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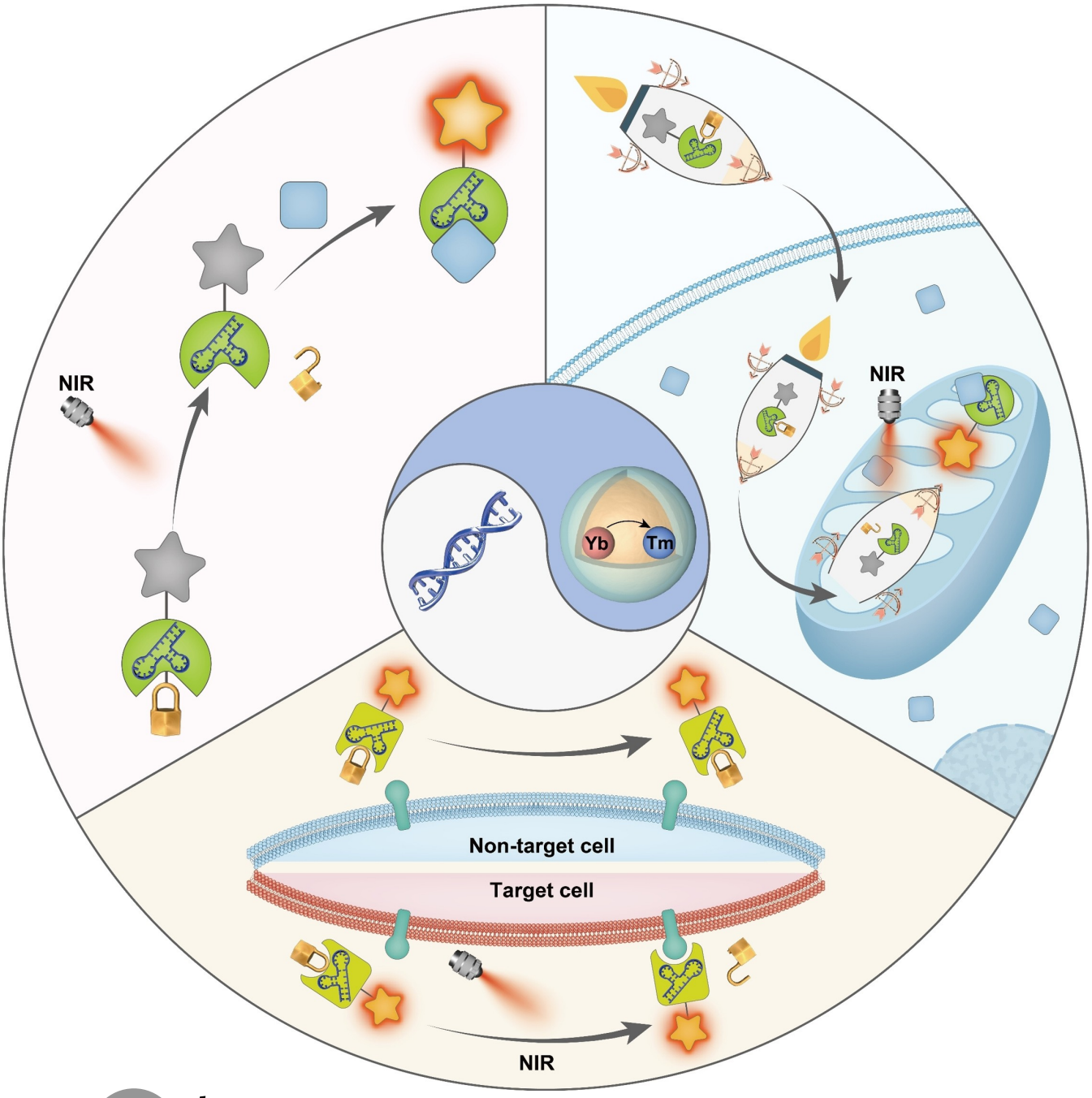
DNA Nanotechnology

Spatiotemporally Selective Molecular Imaging via Upconversion Luminescence-Controlled, DNA-Based Biosensor Technology

Jian Zhao[+], *Zhenghan Di*[+], *and Lele Li**



Abstract: DNA-based biosensor technologies have shown great potential in chemical and biological detection. These biosensors have been actively developed as probes for molecular imaging in live cells and in animals, allowing in situ detection of analytes in complex biological systems, elucidation of the roles of key molecules in biological processes, and the development of non-invasive diagnosis and image-guided surgery. Despite the progress made, improving the spatial-temporal precision remains a challenge in this field. In this Minireview, we describe the concepts behind spatiotemporally selective molecular imaging via the combination of engineered, light-activatable DNA-based biosensors and upconversion nanotechnology. We then highlight the application of the approach for the spatiotemporally controlled imaging of various targets in specific intracellular organelles, signal amplification, as well as the regulation of targeting activity to receptor proteins. We finally discuss the challenges and perspectives for possible future developments in this emerging field.

1. Introduction

The unique properties of DNA, including its unparalleled programmability and easy-to-predict thermodynamics, have led to an explosion in synthetic architectures for far-ranging applications in material science and biology,[1,2] with an increasing focus on designer DNA devices to recognize molecular inputs of interest and transduce them into readable signal outputs.[3] In particular, tremendous efforts have contributed to the design of DNA-based biosensors for the detection and imaging of a broad range of analytes, including metal ions, small molecules, RNA, and proteins.[4–10] Such probes have shown great potential to help figure out fundamental mechanisms of pathophysiological processes at the molecular level and to facilitate disease diagnosis.[6–10] Despite the significant progress achieved, the DNA-based biosensors that respond to analytes through irreversible mechanisms such as DNAzyme-based metal-ion sensing, cleavage-activatable enzyme detection, and signal amplification strategies for RNA analysis, are still limited by spatiotemporal issues because such biosensors could be irreversibly turned on during the delivery process.[10,11] To improve the spatial-temporal precision remains a central theme in the field of molecular sensing and imaging.

Since light has the advantages of noninvasive modality and facile modulation of wavelength and intensity, it has emerged as an attractive tool for the precise regulation of chemical and biological systems.[12] Light-based techniques have allowed remote control of signaling transduction,[13] gene expression,[14] and therapeutic activity.[15] Nevertheless, such photoregulation approaches face predicaments due to the use of ultraviolet (UV) or visible light with limited tissue penetration depth, since most photolabile moieties respond to light only at these wavelengths. Near-infrared (NIR) light with less photon scattering and high tissue penetration is an ideal alternative for the regulation of biological events. Recently, lanthanide-doped upconversion nanoparticles (UCNPs) have been employed for the engineering of NIR light-regulated systems.[16–18] UCNPs can promote photon upconversion, in which two or more lower-energy photons are absorbed and upconverted into high-energy luminescence.[19,20] UCNPs rationally doped with a combination of sensitizers and activators can generate upconversion luminescence (UCL) ranging from UV to NIR upon NIR light excitation. Importantly, numerous studies show that UCNPs are chemically stable and biocompatible with cells and in vivo.[21–23] Therefore, due to their unique anti-Stokes optical property and excellent biosafety, UCNPs have been widely explored for various biomedical applications, including biosensing and imaging, manipulation of neural activity, and drug delivery.[16,17,21–24] In particular, UCNPs have been combined with DNA-based components to construct biosensors for the detection and imaging of various analytes.[25–30] In these designs, acceptors were carefully selected and modified on the surface of UCNPs (as the donor) for the construction of Förster resonance energy transfer (FRET)-based sensors, in which the target detection is based on the changes in UCL intensity.[25]

