JACS Au
AN OPEN ACCESS JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Perspective

Exploring Framework Nucleic Acids: A Perspective on Their Cellular Applications

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Cite This: *JACS Au* 2024, 4, 4110−4128
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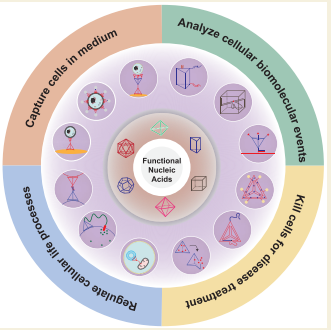
ABSTRACT: Cells are fundamental units of life. The coordination of cellular functions and behaviors relies on a cascade of molecular networks. Technologies that enable exploration and manipulation of specific molecular events in living cells with high spatiotemporal precision would be critical for pathological study, disease diagnosis, and treatment. Framework nucleic acids (FNAs) represent a novel class of nucleic acid materials characterized by their monodisperse and rigid nanostructure. Leveraging their exceptional programmability, convenient modification property, and predictable atomic-level architecture, FNAs have attracted significant attention in diverse cellular applications such as cell recognition, imaging, manipulation, and therapeutic interventions. In this perspective, we will discuss the utilization of FNAs in living cell systems while critically assessing the opportunities and challenges presented in this burgeoning field.



KEYWORDS: *Framework Nucleic Acids, Cell Identification, Cell Imaging, Cell Manipulation, Disease Treatment*

1. INTRODUCTION

Cells are the structural and functional units of life. Abnormal molecular events within cells are linked to the progression of many diseases.[1−5] Analysis and regulation of these cellular processes are crucial for understanding, diagnosing, and treating diseases. However, cells are an exceptionally complex and heterogeneous system.[6−10] Gene and protein expression, along with their post-translational modifications, vary not only between different cell types but also among cells of the same type.[11−14] Additionally, the concentration and distribution of cellular components are highly dynamic, and even minor changes can trigger significantly different cellular responses.[15−17] Moreover, the intricate structure of cells is subdivided into functionally distinct membrane-bound organelles.[18−21] Increasing evidence suggests that identical components can generate entirely different biological effects depending on their subcellular or even suborganellar location.[22−24] In this content, the development of programmable tools and technologies that enable precise cellular studies is highly desired.

As a carrier of genetic information with good biocompatibility,[25] high programmability,[26] and predictable Watson−Crick base pairing,[27] DNA represents one of the most promising materials to develop functional tools for biological applications. Since Seeman's pioneering work on the 4-arm DNA junction,[28] structural DNA nanotechnology has entered a new era. A wide range of DNA nanostructures with intricate shapes, precise sizes, and high addressability have been synthesized, including nanotubes,[29−31] origamis,[32−34] and polyhedra.[35−37] As a whole concept, framework nucleic acids (FNAs) refer to artificially designed nucleic acid structures that possess specific shapes, sizes, and functional groups, enabling spatially controlled patterning of functional biomolecules at the nanoscale.[38] Compared with nucleic acid-functionalized inorganic or organic nanomaterials, such as spherical nucleic acids,[39] DNA-Decorated hydrogels,[40] and DNA block copolymers,[41] FNAs can be integrated with diverse functional motifs, such as small molecules, aptamers, and peptides, with spatially and numerally controlled at the nanoscale. Meanwhile, via self-assembly into rigid three-dimensional (3D) nanostructures, FNAs exhibit higher biostability and higher cellular entry efficiency than linear nucleic acids.[42−46] These properties make them a highly attractive platform for various applications in cellular studies.

Recently, several review papers have discussed the applications of FNAs in the field of biosensing,[47−49] cellular imaging,[49,50] and disease treatment.[51,52] Meanwhile, there is still a need for a comprehensive overview that discusses their developmental trajectory and common challenges from a global perspective. Here, we focus on recent advancements of FNAs in the study of living cells, specifically addressing cell recognition and capture, imaging of specific cellular components, regulation, and mimicry of key molecular processes, and

Received: August 24, 2024
Revised: October 8, 2024
Accepted: October 9, 2024
Published: October 22, 2024

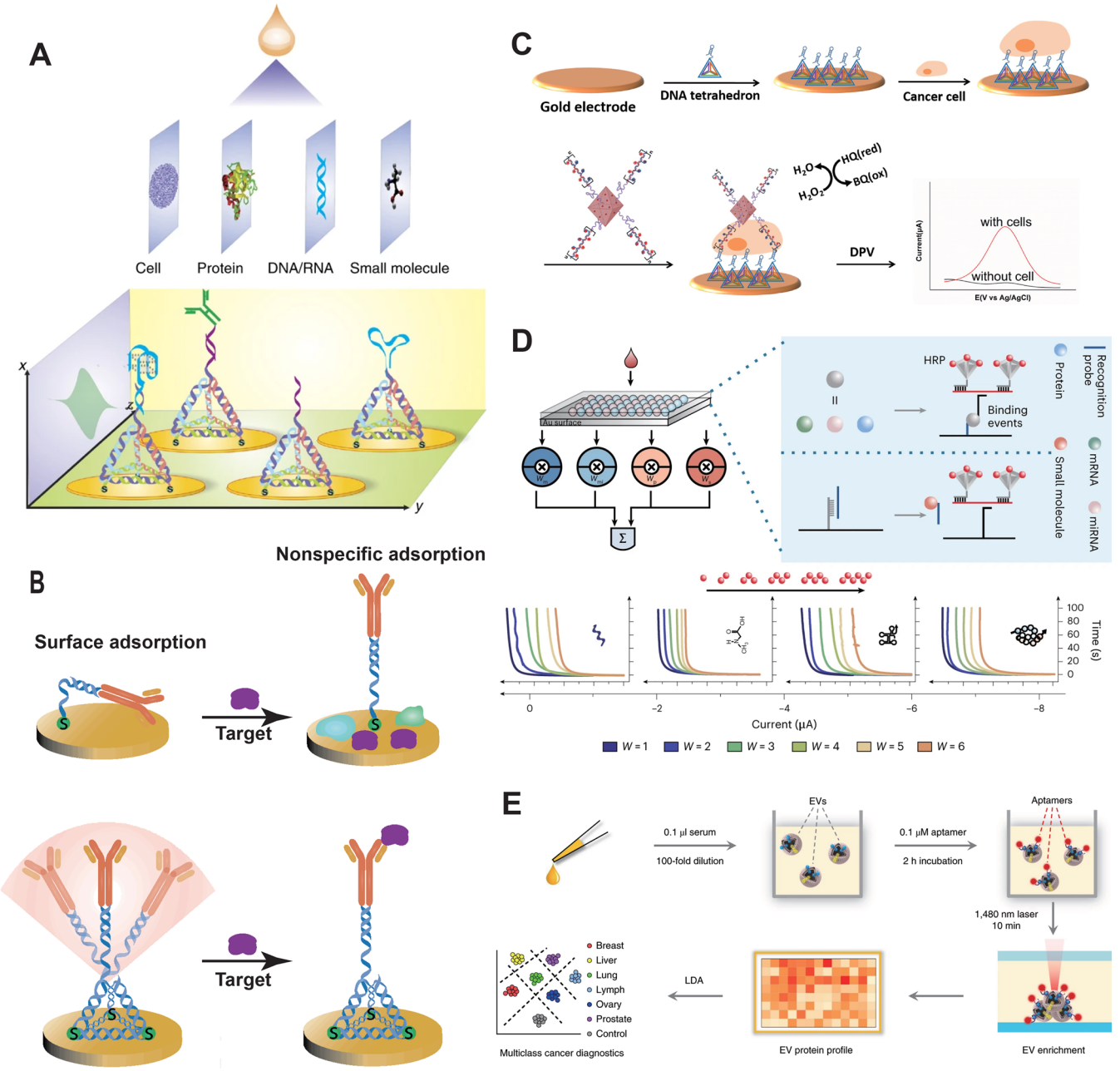


Figure 1. Examples of single-stranded DNA and FNAs in cell identification. (A) FNAs incorporate various molecular recognition ligands for target capture. Reproduced with permission from ref 56. Copyright 2016 Springer Nature. (B) Comparison of the performance of single-chain aptamers and FNA at solid interfaces. Reproduced permission from ref 68. Copyright Annual Reviews. (C) Capture of CTCs using FNAs. Reproduced with permission from ref 71. Copyright 2018 Elsevier B.V. (D) DNA-framework-based programmable atom-like nanoparticles to convert molecular recognition events into numerical information. Reproduced from ref 74. Copyright 2023 The Authors. (E) Aptamer-based thermophoretic sensor for early screening of cancers. Reproduced with permission from ref 77. Copyright 2019 The Authors.

cell-based disease treatments. Also, we discuss the opportunities and challenges inherent in these fields.

