ACS APPLIED BIO MATERIALS

www.acsabm.org Review

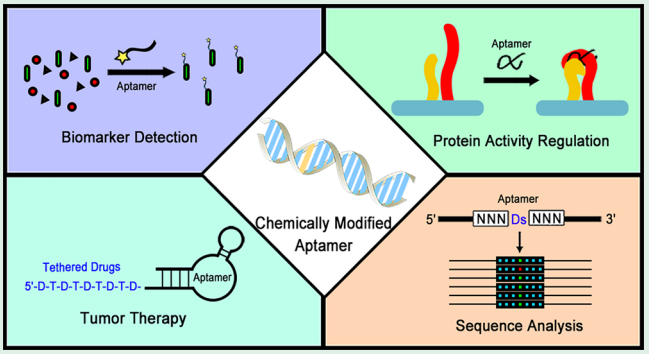
Chemically Modified Aptamers in Biological Analysis

Ruichen Shen, Jie Tan,* and Quan Yuan*

Cite This: *ACS Appl. Bio Mater.* 2020, 3, 2816−2826 Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Recently, nucleic acid aptamers have been widely used in biological analysis due to their high affinity, binding selectivity, and programmability. Researchers have introduced the idea that chemical modification endows aptamers with rich spatial conformations and functions, extending the application of aptamers in biological analysis. Currently, reviews about aptamers in the bioanalytical field usually focus on the complexes of aptamers and functional materials. Few reviews pay attention to the chemically modified aptamers. In this review, we introduce the synthesis and screening of chemically modified nucleic acids. In addition, we summarize the recent works about chemically modified aptamers in the field of bioanalysis, aiming to provide a promising step toward an aptamer-based versatile platform for biological analysis.



KEYWORDS: *aptamer, modified nucleotides, biomarker detection, protein activity regulation, tumor treatment, sequence analysis*

INTRODUCTION

Nucleic acids consist of five kinds of natural nucleotides (A, T, G, C, and U).[1−3] In living organisms, nucleic acids can participate in a variety of physiological procedures, such as genetic information storage, expression, and regulation.[4−8] Chemically modified nucleic acids here refer to the nucleic acids modified with new functional groups that do not exist in natural nucleotides.[9−13] In nature, several chemically modified nucleic acids already exist, producing specific functions that cannot be achieved by natural nucleic acids.[14−18] Therefore, nucleic acids with rational design and chemical modification can be expected to show new properties and functions,[19−22] promoting their application in biological analysis.[23−26]

Recently, nucleic acid aptamers have been widely used in biological analysis because of their high affinity, binding selectivity, and programmability.[27−32] However, the application of many aptamers with excellent properties is limited by defects, such as rapid degradation in vivo and short half-lives.[33,34] To solve these problems, a series of chemically modified aptamers have been investigated. It has been reported that chemical modification enhances the resistance of aptamers to degradation. Chemical modification endows aptamers with abundant spatial conformations and enhanced affinity for the target molecules, thus giving aptamers new functions.[32,35,36] Thus, the rational design of modified aptamers holds great potential to provide a versatile platform for biological analysis.

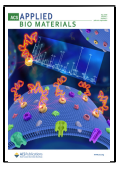
Currently, reviews about aptamers in the bioanalytical field usually focus on the complexes of aptamers and functional materials.[37−41] However, few reviews pay attention to chemically modified aptamers. In this review, we introduce chemically modified aptamers and their applications in the bioanalytical field. Specifically, we introduce the synthesis and screening of chemically modified nucleic acids. In addition, we summarize the studies and applications of chemically modified aptamers in biomarker detection, regulation of protein activity, tumor treatment, and sequence analysis. At the end of this review, we present the opportunities and challenges of chemically modified aptamers in the bioanalytical field, aiming to provide a promising step toward an aptamer-based versatile platform for biological analysis.

SYNTHESIS AND SCREENING OF CHEMICALLY MODIFIED NUCLEIC ACID APTAMERS

At present, aptamers are mainly obtained by SELEX and Cell-SELEX.[42−44] Through polymerase chain reaction (PCR) amplification of the binding sequence, the technology enables evolution from random sequence libraries to sequences with high affinity.[45,46] However, most chemically modified nucleotides cannot be recognized by traditional DNA polymerases (usually Taq enzymes). This phenomenon limits the

Special Issue: Nucleic Acids: Chemistry, Nanotechnology, and Bioapplications

Received: January 15, 2020
Accepted: March 9, 2020
Published: March 9, 2020



https://dx.doi.org/10.1021/acsabm.0c00062
ACS Appl. Bio Mater. 2020, 3, 2816−2826

application of chemically modified nucleotides in PCR amplification. To achieve screening of chemically modified aptamers, we could optimize the nucleotide molecule design and PCR amplification procedures.

Molecular Design of Nucleotide Molecules. From the perspective of molecular design, we need to design a chemically modified nucleotide molecule that can be recognized by traditional DNA polymerases. DNA polymerase uses DNA as a template to catalyze the polymerization of the substrate dNTP to create new DNA strands.[47] However, chemically modified nucleotides and natural nucleotides are hard to complementarily pair due to their structural differences.[48] In addition, it is difficult for traditional DNA polymerases to use chemically modified nucleotides as substrates for DNA synthesis. Therefore, it is necessary to retain the structural characteristics of natural nucleotides when chemically modifying nucleotides, which will facilitate subsequent PCR amplification. For this reason, researchers often introduce amino acid side chains or other biological small molecule fragments into nucleotides to achieve chemical modifications while maintaining the compatibility of the modified nucleotides with DNA polymerase.

For example, Bharat et al. modified carboxamides on cytosine nucleotides and used them to synthesize and screen chemically modified aptamers.[28] Furthermore, the authors also studied the stability of the obtained aptamers, and the results showed that the stability of chemically modified aptamers has been improved. In addition to chemically modified cytosine nucleotides, some researchers have also studied chemically modified uracil nucleotides. Hirotaka et al. synthesized a chemically modified aptamer using a library including chemically modified uracil nucleotides and used the aptamer to detect salivary amylase.[49] Franziska et al. used click-SELEX to introduce chemically modified uracil nucleotides into the original library to obtain chemically modified aptamers.[50]

DNA Polymerase That Is Compatible with Chemically Modified Nucleotides. From the perspective of the PCR amplification procedures, we need to find a DNA polymerase that is compatible with chemically modified nucleotides. Researchers have found that HS Takara Taq DNA polymerase is compatible with nucleotides containing Z (2-amino-3-nitropyridin-6-one) and P [imidazo[1,2-*a*]-1,3,5-triazin-4(8*H*)-one] bases. Therefore, many researchers use libraries containing these two bases to obtain chemically modified aptamers. Both Li et al. of the Tan group and Xiao et al. used libraries containing Z and P bases to synthesize chemically modified aptamers.[51,52] On this basis, researchers refer to the Z and P bases as a six-letter artificial genetic system along with GACT.

Mutual Substitution of Chemically Modified Nucleotides with Natural Nucleotides. In addition, we can convert chemically modified nucleotides into natural nucleotides on the basis of the principle of complementary base pairing to achieve target sequence amplification. There are currently two general methods of nucleotide conversion. The first method requires us to obtain a sequence from the library that can tightly combine with the target and then to obtain the corresponding complementary nucleic acid. Then natural nucleic acids are treated with a sublibrary containing chemically modified nucleotides and T4 ligase to obtain the desired chemically modified aptamer. The second method also requires suitable chemically modified aptamers, then synthesizing complementary natural nucleic acids and PCR amplifying them. However, this method requires attaching two or three natural nucleotides as recognition tags to the 5′ end of the chemically modified nucleotides (Figure 1). Accordingly, we

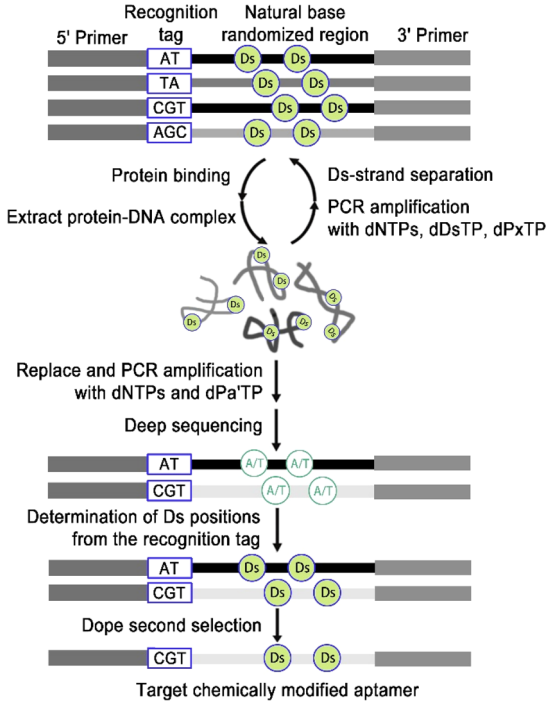


Figure 1. Scheme of the chemically modified aptamer SELEX by nucleotide conversion.

can identify the corresponding positions of chemically modified nucleotides in the sequence based on these tags and convert the natural nucleic acid back to the desired chemically modified aptamer. For example, Michiko et al. synthesized and amplified a natural nucleic acid sequence that was complementary to a chemically modified nucleic acid containing Ds nucleotides and finally obtained chemically modified nucleic acid aptamers based on the recognition tag.[53]

APPLICATION OF CHEMICALLY MODIFIED NUCLEIC ACID APTAMERS

A nucleic acid aptamer is a nucleic acid sequence that specifically binds to a target.[39,54,55] It has advantages of a higher affinity, a lower immunogenicity, and a stronger nuclease stability.[56−58] Therefore, it has attracted considerable attention in the field of biological analysis in recent years.[59−63] However, the targeted binding ability of the aptamer is disturbed by factors such as pH, temperature, and ionic strength, which limits the use of the aptamer in bioanalysis.[64,65] The introduction of new chemical groups is expected to screen out suitable chemically modified nucleic acid aptamers.[66,67] It has been reported that chemically modified aptamers may have stronger affinity and biological functions than conventional aptamers.[68] Therefore, it is expected to extend the application of aptamers in the bioanalytical field. In this section, we introduce the related work with chemically modified nucleic acid aptamers in the bioanalytical field in four realms.