In this Minireview, we summarize the development of UCL-controlled, DNA-based sensor technologies for spatiotemporally selective molecular imaging (Figure 1). Conventional DNA-based sensor designs employ an "always active" principle and the sensors are immediately turned "on" when they encounter the targets (Figure 1a). We present a conceptual methodology for the rational engineering of UV light-activatable DNA sensor probes and their further combination with UCNPs to regulate molecular imaging with the high spatial-temporal precision of NIR light. Notably, in comparison to the previous FRET-UCNP based sensors that employ UCNPs as signal units, here UCNPs are used as NIR-to-UV transducers to achieve NIR light-triggered biosensing. In addition, since naked DNA cannot penetrate the cell membrane, the application of DNA probes for detection in living cells often requires the use of various nanoparticles as carriers for intracellular delivery, including liposomes, gold nanoparticles, and quantum dots.[8,31] In this design, UCNPs also serve as biocompatible carriers to deliver light-responsive DNA probes into cells for molecular imaging. Furthermore, UCL-controlled DNA sensor technology was extended to precise molecular imaging at the subcellular level (Figure 1b). A strategy for the targeting of specific organelles was developed previously

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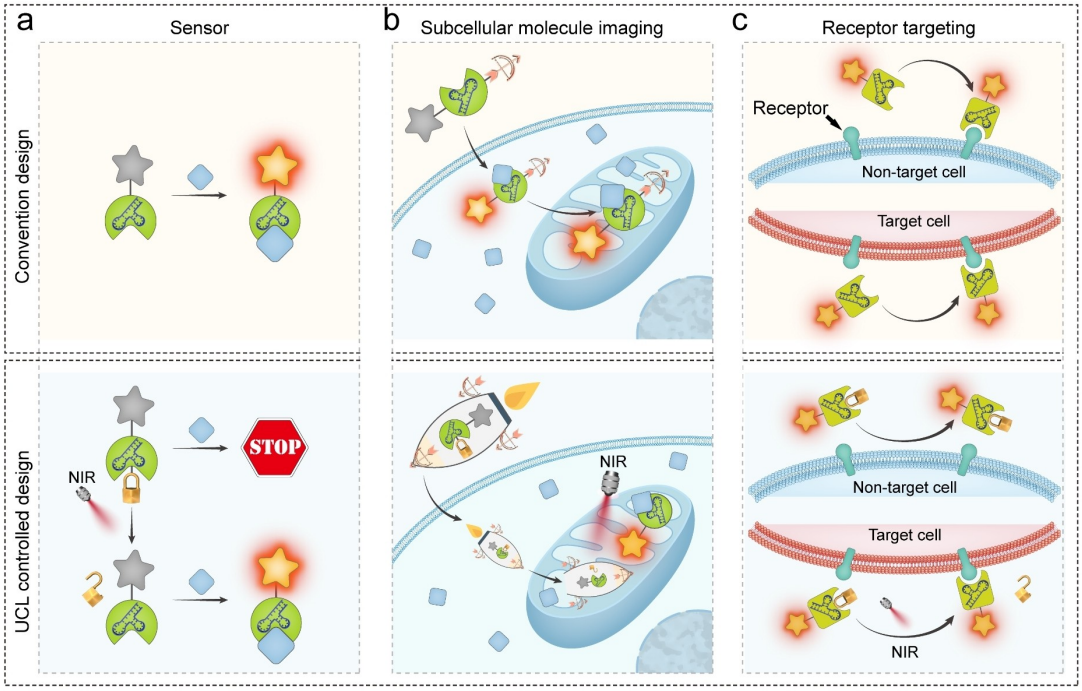

Figure 1. a) Illustration of the UCL-controlled sensor mechanism in comparison to that behind conventional sensors. b) Schematic showing the UCL-controlled sensor technology that permits molecular imaging in an organelle of choice (e.g., mitochondria), while conventional sensors may respond to targets encountered in transit. c) Comparison of UCL-controlled method and the conventional approach for targeting DNA probes to the receptor proteins on target and non-target cells. The locks represent the photosensitive groups. The blue cubes indicate analytes. The rockets represent the UCL-controlled sensor devices that are designed through the conjugation of organelle-targeting ligands on the surface of UCNPs for targeted delivery of light-activatable DNA sensing probes.

for subcellular imaging. In the UCL-controlled design, the sensing activity of the DNA-based sensors is blocked during the targeting and delivery process in order to prevent premature response to the analytes (which are also distrib-

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uted outside cells or in cytosol) before the sensors reach the targeted organelles. After localization to specific organelles, the sensors could be photoactivated with NIR light, enabling precise subcellular molecular imaging. Furthermore, it has been established that it is possible to use upconversion nanotechnology to modulate the biorecognition activity of functional DNA probes to receptor proteins (Figure 1c). In conventional targeting approaches, functional DNA ligands bind receptor proteins on both target and non-target cells. In our design, the biorecognition function of DNA ligands is blocked and then restored by local UCL activation for receptor binding on target cells, leading to enhanced spatial precision for disease diagnosis and therapy. Finally, we give a brief summary and present some insights into current challenges and future opportunities in this emerging field. This Minireview should open up broad perspectives for the merging of DNA-based sensor design with upconversion nanotechnology to improve the spatiotemporal accuracy of molecular imaging, which may further provide guidance for disease characterization and management.

2. Spatiotemporally Selective Molecular Imaging in Live Cells

2.1. Establishment of the Methodology

Traditional chemical and biological sensors lack temporal controllability and can be turned on immediately once they encounter targets during the delivery process. Here we present the conceptual methodology for spatiotemporally controlled molecular imaging based on the design of a UV light-triggered aptamer sensor and its further combination with upconversion nanotechnology (Figure 2a).[32] Aptamers are single-stranded nucleic acids with well-defined conformations that possess specific binding properties toward diverse targets including small molecules, metal ions, and cell surface proteins.[5,6] We chose an anti-ATP aptamer-based, structure-switchable duplex design[33] to demonstrate our approach. The sensing device named Apt-Act/UC is composed of a UV light-activatable aptamer probe and a UCNP. The aptamer probe is a hybrid of a Cy3-labeled aptamer strand and a quencher-labeled complementary DNA containing a photocleavable (PC) linker. In this duplex state, the ability of the aptamer to bind to ATP is blocked and the fluorescence of the probe is quenched due to FRET. As an exogenous regulation tool, UV light enables the photolysis of the PC linker and reduces the hybridization affinity of the complementary DNA to the aptamer strand, allowing the recovery of the fluorescence sensing function for the structure-switch of the aptamer to recognize and detect ATP. Moreover, UCNPs serve as NIR-to-UV transducers to absorb NIR light and produce UV emission locally for remote control of the ATP sensing function with deep-tissue-penetrable NIR light. As shown in Figure 2b, Apt-Act/UCs-treated cells showed a notable signal increase upon NIR light irradiation compared with those without NIR irradiation. As a control, the inactive system (Apt-non/UCs), which has the same structure as Apt-Act/UCs but without a PC linker, showed no NIR light-activatable sensing performance. This work validates UCL-regulated biosensing for spatiotemporally controlled molecular imaging in living cells.

2.2. Imaging of Diverse Key Molecules

After establishment of the conceptual method, it was extended to the spatiotemporally selective imaging of diverse important molecules such as microRNAs (miRNAs), metal ions, and small molecules. miRNAs are short, endogenous noncoding RNAs that are involved in numerous biological processes.[34] Spatially selective visualization of miRNA dynamics would facilitate reveal their pathophysiological roles. We designed an on-demand activatable DNA nanodevice that permits miRNA imaging with the high spatial and temporal precision associated with NIR light (Figure 2c).[35] The DNA nanodevice (denoted as PBc-UN) was constructed by the integration of a UV light-activatable molecular beacon (PBc) and UCNPs. Molecular beacons, characterized by stem-loop hairpin structure, are a straightforward tool for the detection of nucleic acids via the Watson–Crick base paring principle.[36] PBc was designed through the re-engineering of a conventional molecular beacon probe by installation of a PC linker into the hairpin loop, which would temporarily block its functionality to recognize and detect the target miRNA. NIR light-induced generation of ultraviolet upconversion luminescence (UV UCL) from UCNPs would cleave the PC bond and thus enable the target miRNA-binding mediated displacement of the quencher-labeled strand in the cleaved PBc, resulting in fluorescence enhancement for biosensing. It was shown that PBc-UN allowed remote control of its miRNA imaging activity in live cells through NIR light activation at a chosen time (Figure 2d). In addition, we demonstrated that the system can be used to regulate the imaging of miRNAs at tumor sites in animals (Figure 2e,f).

