ChemComm

DNA-modulated dimerization and oligomerization of cell membrane receptors

Journal:	*ChemComm*
Manuscript ID	CC-FEA-06-2024-003077.R1
Article Type:	Feature Article

DNA-modulated dimerization and oligomerization of cell membrane receptors

Ahsan Ausaf Ali [a] and Mingxu You *[a,b]

[a] Department of Chemistry, University of Massachusetts Amherst, Amherst, MA 01003
[b] Molecular and Cellular Biology Graduate Program, University of Massachusetts Amherst, Amherst, MA 01003

* Corresponding author: mingxuyou@umass.edu

Abstract

DNA-based nanostructures and nanodevices have recently been employed for a broad range of applications in modulating the assemblies and interaction patterns of different cell membrane receptors. These versatile nanodevices can be rationally designed with modular structures, easily programmed and tweaked such that they may act as smart chemical biology and cell biology tools to reveal insights into complicated cellular signaling processes. Their outstanding *in vitro* and cellular features have also begun to be further validated for some *in vivo* applications and demonstrated their great biomedical potential. In this review, we will highlight some key current advances in the molecular engineering and biological applications of DNA-based functional nanodevices, with a focus on how these tools have been used to respond and modulate membrane receptor dimerizations and/or oligomerizations, as a way to control cellular signaling processes. Some current challenges and future directions to further develop and apply these DNA nanodevices will also be discussed.

1. Introduction

The cell membrane is a complex mixture of proteins, lipids, and carbohydrates that provides an essential barrier for cells and enables the compartmentalization of the chemistry of life. The membrane structure isolates key intracellular biochemical reactions from the outside environment, which also enables cells to selectively communicate with each other. Signal transduction through the cell membrane is mediated by various membrane transport proteins and receptors. Membrane-associated proteins can interact and work together to form key signaling protein complexes that are critical in maintaining cellular functionality. Complications or dysregulation of these membrane protein interactions are known to lead to various diseases including cancer, diabetes, and central nervous system diseases, etc.[1-3] The ability to detect and modulate these specific membrane interactions, especially on the live cell surfaces, is thus critical for studying cell signaling, identifying new targets for disease diagnostics, as well as providing a new promising approach aid in therapeutics.

Several effective small-molecule ligands (e.g., molecular glues), bispecific antibodies, and aptamers have been developed for modulating membrane protein assemblies.[4-7] Among these different types of modulators emerging in this quickly advancing area, DNA-based self-assembled nanostructures and nanodevices have begun to show some exciting and unique features and functions.[6-8] These functional DNA nanodevices can be applied *in situ* on the cell surfaces via membrane-targeting aptamers, antibodies, covalent linkages, and/or lipid moieties. By working as precise scaffolds and/or reversible linkers, a broad array of DNA nano-assemblies has been recently engineered to control the spatial distributions and/or dynamic interactions among different cell membrane receptors. Such DNA nanodevices can actively engage membrane receptors with high controllability and programmability, used for regulating cellular signaling processes in live systems. This new field of research has begun to allow us to explore, in much greater detail, the dynamic structures and functions of the cell membranes.

In this review article, we will highlight some key advances in this emerging field of DNA-modulated assemblies and interaction patterns of various cell membrane receptors. Starting by introducing different available strategies to specifically modify DNA nanodevices onto cell membranes, we will then summarize a large variety of design principles of these DNA nanodevices as well as the signaling pathways and membrane receptors that have been targeted thus far, with an emphasis on the membrane receptor dimerizations and oligomerizations. Finally, some critical challenges and potential opportunities and directions of this exciting new research area will also be discussed. We hope this review article will inspire more people with diverse backgrounds of chemistry, molecular engineering, nanotechnology, and cell biology, etc., to join this emerging field of study.

2. Modulation of membrane receptor dimerizations with DNA nanodevices

DNA nanodevices have been widely used to develop non-genetically encoded tools to promote or inhibit membrane receptor dimerizations and oligomerizations, which have been successfully used to modulate a range of cellular signaling processes.[6-8] Among the most common membrane receptors explored thus far are integrins, immune cell receptor complexes, and various receptor tyrosine kinases (RTKs) such as the mesenchymal epithelial transition receptor (Met), vascular endothelial growth factor receptor (VEGFR) and the epithelial growth factor receptor family (EGFR). RTKs are essential modulators of cell-to-cell communications,[9-11] which are single-pass transmembrane proteins that mediate signal transduction via lateral dimerizations or oligomerizations in the plasma membrane. The formation of these membrane receptor dimers and oligomers (e.g., through ligand-induced transmembrane domain coupling) can aid in the occurrence of trans-phosphorylation reactions in their cytoplasmic domains. Membrane modulation of these receptor assemblies is known to play a critical role in cancers as well as in regulating normal cellular metabolism and phenotypes such as the cell growth, proliferation, and differentiation.[12-16] Here, we will focus on the versatile mechanisms of highly modular

and responsive DNA nanodevices and showcase how DNA can be an ideal material to achieve membrane modulations with a great degree of precision and efficacy.

2.1. Bivalent aptamers

Aptamers are single-stranded DNAs or RNAs that have been widely used to recognize specific cell membrane receptors. Since the inherent nucleic acid nature of aptamers allows them to be directly incorporated into the DNA nanodevices, these aptamer moieties have been used as one of the most popular ligands to design and implement DNA nanodevices to target certain cell membrane receptors. However, it must be noted that as aptamers often evolved through an *in vitro* systematic evolution of ligands by exponential enrichment (SELEX) process, and compared to antibodies (i.e., their more natural equivalent), the cross reactivities and target specificities of these aptamer molecules should be more carefully examined, as well as their stability and pharmacokinetics for potential *in vivo* applications. The current list of membrane targets that can be specifically recognized by aptamers, especially those with well-characterized binding sites and minimal cross reactivities, is still limited.[17] While still, as shown in the following sections, these available aptamers have already demonstrated their great potential as the target-recognition moieties for engineering functional DNA nanodevices to control membrane receptor functions.

One of the most straightforward ideas in DNA-modulated membrane receptor dimerizations is to directly link two aptamers together, one for each target receptor of interest (Fig. 1A). The first successful demonstration of this strategy was shown by Ramaswamy and collages in 2015,[18] where two VEGFR-targeting DNA aptamers were tethered to each other via an 18-atom hexaethylene glycol spacer. Polyethylene glycol (PEG) has been popularly used to develop these DNA nanodevices, which is because PEG is quite biocompatible and flexible in structures, without imposing steric hindrance on the aptamer-target binding events. After incubating these bivalent VEGFR aptamers with human endothelial cells, targeted phosphorylation of VEGFR and consequently the activation of downstream Akt pathway, as well as the formation of capillary-like tubes, all clearly demonstrated the potential function of these DNA nanodevices as an alternative to the natural cognate ligands of VEGFR in controlling cell signaling.

Similarly, Ueki et al. developed bivalent DNA aptamers to modulate membrane dimerizations of the Met receptors[19] and fibroblast growth factor receptors (FGFRs).[20] Supported self-renewal and pluripotency of stem cells, as well as the enhanced human cell migration and proliferation, can be resulted from these modulated membrane receptor dimerizations. It is worth mentioning that instead of PEG spacers, different lengths of single-stranded or double-stranded DNA linkers were used to conjugate two aptamer moieties. Interestingly, in both studies, a 0-nucleotide “single-stranded linker” was proven to be most efficient in inducing the target receptor phosphorylation. In the case of Met aptamers, the shorter the linkers were, the higher the Met activation levels were observed, which may be due to the proximity or direct contact requirement for the signal activation process. It should also be noted that this is one of the advantages to using DNA nanodevices to develop these bivalent modulators: the relative distance and orientation between two anchoring ligands can be fine-tuned for optimized regulation efficacies. Therefore, the degree of downstream signaling can possibly be controlled very precisely due to these beneficial properties of DNA nanodevices.

Another key advantage of these bivalent aptamer-modulated membrane receptor assemblies is that the binding affinities of aptamers and the corresponding receptor signaling activities can be rationally and easily adjusted, which properties have been well-demonstrated in another study by Akiyama et el.[21] By simply altering the stem sequence of the above-developed Met-targeting bivalent DNA aptamers,[19] they were able to develop partial agonists, with designated maximal receptor phosphorylation level at the saturated ligand concentration. Without sacrificing the half-maximal concentration of these artificial bivalent ligands, the Met dimer stability on the cell membranes and their biological activities in regulating cell proliferation and wound healing can still be dynamically modulated. These bivalent aptamer-based

partial agonists thus may be potentially used as safer and effective therapeutics, owing to their ease of design, flexibility, and high modularity. These properties are often not associated with other therapeutic strategies such as small-molecule drugs.

Bivalent aptamers have indeed been applied *in vivo* as therapeutic tools. For example, the Sando group demonstrated that without chemical modifications or delivery agents, Met-targeting bivalent DNA aptamers can be directly used to activate Met signaling in mouse hepatocytes and alleviate the progression of an *in vivo* fulminant hepatitis model.[22] Interestingly, these bivalent aptamers exhibited high stability in serum, which was owing to its self-contained stem-loop structure near the 3'-end that could minimize the exonuclease digestion. To further increase the biostability and therapeutic potential of these bivalent aptamers, Liang et al. introduced a circular version of Met-targeting bivalent aptamer to induce Met dimerization and *in vivo* repair of analgesic-acetaminophen-induced liver injury in a mice model.[23] Without either 5'- or 3'-free end, these circular bivalent aptamers can indeed exhibit high exonuclease resistance and possess better potential as the therapeutic choice for targeting complicated biological pathways *in vivo* while maintaining long-term stability in the body. For example, this feature can be critical for the *in vivo* recognition of cancer signaling pathways with particular combinations of the RTK expression patterns.

Another nice example indicating the therapeutic potential of bivalent aptamers was shown by Liu et al. in attenuating the assembly and toxicity of amyloid β oligomers (AβO) by inducing membrane dimerizations of cellular prion protein (PrP^C) receptors.[24] In the presence of PrP^C-targeting bivalent aptamers, the formation of PrP^C receptor dimers shedded and protected these membrane receptors from interacting with AβO. By inhibiting these AβO-PrP^C interactions, the downstream mitochondrial dysfunction and caspase-3-induced apoptosis processes can also be prevented, which has been shown as a major pathway involved in Alzheimer's disease. These bivalent DNA aptamers may thus be possibly used for a therapeutic purpose in neurodegenerative diseases given their demonstrated minimal off-target impacts.