2. APPLICATIONS OF FNAs IN CELL IDENTIFICATION

The precise recognition and capture of cells are crucial for the early diagnosis, efficacy evaluation, and prognostic monitoring of diseases.[53−55] However, the low abundance and high heterogeneity of pathological cells pose significant challenges for this task. FNAs, with the capability to incorporate various molecular recognition ligands and DNA computing modules, show great promise for accurate identification of target cells (Figure 1A).[56]

2.1. FNAs in Cell Recognition

For specific cell identification, molecular recognition ligands are needed. Aptamers, oligonucleotides that can selectively bind to target molecules, can be readily integrated with FNAs through hybridization or sequence extension.[57−59] Particularly, the development of the cell-SELEX technology has enabled screening of aptamers against target cells even without prior knowledge of their molecular features, offering a panel of specific ligands for cell recognition.[60,61] For instance, Song et al. used MDA-MB-231 cells as the selection target and screened an aptamer specific for EpCAM, a membrane protein overexpressed on many tumor cells. They immobilized this

https://doi.org/10.1021/jacsau.4c00776
JACS Au 2024, 4, 4110−4128

aptamer on microfluidic chips for specific capture of circulating tumor cells (CTCs) in blood samples, achieving a capture efficiency of 63% and a cell purity of 80%.[62] Meanwhile, Zhao et al. used rolling circle amplification to synthesize an aptamer-incorporated multidimensional DNA network and decorated it onto the surface of microfluidic chips. Based on the multidimensional cell targeting effect, they achieved a cell capture efficiency of 80% at a liquid flow rate of 60 μL/h.[63] To improve the cell binding affinity, Song et al. employed a multivalent effect for cell-surface protein targeting. They developed aptamer-modified nanospheres and immobilized them on microfluidic chips. Compared to monovalent aptamers, their polyvalent probes achieved over 300% enhancement in CTC capture efficiency.[64] Considering the negative interference of the solid interface on the integrity and status of cells, Wu et al. decorated nanovesicles extracted from the membranes of functionalized white blood cells on microfluidic chips. They demonstrated that the mobility of these biofilms could enhance the binding frequency of recognition ligands with CTCs. They also proved that this soft capturing surface could minimize the undesired effects caused by the fixed solid surface on cell activity.[65]

Although a flowing interface can enhance the cell capture frequency to some extent, the self-entanglement of immobilized recognition ligands and their nonspecific interactions with the interfaces remain unavoidable (Figure 1B).[66−68] FNAs, benefiting from its mechanical rigidity and geometric stability, offered a potent scaffold to address this issue.[69,70] For example, Chen et al. constructed a sandwich-type electrochemical biosensor using DNA tetrahedrons as the scaffold for the detection of HepG2 cells (Figure 1C).[71] They proved that this FNA-incorporated biosensor could reduce the nonspecific absorption the cell-targeting ligand by protruding it outward, achieving a low detection limit of five cells per milliliter. To further enhance the cell recognition efficiency, Wang et al. designed a double tetrahedron framework that contained three recognition ligands in one FNA construct. They proved that this multivalent FNA probe achieved a low detection limit of one cell per milliliter.[72] Considering the high heterogeneity of cells, a single parameter is always challenging for their accurate identification, and molecular profiling of cell-surface proteins can offer a potent strategy for precise cellular classification. To address this issue, Xu et al. designed an aptamer-based AND logic operation on multiple cell-surface proteins. By integrating the information on two types of cancer-related membrane proteins, they successfully identified CEM cells from a mixture of other cancer cells.[73] Yin et al. utilized FNA-based programmable atom-like nanoparticles to construct a valence-encoded signal reporters, enabling the conversion of molecular recognition events into numerical information (Figure 1D).[74] Using this classifier, they performed interactive 3D analysis of six types of biological markers including RNA, protein, and metabolite. By assigning weights to the multidimensional molecular information for computational classification, they achieved a nearly deterministic molecular taxonomy of prostate cancer patients. These studies demonstrated the potential of FNAs as a programmable platform to modulate the avidity and specificity of cell-targeting ligands, offering a promising approach to enhancing the accuracy of cell identification. Meanwhile, given the high complexity of cells, such as heterogeneous phenotypes, low-abundance biomarkers, and volatile cellular states, there is ample opportunity to develop suitable tools for precisely identifying target cells.

2.2. FNAs in Identification of Cell-Derived Vehicles

In addition to cell bodies, cell-derived extracellular vehicles (EVs), such as exosomes and microvesicles, carry many biological properties of their parent cells, thus offering valuable information for disease analysis.[75] In order to analyze the information on EVs, Shi et al. developed an aptamer-functionalized tetrahedron-based electrochemical sensor. Their results showed that this sensor could specifically capture EVs and then perform in situ EV fragmentation. Subsequently, the electrode-surface immobilized sensing probes enabled the sensitive detection of exosomal miR-21.[76] Liu et al. developed an aptamer-based thermophoretic sensor for early screening of cancers (Figure 1E).[77] EVs in patient blood samples could be directionally enriched by a temperature gradient generated from light illumination. Using seven aptamers targeting the surface proteins, molecular profiling of EVs was obtained. Besides, by combination with linear discriminant analysis, they achieved effective classification across six cancer types with an accuracy rate of 68%. DNA computing represented a new direction in computational science that employed the sequence information on DNA to perform complex computations.[78] By combining DNA computing and thermophoretic enrichment, Li et al. developed an aptamer-based logic gate operation system for the sensitive and specific profiling of tumor-derived EVs.[79] Using multiple protein markers on individual EV as inputs, this strategy enabled distinguishing two subtypes of breast cancer cells with 90% accuracy.

While FNAs offer a versatile platform for recognizing and analyzing molecular features on the surface of EVs, the concentration of target molecules on these small-sized vesicles, particularly exosomes (diameters of 30−150 nm), is likely to be very low. Herein, the incorporation of FNAs with high-affinity recognition ligands and target-responsive signal amplification strategies would be beneficial for precisely probing their molecular information.

3. APPLICATIONS OF FNAs IN CELL IMAGING

The dynamic imaging of specific molecules in live cells would offer valuable information for cellular studies.[80,81] FNAs can be readily functionalized with multiple detection moieties and organelle-targeting ligands, making them a promising tool for the precise analysis of various cellular components. Additionally, due to their capability for easy internalization by cells, FNAs enable various probes to penetrate the cell membrane barrier,[43,82−84] enabling intracellular imaging of specific molecules or ions.[80−82]

3.1. FNAs in Imaging of the Cell Membrane Interface

The cell membrane interface is the local surrounding of cells for modulating their exchange of substances and information with external environments.[85] Monitoring of specific molecules or ions in this local environment would advance our understanding of many cellular processes, such as cell signaling, communication and metabolism.[80,81] To meet this goal, biosensing probes have been immobilized on the cell surface through covalent conjugation,[86] transfection of recombinant protein,[87] or receptor-targeted attachment.[88] While these cell membrane-anchored biosensing probes showed great promise for the dynamic monitoring of target analytes in this extracellular microenvironment, they generally involved complicated operations and modification of cellular components, possibly leading to conflict results. To address this issue, Liu et al. designed amphiphilic DNA probes by conjugating

https://doi.org/10.1021/jacsau.4c00776
JACS Au 2024, 4, 4110−4128

one end of the DNA strand with a hydrophobic diacyllipid tail.[89] They proved that these amphiphilic probes could efficiently anchor onto the cell membrane through a hydrophobic interaction between the diacyllipid tail and the phospholipid bilayer. They also demonstrated that this cell-surface engineering strategy could be generalized to different cell lines with good biocompatibility. By using this strategy, Qiu et al. developed cell membrane-anchored DNAzyme probes and showed their good performance in monitoring the cellular release of target ions (Figure 2A).[90] Afterward, they found that linear amphiphilic DNA detached rapidly from the cell membrane in serum-containing medium, affecting the reliability of the biosensing platform. To resolve this problem, they synthesized amphiphilic DNA tetrahedrons with an overhang probe at the top vertex and cholesterol tags at the three bottom vertices.[91] With three spaced anchoring motifs, DNA probes could be stably anchored onto the cell membrane with negligible internalization and no more than 20% detachment even after incubating the cells in complete culture medium for 2 h. Meanwhile, Xing et al. incorporated a pH-responsive DNA triplexes on the top vertex of amphiphilic DNA tetrahedron, and achieved dynamic monitoring of pH variations in the extracellular microenvrionment (Figure 2B).[92] In addition to tetrahedron, DNA origami represented another potent platform for membrane decoration with high addressability.[32] By conjugation with multiple hydrophobic tags, amphiphilic origami could be stably anchored on the cell membrane, allowing for the precise incorporation of diverse functional ligands. For example, Shahhosseini et al. anchored rod-shaped DNA origami conjugated with multiple cholesterol tags on the cell membrane, and incorporated two distinct DNA probes onto the outer leaflet of this nanoconstruct (Figure 2C).[93] Their results showed that this amphiphilic origami probe enabled the simultaneous detection of two nucleic acid molecules in their extracellular surroundings. While amphiphilic FNAs provide an effective, stable, and universal platform for incorporating detection probes onto the cell membrane, they fail to specifically label target cells in complex systems. Therefore, the development of FNAs, which enable cell type-specific membrane anchoring with minimal interference on the natural cellular components and status, is highly desired.