Chemically Modified Aptamers for Biomarker Detection. The detection of biomarkers can provide us with a wealth of information that will help us more accurately diagnose disease.[69−73] It is well-known that chemicals in living

https://dx.doi.org/10.1021/acsabm.0c00062
ACS Appl. Bio Mater. 2020, 3, 2816−2826

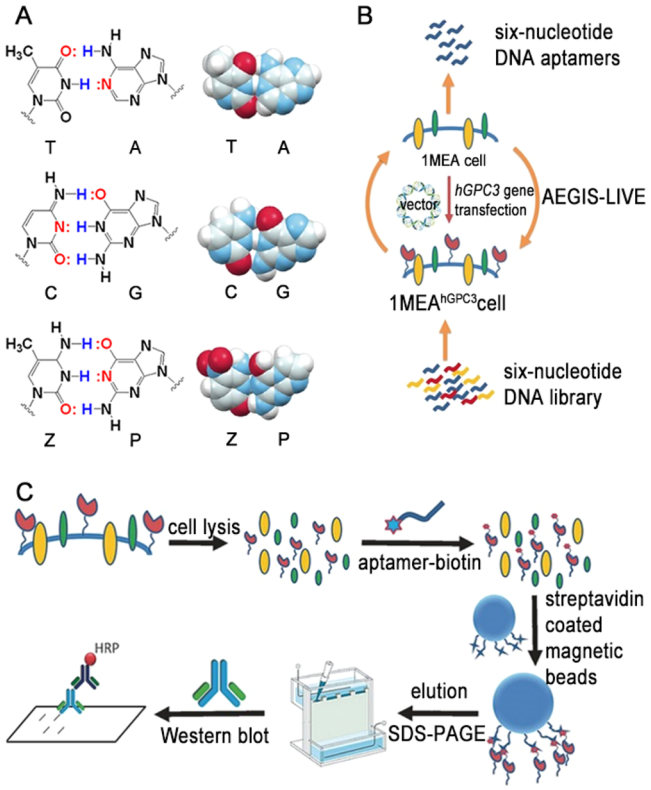


Figure 2. AEGIS-LIVE procedure that screens aptamers and uses aptamers to detect GPC3 protein. (A) Molecular structures (left) and space filling models (right) of A, T, G, C, Z, and P. (B) 1MEA^{hGPC3} cell aptamers were screened using AEGIS-LIVE. (C) Schematic diagram of aptamers for identifying GPC3 protein. Reproduced from ref 51. Copyright 2016 Wiley-VCH Verlag GmbH & Co. KGaA.

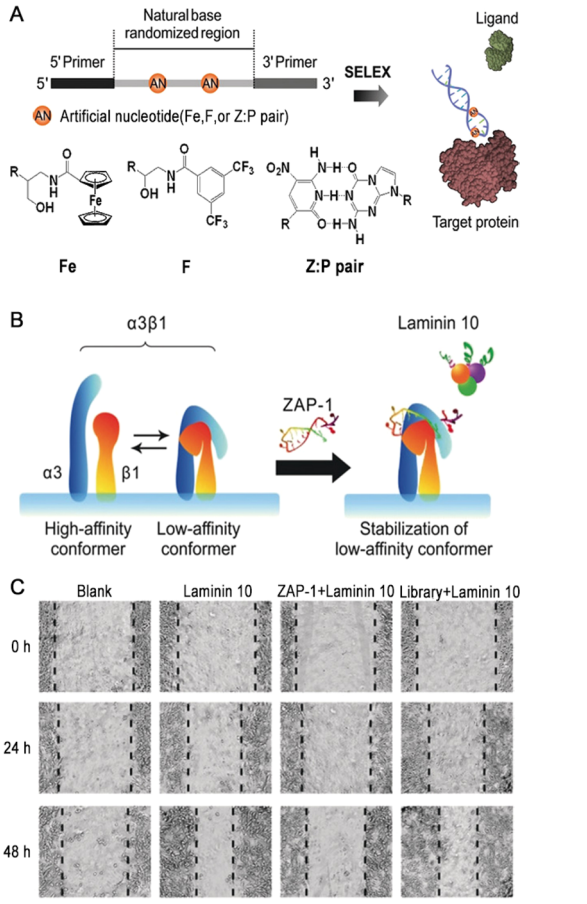


Figure 3. (A) Schematic of the molecular design of aptamers as modulators of protein activity. (B) ZAP-1 binds $\alpha 3\beta 1$ on cells to form a stable low-affinity conformation. (C) The ZAP-1 aptamer inhibits cell migration of adhesion between MDA-MB-231 cells and Laminin 10. Reproduced from ref 99. Copyright 2019 Wiley-VCH Verlag GmbH & Co. KGaA.

organisms are complex.[74,75] Therefore, it is necessary to detect the target substance using reagents and probes with a high level of specific binding.[76−81] Aptamers have the potential to specifically recognize target substances and detect diseases.[82,83] However, some biomarkers have not been able to find the corresponding aptamers by screening natural nucleic acids. The integration of chemical groups allows chemically modified aptamer molecules to have richer spatial conformations.[84,85] This increases the likelihood of screening for desired nucleic acid aptamers.[86−89] In this section, we describe the work of chemically modified aptamers in the detection of biomarkers.

The Tan group has extensively studied the Z and P bases and integrated them into chemically modified nucleic acid aptamers. Li et al. of the Tan group studied the specific biomarker glypcan 3 (GPC3) of hepatocellular carcinoma (HCC).[51] Using the six-letter ATGCZP as a library (Figure 2A), the authors obtained the aptamer of GPC3 by an artificially expanded genetic information system laboratory in vitro evolution (AEGIS-LIVE) procedure (Figure 2B). They then used the aptamers for Western blot analysis of GPC3. The results showed that the obtained aptamer could efficiently detect the abnormal expression of GPC3 protein induced by HCC (Figure 2C).

Bacillus anthracis is a highly toxic spore bacterium that is considered a potential bioterrorism agent.[90] At present, aptamers capable of highly specific binding to *B. anthracis* have not been found in natural nucleic acid libraries. Elisa et al. of the Benner group reported a chemically modified nucleic acid aptamer containing Z and P bases that are capable of targeting the PA63 antigen in *B. anthracis*.[91] The fraction bound of the modified aptamer was increased by ∼10% compared to that of the aptamer that was not modified. The results indicated that the stability of the aptamer after modification of the Z:P base pair was improved. In addition to the construction of new base structures, chemically modified aptamers can also be obtained by chemically modified natural bases. Bharat et al. chemically modified the natural bases C and U to obtain singly modified and doubly modified DNA aptamers.[28] By comparing the affinity of the unmodified, singly modified, and doubly modified nucleic acid aptamers, the authors found that doubly modified aptamers had stronger affinities. Their affinity for PCSK9 is 2 orders of magnitude higher than that of unmodified aptamers. These studies indicate that chemical modification of nucleic acid aptamers will enrich the structure and function of aptamers. Therefore, identification and detection of other biomarkers can be achieved by aptamers.

Chemically Modified Nucleic Acid Aptamers for the Regulation of Protein Activity. Protein is an important component of living organisms, and most life activities involve the participation of proteins.[92−94] Therefore, research of the regulation of protein activity is important for understanding the principles of biological activity,[95] disease processes,[96,97] and disease treatment.[98,99] The current research indicates that

https://dx.doi.org/10.1021/acsabm.0c00062
ACS Appl. Bio Mater. 2020, 3, 2816−2826

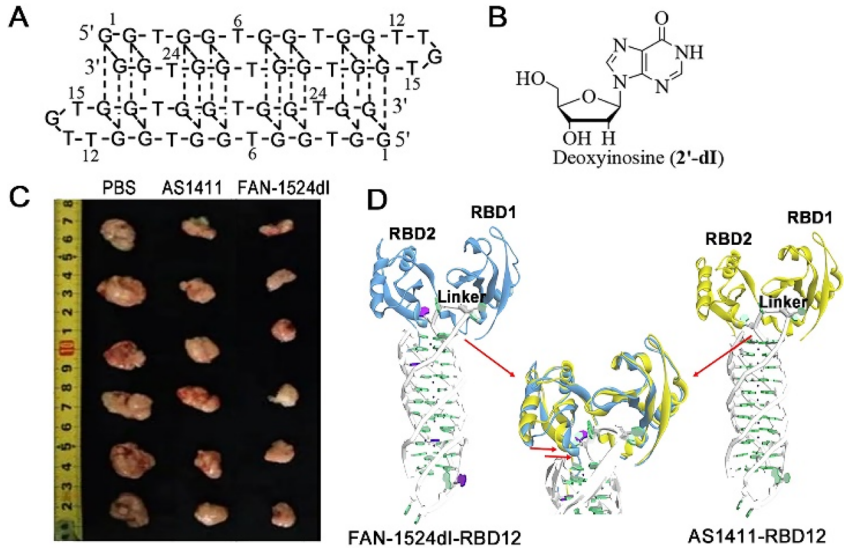


Figure 4. Chemical structures of (A) AS1411 and (B) 2′-deoxynucleosides. (C) FAN-1524dI and AS1411 inhibit the growth of MCF-7 tumors. (D) Comparison of the structures of FAN-1524dI−RBD12 and AS1411−RBD12 complexes. On the left, RBD12 is colored blue and the 2′-dI at position 15 is colored purple. On the right, RBD12 is colored yellow. The middle panel shows the superposition of the two complexes. The red arrow highlights the structural changes of the protein. Reproduced from ref 124. Copyright 2016 Springer Nature.

some nucleic acid aptamers can bind to proteins for labeling and detection.[99,100] However, there has been little research on the regulation of protein activity using traditional nucleic acid aptamers. As mentioned previously, chemically modified aptamers have richer spatial conformations. Thus, they are expected to bind with protein to achieve regulation of protein activity.[101] In this section, we introduce the application of chemically modified nucleic acids in the regulation of protein activity.

In the Tan group, Jie et al. reported the ZAP-1 aptamer by SELEX screening of DNA libraries containing Z and P bases,[99] as shown in Figure 3A. The authors used it for the regulation of integrin $\alpha 3\beta 1$ affinity. $\alpha 3\beta 1$ usually has two types of conformations (high-affinity and low-affinity conformations), and they are normally in a state of rapid equilibrium. ZAP-1 can inhibit the affinity of $\alpha 3\beta 1$ by binding to $\alpha 3\beta 1$ to form a stable low-affinity conformation (Figure 3B). The authors used MDA-MB-231 cells as an example and treated them with ZAP-1 and a random library. The experimental results showed that the migration ability of cancer cells was significantly decreased after ZAP-1 treatment, and the migration ability of cells treated by the random library was not significantly affected (Figure 3C). This indicates that ZAP-1 can effectively inhibit the affinity of $\alpha 3\beta 1$.