2.2. Responsive DNA nanodevices

DNA nanodevices can also be conditionally activated to regulate the dimerizations of membrane receptors. These responsive functional DNA structures have been used to reprogram cellular receptors to respond to non-cognate ligands or user-defined external cues, which can provide a new dimension of control over cell signaling with great flexibility. For example, Ueki et al. devised a bispecific aptamer strategy to modulate Met signaling through external protein ligands such as thrombin and platelet-derived growth factor (PDGF).[25] Using thrombin as an example, in their strategy, Met-targeting aptamer was fused with a thrombin aptamer. Since one thrombin can bind with two orthogonal thrombin aptamers, after adding thrombin, two bispecific aptamers conjugate together to form "bivalent aptamers" to induce the Met dimerization and signal activation (Fig. 1B). As a result, external ligands (e.g., thrombin and PDGF) can now be used to replace cognate ligands (e.g., hepatocyte growth factor for the Met) in modulating membrane receptor dimerizations, without the requirement of genetic engineering. The same system can also be used for the detection of extracellular molecules or for the gene expression regulation.[26]

In another more modular design without the requirement of two aptamer binding sites per external cue, Li et al. introduced a toehold-mediated strand displacement strategy to induce Met membrane dimerizations upon adding small-molecule cues like ATP, histidine, or Zn^{2+}.[27] Their DNA nanodevice comprises two Met-targeting aptamers, one of which was first blocked by a small-molecule-cue-binding aptamer or DNAzyme (Fig. 1C). In the presence of corresponding small molecular trigger, the blocking aptamer or DNAzyme will be removed to expose a toehold region and initiate the hybridization of two Met-targeting aptamers to induce Met dimerization and signal activation. More recently, using a similar design strategy, the Nie group further engineered a smart DNA nanodevice to reprogram non-

mechanosensitive RTK receptors for sensing mechanical forces and achieving intercellular mechanotransduction.[28,29]

In addition to the chemical and mechanical cues, a range of light-response DNA nanodevices have also been developed to modulate cell membrane receptor dimerizations. One dramatic advantage of these optically controlled nanodevices is their extremely high spatial and temporal precision. Chen and colleagues reported one of the first demonstrations of light-induced activation of membrane dimerization.[30] In their design, again using Met dimerization as the example, a photocaged ortho-nitrobenzyl group-containing blocker DNA was initially hybridized with one Met-targeting aptamer. Right after a brief UV light irradiation, the rapid cleavage of the photocage removed the blocker DNA and triggered the assembly of two Met aptamers for the activation of Met signaling. Very similarly, the Sando group applied another 6-nitropiperonyl-methyl photocage to achieve photo-induced membrane dimerization of the Met receptors (Fig. 1D).[31] Intriguingly, by controlling the light irradiation pattern, the dynamics, durations, and efficiencies of Met signaling can be modulated correspondingly. These signal activations can occur only in the area being irradiated, proving the great spatiotemporal precision of such light-modulated cell signaling.

Such light-controlled approaches are also useful for *in vivo* applications as demonstrated by the Nie group.[32] In their work, trigger DNA-modified gold nanorods can absorb near-infrared (NIR) light to achieve photothermally controlled DNA release and bridge two Met-binding aptamers together on the cell membranes. Compared to UV light, NIR light exhibits much deeper tissue penetration depth and is ideally suitable for *in vivo* applications with minimal photodamage. Indeed, in mice models, such photo-controllable DNA nanodevice can promote the migration, proliferation, and differentiation of the muscle satellite cells for the skeletal muscle regeneration. Using different light, chemical, and/or mechanical triggers, we expect some additionally validated and precisely modulated *in vivo* usage of the responsive DNA nanodevices will be followed in future research. These stimuli-responsive tools enable precise temporal control and are of particular interest in the development of targeted and personalized therapeutics, as that shown in the successful clinical applications of light-activated therapeutics especially the photodynamic therapy.[33]

2.3. Reversible and dynamic modulators

An additional key feature of DNA nanodevices is their ability to undergo reversible hybridizations, which is particularly useful for dynamic and controllable cellular regulation. One technique that has gained attention for labelling membranes for such functionality involves the conjugation of nucleic acids with hydrophobic moieties, especially different lipid molecules.[34-36] Cholesterol is the most commonly used lipid for this purpose. Cholesterol-modified oligonucleotides are commercially available and can spontaneously and efficiently insert onto various cell membranes after a simple and brief incubation step. While these lipid-DNA conjugates have been widely used for cell membrane imaging and manipulation,[35] one major limitation is that they cannot specifically label certain receptors and therefore are distributed throughout the whole membranes or within lipid domains. For example, we have previously developed a type of “DNA Zipper” nanodevice to tune the membrane binding durations of two lipid molecules based on short complementary DNA strands.[37,38] By changing the number of base pairs between two lipid-modified DNA strands (Fig. 2A), the binding strength of lipid dimers on live-cell membranes could also be precisely modulated for studying membrane order and heterogeneity, as well as their involvement in processes such as T-cell signaling and epithelial-to-mesenchymal transition of cancer. By using such probes, it was revealed that lipid domain formation precedes the activation of T-cells and then gradually disperses after the activation events. Meanwhile, the T-cell-receptor/CD3 complex colocalizes within liquid-ordered membrane domains upon T-cell activation, indicating a close correlation between lipid domain formation and the stimulation of T-cell signaling.

Another excellent example was demonstrated by the Tan group, where a tweezer-like DNA nanodevice was engineered to dynamically regulate the membrane distance between dimeric CD28 receptors.[39] These "DNA nanotweezers" comprises two 14-nm-long arms interconnected by a single-stranded DNA hairpin in between (Fig. 2B). After adding a complementary DNA, the hairpin structure converts to a rigid DNA duplex and opens the nanotweezer. The complementary DNA can be further removed by another competing strand, and as a result, >10 cycles of alternate open and close switching of the DNA nanotweezer was able to achieve and used to modulate CD28 receptor signaling and T-cell activities. Very similarly, Li et al. devised another smaller hairpin-based "DNA nanorobot" to reversibly modulate the dimerization and de-dimerization of membrane Met receptors by controlling their distances.[40] Composed of only three DNA strands, this switchable DNA nanodevice was capable of dynamically regulating the cell migration process mediated by the Met signaling. Such systems enable us to study cell signaling kinetics in real-time with well-controlled starting, duration, and ending points, which has proven to be a challenge in the past. Another key feature of these reversible modulators is their ability to control membrane receptor dimer cycling in between the off and on states for multiple rounds, which can be useful for validating the cellular responses to these membrane receptor patterns and better mimicking the natural dynamic signal transduction processes.

Such receptor distance-dependent membrane modulation has been more carefully characterized by Wang et al.[41] By introducing between two EGFR-targeting aptamers with a double-stranded DNA bridge of seven different lengths (in the range of 0–100 base pairs, i.e., ~0–34 nm distance), the correlation between the receptor distance and its signal activation efficiency was explored in detail (Fig. 2C). Their results indicated that as the DNA duplex length increases, their effects on the EGFR activation gradually decrease, until after reaching 50 base pairs, where an inhibition effect starts to appear. Owing to the rigidness of DNA duplex, it can act as a rod to prevent two receptors from interacting with each other on the cell membranes. Indeed, DNA nanodevices, with well-controllable hybridization dynamics and spatial distances, can be used to precisely regulate the proximity of membrane receptors for either activation or inhibition of cell signaling, which shows the extent of the sensitivity of cell signaling to the proximity-induced dimer formations.

In another membrane dynamic DNA nanodevice, Li et al. engineered an autonomous DNA walker that can stepwise move along the cell membranes and catalytically drive the formation of multiple Met receptor dimers.[42] In this system, a Zn^{2+}-activated DNAzyme functions as the DNA walker, once activated, it can induce the *trans*-cleavage of a foothold strand and activate one Met-binding aptamer. The activated Met aptamer will then hybridize with another actuator Met aptamer to induce the membrane Met receptor dimerization, while the DNA walker itself will move to the next foothold strand via a toehold-mediated strand displacement reaction (Fig. 2D). The walker strand can continue relocating from one foothold DNA to another, increasing the number of receptor dimers and resulting in significantly enhanced cell migration over time.

2.4. Smart receptor dimer inhibitors

Instead of membrane homodimer-induced signal activation effect, the receptor dimer inhibition function of DNA nanodevices has been nicely demonstrated in another work by the Yang group.[43] In this approach, a bispecific aptamer was used to inhibit the formation of Met dimers by artificially inducing a non-natural transferrin factor (TfR)-Met membrane dimer instead (Fig. 3A). Even in the presence of hepatocyte growth factor (HGF), a cognate ligand for promoting membrane Met dimerization, the TfR-Met-bispecific aptamer can still inhibit Met assembly via a steric hindrance effect and competitive receptor binding. Selective and efficient reprogramming of cell signaling was thus possible here for potential therapeutic functions. For example, while cancerous cells can overexpress Met receptor, these RTKs are also expressed at a lower level during normal cell growth. Smart receptor dimer regulators that function only on a certain Met membrane concentrations can be used for more targeted cancer therapy.

Using the same concept of Met inhibition with the TfR-Met heterodimer, Wu et al. further developed responsive DNA bispecific aptamers that can be specifically activated in the tumor-like acidic and high ATP level environment.[44] A pH-responsive i-motif structure and an ATP-binding aptamer were both employed in their DNA nanodevice to allow the conditional activation of TfR-Met heterodimers only in the presence of both low pH and high ATP conditions. The potential therapeutic precision of the nanodevice was further enhanced as TfR is a tumor cell-specific membrane receptor. Indeed, the targeted inhibition of Met dimerization and reduced cancer cell motility was shown using this smart DNA nanodevice.

The reliability and programmability of engineering logic-gated inhibitory DNA nanodevices was also nicely demonstrated by the Li group,[45] where multiple AND, OR, NOR, and AND/OR logic gates were designed based on DNA- and light-regulated TfR-Met membrane heterodimers. For example, in a two-layered AND/OR logic circuit, the presence of both a short trigger DNA and light irradiation (an AND gate) is needed to remove two block strands, which further induces the dimerization formation between a TfR and Met receptor. Alternatively, a long trigger DNA itself can be enough to remove both block strands, equivalent to a logic OR gate. Using these programmable DNA nanodevices, elaborate and sophisticated membrane assembly control and cell signaling patterns can be expected in the near future. The potential applications of these highly programmable logic-gated devices may be seen in the field of targeted immunotherapy and tissue engineering, where layers of stimuli control are highly valuable and represent one major challenge that the successful incorporation DNA nanodevices can make a giant leap forward in the field.

In another intriguing design of multi-functional DNA nanodevice, Chen et al. visualized the Met signaling-induced VEGF secretion by using a two-armed lipid-anchored aptamer probe. Dimerization of Met triggers the release of VEGF that can be detected by one aptamer arm of the probe to generate fluorescence. Light-responsive photocleavable aptamer on the other arm is used to achieve controllable inhibition of membrane Met dimerizations.[46] After the membrane detection of cellular secreted VEGF using an aptamer probe on one arm of the DNA nanodevice, the UV light was irradiated to rapidly cleave a photocage group and release an activated Met-targeting aptamer (Fig. 3B). This aptamer can then bind to the Met receptor and prevent it from accessing another Met receptor, which thus effectively shuts down Met signaling and consequently reduces VEGF secretion. As shown by these representative examples, owing to their highly predictable hybridization and modularity, DNA-based nanodevices have started to be used as unique tools in a broad range of logic-based smart applications on living cell membranes.

2.5. Proximity-induced dimer stabilizers

Another type of DNA nanodevice has been developed to convert transient membrane receptor dimers into more permanent binding pairs. It is worth noting that different from the above-discussed bivalent aptamers, here, the conditional membrane activation of these dimer stabilizers requires some initial proximity and binding duration between the target receptor pair of interest. These proximity-based DNA stabilizers can be used for both membrane dimer modulation and imaging. One of the first examples was demonstrated by Liang et al. by using a Met dimerization-triggered strand displacement system.[47] In this system, a transient HGF-induced Met homodimer induces the formation of an 8-base-pair-long DNA duplex, which further initiates a toehold-mediated strand displacement reaction to form a stable DNA three-way junction structure (Fig. 3C). In the absence of HGF, this DNA nanodevice will not stabilize the membrane Met dimerization, which is quite different from that shown by directly using a bivalent Met aptamer. By further labeling these DNA strands with a FAM and Cy5 fluorophore pair, membrane Förster resonance energy transfer (FRET) signals can be detected for imaging the spatial distributions and membrane dynamics of HGF-induced Met receptor dimerization events.

