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Mini-Review

Utility of Multivalent Aptamers to Develop Nanoscale DNA Devices against Surface Receptors

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Cite This: *ACS Omega* 2021, 6, 12382−12391

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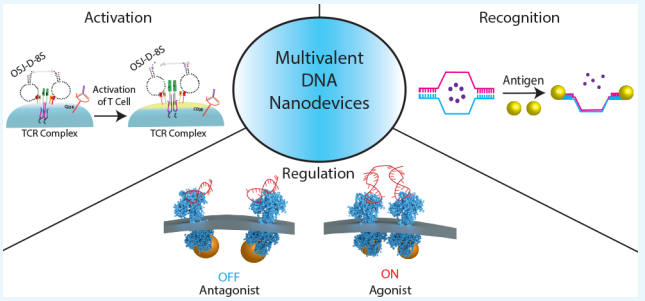
ABSTRACT: DNA nanotechnology is undergoing rapid progress in the assembly of functional devices with biological relevance. In particular, currently, the research attention is more focused on the application of nanodevices at the interface of chemistry and biology, on the cell membrane where protein receptors communicate with the extracellular environment. This review explores the use of multivalent nucleic acid ligands termed aptamers in the design of DNA-based nanodevices to probe cellular interactions followed by a perspective on the untapped utility of XNA and UBP nanotechnology in designing functional nanomaterials with broader structural space.



INTRODUCTION

Ligands, such as neurotransmitters, antibodies, hormones, growth factors, and many other molecules, bind to cell membrane receptors to initiate a cascade of cell signaling processes. Precision and efficiency play an important role since cells respond to these interactions, making decisions that alter the cell's own biological state. The cell surface features multivalency by the interaction among multiple ligands with a cluster of receptors, leading to multiple interactions.[1] An increase in effective ligand concentration in multivalent interactions contributes to enhanced ligand−receptor recognition, leading to, in turn, positive or negative cooperativity while ensuring precise, yet reversible, chemical interactions.[1] When the interaction is between two cells, molecular recognition is governed by highly organized concurrent events between the two cellular entities.

Among ligands, antibodies are unique, in comparison to other types of known ligands, owing to their inherent multivalency, e.g., 2, 4, 6, and 10.[1] The main function of antibodies in the immune system is to identify and neutralize foreign pathogens, such as bacteria and viruses. Therefore, in some instances, the multivalency in antibodies plays a functional role in mediating cellular interactions. For example, immature monomeric IgM exhibits relatively lower affinity toward its antigen; however, secreted soluble pentameric IgM (sIgM) with 10 binding sites shows higher avidity against multiple antigens, resulting in rapid and specific recognition of foreign cells.[1] Serum IgA, for instance, is typically bivalent. However, IgA in mucosa tends to be dimeric, trimeric, or even tetrameric. IgA protects mucosal surfaces from bacteria, viruses, and toxins by direct neutralization or by blocking their binding to mucosal surfaces.[1] Thus, in this case, multivalent IgA has the advantage over invading ligands by the presence of multiple binding sites.

Multivalency is a key property of simultaneous interactions between mixtures of ligand−receptor pairs, leading to specific, precise interactions between two cells.[1] For example, the interaction between a T-cell and an antigen-presenting cell (APC) is mediated by intricate multiple receptor−ligand interactions, resulting in higher precision in cellular recognition.[1] Naturally existing multivalent interactions could offer a guide toward how nature uses multivalency to achieve efficiency, functionality, and precision in biological recognition. Indeed, drawing inspiration from the use of multivalency in nature, it is possible to design synthetic devices using multivalent ligands to mimic natural interactions. To this end, various attempts have been made to develop synthetic materials using peptides and supramolecular chemistry.[2] Herein, we explore the applicability of artificial nucleic acid ligands, also known as aptamers in DNA nanotechnology, in the development of bioinspired materials and devices to mimic and sense biological interactions on the cell surface.

DNA-BASED NANODEVICES

Since the structure and function of a molecule are closely related, nanoscale precision of synthetic structures is essential

Received: March 21, 2021
Accepted: April 20, 2021
Published: May 4, 2021

in designing functional devices. Nucleic acids, one of the essential macromolecules found in organisms, play a dynamic role in encoding a large amount of genetic information. By exploiting the nature of its precise programmability, Seeman showed that DNA could be used as a structural material to assemble ordered nanostructures and ushered in the field of structural DNA nanotechnology.[3] Years later, Rothemund showed how to assemble 2-D DNA structures called origami. Origami structures are based on long, single-stranded DNA folded into well-defined nanoshapes held together by short oligonucleotide staples.[4] More complex designs and further breakthrough developments later followed, expanding structural space and technologies to design exotic shapes.[5] Additionally, functional space of DNA nano-objects has been expanded by the design of biocompatible, programmable assemblies as sensing devices, measurement tools, and DNA-based computation to design smart stimuli-responsive materials for multiple applications.[5b,6] The most attractive feature of DNA is the ability to design materials with precise spatial control facilitated by well-understood Watson−Crick base pairing for a given application.

In the following sections, we will review the self-assembly properties and chemical tagging of DNA, focusing particularly on cell-surface targeting.