Metal ions play key roles in the regulation of diverse cellular functions. Probing intracellular metal ions with high spatial and temporal resolution is helpful to elucidate related physiological and pathological processes. By conjugating photocaged DNAzyme probes on UCNPs, Lu et al. constructed a UCL-regulated biosensor that facilitated optically controlled metal-ion imaging in cells and zebrafish.[37] As shown in Figure 3a, the substrate strand of the Zn^{2+}-specific DNAzyme (termed 8–17 DNAzyme) was dually labeled with a fluorophore (F) and a quencher (Q1) at opposite ends, and the enzyme strand was labeled with another quencher (Q2). Such a construct ensures efficient fluorescence quenching of the fluorophore and low background fluorescence. Classically, Zn^{2+}-binding would trigger the cleavage of the substrate strand and lead to release of the fluorophore-labeled product, inducing an increase of the fluorescence signal. However, the authors modified a 2′-nitrobenzyl photocage group (PG) at the cleavage site (rA), which is able to protect the substrate strand from metal-ion-induced cleavage. Therefore, the Zn^{2+}-sensing function of

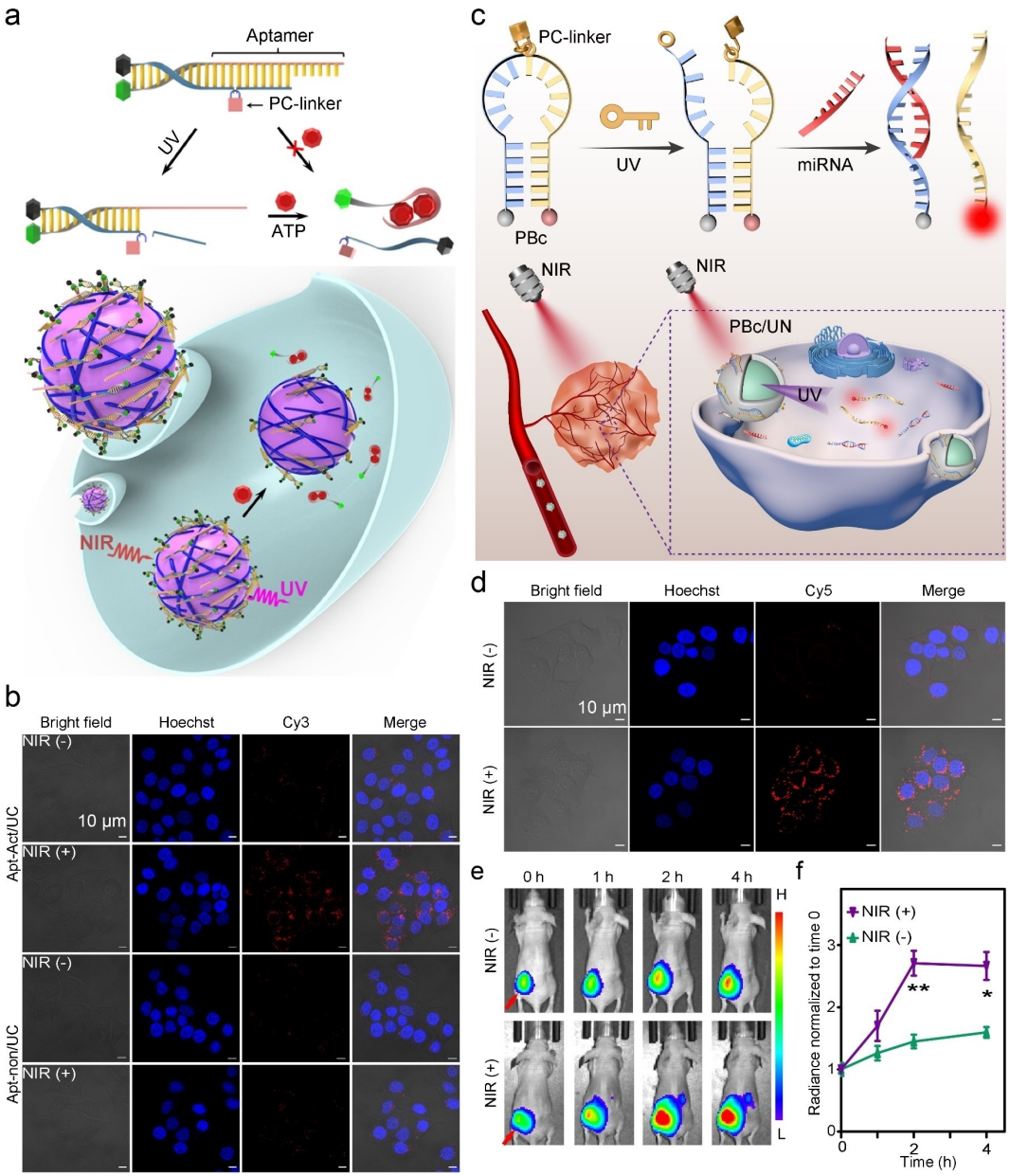

Figure 2. a) Schematic of the working principle of UCL-controlled, spatiotemporally selective ATP imaging based on the integration of a UV light-triggered aptamer probe (Apt-Act) with UCNPs. b) Confocal fluorescence images of HeLa cells treated with Apt-Act/UCs (designed by the loading of Apt-Act probes on UCNPs) and Apt-non/UCs (constructed by the loading of inactive Apt-non probes on UCNPs), followed by activation or no activation with NIR light. Reproduced from Ref. [32] with permission. Copyright 2018 American Chemical Society. c) Schematic of the working principle of UCL-controlled miRNA imaging through the engineering of a photosensitive molecular beacon probe (PBc) and further combination with UCNPs to achieve the sensing device (PBc-UN). d) Confocal fluorescence images of HeLa cells treated with PBc-UN with and without NIR irradiation. e) Fluorescence imaging of tumor-bearing mice injected with PBc-UN with or without subsequent NIR irradiation at the tumor sites. f) The intratumoral fluorescence intensity of mice in (e). Reproduced from Ref. [35] with permission. Copyright 2019 American Chemical Society.

the engineered DNAzyme probe is blocked until UV-mediated photodissociation of the caged group occurs. Through the covalent conjugation of this photocaged DNAzyme probe on the surface of UCNPs, its Zn^{2+}-sensing function can be controlled by 980 nm NIR light. It was reported that the nanoprobes enabled temporally selective imaging of endogenous Zn^{2+} in early embryos of zebrafish via selective irradiation with NIR light (Figure 3b). Ultimately, the method should allow spatiotemporally controlled imaging of diverse targets through the combination of different DNA-based sensing designs with upconversion nanotechnology.[38,39] For example, by applying a cytosine-rich i-motif design, we constructed a remotely controlled DNA nanodevice for time-resolved activation of pH sensing and imaging in living cells.[38]