Nicola et al. used an acyclic nucleoside as a mimetic of the T base and inserted it into the oligodeoxynucleotide sequence, known as thrombin-conjugated aptamer (TBA).[102] The authors preincubated aptamers with fibrinogen and then added thrombin to compare the clotting times of fibrinogen after treatment with different aptamers. Compared with unmodified TBA, the aptamer TBA-T_7b modified with acyclic nucleoside has stronger targeted binding and a shorter binding time, and effectively inhibits thrombin coagulation of fibrinogen. The ratio of clotting was shortened from 0.3 to 0.2. Furthermore, the authors performed CD melting analysis on the aptamers before and after modification, and the melting temperature of the aptamers was increased from 33.0 to 37.5 °C. These researches indicate that the rational introduction of chemically modified groups not only enables the nucleic acid aptamer to have stronger targeted binding ability but also makes the aptamer more stable in complex environments.

Chemically Modified Nucleic Acids for Tumor Therapy. Cancer has a high mortality rate and has been a research hot spot.[103−105] At present, most cancer therapeutic drugs used for clinical treatment cannot target the cancer sites.[106−108] Therefore, the therapeutic drug also has a certain degree of killing effects on healthy cells.[109−111] This will cause serious side effects after the patients take the medicine.[112−114] Nucleic acid aptamers have the ability to specifically recognize targets.[115−117] It can be used to target drug delivery and directly treat tumors.[118,119] However, some cancers cannot obtain suitable aptamers by conventional screening techniques. Chemically modified aptamers have a richer spatial conformation, and screening aptamers suitable for tumor therapy is expected to occur.[120,121] In this section, we highlight the application of chemically modified nucleic acids for tumor therapy.

Aptamers as Tumor Therapeutic Agents. In recent years, studies have shown that chemically modified nucleic acid aptamers could be directly used as therapeutic agents for tumor therapy.

AS1411 is an oligonucleotide containing a large amount of guanine (Figure 4A), which has the ability to inhibit cancer cell proliferation and induce cancer cell death. Moreover, it has little effect on normal cells, and therefore, it has broad development potential in tumor therapy.[122,123] Xin et al. integrated 2′-deoxyinosine (2′-dI) (Figure 4B) into AS1411 to enhance its function.[124] The incorporation of 2′-dI at positions 15 and 24 of AS1411 produces an antiparallel G quadrilateral FAN-1524dI. Thus, there is a structural difference between the FAN-1524dI−RBD12 complex and the AS1411−RBD12 complex. As Figure 4D shows, the protein in the FAN-1524dI−RBD12 complex was significantly shifted to the G quadruplex. Therefore, the binding of FAN-1524dI to proteins is more stable, which can better inhibit the proliferation of cancer cells.

Ken-ichiro et al. of the Ichiro Hirao group integrated hydrophobic Ds bases into DNA aptamers to improve the

https://dx.doi.org/10.1021/acsabm.0c00062
ACS Appl. Bio Mater. 2020, 3, 2816−2826

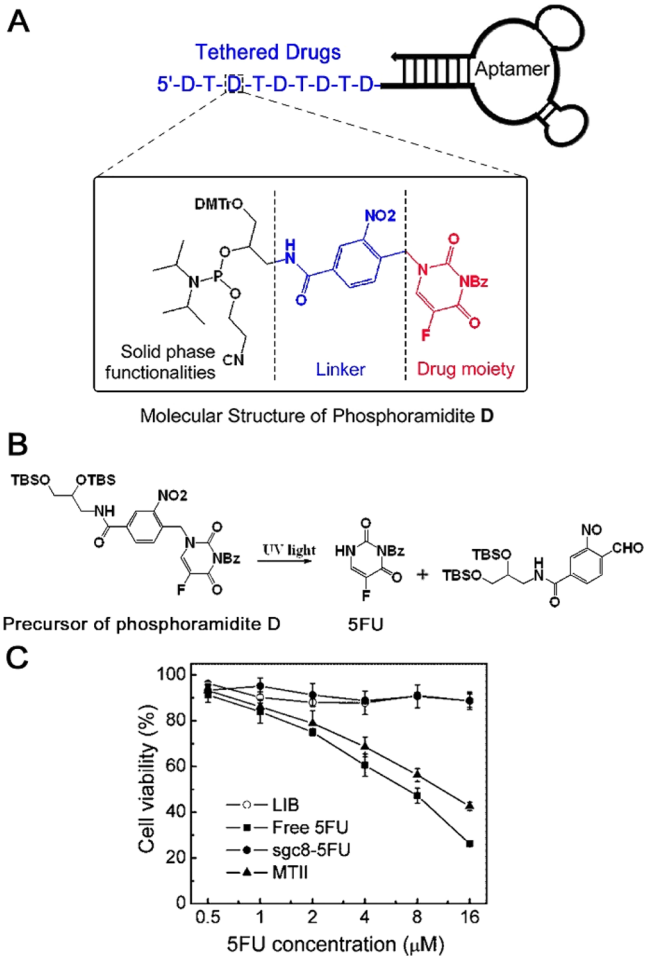


Figure 5. (A) Schematic diagram of aptamer−drug conjugate and therapeutic module phosphoramidite D. (B) Reaction for photocontrolled release of a drug moiety from the aptamer−drug conjugate. (C) Cell viability of LIB-, free 5FU-, sgc8-5FU-, and MTII-treated HTC116 cells. Reproduced from ref 114. Copyright 2014 American Chemical Society.

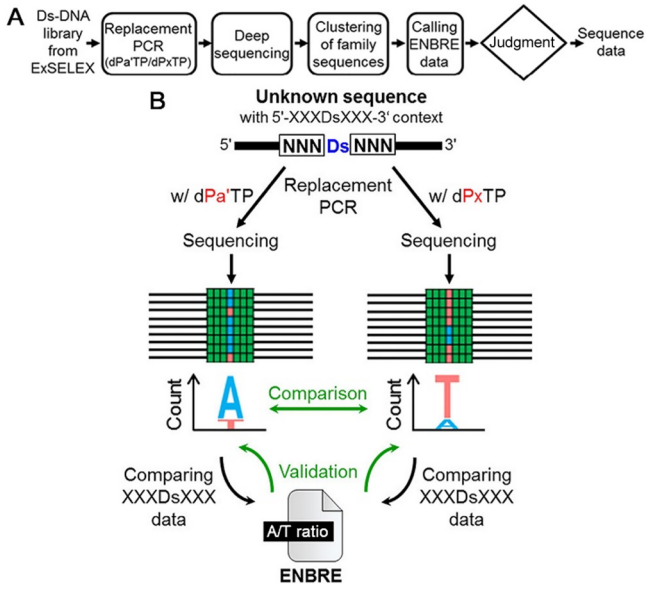


Figure 6. (a) Sequencing procedure and (b) schematic of the Ds-containing aptamer. In the presence of dPa'TPs or dPxTPs, the Ds bases in the sequence are replaced by replacement PCR, and then the sequence data of the chemically modified nucleic acids are obtained by deep sequencing. Reproduced from ref 153. Copyright 2019 American Chemical Society.

affinity of the aptamer for IFN-γ.[125] Then the authors modified the small hairpin DNA on the aptamer to further improve the affinity and stability of the aptamer. The results showed that the native DNA aptamer had a lower affinity for IFN-γ (K_D = 16 nM), while the aptamer integrating Ds bases had a higher affinity (K_D = 46 pM). This demonstrates that the affinity of the aptamer is enhanced after the Ds base is modified. However, the modified aptamer still has the problem of poor stability to nucleases, with a survival rate by 72 h of only 9%. The authors introduced a small hairpin DNA into the aptamer, further enhancing the affinity and stability of the aptamer. After the introduction of small hairpin DNA into the chemically modified aptamer, the K_D was reduced to 33 pM, and the survival rate at 72 h increased to 83%. The research indicated that the chemically modified aptamers have a stronger affinity and inhibit cancer cell proliferation. Furthermore, Sha et al. studied slow off-rate modified aptamers (SOMAmers) containing chemically modified uridine.[126] These modifications promote the high affinity in combination with IL-6 and inhibit tumor cell proliferation. Specifically, the K_D value of the chemically modified aptamer was reduced by nearly 20 times, and the stability was also improved. These studies indicated that chemically modified aptamers are promising therapeutic drugs.

Chemically Modified Nucleic Acids for Targeted Drug Delivery. At present, the most common cancer treatment problems in the clinic are strong side effects and easy recurrence.[127−129] Therefore, a common research idea is to develop a drug delivery platform that can accumulate in diseased areas.[130,131] Then the drug platform releases the drug to effectively inhibit tumor growth.[131,132] Currently, some drug delivery platforms can accumulate around tumor tissue by the EPR effect.[133−136] However, the EPR effect requires attributes such as the dimension and solubility of the drug and drug delivery platform.[137−140] These limit the further progress of drug delivery platforms. Because the nucleic acid aptamers have the ability to specifically bind to the target substance, they are also expected to bind to the tumor site regardless of the EPR effect. Therefore, structurally diverse chemically modified aptamers have greater potential for drug delivery platforms.[141,142] Furthermore, some researchers have introduced photosensitive groups into chemically modified nucleic acid aptamers to achieve targeted release of drugs under specific illumination.[143] In this part, we introduce the related work of chemically modified nucleic acids for targeted drug delivery.

