Li et al. [92]. 2022 American Chemical Society. (b) The design of the artificial-nucleotide-expanded aptamer against integrin alpha3 for regulating cell activities. Source: Reproduced with permission from Tan et al. [93]. © 2018 John Wiley & Sons.

aptamer-signal base conjugates (ApSC) to develop AND-gated molecular tools for tumor-targeted molecular imaging [92]. As depicted in Figure 1.5a, the molecular probe consists of two segments: a DNA aptamer acting as the targeting ligand and tumor microenvironment-responsive artificial ferrocene bases for tumor cell imaging. The resulting ApSC demonstrates targeted accumulation in the tumor cells via aptamer-mediated molecular recognition. Meanwhile, the acidic pH and high levels of H_2O_2 in the tumor microenvironment trigger the *in situ* activation of the synthetic ferrocene bases through the Fenton-like reaction that leads to an amplified MRI signal over normal tissues.

The various synthesized nucleotides can be applied to enlarge the chemical space of the nucleic acid libraries in SELEX processes, thus increasing the diversity of DNA aptamers. Hence, an artificial-nucleotide-expanded SELEX approach was developed to evolve artificial nucleotide-based aptamers [93]. The nucleic acid library of cell-SELEX was extended with three artificial nucleotide

bases: Fe base with a ferrocenyl group, F base with a trifluoromethyl group, and Z:P base pair (Figure 1.5b). And an artificial nucleotide-expanded aptamer named ZAP-1, targeting the integrin alpha3 (ITGA3), was screened through the artificial-nucleotide-expanded cell-SELEX process. The binding of aptamer ZAP-1 with ITGA3 can reduce the interaction between ITGA3 and its natural ligand, which inhibits the triple-negative breast cancer (TNBC) cells' adhesion and migration. We anticipate that the artificial-nucleotide-expanded aptamers evolved from this artificial nucleotide-assisted cell-SELEX approach would enable the elucidation of the molecular basis of biological activities and regulation of biological functions.

1.4.2 Chemical Modifications Through Covalent Conjugation

Covalent conjugation is a valuable technique that creates a solid covalent bond between the aptamer and a non-nucleotide moiety, like a fluorophore, drug, or nanomaterial (Figure 1.4). Covalent conjugation can significantly improve the performance of DNA aptamers *in vitro* and *in vivo*, offering a predicted advantage in various applications. To achieve covalent conjugation, different chemical reactions can be used, such as thiol-maleimide, click chemistry, or amine-reactive crosslinking, depending on the specific properties of the DNA aptamer and the intended applications.

Small-molecule drugs possess several advantages, such as easy storage and transportation, low immunogenicity, and oral administration [94]. However, their low specificity results in low efficacy, relatively high toxicity, and unavoidable side effects. Targeted delivery of small-molecule drugs has been proven to enhance drug efficacy significantly, thus reducing toxicity and side effects. With the efficient molecular recognition capability, DNA aptamers can serve as ideal targeting units when coupled with small-molecule drugs for targeted drug delivery. Upon the covalent conjugation approach, our group exploited the first ApDC in 2009 and then contributed much to the ApDC research [95]. Li et al. presented an Sgc8c-artesunate conjugate with much higher therapeutic efficacy than artesunate alone as a consequence of the DNA aptamer-induced accumulation of artesunate in target cells [96]. And its retention time in tumor cells is much longer than that in control cells, demonstrating the specific targeting capability of the Sgc8c-artesunate conjugate. In addition, Huang et al. investigated the impact of linkers on the properties of ApDC [97]. Combretastatin A4 is conjugated to the Sgc8c aptamer via three different linkers, namely disulfide, phosphodiester, and carbamate bonds, for the investigation of the drug release mechanism and anticancer efficacy, as shown in Figure 1.6. The results indicate that a nucleophilic attack of glutathione can cleave the phosphodiester bond, and the repeated cleavage of the linker endows the ApDC with higher anticancer efficacy. These imply that the design of the linker unit is also critical for the efficacy of ApDC.

TNBC is considered one of the most malignant cancers. Developing effective targeted TNBC therapy has been regarded as an essential research topic in TNBC treatment. He et al. developed an AS1411-triptolide-conjugate to treat TNBC, which reveals high specificity and cytotoxicity against the MDA-MB-231 cell line [98].

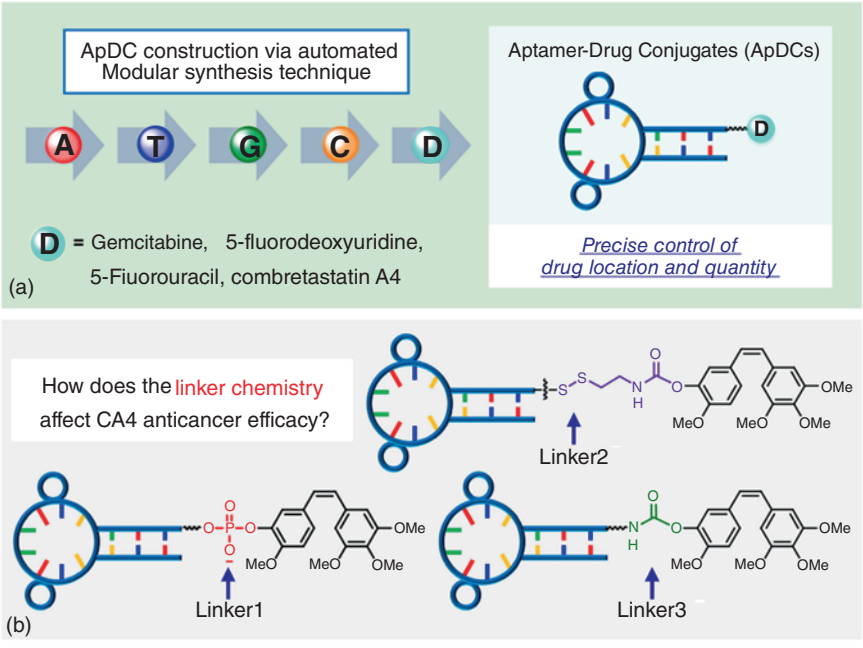

Figure 1.6 Schematic representation of (a) the construction of aptamer–drug conjugates through automated modular synthesis strategy. (b) Three different linkers: a phosphodiester bond (Linker 1), a disulfide bond (Linker 2), and a carbamate (Linker 3) involved in modifying the DNA aptamer. Source: Reproduced with permission from Huang et al. [97]. 2021 American Chemical Society.

More importantly, the AS1411-triptolide-conjugate has excellent *in vivo* anti-tumor efficacy for TNBC and negligible side effects on healthy organs. Compared with monotherapy using a single medication, polytherapy with multiple drugs can simultaneously target multiple mechanisms associated with tumor growth. This strategy can decrease individual drug dosages, enhance therapeutic efficacy against multiple targets, and overcome resistance mechanisms. However, there are challenges to creating effective combination therapies with an accurate tune of the drug ratio. To address these challenges, Zhou et al. developed cyclic bivalent ApDCs (cb-ApDCs) with a tunable drug ratio [99]. The resulting cb-ApDCs, remaining the specific recognition, are stable and can be quickly taken into cells. The drug ratio in cb-ApDCs can be easily controlled to strengthen the synergistic effect without complex chemistry. These cb-ApDCs offer a promising strategy for targeted anticancer therapy and hold the potential to stimulate the advancement of novel drug combinations for combinatorial cancer therapy.