DNA NANOTECHNOLOGY ON THE CELL SURFACE

Cellular membranes are composed of hydrophobic membrane structures; therefore, given the polyanionic nature of the nucleic acid backbone, the application of DNA nanodevices on the cell surface is usually challenging. One way of developing DNA-based nanodevices against the cell-surface membrane is by appending various hydrophobic groups to the DNA molecule. This avenue has been facilitated by the chemical versatility of DNA and availability of a wide range of functionalized phosphoramidites. Thus, by designing DNA nanodevices with lipophilic anchors, for example, several groups have demonstrated that complex DNA nanostructures could be assembled for a specific function on the cell-surface membrane. For instance, Langecker et al. engineered nanometer-scale transmembrane channels using scaffolded DNA origami decorated with 26 cholesterol moieties.[7a] Using single-channel electrophysiological measurements, this study reported that the DNA device acts in a manner similar to that of the natural ion channel. In another study, Suzuki et al. introduced a dynamic photoresponsive DNA origami nanostructure inserted in the cell membrane.[7b] The designed hexagonal structures decorated with azobenzene enabled assembly and disassembly in response to irradiation of light. To observe isotropic−nematic transition in two dimensions, Czogalla et al. introduced nanoneedles anchored by cholesterol and fluorescent labeling at defined positions as a tool.[7c] Apart from engineering devices, Li et al. developed a three-dimensional amphiphilic pyramidal DNA scaffold appended to cholesterol, as a membrane anchor, to decorate the cell surface and promote cell−cell interactions.[7d] Other DNA modifications to enhance membrane interactions include adding stearyl, diacyl lipids, and multichain lipid molecules to DNA to develop amphiphilic DNA. For instance, Liu et al. demonstrated that amphiphilic DNA decorated with pyrene could be assembled into micellar structures with optical activity.[7e] To incorporate biological functionality to a DNA device, Jin et al. engineered alkaline phosphatase-dependent cell membrane adhesion molecules from DNA scaffolds linked with phosphorylated lipid molecules.[7f] The high expression of alkaline phosphatase cleaves the phosphate group from the lipid molecule, allowing rapid and selective insertion of the DNA device based on elevated alkaline phosphatase levels. This study demonstrated how to exploit the unique cellular environment in designing functional DNA-based nanodevices. Furthermore, using a protein-based ligand, ephirin-A5 toward receptor EphA2, DNA nanocalipers composed of the ligand and DNA origami was used for regulation of the EphA2 receptor expressed on MDA-MB-231 breast cancer by a team led by Högberg and Teixeira. In this device, the distance between the ligands was varied from 100.1 to 42.9 nm in dimeric structures and to 14.3 nm in a saturated polyvalent structure, demonstrating the ability to engineer precise structures combining protein ligands and DNA origami to regulate a specific receptor in its native conformational state.[7g] Many reports in the literature show that DNA-based nanodevices consist of both simple or complex molecular scaffolds engineered as functional tools to manipulate the cell-surface membrane for biomedicine.[7h] However, most of these demonstrations are restricted owing to the limited specificity of the device. For example, DNA devices anchored to hydrophobic moieties are not specific toward one set of cells or a specific protein expressed on the cell membrane, thus limiting the scope of the DNA device. Moreover, in response to external stimuli, it is well-known that membrane protein expression levels can vary, leading to an altered biological outcome. In order to study such alterations and develop devices to recognize, sense, and manipulate subsets of cells with variable cell-surface receptor profiles, a specific targeting moiety is essential. In the following sections, we explore the application of multimeric−multifunctional nucleic acid ligands termed aptamers as a recognition, regulating, activating, and sensing moiety, as well as an anchoring moiety for DNA-based devices, to study cell-surface receptors and their biological interactions.

APPLICABILITY OF APTAMERS (ARTIFICIAL NUCLEIC ACID LIGANDS) TO DESIGN DNA NANODEVICES

The application of DNA nanodevices to probe cell-surface receptor interactions relies on the introduction of robust, versatile, and synthetically compatible ligands against biologically and functionally critical cellular targets. Typical ligands available in biological interactions are antibodies, protein-based ligands, such as growth factors and peptides, or other small-molecule ligands. However, attaching protein-based ligands or small molecules to a DNA nanodevice could involve cumbersome, complex chemistry.

The introduction of functional capacity into a DNA device would be more feasible if the ligand is also made from nucleic acids. Accordingly, artificial nucleic acid ligands, also termed aptamers, would be the most appropriate candidate to design functional DNA-based nanodevices. Aptamers consist of single-stranded DNA/RNA/XNA/UBP (X = Xeno nucleic acids, UBP = unnatural base pairs) molecules that specifically bind to a target with high affinity and specificity. Aptamers can be selected against a wide range of targets, including small molecules, proteins, whole cells, and tissues. Aptamers are often compared to antibodies; interestingly, however, they are synthetic nucleic acids with properties resembling both small molecules and antibodies. These properties, as outlined below, show the advantages of utilizing aptamers in DNA-based nanodevices.