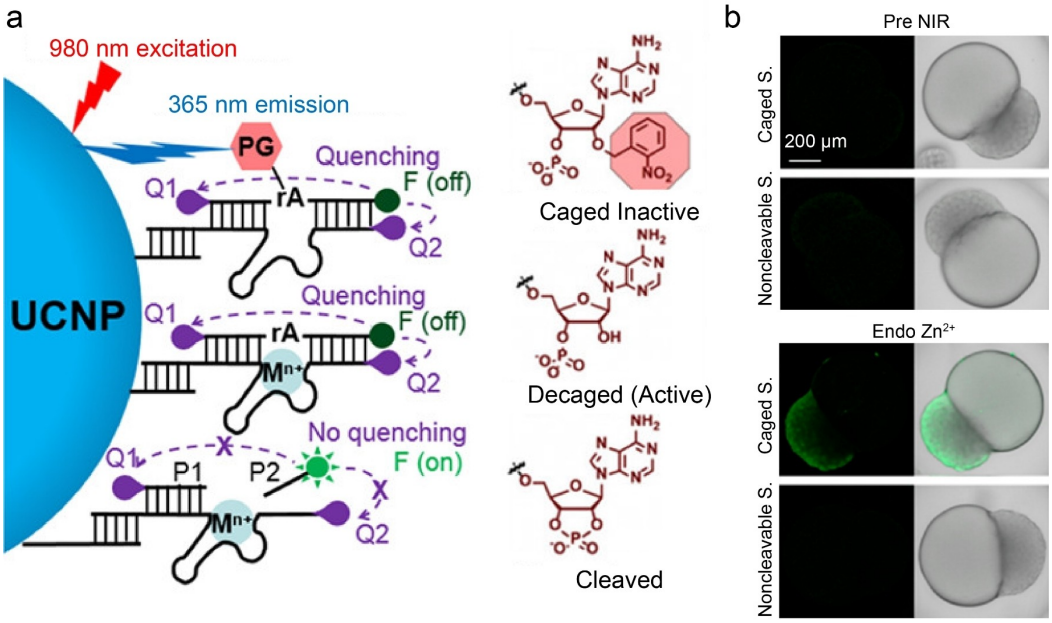

Figure 3. a) Schematic of the UCL-controlled, DNAzyme-based nanoprobe for optically controlled metal-ion imaging. The designed nanoprobe consists of UCNP conjugated with photocaged DNAzyme probes, in which a 2′-nitrobenzyl photocage group (PG) is modified at the ribonucleotide adenosine (rA) site in the substrate strand to protect it from being cleaved. b) Confocal fluorescence images of zebrafish embryos treated with the nanoprobes, with and without subsequent NIR light irradiation. Reproduced from Ref. [37] with permission. Copyright 2018 American Chemical Society.

2.3. Multiplexed Molecular Imaging

Most physiological and pathological processes are attributed to complicated interactions between a series of components. The ability to simultaneously measure a multitude of molecular signatures in living organisms is thus critical to understanding the dynamics of complex networks, as well as disease diagnosis and treatment. In particular, DNA computation offers intriguing opportunities for multiplex molecular detection, where engineered DNA circuits can output signals in a programmable manner in response to different molecular inputs.[3] However, conventional DNA circuits are constitutively in an "ON" state, and less spatial-temporal control over DNA computation has been achieved, leading to low precision for the intended control of the timing and site of molecular computation in complex environments.

Recently, we extended the aforementioned approach to design photonic nanocircuits that made DNA computation possible with high spatiotemporal selectivity (Figure 4a).[40] To realize molecular imaging in an "AND" Boolean logic manner, an anti-ATP aptamer was combined with photoactivated design and toehold-mediated strand displacement reaction (SDR) for the construction of the DNA circuit. When the DNA nanocircuit was triggered remotely with NIR light, UCL-mediated cleavage of the PC linker enabled the binding of the ATP to aptamer (step 1) and thus exposure of the toehold in the P2 strand (step 2). Then, the toehold-mediated SDR between the target miRNA and the P2 strand resulted in the release of a signaling output (step 3). This approach employs an exogenous NIR light input to achieve intended control over the spatiotemporal selectivity of imaging of two types of cancer biomarkers (ATP and miRNA) (Figure 4b). Spatial control of DNA computation in animals was also demonstrated using tumor-bearing mice via the administration of the nanocircuits by tail vein injection. In addition, we further designed an "OR–AND"-gated nanocircuit of higher complexity to show the versatility of this method. This gain-of-function strategy paves a new way for precise information processing in biological systems.

Apart from the above-mentioned circuit-based multiplexed detection that gives a one-channel signal upon multiple molecular inputs, the strategy of multicolor multiplexed imaging allows the parallel detection of multiple independent targets through corresponding multiple-channel signals.[41] Recently, Luo et al. constructed a UCL-activated nanoprobe for the multicolor multiplexed imaging of three miRNAs in living cells (Figure 4c).[42] First, a UV light-activated triangular DNA nano sucker (TDS) was designed via the assembly of three PC linker-containing DNA hairpins (PH1, PH2, and PH3) with three DNA strands (Q1, Q2, and Q3), in which three FRET pairs of different fluorescent wavelength were labeled. In this manner, a multiplexed three-color FRET sensor was achieved for UV light-triggered, simultaneous detection of three different miRNAs. Then, the nanoprobe was constructed by integration of TDS with UCNPs. When the nanoprobes entered the cells, NIR light irradiation could activate the sensing functions, allowing for simultaneous imaging of miRNA-21, miRNA-373, and miRNA-155 in HeLa cells through the fluorescent channels of FAM, TAMRA and Cy5, respectively (Figure 4d). This technology provides novel strategies

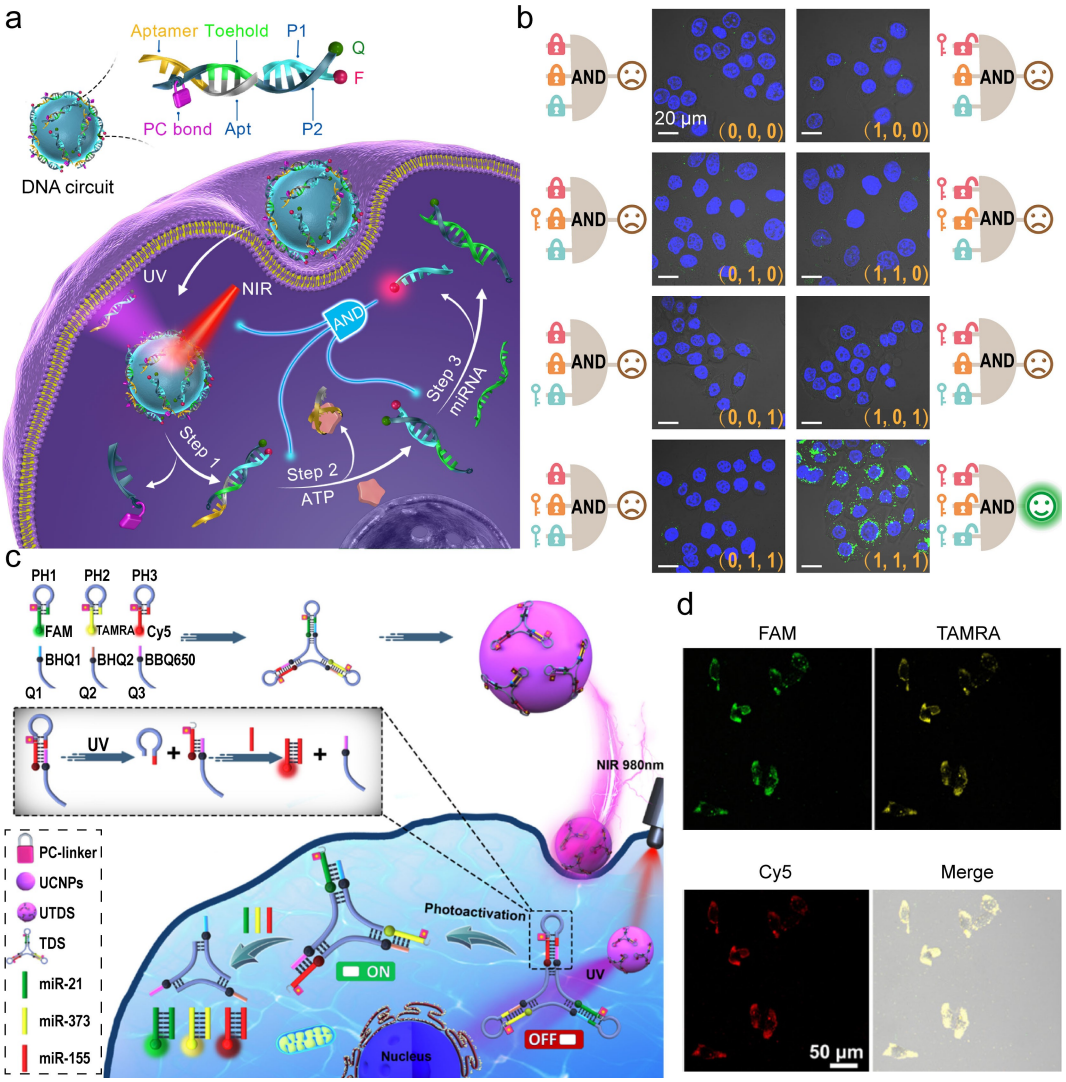

Figure 4. a) Schematic of UCL-activated DNA computation for multiplexed molecular imaging. b) Confocal fluorescence images representing molecular imaging in HeLa cells following "AND" Boolean logic. The red, brown, and green lock stands for PC linker, anti-ATP aptamer, and the miR-21 binding sequence, which respond to light, ATP, and target RNA, respectively. Reproduced from Ref. [40] with permission. Copyright 2021 American Chemical Society. c) Schematic of the UCL-activated DNA nanoprobe for multichannel multiplexed imaging of three intracellular miRNAs. d) Confocal fluorescence images of HeLa cells treated with the nanoprobes, with or without subsequent NIR light activation. The fluorescent channels of FAM, TAMRA, and Cy5 corresponds to response signals of miRNA-21, miRNA-373, and miRNA-155, respectively. Reproduced from Ref. [42] with permission. Copyright 2021 American Chemical Society.

for multiple biomarker diagnostics with high spatial-temporal resolution.