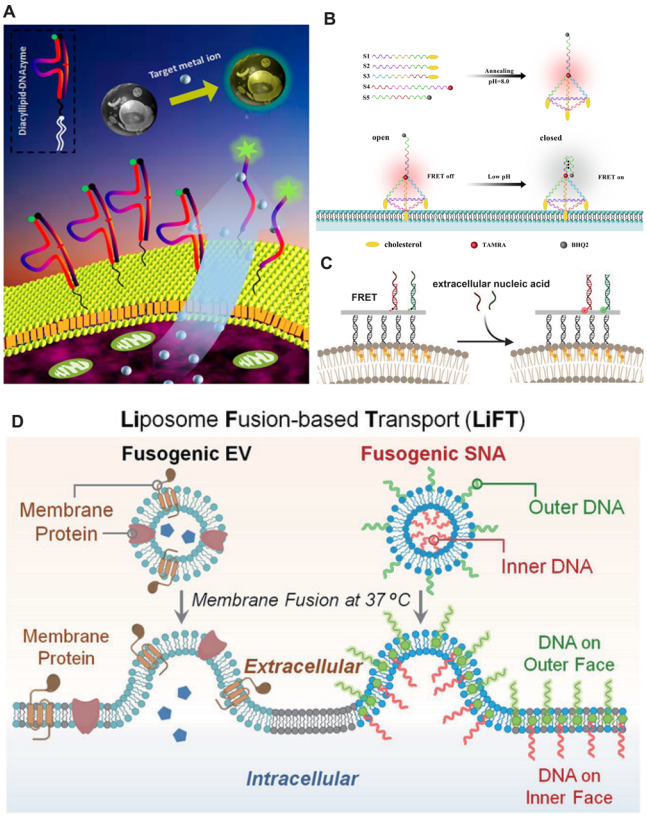


Figure 2. Functional nucleic acids modified to the cell membrane surface for monitoring cell life activities. (A) Cell membrane-anchored DNAzyme probes to monitor the cellular release of target Mg^{2+}. Reproduced from ref 90. Copyright 2014 American Chemical Society. (B) pH-responsive DNA triplexes on the amphiphilic DNA tetrahedron for monitoring the dynamic variation of extracellular pH. Reproduced with permission from ref 92. Copyright 2023 Elsevier B.V. (C) Rod-shaped DNA origami decorated with multiple cholesterol tags on the cell membrane sensitive for the detection of two extracellular nucleic acid molecules simultaneously. Reproduced from ref 93. Available under a CC-BY-NC-ND 4.0 license. Copyright 2022 The Authors. (D) Fusion of vesicles and cell membranes to modify probes to the intracellular membrane. Reproduced with permission from ref 96. Copyright 2021 Wiley-VCH GmbH.

Beyond the outer surrounding, the inner-leaflet membrane interface is a crucial region, where numerous signaling pathways are initiated.[94,95] Precise monitoring of molecules or ions within this microregion provides valuable insights into the underlying molecular mechanisms of cellular signal transduction processes. However, the negatively charged lipid bilayer poses significant challenges for the membrane translocation of many probes. As inspired by the fusion of exosomes with the cell membrane, liposomes decorated with DNA probes in the luminal surface was developed to deliver DNA onto the inner-leaflet of the cell membrane. By using this strategy, Lin et al. developed inner-leaflet membrane-anchored cholesterol-conjugated ATP aptamer probe, which allowed detection of ATP within the membrane-proximal cytosolic region (Figure 2D).[96] While this liposome fusion strategy showed great potential for the delivery of nucleic acid probes to the inner-leaflet membrane interface, there remain significant challenges, such as low membrane-anchoring stability, sensitivity to enzyme digestion, and difficulties in signal amplification, in biosensing of this closed and localized region. Besides, the interference of liposomes on the natural lipid components of the cell membrane and thus the cellular status remain an open research question.

3.2. FNAs in Imaging of Cytosolic Molecules

Cytosol is the aqueous component of the cytoplasm that supports various metabolic processes, enzyme activities, and cell signaling. Detecting specific molecules or ions in the cytosol is crucial for assessing cellular health, diagnosing diseases, and developing targeted therapies. Whereas, the plasma membrane serves as a barrier for the cellular delivery of biosensing probes. DNA molecules, as negatively charged macromolecules, generally fail to penetrate the plasma membrane. However, by folding into 3D nanostructures, FNAs can efficiently enter cells, offering a promising delivery platform for intracellular analysis. To investigate the possible mechanisms underlying the cell entry of these FNAs, Peng et al. used both experimental and simulation approaches to explore the interaction between cells and three typical FNAs with different shapes.[43] Their results proved that the corner-angle-mediated molecular interaction with scavenger receptors determined the cellular entry frequency of these FNAs. Ding et

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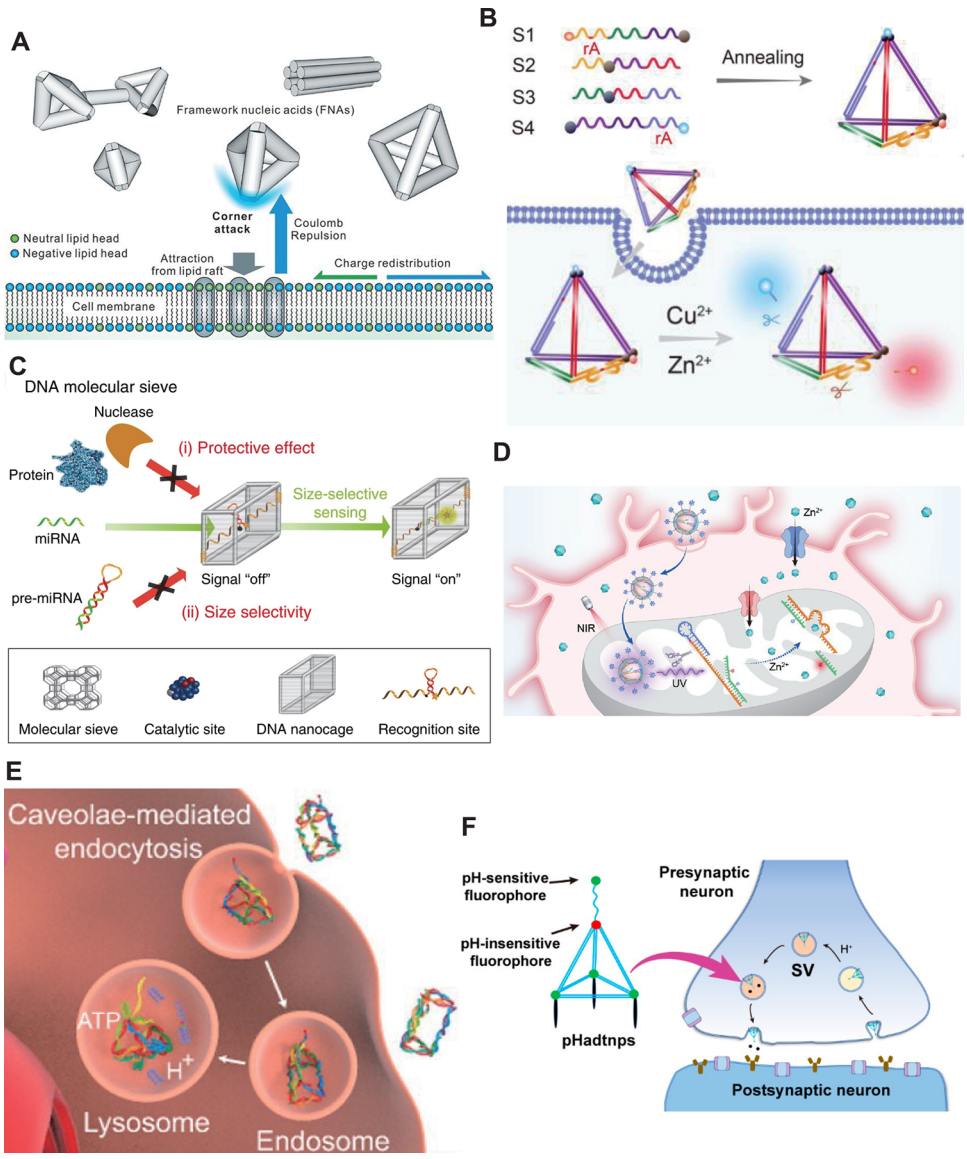

Figure 3. Examples of FNAs for intracellular imaging. (A) Tetrahedra FNAs use a "vertex attack" to penetrate cells. Reproduced from ref 97. Copyright 2018 American Chemical Society. (B) DNAzyme probes incorporated into the backbone of FNAs for the detection of Cu^{2+} and Zn^{2+} simultaneously. Reproduced with permission from ref 100. Available under a CC-BY 4.0 license. Copyright 2023 The Authors. (C) The confinement effect present in FNAs. Reproduced from ref 102. Available under a CC-BY 4.0 license. Copyright 2020 The Authors. (D) TPP-functionalized DNA nanoparticle to deliver DNAzyme into mitochondria for the detection of Zn^{2+}. Reproduced from ref 115. Copyright 2022 American Chemical Society. (E) Triangular FNAs for simultaneous imaging of both pH and ATP in lysosomes.[118] Reproduced from ref 118. Copyright 2019 American Chemical Society. (F) pH-responsive ratiometric fluorescent tetrahedra FNA for dynamically quantifying pH variations in synaptic vesicles.[124] Reproduced from ref 124. Copyright 2020 American Chemical Society.

al. reported that tetrahedra used a "vertex attack" to penetrate cells by minimizing their contact with the plasma membrane (Figure 3A).[97] These two studies proved that FNAs with sharp angular structures were more favorable for cellular internalization, offering new clues for the rational design of DNA-based delivery platforms.