https://doi.org/10.1021/acsomega.1c01513
ACS Omega 2021, 6, 12382−12391

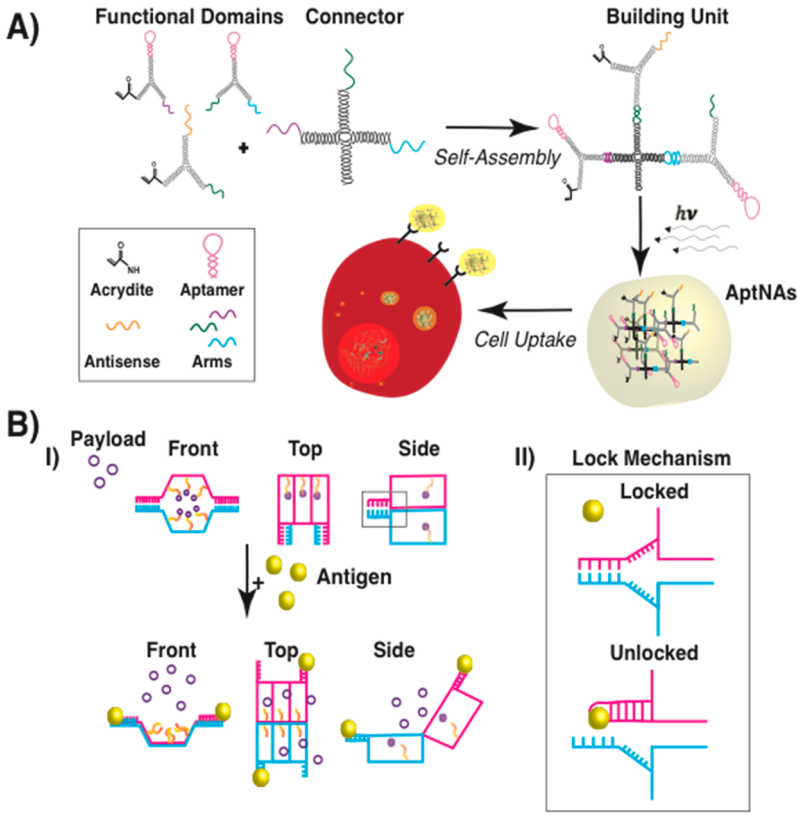


Figure 1. Illustrations of aptamer-based multivalent DNA nanodevices. (A) Schematic of the self-assembly and photo-cross-linking processes to form aptamer-based nanoassemblies (AptNAs). Y-shaped functional domains made from DNA sequences, including aptamers, antisense oligonucleotides, and acrydite-modified ssDNA, hybridize with the cDNA of the X-shaped connectors to create building units. Through photopolymerization, hundreds of these building units are cross-linked via the acrydite-modified ssDNA to form multifunctional, programmable AptNAs. (B) Design and mechanism of aptamer-locked DNA nanorobot: (I) front, top, and side views of the DNA nanorobot in closed conformation, switching to open conformation once the aptamer locks are activated in the presence of antigen (yellow); (II) aptamer lock mechanism consisting of DNA aptamer (pink) and a partial complementary strand (blue). When the antigen key (yellow) is within range of the lock mechanism, the aptamer dissociates from the complementary strand to form an aptamer−target complex, leaving the system unlocked.

First, both aptamer and nanomaterial can be synthesized by well-established solid-state chemistry using a standard DNA/RNA synthesizer in only one step. This offers precise synthesis, while ensuring proper quality control of the end products. Second, aptamers are inherently monovalent, and antibodies are typically multivalent. The monovalent nature of aptamers leads to relatively lower affinities for aptamers. However, in designing multivalent DNA-based nanodevices, such lower affinities would allow the fine-tuning of avidity toward antigen to attain the desired biological outcome. This is not possible using antibodies. Third, aptamers are smaller in size, and their pharmacokinetic properties resemble those of a small molecule with rapid renal clearance. Generally, small-molecule ligands are challenging to modify without interfering with their biological function. While aptamers show rapid renal clearance, they are, structurally, polymeric, consisting of 40−50 DNA/RNA/XNA/UBP molecules, allowing the strategic manipulation of chemical functionality without compromising target binding. This polymeric nature of aptamers makes the structure−activity relationship (SAR) studies feasible by systematic truncation, followed by functional binding assays.

Importantly, aptamers are identified using an in vitro screening method called systematic evolution of ligands by exponential enrichment (SELEX). Using SELEX, aptamers can be selected against a wide variety of target molecules. Since the original introduction of SELEX in 1990 by Szostak and Ellington, Gold and Turek, and Joyce, a number of variants have been developed to increase specificity of aptamers toward the target.[8] Among them is one of the newest technologies called ligand-guided selection (LIGS).[8b,c] Using LIGS, aptamers can be identified against cell-surface proteins in their native conformation. LIGS, as a variant of SELEX, exploits the combinatorial nature and the competition of weak and strong binders of a SELEX library to identify highly selective aptamers. We used LIGS to identify aptamers against membrane IgM and the CD3 complex required for TCR surface expression, demonstrating the capacity of LIGS to generate highly specific functional nucleic acid ligands against highly complex key receptors expressed in B-cells and T-cells.[8c] Furthermore, we recently diversified the SELEX library by incorporating Benner's artificially expanded genetic information system (AEGIS) bases dZ and dP, which led to the identification of AEGIS DNA ligands against T-cell receptor complex CD3ε.[8b] The aptamers generated by AEGIS-LIGS highlight the simplicity of LIGS to identify AEGIS ligands against highly complex protein receptors in their native conformation, allowing the use of aptamers with better functional capacity and folding to be incorporated into device development. Collectively, the characteristics of aptamers make them ideal for generating functional molecular devices based on nucleic acids. Thus, the combination of nucleic acid nanotechnology and highly specific aptamers against known

https://doi.org/10.1021/acsomega.1c01513
ACS Omega 2021, 6, 12382−12391

cell-surface receptors enables the design and engineering of devices with precise structural parameters to introduce functionality.