3. Spatiotemporally Selective Molecular Imaging at the Subcellular Level

Spatial organization of biomolecules in functionally distinct organelles is crucial to critical cellular processes, including signaling, metabolism and apoptosis.[43,44] Indeed, precise detection of such molecules at subcellular resolution remains a central theme in the field of molecular imaging.[45] Despite significant advances in the developments of diverse DNA-based probes for molecular sensing and imaging, the available approaches for subcellular imaging are severely limited. This is because conventional DNA probes generally lack localization specificity to target organelles. Moreover, such probes lack temporal control and thus can respond to the encountered targets (e.g., in the cytosol) before they reach the specific organelles.

3.1. Imaging of Mitochondrial MiRNAs (MitomiRs)

The localization of miRNAs in mitochondria, a critical organelle responsible for energy metabolism, plays a role in diverse physiological and pathological processes.[46] Deregulated expression of mitomiRs is involved in a series of pathologies, such as neurodegenerative disorders, metabolic

diseases, and cancer.[47,48] Most recently, we developed a DNA sensor technology for correlated imaging of dual mitomiRs in vitro and in vivo via UCL-controlled, toehold-mediated SDRs (Figure 5a).[49] In our design, the sensing device (PD-mUC) was established via equipping rationally designed DNA probes (termed as PD) with UCNPs and further modification with a mitochondria localization signal. The miRNA-responsive function of the PC linker-containing PD was inhibited ("OFF"). In addition, a mitochondria-targeting ligand (triphenylphosphonium (TPP)) was modified on the system to achieve specific localization. After programmable mitochondrial targeting, remotely controlled NIR light irradiation could trigger two toehold-mediated SDRs of PD with miR-10b and miR-21 miRNAs, respectively, leading to release of a signaling strand for dual mitomiR imaging with improved subcellular accuracy. The well overlapping sensing signals of PD-mUC and Mito-Tracker verified the precise mitomiR imaging in live cells. Furthermore, the system enables probing of mitomiRs in cancer cells and normal cells with different expression levels (Figure 5b). Spatiotemporally controlled imaging of the mitomiRs in vivo was also demonstrated.

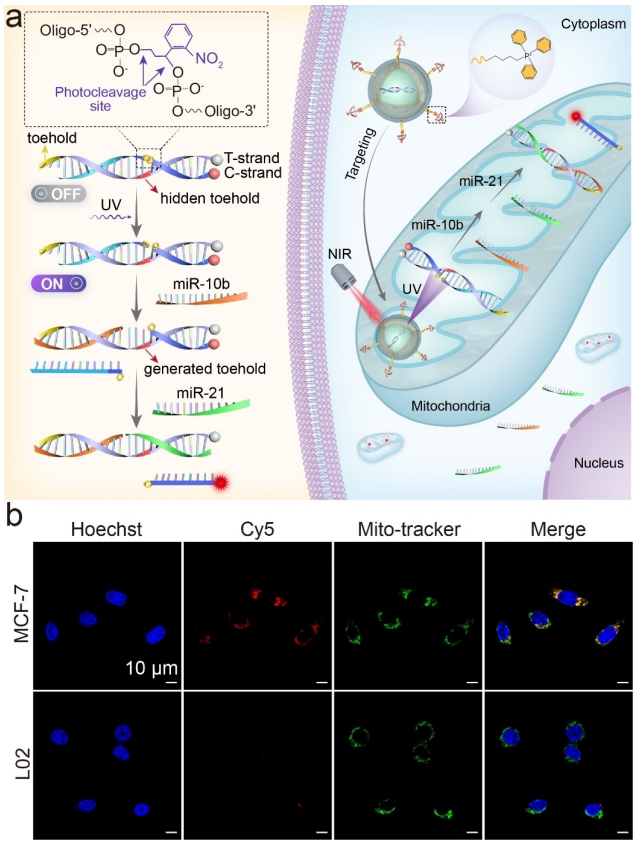

Figure 5. a) Schematic illustration of the design of the DNA sensing device (PD-mUC) for dual mitomiR imaging via UCL-controlled SDRs. b) Confocal fluorescence images of MCF-7 cancer cells and L02 normal cells treated with PD-mUC and NIR light irradiation. Reproduced from Ref. [49] with permission. Copyright 2021 Wiley-VCH GmbH.

3.2. Imaging of Subcellular Enzymes

Considering that subcellular enzymes have crucial functions in cells, we subsequently applied this strategy to their precise imaging in specific organelles. Human apurinic/apyrimidinic endonuclease 1 (APE1), a ubiquitous enzyme present in different organelles, is responsible for distinct physiological processes including DNA repair, transcription regulation, and redox pathways.[50] In addition, the dynamics of APE1 in subcellular localizations is correlated with tumor progression, metastasis, and therapeutic outcomes.[51,52] Given the critical function of APE1, we validated the feasibility of our method for in situ APE1 imaging in an organelle of choice (Figure 6a).[53] The sensing device contains a UV light-activatable APE1-sensing module (AP-P), a UCNP as light transducer, and an organelle-targeting ligand. UV light-induced photolysis of the PC linker in AP-P facilitated the formation of a molecular beacon structure with an apurinic/apyrimidinic (AP) site in its stem. Then, APE1 recognized and cleaved the AP site, leading to the disruption of the molecular beacon and thus fluorescence signal output. Furthermore, a mitochondria or nucleus localization signal was employed for the NIR light-controlled, spatiotemporally selective imaging of APE1 in a specific organelle (mitochondria or nucleus). Precise profiling of enzymatic activity in mitochondria was achieved through TPP-mediated, targeted delivery of the system (AP-P/UC_M) to mitochondria (Figure 6b). This platform could also be applied to image enzymatic activity in the nucleus through the introduction of TAT peptide as a nucleus-targeted ligand to construct nanosensors (AP-P/UC_N) (Figure 5c). Furthermore, the system enabled the visualization of APE1 translocation at the subcellular level in response to oxidative stress.

4. Spatiotemporally Selective Signal Amplification

Conventional sensing probes are designed based on a signal output-to-input ratio of 1:1, which results in insufficient sensitivity for the detection of low-abundance targets in biological milieu. For this reason, signal amplification techniques have been widely developed to overcome the limitation and increase signal gain and sensitivity.[9] Despite the progress made, such approaches with little temporal control will passively provide amplified signal once they meet the targets, limiting signal amplification at the chosen time and position.