For imaging cytosolic mRNA, Chor et al. conjugated a molecular beacon onto the vertex of tetrahedral FNA.[98] Their results showed that this FNA probe could effectively enter living cells and enable the specific detection of GAPDH mRNA in the cytosol. In addition to the vertex, the sides of FNAs could be incorporated with biosensing probes.[99] For example, Zhu et al. incorporated DNAzyme probes onto the sides of FNAs for the detection of cytosolic Cu^{2+} and Zn^{2+} in Hela cells (Figure 3B).[100] These sensors typically report only the total target signals, lacking dynamic information about target fluctuations. To address this issue, Wang et al. developed a transformable tetrahedral FNA for probing RNA dynamics in live cells.[101] Unlike static DNA nanostructures, the probe is transformed into a 3D tetrahedral FNA upon binding with a target RNA, which brought the donor−acceptor pair into close proximity to generate a FRET signal. Using this method, they investigated the dynamics of delta-like ligand 4 (Dll4) mRNA and uncovered previously unrecognized subpopulations of Dll4 mRNA.

Given that the concentration of some cytosolic analytes is rather low, signal amplification strategies to enhance the detection sensitivity would be beneficial. To achieve this goal, Zhang et al. found that the confinement effect in the cavity of FNAs could enhance the sensitivity of detection probes

https://doi.org/10.1021/jacsau.4c00776
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(Figure 3C).[102] By localizing DNAzyme probes within orthorhombic FNAs, nonspecific interference from large molecules on their sensing performance could be minimized, thereby reducing background noise. Herein, the detection sensitivity against target histidine inside was enhanced by more than 5000 times.[46] To distinguish target molecules with similar structures, Zhao et al. utilized the size-selective ability of DNA cages to exclude large-sized immature miRNA, and achieved the selective detection of small-sized mature miRNAs, which contained the same target fragment as their immature counterparts.[103]

The concentrations and distributions of intracellular components are highly dynamic. Herein, capability to analyze these components with biologically comparable spatial and temporal precision would be critical for understanding their molecular mechanisms in complex cellular processes.[104−106] The Li group constructed a series of photoactivated DNA biosensors for the detection of intracellular analysts with spatiotemporal control.[107] For instance, they synthesized an ATP aptamer probe, in which the ATP recognition fragment was partially hybridized with a complementary DNA that included a photocleavable group in the middle.[108] Only upon exposure to ultraviolet light would the complementary DNA be cleaved to recover the ATP sensing capability of the aptamer probe, achieving live-cell imaging of ATP with high spatiotemporal resolution. The need for light irradiation limits the *in vivo* use of this method due to poor tissue penetration. To overcome this issue, Yi et al. developed DNAzyme sensors activated by endogenous enzymes.[109] The enzyme strand of this sensor was modified with an abasic site and formed a stable duplex structure to block its catalytic ability. Only in cancer cells with a high expression level of apurinic/apyrimidinic endonuclease 1, the abasic site was cleaved to recover the activity of the DNAzyme probe, enabling the cell type-specific detection for target metal ions in vivo. Although FNAs can be internalized by living cells to some extent, their efficiency of cell entry, particularly in terms of endolysosomal escape, still needs improvement. Besides, due to the presence of numerous nucleases in the cytosol, enhancing the biostability of FNAs is essential for reliable intracellular analysis in vivo.

3.3. FNAs in Organellar Imaging

Cells are complex entities composed of diverse organelles. Dynamically coordinating the activity and organellar localization of specific biomolecules is fundamentally required for various cellular processes.[110] Herein, precise imaging of target components at the organellar level would be crucial.[111−114] As a typical example, mitochondria are the key organelles in maintaining cellular energy metabolism processes. Xin et al. designed a triphenylphosphonium (TPP)-functionalized DNA nanosensor for mitochondria-targeted delivery of biosensing probes, which composed of an ATP aptamer and a complementary DNA strand linked by a glutathione (GSH)-cleavable disulfide bond.[107] By using this design, they achieved GHS-activated imaging of ATP in mitochondria. Yi et al. utilized TPP-functionalized nanoparticle to deliver DNAzyme probes into mitochondria, enabling the organellar detection of Zn^{2+} (Figure 3D).[115] In addition to mitochondria, lysosomes are the metabolic center of cells, containing more than 60 types of hydrolytic enzymes in acidic lumen for degradation of macromolecules.[116] Their dysfunction is closely related to the occurrence and development of various diseases. Gao et al. found that tetrahedral FNA could effectively target lysosomes.[117] They developed a Zn^{2+} fluorescent probe and incorporated it into the FNA scaffold. Using this lysosome-localized FNA probe, we could sensitively detect the lysosomal zinc ions with a low detection limit of 31.9 nM.

To achieve multiplex detection, Du et al. constructed a DNA nanodevice by integrating an i-motif probe and an ATP-binding aptamer into the triangular FNAs (Figure 3E).[118] Their results proved that this nanodevice enabled simultaneous imaging of both the pH and ATP in lysosomes. While we could integrate different detection probes on the same FNA for simultaneous imaging of multiple components, the quantitative measurement of organellar variations was still challenging. Ratiometric fluorescence probes, with the capability to correct variations in probe concentration, instrument fluctuations, and environmental conditions, represent an attractive alternative for quantitative measurement. Saha et al. developed a double-stranded DNA structure consisting of a Cl^--sensitive probe, a Cl^--insensitive fluorophore for normalization, and a targeting module for endolysosomal pathways.[119] Using this nanodevice, they quantitatively measured Cl^- along with the endolysosomal pathways and demonstrated that lysosomal Cl^- had a far-reaching function beyond pH regulation. Leveraging the programmability and modularity of the DNA scaffolds, this research group further incorporated the DNA nanodevices with different sensing probes, achieving precise quantitative analysis of Ca^{2+},[120] Na^+,[121] K^+,[122] and electric potential[123] in specific organelles. Due to the interconnections among the variations of multiple ions and molecules within organelles, it is of significant importance to conduct precise simultaneous detection of these entities. However, the multiplex detection capability of these DNA duplex probes is constrained by their relatively flexible structure and limited size (generally <80 bp). FNAs, with higher rigidity and broader dimension range, present an excellent solution to this issue. Liu et al. utilized amphiphilic tetrahedron FNAs as the membrane-anchored nanoscaffold and incorporated with three pH-sensitive and one pH-insensitive dyes to develop a ratiometric fluorescent probe. When the plasma membrane of neurons invaginated to form synaptic vesicles (SVs), the FNA probe was loaded into the SV lumen, enabling the dynamic monitoring of pH variations during the exocytosis and retrieval of SVs (Figure 3F).[124]

Currently, cell biology is shifting from a holistic perspective to a more localized focus, emphasizing the importance of understanding cellular processes on a finer scale. The quantitative measurement of signaling molecules and ions in specific micro- and nanostructural domains of cells or organelles can offer valuable insights into related molecular mechanism study. FNAs can be precisely integrated with a variety of sensing probes, providing an excellent platform for analyzing cellular processes at unprecedented resolutions. Yet this exciting field is still in its early stages, offering substantial opportunities for further exploration and innovation.