Solid-phase synthesis is an automated and highly controllable method of molecular synthesis.[144] In the Tan group, Ruo et al. used a solid-phase technique to integrate the 5FU drug into the aptamer to obtain an aptamer−drug conjugate (termed MTII),[114] as shown in Figure 5A. Because the conjugate has a photocontrolled group, the drug 5FU can be effectively released under ultraviolet (UV) irradiation. The relevant reaction formula is shown in Figure 5B. The authors then analyzed the inhibition of LIB, free 5FU, sgc8-5FU (without photocleavable linkers between 5FU and backbones), and MTII on HTC116 cells under UV light. The results indicate that MTII is capable of effectively releasing 5FU by light control (Figure 5C).

https://dx.doi.org/10.1021/acsabm.0c00062
ACS Appl. Bio Mater. 2020, 3, 2816−2826

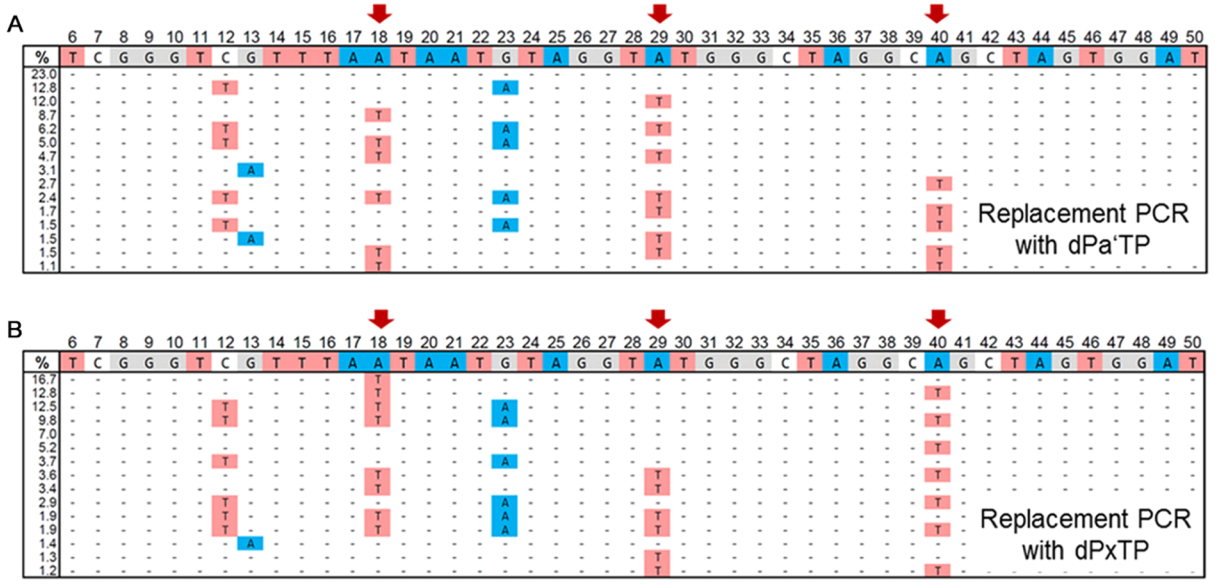


Figure 7. Sequence data obtained by deep sequencing of chemically modified nucleic acids when (A) dPa'TPs and (B) dPxTPs are used as non-natural base substrates. Reproduced from ref 153. Copyright 2019 American Chemical Society.

In addition, Ruo et al. reported the design and synthesis of photoresponsive bases and used the bases to construct DNA nanomolecules.[143] This base is formed by fusing azobenzene with a natural T base (zT). The results indicate that the *trans*-zT base can complement the A base to form a stable double-stranded DNA. However, the *trans*-zT base will be converted to *cis*-zT under UV light. Because *cis*-zT cannot form a hydrogen bond with the A base, it can be effectively dissociated by light irradiation. These researches indicate that introduction of photosensitive groups into aptamers of nucleic acids can change the structure of the DNA molecules via irradiation with light of a specific wavelength. Therefore, the introduction of a photoresponsive group into an aptamer is a latent drug delivery strategy.

In 2015, Gui et al. of the Tan group described an aptamer–drug conjugate DDA with nuclease resistance.[127] The authors subsequently investigated the delivery efficiency of targeted drugs both in vitro and in vivo. The results demonstrated that the aptamer–drug conjugate can effectively inhibit CEM cell growth, while being less toxic to other cells. Furthermore, Fang et al. also synthesized a circular bivalent aptamer–drug conjugate.[145] The conjugate was purified by HPLC to remove residual drug modules and linkers. Further investigation indicated that the aptamer–drug conjugate showed potent therapeutic efficacy on target DU145 cells. These studies indicate that chemically modified aptamers are promising delivery platforms.

Sequence Analysis of Chemically Modified Aptamers. As more and more researchers have begun to study chemically modified nucleic acids in recent years,[146−148] the genetic alphabet expansion technology has been rapidly developed and many unnatural bases have been produced.[149−152] Sequence analysis of chemically modified nucleic acids helps in better understanding and improving their properties.[153−155] Therefore, the demand for analysis of unnatural base positions in chemically modified nucleic acid aptamers is rapidly increasing.[156,157] In this section, the relevant work in the study of sequence analysis of chemically modified aptamers will be described.

Kiyofumi et al. of the Ichiro Hirao group recently reported a chemically modified nucleic acid containing Ds bases.[153] They used a library with a non-natural base substrate (dPa'TPs or dPxTPs) and four natural base substrates (dNTPs) for replacement PCR. Then, the Ds bases in the original sequence were substituted with a mixture of A-T and G-C (Figure 6). Therefore, researchers can distinguish the original positions of unnatural bases in chemically modified nucleic acids by analyzing the positions and numbers of A-T and G-C base pairs in the sequences obtained by replacement PCR under different non-natural base substrates (Figure 7).

Similarly, Ken-ichiro et al. also constructed a nucleic acid aptamer containing Ds bases.[156] In addition to analyzing the sequence of chemically modified aptamers, the authors also studied the affinity and stability of the aptamers. The research has indicated that the natural aptamers have a lower affinity for target vWF (K_D = 326 pM), while chemically modified aptamers have a higher affinity (K_D = 75 pM). Furthermore, the authors modified a small hairpin DNA into the aptamer to further improve the affinity and stability of the aptamer. Among them, the K_D value of the aptamer that modified two small hairpin DNAs reached 61.3 pM, and the T_m increased from 63 to 76.5 °C. These values indicated that sequence analysis of chemically modified aptamers not only provides an understanding of their properties but also provides the foundation for further application of aptamers.

CONCLUSION AND OUTLOOK

In the past decade, nucleic acid aptamers have attracted widespread attention of researchers because of their unique binding capabilities. They are biological materials with great development potential. In the study presented here, we used a chemical method to introduce new functional groups to obtain chemically modified nucleic acid aptamers. Then the aptamer's affinity, stability, and light responsiveness were analyzed. The results show that chemically modified aptamers have an affinity and a stability that are higher than those of traditional aptamers. The reason for these changes is the introduction of new functional groups that allow the aptamers to form stable

https://dx.doi.org/10.1021/acsabm.0c00062
ACS Appl. Bio Mater. 2020, 3, 2816−2826

hydrogen bonds and different active sites. For chemically modified aptamers that introduce specific functional groups, they cause conformational change or dissociation under irradiation with light of a specific wavelength. Therefore, the introduction of functional groups expands the function of the aptamer, further enhancing its use in the field of bioanalysis.

In this review, we assess the research and application of chemically modified aptamers in biomarker detection, protein activity regulation, tumor therapy, and sequence analysis. These studies indicate that chemically modified nucleic acid aptamers have made great technological advances in the field of bioanalysis. However, the clinical research on nucleic acid aptamers is still in initial stage, which is reflected in the following. (i) In this review, the kinds of functional groups and artificial bases used to chemically modified nucleic acid aptamers are very limited. (ii) The number of chemically modified aptamers studied is very low. (iii) Although chemically modified aptamers have great potential for development, they still lack a large number of clinical trials. In the future, many differently modified nucleic acid aptamers will be developed, which is a promising step toward an aptamer-based versatile biological analysis platform and its clinical applications.

AUTHOR INFORMATION

Corresponding Authors

Quan Yuan − *Institute of Chemical Biology and Nanomedicine (ICBN), State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, China; Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, China;* orcid.org/0000-0002-3085-431X; Email: yuanquan@whu.edu.cn

Jie Tan − *Institute of Chemical Biology and Nanomedicine (ICBN), State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, China*; Email: tanjie0416@hnu.edu.cn

Author

Ruichen Shen − *Institute of Chemical Biology and Nanomedicine (ICBN), State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsabm.0c00062

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (21925401 and 21904037).

REFERENCES

(1) Gomez, E. F.; Venkatraman, V.; Grote, J. G.; Steckl, A. J. Exploring the Potential of Nucleic Acid Bases in Organic Light Emitting Diodes. *Adv. Mater.* **2015**, *27* (46), 7552−62.

(2) Heidari, A. An analytical and computational infrared spectroscopic review of vibrational modes in nucleic acids. *Austin J. Anal. Pharm. Chem.* **2016**, *3* (1), 1058.

(3) Zhirnov, V.; Zadegan, R. M.; Sandhu, G. S.; Church, G. M.; Hughes, W. L. Nucleic acid memory. *Nat. Mater.* **2016**, *15* (4), 366−70.

(4) Wang, K.; Hu, Q.; Zhu, W.; Zhao, M.; Ping, Y.; Tang, G. Structure-Invertible Nanoparticles for Triggered Co-Delivery of Nucleic Acids and Hydrophobic Drugs for Combination Cancer Therapy. *Adv. Funct. Mater.* **2015**, *25* (22), 3380−3392.

(5) Tzeng, S. Y.; Green, J. J. Polymeric Nucleic Acid Delivery for Immuno-engineering. *Curr. Opin. Biomed. Eng.* **2018**, *7*, 42−50.

(6) Flingai, S.; Plummer, E. M.; Patel, A.; Shresta, S.; Mendoza, J. M.; Broderick, K. E.; Sardesai, N. Y.; Muthumani, K.; Weiner, D. B. Protection against dengue disease by synthetic nucleic acid antibody prophylaxis/immunotherapy. *Sci. Rep.* **2015**, *5*, 12616.

(7) Chen, C.; Yang, Z.; Tang, X. Chemical modifications of nucleic acid drugs and their delivery systems for gene-based therapy. *Med. Res. Rev.* **2018**, *38* (3), 829−869.

(8) Hendel, A.; Bak, R. O.; Clark, J. T.; Kennedy, A. B.; Ryan, D. E.; Roy, S.; Steinfeld, I.; Lunstad, B. D.; Kaiser, R. J.; Wilkens, A. B.; Bacchetta, R.; Tsalenko, A.; Dellinger, D.; Bruhn, L.; Porteus, M. H. Chemically modified guide RNAs enhance CRISPR-Cas genome editing in human primary cells. *Nat. Biotechnol.* **2015**, *33* (9), 985−989.

(9) Chen, K.; Zhao, B. S.; He, C. Nucleic Acid Modifications in Regulation of Gene Expression. *Cell Chem. Biol.* **2016**, *23* (1), 74−85.