In this regard, we reported a spatially and temporally controlled signal amplification method through the re-engineering of the hybridization chain reaction (HCR) and further integration with upconversion nanotechnology (Figure 7a).[54] HCR, developed by Dirks and Pierce in 2004, represents a powerful approach for signal amplification in sensing and imaging applications.[55,56] As an isothermal amplification process, HCR involves an initiator sequence to trigger a cascade of hybridization events between two metastable hairpins to form a long double-strand DNA product.[55] In our design, the toehold of one of the hairpins, H1, was sheltered with a short six-base tail linked by a PC

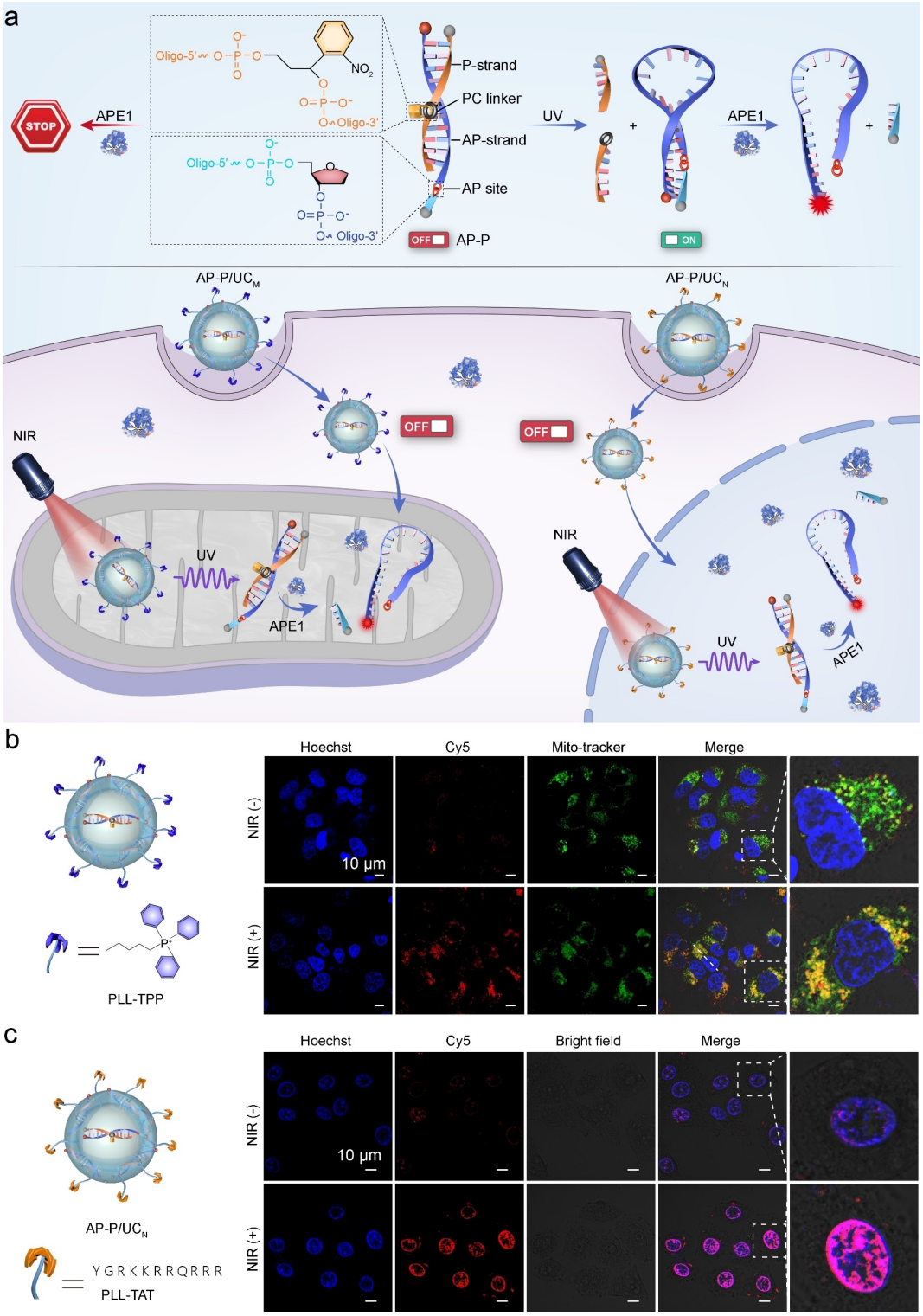

Figure 6. a) Schematic showing the design of DNA nanosensors for UCL-activatable imaging of APE1 in an organelle of choice. The nanosensor consists of a UV light-activatable DNA reporter (AP-P), a UCNP as a light transducer, and an organelle-targeting ligand. b) Structure of AP-P/UC_M that employs TPP as a mitochondria-targeting ligand (left) and confocal fluorescence images of Huh7 cells treated with AP-P/UC_M with or without NIR laser irradiation (right). c) Structure of AP-P/UC_N that employs TAT peptide as a nucleus-targeting ligand (left) and confocal fluorescence images of HeLa cells treated with AP-P/UC_N with or without NIR irradiation. Reproduced from Ref. [53] with permission. Copyright 2021 Wiley-VCH GmbH.

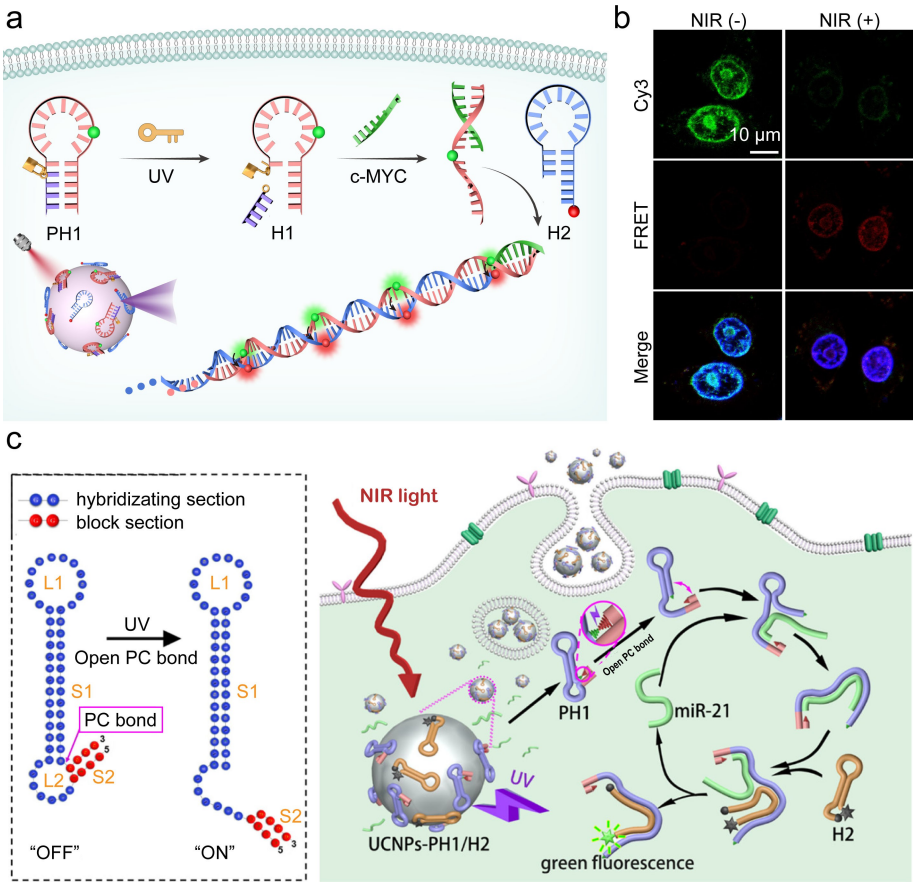

Figure 7. a) Schematic showing UCL-controlled HCR in living cells for amplified imaging of mRNA with high spatiotemporal resolution. The system (UC-PH1/H2) was constructed by the loading of light-responsive PH1 and H2 on UCNPs. b) Confocal fluorescence images of MCF-7 cells treated with TAT-modified UC-PH1/H2 with or without subsequent NIR irradiation. Reproduced from Ref. [54] with permission. Copyright 2019 Wiley-VCH GmbH. c) Schematic showing UCL-controlled CHA for amplified miRNA imaging. Left: a DNA dumbbell structure probe (PH1) was designed for UV light-activatable CHA. Right: NIR light-triggered CHA in cells using UCNPs-PH1/H2 that is designed by the loading of PH1 and H2 on UCNPs. Reproduced from Ref. [59] with permission. Copyright 2020 American Chemical Society.

linker. As a result, the capability of the resulting PH1 to react with the initiator mRNA is prevented. Upon photolysis of the PC group by UV UCL, the toehold was generated, allowing for the target mRNA-initiated reaction and subsequently successive hybridization with hairpin H2. Since each hybridization event induces FRET between Cy3 and Cy5 labeled in H1 and H2, respectively, the approach enables spatiotemporally controlled amplification of the ratiometric fluorescence signal and thus sensitive imaging of mRNA. Remote control over amplified imaging of c-MYC mRNA in living cells was demonstrated through NIR light activation of the system (UC-PH1/H2). Furthermore, a nuclear localization TAT peptide was exploited to combine with the approach for spatially selective signal amplification in the cell nucleus (Figure 7b).