In addition to small molecular drugs, various functional groups, such as fluorescent dyes, nanoparticles, enzymes, antibodies, and peptides, have been conjugated into DNA aptamers, which enriches their diversity and functionality. For example, by attaching fluorescent dyes to a DNA aptamer, the binding of the DNA aptamer with its target can be visualized and quantified in real time. This approach contributes to surveying the localization and dynamics of proteins in cells and

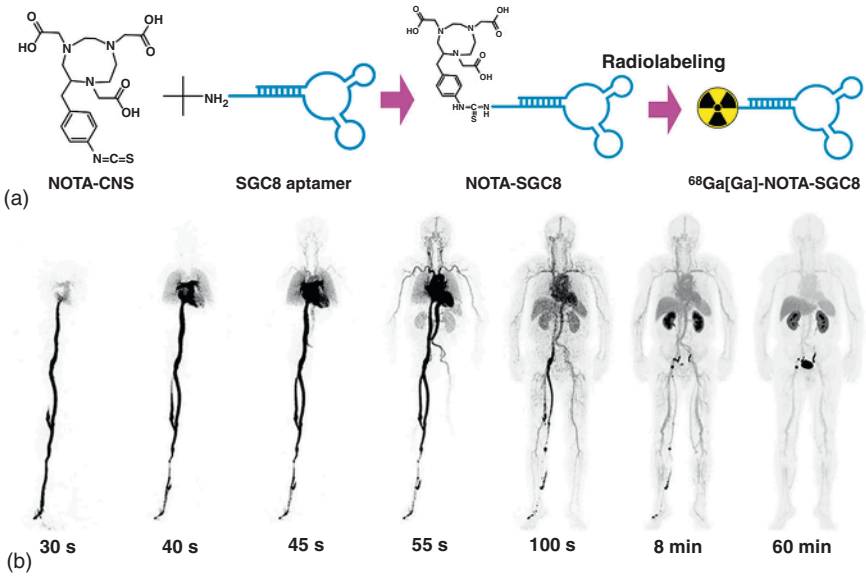

Figure 1.7 (a) Schematic illustration of chemical modification of Sgc-8 aptamer with gallium-68 radiolabel. (b) Whole-body dynamic imaging of the gallium-68 (^{68}Ga) radiolabeled aptamer-injected patient at different time points post administration. Source: Ding et al. [100]/American Association for the Advancement of Science/CC BY 4.0.

provides insights into cellular signaling pathways [60], significantly promoting our understanding of cellular processes and novel discoveries in cell biology. Also, fluorescent dye-conjugated DNA aptamers can be used to detect specific molecules in complex systems as biosensing devices.

Recently, Ding et al. developed a gallium-68 (^{68}Ga) radiolabeled aptamer (Figure 1.7a) to address the limited knowledge of the biosafety and metabolism patterns of DNA aptamers in the human body, which has impeded their clinical application in precision medicine [100]. The first-in-human pharmacokinetics study of the Sgc-8 aptamer was conducted using state-of-the-art total-body positron emission tomography (PET) technology. As shown in Figure 1.7b, the study captured dynamic distribution patterns of the aptamer in the human body, and reveals that the radiolabeled aptamer is safe for normal organs. The majority of the aptamer accumulated in the kidneys and was subsequently eliminated from the body through urine. Furthermore, a physiologically based pharmacokinetic model for the aptamer was developed, demonstrating its potential for predicting therapeutic responses and facilitating personalized treatment strategies.

1.4.3 Self-assembly Systems Based on Chemically Modified DNA Aptamers

Besides the simple covalent modification of aptamers, self-assembly systems based on chemically modified aptamers hold promise to enhance the cellular uptake, stability, and specificity of aptamers toward their targets. Such systems have been

exploited for their potential in targeted drug delivery and imaging applications. The conjugation of highly hydrophilic DNA aptamers with hydrophobic small-molecule drugs results in the formation of amphiphilic ApDC. These amphiphilic conjugates can self-assemble into DNA aptamer-based micelle structures with a hydrophobic drug core and a hydrophilic DNA aptamer shield, suggesting a multivalent effect and an enhanced aptamer-target binding ability [101]. Such a supramolecular assembly could be a high-efficacy transport platform for cell imaging and drug delivery. Besides the multivalent effect induced by molecular self-assembly, Geng et al. reported a multivalent aptamer drug conjugate (ApMDC) [102] synthesized upon the coupling of a hydrophilic DNA aptamer with a hydrophobic monodendron anchored with four anticancer drugs (Figure 1.8). Amphiphilic ApMDC and its PEG-substituted analog synergistically assemble into nanomicelles, which display precise drug loading and tunable surface density of aptamers for optimal complementation between blood circulation and tumor-targeting ability. The released drug from the consequent degradation of nanomicelles induced by the acidic tumor microenvironment reinforces the immunogenic cell death of tumor cells. With these merits, ApMDC nanomicelles represent a robust platform for structure-function optimization of drug conjugates and nanomedicines.

Steadily increasing attention has been paid to the orchestration of supramolecular assemblies by using amphiphilic molecules due to their broad biological applications [103]. Upon the covalent coupling of hydrophilic DNA aptamer with hydrophobic lipids, the generated amphiphilic aptamer-lipid conjugates can self-assemble into DNA aptamer-based micelles, vesicles, and other complex assemblies, exhibiting enhanced target binding ability as a result of their multivalent effect, trim sizes, and increased cell permeability and carrier capacity. Therefore, DNA aptamer-based assemblies could be broadly applicable in nanobiotechnology, cell biology, and drug delivery systems. Our group pioneered the construction of DNA aptamer-based micelles. In 2009, they reported the development of TDO5 aptamer diacyllipid amphiphilic micelles [104] composed of a hydrophilic DNA aptamer head and a hydrophobic lipid tail. This study implies their potential as a targeted drug delivery system and a selective detection tool. One of the advantages of DNA aptamer-conjugated assemblies is their modularity and tunability. By altering assemblies' composition, size, and shape as well as the sequence and orientation of DNA aptamers, assemblies' properties can be fine-tuned to meet specific requirements. Recently, Li et al. presented a new strategy for constructing stable and specific aptamer-lipid micelles [105]. The DNA aptamer and lipid fragments were linked to a methacrylamide branch, which covalently crosslinks the aptamer-lipid micelle under photoirradiation, to increase the DNA aptamer's biostability.

Another advantage of DNA aptamer-conjugated assemblies is their biocompatibility and biodegradability. Unlike other nanoparticle-based systems, DNA aptamer-conjugate assemblies are usually non-toxic and metabolizable via normal metabolic pathways in the body, providing an ideal platform for various biomedical applications, including *in vivo* imaging and targeted drug delivery. For example, an aptamer-diacyllipid conjugate was synthesized by covalent coupling of an Sgc8 aptamer with a diacyllipid tail via a PEG linker [106]. Exosomes loaded