(10) Khvorova, A.; Watts, J. K. The chemical evolution of oligonucleotide therapies of clinical utility. *Nat. Biotechnol.* **2017**, *35* (3), 238−248.

(11) Kuwahara, M.; Sugimoto, N. Molecular evolution of functional nucleic acids with chemical modifications. *Molecules* **2010**, *15* (8), 5423−44.

(12) Mayer, G. The chemical biology of aptamers. *Angew. Chem., Int. Ed.* **2009**, *48* (15), 2672−89.

(13) E. Wang, R.; Wu, H.; Niu, Y.; Cai, J. Improving the stability of aptamers by chemical modification. *Curr. Med. Chem.* **2011**, *18* (27), 4126−38.

(14) Ngo, T. T.; Yoo, J.; Dai, Q.; Zhang, Q.; He, C.; Aksimentiev, A.; Ha, T. Effects of cytosine modifications on DNA flexibility and nucleosome mechanical stability. *Nat. Commun.* **2016**, *7*, 10813.

(15) Park, J. H.; Yang, S. W.; Park, J. M.; Ka, S. H.; Kim, J. H.; Kong, Y. Y.; Jeon, Y. J.; Seol, J. H.; Chung, C. H. Positive feedback regulation of p53 transactivity by DNA damage-induced ISG15 modification. *Nat. Commun.* **2016**, *7*, 12513.

(16) Tosun, G.; Muratoglu, M. The drilling of an Al/SiCp metal-matrix composites. Part I: microstructure. *Compos. Sci. Technol.* **2004**, *64* (2), 299−308.

(17) Bugaut, A.; Toulm, J.-J.; Rayner, B. SELEX and dynamic combinatorial chemistry interplay for the selection of conjugated RNA aptamers. *Org. Biomol. Chem.* **2006**, *4* (22), 4082.

(18) Xie, Q.; Wu, T. P.; Gimple, R. C.; Li, Z.; Prager, B. C.; Wu, Q.; Yu, Y.; Wang, P.; Wang, Y.; Gorkin, D. U.; Zhang, C.; Dowiak, A. V.; Lin, K.; Zeng, C.; Sui, Y.; Kim, L. J. Y.; Miller, T. E.; Jiang, L.; Lee, C. H.; Huang, Z.; Fang, X.; Zhai, K.; Mack, S. C.; Sander, M.; Bao, S.; Kerstetter-Fogle, A. E.; Sloan, A. E.; Xiao, A. Z.; Rich, J. N. N(6)-methyladenine DNA Modification in Glioblastoma. *Cell* **2018**, *175* (5), 1228−1243.

(19) Yang, X.; Bassett, S. E.; Li, X.; Luxon, B. A.; Herzog, N. K.; Shope, R. E.; Aronson, J.; Prow, T. W.; Leary, J. F.; Kirby, R. Construction and selection of bead-bound combinatorial oligonucleoside phosphorothioate and phosphorodithioate aptamer libraries designed for rapid PCR-based sequencing. *Nucleic Acids Res.* **2002**, *30* (23), 132e.

(20) Gallo, M.; Montserrat, J.; Iribarren, A. Design and applications of modified oligonucleotides. *Braz. J. Med. Biol. Res.* **2003**, *36* (2), 143−151.

(21) Ferreira, C. S.; Missailidis, S. Aptamer-based therapeutics and their potential in radiopharmaceutical design. *Braz. Arch. Biol. Technol.* **2007**, *50* (SPE), 63−76.

(22) Lee, J.; Lee, K. H.; Jeon, J.; Dragulescu-Andrasi, A.; Xiao, F.; Rao, J. Combining SELEX Screening and Rational Design to Develop

Light-Up Fluorophore− RNA Aptamer Pairs for RNA Tagging. *ACS Chem. Biol.* **2010**, *5* (11), 1065−1074.
(23) Huang, R.; Xi, Z.; He, N. Applications of aptamers for chemistry analysis, medicine and food security. *Sci. China: Chem.* **2015**, *58* (7), 1122−1130.
(24) Banerjee, J.; Nilsen-Hamilton, M. Aptamers: multifunctional molecules for biomedical research. *J. Mol. Med.* **2013**, *91* (12), 1333−42.
(25) Avino, A.; Fabrega, C.; Tintore, M.; Eritja, R. Thrombin binding aptamer, more than a simple aptamer: chemically modified derivatives and biomedical applications. *Curr. Pharm. Des.* **2012**, *18* (14), 2036−2047.
(26) Sun, H.; Zu, Y. A Highlight of Recent Advances in Aptamer Technology and Its Application. *Molecules* **2015**, *20* (7), 11959−80.
(27) Blind, M.; Blank, M. Aptamer Selection Technology and Recent Advances. *Mol. Ther.–Nucleic Acids* **2015**, *4*, e223.
(28) Gawande, B. N.; Rohloff, J. C.; Carter, J. D.; von Carlowitz, I.; Zhang, C.; Schneider, D. J.; Janjic, N. Selection of DNA aptamers with two modified bases. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114* (11), 2898−2903.
(29) Zhu, G.; Niu, G.; Chen, X. Aptamer-Drug Conjugates. *Bioconjugate Chem.* **2015**, *26* (11), 2186−97.
(30) Tan, W.; Donovan, M. J.; Jiang, J. Aptamers from cell-based selection for bioanalytical applications. *Chem. Rev.* **2013**, *113* (4), 2842−62.
(31) Zhao, Y.; Xu, D.; Tan, W. Aptamer-functionalized nano/micro-materials for clinical diagnosis: isolation, release and bioanalysis of circulating tumor cells. *Integr. Biol.* **2017**, *9* (3), 188−205.
(32) Li, J.; Mo, L.; Lu, C. H.; Fu, T.; Yang, H. H.; Tan, W. Functional nucleic acid-based hydrogels for bioanalytical and biomedical applications. *Chem. Soc. Rev.* **2016**, *45* (5), 1410.
(33) Zhou, J.; Rossi, J. Aptamers as targeted therapeutics: current potential and challenges. *Nat. Rev. Drug Discovery* **2017**, *16* (3), 181.
(34) Sassanfar, M.; Szostak, J. W. An RNA motif that binds ATP. *Nature* **1993**, *364* (6437), 550−553.
(35) Orbach, R.; Willner, B.; Willner, I. Catalytic nucleic acids (DNAzymes) as functional units for logic gates and computing circuits: from basic principles to practical applications. *Chem. Commun.* **2015**, *51* (20), 4144−60.
(36) Sprangers, A. J.; Hao, L.; Banga, R. J.; Mirkin, C. A. Liposomal Spherical Nucleic Acids for Regulating Long Noncoding RNAs in the Nucleus. *Small* **2017**, *13* (10), 1602753.
(37) Kwon, P. S.; Ren, S.; Kwon, S.-J.; Kizer, M. E.; Kuo, L.; Xie, M.; Zhu, D.; Zhou, F.; Zhang, F.; Kim, D.; et al. Designer DNA architecture offers precise and multivalent spatial pattern-recognition for viral sensing and inhibition. *Nat. Chem.* **2020**, *12* (1), 26−35.
(38) Dehghani, S.; Nosrati, R.; Yousefi, M.; Nezami, A.; Soltani, F.; Taghdisi, S. M.; Abnous, K.; Alibolandi, M.; Ramezani, M. Aptamer-based biosensors and nanosensors for the detection of vascular endothelial growth factor (VEGF): A review. *Biosens. Bioelectron.* **2018**, *110*, 23−37.
(39) Meng, H. M.; Liu, H.; Kuai, H.; Peng, R.; Mo, L.; Zhang, X. B. Aptamer-integrated DNA nanostructures for biosensing, bioimaging and cancer therapy. *Chem. Soc. Rev.* **2016**, *45* (9), 2583−602.
(40) Jiang, Y.; Shi, M.; Liu, Y.; Wan, S.; Cui, C.; Zhang, L.; Tan, W. Aptamer/AuNP Biosensor for Colorimetric Profiling of Exosomal Proteins. *Angew. Chem., Int. Ed.* **2017**, *56* (39), 11916−11920.
(41) Song, P.; Ye, D.; Zuo, X.; Li, J.; Wang, J.; Liu, H.; Hwang, M. T.; Chao, J.; Su, S.; Wang, L.; et al. DNA hydrogel with aptamer-toehold-based recognition, cloaking, and decloaking of circulating tumor cells for live cell analysis. *Nano Lett.* **2017**, *17* (9), 5193−8.
(42) Gao, S.; Zheng, X.; Jiao, B.; Wang, L. Post-SELEX optimization of aptamers. *Anal. Bioanal. Chem.* **2016**, *408* (17), 4567−4573.
(43) Quang, N. N.; Miodek, A.; Cibiel, A.; Ducongé, F. Selection of Aptamers Against Whole Living Cells: From Cell-SELEX to Identification of Biomarkers. *Methods Mol. Biol.* **2017**, *1575*, 253−272.
(44) Wu, Y. X.; Kwon, Y. J. Aptamers: The "evolution" of SELEX. *Methods* **2016**, *106*, 21−28.
(45) Kusser, W. Chemically modified nucleic acid aptamers for in vitro selections: evolving evolution. *Rev. Mol. Biotechnol.* **2000**, *74* (1), 27−38.
(46) Elenko, M. P.; Szostak, J. W.; van Oijen, A. M. Single-molecule imaging of an in vitro-evolved RNA aptamer reveals homogeneous ligand binding kinetics. *J. Am. Chem. Soc.* **2009**, *131* (29), 9866−9867.
(47) Tan, C.-K.; Castillo, C.; So, A.; Downey, K. An auxiliary protein for DNA polymerase-delta from fetal calf thymus. *J. Biol. Chem.* **1986**, *261* (26), 12310−6.
(48) Alberts, B. DNA replication and recombination. *Nature* **2003**, *421* (6921), 431−435.
(49) Minagawa, H.; Onodera, K.; Fujita, H.; Sakamoto, T.; Akitomi, J.; Kaneko, N.; Shiratori, I.; Kuwahara, M.; Horii, K.; Waga, I. Selection, Characterization and Application of Artificial DNA Aptamer Containing Appended Bases with Sub-nanomolar Affinity for a Salivary Biomarker. *Sci. Rep.* **2017**, *7*, 42716.
(50) Pfeiffer, F.; Rosenthal, M.; Siegl, J.; Ewers, J.; Mayer, G. Customised nucleic acid libraries for enhanced aptamer selection and performance. *Curr. Opin. Biotechnol.* **2017**, *48*, 111−118.
(51) Zhang, L.; Yang, Z.; Le Trinh, T.; Teng, I. T.; Wang, S.; Bradley, K. M.; Hoshika, S.; Wu, Q.; Cansiz, S.; Rowold, D. J.; McLendon, C.; Kim, M. S.; Wu, Y.; Cui, C.; Liu, Y.; Hou, W.; Stewart, K.; Wan, S.; Liu, C.; Benner, S. A.; Tan, W. Aptamers against Cells Overexpressing Glypican 3 from Expanded Genetic Systems Combined with Cell Engineering and Laboratory Evolution. *Angew. Chem., Int. Ed.* **2016**, *55* (40), 12372−5.
(52) Wang, X.; Hoshika, S.; Peterson, R. J.; Kim, M. J.; Benner, S. A.; Kahn, J. D. Biophysics of Artificially Expanded Genetic Information Systems. Thermodynamics of DNA Duplexes Containing Matches and Mismatches Involving 2-Amino-3-nitropyridin-6-one (Z) and Imidazo[1,2-a]-1,3,5-triazin-4(8H)one (P). *ACS Synth. Biol.* **2017**, *6* (5), 782−792.
(53) Kimoto, M.; Yamashige, R.; Matsunaga, K.; Yokoyama, S.; Hirao, I. Generation of high-affinity DNA aptamers using an expanded genetic alphabet. *Nat. Biotechnol.* **2013**, *31* (5), 453−7.
(54) Seok Kim, Y.; Ahmad Raston, N. H.; Bock Gu, M. Aptamer-based nanobiosensors. *Biosens. Bioelectron.* **2016**, *76*, 2−19.
(55) Abbaspour, A.; Norouz-Sarvestani, F.; Noori, A.; Soltani, N. Aptamer-conjugated silver nanoparticles for electrochemical dual-aptamer-based sandwich detection of staphylococcus aureus. *Biosens. Bioelectron.* **2015**, *68*, 149−155.
(56) Meek, K. N.; Rangel, A. E.; Heemstra, J. M. Enhancing aptamer function and stability via in vitro selection using modified nucleic acids. *Methods* **2016**, *106*, 29−36.
(57) Lei, Y.; Qiao, Z.; Tang, J.; He, X.; Shi, H.; Ye, X.; Yan, L.; He, D.; Wang, K. DNA nanotriangle-scaffolded activatable aptamer probe with ultralow background and robust stability for cancer theranostics. *Theranostics* **2018**, *8* (15), 4062−4071.
(58) Li, K.; Deng, J.; Jin, H.; Yang, X.; Fan, X.; Li, L.; Zhao, Y.; Guan, Z.; Wu, Y.; Zhang, L.; Yang, Z. Chemical modification improves the stability of the DNA aptamer GBI-10 and its affinity towards tenascin-C. *Org. Biomol. Chem.* **2017**, *15* (5), 1174−1182.
(59) Shahdordizadeh, M.; Yazdian-Robati, R.; Ramezani, M.; Abnous, K.; Taghdisi, S. M. Aptamer application in targeted delivery systems for diagnosis and treatment of breast cancer. *J. Mater. Chem. B* **2016**, *4* (48), 7766−7778.
(60) Robati, R. Y.; Arab, A.; Ramezani, M.; Langroodi, F. A.; Abnous, K.; Taghdisi, S. M. Aptasensors for quantitative detection of kanamycin. *Biosens. Bioelectron.* **2016**, *82*, 162−72.
(61) Eissa, S.; Siaj, M.; Zourob, M. Aptamer-based competitive electrochemical biosensor for brevetoxin-2. *Biosens. Bioelectron.* **2015**, *69*, 148−54.
(62) Lian, Y.; He, F.; Wang, H.; Tong, F. A new aptamer/graphene interdigitated gold electrode piezoelectric sensor for rapid and specific detection of Staphylococcus aureus. *Biosens. Bioelectron.* **2015**, *65*, 314−9.
(63) Zhou, W.; Huang, P. J.; Ding, J.; Liu, J. Aptamer-based biosensors for biomedical diagnostics. *Analyst* **2014**, *139* (11), 2627−40.