Since many signal amplification methods have been developed for amplified detection,[9,57] the UCL-mediated design provides a general methodology for sensitive imaging of a wide range of targets with high spatial-temporal resolution based on the integration upconversion nanotechnology with such amplification strategies. For example, as an enzyme-free amplification technique, catalytic hairpin assembly (CHA) has shown great potential for the amplified detection of RNA analytes.[9,58] By extending the H1 strand in the conventional CHA design at both sides and introducing a PC linker into one of the extended segments (Figure 7c), Luo et al. designed an UV light-responsive DNA dumbbell structure probe (PH1), which allowed for photoactivatable CHA and sensitive miRNA detection.[59] They further integrated this pre-blocked sensing system with UCNPs and demonstrated NIR light-controlled imaging of target miRNA in live cells.

As a type of DNA nanomachine, DNA walkers have been widely developed to enhance signal transduction and amplify molecular imaging signals.[60,61] Through the integration of UCNPs with a DNA walker fueled by intracellular ATP, Xian and co-workers constructed a NIR light-triggered sensor system for amplified miRNA imaging (Figure 8a).[62] In the design, a PC linker is inserted into the loop of a hairpin-structured DNA probe (Hp) loaded on the UCNPs' surface. NIR light-induced UCL enables photolysis of the PC linker and thus a SDR of the cleaved Hp probe with target miRNA, leading to release of a Hp-1 strand as the "initiator" of the downstream signal amplification on

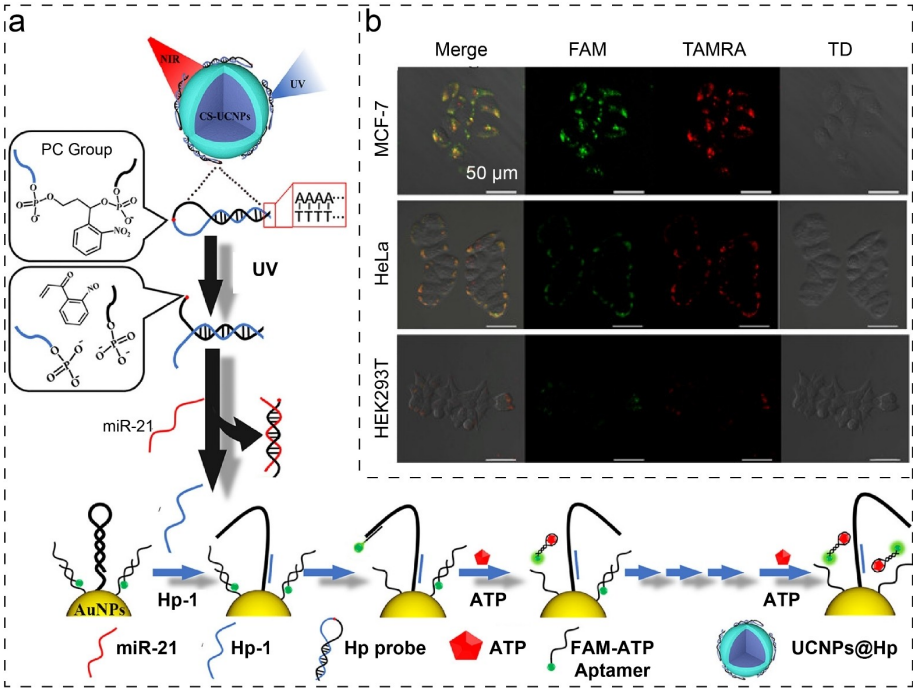

Figure 8. a) Schematic showing UCL-controlled DNA walkers for amplified imaging of miRNA. The system is made of UCNPs loaded with the light-responsive Hp probe and the AuNPs-based DNA walker structure. b) Confocal fluorescence images of intracellular miR-21 expression in MCF-7, HeLa, and HEK293T cells treated with the nanosystems and NIR irradiation. Reproduced from Ref. [62] with permission. Copyright 2021 American Chemical Society.

gold nanoparticles (AuNPs). The hairpin-stable walking stand is activated by the Hp-1 and then hybridizes with the FAM-labeled anti-ATP aptamer stand. Then, the binding with intracellular ATP leads to a release of FAM-labeled aptamer strand from AuNPs and thus fluorescence increase. Importantly, such a competitive binding is able to recycle the walking stand for continuous release of the FAM-labeled aptamer strand, resulting in the generation of a number of fluorescence signals for signal amplification. The authors demonstrated the utility of the NIR-triggered DNA walker system for target miRNA analysis in different cell lines (Figure 8b).

5. Spatiotemporally Selective Targeting of Receptor Protein

Cell-associated protein receptors are well-known targets for many diseases such as cancer. Through specific biorecognition of such receptors, targeted imaging and treatment can be achieved. Unfortunately, such receptors that often have a high expression in diseased cells are also expressed in normal cells at a relatively low level.[63] Therefore, conventional targeting design can also lead to the interaction with the same target on normal cells, resulting in "on-target, off-tumor" effects.

Recently, we developed an orthogonal UCL-controlled biorecognition strategy that permits spatiotemporally controlled tumor targeting and treatment (Figure 9a).[64] The system (PT-UN) was built by the modification of a light-responsive DNA aptamer module (L-Apt) and photosensitizers (PSs) on the surface of UCNPs. L-Apt is a hybrid of a specific aptamer and its complementary strand bearing two PC linkers, in which the nucleolin (NCL) receptor recognition capability of the aptamer is inhibited until the UV light-mediated cleavage of PC linkers and thus liberation of the aptamer. As orthogonal light transducers, UCNPs could convert two distinct NIR lights of different wavelengths (808 and 980 nm) into UV and green UCL, respectively. After the nanodevices enter the tumor microenvironment, UV UCL generated by 808 nm NIR light irradiation allowed the liberation of the aptamers for targeting NCL on tumor cells, while green UCL induced by 980 nm NIR light irradiation enables excitation of PSs for generation of ROS. 808 nm NIR light-mediated irradiation of the tumor site in the mice treated with PT-UN showed significantly enhanced intratumoral fluorescence (Figure 9b), indicative of photoactivated targeting. In addition, we directly modified aptamers on UCNPs to achieve conventional targeting system (termed as T-UN). In bioimaging studies the PT-UN + 808 nm group showed 2.0-fold higher fluorescence ratio of tumor to liver than that in the T-UN group, confirming the UCL-mediated design enables an improved tumor-targeting specificity compared with the conventional targeting system (Figure 9c). This is because NIR-mediated local activation at tumor sites could reduce the nonspecific target binding in normal tissues and thus decrease "on-target, off-tumor" effects. As another control, NIR-irradiation of inactive nPT-UN, which had the same structure with PT-UN but without