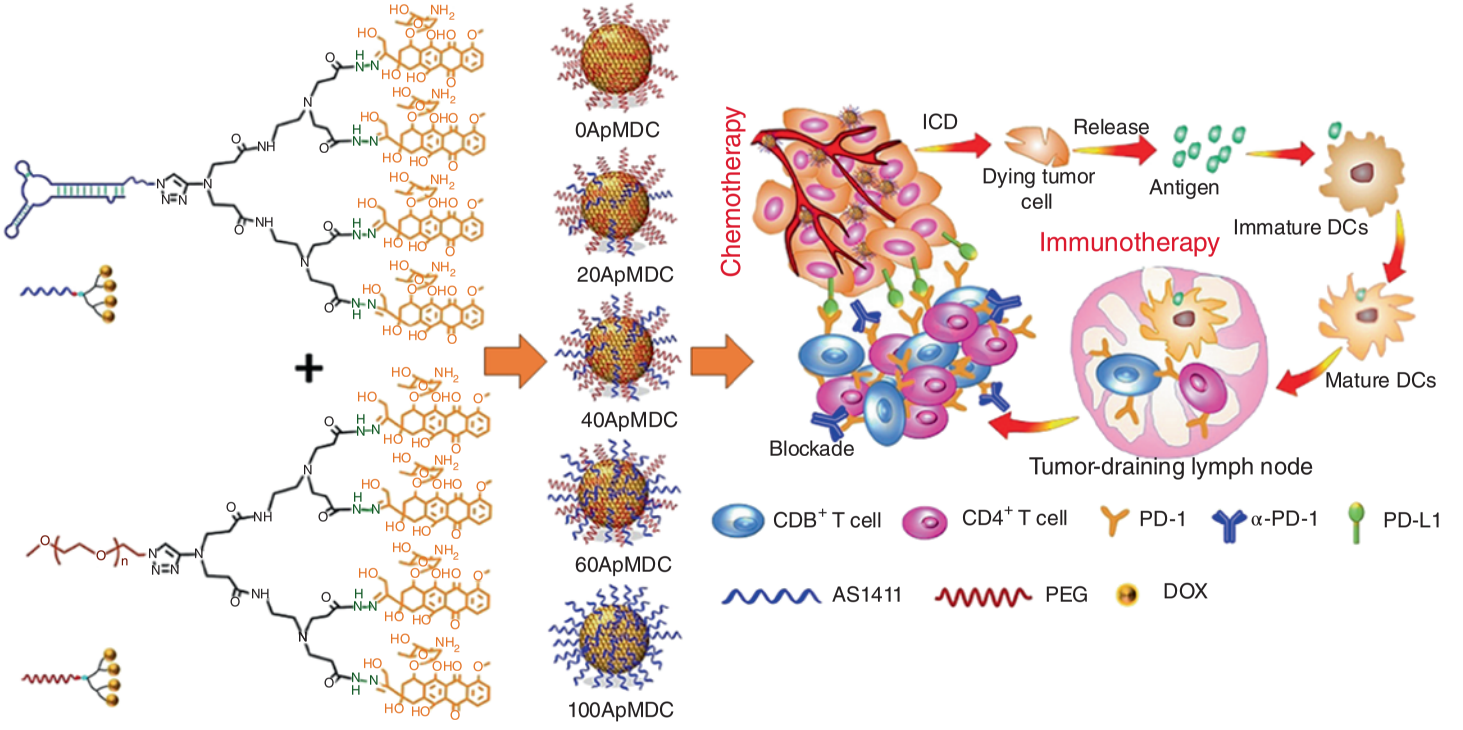

Figure 1.8 Schematic representation of the co-assemblies of a multivalent aptamer drug conjugate (ApMDC) and its PEG-substituted analog into nanomicelles for reinforcing the immunogenic cell death of tumor cells. Source: Reproduced with permission from Geng et al. [102]. © 2021 John Wiley & Sons.

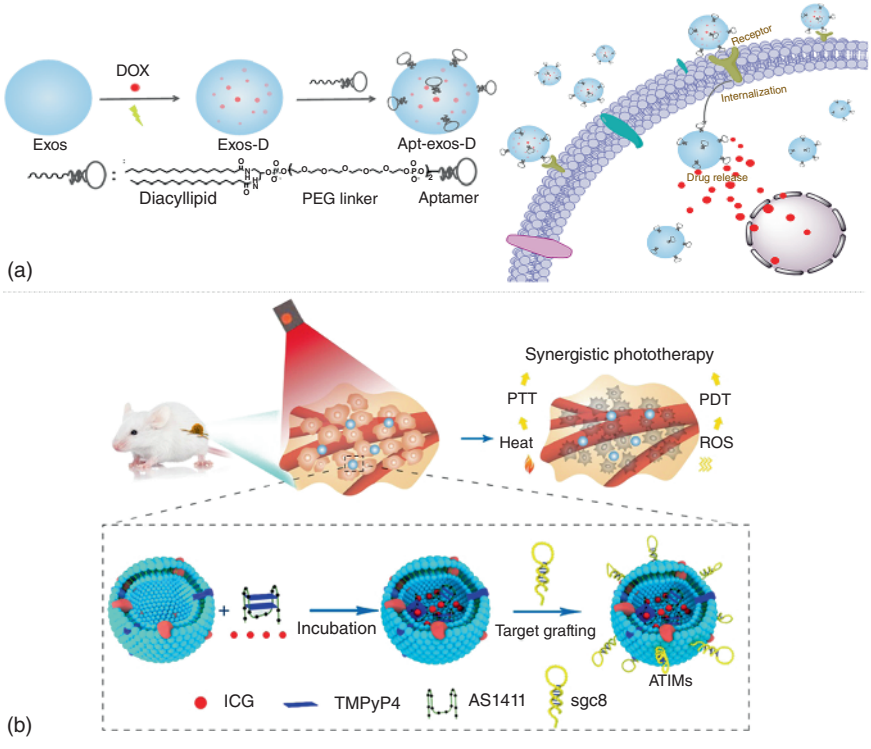

Figure 1.9 Schematic illustration of the design of (a) aptamer-functionalized exosomes (Apt-Exos) and (b) aptamer-cholesterol-modified vesicle for targeting delivery to cancer cells. Source: (a) Reproduced with permission from Zou et al. [106]. 2019 American Chemical Society. (b) Reproduced with permission from Luo et al. [107]. 2019 American Chemical Society.

with chemotherapeutic drugs were functionalized with this aptamer-diacyllipid conjugate, leading to aptamer-functionalized exosomes (Apt-Exos) for targeting delivery to cancer cells. Due to the natural delivery advantages of exosomes and specific molecular recognition properties of DNA aptamers, Apt-Exos can act as an efficient delivery tool for targeted cancer theranostics, Figure 1.9a.

Beyond the natural exosomes, biomimetic liposomes were also integrated with DNA aptamers for targeted therapeutics [108]. Liposomes self-assemble into vesicles with giant cavities mimicking the living cell membranes. They can be readily loaded with different kinds of cargo, from imaging reagents, drugs, and genes to biomolecule tools, aiming for various applications. However, the non-selectivity of liposome delivery is considered unfavorable for its practical applications. DNA aptamers' splendid molecular recognition properties make them ideal targeting modules to decorate with liposomes toward multifunctional target-specific delivery systems. Luo et al. exploited a new aptamer-cholesterol-modified vesicle loaded with therapeutic agents for cancer therapy, Figure 1.9b [107]. DNA aptamer-functionalized liposomes can precisely deliver the cargo into targeted cells. More

promising applications of the DNA aptamer-based liposomes involve the delivery of miRNAs and CRISPR/Cas9 complex into specific cells to selectively manipulate cellular activities.

DNA aptamer-based assemblies represent a versatile and promising platform for various biomedical and biotechnological applications. They hold great potential for advancing our understanding of *in vivo* self-assembly principles and target delivery processes and improving the treatment of various diseases.

1.4.4 DNA Aptamers Engineered with Nanotechnology

DNA nanotechnology, where nucleic acids can be regarded as building blocks to orchestrate nanostructures and nanodevices with tunable sizes and shapes, significantly improves the capability to control molecular self-assembly [40]. Integrating DNA aptamers into nanotechnology has opened new possibilities for developing highly sensitive and specific biosensors, targeted drug delivery systems, and diagnostic molecular tools.

