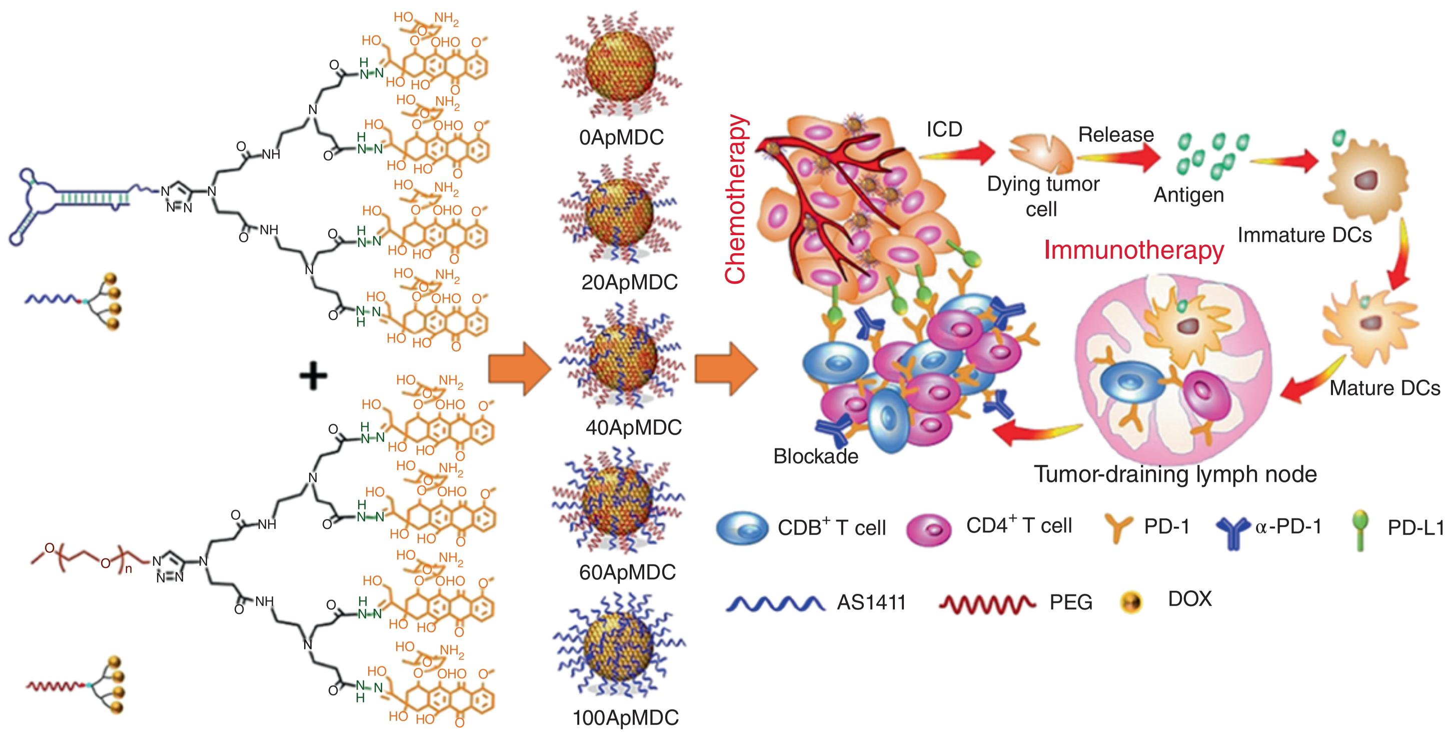

Figure 1.8 Schematic representation of the co-assemblies of a multivalent aptamer drug conjugate (ApMDC) and its PEG-substituted analog into nanomicelles for reinforcing the immunogenic cell death of tumor cells. Source: Reproduced with permission from Geng et al. [102]. © 2021 John Wiley & Sons.