4. APPLICATION OF FNAs IN CELLULAR REGULATION

Artificial manipulation of cellular functions and behaviors offers a new approach for understanding specific cellular processes and advancing disease theranostics.[125−127] The predictable and switchable structure of DNA molecules demonstrates significant potential in cellular regulation. In particular, by adopting a 3D configuration, FNAs provide a versatile platform, a highly adaptable platform characterized by

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JACS Au 2024, 4, 4110−4128

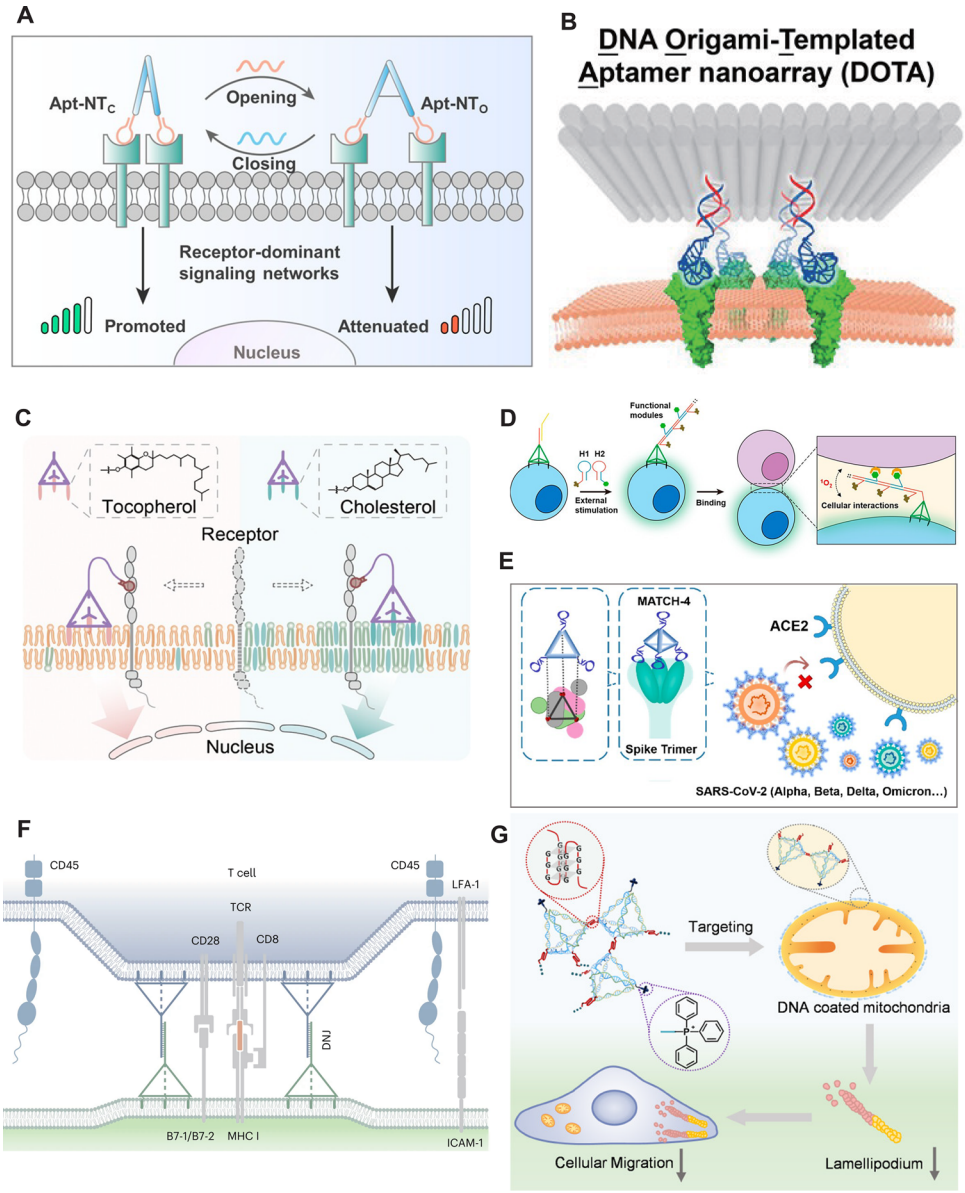

Figure 4. Examples of FNAs in manipulating cellular activities. (A) DNA nanodevice for dynamically regulating the lateral distance between target membrane receptors in a dimer. Reproduced from 131. Copyright 2022 American Chemical Society. (B) A DNA origami-templated aptamer nanoarray allowing precise programming of receptor tyrosine kinase oligomerization with defined valency, distribution, and stoichiometry at the ligand−receptor interface. Reproduced from 132. Copyright 2022 American Chemical Society. (C) Two tetrahedron FNAs for separately targeting the lipid raft and nonraft domains of the cell membrane. Reproduced from ref 139. Copyright 2024 American Chemical Society. (D) Two tetrahedron FNAs for separately targeting the lipid raft and nonraft domains of the cell membrane. Reproduced from ref 141. Copyright 2021 American Chemical Society. (E) Tetrahedron FNA with a side length of 5.4 nm to seal the upper surface of the SARS-CoV-2 spike trimer with nanoscale precision. Reproduced from ref 144. Copyright 2022 American Chemical Society. (F) DNA nanojunctions for regulating the intermembrane spacing between antigen-presenting cells and T cells. Reproduced with permission from ref 145. Copyright 2023 The Authors. (G) Tetrahedron FNA that forms a polyanionic barrier and causes mitochondrial dysfunction. Reproduced from ref 152. Copyright 2022 American Chemical Society.

extensive programming, nanoscale resolution, and superior reliability, thereby aligning with this objective.

4.1. FNAs in Manipulation of Natural Cell-Surface Proteins

The clustering of membrane proteins is an important way for regulating various cellular processes, such as signaling transduction, material transportation, and cellular communications.[128] Capability to artificially manipulate the clustering of target proteins is beneficial for exploring related biological processes. To control the interactions of different membrane proteins, Liu et al. developed a bivalent linker composed of two different aptamers, which modulated the heterodimerization of receptor tyrosine kinases from different families, thus enabling the stimulation of EGFR1 and c-Met signal transduction pathways.[129] Yang et al. constructed a nongenetically engineered artificial DNA mechanoreceptor, which could sense the exerted tensile forces through an allosteric DNA switch. This design implemented a force-triggered dynamic DNA assembly that promoted receptor dimerization and enhanced downstream signaling in the force-receiving cells.[130] In addition to protein dimerization, the molecular distance

https://doi.org/10.1021/jacsau.4c00776
JACS Au 2024, 4, 4110−4128

between receptors in a dimer may be another important factor in modulating the downstream signaling output. To test this hypothesis, Wang et al. developed an aptamer-functionalized DNA nanotweezer for dynamically regulating the lateral distance between target membrane receptors in a dimer(Figure 4A).[131] Via control over the open or closed states of the nanotweezer, they modulated the lateral distance between CD28 receptors, which led to regulation of the T cell activation process. To study how receptor oligomerization governed the signaling response, Wang et al. constructed a DNA origami-templated aptamer nanoarray, which allowed precise programming of receptor tyrosine kinase oligomerization with defined valency, distribution, and stoichiometry at the ligand−receptor interface (Figure 4B).[132] They revealed that varying the valence of receptor oligomerization could trigger distinct signaling activation pathways. Additionally, the proper spatial arrangement of proteins is essential for achieving desired functional outcomes.[133] Zhu et al. found that simple protein ligation using a bivalent aptamer was insufficient to induce glycosylation reactions.[134] Instead, optimizing the linker design between the two aptamers to position the target proteins in an appropriate spatial arrangement was necessary to facilitate the glycosylation reaction. To finely tune the angle of protein interaction, Zhou et al. designed tetrahedral FNAs incorporated with a series of linker length ranging from 8 to 57 Å, allowing control over the distance of interaction between two proteins.[135] Based on this, they developed covalent DNA framework-based PROTACs (DbTACs). By precisely positioning ligands of proteins of interest and E3 ligase on the FNAs, they found that DbTACs with optimal linker lengths between ligands achieved higher degradation rates and enhanced binding affinity. Simultaneously, they also designed bispecific DbTACs with trivalent ligand assembly, enabling multitarget depletion while maintaining highly selective degradation of specific protein subtypes.

Apart from protein interactions, the membrane distribution may be an important factor for regulating their biological functions.[136] Increasing evidence proved that the components of the cell membrane was not uniformly distributed, but segregated into distinct lipid-ordered (lipid raft) and lipid-disordered (nonraft) domains, displaying a laterally heterogeneous pattern.[137] Manipulating the partitioning of proteins in these membrane domains would provide a new perspective for studying related biological processes. Sun et al. found that two complementary cholesterol-functionalized DNA strands could cause the rearrangement of lipid rafts in the cell membrane. Based on this experimental phenomenon, they reported a DNA nanotweezer composed of a cholesterol-functionalized DNA duplex for stabilizing transient lipid rafts and inducing clustering of raft-associated components.[138] They demonstrated the potential of this nanotweezer for effectively capturing and expanding cholesterol-rich raft domains. By using these nanotweezers, they successfully included the recruitment of TCR, and stimulated T cell activation. Meanwhile, Ma et al. designed two DNA tetrahedrons for separately targeting the lipid raft and nonraft domains on the cell membrane. They also used these two tetrahedrons for regulating the membrane partitioning of PTK7 on HCT116 cells and CD45 on Jurkat T cells (Figure 4C).[139] Their results demonstrated that different membrane partitionings of these two proteins could induce completely different cellular responses.