The major advantage of DNA aptamer-based nanotechnology lies in the molecular recognition ability of DNA aptamers. DNA aptamers screened by SELEX or cell-SELEX can bind to specific receptors, making them ideal probes for visualizing and detecting these receptors. This feature allows the advanced design of DNA aptamer-based nanodevices to detect and capture target molecules in complex biological samples precisely. In the study reported by Chen et al. [109], a DNA aptamer tool was developed for one-step fluorescence detection of antibody production and quality control. Trastuzumab, a humanized IgG1 antibody to the human epidermal growth factor 2 receptor, is selected as the model antibody drug. A DNA aptamer against trastuzumab is screened and identified by *in vitro* SELEX process. By using this DNA aptamer tool, the quality control and traceless purification of antibody drugs are demonstrated, which can support and accelerate the manufacture of antibody drugs.

Also, DNA aptamers can be engineered to undergo conformational changes to switch their molecular recognition abilities, which allows for the creation of "smart" nanomachines responding to specific environmental cues, such as pH changes or specific molecules. For example, Huang et al. designed a logic-gated nanodevice [110], as shown in Figure 1.10a. This nanodevice consists of a tetrahedron modified with a Sgc8 aptamer tail (Sgc8-CT) and a pH-responsive C-rich nucleic acid complementary with Sgc8 aptamer (i-motif/Sgc8-CT) to block the molecular recognition between Sgc8-CT and the target cell. Then the logic-gated DNA nanodevice is immobilized on the surface of nanovesicles filled with gold carbon dots (GCDs), forming a logic-gated nanovesicle capable of controlling the transportation of GCDs into the target cell. Initially, the C-rich domain of the nanodevice adopts a conformation interacting with the Sgc8 aptamer, which hinders its binding to target cells. Once the logic-gated nanovesicle is exposed to an acidic environment, the C-rich domain reconfigures into an i-motif structure, leading to its dissociation from Sgc8 aptamer and the recovery of Sgc8 aptamer's targeting ability. Thus, the nanovesicle can stimulate cargo delivery in the presence of an acidic environment and is a target

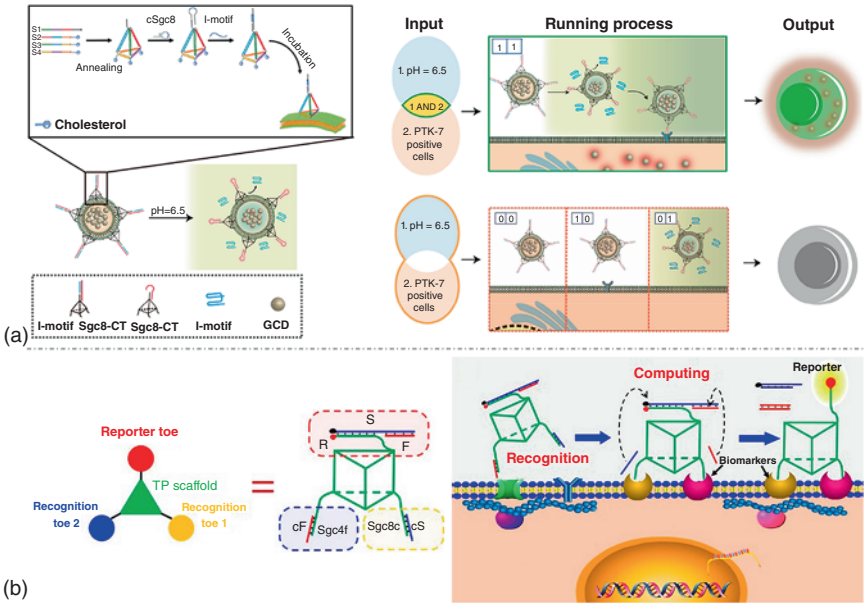

Figure 1.10 Schematic diagram of (a) the design of a logic-gated DNA nanodevice and its targeted GCD delivery process induced by an acidic environment and the overexpression of PTK-7. Source: Reproduced with permission from Huang et al. [110]. 2021 American Chemical Society. (b) A logic gate-guided DNA nanomachine for bispecific recognition and computing on cell surfaces. Source: Reproduced with permission from Peng et al. [111]. 2018 American Chemical Society.

biomarker for Sgc8-CT. As GCDs delivered into target cells, the intracellular redox status variation is monitored by the fluorescence changes of GCDs.

Furthermore, DNA aptamer-based nanotechnology has demonstrated enormous potential in DNA computing. Instead of traditional silicon-based computer chips, nucleic acids can be leveraged for implementing computing functions in living systems. Using DNA aptamers as molecular recognition elements, researchers have developed precise and efficient algorithms for solving logic problems in complex biological environments. Peng et al. fabricated a logic gate-guided DNA nanomachine for bispecific recognition and computing on cell surfaces [111], Figure 1.10b. A DNA triangular prism is decorated with two DNA aptamers, namely, Sgc8c and Sgc4f. These aptamers are designed to target two different overexpressed cancer biomarkers. Initially, their binding abilities are blocked by two specific single-stranded DNAs: cF (blocking Sgc4f) and cS (blocking Sgc8c). When two DNA aptamers interact with the respective biomarkers, two single-stranded DNAs, cF and cS, are released and tend to replace strand S from the DNA duplex structure (R/F/S) cooperatively. The coexistence of cF and cS turns the Boolean operator into an "AND" state, where the logic gate-guided nanomachine generates a true value. As a result, the released S strand triggers the fluorescence of the system, which indicates the simultaneous overexpression of both target biomarkers on the cancer cell, thus offering valuable information for cancer cell analysis.

In addition, the combination of DNA aptamers with nanotechnology provides exciting prospects for constructing complex nucleic acid-based dynamic networks [112–115]. These artificial networks aim at mimicking complex signaling dynamic behaviors and emerging functions observed in living systems. They are typically constructed using modular design principles, where a DNA aptamer is facilely integrated to generate a more extensive network. In the network, DNA aptamers can provide promising signal-recognizing and transmitting tools for the reception, processing, and feedback of biological signals. High binding affinity and specificity of DNA aptamers enable the network to function in the presence of low signal molecule concentration and substantial interference, which is essential for signal sensing, amplification, and processing as well as functional regulation in complex biological systems. He et al. presented a DNA-based signal transducer module that converts complex signal information into easy-to-read temperature output [116]. In this study, a switchable DNA G4 aptamer–Hemin complex (DGAH) was designed as a temperature-output DNA transducer. When DGAH is switched on to catalyze the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) in the presence of hydrogen peroxide, the system's color changes from colorless to deep blue. Since the oxidized form of TMB exhibits strong and broad absorption of complementary colors across the yellow to near-infrared (NIR) regions, strong thermal conversion can be anticipated upon absorbing photons. Upon incorporating the temperature-output DNA transducer module into DNA reaction networks, the information encoded in nucleic acids can be successfully received, processed, amplified, and transduced into a high-sensitivity temperature output.

Integrating DNA aptamers into nanotechnology promotes the development of a versatile DNA aptamer-based toolbox. Combining controllable physicochemical properties and precise addressability of DNA nanotechnology with high binding specificity and affinity of DNA aptamers provides molecular recognition accessories for nanostructures and nanomaterials to target biomolecules, cells, or tissues with ultra-high sensitivity and specificity. These outstanding performances advance their applications in biosensing, bioimaging, targeted drug delivery, bioregulation, and biomimicry.