NANODEVICES USING APTAMERS (ARTIFICIAL NUCLEIC ACID LIGANDS) AS RECOGNITION ARMS

Engineering DNA-based nanodevices using aptamers as recognition arms has been reported. For example, Wu et al. elegantly demonstrated that a multivalent aptamer platform could be designed from polymerized DNA architectures (Figure 1A)[9] without the use of complex DNA origami. Termed multifunctional aptamer-based nanoassemblies (AptNAs), this study showed the potential of AptNAs in tumor cell targeting and therapeutic development.[9] The first unit of the nanoassembly consisted of a trivalent Holliday junction Y-shaped functional DNA domain that could be used for conjugation to aptamers, anticancer drugs, cDNA, acrydite-modified ssDNA, or antisense oligonucleotides. The second unit was made of a tetravalent Holliday junction X-shaped DNA core connector with strands complementary to the Y-shaped DNA domain. Using the programmability of DNA, hundreds of these building blocks were hybridized and further cross-linked via the photopolymerization of the acrydite-modified ssDNA to form AptNAs with controllable size and target capability for increased precision in drug delivery by promoting increased cell uptake and decreased drug resistance in tumor cells.

Douglas and Church showed one of the first examples of utilizing heteromultivalent aptamers in a DNA origami nanodevice to achieve higher precision (Figure 1B).[10] Here, Douglas et al. designed an autonomous nanorobot with the multiple functions of transporting payloads, sensing specific cell-surface markers using multiple aptamer molecules, responding to a stimulus, and switching conformation.[10] The nanorobot is controlled through two aptamer-encoded logic gates, which allows aptamers to be chosen for a wide selection of targets, not solely cell-surface markers. The lock mechanism on this nanorobot is based on a target-induced conformational change of the aptamer−complement duplex switching to an aptamer−target complex, releasing the complementary strand from the aptamer and opening one side of the nanorobot. This DNA origami nanorobot demonstrated autonomous recognition of combinations of target antigens on cells using an AND Boolean operator for added selectivity in drug delivery.[10]

In addition to the aptamer−target complex used to open the locks, a nanorobot could also be activated by a DNA key that hybridizes with the lock's complementary strand to release the aptamer strand via toehold-mediated migration.[11] The DNA key is then able to access the lock of an adjacent robot, altering its state to active and open. The presence of the DNA key assigns a "positive regulator" phenotype to the nanorobot, while DNA clasps that prevent the opening of the nanorobot, even with target antigens present, can be added to assign a "negative regulator" phenotype to the robot. In mixing different ratios of different phenotypes of robots, logic gates, including AND, OR, XOR, NAND, NOT, CNOT, and a half adder, were assembled for cell targeting.[11]

Furthermore, to illustrate the application of autonomous nanorobots, Li et al. constructed an autonomous nanorobot through DNA origami with multifunctionality in targeting tumor cells and transporting payloads.[12] Aptamer AS1411, which specifically recognizes nucleolin expressed on tumor-associated endothelial cells, was integrated into the DNA nanostructure. Additionally, the blood coagulation protease thrombin was conjugated to the inner cavity of the nanorobot.[12] In this design, the aptamer responds to the presence of the target cell-surface marker and simultaneously triggers the conformational switch in the nanorobot to expose the protease thrombin necessary for coagulation. In turn, intravascular thrombosis results in tumor necrosis and inhibition of further tumor growth in mice models, proving the vast potential for DNA nanotechnology in cancer therapies.[12]

MULTIVALENT FUNCTIONAL APTAMERS AS DEVICES TO REGULATE RECEPTOR BIOLOGY

Another area of aptamer development is the engineering of multivalent aptamers tethered by a polyethylene glycol (PEG) linker or double-stranded DNA segment without the use of complex origami assemblies. Cell-surface biomarkers are functionally essential molecules involved in biological processes, such as signal transduction, cell adhesion and migration, cell−cell interactions, and communication between the intra- and extracellular environments. In the past decade, many aptamers targeting cell-surface biomarkers have been developed for tumor therapy,[13] including those inducing antitumor immune response, inhibiting tumor growth, inhibiting viral infection, and engaging immune cells for tumor cell lysis. Structural design and modulation of activity at the nanoscale are hallmarks of DNA nanotechnology, allowing scientists to engineer DNA and RNA aptamer systems that mimic antibody functions.