The spatial distribution of membrane proteins is closely linked to their biological functions, making their precise arrangement critical for cellular processes. FNAs provide unique advantages in controlling the aggregation, distance, and spatial arrangement of protein molecules, offering an attractive platform for the precise manipulation of protein-related processes. However, to date, FNA-based strategies for protein manipulation have primarily focused on membrane proteins, arising from the challenges associated with delivering complex nucleic acid constructs to specific intracellular regions. Herein, there is a pressing need for innovative approaches that can enhance the delivery and application of FNAs for manipulating the interactions among intracellular proteins.

4.2. FNAs in Regulating Cellular Interactions

Cell−cell interactions play a crucial role in regulating various physiological processes, such as tissue development, immune response, and homeostasis. The capability to manipulate the interactions between cells would offer many opportunities for the development of cell-based theranostics and tissue engineering. Yang et al. designed a circular bispecific aptamer for cellular connection.[140] By creating artificial recognition between native T cells and tumor cells, they achieved an enhanced accumulation of T cells in tumor tissue and consequently improved their cytotoxic effects. To manipulate the dynamic cellular interactions in coordination with their intrinsic activity, Li et al. designed a membrane-anchored FNAs which could be activated by cell-secreted signaling molecules (Figure 4D).[141] Consequently, multiple functional modules could be assembled on the activated FNAs through the hybridization chain reaction to arm the cells with the binding and killing capability against target cells. To reversibly regulate the cellular interactions, Lu et al. incorporated the cell recognition ligands with the i-motif, G4 or DNAzymes to manipulate the aggregation and disaggregation of cells.[142,143] In addition to establishing new connections between cells, DNA was also used to block the cellular interactions. Wan et al. used a DNA tetrahedron with a side length of 5.4 nm to block the SARS-CoV-2 spike trimer with nanoscale precision (Figure 4E).[144] This approach effectively prevented interaction between the spike trimer and ACE2, thereby inhibiting SARS-CoV-2 infection and fusion with host cells.

Beyond membrane receptor-based molecular recognition, the physical parameters at the cellular interface would play an essential role in modulating cell−cell interactions. To evaluate the impact of intermembrane spacing on T cell activity, Du et al. constructed three DNA nanojunctions with distinct sizes through overhang hybridization between two membrane-anchored tetrahedral FNAs (Figure 4F).[145] Their results demonstrated that the intermembrane distance plays a crucial role in modulating the T cell activation process, presumably by modulating protein reorganization and mechanical forces. They also revealed that shortening this distance with small DNA nanojunctions could significantly enhance T cell activity. In addition to the physical distance, the molecular forces generated during cellular interactions are another critical parameter. Based on the predictable thermodynamics of base pairing, You et al. developed a series of DNA mechanical probes to measure the tensile forces generated during cell−cell interactions.[146−148] As interest in studying the molecular forces involved in cellular interactions has grown, DNA mechanical sensors have evolved from simply detecting the magnitude of the force to also responding to the rate and duration of the applied force.[149]

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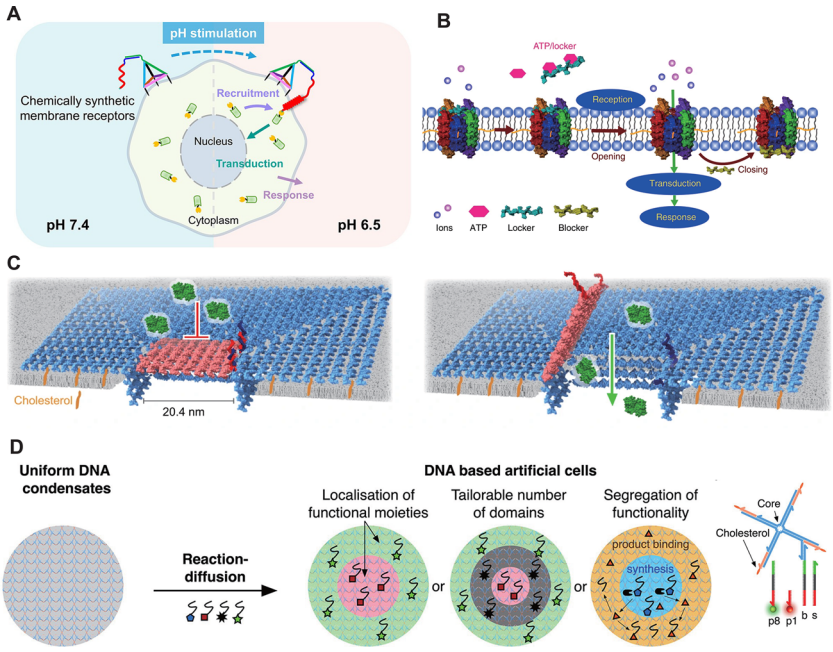

Figure 5. Examples of artificial cell receptor construction. (A) pH-responsive artificial cell membrane receptor building by amphiphilic tetrahedral FNAs and pHLIP. Reproduced from ref 154. Copyright 2023 American Chemical Society. (B) Transmembrane DNA nanogate building by DNA 6-helix bundles. Reproduced from ref 155. Available under a CC-BY 4.0 license. Copyright 2020 The Authors. (C) Transmembrane channel building by DNA origami. Reproduced from ref 156. Available under a CC-BY 4.0 license. Copyright 2022 The Authors. (D) Membraneless artificial cells based on DNA frameworks. Reproduced from ref 159. Available under a CC-BY 4.0 license. Copyright 2022 The Authors.

In addition to interactions between intact cells, the interplay of intracellular membrane-bound organelles also plays a fundamental role in maintaining cellular homeostasis. For example, fusion of autophagosomes and lysosomes can lead to degradation of age-damaged organelles; contact between the endoplasmic reticulum and damaged lysosomes can cause lysosomal repair; mitochondrial aggregation triggers localized increase of reactive oxygen species (ROS) to enhance mitochondrial stress. To artificially induce organellar aggregation in cells, Lu et al. designed a DNAzyme probe incorporated with mitochondria-targeting peptides. They proved that this probe could bring two mitochondria closer, and thus induce mitochondrial damage.[143] Zhu et al. synthesized a long-chain DNA conjugated with multiple mitochondria-targeting modules to trigger the clustering and then fusion of mitochondrial.[150] Using this approach, they successfully repaired ROS-stressed neuronal cells. These DNA probs were generally introduced into cells using transfection reagents. Instead, FNAs themselves could rapidly enter cells (<2 h) with high biostability, offering an attractive alternative.[45] Dong et al. used split i-motif structure to construct a H^+-responsive FNA prob.[151] Their results showed that lysosomal acidity could trigger the aggregation of these FNA probes, which led to the consumption of H^+ content within lysosomes, thereby regulating luminal enzyme activity. Based on the difference of the K^+ concentration between lysosomes and cytosol, Li et al. designed a K^+-mediated 3D assembly of tetrahedral FNAs to a polyanionic barrier around mitochondrial (Figure 4G).[152] After entry into lysosomes with a low K^+ concentration, FNAs maintained a dispersed pattern in a monomer form. Upon escape into the K^+-abundant cytosol, the G-quadruplex was formed to facilitate the 3D assembly of the FNA monomer. By incorporation of TPP, these DNA nanostructures were able to specifically target mitochondria and influence mitochondrial respiration. As a result, they suppressed the migration capacity of the MCF-7 cells by as much as 50%.

Currently, the applications of FNAs in regulating cellular interactions have advanced from merely bringing cells closer together to precisely controlling the physical and chemical parameters at cellular interfaces. Meanwhile, using FNAs to modulate the interactions between intracellular organelles represents a new direction in cellular research. However, significant challenges remain in effectively decorating functional DNA constructs with specific organelles. To achieve these goals, innovative strategies are needed to enhance the design, specificity, and functionality of FNAs.