1.5 Summary and Outlook

Cells are highly complex systems whose structures and functionalities have been studied from many perspectives for decades. However, we are still far from a comprehensive understanding of their inner workings. In recent years, the advent of cell-SELEX technology has revolutionized the research field of DNA aptamers, making them valuable tools for molecular recognition and cell targeting. Also, the high affinity and specificity, programmable molecular structures, and facile chemical modification of DNA aptamers render them highly versatile tools for cellular applications. The development of the DNA aptamer toolbox has rapidly progressed through the incorporation with chemical modifications and advanced nanotechnology. These advancements have created aptamer-based molecular tools

with exceptional versatility, such as ApDCs, aptamer-based molecular probes, aptamer-based nanodevices, and aptamer-based molecular computers, holding tremendous potential for numerous cellular applications, including sensing, imaging, targeted drug delivery, bioregulation, and biomimicry.

Despite the enormous potential and impressive advancements in DNA aptamer-based tools, their actual impact on biological and biomedical applications is yet to be fully realized. A few challenges remain in this emerging field, including complex DNA aptamer discovery strategies, limited understanding of the binding mechanisms between aptamers and bio-targets, low stability and efficacy of aptamer tools in biological research, and concerns regarding biosecurity in their applications. To push this emerging field, persistent efforts are required to address these fundamental gaps and challenges, such as the exploration of new aptamer screening methods and instruments, the investigation of the structural information of aptamer-target in complex physiological environments, and the development of the DNA aptamer toolbox to enhance the biological performance and biosecurity. With these efforts, it is conceivable that DNA aptamer tools will open up new ways for cell research, ultimately realizing clinical applications in the future.

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References

1 Deatherage, F.E. (1975). Cells, the fundamental biological units. In: *Food for Life* (ed. F.E. Deatherage), 308–311. Boston: Springer. https://doi.org/10.1007/978-1-4684-0748-8_3.

2 Ellinger, I. and Ellinger, A. (2014). Smallest unit of life: cell biology. In: *Comparative Medicine* (ed. E. Jensen-Jarolim), 19, 19–33, 33. Vienna: Springer. https://doi.org/10.1007/978-3-7091-1559-6_2.

3 Alberts, B., Johnson, A., Lewis, J. et al. (2002). The chemical components of a cell. In: *Molecular Biology of the Cell*, 4e, 44–125. New York: Garland Science. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26883/.

4 Wolkenhauer, O. and Muir, A. (2011). The complexity of cell-biological systems. In: *Philosophy of Complex Systems, Volume 10 in Handbook of the Philosophy of Science* (ed. D.M. Gabbay, P. Thagard, and J. Woods), 355–385. Elsevier. https://doi.org/10.1016/B978-0-444-52076-0.50013-4.

5 Barabási, A.L. and Oltvai, Z. (2004). Network biology: understanding the cell's functional organization. *Nature Reviews Genetics* 5: 101–113. https://doi.org/10.1038/nrg1272.

6 Trapnell, C., Williams, B.A., Pertea, G. et al. (2010). Transcript assembly and quantification by RNA-seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nature Biotechnology* 28: 511–515. https://doi.org/10.1038/nbt.1621.

7 Yosef, N., Shalek, A.K., Gaublomme, J.T. et al. (2013). Dynamic regulatory network controlling TH17 cell differentiation. *Nature* 496: 461–468. https://doi.org/10.1038/nature 11981.

8 Whitfield, M., George, L., Grant, G. et al. (2006). Common markers of proliferation. *Nature Reviews Cancer* 6: 99–106. https://doi.org/10.1038/nrc1802.

9 Purvis, J.E. and Lahav, G. (2013). Encoding and decoding cellular information through signaling dynamics. *Cell* 152 (5): 945–956. https://doi.org/10.1016/j.cell.2013.02.005.

10 Strzyz, P. (2018). PrEView of cell–cell communication. *Nature Reviews Molecular Cell Biology* 19: 752–753. https://doi.org/10.1038/s41580-018-0073-3.

11 Kholodenko, B. (2006). Cell-signalling dynamics in time and space. *Nature Reviews Molecular Cell Biology* 7: 165–176. https://doi.org/10.1038/nrm1838.

12 Guimerà, R. and Nunes Amaral, L.A. (2005). Functional cartography of complex metabolic networks. *Nature* 433: 895–900. https://doi.org/10.1038/nature03288.

13 Jeong, H., Tombor, B., Albert, R. et al. (2000). The large-scale organization of metabolic networks. *Nature* 407: 651–654. https://doi.org/10.1038/35036627.

14 Hens, C., Harush, U., Haber, S. et al. (2019). Spatiotemporal signal propagation in complex networks. *Nature Physics* 15: 403–412. https://doi.org/10.1038/s41567-018-0409-0.

15 Goldbeter, A. (2017). Dissipative structures and biological rhythms. *Chaos: An Interdisciplinary Journal of Nonlinear Science* 27 (10): 104612. https://doi.org/10.1063/1. 4990783.

16 Andrews, L.B., Nielsen, A.A.K., and Voigt, C.A. (2018). Cellular checkpoint control using programmable sequential logic. *Science* 361 (6408): eaap8987. https://doi.org/10.1126/science.aap898.

17 Lezia, A., Csicsery, N., and Hasty, J. (2022). Design, mutate, screen: Multiplexed creation and arrayed screening of synchronized genetic clocks. *Cell Systems* 13 (5): 365–375. https://doi.org/10.1016/j.cels.2022.02.005.

18 Boya, P., Reggiori, F., and Codogno, P. (2013). Emerging regulation and functions of autophagy. *Nature Cell Biology* 15: 713–720. https://doi.org/10.1038/ncb2788.

19 Zhang, Y., Tian, Z., Ye, H. et al. (2022). Emerging functions of circular RNA in the regulation of adipocyte metabolism and obesity. *Cell Death Discovery* 8: 268. https://doi.org/10.1038/s41420-022-01062-w.

20 Ganser, L.R., Kelly, M.L., Herschlag, D. et al. (2019). The roles of structural dynamics in the cellular functions of RNAs. *Nature Reviews Molecular Cell Biology* 20: 474–489. https://doi.org/10.1038/s41580-019-0136-0.

21 Pantsar, T. (2019). The current understanding of KRAS protein structure and dynamics. *Computational and Structural Biotechnology Journal* 18 (2020): 189–198. https://doi.org/10.1016/j.csbj.2019.12.004.

22 Gorka, M., Swart, C., Siemiatkowska, B. et al. (2019). Protein complex identification and quantitative complexome by CN-PAGE. *Scientific Reports* 9: 11523. https://doi.org/10.1038/s41598-019-47829-7.

23 Alivisatos, P. (2004). The use of nanocrystals in biological detection. *Nature Biotechnology* 22: 47–52. https://doi.org/10.1038/nbt927.

24 Ko, J., Wilkovitsch, M., Oh, J. et al. (2022). Spatiotemporal multiplexed immunofluorescence imaging of living cells and tissues with bioorthogonal cycling of fluorescent probes. *Nature Biotechnology* 40: 1654–1662. https://doi.org/10.1038/s41587-022-01339-6.

25 Stehr, F., Stein, J., Bauer, J. et al. (2021). Tracking single particles for hours via continuous DNA-mediated fluorophore exchange. *Nature Communications* 12: 4432. https://doi.org/10.1038/s41467-021-24223-4.

26 Hou, S., Exell, J., and Welsher, K. (2020). Real-time 3D single molecule tracking. *Nature Communications* 11: 3607. https://doi.org/10.1038/s41467-020-17444-6.

27 Alon, U. (2007). Network motifs: Theory and experimental approaches. *Nature Reviews Genetics* 8: 450–461. https://doi.org/10.1038/nrg2102.

28 Lambiotte, R., Rosvall, M., and Scholtes, I. (2019). From networks to optimal higher-order models of complex systems. *Nature Physics* 15: 313–320. https://doi.org/10.1038/s41567-019-0459-y.