For instance, aptamers have been widely applied in immunomodulation. One of the central co-stimulatory receptors responsible for the proper activation of T lymphocytes is the CD28 receptor. Pastor et al. reported on the isolation dimerization of the anti-CD28 receptor aptamer to cross-link CD28 receptors. Two dimeric variants were designed using two aptamers, CD28Apt7 and CD28Apt2, linking with a 21-nucleotide linker, reflecting the average distance between two Fc domains of IgG molecules approximately equal to 65 Å. Using the same monomers, Pastor et al. explored the functionality of dimeric variants without a linker by directly connecting monomers. While the affinities of the dimeric versions were similar to those of the monomeric versions, monomeric aptamers acted as antagonists, whereas, interestingly, the dimeric variants acted as agonists. The monomeric aptamer blocked the binding of the bivalent antibody to the CD28 receptor, leading to a robust inhibitory effect on the proliferation of purified CD4 lymphocytes. On the other hand, the dimeric variants provided an artificial co-stimulatory signal to enhance the antitumor immune response. Strong cell proliferation was achieved with the directly linked dimer, CD28 Apt7-dimer, which showed the highest proliferation, 52.1−58%, compared to proliferation of 37−39.4% with the CD28 Apt7-dimer AB, which contained a linker. As a source of comparison, the cell proliferation ratio of anti-CD28 antibody was 37−40.8%. In addition, a strong cellular response was achieved with the CD28 Apt7-dimer, where the treated mice generated a cellular immune response stronger than those receiving the control vaccinations.[14] This behavior, whereby the monomeric aptamer acts as the antagonist, while the dimeric version shows agonistic behavior, is not surprising, as similar behavior has been previously reported in the literature for corresponding antibodies and antibody fragments, suggesting that the aptamer could be

https://doi.org/10.1021/acsomega.1c01513
ACS Omega 2021, 6, 12382−12391

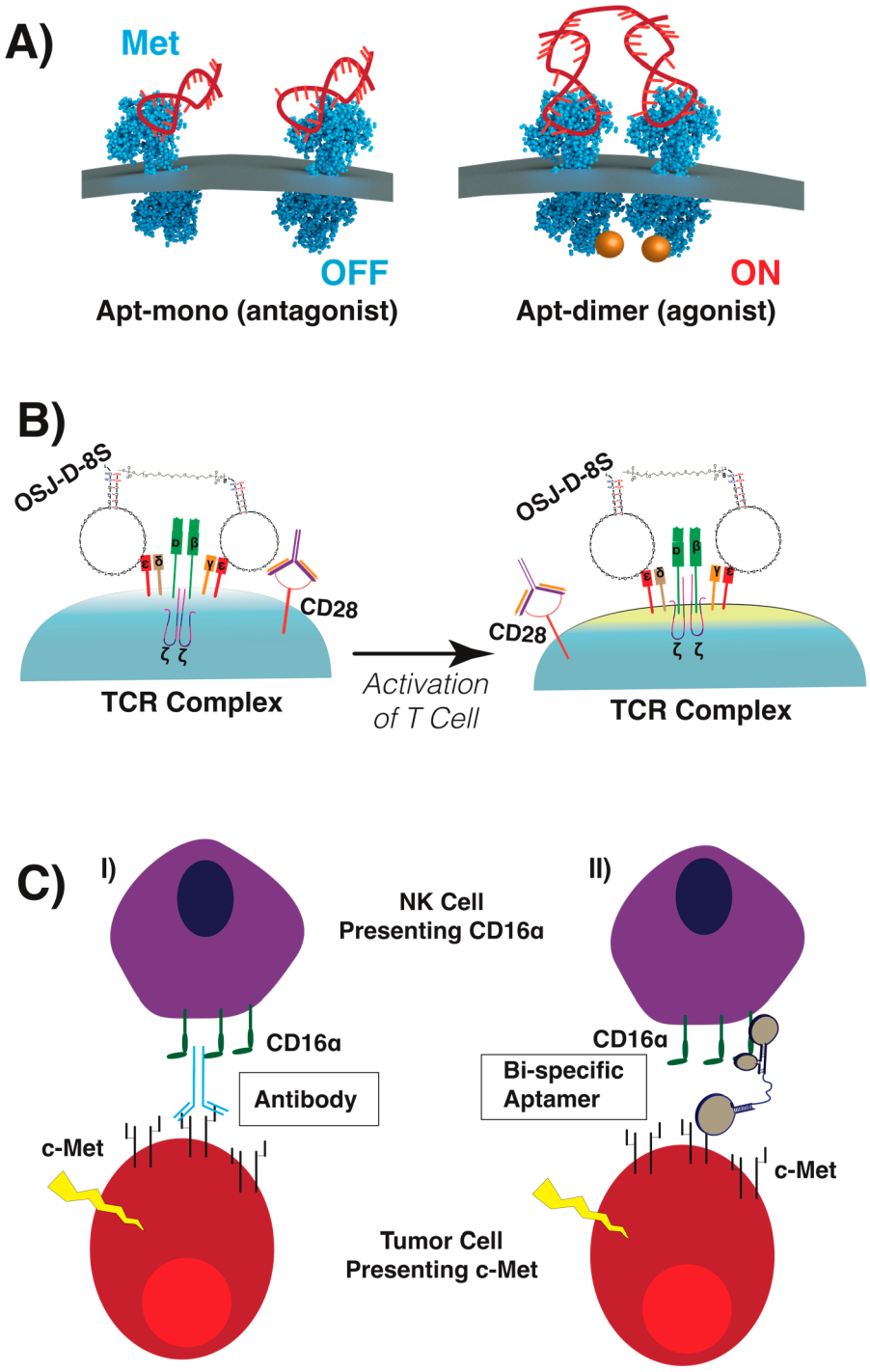


Figure 2. Schematic representation of three different systems that use dimerized aptamers to activate biological function. (A) Met activation in the presence of monomeric aptamer (Apt-mono) and dimeric aptamer (Apt-dimer). Apt-mono acted as an antagonist with no activation, whereas Apt-dimer acted as an agonist that activated the Met receptor. (B) LNA- and OMe-RNA bases-modified dimer OSJ-D-8S, binding to CD3 complex. The dimer OSJ-D-8S activated T-cells after 6 h of incubation at 37 °C in the presence of the co-stimulatory CD28 antibody that bound to CD28 receptor on the T-cell surface. (C) Tumor cell lysis induced by (I) antibody-dependent recruitment of natural killer cells, using CD16 receptor, to target overexpression of c-Met receptor on tumor cells and (II) heterodimer aptamer binding to mediate this same natural killer cell recruitment pathway.