https://dx.doi.org/10.1021/acsabm.0c00062
ACS Appl. Bio Mater. 2020, 3, 2816−2826

(64) Hianik, T.; Ostatna, V.; Sonlajtnerova, M.; Grman, I. Influence of ionic strength, pH and aptamer configuration for binding affinity to thrombin. *Bioelectrochemistry* **2007**, *70* (1), 127−33.
(65) Huang, L.; Yang, X.; Qi, C.; Niu, X.; Zhao, C.; Zhao, X.; Shangguan, D.; Yang, Y. A label-free electrochemical biosensor based on a DNA aptamer against codeine. *Anal. Chim. Acta* **2013**, *787*, 203−10.
(66) Tolle, F.; Brandle, G. M.; Matzner, D.; Mayer, G. A Versatile Approach Towards Nucleobase-Modified Aptamers. *Angew. Chem., Int. Ed.* **2015**, *54* (37), 10971−4.
(67) Ames, T. D.; Breaker, R. R. Bacterial aptamers that selectively bind glutamine. *RNA Biol.* **2011**, *8* (1), 82−9.
(68) Levay, A.; Brenneman, R.; Hoinka, J.; Sant, D.; Cardone, M.; Trinchieri, G.; Przytycka, T. M.; Berezhnoy, A. Identifying high-affinity aptamer ligands with defined cross-reactivity using high-throughput guided systematic evolution of ligands by exponential enrichment. *Nucleic Acids Res.* **2015**, *43* (12), e82.
(69) Golubnitschaja, O.; Flammer, J. What are the biomarkers for glaucoma? *Surv. Ophthalmol.* **2007**, *52* (6), S155−61.
(70) Wu, L.; Qu, X. Cancer biomarker detection: recent achievements and challenges. *Chem. Soc. Rev.* **2015**, *44* (10), 2963−97.
(71) Li, Y.; Zhang, Z.; Liu, B.; Liu, J. Incorporation of Boronic Acid into Aptamer-Based Molecularly Imprinted Hydrogels for Highly Specific Recognition of Adenosine. *ACS Applied Bio. Materials.* **2019**, DOI: 10.1021/acsabm.9b00936.
(72) Wu, Q.; Wu, L.; Wang, Y.; Zhu, Z.; Song, Y.; Tan, Y.; Wang, X. F.; Li, J.; Kang, D.; Yang, C. J. Evolution of DNA aptamers for malignant brain tumor gliosarcoma cell recognition and clinical tissue imaging. *Biosens. Bioelectron.* **2016**, *80*, 1−8.
(73) Li, D.; Liang, L.; Tang, Y.; Fu, L.; Xiao, S.; Yuan, Q. Direct and single-step sensing of primary ovarian cancers related glycosidases. *Chin. Chem. Lett.* **2019**, *30* (5), 1013−1016.
(74) Borrmann, A.; van Hest, J. C. M. Bioorthogonal chemistry in living organisms. *Chem. Sci.* **2014**, *5* (6), 2123.
(75) Scarr, G. Simple geometry in complex organisms. *J. Bodyw. Mov. Ther.* **2010**, *14* (4), 424−44.
(76) Sheng, P.; Yang, Z.; Kim, Y.; Wu, Y.; Tan, W.; Benner, S. A. Design of a novel molecular beacon: modification of the stem with artificially genetic alphabet. *Chem. Commun.* **2008**, No. 41, 5128−30.
(77) Sun, W.; Du, L.; Li, M. Aptamer-based carbohydrate recognition. *Curr. Pharm. Des.* **2010**, *16* (20), 2269−2278.
(78) Gopinath, S. C. Methods developed for SELEX. *Anal. Bioanal. Chem.* **2006**, *387* (1), 171−82.
(79) Qing, Z.; Xu, J.; Hu, J.; Zheng, J.; He, L.; Zou, Z.; Yang, S.; Tan, W.; Yang, R. In Situ Amplification-Based Imaging of RNA in Living Cells. *Angew. Chem., Int. Ed.* **2019**, *58* (34), 11574−11585.
(80) Lin, Q.; Li, Z.; Yuan, Q. Recent advances in autofluorescence-free biosensing and bioimaging based on persistent luminescence nanoparticles. *Chin. Chem. Lett.* **2019**, *30* (9), 1547−1556.
(81) Liu, M.; Li, Z.; Li, Y.; Chen, J.; Yuan, Q. Self-assembled nanozyme complexes with enhanced cascade activity and high stability for colorimetric detection of glucose. *Chin. Chem. Lett.* **2019**, *30* (5), 1009−1012.
(82) Lao, Y.-H.; Phua, K. K.; Leong, K. W. Aptamer nanomedicine for cancer therapeutics: barriers and potential for translation. *ACS Nano* **2015**, *9* (3), 2235−2254.
(83) Kong, H. Y.; Byun, J. Nucleic Acid aptamers: new methods for selection, stabilization, and application in biomedical science. *Biomol. Ther.* **2013**, *21* (6), 423−34.
(84) Grytz, C. M.; Marko, A.; Cekan, P.; Sigurdsson, S. T.; Prisner, T. F. Flexibility and conformation of the cocaine aptamer studied by PELDOR. *Phys. Chem. Chem. Phys.* **2016**, *18* (4), 2993−3002.
(85) Patel, D. J. Structural analysis of nucleic acid aptamers. *Curr. Opin. Chem. Biol.* **1997**, *1* (1), 32−46.
(86) Liu, J.; Wei, T.; Zhao, J.; Huang, Y.; Deng, H.; Kumar, A.; Wang, C.; Liang, Z.; Ma, X.; Liang, X. J. Multifunctional aptamer-based nanoparticles for targeted drug delivery to circumvent cancer resistance. *Biomaterials* **2016**, *91*, 44−56.
(87) Stoltenburg, R.; Krafcikova, P.; Viglasky, V.; Strehlitz, B. G-quadruplex aptamer targeting Protein A and its capability to detect Staphylococcus aureus demonstrated by ELONA. *Sci. Rep.* **2016**, *6*, 33812.
(88) Zhang, P.; Zhao, Z.; Li, C.; Su, H.; Wu, Y.; Kwok, R. T. K.; Lam, J. W. Y.; Gong, P.; Cai, L.; Tang, B. Z. Aptamer-Decorated Self-Assembled Aggregation-Induced Emission Organic Dots for Cancer Cell Targeting and Imaging. *Anal. Chem.* **2018**, *90* (2), 1063−1067.
(89) Ulrich, H.; Martins, A. H.; Pesquero, J. B. RNA and DNA aptamers in cytomics analysis. *Cytometry* **2004**, *59A* (2), 220−31.
(90) He, L.; Deen, B. D.; Pagel, A. H.; Diez-Gonzalez, F.; Labuza, T. P. Concentration, detection and discrimination of Bacillus anthracis spores in orange juice using aptamer based surface enhanced Raman spectroscopy. *Analyst* **2013**, *138* (6), 1657−9.
(91) Biondi, E.; Lane, J. D.; Das, D.; Dasgupta, S.; Piccirilli, J. A.; Hoshika, S.; Bradley, K. M.; Krantz, B. A.; Benner, S. A. Laboratory evolution of artificially expanded DNA gives redesignable aptamers that target the toxic form of anthrax protective antigen. *Nucleic Acids Res.* **2016**, *44* (20), 9565−77.
(92) Rodriguez-Sinovas, A.; Ruiz-Meana, M.; Denuc, A.; Garcia-Dorado, D. Mitochondrial Cx43, an important component of cardiac preconditioning. *Biochim. Biophys. Acta, Biomembr.* **2018**, *1860* (1), 174−181.
(93) Drommer, J.; Schneider, H.; Alteheld, B.; Stehle, P.; Meier, R. Protein is an important component of nutritional support predicting complications in malnourished hospitalised patients - Details of our previous randomised controlled trial (RCT). *Clin. Nutr. ESPEN.* **2015**, *10* (3), e124−e128.
(94) Hol, E. M.; Pekny, M. Glial fibrillary acidic protein (GFAP) and the astrocyte intermediate filament system in diseases of the central nervous system. *Curr. Opin. Cell Biol.* **2015**, *32*, 121−30.
(95) Tzeng, S. R.; Kalodimos, C. G. Protein activity regulation by conformational entropy. *Nature* **2012**, *488* (7410), 236−40.
(96) Kuenzi, B. M.; Remsing Rix, L. L.; Stewart, P. A.; Fang, B.; Kinose, F.; Bryant, A. T.; Boyle, T. A.; Koomen, J. M.; Haura, E. B.; Rix, U. Polypharmacology-based ceritinib repurposing using integrated functional proteomics. *Nat. Chem. Biol.* **2017**, *13* (12), 1222−1231.
(97) Fan, Y. F.; Zhang, W.; Zeng, L.; Lei, Z. N.; Cai, C. Y.; Gupta, P.; Yang, D. H.; Cui, Q.; Qin, Z. D.; Chen, Z. S.; Trombetta, L. D. Dacomitinib antagonizes multidrug resistance (MDR) in cancer cells by inhibiting the efflux activity of ABCB1 and ABCG2 transporters. *Cancer Lett.* **2018**, *421*, 186−198.
(98) Zhang, J.; Jiang, X.; Jiang, Y.; Guo, M.; Zhang, S.; Li, J.; He, J.; Liu, J.; Wang, J.; Ouyang, L. Recent advances in the development of dual VEGFR and c-Met small molecule inhibitors as anticancer drugs. *Eur. J. Med. Chem.* **2016**, *108*, 495−504.
(99) Tan, J.; Zhao, M.; Wang, J.; Li, Z.; Liang, L.; Zhang, L.; Yuan, Q.; Tan, W. Regulation of Protein Activity and Cellular Functions Mediated by Molecularly Evolved Nucleic Acids. *Angew. Chem., Int. Ed.* **2019**, *58* (6), 1621−1625.
(100) Harcourt, E. M.; Kietrys, A. M.; Kool, E. T. Chemical and structural effects of base modifications in messenger RNA. *Nature* **2017**, *541* (7637), 339−346.
(101) Wang, J.; Wei, Y.; Hu, X.; Fang, Y. Y.; Li, X.; Liu, J.; Wang, S.; Yuan, Q. Protein Activity Regulation: Inhibition by Closed-Loop Aptamer-Based Structures and Restoration by Near-IR Stimulation. *J. Am. Chem. Soc.* **2015**, *137* (33), 10576−84.
(102) Borbone, N.; Bucci, M.; Oliviero, G.; Morelli, E.; Amato, J.; D'Atri, V.; D'Errico, S.; Vellecco, V.; Cirino, G.; Piccialli, G.; Fattorusso, C.; Varra, M.; Mayol, L.; Persico, M.; Scuotto, M. Investigating the role of T7 and T12 residues on the biological properties of thrombin-binding aptamer: enhancement of anticoagulant activity by a single nucleobase modification. *J. Med. Chem.* **2012**, *55* (23), 10716−28.
(103) Miller, K. D.; Siegel, R. L.; Lin, C. C.; Mariotto, A. B.; Kramer, J. L.; Rowland, J. H.; Stein, K. D.; Alteri, R.; Jemal, A. Cancer treatment and survivorship statistics, 2016. *Ca-Cancer J. Clin.* **2016**, *66* (4), 271−89.