To further increase the sensitivity of these proximity-induced dimer stabilizers and also test their potential *in vivo* usage, Wang et al. employed a similar strand displacement-induced three-way junction formation design to assemble Met receptor dimers, but also additionally combined with a hybridization chain reaction (HCR) strategy.[48] HCR is a widely used enzyme-free isothermal signal amplification method. In this design, after Met proximity-induced strand displacement reaction, an HCR initiator was exposed at the site of dimerization, which triggered the sequential hybridizations among two types of HCR hairpin probes to produce amplified *in situ* fluorescence signals (Fig. 3D). Such a sensitive nanodevice was later also proven to be effective for imaging Met receptor dimers in zebrafish embryos. With a similar goal of achieving highly sensitive dimer imaging and stabilization, even at the single-molecule level, the Xu group developed a DNA "plasmon ruler" using Au nanoparticle-modified DNA strands.[49] Instead of using a FRET or fluorophore-quencher pair, the Met receptor proximity-induced plasmon coupling between a 50 nm and 20 nm Au nanoparticle results in a bright scattering signal change under dark-field microscopy. By applying these DNA plasmon rulers, real-time manipulation of Met membrane assembly and disassembly, as well as high-resolution long-term imaging of Met dimers were successfully achieved on living cell surfaces.

Bioorthogonal functional groups (e.g., azides, alkynes, and ketones) can also be introduced into membrane receptors through metabolic labeling and/or genetic code expansion.[50] Afterwards, DNAs will selectively conjugate to these functional groups to achieve targeted membrane labeling. Similarly, membrane receptors can also be genetically engineered with active peptide or protein tags (e.g., His tag, SNAP, and HaloTag), and then immobilize DNAs at the active sites of these peptide tags. These two-step modification strategies can often result in a very efficient and robust site-specific incorporation of DNA nanodevices onto proteins of interest, although these tags can only used for genetically engineered membrane receptors, rather than endogenous protein targets. Considering these advantages, a covalent DNA-based proximity modulation method has been reported by Yang et al.[51] In their method, a dibenzo cyclooctyne-modified DNA was covalently conjugated with azide-bearing membrane glycoprotein receptors as introduced via metabolic glycan labeling (Fig. 3E). Further guided by Met-, EGFR- or HER2-targeting aptamers, such covalent modification can be achieved onto specific membrane receptor of interest for imaging and modulating different receptor homo- and heterodimers.

Ogorek et al. also developed a proximity-based strategy to covalently label membrane protein receptor dimers with the biotin moieties.[52] The system works similar to the above-mentioned dimerization-triggered strand displacement reaction,[47] but instead by activating a $Ru(bpy)_2$(phenanthroline) photocatalyst to induce localized protein oxidation with a biotin phenol molecule. Using both aptamer-based Met dimer labeling and antibody-DNA conjugate-mediated HER2-HER3 heterodimer conjugation as the examples, the authors validated a powerful DNA nanodevice for dimer-specific covalent modulation, and also potentially for the identification of new binding partners of membrane protein receptors. Therefore, as highlighted above, antibodies have also been used to anchor DNA nanodevices onto cell membrane protein receptors with high specificity. These membrane anchorings occur based on either reversible non-covalent binding or more permenant covalent modification of target proteins. Since covalent modification can likely be more effective in allowing long-term membrane DNA anchoring even after washing away free DNA strands to reduce non-specific interactions, these covalent DNA-protein linkages do seem to be an attractive alternative to the common noncovalent assemblies. Still, the covalent linkage between DNA and endogenous membrane receptors can now be achieved via ligand-guided proximity labeling, such as by using thiol alkylation of cysteines, primary amine modification, click chemistry or photo-crosslinkers.[53-56]

3. Modulation of membrane receptor oligomerizations using DNA nanodevices

Cell membrane signaling is clearly not only reliant upon receptor dimerizations. More complex membrane signaling networks also involve the modulation of larger-scale receptor assemblies. For

example, the formation of membrane receptor oligomers and complexes among various G-protein-coupled receptors are known to play a critical role in regulating cellular ligand binding, receptor maturation and internalization in neuron and cancer cells.[57-59] In addition to the above-discussed dimerization controls, DNA-based nanodevices have also begun to show versatile usage in regulating the membrane oligomerization and crosslinking of different receptor molecules.

3.1. Polymeric DNA scaffolds

DNA oligonucleotides can be self-assembled into larger polymerized structures. As multiple receptor-binding sites can be potentially modified on these DNA polymers, one simple approach to modulate receptor oligomerization is to directly use ligand-modified DNA polymers as scaffolds to guide the assembly of membrane receptors. In one of the earliest examples, Zhang et al. demonstrated the synthesis of a DNA polymer scaffold that contains multiple anti-CD20 antibodies, which can efficiently induce membrane CD20 clustering to mediate the apoptosis of B-cell lymphoma.[60] This DNA polymer was prepared based on an isothermal rolling circle amplification (RCA) reaction, where a circular DNA template is used to produce a long sequence-repeat single-stranded DNA product (Fig. 4A).

Another similar RCA-based DNA polymeric nanodevice was later reported by the Li group, who used a multi-Arg-Gly-Asp (RGD)-modified DNA scaffold to reversibly modulate the membrane distance and aggregation status of the integrin receptors.[61] The reversibility of the DNA nanodevice was achieved based on the repeated hairpin structures in the DNA polymer, which can be open and closed via the addition of complementary DNA strands (Fig. 4B). Once opened, the distance between two RGD ligands (as well as between integrins) increased significantly, which further activated the phosphoinositide 3-kinase (PI3K) and Rac family small GTPase 1 (Rac1) signaling and impacted the cellular morphology. Compared to the above-mentioned single-stranded RCA DNA nanodevice,[60] another major difference here is the RGD ligands were introduced via a complementary DNA sequence to hybridize with the single-stranded RCA product. This synthesis route can have better modularity and be used to produce a more rigid DNA nanodevice (with double-stranded regions) to control the spatial distances among different membrane receptors.

Instead of adding complementary DNA strands, Sethi et al. developed a more convenient light-controlled DNA nanodevice, using such RCA reaction-produced hairpin-containing DNA nanodevice.[62] Photoswitchable azobenzene moieties were incorporated in the DNA strands here. Under UV or visible light irradiation, azobenzene can switch between the *cis*- and *trans*-conformation, which consequently inhibits or promotes DNA hybridizations and regulates the shrinking and relaxation states of the whole nanodevice (Fig. 4C). By modifying with the RGD ligands, such photo-controlled shrunken or relaxed spring-like DNA nanodevice was able to dynamically control the membrane aggregation status of the integrin receptors and used to fine-tune the morphology of human mesenchymal stem cells.

Rather than using the RCA reaction to prepare single-stranded DNA scaffold, the above-mentioned HCR system has also been applied to produce double-stranded DNA polymeric membrane receptor scaffold. In one study by Yang et al,[63] the HCR trigger and two hairpin strands were first conjugated via aptamers to specific membrane receptors, such as the human protein tyrosine kinase 7 (PTK7) (Fig. 4D). Once adding an activator DNA, a membrane-anchored HCR interaction occurs that can either induce a homogenous oligomerization of the same PTK7 receptor protein or trigger the heterogenous clustering of two types of membrane receptors targeted by different DNA aptamers. These versatile DNA polymeric nanodevices have been used to modulate cellular migration, proliferation, and survival capabilities.

3.2. DNA origami-guided spatial receptor patterns

In addition to the “two-dimensional” DNA polymeric scaffolds formed via HCR or RCA reactions, more precise control over the membrane receptor distance, three-dimensional geometry, and stoichiometry can be achieved using designed DNA origami nanostructures. Since the initial development of DNA origami by Rothemund in 2006,[64] these self-assembled DNA architectures of complicated shapes and patterns have now been broadly used to study and control biological systems.[3] One of the first DNA origami design used for membrane receptor organization and cell signaling modulation was reported by Shaw et al.[65] By decorating an 18-helix bundle DNA origami with ephrin-A5 ligands of varied distance from ~14 nm, to ~100 nm, the binding strength and signal activation efficiency of the EphA2 receptors on breast cancer cell membranes can be modulated differentially (Fig. 5A). Interestingly, when separated at a ~100 nm distance, the ephrin-A5 ligands exhibited similar efficiency as a single ephrin-A5 in activating EphA2 clusters. While at a ~43 nm distance, a more significant effect on modulating cell migration was observed, indicating the importance of ligand spacing in regulating membrane receptor clusters. Further decrease in the ligand distance (such as at ~14 nm) or increase in the number of ligands didn’t really impact the EphA2 signaling outcomes.

In another work by the Högberg group, both a hexagon- and a square-shaped wireframe DNA origami flat sheets were prepared as the templates to organize the spatial pattern and stoichiometry of a 17-amino-acid-long cyclic peptide to mimic apoptosis-inducing ligands for modulating oligomerization of the death receptor 5 (DR5).[66] The authors first confirmed the successful conjugation of six peptide ligands (>75%) on each DNA origami structure using both gel electrophoresis and a single-molecule localization microscopy technique, “DNA-PAINT”. By changing the inter-ligand spacing from ~5.7 nm to ~25.5 nm on hexagon- and from ~6.3 nm to ~11.1 nm on square-shaped flat sheets (Fig. 5B), the most effective DR5 clustering and cell apoptosis was observed when these ligands were separated with a <10 nm spacing. Such modulations worked on all three types of human breast cancer cell lines being tested, including the ones previously deemed to be resistant to the natural DR5 ligand treatment. Similarly, the Zhao group applied flat rectangular DNA origami to explore how the inter-ligand spacing of tumor necrosis factor-related apoptosis inducing ligand (TRAIL) trimers could affect the clustering of death receptors and lead to apoptosis.[67] By varying the distance of the TRAIL trimer in the range of 15–60 nm, the most effective cytotoxicity and antitumor effect in a COLO205 xenograft-bearing mouse model at a spacing of ~40 nm. A DR5 clustering “active unit” model has been further proposed in this study, providing additional insights regarding how improved therapeutics may be designed by using optimum spatial orientations of the TRAIL trimers to achieve maximum cell death.

An intriguing triangular DNA origami scaffold was employed by the Zhang group to spatially arrange three types of membrane receptors together.[68] Aptamers targeting EpCAM, HER2, and EGFR were attached at different corners of three triangles with distances of ~30, 70, and 100 nm (Fig. 5C). These inter-ligand distances are associated with the average diameters of the corresponding receptor clusters on natural cancer cell surfaces. Such DNA nanodevice exhibits much enhanced binding affinity against target breast cancer cell lines, indicating the importance of matching membrane receptor patterns for the improved ligand binding on the cell surfaces. Meanwhile, these triangular aptamer patterns can also be used for the efficient separation and enrichment of rare circulating tumor cells from peripheral blood samples, suggesting their potential function for clinical diagnosis.