assembled in multivalent structures to achieve specific functionality.

4-1BB is another primary co-stimulatory receptor that promotes the survival and expansion of activated T-cells such that the agonistic anti-4-1BB antibody enhances tumor immunity in mice. McNamara et al. sought to achieve similar agonistic activity using an anti-4-1BB aptamer with co-stimulated $CD8^+$ T-cells in vitro to enhance the proliferation of suboptimally stimulated $CD8^+$ T-cells. They designed a multimeric aptamer that exhibited a high co-stimulatory effect and increased T-cell proliferation by 46% compared to the monomeric 4-1BB aptamer. In a more clinically relevant

https://doi.org/10.1021/acsomega.1c01513
ACS Omega 2021, 6, 12382−12391

Table 1. Multivalent Aptamers Designed for Improved Functionality

target	function after dimerization	valency	distance between the monomers/linker type	dissociation constant of bivalent aptamer	ref
CD28$^+$ on T-lymphocytes	enhanced cell proliferation; promoted cellular immune humoral response	bivalent RNA aptamer	21 bp RNA duplex strand (∼65 Å) direct linking; same single strand	same as monomeric	14
4-1BB$^+$ on CD8 $^+$ T-cells	enhanced cell proliferation and antitumor immunity; increased IFN-γ secretion	bivalent RNA aptamer	21 bp RNA duplex strand (71 Å)	not reported	13a
4-1BB$^+$PSMA$^+$ on CD8 $^+$ T-cells	co-stimulation at the site of disseminated tumor; enhanced antitumor immunity	heterotrivalent RNA aptamer	21 bp RNA duplex strand	not reported	15
CTLA-4$^+$ on CD25$^+$CD4$^+$ T-cells	increased cell proliferation; enhanced antitumor immunity	tetravalent RNA aptamer	20 bp DNA duplex strand (68 Å) with four single-stranded ends complementary to aptamers	not reported	16
OX40$^+$ on T-lymphocytes	enhanced T-cell proliferation ratio; increased IFN-γ secretion	bivalent RNA aptamer	two 20 nt DNA scaffolds complementary to aptamer, connected by a PEG spacer (18-carbon)	same as monomeric	13b
Met$^+$ on parenchymal cells	activated growth factor receptor; reduced progression of Fas-induced fulminant hepatitis	bivalent DNA aptamer	direct linking, 3′ to 5′ without a spacer	not reported	17
HER2$^+$ on breast cancer cells	enhanced antitumor activity by silencing EGFR in vivo and in vitro	heterobivalent RNA aptamer	21 bases of EGFR siRNA with 2−4 unpaired base linkers	not reported	18
hTFR$^+$ on New World hemorrhagic fever mammarena viruses	inhibited the binding site of the human transferrin receptor (hTFR)	trivalent RNA aptamer	biotinylated aptamers assembled on streptavidin	trivalent aptamer: WAZ, multi: 12 ± 1 nM	19
mIgM$^+$ on B-cells	specifically, recognized mIgM- expressing cells	bivalent DNA aptamer	3, 5, and 7 repeats of spacer 18 (consists of 6 PEG units)	bivalent aptamers at 25 °C: DR 1.2-3S: 11.4 ± 1.39 nM DR 1.2-5S: 20.8 ± 3.21 nM DR 1.2-7S: 48.6 ± 3.28 nM	21
mIgM$^+$ on B-cells	increased avidity at physiological condition in vivo and in vitro	1-bivalent DNA aptamer 2-trivalent DNA aptamer 3-tetravalent DNA aptamer	6, 8, and 12 repeats of spacer 18 (12.6, 16.8, and 25.23 nm)	bivalent aptamers at 37 °C: L-BVA.8S: 6222 nM L-TVA.8S: 256 nM L-TetVA.8S: 272 nM	22
CD3$^+$ on T-cells	activated T-cells	bivalent DNA aptamer	2, 4, 6, and 8 repeats of spacer 18; consists of 6 PEG units	bivalent aptamers: OSJ-dimer-2S: 0.5 nM OSJ-dimer-4S: 0.3 nM OSJ-dimer-6S: 0.4 nM OSJ-dimer-8S: 1.7 nM	23
CD16α^+cMet$^+$ on natural killer cells and tumor cells	enhanced intercellular communication; enhanced antibody-dependent cellular cytotoxicity (ADCC)	heterobivalent DNA aptamer	single-stranded DNA oligonucleotides with various linkers—15-deoxyadenosine, up to 44 nt, or short PEG chains—with lengths ranging from 0 to 217 Å	bivalent aptamers: targeting CD16: 15 to 197 nM targeting c-Met: 0.16 to 370 nM	24

https://doi.org/10.1021/acsomega.1c01513
ACS Omega 2021, 6, 12382−12391

format, they utilized a bivalent aptamer that also co-stimulated the $CD8^+$ T-cells, increasing proliferation, both in vivo and in vitro, promoting tumor immunity.[13a] These results demonstrate how engineering aptamers into multimeric scaffolds can transform aptamer functionality.