29 Ludwig, J. and Weinstein, J. (2005). Biomarkers in cancer staging, prognosis and treatment selection. *Nature Reviews Cancer* 5: 845–856. https://doi.org/10.1038/nrc1739.

30 Kwong, G.A., Ghosh, S., Gamboa, L. et al. (2021). Synthetic biomarkers: a twenty-first century path to early cancer detection. *Nature Reviews Cancer* 21: 655–668. https://doi.org/10.1038/s41568-021-00389-3.

31 Black, J.R.M. and McGranahan, N. (2021). Genetic and non-genetic clonal diversity in cancer evolution. *Nature Reviews Cancer* 21: 379–392. https://doi.org/10.1038/s41568-021-00336-2.

32 Weis, S. and Cheresh, D. (2011). Tumor angiogenesis: molecular pathways and therapeutic targets. *Nature Medicine* 17: 1359–1370. https://doi.org/10.1038/nm.2537.

33 Fares, J., Fares, M.Y., Khachfe, H.H. et al. (2020). Molecular principles of metastasis: a hallmark of cancer revisited. *Signal Transduction and Targeted Therapy* 5: 28. https://doi.org/10.1038/s41392-020-0134-x.

34 Komarova, N., Panova, O., Titov, A. et al. (2022). Aptamers targeting cardiac biomarkers as an analytical tool for the diagnostics of cardiovascular diseases: a review. *Biomedicines* 10 (5): 1085. https://doi.org/10.3390/biomedicines10051085.

35 Micura, R. and Höbartner, C. (2020). Fundamental studies of functional nucleic acids: aptamers, riboswitches, ribozymes and DNAzymes. *Chemical Society Reviews* 49 (20): 7331–7353. https://doi.org/10.1039/D0CS00617C.

36 Felsenfeld, G. and Miles, H.T. (1967). The physical and chemical properties of nucleic acids. *Annual Review of Biochemistry* 36: 407–448. https://doi.org/10.1146/annurev.bi.36.070167.002203.

37 Xu, W., He, W., Du, Z. et al. (2021). Functional nucleic acid nanomaterials: development, properties, and applications. *Angewandte Chemie International Edition* 60 (13): 6890–6918. https://doi.org/10.1002/anie.201909927.

38 Zhang, J., Lan, T., and Lu, Y. (2019). Molecular engineering of functional nucleic acid nanomaterials toward in vivo applications. *Advanced Healthcare Materials* 8 (6): e1801158. https://doi.org/10.1002/adhm.201801158.

39 Peng, T., Deng, Z., He, J. et al. (2020). Functional nucleic acids for cancer theranostics. *Coordination Chemistry Reviews* 403: 213080. https://doi.org/10.1016/j.ccr.2019.213080.

40 Seeman, N. and Sleiman, H. (2018). DNA nanotechnology. *Nature Reviews Materials* 3: 17068. https://doi.org/10.1038/natrevmats.2017.68.

41 Ku, T.H., Zhang, T., Luo, H. et al. (2015). Nucleic acid aptamers: an emerging tool for biotechnology and biomedical sensing. *Sensors* 15 (7): 16281–16313. https://doi.org/10.3390/s150716281.

42 Wang, H., Cheng, H., Wang, J. et al. (2016). Selection and characterization of DNA aptamers for the development of light-up biosensor to detect Cd(II). *Talanta* 154: 498–503. https://doi.org/10.1016/j.talanta.2016.04.005.

43 Baker, B.R., Lai, R.Y., Wood, M.S. et al. (2006). An electronic, aptamer-based small-molecule sensor for the rapid, label-free detection of cocaine in adulterated samples and biological fluids. *Journal of the American Chemical Society* 128 (10): 3138–3139. https://doi.org/10.1021/ja056957p.

44 Cai, H., Lee, T.M.H., and Hsing, I.M. (2006). Label-free protein recognition using an aptamer-based impedance measurement assay. *Sensors and Actuators, B: Chemical* 114 (1): 433–437. https://doi.org/10.1016/j.snb.2005.06.017.

45 Labib, M., Zamay, A.S., Muharemagic, D. et al. (2012). Aptamer-based viability impedimetric sensor for viruses. *Analytical Chemistry* 84 (4): 1813–1816. https://doi.org/10.1021/ac203412m.

46 Dunn, M., Jimenez, R., and Chaput, J. (2017). Analysis of aptamer discovery and technology. *Nature Reviews Chemistry* 1: 0076. https://doi.org/10.1038/s41570-017-0076.

47 Shangguan, D., Li, Y., Tang, Z. et al. (2006). Aptamers evolved from live cells as effective molecular probes for cancer study. *Proceedings of the National Academy of Sciences of the United States of America* 103 (32): 11838–11843. https://doi.org/10.1073/pnas.0602615103.

48 Huang, Z.X., Xie, Q., Guo, Q.P. et al. (2017). DNA aptamer selected for specific recognition of prostate cancer cells and clinical tissues. *Chinese Chemical Letters* 28 (6): 1252–1257. https://doi.org/10.1016/j.cclet.2017.01.002.

49 Mao, X., Liu, M., Yan, L. et al. (2020). Programming biomimetically confined aptamers with DNA frameworks. *ACS Nano* 14 (7): 8776–8783. https://doi.org/10.1021/acsnano.0c03362.

50 Yang, Y., Xu, J., Sun, Y. et al. (2021). Aptamer-based logic computing reaction on living cells to enable non-antibody immune checkpoint blockade therapy. *Journal of the American Chemical Society* 143 (22): 8391–8401. https://doi.org/10.1021/jacs.1c02016.

51 Park, J., Lee, S., Kim, Y. et al. (2021). Methods to generate site-specific conjugates of antibody and protein. *Bioorganic and Medicinal Chemistry* 30: 115946. https://doi.org/10.1016/j.bmc.2020.115946.

52 Brazier, J. (2023). Chemical synthesis of oligonucelotide sequences: phosphoramidite chemistry. In: *DNA Manipulation and Analysis* (ed. G. Scarlett), 185–193. New York: Humana. https://doi.org/10.1007/978-1-0716-3004-4_14.

53 Wang, R.W., Zhu, G., Mei, L. et al. (2014). Automated modular synthesis of aptamer–drug conjugates for targeted drug delivery. *Journal of the American Chemical Society* 136 (7): 2731–2734. https://doi.org/10.1021/ja4117395.

54 Wen, J., Tao, W., Hao, S. et al. (2016). A unique aptamer-drug conjugate for targeted therapy of multiple myeloma. *Leukemia* 30: 987–991. https://doi.org/10.1038/leu.2015.216.

55 Hampton, T. (2008). Researchers create artificial DNA bases. *Journal of the American Medical Association* 299 (11): 1251–1251. https://doi.org/10.1001/jama.299.11.1251.

56 Meng, H.M., Liu, H., Kuai, H. et al. (2016). Aptamer-integrated DNA nanostructures for biosensing, bioimaging and cancer therapy. *Chemical Society Reviews* 45 (9): 2583–2602. https://doi.org/10.1039/C5CS00645G.

57 Walia, S., Chandrasekaran, A.R., Chakraborty, B. et al. (2021). Aptamer-programmed DNA nanodevices for advanced, targeted cancer theranostics. *ACS Applied Bio Materials* 4 (7): 5392–5404. https://doi.org/10.1021/acsabm.1c00413.

58 Zhao, S., Tian, R., Wu, J. et al. (2021). A DNA origami-based aptamer nanoarray for potent and reversible anticoagulation in hemodialysis. *Nature Communications* 12: 358. https://doi.org/10.1038/s41467-020-20638-7.