Moving on from 2D assemblies to 3D, a type of RGD peptide-modified DNA nanotubes has been constructed to enhance the differentiation of neural stem cells into neurons.[69] In this study, the Stupp group synthesized a ~14-nm-long and ~20-nm-in-diameter DNA nanotube unit that can end-to-end conjugate together to form micron-long fiber structures. Containing 7–10 RGD peptides in each nanotube unit, this large self-assembled DNA device can efficiently promote the adhesion and selective differentiation of neural stem cells. The Wei group has also made use of tube-like DNA nanostructures to control the distance between two EGF ligands at either ~25 or 100 nm for the differentiation of wild-type and mutated EGFR-expressing lung cancer cells.[70] The general spacing between EGFR receptors

appears to vary significantly between these two cell types, with mutated EGFR cells (as that shown in lung adenocarcinoma patients) exhibiting narrower receptor distances. By comparing the EGFR mutation differentiation results using DNA nanodevices with next-generation sequencing data, good concordance was observed within clinical samples, demonstrating a rapid diagnostic approach for detecting a common subtype of lung cancer.

Rod-like insulin-containing DNA origami has also been reported by the Teixeria group to achieve multivalent activation of insulin receptors.[71] A 140-nm-long DNA nanorod was modified with 1, 2, 4, 7, or 15 insulins with a constant (7 nm) or different spacing (Fig. 5D). Both valency and spacing were found to be critical in the activation of insulin receptor pathways as well as the modulation of transcriptional responses. The optimal multivalent 7-insulin-containing DNA nanorod (NR-7) can also be used to reduce free glucose levels in a zebrafish model. Considering the importance of insulin therapeutics in the treatment of diabetes, these data may suggest a potential new DNA-based therapeutic approach by controlling both the valency and spatial orientation of the insulin ligands.

In another study, the Högberg group demonstrated a force-independent Notch activation model using Jag1-modified DNA origami nanorod.[72] Notch signaling is well-conserved in development and has been used as targets for cancer therapy. A 4–12 pN range pulling force has been thought as necessary for the activation of membrane Notch receptors. Interestingly here, by increasing the number of Jag1 on the DNA nanorod, or even using only one Jag1 together with other membrane binders, significant activation of the Notch signaling was observed without the involvement of pulling forces (Fig. 5E). Two possible mechanisms have been proposed: due to either the increased ligand-receptor membrane association time or that combined with the mimic of neighboring cell environment from the bulk DNA nanostructure. While further validations are still needed, this work demonstrated the great potential of DNA nanodevices for developing new soluble Notch agonists for cancer or regenerative medicine.

A pH-responsive DNA origami has also been recently developed by the Chen group for controlling CD95 signaling on the surface of immune cells.[73] In this device, an i-motif sequence was used to control the reversible transition of a CD95 ligand-incorporated DNA origami between an open and closed pattern (Fig. 5F). Under acidic condition like that within inflamed synovial tissues, the CD95 ligand clusters will be exposed for the conditional activation of CD95 receptor-induced death signaling. While at neural pH, the CD95 ligands can be protected inside the DNA nanodevice to minimize their off-target toxicity. Such a smart and programmable system has also been validated *in vivo* in a collagen-induced arthritis mouse model after intravenous injection. This is an excellent example demonstrating the promise of DNA nanotechnology for the development of intelligent targeted therapeutics.

3.3. Lipid raft modulators

Lastly, instead of directly targeting a specific membrane protein receptor, DNA nanodevices have also been developed to modulate the formation of membrane lipid domains or lipid rafts, which can function as the reaction hubs for different membrane receptors. As a result, these DNA-based lipid raft modulators can provide an alternative avenue for regulating the dynamic aggregations of membrane receptors and thus for controlling cell signaling. For example, the Li group synthesized cholesterol-modified DNA probes to stabilize transient lipid rafts and used them for activating T cells.[74] In their design, a 5'-cholesterol-functionalized DNA strand can hybridize with another 5'-cholesterol DNA (Fig. 6A). After hybridization, a large cluster of cholesterol-DNA conjugates can be aggregated on the cell membrane via hydrophobic interactions. These cholesterol-enriched membrane regions can further recruit raft-associated lipids and protein receptors to facilitate the formation of membrane lipid rafts. Meanwhile, by adding a longer complementary DNA to displace and disrupt the hybridizations between two cholesterol-modified DNA strands, these lipid rafts can be *in situ* redistributed and dispersed on the membranes.

In a more advanced design, Su et al. developed an ATP-triggered HCR DNA nanodevice that contains multiple cholera toxin B (CTxB)-targeting DNA aptamers to control the membrane assembly of ganglioside (GM1), another key component of lipid rafts.[75] In the presence of ATP, HCR-produced DNA polymer fluorescence can be clearly visualized on the cell membranes (Fig. 6B), which was gradually transformed into clusters and associated with membrane lipid rafts. Interestingly, clustering of cell surface CD44 adhesion receptors was also found to be colocalized in these DNA-enriched raft regions, which lead to the inhibition of F-actin and integrin and result in an attenuated cell mobility. Such a system was further validated *in vivo* using a melanoma mouse tumor model. The tumor growth was indeed effectively inhibited after an intratumoral injection of DNA nanodevices. These results indicate that the modulation of lipid rafts can be directly used to impact cell signaling and disease models, which appears to be an emerging strategy for therapeutics with the ability to control a broad range of receptor targets.

DNA origami-based nanodevices have also been employed to manipulate the cell membrane local lipid environment and phase separation. For example, Chen et al. recently developed a 90 nm × 60 nm rectangular DNA origami platform, which was decorated with aptamers either targeting CTxB or transferrin receptor, which are located in the raft and non-raft regions of the cell membrane, respectively.[76] The authors also anchored a gold nanorod onto each origami assembly in order to generate localized heat upon an NIR light irradiation and also to explore how these domain-specific heat will impact membrane phase separation and dynamics. After raft- and non-raft-targeted heating treatment, proteomics analysis was performed using MCF-7 cells, where a number of proteins were discovered to be up- or down-regulated in both cases. Further pathway analysis demonstrated that both raft- and non-raft-targeted treatment can result in cell signal transduction related to the migration process. Such a remotely controlled nanodevice has also been used in an *in vivo* mouse wound healing model, demonstrating their potential usage in advancing our understanding and ability to modulate lipid rafts as potential therapeutic strategies, opposed to traditional techniques.

4. Conclusions and perspectives

As demonstrated by the multiple examples shown in this review, DNA-based nanodevices, with highly predictable and modular structures, have enabled a range of design and applications in modulating the dimerization and oligomerization status of cell membrane receptors. These functional nanodevices can respond to various stimuli inputs and consequently reprogram cell signaling outputs with high spatial and temporal precision. A dynamic, reversible, and logical modulation of the interaction patterns among various homogenous and heterogenous membrane receptors can be rationally achieved by using these DNA nanodevices. Having such versatile properties to directly influence and modulate cellular signaling is a very attractive feature and a significant advance in understanding cellular systems. These systems have begun to advance our understanding of cell membrane structures, the biophysics of receptor dimerization, and their associated signaling pathways, all of which fields are of paramount importance in cellular biology and drug discovery.

Meanwhile, these cell membrane DNA nanodevices have also begun to be used for some *in vivo* applications, for the potential therapeutic and/or diagnostic purposes. Without the need for genetic encoding, these highly controllable and precise DNA nano-assemblies may be potentially used for developing much safer and more effective therapeutics. Such DNA-based nanodevices can be easily anchored onto cell membranes with minimal immunogenicity or toxic impacts from the DNA nanostructures themselves. While owing to their modular design and programmable features, once recognizing some particular cellular receptor targets, DNA nanodevices can enable the rapid modulation and perturbation of specific cell signaling events. In addition, different drug and stimuli-responsive (e.g., photocage) moieties can be modified within these DNA nanodevices to further enhance their therapeutic power. Meanwhile, by incorporating with some reporter units, such as fluorescent dyes, DNA-based systems can serve as a great platform for monitoring receptor modulated live-cell signaling events, as

well as for developing vital drug screening assays. The recent rapid progress in this emerging field is really exciting. But meanwhile, we would like to highlight some current challenges and potential directions that will need some immediate or long-term efforts.

First of all, most of these current studies have been based on some similar and well-characterized model membrane receptor system, like the Met dimers. However, the human interactome has been estimated to involve ~130,000–650,000 different protein pairs, including many membrane protein receptors, like ~800 transmembrane GPCRs.[2,77,78] The cellular functions of most majority of these interactions remain elusive and barely understood. The modulation of these unknown or less-studied membrane receptor assemblies can be potentially important for interpreting the fundamental properties of cellular networks and for identifying new potential therapeutic targets in diseases. DNA nanodevices may potentially play a critical role here.

Secondly, most current DNA nanodevices function by using specific ligand-receptor interactions to bring multiple receptors into close proximity. However, proximity itself may not induce the formation of native or desired protein conformations or interfaces for the receptor assembly. Meanwhile, we have to consider the steric effects induced by these DNA nanodevices, especially within some rigid nanostructure scaffolds. This challenge has been further complicated as the detailed crystal structures of most ligand-receptor pair and/or receptor aggregates are still missing. In addition, the popular use of aptamers as the ligand molecules in developing DNA nanodevices, though stems from their nucleic acid nature, precautions are indeed needed to ensure minimal cellular or *in vivo* cross reactivities of these mostly *in vitro* identified ligand molecules.

In addition, even though the *in vivo* functionality of these DNA nanodevices have been demonstrated in several preliminary studies, the detailed pharmacokinetic characteristics of these nucleic acid molecules are still largely lacking. We have limited knowledge about the biostability, distribution, *and in vivo* target protein specificity and modulation efficacy of these DNA nanodevices, as well as their impacts on the metabolism, proteome, and transcriptome, etc. Nevertheless, as a rapidly growing field of research, all these current challenges represent great potential opportunities to really convert these versatile membrane-modulating DNA nanodevices into real clinically effective therapeutic tools.

Conflicts of interest

There are no conflicts to declare.

Acknowledgments

The authors gratefully acknowledge the support from NSF 2425057, NIH R35GM133507 and Camille Dreyfus Teacher-Scholar Award to M.Y. and support of Paul Hatheway Terry Scholarship to A.A.A.