4.3. FNAs in Construction of Artificial Receptors

DNA is used not only to regulate the functional activities of natural proteins but also to create constructs that mimic protein functions and establish new signaling pathways. Inspired by the RTK receptor dimer, Chen et al. designed an artificial membrane receptor consisting of two transmembrane units, where two cholesterol tags were modified in the middle site of the DNA construct. Meanwhile, this artificial receptor was consist of: a pH-responsive split i-motif as the top signal recognition module and a split G-quadruplex that performed peroxidase-like catalytic reactions as the bottom signal output module.[153] As the signal input, protons triggered the formation of the i-motif at the top, leading to the dimerization of the artificial receptor, which in turn formed the bottom G-quadruplex to initiate downstream signal reactions. While it was an interesting design, the linear DNA structure would restrict the application scope of the artificial membrane receptors. Introduction of FNAs was expected to enhance the functionality of artificial receptors. For example, Wu et al. constructed a pH-responsive cell membrane receptor by immobilizing amphiphilic tetrahedral FNAs incorporated with a pH (low) insertion peptide (pHLIP) (Figure 5A).[154]

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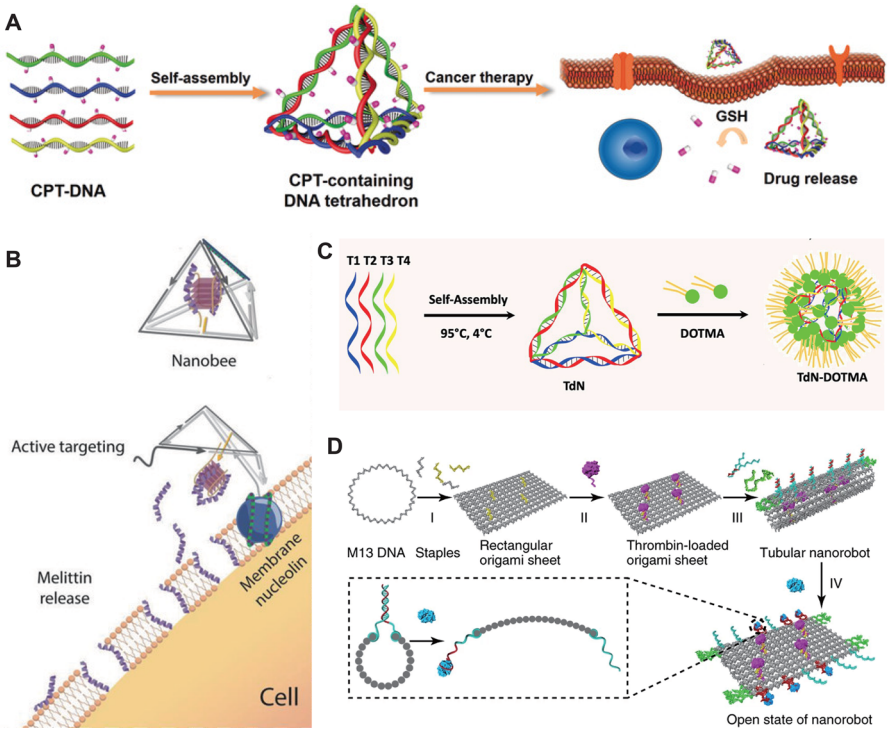

Figure 6. Examples of FNAs used in disease treatment. (A) Covalent bonding of camptothecin on tetrahedral FNAs. Reproduced with permission from ref 170. Copyright 2019 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (B) Tetrahedral FNA loaded with bee venom peptides. Reproduced with permission from ref 172. Copyright 2020 Wiley-VCH GmbH. (C) Complexes of cationic lipids and tetrahedra to improve the stability in the zebrafish system and enhance the cellular uptake efficiency. Reproduced from ref 174. Copyright 2023 American Chemical Society. (D) Aptamer-functionalized origami nanotube encapsulated with thrombin. Reproduced with permission from ref 178. Copyright 2018 Springer Nature America.

Upon exposure to a low-pH environment, the pHLIP module rapidly formed a rigid α-helical structure and then actively inserted into the cell membrane, delivering the C-terminal fragment carrying a protein recruiter to the cytoplasmic leaflet. This led to the recruitment of specific cytosolic proteins to the membrane-proximal region, which then initiated downstream signaling events. In addition to mimicking cell-surface receptors, FNAs have also been employed to simulate transmembrane ion channels. For example, Peng et al. constructed a transmembrane DNA nanogate, which was formed by six-helix DNA bundles conjugated with cholesterol at the middle of the side edge (Figure 5B).[155] They used cell-derived giant vesicles as a protocell model and demonstrated the capability of this artificial channel to modulate the transmembrane flux of ions and small molecules. To develop DNA nanostructures with higher addressability, Dey et al. used DNA origami to design a transmembrane channel that could be precisely controlled by external stimulation (Figure 5C).[156] This artificial channel had an open cross-sectional region that allowed impermeable proteins, such as trypsin and GFP, to enter the cell upon exposure to external stimuli. Furthermore, the construction of artificial organelles in the cytoplasm would offer a new paradigm for correcting organellar impairments in defective cells. Zhang et al. constructed dual-enzyme-loaded microdroplets for correcting cellular nitric oxide deficiency.[157] Yao et al. utilized a dynamic DNA assembly system to *in situ* construct peroxisomes within cells for removal of ROS.[158]

Reproducing life-like characteristics synthetically would enhance our understanding of biological processes and lead to development of next-generation tools in biotechnology and pharmaceuticals. Leathers et al. constructed membraneless artificial cells using the self-assembly of synthetic DNA nanostructures (Figure 5D).[159] By modifying various functional groups on nucleic acids, it is easy to modify the artificial cell system and simulate specific biological chemical reactions in cells. However, it is challenging to balance the trade-offs among structural stability, versatility, and molecular communication in artificial cell systems. To overcome this challenge, Zhang et al. developed a self-stabilizing and fastener-bound gain-of-function DNA membraneless organelle with high stability and controllable bioactivity.[160] This artificial organellar scaffold, generated by rolling circle amplification of long single-stranded DNA, would assemble into membraneless coacervates via phase separation, leading to recruitment of Mg^{2+} for self-stabilization. Furthermore, by binding with other fasteners, designated functionalities could be readily integrated into this artificial organelle.

The development of artificial receptors, organelles, and cells is a key focus of "bottom-up" synthetic biology. FNAs showed potential in the construction of artificial systems to mimic the biological structures and functions of these entities but are significantly challenged by the high complexity of these biological systems. Particularly, the adaptive response capacity of living cells is established by a complex signaling network, which involves not only diverse molecules but also different membrane-bound compartments.

5. APPLICATION OF FNAs IN DISEASE TREATMENT

Effective drug delivery into cells is often a primary step for the successful treatment of various diseases, but this process is frequently impeded by the barrier posed by the cell membrane.[161] FNAs, with their enhanced ability to penetrate

https://doi.org/10.1021/jacsau.4c00776
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the cell membrane, can significantly improve the efficiency of drug delivery. Additionally, FNAs are highly versatile, allowing for the integration of various functional modules tailored to specific therapeutic needs. This adaptability makes them a promising platform for the development of advanced dis-ease therapies.

5.1. FNAs as Therapeutics in Disease Treatment

Many studies suggested that tetrahedral FNAs themselves could serve as therapeutics for many diseases. For example, Li et al. found that tetrahedral FNAs could inhibit cisplatin-induced iron death in renal tubular cells by reversing the down-regulation of glutathione peroxidase 4.[162] Wang et al. found that tetrahedral FNAs could affect ectopic ossification through modulating the II-17 pathway.[163] Li et al. found that tetrahedral FNAs could modulate the PI3K/AKT pathway, and thus inhibited the progression of type 2 diabetes.[164] Cui et al. found that tetrahedral FNAs could alleviate the symptoms of Parkinson's disease by reducing the accumulation of α-synuclein to inhibit the apoptosis of PC12 cells.[165] Shao et al. also reported that tetrahedral FNAs could protect PC12 cells from the toxicity of amyloid β-peptide.[166] Zhao et al. found that tetrahedral FNAs could promote the growth and migration of epidermal cells through the Notch pathway.[167] Furthermore, the structures and sizes of FNAs would cause different biological effect. Shi et al. detailly investigated the biological effects of tetrahedral FNAs with different sizes.[168] They proved that smaller FNAs tended to promote cell proliferation and migration, potentially promoting tissue regeneration and wound healing. Besides, Hu et al. reported that tetrahedral FNAs of different sizes or different shapes exhibited different activation levels on immune cells. Besides, this study showed that FNAs different sizes could modulate the molecular distance of the conjugated CpG ligands and thus trigger different degrees of immune response.[169]

FNAs have demonstrated a variety of advantages in disease treatment, but to enhance therapeutic outcomes and broaden their applications, it is crucial to gain a deeper understanding of their interactions with cells. Investigating the mechanisms by which FNAs engage with cellular components can provide valuable insights into their efficacy and safety profiles. This understanding may lead to the optimization of FNA designs, enabling more effective targeting of specific cell types and disease states.