https://dx.doi.org/10.1021/acsabm.0c00062
ACS Appl. Bio Mater. 2020, 3, 2816−2826

(104) Siegel, R. L.; Miller, K. D.; Jemal, A. Cancer statistics, 2016. *Ca-Cancer J. Clin.* **2016**, *66* (1), 7−30.
(105) Liu, Z.; Zhao, H.; He, L.; Yao, Y.; Zhou, Y.; Wu, J.; Liu, J.; Ding, J. Aptamer density dependent cellular uptake of lipid-capped polymer nanoparticles for polyvalent targeted delivery of vinorelbine to cancer cells. *RSC Adv.* **2015**, *5* (22), 16931−16939.
(106) Maeda, H. Macromolecular therapeutics in cancer treatment: the EPR effect and beyond. *J. Controlled Release* **2012**, *164* (2), 138−44.
(107) Danhier, F.; Préat, V. Strategies to improve the EPR effect for the delivery of anti-cancer nanomedicines. *Cancer Cell Microenviron.* **2015**, *2* (3), e808.
(108) Wei, T.; Chen, C.; Liu, J.; Liu, C.; Posocco, P.; Liu, X.; Cheng, Q.; Huo, S.; Liang, Z.; Fermeglia, M.; et al. Anticancer drug nanomicelles formed by self-assembling amphiphilic dendrimer to combat cancer drug resistance. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112* (10), 2978−83.
(109) Money, S.; Garber, B. Management of Cancer Pain. *Curr. Emerg. Hosp. Med. Rep.* **2018**, *6* (4), 141−146.
(110) Zabernigg, A.; Gamper, E. M.; Giesinger, J. M.; Rumpold, G.; Kemmler, G.; Gattringer, K.; Sperner-Unterweger, B.; Holzner, B. Taste alterations in cancer patients receiving chemotherapy: a neglected side effect? *Oncologist* **2010**, *15* (8), 913−20.
(111) Georg, P.; Potter, R.; Georg, D.; Lang, S.; Dimopoulos, J. C.; Sturdza, A. E.; Berger, D.; Kirisits, C.; Dorr, W. Dose effect relationship for late side effects of the rectum and urinary bladder in magnetic resonance image-guided adaptive cervix cancer brachytherapy. *Int. J. Radiat. Oncol., Biol., Phys.* **2012**, *82* (2), 653−7.
(112) Minotti, G.; Menna, P.; Salvatorelli, E.; Cairo, G.; Gianni, L. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. *Pharmacol. Rev.* **2004**, *56* (2), 185−229.
(113) Lyu, Y.; Chen, G.; Shangguan, D.; Zhang, L.; Wan, S.; Wu, Y.; Zhang, H.; Duan, L.; Liu, C.; You, M.; Wang, J.; Tan, W. Generating Cell Targeting Aptamers for Nanotheranostics Using Cell-SELEX. *Theranostics* **2016**, *6* (9), 1440−52.
(114) Wang, R.; Zhu, G.; Mei, L.; Xie, Y.; Ma, H.; Ye, M.; Qing, F.-L.; Tan, W. Automated Modular Synthesis of Aptamer−Drug Conjugates for Targeted Drug Delivery. *J. Am. Chem. Soc.* **2014**, *136* (7), 2731−2734.
(115) Tang, J.; Xie, J.; Shao, N.; Yan, Y. The DNA aptamers that specifically recognize ricin toxin are selected by two in vitro selection methods. *Electrophoresis* **2006**, *27* (7), 1303−11.
(116) Shangguan, D.; Meng, L.; Cao, Z. C.; Xiao, Z.; Fang, X.; Li, Y.; Cardona, D.; Witek, R. P.; Liu, C.; Tan, W. Identification of liver cancer-specific aptamers using whole live cells. *Anal. Chem.* **2008**, *80* (3), 721−728.
(117) Tan, W.; Wang, H.; Chen, Y.; Zhang, X.; Zhu, H.; Yang, C.; Yang, R.; Liu, C. Molecular aptamers for drug delivery. *Trends Biotechnol.* **2011**, *29* (12), 634−40.
(118) Huang, Y. F.; Shangguan, D.; Liu, H.; Phillips, J. A.; Zhang, X.; Chen, Y.; Tan, W. Molecular assembly of an aptamer-drug conjugate for targeted drug delivery to tumor cells. *ChemBioChem* **2009**, *10* (5), 862−8.
(119) Shieh, Y.-A.; Yang, S.-J.; Wei, M.-F.; Shieh, M.-J. Aptamer-based tumor-targeted drug delivery for photodynamic therapy. *ACS Nano* **2010**, *4* (3), 1433−1442.
(120) Gyi, J. I.; Gao, D.; Conn, G. L.; Trent, J. O.; Brown, T.; Lane, A. N. The solution structure of a DNA*RNA duplex containing 5-propynyl U and C; comparison with 5-Me modifications. *Nucleic Acids Res.* **2003**, *31* (10), 2683−93.
(121) Liu, B.; Li, D. Structural transformation induced by locked nucleic acid or 2′−O-methyl nucleic acid site-specific modifications on thrombin binding aptamer. *Chem. Cent. J.* **2014**, *8* (1), 19.
(122) Girvan, A. C.; Teng, Y.; Casson, L. K.; Thomas, S. D.; Juliger, S.; Ball, M. W.; Klein, J. B.; Pierce, W. M. Jr; Barve, S. S.; Bates, P. J. AGRO100 inhibits activation of nuclear factor-kappaB (NF-kappaB) by forming a complex with NF-kappaB essential modulator (NEMO) and nucleolin. *Mol. Cancer Ther.* **2006**, *5* (7), 1790−9.
(123) Bates, P. J.; Laber, D. A.; Miller, D. M.; Thomas, S. D.; Trent, J. O. Discovery and development of the G-rich oligonucleotide AS1411 as a novel treatment for cancer. *Exp. Mol. Pathol.* **2009**, *86* (3), 151−64.
(124) Fan, X.; Sun, L.; Wu, Y.; Zhang, L.; Yang, Z. Bioactivity of 2′-deoxyinosine-incorporated aptamer AS1411. *Sci. Rep.* **2016**, *6*, 25799.
(125) Matsunaga, K.; Kimoto, M.; Hanson, C.; Sanford, M.; Young, H. A.; Hirao, I. Architecture of high-affinity unnatural-base DNA aptamers toward pharmaceutical applications. *Sci. Rep.* **2016**, *5*, 18478.
(126) Gupta, S.; Hirota, M.; Waugh, S. M.; Murakami, I.; Suzuki, T.; Muraguchi, M.; Shibamori, M.; Ishikawa, Y.; Jarvis, T. C.; Carter, J. D.; Zhang, C.; Gawande, B.; Vrkljan, M.; Janjic, N.; Schneider, D. J. Chemically modified DNA aptamers bind interleukin-6 with high affinity and inhibit signaling by blocking its interaction with interleukin-6 receptor. *J. Biol. Chem.* **2014**, *289* (12), 8706−19.
(127) Zhu, G.; Cansiz, S.; You, M.; Qiu, L.; Han, D.; Zhang, L.; Mei, L.; Fu, T.; Chen, Z.; Tan, W. Nuclease-resistant synthetic drug-DNA adducts: programmable drug-DNA conjugation for targeted anti-cancer drug delivery. *NPG Asia Mater.* **2015**, *7* (3), e169−e169.
(128) Ray, P.; White, R. R. Aptamers for Targeted Drug Delivery. *Pharmaceuticals* **2010**, *3* (6), 1761−1778.
(129) Song, X.; Ren, Y.; Zhang, J.; Wang, G.; Han, X.; Zheng, W.; Zhen, L. Targeted delivery of doxorubicin to breast cancer cells by aptamer functionalized DOTAP/DOPE liposomes. *Oncol. Rep.* **2015**, *34* (4), 1953−60.