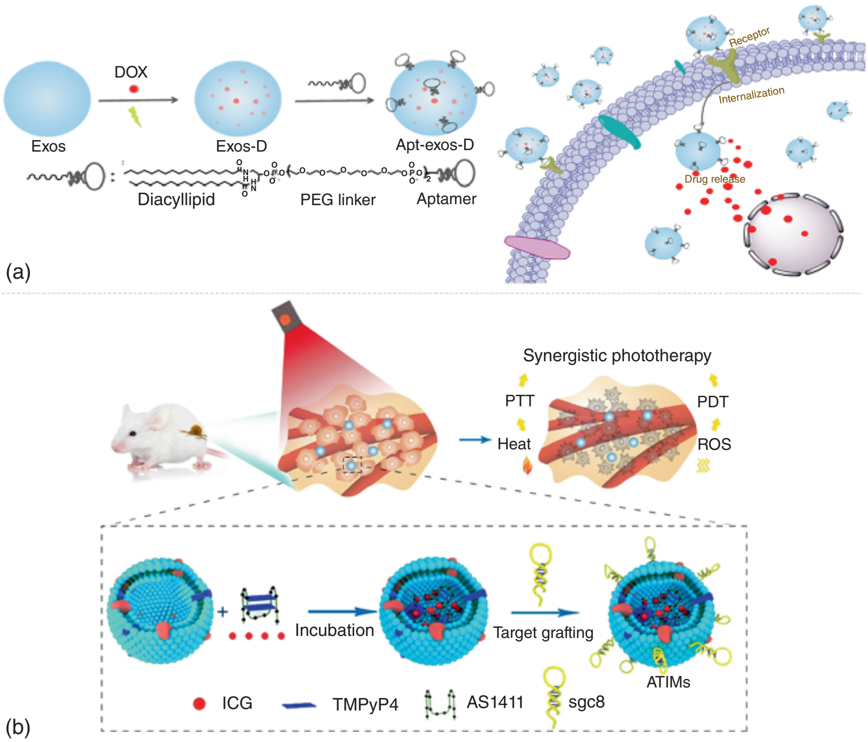

Figure 1.9 Schematic illustration of the design of (a) aptamer-functionalized exosomes (Apt-Exos) and (b) aptamer-cholesterol-modified vesicle for targeting delivery to cancer cells. Source: (a) Reproduced with permission from Zou et al. [106]. 2019 American Chemical Society. (b) Reproduced with permission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

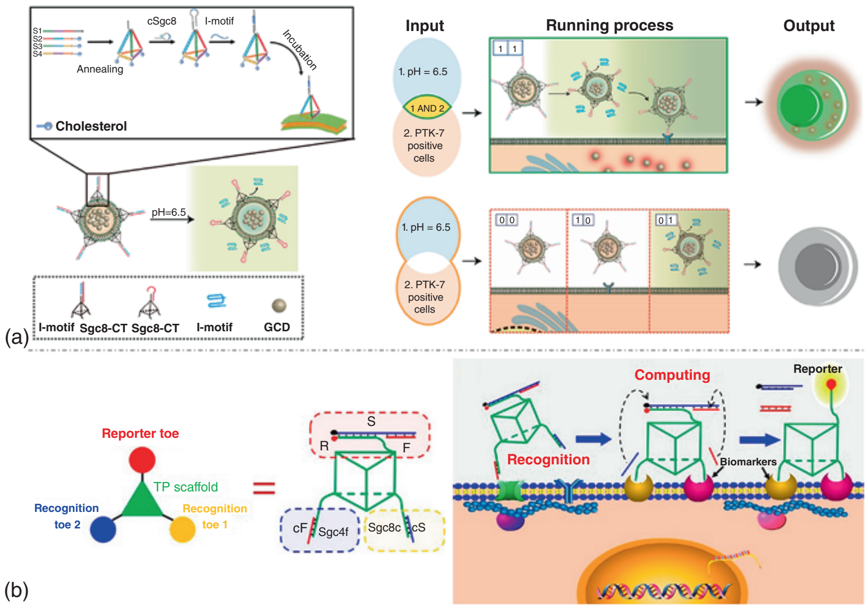

Figure 1.10 Schematic diagram of (a) the design of a logic-gated DNA nanodevice and its targeted GCD delivery process induced by an acidic environment and the overexpression of PTK-7. Source: Reproduced with permission from Huang et al. [110]. 2021 American Chemical Society. (b) A logic gate-guided DNA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