5.2. FNAs as Drug Carriers in Disease Treatment

Taking advantages of enhanced cell entry efficiency, ease of modification, and good biocompatibility, FNAs have been used as carriers of therapeutics for the treatment of various diseases, such as tumors, neurodegenerative disorder, and senescence. For example, Zhang et al. conjugated camptothecin onto tetrahedral FNAs through disulfide bonding (Figure 6A).[170] After being delivered into the cytoplasm, camptothecin could be liberated from FNAs through glutathione-induced reduction of the disulfide, thereby leading to effective cell killing. While covalent conjugation of therapeutics offered high stability and controllable stoichiometric ratios, it generally suffered from limitations of low payload and complex operations. To enhance drug payload, Ren et al. synthesized tetrahedral FNAs embedded with doxorubicin (DOX) for cancer treatment.[171] Via electrostatic and van der Waals forces, DOX molecules were loaded into the C-G base pairs of the DNA duplex, and over 80 DOX molecules were embedded in each tetrahedral FNA. In addition to small molecules, tetrahedral FNAs could be used for the delivery of therapeutic peptides and miRNAs. For example, Tian et al. developed a tetrahedral FNA loaded with bee venom peptides (Figure 6B).[172] Upon binding to the target surface receptor, the FNA would undergo a conformational change, releasing the peptide to induce cell perforation and death. Gao et al. designed tetrahedral FNAs containing a low pH-responsive i-motif hybridized with siRNA.[173] After entry into endolysosomes, one side of the FNA would undergo a conformational change to form an i-motif structure, thus leading to the release of siRNA for silencing tumor-associated genes. The biostability of FNAs is one of the critical factors influencing therapeutic efficacy. To address this issue, Kansara et al. reported that the complexes of cationic lipids and tetrahedra could significantly improve their biostability and cellular uptake efficiency (Figure 6C).[174] Positively charged drug molecules can also improve the internalization efficiency of FNAs. Yan et al. incorporated tetrahedral FNAs with positively charged DOX and proved that these DNA nanostructures could cross the cell membrane directly without entering the endocytosis pathway.[175] They also proved that this positively charged FNA had a high tumor penetration depth, offering a potential delivery system for tumor elimination. To explore the potential of FNAs as drug delivery platform, Zhang et al. evaluated the cellular uptake and *in vivo* stability of tetrahedral FNAs with four different modifications: replacing DNA strands with antisense peptide nucleic acids, attaching aptamers at the vertex, directly mixing with small molecular drugs (e.g., paclitaxel and baicalein), and coating with protective reagents (e.g., cationic polymers).[176] These results offered instructive information for the rational design of drug nanocarriers.

Due to the high complexity of tumors, the efficacy of treatments against a single therapeutic target was often limited. Benefiting from high programmability, FNAs could be integrated with multiple therapeutic agents or modalities into a single platform. For example, Li et al. constructed a tetrahedral FNA, where the vertices were extended with three miR-132.[177] Their results demonstrated that the combination of three miR-132 molecules could effectively delay skin aging. To address the off-target effect, Li et al. developed an aptamer-functionalized origami nanotube encapsulated with thrombin (Figure 6D).[178] Upon aptamer-based binding to nucleolin overexpressed on tumor cells, the hybridized duplex would be unzipped to release thrombin, leading to vascular occlusion and then starvation death of the tumors. Wu et al. modified an EGFP-specific nanoantibody on the tetrahedral skeleton embedded with the drug 56MESS for tumor-targeted chemotherapy.[179] Compared with single therapy, combination therapy could offer a more comprehensive approach to improving tumor treatment efficacy. Tang et al. designed an aptamer-modified DNA hydrogel that embedded with a chemotherapeutic drug and incorporated antisense oligonucleotide therapeutics as linkers between two tetrahedra (Figure 6E).[180] Their results demonstrated that the combination of targeted gene therapy with chemotherapy could significantly enhance the therapeutic efficacy against multidrug-resistant tumors.

Drug delivery is a critical aspect of effective disease treatment. Over the years, various drug delivery vehicles have been developed, including lipid nanoparticles,[181,182] inorganic nanoparticles,[183−185] liposomes,[186,187] viral vectors,[188,189] and biological vesicles.[190,191] Despite significant progresses, many of these vehicles still face several limitations, such as low

https://doi.org/10.1021/jacsau.4c00776
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biocompatibility,[192] inaccurate drug loading,[193] and unclear delivery mechanisms.[194−196] FNAs offer a promising alternative due to their high programmability, excellent biocompatibility, and predictable structures. On the other hand, the practical applications of FNAs are still limited by low production yield and poor biostability. In addition, the potential biological effects of FNAs remain largely unexplored and require further investigation.

SUMMARY AND OUTLOOK

Taking advantage of convenient modification, high programmability, good biocompatibility, and structural rigidity, FNAs have attracted significant interest in cell research. By programming the valence and types of cell-targeting ligands, FNAs offer a potent platform for specific cell recognition and capture. Meanwhile, by integrating molecular recognition with DNA-based computation, FNAs enable the development of intelligent strategies for accurate cell identification. Additionally, they can be modified with various functional groups to target specific subcellular domains. For example, FNAs have been modified with hydrophobic tags, allowing them to be effectively anchored onto the cell membrane to monitor specific molecules and ions at the membrane interface. By folding into 3D nanostructures, FNAs can enter living cells without the need for transfection agents, providing predictable and versatile vehicles for the intracellular delivery of sensing probes and therapeutics. This ability significantly enhances the precision and efficacy of disease theranostics. Moreover, FNAs hold great potential for manipulating and mimicking cellular functions and behaviors, opening up numerous opportunities in the realms of intelligent synthetic biology and cell-based therapies. Their applications can lead to the development of advanced biotechnological tools, such as synthetic receptors, organelles, and even cells, that can interact with and respond to external stimuli in a highly controlled manner. As research advances, these innovations in FNA technology have the potential to revolutionize cell-based treatments and contribute to the development of novel therapeutic strategies, ultimately improving patient outcomes.

On the other hand, several challenges remain to be addressed. First, natural nucleic acids can be rapidly degraded by ubiquitous nucleases in complex biological systems. Intensive efforts have been made to improve the biostability of nucleic acids. For example, the phosphate backbone of nucleic acids has been chemically modified with phosphorothioate-based nucleotides,[197] and alkyl phosphonate nucleic acids,[198] to enhance their resistance to nucleases.[199−201] Also, chemical modification on the sugar ring and nucleobase, such as L-nucleotide,[202] 2′-*O*-methyl groups,[203] and 7-deaza-7-modified guanosine base analogues,[204] has been performed. Given that most nucleases digest nucleic acids from the free ends, various end-blocking strategies have been developed, including strand cyclization,[205] terminal conjugation with chemical groups (e.g., amino, carboxyl, and dibenzocyclooctyne, etc.),[206] polymers,[207,208] and inorganic nanoparticles.[209] While improved resistance has been achieved, these modifications may alter the original biological functions of these nucleic acid probes. Thus, tedious optimization and evaluation would be needed to obtain the optimal design. Second, due to the flexible configurations of nucleic acids, the construction efficiency of uniform FNAs is still insufficient, especially for those large-size and complex pairing structures. The relatively low purity of DNA nanostructures may hinder their practical applications. Herein, tedious optimization of experimental conditions such as reaction temperature, solvent types, sequence design, and strand ratios are often required. Zhu et al. reported that rapidly freezing the DNA and slowly thawing it could increase the local DNA concentration within the ice crystal, thereby enhancing DNA hybridization efficiency by 120 times.[210] This approach may prove beneficial in facilitating the formation of FNA structures by selecting an appropriate ice crystal gap size. Third, the comprehensive biological impact of FNAs on cells remains largely unexplored. Increasing evidence suggests that FNAs can affect the cellular status and activity. For example, Liu et al. found that tFNA can activate the P38 and ERK1/2 signaling pathways in human corneal epithelial cells, thereby enhancing their proliferation and migration abilities.[211] Other studies also found FNAs can affect various signaling pathways, such as the interleukin-17 pathway,[165] PI3K/Akt pathway,[166] and Wnt pathway.[212] This characteristic of FNAs is a double-edged sword. On one hand, their biological influence may potentially synergize with the carried therapeutics, thereby enhancing therapeutic efficacy in disease treatment. On the other hand, when employing FNAs as vehicles for delivering biosensing probes in cellular analysis, their uncertain influence may result in conflicting outcomes.

Collectively, the cellular applications of FNAs are still in their infancy, and there is much work to be done to address the associated challenges. At the same time, we are encouraged to see that growing efforts from various fields are advancing this emerging area of research. We can optimistically anticipate that FNAs will play an increasingly significant role in cell-based scientific studies.

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https://doi.org/10.1021/jacsau.4c00776
JACS Au 2024, 4, 4110−4128

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work is supported by the National Key Research Program (2021YFA0910100), the National Natural Science Foundation of China (NSFC 21922404, 22174039, and 21827811), the Science and Technology Project of Hunan Province (2021RC4022, 2019SK2201 and 2017XK2103), and the Health Commission of Zhejiang Province Grant (WKJ-ZJ-2103).

■ ABBREVIATIONS

FNA, framework nucleic acid; CTC, circulating tumor cell; EV, extracellular vesicle; ROS, reactive oxygen species; pHLIP, pH (low) insertion peptide

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https://doi.org/10.1021/jacsau.4c00776
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