Although these studies show promising results, insufficient co-stimulation within an actual tumor site remains a significant reason why tumors are not responsive to therapeutics. To address this, Pastor at al. reported a new clinically feasible multivalent aptamer design combining the 4-1BB bivalent aptamer and a prostate-specific membrane antigen-binding aptamer (PSMA) to localize and promote in situ co-stimulation of disseminated tumor cells. This led to significant inhibition of tumor growth and long-term tumor rejection.[15]

Similarly, Santulli-Marotto et al. reported the development of an RNA aptamer that binds to receptor CTLA-4 with high affinity and specificity, leading to inhibition of CTLA-4 function. Although the monomeric variant of the aptamer did demonstrate CTLA-4 inhibition, the tetrameric variant of the aptamer, which was generated by in vitro assembly, significantly enhanced its avidity, causing inhibition in vivo and in vitro.[16]

In addition to the ability of multivalent aptamers to increase binding attributes and co-stimulate cell activity, they can also be designed toward cell activation without the need for antibody co-stimulation. The activation of receptors often occurs when receptors are multimerized on the cell surface through ligand binding. As opposed to co-stimulatory agents, which are designed to mimic antibody domain spacing, Dollins et al. introduced a dimeric aptamer against OX40 that links receptor domains over their standard spacing. A flexible DNA scaffold containing a polyethylene glycol spacer was used to bind two copies of the aptamer, to match spacing within the natural receptor−ligand complex. The introduced flexibility between the aptamers acts to model the OX40 ligand when complexed with the OX40 receptor, where the overall ligand spacing can potentially span between 39 and 76 Å. The activation of OX40 by the engineered dimerized aptamer induced nuclear localization signal NFκB, cytokine production, cell proliferation, and enhanced immunotherapy when systemically delivered to mice.[13b]

In the same manner, Ueki et al. reported a dimerized aptamer to bind and induce dimerization of two subunits of growth factor receptor (Met), mitigating the progression of Fas-induced fulminant hepatitis in a mouse model.[17] The bivalent agonistic aptamer features high stability owing to a sequence added to the 3′-terminus of the dimer that serves as a protective motif against 3′ exonuclease activity by adopting a protective stem-loop structure (Figure 2A).

The multimerized aptamer can accommodate more than one type of aptamer to target different receptors and, as a result, induce multiple biological responses. For example, Yu et al. designed a three-in-one nucleic acid aptamer−small interfering RNA (siRNA) chimera by fusing HER2 and HER3 aptamers together with a double-stranded EGFR siRNA. The siRNA acted as a linker, as well as an additional therapeutic compound. Both aptamers HER2 and HER3 act as antagonistic molecules for blocking the HER2 and HER3 signaling pathways, aiding in tumor uptake of siRNA by inducing specific internalization.[18]

While many aptamers are focused on immunomodulatory applications, aptamers can also be used as therapeutic compounds through the blocking of receptors. Maier at al. describe the development of an aptamer against the human transferrin receptor, which plays a significant role in human infection. The 48-nucleotide aptamer, termed WAZ, could bind the receptor's apical domain without interfering with transferrin binding and inhibit infection of human cells by five recombinant New World clade B hemorrhagic fever mammarenaviruses in culture. Aptamer multimerization further enhanced this inhibition >10-fold compared to the monomeric variant.[19]

As discussed previously, dimerization can be used to increase aptamer co-stimulation and activation, and this procedure can also be used with a truncated system to further enhance aptamer function. For example, dimerization of a previously truncated aptamer toward B-cell IgM, termed R1,[20] was shown to improve avidity at 25 °C, as presented by Batool et al. Different linker lengths were tested to improve the affinity of the dimer toward B-cells without compromising specificity. Upon testing B- and T-cells from a healthy mononuclear sample, the dimers bound to B-cells but showed no binding affinity toward T-cells. The failure of dimeric R1.2 to bind with T-cells obtained from PBMCs suggests that the dimeric aptamer could distinguish between IgM expressed on the B-cell membrane from other Igs expressed on non-B-cells.[21]

Similarly, Mallikaratchy et al. systematically truncated and modified aptamer TD05 with locked nucleic acids (LNA) as a tool to increase conformational stability and nuclease resistance. Following this, the aptamer was multimerized into dimeric, trimeric, and tetrameric versions with optimized PEG linker lengths. In addition to increasing aptamer valency to assist the binding affinity, the effect turned out to be saturated at a certain point. The trivalent and tetravalent aptamers showed binding significantly higher than that of the dimer, but they were not significantly different from each other. This result suggests that receptor density on the cell surface limits increase in the dissociation constant. Testing the dimeric aptamer with B-cell lymphoma using fresh mononuclear cells from patients with chronic lymphocytic leukemia demonstrated that multimerization of the aptamer improved avidity while, at the same time, retaining its specificity at physiological conditions both in vivo and in vitro.[22]