References

1. D. E. Scott, A. R. Bayly, C. Abell and J. Skidmore, Small molecules, big targets: drug discovery faces the protein-protein interaction challenge, *Nat. Rev. Drug Discov.*, 2016, **15**, 533-550.
2. T. L. Nero, C. J. Morton, J. K. Holien, J. Wielens and M. W. Parker, Oncogenic protein interfaces: small molecules, big challenges, *Nat. Rev. Cancer*, 2014, **14**, 248-262.
3. L. G. Milroy, T. N. Grossmann, S. Hennig, L. Brunsveld and C. Ottmann, Modulators of protein-protein interactions, *Chem. Rev.*, 2014, **114**, 4695-4748.
4. L. Kelly, K. E. Maier, A. Yan and M. Levy, A comparative analysis of cell surface targeting aptamers, *Nat. Commun.*, 2021, **12**, 6275.
5. M. Takahashi, Aptamers targeting cell surface proteins, *Biochimie*, 2018, **145**, 63-72.
6. J. Li, L. Wang, J. Tian, Z. Zhou and H. Yang, Nongenetic engineering strategies for regulating receptor oligomerization in living cells, *Chem. Soc. Rev.*, 2020, **49**, 1545-1568.
7. K. Zhang, H. Gao, R. Deng and J. Li, Emerging applications of nanotechnology for controlling cell-surface receptor clustering, *Angew. Chem. Int. Ed.*, 2019, **58**, 4790-4799.
8. H. Dong, L. Liu, J. Wang, J. Fan, H. H. Wang and Z. Nie, DNA-based reprogramming strategy of receptor-mediated cellular behaviors: from genetic encoding to nongenetic engineering, *ACS Appl. Bio. Mater.*, 2020, **3**, 2796-2804.
9. G. Manning, D. B. Whyte, R. Martinez, T. Hunter and S. Sudarsanam, The protein kinase complement of the human genome, *Science*, 2002, **298**, 1912-1934.
10. M. A. Lemmon and J. Schlessinger, Cell signaling by receptor tyrosine kinases, *Cell*, 2010, **141**, 1117-1134.
11. Z. Du and C. M. Lovly, Mechanisms of receptor tyrosine kinase activation in cancer, *Mol. Cancer*, 2018, **17**, 58.
12. Y. Yarden and G. Pines, The ERBB network: at last, cancer therapy meets systems biology, *Nat. Rev. Cancer*, 2012, **12**, 553-563.
13. Y. Yarden and M. X. Sliwkowski, Untangling the ErbB signalling network, *Nat. Rev. Mol. Cell. Biol.*, 2001, **2**, 127-137.
14. M. A. Lemmon, J. Schlessinger and K. M. Ferguson, The EGFR family: not so prototypical receptor tyrosine kinases, *Cold Spring Harb Perspect. Biol.*, 2014, **6**, a020768.
15. J. Baselga and S. M. Swain, Novel anticancer targets: revisiting ERBB2 and discovering ERBB3, *Nat. Rev. Cancer*, 2009, **9**, 463-475.
16. M. J. Wieduwilt and M. M. Moasser, The epidermal growth factor receptor family: biology driving targeted therapeutics, *Cell. Mol. Life Sci.*, 2008, **65**, 1566-1584.
17. N. Kara, N. Ayoub, H. Ilgu, D. Fotiadis and M. Ilgu, Aptamers targeting membrane proteins for sensor and diagnostic applications, *Molecules*, 2023, **28**, 3728.
18. V. Ramaswamy, A. Monsalve, L. Sautina, M. S. Segal, J. Dobson and J. B. Allen, DNA aptamer assembly as a vascular endothelial growth factor receptor agonist, *Nucleic Acid Ther.*, 2015, **25**, 227-234.
19. R. Ueki, A. Ueki, N. Kanda and S. Sando, Oligonucleotide-based mimetics of hepatocyte growth factor, *Angew. Chem. Int. Ed.*, 2016, **55**, 579-582.
20. R. Ueki, S. Atsuta, A. Ueki, J. Hoshiyama, J. Li, Y. Hayashi and S. Sando, DNA aptamer assemblies as fibroblast growth factor mimics and their application in stem cell culture, *Chem. Commun.*, 2019, **55**, 2672-2675.
21. M. Akiyama, R. Ueki, M. Yanagawa, M. Abe, M. Hiroshima, Y. Sako and S. Sando, DNA-based synthetic growth factor surrogates with fine-tuned agonism, *Angew. Chem. Int. Ed.*, 2021, **60**, 22745-22752.
22. R. Ueki, S. Uchida, N. Kanda, N. Yamada, A. Ueki, M. Akiyama, K. Toh, H. Cabral and S. Sando, A chemically unmodified agonistic DNA with growth factor functionality for in vivo therapeutic application, *Sci. Adv.*, 2020, **6**, eaay2801.

23. H. Liang, Z. Yan, Y. Tong, S. Chen, J. Li, L. Chen and H. Yang, Circular bivalent aptamers enhance the activation of the regenerative signaling pathway for repairing liver injury in vivo, *Chem. Commun.*, 2023, **59**, 1621-1624.
24. S. Liu, S. Li, J. Lin, J. Li and H. Yang, Aptamer-induced-dimerization strategy attenuates AβO toxicity through modulating the trophic activity of PrP(C) signaling, *J. Am. Chem. Soc.*, 2022, **144**, 9264-9270.
25. R. Ueki, S. Atsuta, A. Ueki and S. Sando, Nongenetic reprogramming of the ligand specificity of growth factor receptors by bispecific DNA aptamers, *J. Am. Chem. Soc.*, 2017, **139**, 6554-6557.
26. H. Liu, T. A. Baeumler, K. Nakamura, Y. Okada, S. Cho, A. Eguchi, D. Kuroda, K. Tsumoto, R. Ueki and S. Sando, An engineered synthetic receptor-aptamer pair for an artificial signal transduction system, *ACS Nano*, 2023, **17**, 9039-9048.
27. H. Li, M. Wang, T. Shi, S. Yang, J. Zhang, H. H. Wang and Z. Nie, A DNA-mediated chemically induced dimerization (D-CID) nanodevice for nongenetic receptor engineering to control cell behavior, *Angew. Chem. Int. Ed.*, 2018, **57**, 10226-10230.
28. S. Yang, M. Wang, D. Tian, X. Zhang, K. Cui, S. Lü, H. H. Wang, M. Long and Z. Nie, DNA-functionalized artificial mechanoreceptor for de novo force-responsive signaling, *Nat. Chem. Biol.*, 2024, **20**, 1066-1077.
29. A. A. Ali, M. A. Tabrizi and M. You, Forced rewiring of RTK signaling, *Nat. Chem. Biol.*, 2024, **20**, 946-947.
30. S. Chen, J. Li, H. Liang, X. H. Lin and H. H. Yang, Light-induced activation of c-Met signalling by photocontrolled DNA assembly, *Chemistry*, 2018, **24**, 15988-15992.
31. R. Ueki, S. Hayashi, M. Tsunoda, M. Akiyama, H. Liu, T. Ueno, Y. Urano and S. Sando, Nongenetic control of receptor signaling dynamics using a DNA-based optochemical tool, *Chem. Commun.*, 2021, **57**, 5969-5972.
32. M. Wang, F. He, H. Li, S. Yang, J. Zhang, P. Ghosh, H. H. Wang and Z. Nie, Near-infrared light-activated DNA-agonist nanodevice for nongenetically and remotely controlled cellular signaling and behaviors in live animals, *Nano Lett.*, 2019, **19**, 2603-2613.
33. B. M. Vickerman, E. M. Zywot, T. K. Tarrant and D. S. Lawrence, Taking phototherapeutics from concept to clinical launch, *Nat. Rev. Chem.*, 2021, **5**, 816-834.
34. Y. Bagheri, S. Chedid, F. Shafiei, B. Zhao and M. You, A quantitative assessment of the dynamic modification of lipid-DNA probes on live cell membranes, *Chem. Sci.*, 2019, **10**, 11030-11040.
35. A. A. Ali, Y. Bagheri and M. You, Digging into the biophysical features of cell membranes with lipid-DNA conjugates, *Q. Rev. Biophys.*, 2022, **55**, e5.
36. B. Zhao, Q. Tian, Y. Bagheri and M. You, Lipid-Oligonucleotide Conjugates for simple and efficient cell membrane engineering and bioanalysis, *Curr. Opin. Biomed. Eng.*, 2020, **13**, 76-83.
37. Y. Bagheri, A. A. Ali, P. Keshri, J. Chambers, A. Gershenson and M. You, Imaging membrane order and dynamic interactions in living cells with a DNA Zipper probe, *Angew. Chem. Int. Ed.*, 2022, **61**, e202112033.
38. A. A. Ali, Y. Bagheri, Q. Tian and M. You, Advanced DNA Zipper probes for detecting cell membrane lipid domains, *Nano Lett.*, 2022, **22**, 7579-7587.
39. Z. Wang, S. Xie, L. Wu, F. Chen, L. Qiu and W. Tan, Aptamer-functionalized nanodevices for dynamic manipulation of membrane receptor signaling in living cells, *Nano Lett.*, 2022, **22**, 7853-7859.
40. B. Li, Y. Wang and B. Liu, Transformable DNA nanorobots reversibly regulating cell membrane receptors for modulation of cellular migrations, *ACS Nano*, 2023, **17**, 22571-22579.
41. Y. Wang, Y. Xiong, L. Song, S. He, F. Yao, Y. Wu, K. Shi and L. He, Spatial control of receptor dimerization using programmable DNA nanobridge, *Biomacromolecules*, 2023, **24**, 3228-3236.
42. H. Li, J. Gao, L. Cao, X. Xie, J. Fan, H. Wang, H. H. Wang and Z. Nie, A DNA molecular robot that autonomously walks on the cell membrane to drive cell motility, *Angew. Chem. Int. Ed.*, 2021, **60**, 26087-26095.