59 Li, L., Jiang, Y., Cui, C. et al. (2018). Modulating aptamer specificity with pH-responsive DNA bonds. *Journal of the American Chemical Society* 140 (41): 13335–13339. https://doi.org/10.1021/jacs.8b08047.

60 Xie, S., Du, Y., Zhang, Y. et al. (2020). Aptamer-based optical manipulation of protein subcellular localization in cells. *Natture Communications* 11: 1347. https://doi.org/10.1038/s41467-020-15113-2.

61 Goda, T. and Miyahara, Y. (2011). Thermo-responsive molecular switches for ATP using hairpin DNA aptamers. *Biosensors and Bioelectronics* 26 (9): 3949–3952. https://doi.org/10.1016/j.bios.2011.02.041.

62 Beaucage, S.L. and Caruthers, M.H. (1981). Deoxynucleoside phosphoramidites—a new class of key intermediates for deoxypolynucleotide synthesis. *Tetrahedron Lett.* 22: 1859–1862. https://doi.org/10.1016/S0040-4039(01)90461-7.

63 Tuerk, C. and Gold, L. (1990). Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* 249 (4968): 505–510. https://doi.org/10.1126/science.2200121.

64 Ellington, A. and Szostak, J. (1990). *In vitro* selection of RNA molecules that bind specific ligands. *Nature* 346: 818–822. https://doi.org/10.1038/346818a0.

65 Yu, H., Alkhamis, O., Canoura, J. et al. (2021). Advances and challenges in small-molecule DNA aptamer isolation, characterization, and sensor

development. *Angewandte Chemie International Edition* 60 (31): 16800–16823. https://doi.org/10.1002/anie.202008663.

66 Huizenga, D.E. and Szostak, J.W. (1995). A DNA aptamer that binds adenosine and ATP. *Biochemistry* 34 (2): 656–665. https://doi.org/10.1021/bi00002a033.

67 Stojanovic, M.N., de Prada, P., and Landry, D.W. Fluorescent sensors based on aptamer self-assembly. *Journal of the American Chemical Society* 122 (46): 11547–11548. https://doi.org/10.1021/ja0022223.

68 Guo, X., Wen, F., Zheng, N. et al. (2020). Aptamer-based biosensor for detection of mycotoxins. *Frontiers in Chemistry* 8: 195. https://doi.org/10.3389/fchem.2020.00195.

69 Krauss, I.R., Merlino, A., Giancola, C. et al. (2011). Thrombin–aptamer recognition: a revealed ambiguity. *Nucleic Acids Research* 39 (17): 7858–7867. https://doi.org/10.1093/nar/gkr522.

70 Hongjie, X., Jianhua, Y., Cai, S. et al. (2019). Cancer protein biomarker discovery based on nucleic acid aptamers. *International Journal of Biological Macromolecules* 132: 190–202. https://doi.org/10.1016/j.ijbiomac.2019.3.165.

71 Fang, X. and Tan, W. (2010). Aptamers generated from cell-SELEX for molecular medicine: a chemical biology approach. *Accounts of Chemical Research* 43 (1): 48–57. https://doi.org/10.1021/ar900101s.

72 Sefah, K., Shangguan, D., Xiong, X. et al. (2010). Development of DNA aptamers using Cell-SELEX. *Nature Protocols* 5: 1169–1185. https://doi.org/10.1038/nprot.2010.66.

73 Dua, P., Kim, S., and Lee, D.K. (2011). Nucleic acid aptamers targeting cell-surface proteins. *Methods* 54 (2): 215–225. https://doi.org/10.1016/j.ymeth.2011.02.002.

74 Jin, C., Qiu, L., Li, J. et al. (2016). Cancer biomarker discovery using DNA aptamers. *The Analyst* 141 (2): 461–466. https://doi.org/10.1039/C5AN01918D.

75 Wu, X., Liu, H., Han, D. et al. (2019). Elucidation and structural modeling of CD71 as a molecular target for cell-specific aptamer binding. *Journal of the American Chemical Society* 141 (27): 10760–10769. https://doi.org/10.1021/jacs.9b03720.

76 Hou, Z., Meyer, S., Propson, N. et al. (2015). Characterization and target identification of a DNA aptamer that labels pluripotent stem cells. *Cell Research* 25: 390–393. https://doi.org/10.1038/cr.2015.7.

77 Zhou, W., Huang, P.J., Ding, J. et al. (2014). Aptamer-based biosensors for biomedical diagnostics. *Analyst* 139 (11): 2627–2640. https://doi.org/10.1039/C4AN00132J.

78 Akki, S.U. and Werth, C.J. (2018). Critical review: DNA aptasensors, are they ready for monitoring organic pollutants in natural and treated water sources? *Environmental Science & Technology* 52 (16): 8989–9007. https://doi.org/10.1021/acs.est.8b00558.

79 Huang, Z., Qiu, L., Zhang, T. et al. (2021). Integrating DNA nanotechnology with aptamers for biological and biomedical applications. *Matter* 4 (2): 461–489. https://doi.org/10.1016/j.matt.2020.11.002.

80 Bashir, A., Yang, Q., Wang, J. et al. (2021). Machine learning guided aptamer refinement and discovery. *Nature Communications* 12: 2366. https://doi.org/10.1038/s41467-021-22555-9.

81 Elskens, J.P., Elskens, J.M., and Madder, A. (2020). Chemical modification of aptamers for increased binding affinity in diagnostic applications: current status and future prospects. *International Journal of Molecular Sciences* 21 (12): 4522. https://doi.org/10.3390/ijms21124522.

82 Thirunavukarasu, D., Chen, T., Liu, Z. et al. (2017). Selection of 2′-fluoro-modified aptamers with optimized properties. *Journal of the American Chemical Society* 139 (8): 2892–2895. https://doi.org/10.1021/jacs.6b13132.

83 Gao, S., Zheng, X., Jiao, B. et al. (2016). Post-SELEX optimization of aptamers. *Analytical and bioanalytical chemistry* 408 (17): 4567–4573. https://doi.org/10.1007/s00216-016-9556-2.

84 Odeh, F., Nsairat, H., Alshaer, W. et al. (2019). Aptamers chemistry: chemical modifications and conjugation strategies. *Molecules* 25 (1): 3. https://doi.org/10.3390/molecules25010003.

85 Arangundy-Franklin, S., Taylor, A.I., Porebski, B.T. et al. (2019). A synthetic genetic polymer with an uncharged backbone chemistry based on alkyl phosphonate nucleic acids. *Nature chemistry* 11 (6): 533–542. https://doi.org/10.1038/s41557-019-0255-4.

86 Thiviyanathan, V. and Gorenstein, D.G. (2012). Aptamers and the next generation of diagnostic reagents. *Proteomics Clinical Applications* 6 (11–12): 563–573. https://doi.org/10.1002/prca.201200042.

87 Li, X., Li, Z., and Yu, H. (2020). Selection of threose nucleic acid aptamers to block PD-1/PD-L1 interaction for cancer immunotherapy. *Chemical communications* 56 (93): 14653–14656. https://doi.org/10.1039/d0cc06032a.

88 Chen, J., Chen, M., and Zhu, T.F. (2022). Directed evolution and selection of biostable L-DNA aptamers with a mirror-image DNA polymerase. *Nature Biotechnology* 40: 1601–1609. https://doi.org/10.1038/s41587-022-01337-8.

89 Lv, C., Yang, C., Ding, D. et al. (2019). Endocytic pathways and intracellular transport of aptamer-drug conjugates in live cells monitored by single-particle tracking. *Analytical chemistry* 91 (21): 13818–13823. https://doi.org/10.1021/acs.analchem.9b03281.