Linker length plays a vital role in the activation of cells induced by multivalent systems. For instance, Freage et al. explored the effect of truncation and LNA and 2′-OMe RNA base modification on anti-TCR-CD3ε aptamers as T-cell activators (Figure 2B). Here, using different dimer linker lengths of PEG, Freage et al. designed and tested dimeric TCR-CD3ε aptamer variants of different lengths. Interestingly, though none of the dimeric aptamers with shorter linkers activated TCR-CD3ε, the construct with eight PEG spacers with dimensions similar to those of an antibody could activate TCR-CD3. This suggests that the design of functional dimeric aptamer scaffolds is predominantly governed by the space between the aptamers and directly related to the linker length, particularly against TCR-CD3ε.[23] Summary of multivalent aptamers and their functions is listed in Table 1.

This work offers insight into how DNA structure can be used to organize functional moieties, such as aptamers, although cell−cell binding can also be mediated by aptamer constructs organized through different linking methods. Boltz et al. aimed to guide immune response at the tumor site using various linkers to dimerize the c-Met aptamer, targeting cancer cells with CD16α aptamer, which interacts with natural killer cells expressing receptor CD16α (Figure 2C). Through

https://doi.org/10.1021/acsomega.1c01513
ACS Omega 2021, 6, 12382−12391

selecting an optimized linker, this study successfully showed that an immune response could be raised through natural killer cells that can target cancer cells.[24]

FUTURE PERSPECTIVES

From DNA-Based Nanotechnology to XNA or UBP-Nanotechnology. The growth of DNA-based nanodevices, in particular, has been accelerated by the availability of structural data on DNA. The introduction of relatively new XNAs and artificial nucleic acid pairs composed of XNA/UBP:XNA/UBP, XNA/UBP:DNA, and XNA/UBP:RNA polymers is rapidly growing.[25] However, so far, the utilities of XNA and UBP are mainly aimed at synthetic genomic, prebiotic chemistry, and biology applications. The use of XNA in device or ligand design, i.e., XNA or UBP nanotechnology, could offer a broad range of nucleic acid fold space. We still do not know enough about achievable prospective fold space or XNA/UBP polymers' ability to transform the design and development of molecular devices. Benner and others' efforts yielded a variety of XNA bases and unnatural base pairs, and these XNAs bases could be incorporated into nucleic acid device designs.[25a−c,e,f] Still, the XNA and UBP ligand/device development faces its challenges. For example, while structural data are available for some base pairs, extensive structural exploration of the chimeric UBP:DNA, UBP:RNA, XNA:DNA, and XNA:RNA duplexes are not fully explored. Lack of structural data might hinder the predictability of the shape of the device. Furthermore, XNA and UBP are not compatible with current high-throughput sequencing strategies.[25] Moreover, the lack of evolved polymerases to amplify XNA and UBP nucleic acid libraries restricts the facile identification of aptamer using LIGS or SELEX.[8b,25] The lack of commercial availability of the UBP- and XNA-modified oligo strands restricts its wide range of applicability in laboratories with limited synthesis capabilities.[25] Nevertheless, the relevance of UBP-aptamer-based multivalent molecular tool development and the applicability of XNAs and UBP for device development is an exciting avenue to be explored. The generation of highly functional ligands using in vitro selection approaches, such as LIGS and SELEX, against functionally important, already established cellular or subcellular surface targets offers ligands that could be fine-tuned to achieve specific functions on the membrane surface. Combining these ligands with available tools in DNA/RNA/XNA/UBP nanotechnology will allow us to engineer new kinds of programmable artificial synthetic nanomachines. We anticipate that such nanomachines will offer versatile structures, functions, and flexibility, widening the scope of nucleic acid−based nanodevices in molecular analysis on the cell surface.

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Notes

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Natalie Boykoff received her Bachelor of Science in Chemistry from New York University in 2018. She is currently a graduate student with a specialization in Nanotechnology at the Ph.D. program in Chemistry, The CUNY Graduate Center. Her projects involve the design of programmable DNA nanodevices using nucleic acid aptamers against the T-cell receptor complex. If successful, these devices will enable selective activation of T-cells that could be used to regulate cell−cell interactions.

Prabodhika Mallikaratchy, Ph.D., is an associate professor in the Department of Chemistry at Lehman College. She earned her Ph.D. in 2008 from the laboratory of Prof. Weihong Tan at University of Florida, Gainesville, FL. She joined the laboratory of Physician-Scientist David Scheinberg's in 2008 a postdoctoral fellow. At Scheinberg lab, she was awarded the Lauri Strauss Leukemia Research Fellowship and the Lymphoma Research Foundation Research Fellowship to support her research on aptamers against B-cell receptor complex. She joined the department of chemistry, Lehman College at The City University of New York, in 2012. She leads an NIGMS-funded laboratory at Lehman to discover and develop artificial nucleic acid ligands and nanodevices to control cellular interactions. She has been a recipient of the Junior Faculty Research Award in Science and Engineering and, recently, the Maximizing Investigators' Research Award (MIRA).

ACKNOWLEDGMENTS

Authors are grateful for National Institute of Health General Medical Sciences Grant Nos. SC1 GM122648 and R35 GM139336 for financial support.

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