43. L. Wang, H. Liang, J. Sun, Y. Liu, J. Li and H. Yang, Bispecific aptamer induced artificial protein-pairing: a strategy for selective inhibition of receptor function, *J. Am. Chem. Soc.*, 2019, **141**, 12673-12681.
44. Y. Wu, J. Huang, H. He, M. Wang, G. Yin, L. Qi, X. He, H. H. Wang and K. Wang, Logic nanodevice-mediated receptor assembly for nongenetic regulation of cell behavior in tumor-like microenvironment, *Nano Lett.*, 2023, **23**, 1801-1809.
45. S. Chen, Z. Xu, W. Yang, X. Lin, J. Li and H. Yang, Logic-gate-actuated DNA-controlled receptor assembly for the programmable modulation of cellular signal transduction, *Angew. Chem. Int. Ed.*, 2019, **58**, 18186-18190.
46. S. Chen, Z. Xu, S. Li, H. Liang, C. Zhang, Z. Wang, J. Li and H. Yang, Systematic interrogation of cellular signaling in live cells using a membrane-anchored DNA multitasking processor, *Angew. Chem. Int. Ed.*, 2022, **61**, e202113795.
47. H. Liang, S. Chen, P. Li, L. Wang, J. Li, H. H. Yang and W. Tan, Nongenetic approach for imaging protein dimerization by aptamer recognition and proximity-induced DNA assembly, *J. Am. Chem. Soc.*, 2018, **140**, 4186-4190.
48. L. Wang, W. Li, J. Sun, S. Y. Zhang, S. Yang, J. Li and H. H. Yang, Imaging of receptor dimers in zebrafish and living cells via aptamer recognition and proximity-induced hybridization chain reaction, *Anal. Chem.*, 2018, **90**, 14433-14438.
49. J. Wang, J. Song, X. Zhang, S. M. Wang, B. Kang, X. L. Li, H. Y. Chen and J. J. Xu, DNA-programed plasmon rulers decrypt single-receptor dimerization on cell membrane, *J. Am. Chem. Soc.*, 2023, **145**, 1273-1284.
50. K. Lang and J. W. Chin, Cellular incorporation of unnatural amino acids and bioorthogonal labeling of proteins, *Chem. Rev.*, 2014, **114**, 4764-4806.
51. W. Yang, H. Nan, Z. Xu, Z. Huang, S. Chen, J. Li and H. Yang, DNA-templated glycan labeling for monitoring receptor spatial distribution in living cells, *Anal. Chem.*, 2021, **93**, 12265-12272.
52. A. N. Ogorek, X. Zhou and J. D. Martell, Switchable DNA catalysts for proximity labeling at sites of protein-protein interactions, *J. Am. Chem. Soc.*, 2023, **145**, 16913-16923.
53. N. Y. Tretyakova, A. t. Groehler and S. Ji, DNA-protein cross-links: formation, structural identities, and biological outcomes, *Acc. Chem. Res.*, 2015, **48**, 1631-1644.
54. J. B. Trads, T. Torring and K. V. Gothelf, Site-selective conjugation of native proteins with DNA, *Acc. Chem. Res.*, 2017, **50**, 1367-1374.
55. T. Tamura and I. Hamachi, Chemistry for covalent modification of endogenous/native proteins: from test tubes to complex biological systems, *J. Am. Chem. Soc*,. 2019, **141**, 2782-2799.
56. J. L. Vinkenborg, G. Mayer and M. Famulok, Aptamer-based affinity labeling of proteins, *Angew. Chem. Int. Ed.*, 2012, **51**, 9176-9180.
57. S. Terrillon and M. Bouvier, Roles of G-protein-coupled receptor dimerization, *EMBO Rep.*, 2004, **5**, 30-34.
58. J. F. White, J. Grodnitzky, J. M. Louis, L. B. Trinh, J. Shiloach, J. Gutierrez, J. K. Northup and R. Grisshammer, Dimerization of the class A G protein-coupled neurotensin receptor NTS1 alters G protein interaction, *Proc. Natl. Acad. Sci. U.S.A.*, 2007, **104**, 12199-12204.
59. S. Gahbauer and R. A. Böckmann, Membrane-mediated oligomerization of G protein coupled receptors and its implications for GPCR function, *Front. Physiol.*, 2016, **7**, 494.
60. Z. Zhang, M. A. Eckert, M. M. Ali, L. Liu, D. K. Kang, E. Chang, E. J. Pone, L. S. Sender, D. A. Fruman and W. Zhao, DNA-scaffolded multivalent ligands to modulate cell function, *ChemBioChem*, 2014, **15**, 1268-1273.
61. K. Zhang, R. Deng, Y. Sun, L. Zhang and J. Li, Reversible control of cell membrane receptor function using DNA nano-spring multivalent ligands, *Chem. Sci.*, 2017, **8**, 7098-7105.
62. S. Sethi, K. Hidaka, H. Sugiyama and M. Endo, Non-invasive regulation of cellular morphology using a photoswitchable mechanical DNA polymer, *Angew. Chem. Int. Ed.*, 2021, **60**, 20342-20349.

63. X. Yang, L. Yang, D. Yang, M. Li and P. Wang, In Situ DNA self-assembly on the cell surface drives unidirectional clustering of membrane proteins for the modulation of cell behaviors, *Nano Lett.*, 2022, **22**, 3410-3416.
64. P. W. Rothemund, Folding DNA to create nanoscale shapes and patterns, *Nature*, 2006, **440**, 297-302.
65. A. Shaw, V. Lundin, E. Petrova, F. Fördős, E. Benson, A. Al-Amin, A. Herland, A. Blokzijl, B. Högberg and A. I. Teixeira, Spatial control of membrane receptor function using ligand nanocalipers, *Nat. Methods*, 2014, **11**, 841-846.
66. Y. Wang, I. Baars, F. Fördös and B. Högberg, Clustering of Death Receptor for Apoptosis Using Nanoscale Patterns of Peptides, *ACS Nano*, 2021, **15**, 9614-9626.
67. N. Ma, K. Cheng, Q. Feng, G. Liu, J. Liang, X. Ma, Z. Chen, Y. Lu, X. Wang, W. He, H. Xu, S. Wu, J. Zou, Q. Shi, G. Nie and X. Zhao, Nanoscale organization of TRAIL trimers using DNA origami to promote clustering of death receptor and cancer cell apoptosis, *Small*, 2023, **19**, e2206160.
68. M. Mao, Z. Lin, L. Chen, Z. Zou, J. Zhang, Q. Dou, J. Wu, J. Chen, M. Wu, L. Niu, C. Fan and Y. Zhang, Modular DNA-origami-based nanoarrays enhance cell binding affinity through the "lock-and-key" interaction, *J. Am. Chem. Soc.*, 2023, **145**, 5447-5455.
69. N. Stephanopoulos, R. Freeman, H. A. North, S. Sur, S. J. Jeong, F. Tantakitti, J. A. Kessler and S. I. Stupp, Bioactive DNA-peptide nanotubes enhance the differentiation of neural stem cells into neurons, *Nano Lett.*, 2015, **15**, 603-609.
70. K. Zhang, Z. Huang, Y. Wang, J. Xue, N. Liang and Z. Wei, Rapid determination of the presence of EGFR mutations with DNA-based nanocalipers, *Nanoscale*, 2023, **15**, 13834-13841.
71. J. Spratt, J. M. Dias, C. Kolonelou, G. Kiriako, E. Engström, E. Petrova, C. Karampelias, I. Cervenka, N. Papanicolaou, A. Lentini, B. Reinius, O. Andersson, E. Ambrosetti, J. L. Ruas and A. I. Teixeira, Multivalent insulin receptor activation using insulin-DNA origami nanostructures, *Nat. Nanotechnol.*, 2024, **19**, 237-245.
72. I. Smyrlaki, F. Fördős, I. Rocamonde-Lago, Y. Wang, B. Shen, A. Lentini, V. C. Luca, B. Reinius, A. I. Teixeira and B. Högberg, Soluble and multivalent Jag1 DNA origami nanopatterns activate Notch without pulling force, *Nat. Commun.*, 2024, **15**, 465.
73. L. Li, J. Yin, W. Ma, L. Tang, J. Zou, L. Yang, T. Du, Y. Zhao, L. Wang, Z. Yang, C. Fan, J. Chao and X. Chen, A DNA origami device spatially controls CD95 signalling to induce immune tolerance in rheumatoid arthritis, *Nat. Mater.*, 2024, **23**, 993-1001.
74. L. Sun, Y. Su, J. G. Wang, F. Xia, Y. Xu and D. Li, DNA nanotweezers for stabilizing and dynamically lighting up a lipid raft on living cell membranes and the activation of T cells, *Chem. Sci.*, 2020, **11**, 1581-1586.
75. Y. Su, X. Chen, H. Wang, L. Sun, Y. Xu and D. Li, Enhancing cell membrane phase separation for inhibiting cancer metastasis with a stimuli-responsive DNA nanodevice, *Chem. Sci.*, 2022, **13**, 6303-6308.
76. X. Chen, Q. Yang, W. Kong, Y. Ge, J. He, A. Yan and D. Li, High spatial-resolved heat manipulating membrane heterogeneity alters cellular migration and signaling, *Proc. Natl. Acad. Sci. U.S.A.*, 2023, **120**, e2312603120.
77. K. Venkatesan, J. F. Rual, A. Vazquez, U. Stelzl, I. Lemmens, T. Hirozane-Kishikawa, T. Hao, M. Zenkner, X. Xin, K. I. Goh, M. A. Yildirim, N. Simonis, K. Heinzmann, F. Gebreab, J. M. Sahalie, S. Cevik, C. Simon, A. S. de Smet, E. Dann, A. Smolyar, A. Vinayagam, H. Yu, D. Szeto, H. Borick, A. Dricot, N. Klitgord, R. R. Murray, C. Lin, M. Lalowski, J. Timm, K. Rau, C. Boone, P. Braun, M. E. Cusick, F. P. Roth, D. E. Hill, J. Tavernier, E. E. Wanker, A. L. Barabási and M. Vidal, An empirical framework for binary interactome mapping, *Nat. Methods*, 2009, **6**, 83-90.
78. M. P. Stumpf, T. Thorne, E. de Silva, R. Stewart, H. J. An, M. Lappe and C. Wiuf, Estimating the size of the human interactome, *Proc. Natl. Acad. Sci. U.S.A.*, 2008, **105**, 6959-6964.

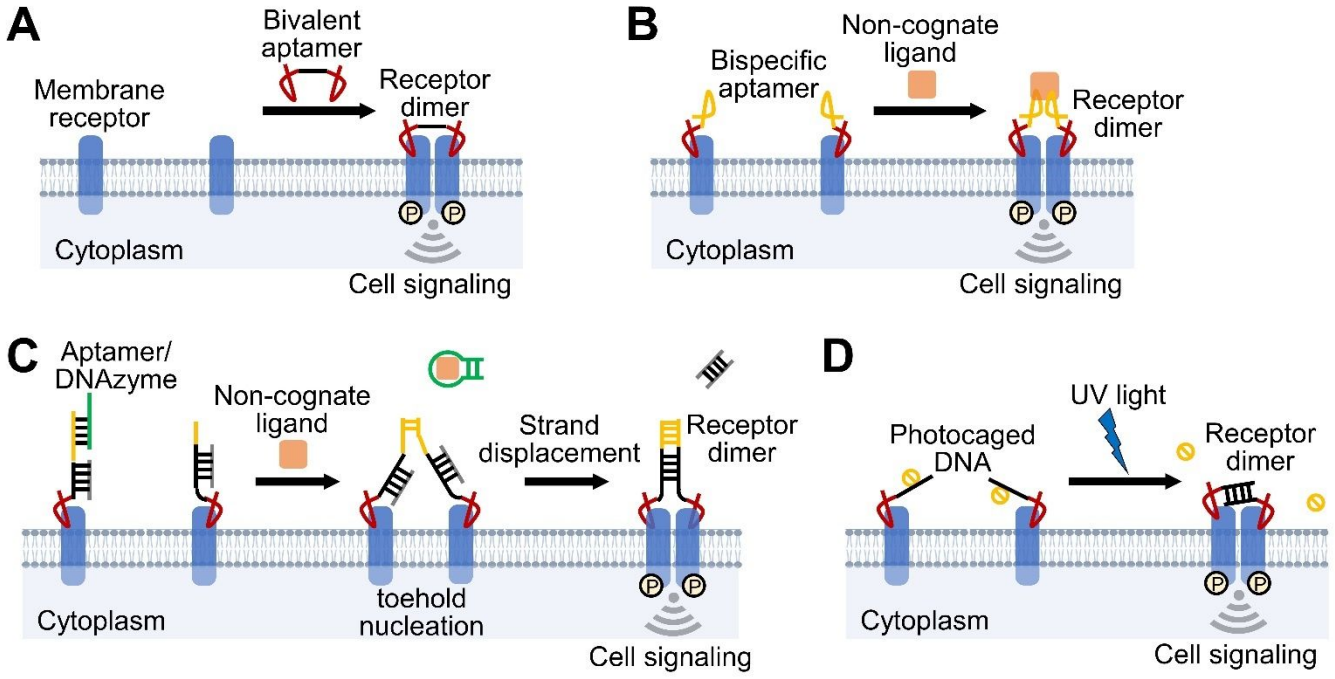

Fig. 1 (A) Schematic of a bivalent DNA aptamer-induced membrane receptor dimerization that consequently activates trans-phosphorylation and cell signaling. (B) Schematic of a bispecific aptamer that can bind non-cognate ligands and then trigger membrane receptor dimerization. (C) Schematic of a non-cognate ligand-triggered strand displacement reaction that induces membrane receptor dimerization. (D) Schematic of a photocaged DNA that can hybridize with each other after UV light-induced cleavage and then trigger membrane receptor dimerization.