(130) Liu, Y.; Miyoshi, H.; Nakamura, M. Nanomedicine for drug delivery and imaging: a promising avenue for cancer therapy and diagnosis using targeted functional nanoparticles. *Int. J. Cancer* **2007**, *120* (12), 2527−37.
(131) Lu, J.; Liong, M.; Li, Z.; Zink, J. I.; Tamanoi, F. Biocompatibility, biodistribution, and drug-delivery efficiency of mesoporous silica nanoparticles for cancer therapy in animals. *Small* **2010**, *6* (16), 1794−805.
(132) Chari, R. V. Targeted cancer therapy: conferring specificity to cytotoxic drugs. *Acc. Chem. Res.* **2008**, *41* (1), 98−107.
(133) Xiao, Z.; Farokhzad, O. C. Aptamer-functionalized nanoparticles for medical applications: challenges and opportunities. *ACS Nano* **2012**, *6* (5), 3670−3676.
(134) Kamaly, N.; Xiao, Z.; Valencia, P. M.; Radovic-Moreno, A. F.; Farokhzad, O. C. Targeted polymeric therapeutic nanoparticles: design, development and clinical translation. *Chem. Soc. Rev.* **2012**, *41* (7), 2971−3010.
(135) Guo, J.; Gao, X.; Su, L.; Xia, H.; Gu, G.; Pang, Z.; Jiang, X.; Yao, L.; Chen, J.; Chen, H. Aptamer-functionalized PEG-PLGA nanoparticles for enhanced anti-glioma drug delivery. *Biomaterials* **2011**, *32* (31), 8010−20.
(136) Alibolandi, M.; Ramezani, M.; Abnous, K.; Sadeghi, F.; Atyabi, F.; Asouri, M.; Ahmadi, A. A.; Hadizadeh, F. In vitro and in vivo evaluation of therapy targeting epithelial-cell adhesion-molecule aptamers for non-small cell lung cancer. *J. Controlled Release* **2015**, *209*, 88−100.
(137) Tan, J.; Li, H.; Hu, X.; Abdullah, R.; Xie, S.; Zhang, L.; Zhao, M.; Luo, Q.; Li, Y.; Sun, Z.; Yuan, Q.; Tan, W. Size-Tunable Assemblies Based on Ferrocene-Containing DNA Polymers for Spatially Uniform Penetration. *Chem.* **2019**, *5* (7), 1775−1792.
(138) Wong, C.; Stylianopoulos, T.; Cui, J.; Martin, J.; Chauhan, V. P.; Jiang, W.; Popović, Z.; Jain, R. K.; Bawendi, M. G.; Fukumura, D. Multistage nanoparticle delivery system for deep penetration into tumor tissue. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108* (6), 2426−2431.
(139) Nishiyama, N.; Matsumura, Y.; Kataoka, K. Development of polymeric micelles for targeting intractable cancers. *Cancer. Sci.* **2016**, *107* (7), 867−74.
(140) Fuse, T.; Tagami, T.; Tane, M.; Ozeki, T. Effective light-triggered contents release from helper lipid-incorporated liposomes co-encapsulating gemcitabine and a water-soluble photosensitizer. *Int. J. Pharm.* **2018**, *540* (1−2), 50−56.
(141) Zhou, J.; Rossi, J. J. Cell-specific aptamer-mediated targeted drug delivery. *Oligonucleotides* **2011**, *21* (1), 1−10.

https://dx.doi.org/10.1021/acsabm.0c00062
ACS Appl. Bio Mater. 2020, 3, 2816−2826

(142) Kanwar, J. R.; Roy, K.; Kanwar, R. K. Chimeric aptamers in cancer cell-targeted drug delivery. *Crit. Rev. Biochem. Mol. Biol.* **2011**, *46* (6), 459−77.
(143) Wang, R.; Jin, C.; Zhu, X.; Zhou, L.; Xuan, W.; Liu, Y.; Liu, Q.; Tan, W. Artificial Base zT as Functional "Element" for Constructing Photoresponsive DNA Nanomolecules. *J. Am. Chem. Soc.* **2017**, *139* (27), 9104−9107.
(144) Beaucage, S. L.; Iyer, R. P. Advances in the synthesis of oligonucleotides by the phosphoramidite approach. *Tetrahedron* **1992**, *48* (12), 2223−2311.
(145) Zhou, F.; Wang, P.; Peng, Y.; Zhang, P.; Huang, Q.; Sun, W.; He, N.; Fu, T.; Zhao, Z.; Fang, X.; Tan, W. Molecular Engineering-Based Aptamer-Drug Conjugates with Accurate Tunability of Drug Ratios for Drug Combination Targeted Cancer Therapy. *Angew. Chem., Int. Ed.* **2019**, *58* (34), 11661−11665.
(146) Shakeel, S.; Karim, S.; Ali, A. Peptide nucleic acid (PNA) — a review. *J. Chem. Technol. Biotechnol.* **2006**, *81* (6), 892−899.
(147) Sridharan, K.; Gogtay, N. J. Therapeutic nucleic acids: current clinical status. *Br. J. Clin. Pharmacol.* **2016**, *82* (3), 659−72.
(148) Tian, T.; Wang, J.; Zhou, X. A review: microRNA detection methods. *Org. Biomol. Chem.* **2015**, *13* (8), 2226−38.
(149) Hamashima, K.; Kimoto, M.; Hirao, I. Creation of unnatural base pairs for genetic alphabet expansion toward synthetic xenobiology. *Curr. Opin. Chem. Biol.* **2018**, *46*, 108−114.
(150) Lee, K. H.; Hamashima, K.; Kimoto, M.; Hirao, I. Genetic alphabet expansion biotechnology by creating unnatural base pairs. *Curr. Opin. Biotechnol.* **2018**, *51*, 8−15.
(151) Dien, V. T.; Morris, S. E.; Karadeema, R. J.; Romesberg, F. E. Expansion of the genetic code via expansion of the genetic alphabet. *Curr. Opin. Chem. Biol.* **2018**, *46*, 196−202.
(152) Karalkar, N. B.; Benner, S. A. The challenge of synthetic biology. Synthetic Darwinism and the aperiodic crystal structure. *Curr. Opin. Chem. Biol.* **2018**, *46*, 188−195.
(153) Hamashima, K.; Soong, Y. T.; Matsunaga, K. I.; Kimoto, M.; Hirao, I. DNA Sequencing Method Including Unnatural Bases for DNA Aptamer Generation by Genetic Alphabet Expansion. *ACS Synth. Biol.* **2019**, *8* (6), 1401−1410.
(154) Chen, J. W.; Liu, X. P.; Feng, K. J.; Liang, Y.; Jiang, J. H.; Shen, G. L.; Yu, R. Q. Detection of adenosine using surface-enhanced Raman scattering based on structure-switching signaling aptamer. *Biosens. Bioelectron.* **2008**, *24* (1), 66−71.
(155) Wei, H.; Li, B.; Li, J.; Wang, E.; Dong, S. Simple and sensitive aptamer-based colorimetric sensing of protein using unmodified gold nanoparticle probes. *Chem. Commun.* **2007**, No. 36, 3735−7.
(156) Matsunaga, K. I.; Kimoto, M.; Hirao, I. High-Affinity DNA Aptamer Generation Targeting von Willebrand Factor A1-Domain by Genetic Alphabet Expansion for Systematic Evolution of Ligands by Exponential Enrichment Using Two Types of Libraries Composed of Five Different Bases. *J. Am. Chem. Soc.* **2017**, *139* (1), 324−334.
(157) Šmuc, T.; Ahn, I.-Y.; Ulrich, H. Nucleic acid aptamers as high affinity ligands in biotechnology and biosensorics. *J. Pharm. Biomed. Anal.* **2013**, *81*−*82*, 210−217.

https://dx.doi.org/10.1021/acsabm.0c00062
ACS Appl. Bio Mater. 2020, 3, 2816−2826