90 Xuan, W., Xia, Y., Li, T. et al. (2020). Molecular self-assembly of bioorthogonal aptamer-prodrug conjugate micelles for hydrogen peroxide and pH-independent cancer chemodynamic therapy. *Journal of the American Chemical Society* 142 (2): 937–944. https://doi.org/10.1021/jacs.9b10755.

91 Sefah, K., Yang, Z., Bradley, K.M. et al. (2014). In vitro selection with artificial expanded genetic information systems. *Proceedings of the National Academy of Sciences of the United States of America* 111 (4): 1449–1454. https://doi.org/10.1073/pnas.1311778111.

92 Li, Y., Li, T., Chen, H. et al. (2022). Engineering AND-gate aptamer-signal base conjugates for targeted magnetic resonance molecular imaging of metastatic cancer. *ACS applied materials & interfaces* 14 (15): 17032–17041. https://doi.org/10.1021/acsami.1c24048.

93 Tan, J., Zhao, M., Wang, J. et al. (2019). Regulation of protein activity and cellular functions mediated by molecularly evolved nucleic acids. *Angewandte Chemie International Edition* 58 (6): 1621–1625. https://doi.org/10.1002/anie.201809010.

94 Robert, A., Benoit-Vical, F., Liu, Y. et al. (2019). Small molecules: the past or the future in drug innovation? In: *Essential Metals in Medicine: Therapeutic Use and Toxicity of Metal Ions in the Clinic* (ed. L. Peggy), 17–48. Boston: De Gruyter. https://doi.org/10.1515/9783110527872-002.

95 Huang, Y.F., Shangguan, D., Liu, H. et al. (2009). Molecular assembly of an aptamer-drug conjugate for targeted drug delivery to tumor cells. *ChemBioChem* 10 (5): 862–868. https://doi.org/10.1002/cbic.200800805.

96 Li, Y., Peng, Y., Tan, Y. et al. (2021). A new paradigm for artesunate anticancer function: considerably enhancing the cytotoxicity via conjugating artesunate with aptamer. *Signal Transduction and Targeted Therapy* 6: 327. https://doi.org/10.1038/s41392-021-00671-8.

97 Huang, Z., Wang, D., Long, C.Y. et al. (2021). Regulating the anticancer efficacy of Sgc8–Combretastatin A4 conjugates: a case of recognizing the significance of linker chemistry for the design of aptamer-based targeted drug delivery strategies. *Journal of the American Chemical Society* 143 (23): 8559–8564. https://doi.org/10.1021/jacs.1c03013.

98 He, J., Peng, T., Peng, Y. et al. (2020). Molecularly engineering triptolide with aptamers for high specificity and cytotoxicity for triple-negative breast cancer. *Journal of the American Chemical Society* 142 (6): 2699–2703. https://doi.org/10.1021/jacs.9b10510.

99 Zhou, F., Wang, P., Peng, Y. et al. (2019). Molecular engineering-based aptamer–drug conjugates with accurate tunability of drug ratios for drug combination targeted cancer therapy. *Angewandte Chemie International Edition* 58 (34): 11661–11665. https://doi.org/10.1002/anie.201903807.

100 Ding, D., Zhao, H., Wei, D. et al. (2023). The first-in-human whole-body dynamic pharmacokinetics study of aptamer. *Research* 6: 0126. https://doi.org/10.34133/research.0126.

101 Xuan, W., Peng, Y., Deng, Z. et al. (2018). A basic insight into aptamer-drug conjugates (ApDCs). *Biomaterials* 182: 216–226. https://doi.org/10.1016/j.biomaterials.2018.08.021.

102 Geng, Z., Wang, L., Liu, K. et al. (2021). Enhancing anti-PD-1 immunotherapy by nanomicelles self-assembled from multivalent aptamer drug conjugates. *Angewandte Chemie International Edition* 60 (28): 15459–15465. https://doi.org/10.1002/anie.202102631.

103 Xie, S., Ai, L., Cui, C. et al. (2021). Functional aptamer-embedded nanomaterials for diagnostics and therapeutics. *ACS Applied Materials & Interfaces* 13 (8): 9542–9560. https://doi.org/10.1021/acsami.0c19562.

104 Wu, Y., Sefah, K., Liu, H. et al. (2010). DNA aptamer-micelle as an efficient detection/delivery vehicle toward cancer cells. *Proceedings of the National Academy of Sciences of the United States of America* 107 (1): 5–10. https://doi.org/10.1073/pnas.0909611107.

105 Li, X., Figg, C.A., Wang, R. et al. (2018). Crosslinked aptamer-lipid micelles for excellent stability and specificity in target-cell recognition. *Angewandte Chemie International Edition* 57 (36): 11589–11593. https://doi.org/10.1002/anie.201804682.

106 Zou, J., Shi, M., Liu, X. et al. (2019). Aptamer-functionalized exosomes: Elucidating the cellular uptake mechanism and the potential for cancer-targeted chemotherapy. *Analytical chemistry* 91 (3): 2425–2430. https://doi.org/10.1021/acs.analchem.8b05204.

107 Luo, C., Hu, X., and Peng, R. (2019). Biomimetic carriers based on giant membrane vesicles for targeted drug delivery and photodynamic/photothermal synergistic therapy. *ACS applied materials & interfaces* 11 (47): 43811–43819. https://doi.org/10.1021/acsami.9b11223.

108 Moosavian, S.A. and Sahebkar, A. (2019). Aptamer-functionalized liposomes for targeted cancer therapy. *Cancer letters* 448: 144–154. https://doi.org/10.1016/j.canlet.2019.01.045.

109 Chen, K., Zhou, J., Shao, Z. et al. (2020). Aptamers as versatile molecular tools for antibody production monitoring and quality control. *Journal of the American Chemical Society* 142 (28): 12079–12086. https://doi.org/10.1021/jacs.9b13370.

110 Huang, H., Guo, Z., Zhang, C. et al. (2021). Logic-gated cell-derived nanovesicles via DNA-based smart recognition module. *ACS applied materials & interfaces* 13 (26): 30397–30403. https://doi.org/10.1021/acsami.1c07632.

111 Peng, R., Zheng, X., Lyu, Y. et al. (2018). Engineering a 3D DNA-logic gate nanomachine for bispecific recognition and computing on target cell surfaces. *Journal of the American Chemical Society* 140 (31): 9793–9796. https://doi.org/10.1021/jacs.8b04319.

112 Yue, L., Wang, S., Wulf, V. et al. (2019). Consecutive feedback-driven constitutional dynamic networks. *Proceedings of the National Academy of Sciences of the United States of America* 116 (8): 2843–2848. https://doi.org/10.1073/pnas.1816670116.

113 Wang, S., Yue, L., Wulf, V. et al. (2020). Dissipative constitutional dynamic networks for tunable transient responses and catalytic functions. *Journal of the American Chemical Society* 142 (41): 17480–17488. https://doi.org/10.1021/jacs.0c06977.

114 Yue, L., Wang, S., Zhou, Z. et al. (2020). Nucleic acid based constitutional dynamic networks: from basic principles to applications. *Journal of the American Chemical Society* 142 (52): 21577–21594. https://doi.org/10.1021/jacs.0c09891.

115 Wang, D., Yang, Y., Chen, F. et al. (2022). Network topology-directed design of molecular CPU for cell-like dynamic information processing. *Science advances* 8 (32): eabq0917. https://doi.org/10.1126/sciadv.abq0917.

116 He, L., Chen, F., Zhang, D. et al. (2020). Transducing complex biomolecular interactions by temperature-output artificial DNA signaling networks. *Journal of the American Chemical Society* 142 (33): 14234–14239. https://doi.org/10.1021/jacs.0c05453.