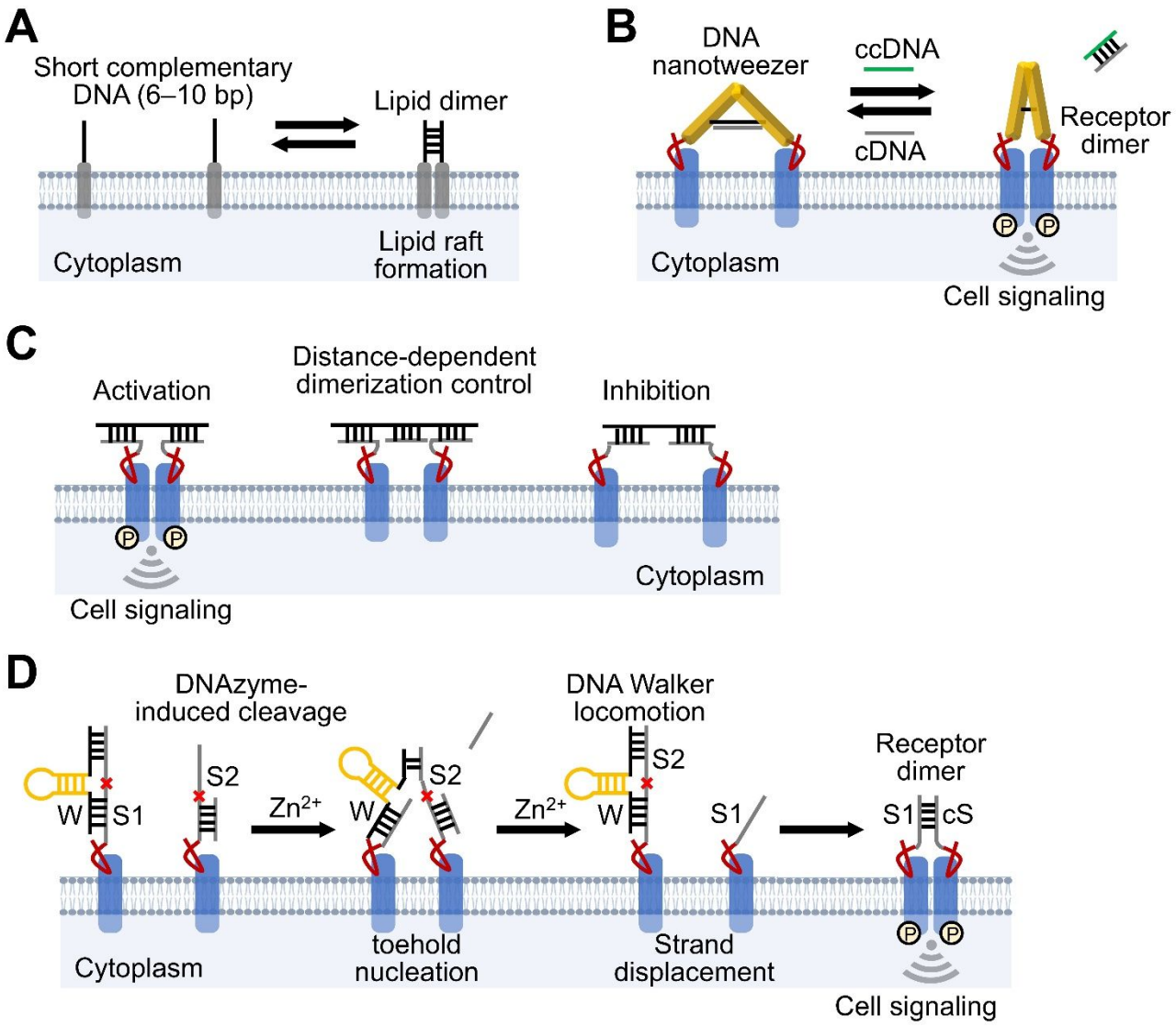

Fig. 2 (A) Schematic of a DNA Zipper nanodevice that dynamically modulates the binding strength of membrane lipid dimers to regulate membrane lipid raft formation and cell signaling. (B) Schematic of a tweezer-like DNA nanodevice that can reversibly modulate membrane receptor dimerization via adding a complementary DNA (cDNA) to open the hairpin or further adding a complementary DNA to the cDNA (ccDNA) to reverse the effect. (C) Schematic of the distance-dependent control of receptor dimerization on live cell membranes using rigid duplex DNA strands. (D) Schematic of a Zn^{2+}-activated DNAzyme walker that can induce the *trans*-cleavage of foothold strands (S1, S2…Sn) and activate receptor-binding aptamers. The activated aptamer (e.g., S1 here) will further hybridize with another actuator aptamer (cS) to induce receptor dimerizations.

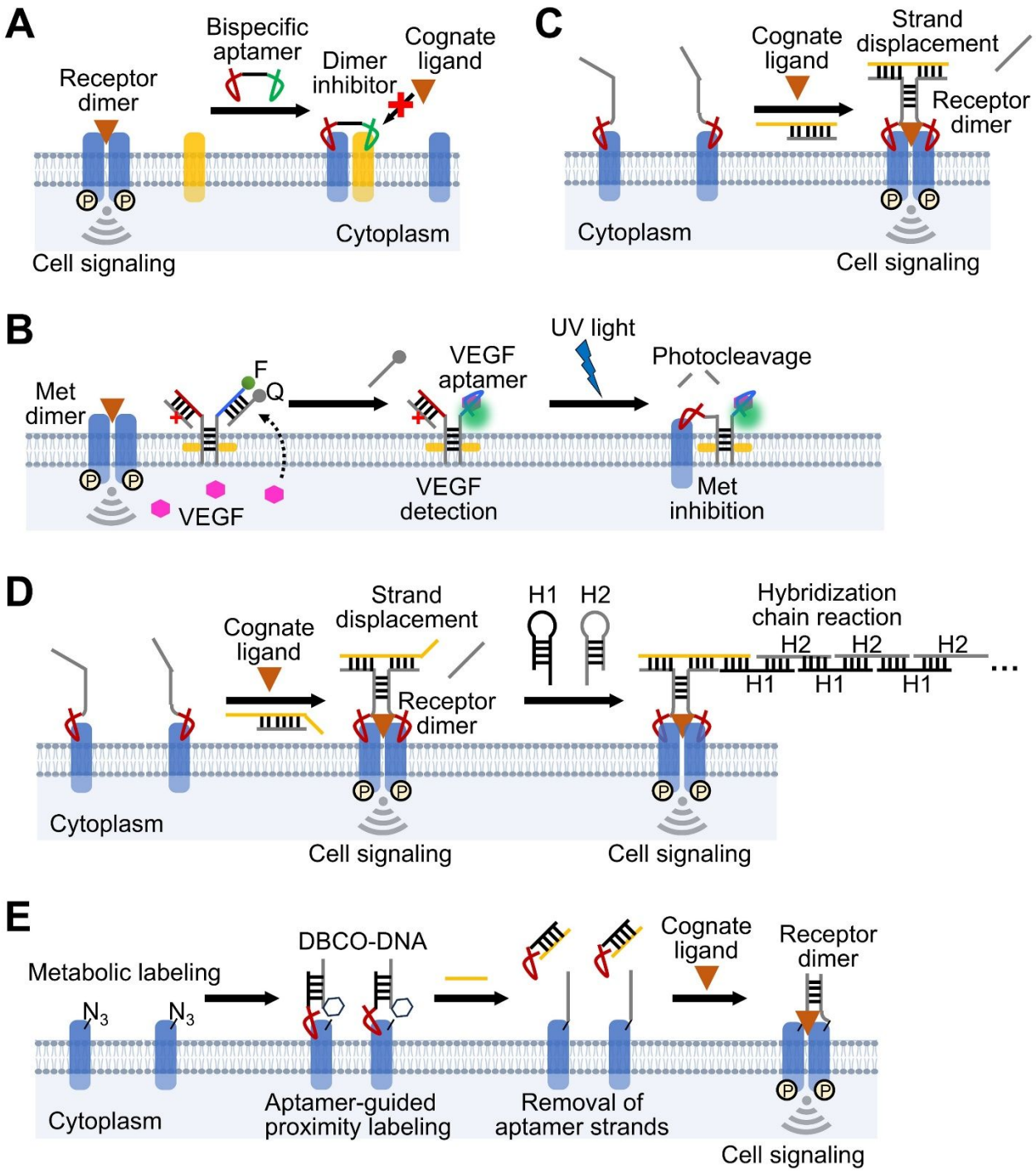

Fig. 3 (A) Schematic of a bispecific aptamer that can inhibit the formation of membrane receptor dimers by forming a non-natural dimer instead. (B) Schematic of a two-armed lipid-DNA probe that can recognize VEGF secretion from Met activation and then inhibition of Met dimerization upon light irradiation. (C) Schematic of a ligand-induced transient receptor dimer that further initiates a toehold-mediated strand displacement reaction to form a stable DNA three-way junction structure. (D) Schematic of a ligand-induced receptor proximity that triggers the sequential hybridizations among two hybridization chain reaction hairpins, H1 and H2, to produce a stable DNA polymer for the modulation and imaging of membrane receptor dimers. (E) Schematic of an azide-DBCO click chemistry approach after metabolic labeling to targeted label and monitor membrane receptor dimerization in real-time.