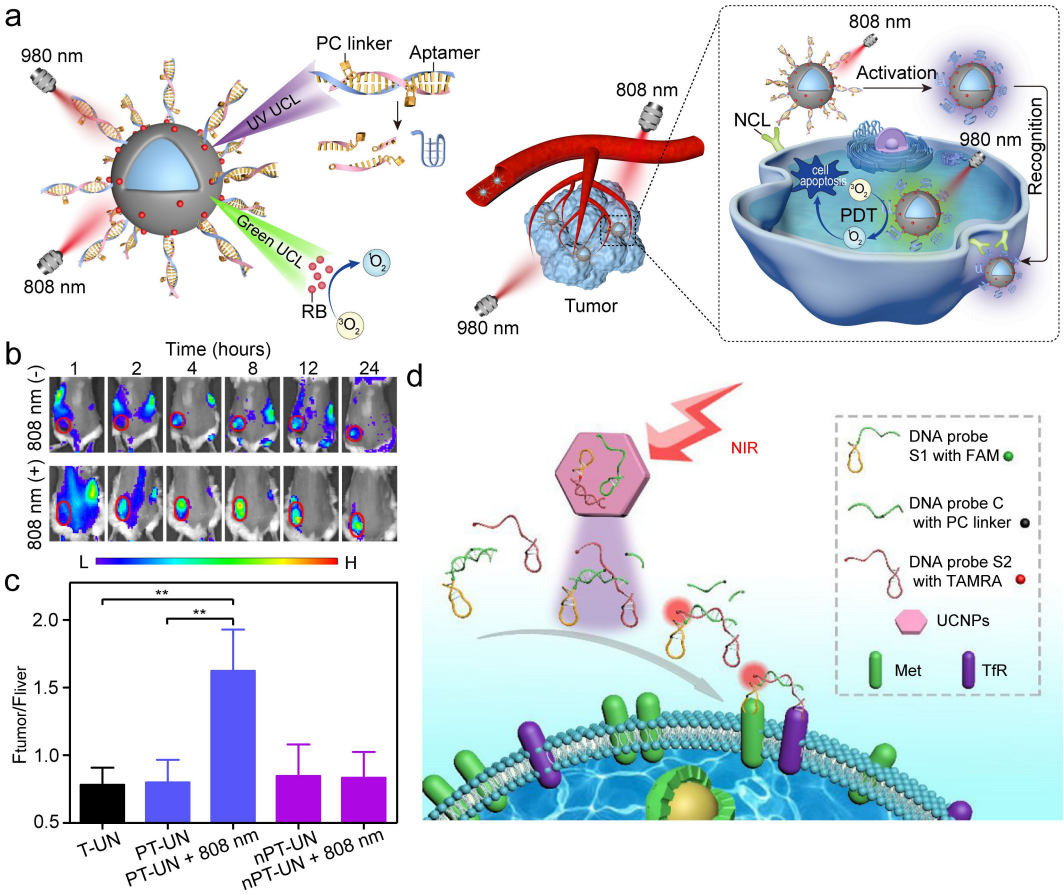

Figure 9. a) Design and working mechanism of an orthogonally regulatable DNA nanodevice (PT-UN), which is constructed by modification of a UV light-responsive DNA aptamer module and rose bengal (RB) photosensitizers on the surface of UCNPs. b) Fluorescence images of tumor-bearing mice intravenously treated with PT-UN (in which RB photosensitizers were replaced with Cy5 fluorophores for imaging) with or without subsequent 808 nm NIR light irradiation. c) The ratio of fluorescence intensity in tumors to that in livers for mice after different treatments. UCNPs were modified with anti-NCL aptamers to yield a conventional targeting system (termed as T-UN). nPT-UN, which has the same structure as PT-UN but lacks the PC linker, was designed as a control. Reproduced from Ref. [64]. Copyright 2020 AAAS. d) Design of DNA nanoclamps for single-molecule imaging of receptor protein oligomerization on cancer cells. Reproduced from Ref. [65] with permission. Copyright 2021 American Chemical Society.

a PC linker, led to no obvious tumor targeting enhancement (Figure 9c).

Recently, Ouyang et al. developed a UCL-controlled DNA nanoclamp for spatiotemporally controlled single-molecule imaging of receptor protein oligomerization (Figure 9d).[65] The system consisted of three DNA strands: 1) FAM-labeled DNA probe S1; 2) TAMRA-labeled DNA probe S2; and 3) DNA probe C containing a PC linker. Probe S1 contains an anti-Met aptamer domain and an extended domain which is complementary to the probe C, while Probe S2 contains an anti-TfR aptamer domain and an extended domain which possesses identical sequences to probe C. By equipping the designed DNA probes on UCNPs, the DNA nanoclamp facilitates NIR light-induced photolysis of the PC linker in probe C that is pre-hybridized with the extended domain of probe S1, making probe S1 accessible to probe S2 for hybridization of the two extended regions which are complementary to each other. Consequently, a high FRET efficiency between FAM and TAMRA can be observed. Furthermore, owing to the fluidity of the cell membrane, the two target receptors, Met and TfR, can be pulled close to each other when they are bound to the aptamer domains of probes S1 and S2. Importantly, the spatiotemporal control could reduce background signals and improve the sensitivity, allowing imaging of protein dimers on individual cell membranes via the combination with the single-molecule counting technique.

Considering the availability of diverse protein-binding aptamers, the strategy can be applied to NIR light-mediated control over the activities of various proteins for diagnostic and therapeutic applications. Recently, Tan, Jiang, and Qiu et al. showed that the combination of UCL technology with specific aptamers made it possible to regulate protein function in cells.[66] An aptamer and a photoresponsive double-stranded DNA containing a PC linker were modified on the surface of UCNPs to achieve the nanoplatform.[66] UCL is capable of unwinding the double-stranded DNA and releasing one strand to hybridize with adjacent aptamer, thereby allowing to inhibition of the aptamer function and thus regulate the translocation behavior of target proteins.

6. Conclusion and Perspectives

In this Minireview, we have presented a conceptual methodology for the spatiotemporal control over the sensing function of DNA-based biosensors, in which the sensing activity remains silent but can be switched on by UCL generated by UCNPs upon deep-tissue-penetrable NIR light illumination. This revolutionary method is still in its infancy but has already demonstrated tremendous potential. With this strategy, a series of DNA-based biosensors have been conceived for remotely controlled imaging of diverse molecular targets (e.g., small molecules, metal ions, RNA, and proteins), and even multiplexed molecular imaging has been achieved via NIR light-regulated DNA computation. Furthermore, spatiotemporally controlled sensor technologies have been developed for the in situ imaging of specific target molecules in an intracellular organelle of choice (e.g., mitochondria and nucleus) through UCL-mediated photoactivation after targeted subcellular localization. Spatially and temporally resolved signal amplification was further achieved via UCL-initiated HCR, CHA, and DNA walkers, which allow amplified imaging of RNA with high spatiotemporal resolution. In addition, the strategy was expanded to regulate the biorecognition activity of functional DNA to receptor proteins, achieving improved spatial specificity and thus mitigating the "off-tumor" effect.

Despite progress made, there are issues that should be taken into consideration in future research. The NIR light-mediated photoactivation of biosensing is closely related to the UCL efficiency. Aiming to improve the activation efficiency, the development of synthetic approaches for UCL enhancement via remotely controlled sensor design is expected. For example, modification of suitable fluorophores on the surface of UCNPs can significantly increase photon upconversion efficiency.[67] In addition, although NIR light has obvious advantages over UV and visible light, its penetration capability is still limited to superficial tissues. The integration with invasive optical fiber implants will improve their clinical potential.[68] Alternatively, since X-ray are more capable of tissue penetration, the introduction of scintillating materials as transducers that can convert X-rays into UV/Visible emission[69] may significantly enhance the efficiency of photoactivation in deep tissues. Moreover, given that many disease-specific biomarkers have been characterized, we envision that they could provide promising endogenous stimuli for controlling the spatiotemporal selectivity of molecular sensing and imaging. For example, most recently, a cancer cell-specific endogenous enzyme was exploited for the spatiotemporal control of DNAzyme-based sensors, enabling cancer cell-selective metal-ion sensing and imaging.[70] Since many proteases have been recognized as cancer biomarkers, we introduced peptide nucleic acid to bridge the gap between peptide and functional DNA and we thus designed a protease-activatable aptamer sensor, which allows for improved molecular imaging specificity to tumor cells.[71] Overall, the development of conditionally controlled, DNA-based sensor technologies opens an avenue for spatiotemporally selective precision imaging. Looking forward, we expect that these methods will be broadly applied for investigating the spatial and temporal dynamics of key molecules in diverse biological and biomedical processes.

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Conflict of Interest

The authors declare no conflict of interest.

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