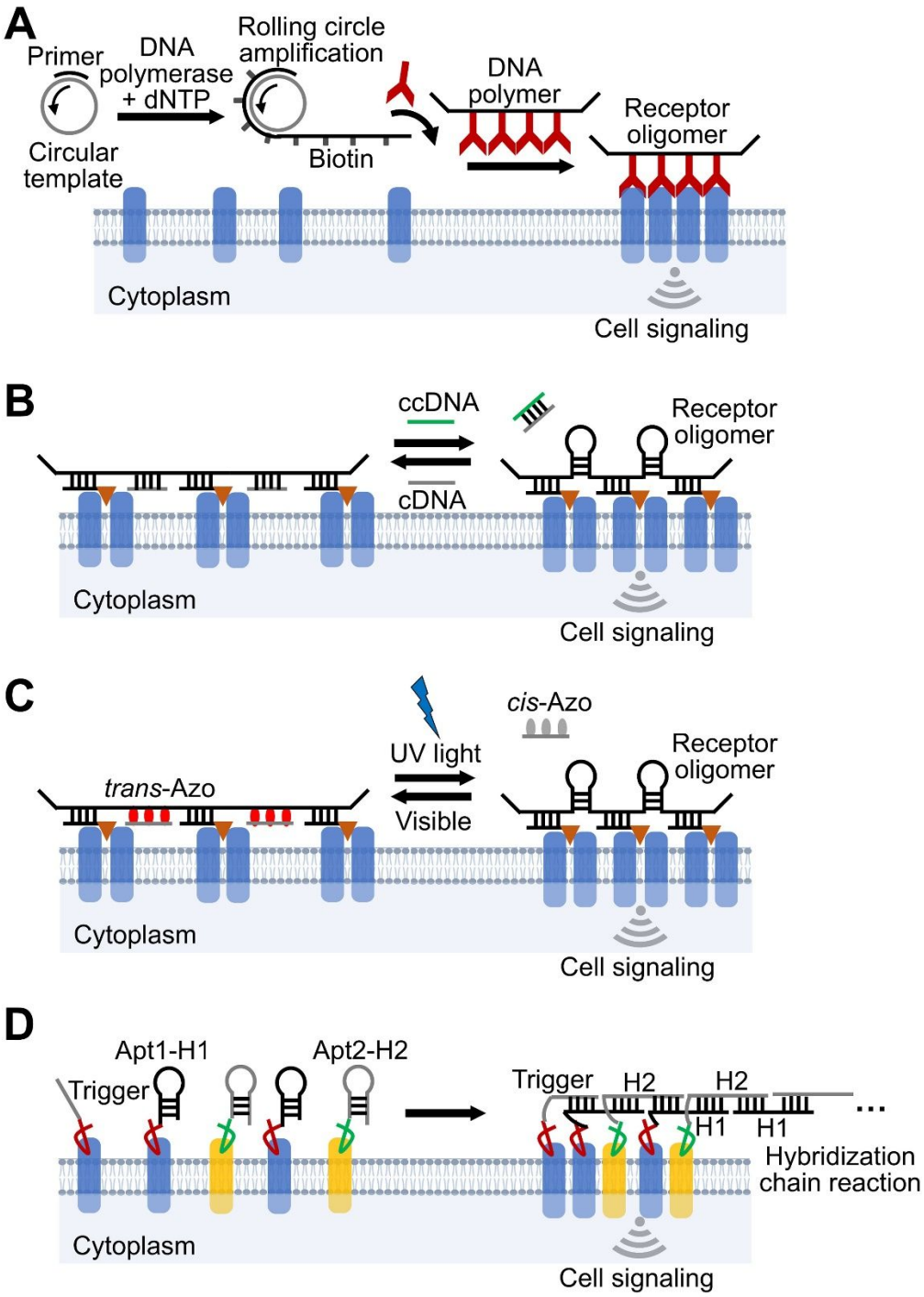

Fig. 4 (A) Schematic of a rolling circle amplification-produced DNA polymer that contains multiple biotinylated sites to modify with neutravidin and biotinylated antibody, which is used to mediate receptor oligomerization. (B) Schematic of a ligand-modified rolling circle amplification-produced DNA polymer that can reversibly modulate membrane receptor oligomerization via adding a complementary DNA (cDNA) to open the hairpin or further adding a complementary DNA to the cDNA (ccDNA) to reverse the effect. (C) Schematic of a ligand-modified rolling circle amplification-produced DNA polymer that contains photoswitchable azobenzene moieties. Azobenzene undergoes *cis*- and *trans*-conformation after UV or visible light irradiation, which reversibly control the close and open of DNA hairpins to dynamically control membrane receptor oligomerization status. (D) schematic of a hybridization chain reaction-based hetero-clustering of two types of receptors on live cell membranes.

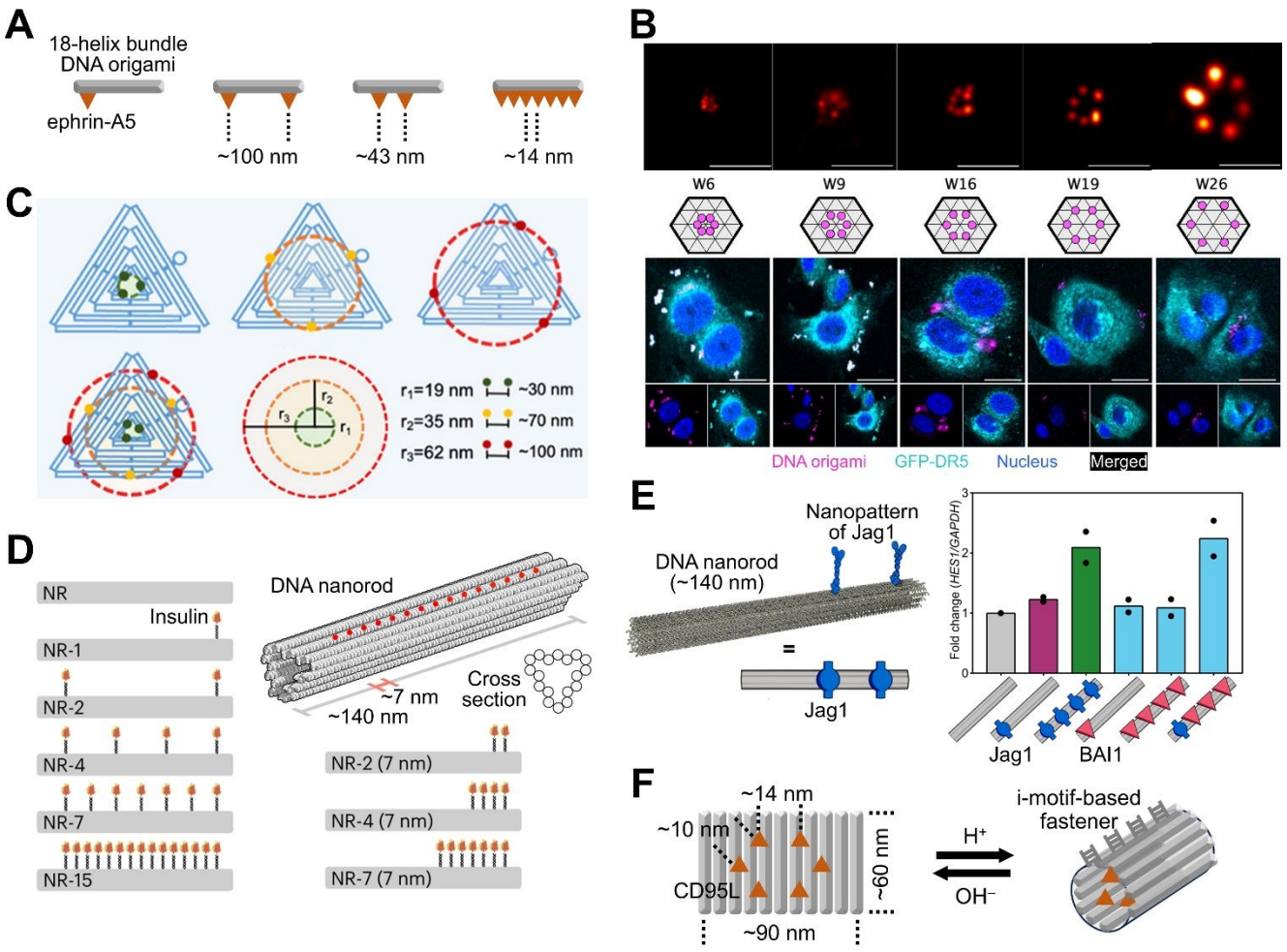

Fig. 5 (A) Schematic of ephrin-A5 ligand-modified bundle DNA origami with varied inter-ligand distance. (B) DNA-PAINT images of the differently sized six 17-amino-acid-long cyclic peptide patterns on hexagon-shaped flat sheets, spacing from ~5.7 nm to ~25.5 nm. Scale bar, 50 nm. Colocalization of hexagon-shaped flat sheets (magenta) with GFP-labeled DR5 receptor (cyan) clusters on MCF-7 cell membranes. Scale bar, 20 μm. Reproduced with permission from Reference 66. Copyright (2021) Björn Högberg. (C) Schematic of triangular DNA origami scaffold attached with EpCAM-, HER2-, and EGFR-targeting aptamers at different distances. Reproduced with permission from Reference 68. Copyright (2023) American Chemical Society. (D) Schematic of rod-like DNA origami containing 0, 1, 2, 4, 7, or 15 insulins at a constant (7 nm) or different spacing. Reproduced with permission from Reference 71. Copyright (2024) Ana I. Teixeira. (E) Jag1-modified DNA origami nanorod, even using only one Jag1 together with other membrane binders like BAI1, can activate Notch signaling in the absence of forces. Reproduced with permission from Reference 72. Copyright (2024) Björn Högberg. (F) Schematic of a pH-responsive DNA origami with reversible exposure of CD95 ligands by transiting between an open and closed DNA pattern.

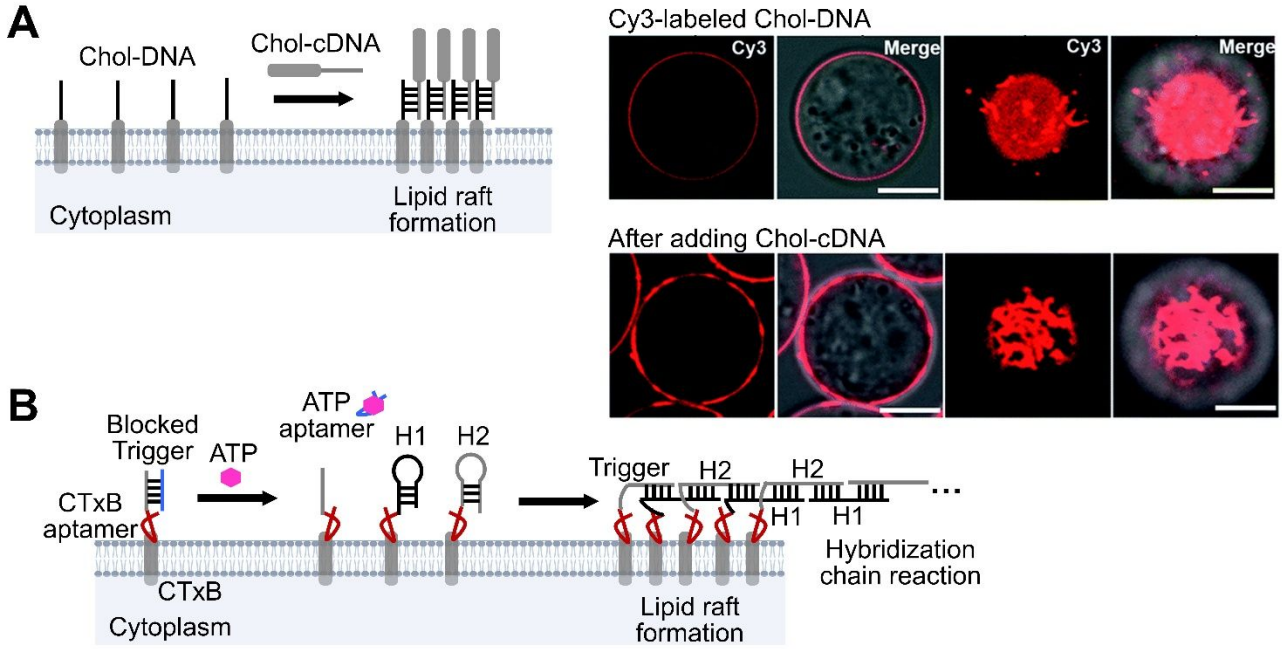

Fig. 6 (A) The use of cholesterol-modified DNA probes to cluster the cell membrane into lipid-ordered or raft domains. Fluorescence microscopy images were taken from cross and bottom view, scale bar, 5 μm. Reproduced with permission from Reference 74. Copyright (2020) Royal Society of Chemistry. (B) Schematic of an HCR-induced cholera toxin B clustering strategy to induce the formation of lipid domains on cell membranes.

Data availability Statement

No primary research results, software or code have been included and no new data were generated or analyzed as part of this review.